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Molecular segregation at multiple scales in perylene-based systems for the preparation advanced materials

Adrià Pérez Calm

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UNIVERSITAT DE BARCELONA

FACULTAT DE FARMÀCIA I CIÈNCIES DE L'ALIMENTACIÓ

MOLECULAR SEGREGATION AT MULTIPLE SCALES IN PERYLENE-BASED
SYSTEMS FOR THE PREPARATION OF ADVANCED MATERIALS

ADRIÀ PÉREZ CALM
2023

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DOCTORAT EN NANOCIÈNCIES

MOLECULAR SEGREGATION AT MULTIPLE SCALES IN PERYLENE-BASED
SYSTEMS FOR THE PREPARATION OF ADVANCED MATERIALS

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Abstract

This thesis addresses molecular segregation in perylene-based systems, including lyotropic chromonic liquid crystals and water-in-water emulsions. These systems offer several advantages compared to conventional surfactant- and oil-based systems, such as lower toxicity and environmental impact, as well as reduced processing constraints, e.g. reduced foam formation. In particular, this work focuses on the formation of chromonic mesophases by water-soluble perylene diimide dyes, which self-assemble forming columnar aggregates that evolve to chromonic liquid crystals at high concentrations. Perylene diimides are compounds with high technological interest due to their unique optical and semiconducting properties, chemical stability and versatility in terms of organic synthesis and self-assembly. In this thesis, the chromonic behavior of several nonionic, cationic and anionic perylene diimides has been systematically studied by polarized optical microscopy (POM) and Small-Angle X-Ray Scattering (SAXS). The chromonic behavior was found to be significantly influenced by the chemical structure of the perylene diimides, and some conclusions about the structure-phase behavior relationship were drawn.

Additionally, the thesis reports the emulsification of these chromonic mesophases in all-aqueous two- and three-phase emulsions, which yielded several interesting confined chromonic structures and complex drop morphologies such as Janus and snowman.

Furthermore, this work explores the preparation of advanced materials from the perylene-based chromonic aggregates. These include anisotropic gels, microporous silica materials with interesting applications as drug carriers, and nitrogen-doped carbon nanofibers with high performance supercapacitor properties. The silica materials revealed a gradual release of small-molecule drugs (ibuprofen) and functioned as substrate for hydroxyapatite growth forming silica-hydroxyapatite composites. Besides, the carbon nanofibers registered high specific capacitances over $250 \text{ F} \cdot \text{g}^{-1}$ with very high life cycles and outstanding capacitance retentions at high current densities, in three-electrode cell systems, which is promising as electrochemical energy storage applications where high power is required. In addition, the carbon nanofibers functioned as efficient materials for carbon dioxide capture.

Moreover, perylene-DNA interactions were studied. DNA binding was observed for positively charged perylenes but not for the anionic and nonionic variants.

In summary, this thesis contributes to a better understanding of the behavior of perylene-based chromonic liquid crystal and chromonic-based all-aqueous emulsions, and demonstrates the high potential of these systems for the preparation of advanced materials with applications in various fields, such as drug delivery and energy storage.

Publications

The work described in this thesis is partially published in:

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- Magana, J.R., Pérez-Calm, A. & Rodríguez-Abreu, C. (2022). **Chromonic nematic liquid crystals in a room-temperature ionic liquid.** *Chemical Communications*, 58(11), 1724-1727. <https://doi.org/10.1039/D1CC05800B>
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List of abbreviations and symbols

Abbreviations

AC: Activated carbon
Ala: Alanine
ATPS: Aqueous two-phase system
BET: Brunauer-Emmett-Teller (for specific surface area)
BF: Bright field
BJH: Barrett-Joyner-Halenda (for pore size distribution)
br: Broad
CD: Circular dichroism
CE: Coulombic efficiency
cmc: Critical micelle concentration
CNF: Carbon nanofiber
CNT: Carbon nanotube
CoIh: Columnar hexagonal
CoIr: Columnar rectangular
CSIC: Consejo Superior de Investigaciones Científicas
CV: Cyclic voltammetry
CTAB: Cetyltrimethylammonium bromide
DAN: 1,5-dialkoxynaphthalene
Dex: Dextran
DMEM: Dulbecco's modified eagle medium
DMSO: Dimethyl sulfoxide
DNA: Deoxyribonucleic acid
D_p: Mesopore diameter
DSC: Differential scanning calorimetry
DSCG: Disodium cromoglycate
EDLC: Electrical double-layer capacitor
EDX: Energy-dispersive X-ray
EE: Energy efficiency
EIS: Electrochemical impedance spectroscopy
ESR: Equivalent series resistance
EtOAc: Ethyl acetate
EtOH: Ethanol

FA: Furfuryl alcohol

FESEM: Field emission scanning electron microscopy

FFT: Fast-Fourier transform

FTIR: Fourier-transform infrared spectroscopy

GCD: Galvanostatic charge-discharge

GCE: Glassy carbon electrode

GdL: Glucono- δ -lactone

H₁: Hexagonal

H₂: Inversed hexagonal

HDTMS: Hexadecyl trimethoxy silane

HK: Horvath-Kawazoe (for micropore size distribution)

HR-TEM: High-resolution transmission electron microscopy

H-bond: Hydrogen bond

I: Isotropic

I₁: Cubic

I₂: Inversed cubic

Ibu: Ibuprofen

IbuEE: Ibuprofen encapsulation efficiency

Ibu_{Load}: Amount of ibuprofen loaded/encapsulated

Ibu_{Total}: Amount of ibuprofen used in the loading/encapsulating method

ICD: Induced circular dichroism

INL: International Iberian Nanotechnology Laboratory

IQAC: Instituto de Química Avanzada de Cataluña

L _{α} : Lamellar

LCLC: Lyotropic chromonic liquid crystal

LCST: Lower critical solution temperature

LMWG: Low molecular weight gel

M: Hexagonal (chromonic liquid crystals)

ML: Modified lignin

MM: Melting method

Mon: Monolith

Mon_{Load}: Weight of a silica monolith loaded with ibuprofen

Mon_w: Weight of a silica monolith

MS: Magnetic stirring

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide

MW: Molecular weight

MWCNT: Multiple-walled carbon nanotube

MWCO: Molecular weight cut-off

N: Nematic

N*: Chiral nematic

N_{Col}: Nematic columnar

N_D: Nematic discotic

NDI: Naphthalene diimide

NIMS: National Institute for Materials Science

NLDFT: Non-local density functional theory

NTCDA: Naphthalene tetracarboxylic acid dianhydride

O: Orthorhombic or rectangular (chromonic liquid crystals)

O/O: Oil-in-oil (emulsion)

O/W: Oil-in-water (emulsion)

PAH: Polyaromatic hydrocarbon

PAN: Polyacrylonitrile

PANI: Polyaniline

PBS: Phosphate buffered saline

PDI: Perylene diimide

PEDOT: Poly(3,4-ethylenedioxythiophene) polystyrene sulfonate

PEG: Polyethylene glycol

PEI: Polyethylene imine

POM: Polarized optical microscopy

PTCDA: Perylene tetracarboxylic acid dianhydride

QDI: Quaterylene diimide

RBI: Rylene bisimide

RCF: Relative centrifuge force

RDI: Rylene diimide

%Rel: Percentage of released drug

rGO: Reduced graphene oxide

SAXS: Small-angle X-ray scattering

SBF: Simulated body fluid

SDM: Solvent + drying method

SEM: Scanning electron microscopy
SmA: Smectic A
SmC: Smectic C
 S_{Micro} : Micropore surface area
SSA: Specific surface area
TDI: Terrylene diimide
TEM: Transmission electron microscopy
TEOS: Tetraethyl orthosilicate
TGA: Thermogravimetric analysis
TMOS: Tetramethyl orthosilicate
Tris: Tris(hydroxymethyl)aminomethane
TSI: Turbiscan stability index
US: Ultrasound
UV: Ultraviolet
Vis: Visible
 V_{Micro} : Micropore volume
 V_{P} : Total pore volume
WAXS: Wide-angle X-ray scattering
 W_{P} : Pore width (from NLDFT)
wt%: Percentage by weight
W/O: Water-in-oil (emulsion)
W/W: Water-in-water (emulsion)
W/W/O: Water-in-water-in-oil (emulsion)
W/W/W: Water-in-water-in-water (emulsion)
XPS: X-ray photoelectron spectroscopy
XRD: X-ray diffraction

Parameters

a : Hexagonal (or rectangular) lattice parameter
 C_s : Specific capacitance
 d_0 : First-order Bragg spacing
 d_H : Hexagonal first-order spacing

Symbols

θ : Angle
 χ : Flory-Huggins parameter

v : Vibronic energy level

λ : Wavelength

1. INTRODUCTION AND LITERATURE REVIEW

Molecular segregation is a phenomenon in which there is a local enrichment of certain molecular component(s) with simultaneous depletion in other regions of the system (Figure 1.1). Segregation includes both aggregation and phase separation systems, where the former refers to the self-assembly of building blocks, while the latter refers the separation between two immiscible phases, such as oil and water. Segregation processes typically occur due to intermolecular forces and solvophobic interactions, that induce segregation by self-assembly and phase separation within the system.

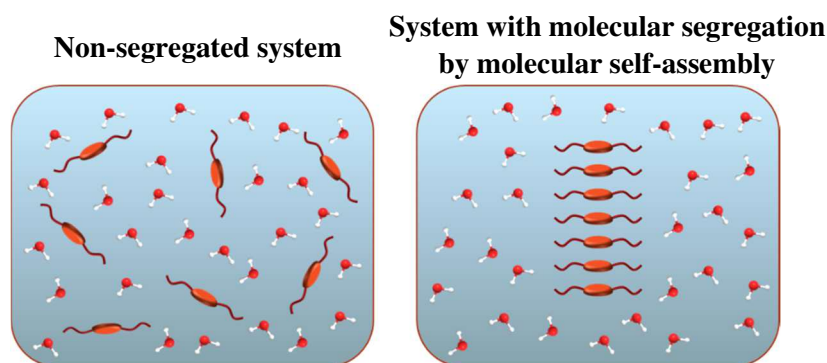


Figure 1.1. Comparison between a non-segregated system, consisting in a solute homogeneously dispersed in the solvent (left), and a system with molecular segregation, in which the solute self-assembles forming a solute-rich region (the aggregate) and a solute-depleted region.

Examples of aqueous systems that show molecular segregation are soap bubbles, lyotropic liquid crystals, vesicles such as micelles, liposomes and hexosomes, and emulsions (Figure 1.2). In all these systems, their forming components (surfactants, chromonic mesogens, polymers, water, oil...) self-assemble and/or phase separate yielding molecularly segregated systems. Note that the molecular segregation can occur at multiple scales. For example, in liquid crystal systems, the structures formed are nanometric, whereas in emulsions the droplets are typically between 100 nm and several micrometers.

The development of aqueous systems exhibiting molecular segregation is of high scientific and technological interest as they allow to organize matter in liquid systems, at the molecular level, through low energy methods, and in a bottom-up approach (i.e. matter spontaneously self-assembling into complex nanostructures through intermolecular interactions, in contrast to top-down approaches, which involve breaking up large pieces of materials to obtain the desired nanostructures). The bottom-up approach allows the preparation of nanostructures with higher resolution compared to the top-down approach ^[1–3].

These systems are of high interest in several areas such as pharmacy, food science, cosmetics, optics, electronics and material science. For instance, liposomes, cubosomes and hexosomes are very promising drug carriers ^[4–8], and oil-in-water (O/W) and water-in-oil (W/O) emulsions have been widely used in pharmacy, cosmetic and food industries ^[9–12]. Moreover, lyotropic amphiphilic liquid crystals are unique templates to prepare complex porous materials with applications in several fields ^[13–17]. However, all these systems share the requirement of using either a surfactant, an oil or both to be formulated, which may be non-desired for some applications due to their potential toxicity, environmental impact, and processing limitations. For example, incorporating proteins in these systems may be complicated as surfactants tend to unfold these biomolecules affecting their biological properties ^[18–20].

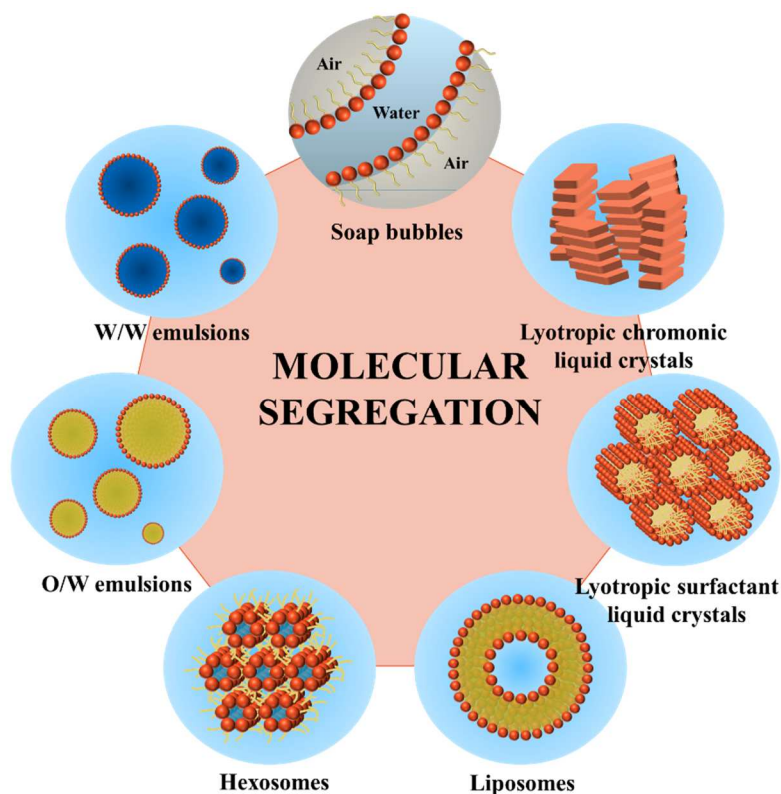


Figure 1.2. Examples of molecularly segregated systems in water. The blue and dark blue regions represent water, the dark-yellow regions represent hydrophobic moieties. O/W and W/W emulsions mean oil-in-water and water-in-water emulsions, respectively.

In this context, developing all-aqueous non-surfactant systems with molecular segregation is highly desirable to complement the surfactant-based formulations in situations where surfactant and oils should be avoided. These systems mainly include lyotropic chromonic liquid crystals (LCLC) and their water-in-water (W/W) emulsions, which unfortunately have been much less studied than their surfactant- and oil-based counterparts ^[21,22]. Accordingly, this thesis aims to study the formulation of LCLC and chromonic-based W/W emulsions, and produce complex materials with potential applications in the pharmaceutical and energy fields. Thus, this work is expected to contribute to a better understanding of both chromonic liquid crystals and W/W emulsions, and reveal the potential applications of these systems to template advanced materials for drug delivery and energy applications.

The following sections of the Introduction chapter provide the fundamental information to understand the formation and properties of both chromonic liquid crystals and water-in-water emulsions, giving context about their history and current state. Furthermore, the templating of advanced materials using these systems as templates is also covered.

In this context, the introduction is divided as follows: First, the main intermolecular interactions leading to molecular segregation are described, including $\pi - \pi$ interactions and solvophobic effects (Section 1.1). Afterwards, a section covering the formation, properties and applications of LCLC, alongside with a comparison of the chromonics with the other known thermotropic and lyotropic liquid crystals, is presented (Sections 1.2 and 1.3). Finally, this introduction provides a section covering W/W emulsions, with particular focus in chromonic liquid crystal-based W/W emulsions (Sections 1.4 – 1.6).

1.1 Intermolecular forces directing molecular segregation

Intermolecular interactions are the primary cause leading to molecular segregation. These are non-covalent attractive or repulsive forces that appear between molecules, including ionic forces, dipole – dipole and ion – dipole interactions, hydrogen bonds, dipole – induced dipole and dispersion interactions, $\pi - \pi$ interactions, and hydrophobic (or solvophobic) effects. All these intermolecular interactions are depicted in Figure 1.3.

Ionic or coulombic forces are one of the strongest intermolecular interactions^[23]. They consist in non-directional long-range attractive or repulsive forces between oppositely or equally charged ions, respectively; and their strength respond to the Coulomb's equation for the electric force between two punctual charges^[24]. Notably, ionic self-assembly has been extensively used to synthesize supramolecular materials^[25,26].

Dipole – dipole and ion – dipole interactions occur between two molecules with a permanent dipole or a dipolar molecule and an ion, respectively. Similar to ion – ion interactions, these forces consist in electric attraction or repulsion, although they cannot be considered as punctual charges, and thus are directional^[27]. Moreover, the strength of dipole – dipole and ion – dipole interactions is lower than for ion – ion bonding^[23]. Dipole – dipole interactions are also known as Keesom forces.

Hydrogen-bonds (H-bonds) are a particular case of dipolar interactions involving an electronegative atom (also called donor or D, most often O, N or F) bound to a hydrogen atom and a second electronegative atom with lone electron pair (also called acceptor or A, typically O, N, F, Cl, Br, I or S), forming a three-center four-electron D – H --- A intermolecular bond^[28,29]. H-bonds show overlapping between the orbitals involved in the bond^[30], are stronger than conventional dipole-dipole interactions (up to 40 kcal/mol^[31]), and highly directional (being D – H – A angles optimally close to 180°), although this may not always be the case for some systems such as H-bonds with acceptor halogens in crystal structures, bifurcated H-bonds or H-bonds involving aromatic moieties as acceptors^[32].

Dispersion forces, also known as London forces, are intermolecular interactions appearing for all molecules, including non-polar molecules^[33]. They consist in the formation of an instantaneous dipole in a molecule that induces a dipole in a polarizable neighbor molecule^[34]. Dispersion forces are the weakest intermolecular interactions for one pair interaction (i.e. a single A – B dispersion intermolecular interaction). However, since they can appear anywhere, the cumulative effect makes them very significant in bigger molecules^[34]. For example, dispersion forces can explain why alkanes with higher molecular weights (hexane, heptane, octane...) are liquid at room temperature, whereas low molecular weight alkanes such as methane, ethane, propane and butane are gases.

Besides, there are intermediate interactions in between Keesom (dipole – dipole) and London (instantaneous dipole – induced dipole) consisting in a permanent dipole inducing a dipole in a polarizable non-polar molecule. These forces are known as permanent dipole – induced dipole or Debye interactions, and are characterized for having an intermediate strength between the Keesom and London forces and being always attractive^[35].

In addition to these attractive forces, molecules in close proximity also experience a powerful repulsive force resulting from the repulsion between their atomic nuclei and the overlapping of their electron clouds. Such overlapping violates the Pauli's exclusion principle, hence producing a strong repulsive force. The overall interplay between these attractive and repulsive interactions is commonly referred to as Van der Waals forces.

$\pi - \pi$ interactions, also called π -stacking, are a widely used intermolecular force to describe the self-assembly of aromatic systems. In general, $\pi - \pi$ interactions refer to the formation of molecular aggregates led by the intermolecular interaction between aromatic moieties. These interactions can be either parallel face-centered (face-to-face), parallel offset or perpendicular edge-to-face T-shaped (see Figure 1.3), generally being the parallel offset and perpendicular conformations more energetically favorable^[36], meanwhile the parallel face-centered is typically preferred when mixing electron-poor and electron-rich aromatic compounds^[37,38]. This is in agreement with the Hunter and Sanders model for $\pi - \pi$ interactions^[39], which considers that electron-rich aromatic molecules exhibit partial negative charge density in the center, but positive in the periphery, whereas electron-poor aromatic compounds show the opposite charge distribution. Accordingly, two aromatic molecules with similar charge distribution will prefer either a parallel offset or edge-to-face conformation to prevent the electrostatic repulsion of a parallel face-centered stacking. In contrast, for 1:1 mixtures of aromatic electron-rich and electron-poor molecules, a face-centered sandwich stack of alternating electron-rich and electron-poor molecules will be preferred. Despite this, more recent studies claimed that the incorporation of electron-donor or acceptor substituents not only affects the stacking by changes in the charge density of the aromatic core but also by direct interaction between the substituent and the adjacent stacked aromatic molecule^[40–42].

As a result of this complex behavior, the terms $\pi - \pi$ interactions and π -stacking are very controversial^[43,44], and some authors have claimed that they are misleading and hence should be avoided^[45], since they can mislead non-experts to the belief that aromatic molecules tend to stack in parallel face-centered aggregates independently of the charge distribution and substitution groups, which is not true. Additionally, it is not clear how significant $\pi - \pi$ interactions are in small aromatic compounds such as benzene^[44], although there is some evidence of $\pi - \pi$ interactions being important for aggregation of polyaromatic hydrocarbons (PAHs)^[46].

Furthermore, the solvent effects have also demonstrated to play a fundamental role in $\pi - \pi$ interactions, especially in polar protic solvents such as water. This is clearly exemplified in a study by Iverson et al.^[47], about the self-assembly in different solvents (including water) of an electron-rich 1,5-dialkoxynaphthalene (DAN) and an electron-poor naphthalene diimide (NDI). They determined the aggregation constants of DAN, NDI and a DAN-NDI 1:1 mixture in chloroform, acetone, DMSO, acetonitrile, methanol, water and methanol-water mixtures, and observed that the aggregation constants were higher for the DAN-NDI 1:1 mixture in all solvents, indicating the presence of an electrostatic interaction between the two compounds. However, the study also revealed that the aggregation constants increased with the solvent polarity for the three studied systems, especially when protic polar solvents were used, which contrasts with a purely electrostatic interaction, and suggests a strong solvent effect. Note that polar solvents should screen electrostatic interactions, thus reducing the aggregation constants. Similar results were obtained by Shimizu et al.^[48], confirming that the solvent plays a major role in π -stacking^[49].

The effect behind the solvent-dependent π -stacking is called solvophobic effect. It is very significant for the aggregation of hydrophobic molecules (of partially hydrophobic molecules) in water. Therefore, it is often referred to as hydrophobic effect. Note that the term solvophobic is more general and includes all solvents whereas hydrophobic particularly refers to water as the solvent. The hydrophobic effect is typically described as the tendency of water to exclude hydrophobic molecules provoking their aggregation, due to an apparent repulsion between the highly polar solvent and the non-polar solute^[50,51]. Historically, the hydrophobic effect has been explained with the clathrate or “iceberg” model proposed by Frank and Evans back in 1945^[52], which proposes that upon adding a non-polar solute in water, the solvent molecules structure

around it adopting a quasi-solid state, which is entropically expensive, and hence thermodynamically unfavorable. Consequently, the non-polar solute will tend to aggregate in order to reduce its contact with water. However, today this model is controversial, as there is no clear evidence of the water molecules adopting ice-like structures around non-polar solutes [53].

Alternatively, other models have emerged to complement the “iceberg” model [54,55], which take into consideration other parameters, such as a higher number of H-bonds between water in bulk compared to water surrounding a hydrophobic surface, which would lead to an enthalpy gain, by releasing water clustering the solute, upon aggregation [56,57]. Additionally, the importance of the non-polar solute size [55,58] and shape [59] has also been evaluated, observing that hydrating big solutes is more energetically expensive and hence the hydrophobic effect is more evident for high-molecular weight hydrophobic compounds [58]. In summary, the nature of hydrophobic effect nature is still on debate and most likely occurs due to a sum of different enthalpic and entropic factors.

Besides these, there are other intermolecular interactions involving aromatic moieties such as cation- π , which consist in an electrostatic interaction between the positively charged cation and the center of electron-rich aromatic compounds (not necessarily annular) such as benzene [60] or tryptophan [61]. Notably, the polarizability of the π -clouds plays a major role in cation- π interactions [62]. Additionally, the less intuitive anion- π interactions have also been described, occurring either between anions and the center of electron-poor aromatic cores [63,64] or through charge-transfer from the anions to the periphery of an aromatic moiety [65].

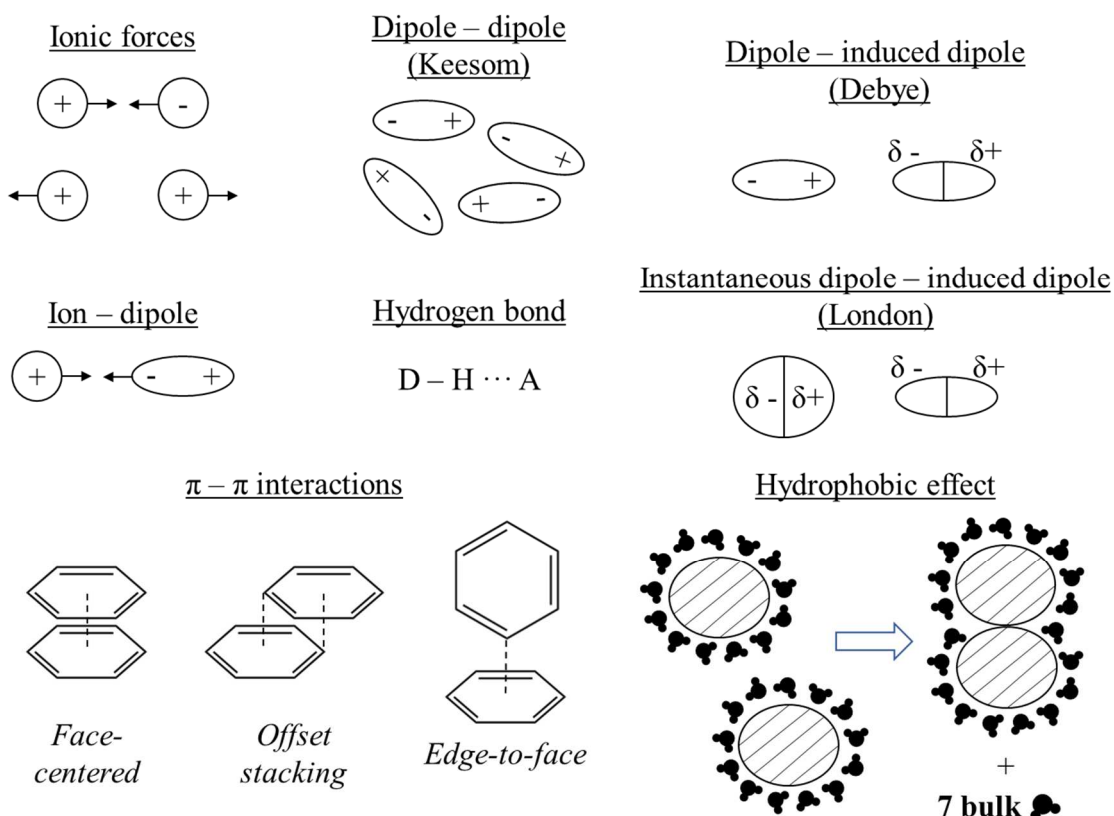


Figure 1.3. Scheme of the main intermolecular interactions.

In summary, it is the interplay between all the forces described in these last paragraphs that direct the aggregation of compounds in solution and trigger the formation of systems with molecular segregation, such as liquid crystals and emulsions.

1.2 Overview of liquid crystals

Liquid crystals are materials that exist in a state of matter in between solids and liquids. In materials science, the term crystal refers to solid materials composed of packed atoms, ions or molecules with tridimensional long-range positional and orientational order; whereas liquids are isotropic materials without any long-range order. This is translated into different macroscopic properties for crystals and liquids. For example, crystal solid materials are typically resistant to deformation, whereas liquids easily deform under the application of a force, hence flowing.

Liquid crystals are a unique state of matter that shares properties of both crystals and liquids, both at the atomic and macroscopic scales. Liquid crystals are typically made up of molecules (hereinafter called *mesogens*, from the Ancient Greek *meso* and *genés*, which mean middle and producer, respectively). These mesogens self-assemble forming structures that possess either long-range orientational order, positional order, or both (like a crystal), but reduced as compared to solid crystals. This gives the mesogens freedom to move in at least one dimension, allowing the liquid crystal materials to flow (like a liquid) at the macroscopic scale (Figure 1.4). The liquid-crystalline phases are also called *mesophases*.

Additionally, liquid crystals are generally anisotropic, which means that they exhibit different mechanical, thermal, optical or electrical properties along different axes of the material. Anisotropy arises due to the different structure of a material as a function of direction. For instance, in the liquid crystal structure shown in Figure 1.4, the arrangement of mesogens is different along the vertical axis compared to the perpendicular directions, which result in the liquid crystal properties varying depending on the direction within the material.

This is further illustrated by the optical properties of liquid crystals. Liquid crystals exhibit an optical property called birefringence, which consists in the material showing different refractive indexes depending on the direction of propagation of the light within its structure. This property is also observed in most crystalline solids, which are also anisotropic, but not in isotropic liquids. The only exception are cubic crystalline solids and liquid crystals, since the cubic lattice only shows positional order, but not orientational order nor anisotropy. Note that orientational order refers to the oriented distribution of molecules towards one direction, whereas positional order consists in a translational symmetry of atoms, ions or molecules within a material structure.

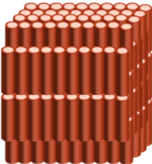
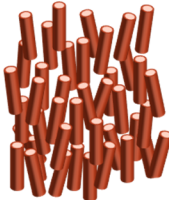
Crystalline solid	Liquid crystal	Isotropic liquid
		
• Packed constituents • Long range 3D orientational and positional order • Do not flow • Typically anisotropic	• Less-packed constituents • Long range orientational and/or positional order • Flow (at least in one direction) • Typically anisotropic	• Non-packed constituents • No long-range orientational nor positional order • Flow • Isotropic

Figure 1.4. General structure and properties of a crystalline solid, a liquid crystal and an isotropic liquid.

Historically, liquid crystals were first observed by Heintz (1849-1855), who described “a second melting point” for stearin samples ^[66]. In his article, Heintz wrote “Stearin becomes cloudy at 51-

52°C, at higher temperatures it opalizes, and at 58°C it is completely opaque. Above 62.5°C it is molten and quite clear”^[67]. Almost simultaneously, Virchow described a soft floating substance surrounding the nerve core, and called it myelin^[68,69], and Mettenheimer observed that myelin was birefringent^[70]. Almost 40 years later, in 1888, the Austrian scientist Friedrich Reinitzer described a similar behavior for cholesterol derivatives extracted from carrots, observing that “the substance melted to a cloudy liquid at 145.5 °C and became a clear liquid at 178.5°C”^[71]. Further research about these materials by Reinitzer and Otto Lehman ended with the latter discovering that the cloudy liquids described by Heintz and Reinitzer were birefringent liquids^[72]. Otto Lehmann greatly contributed to the development of the liquid crystal field during the 1890 – 1900 decade^[73], and published a compilation of his research in 1904, which included the term “Flüssige Kristalle” (liquid crystal in German) in the title^[74]. Note that the term “Flüssige Kristalle” was first used by Gattermann and Ritschke in 1890^[75], and later on popularized by Lehmann.

Today, our knowledge about liquid crystals has expanded drastically, and several different types of these materials have been described. Liquid crystals are traditionally classified as thermotropic and lyotropic. Thermotropic liquid crystals are formed only as a function of temperature, existing between the solid and the liquid phases. As a consequence, materials exhibiting thermotropic liquid crystal phases have at least two melting points between the solid and the liquid phase, one corresponding to the solid → liquid crystal transition and one to the liquid crystal → liquid transition. Both the stearin and the cholesterol derivatives observed by Heintz and Reinitzer are thermotropic liquid crystals.

In general, thermotropic liquid crystals are formed either by rod-like (calamitic liquid crystals) or disk-like (discotic liquid crystals) mesogens (Figure 1.5a,b). Rod-like mesogens are commonly composed by side chain (R), two aromatic groups (A and A') connected with a linkage (X) and another side chain (R') in the other side (Figure 1.5a)^[76,77]. Calamitic liquid crystals form various mesophases, which are normally classified as nematic and smectic liquid crystal phases (Figure 1.5c). In nematic phases, all the mesogens orient towards one direction, the so-called director axis, but they lack of positional order. On the other hand, smectic phases are formed when the mesogens not only are orientationally correlated but also have some degree of positional order, although reduced compared to solids. Notably, smectic mesophases are subcategorized in, mainly, smectic A and smectic C phases, where the main difference is that in smectic A mesophases the mesogens are oriented parallelly to the director axis meanwhile in smectic C phases they are tilted.

Additionally, calamitic liquid crystals also form the so-called cholesteric mesophase^[78], which is thermodynamically equivalent to the nematic phase, but present a helical superstructure due to the presence of chiral groups in the mesogens or by the addition of chiral dopants to the nematic phase (Figure 1.5d).

In contrast, disk-like mesogens are composed by a planar polyaromatic core functionalized with flexible aliphatic chains at the periphery^[79]. Examples are the benzene hexa-n-alkanoates^[80] and triphenylene derivatives^[81,82] (Figure 1.5b), among others like perylene diimides^[83], truxene^[84], phthalocyanines^[85] and hexabenzocoronene derivatives^[86]. Discotic liquid crystals also form a variety of mesophases (Figure 1.5e). At lower temperatures, they show columnar rectangular and hexagonal phases, which consists in stacks of the disk-like mesogens forming columns that organize into rectangular and hexagonal lattices, showing both orientational and positional ordering. At higher temperatures, the positional ordering is lost and a columnar nematic phase appears. Additionally, these mesogens form discotic nematic phases, which consist in all the mesogens orientationally correlated but without forming columnar stacks.

In contrast to thermotropics, lyotropic liquid crystals only occur in solution, and their formation is governed not only by the temperature but also by the concentration of the mesogen in the solvent, which is commonly water ^[87]. The first known example of a lyotropic mesophase is myelin, described by Virchow and Mettenheimer. Afterwards, Lehmann observed that ammonium oleate formed liquid crystal phases with low amounts of water, although he never distinguished this compound from the more common thermotropic compounds. In 1922, the French crystallographer George Friedel made one of the greatest contributions to the lyotropic liquid crystal field ^[88], recognizing the lyotropic mesophases as a distinct and particular type of liquid crystals and hence dividing the liquid crystal field into thermotropic and lyotropic liquid crystals. Friedel also coined the terms mesogen and mesophase.

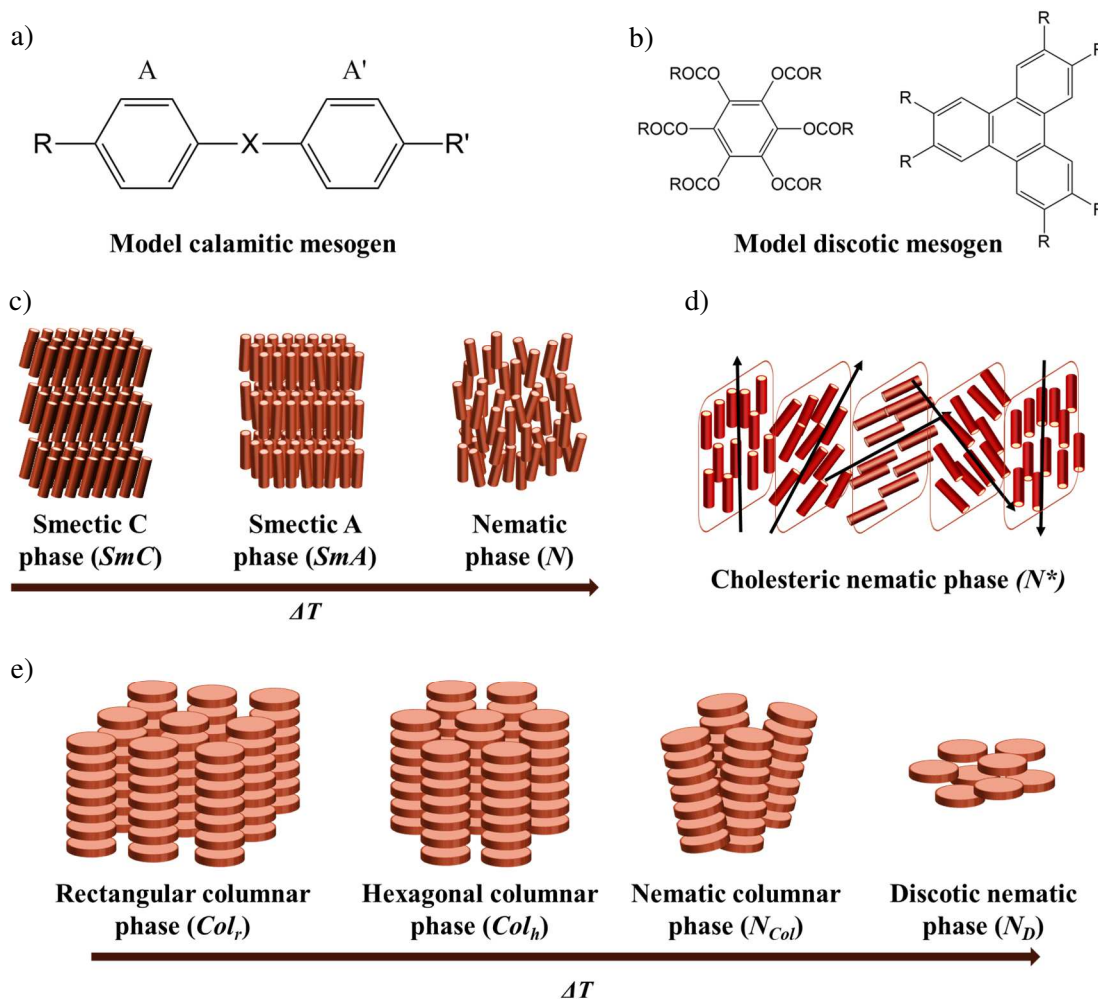


Figure 1.5. Model chemical structure of thermotropic a) rod-like mesogens and b) disk-like mesogens. Structure of c) conventional calamitic liquid crystal phases, d) a cholesteric nematic liquid crystal phase, formed by adding a chiral center or dopant to a calamitic nematic phase and e) conventional discotic liquid crystal phases.

Lyotropic mesogens exhibit a “biphilic” character in their chemical structure, with hydrophilic and hydrophobic parts that interact differently with the solvent, resulting in the formation of complex structures with liquid crystalline behavior. These mesophases are generally formed by amphiphilic molecules in water. Amphiphilic compounds (Figure 1.6a) are composed by a hydrophilic head (glycerol, polyethylene glycol chains, quaternary amines, carboxylates, or phosphates) covalently bonded to a long hydrophobic tail (normally long aliphatic chains). Examples of amphiphiles are soaps, detergents, phospholipids or glycolipids, among others ^[87].

When water is added to amphiphilic compounds, these can either be insoluble (due to very big or rigid hydrophobic moieties), only partially dissolve forming low-concentration micellar aqueous solutions or generate lyotropic liquid crystalline phases at high concentration in aqueous solutions^[89]. At very low concentrations in water, amphiphilic molecules typically adsorb at the air-water interface reducing the solvent surface tension, with the hydrophilic head in the water phase and the hydrophobic tails in the air phase, in an attempt to prevent the repulsive interactions between the water and the hydrophobic chains. This behavior arises from the thermodynamically unfavorable hydration of the hydrophobic moieties of the amphiphiles (hydrophobic effect).

As the amphiphile concentration increases, the air-water interface becomes saturated, and the amphiphilic molecules must penetrate the bulk of the solvent (water). To prevent the hydration of the hydrophobic chains, the amphiphiles spontaneously self-assemble forming spherical nanostructures called micelles (Figure 1.6b), where the hydrophilic heads stay in the outer area of the sphere “hiding” the hydrophobic chains inside. The concentration at which the interface gets saturated and micelles start to form is called critical micelle concentration (*cmc*). Interestingly, the formation of micelles is an entropically-driven process, as it is mainly directed by the exclusion of the hydrophobic chains from water. Moreover, the formation of micelles is not only concentration-dependent but also temperature-dependent, as there is a lower-temperature limit below which the hydrophobic chains become too rigid and cannot form micelles, known as Krafft point^[90].

At higher amphiphile concentrations in water, micelles self-assemble into more complex structures with orientational and/or positional order forming lyotropic mesophases. There is a big variety of lyotropic mesophases, being cubic, hexagonal and lamellar phases the most well-established (Figure 1.6b)^[89]. Notably, both hexagonal and lamellar phases are anisotropic structures that exhibit both positional and orientational order, and thus are birefringent. In contrast, cubic phases only show positional order due to the high symmetry of the cubic lattice.

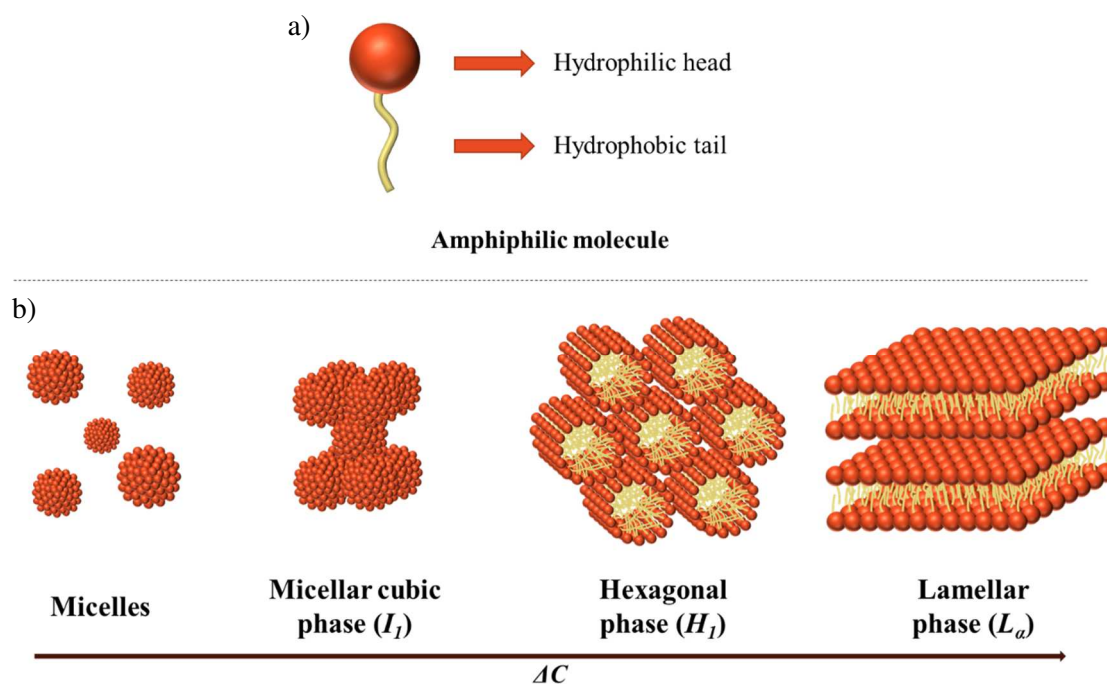


Figure 1.6. a) Structure of amphiphilic molecules. b) Structure of micelles and three lyotropic mesophases (cubic, hexagonal and lamellar).

In general, amphiphilic lyotropic mesogens form cubic mesophases (I_1) at lower concentrations, that evolve to hexagonal (H_1) and lamellar (L_α) as concentration increases (Figure 1.6b). However, inverted hexagonal (H_2) and inverted cubic (I_2) phases can also appear at even higher concentrations, which are equivalent to H_1 and I_1 phases but with the hydrophilic heads and the hydrophobic tails inverted. Additionally, the formation of these amphiphilic mesophases not only depends on the mesogen concentration but also in the structure of the amphiphile. The influence of the structure in the mesogenic behavior is typically described by the packing parameter ^[91], which is calculated as the volume of the hydrophobic part divided by the length of the hydrophobic chain and the area of the hydrophilic head. Therefore, amphiphiles with short and non-branched hydrophobic chains show low packing parameters, whereas bigger hydrophobic moieties exhibit higher packing parameters. In general, low packing parameters (< 1) favor normal structures with high curvature (such as cubic I_1), packing parameters about 1 generate structures with less curvature (hexagonal H_1 and lamellar L_α) and high packing parameters (> 1) induce the formation of inverted structures.

In addition to conventional amphiphiles with the structure shown in Figure 1.6a, other compounds also exhibit the ability to form lyotropic mesophases. Some examples are proteins, synthetic polymers, nanoparticles or rod-like viruses, such as the tobacco mosaic virus ^[92]. Furthermore, there is another group of biphilic molecules formed by small drugs, dyes and nucleic acids that give rise to a new family of lyotropic mesophases known as lyotropic chromonic liquid crystals (LCLC). LCLCs are the main topic of this thesis and, consequently, will be described in detail in the next section.

1.3 Lyotropic chromonic liquid crystals (LCLC)

1.3.1 Chromonic self-assembly

Chromonic liquid crystals are lyotropic mesophases formed plank-like or disk-like biphilic molecules, composed of a hydrophobic planar aromatic core functionalized with hydrophilic moieties at the periphery (Figure 1.7a), which impart water-solubility to the molecules, whereas the hydrophobic core facilitates the self-assembly of these molecules to form chromonic mesophases ^[93–96]. Accordingly, chromonic mesophases are typically formed by small anti-asthmatic and anti-allergy drugs ^[97–99], water-soluble dyes such as perylene diimides ^[100], and nucleic acids (Figure 1.7b) ^[101].

LCLC exhibit several properties that clearly differentiate them from the conventional amphiphilic lyotropic liquid crystals in almost every aspect besides being lyotropic and typically existing in water (Table 1.1) ^[102]. For instance, chromonic molecules do not form micelles but stack like a pile of cards forming columnar aggregates that grow as the mesogen concentration increases, and possess hydrophobic rigid aromatic cores, rather than flexible aliphatic chains.

Moreover, the column elongation in chromonic systems is an additive process, in contrast to the cooperative nature of surfactant micellization. This means that there is no optimal column length and increasing the aggregation number (the number of molecules in an aggregate) only slightly reduces the hydrophobic surface in contact with water, gradually reducing the free energy (Figure 1.8). In contrast, surfactant-based micelles have an optimal size, and consequently an aggregation number of minimum free energy (Figure 1.8) and a critical micelle concentration (cmc). Furthermore, the formation of micelles is an entropically-driven process, whereas the chromonic self-assembly presumably results from the attractive interactions between the π -systems of the planar aromatic groups, and it is exothermic (enthalpically-driven) ^[103–105], although there is also a contribution from the exclusion of the hydrophobic moieties from the solvent (hydrophobic

effect), indicating that the chromonic self-assembly also has significant entropic contributions [106–108]. In addition, there is no chromonic equivalent to the amphiphile Krafft point, and the existence of chromonic mesophases at low temperatures is limited by the formation of ice.

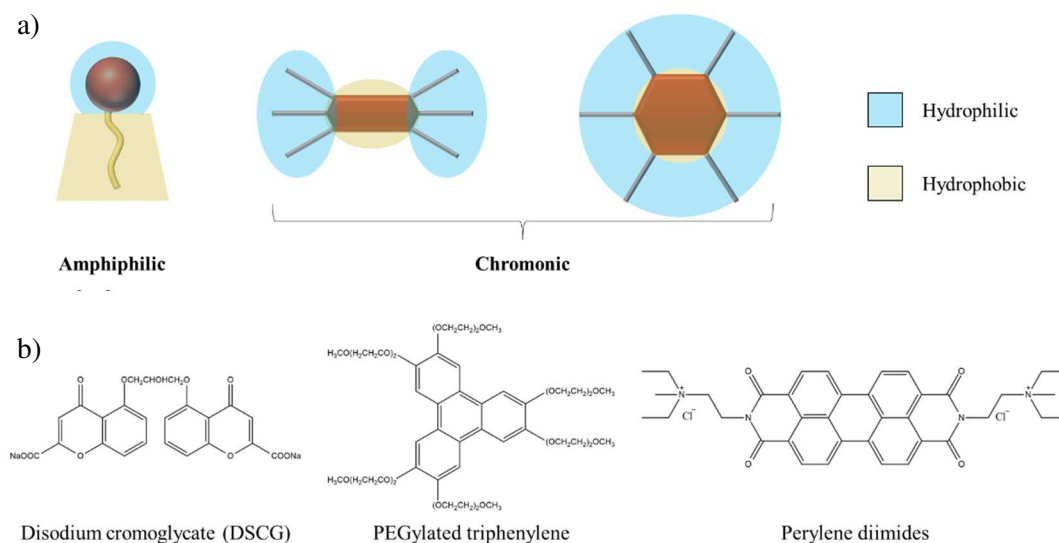
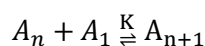


Figure 1.7. a) General structure of amphiphilic and chromonic molecules. The hydrophilic (blue) and hydrophobic (yellow) areas are marked. b) Some examples of chemical compounds forming chromonic mesophases.

Notably, the free energy reduction of adding a chromonic mesogen (A_1) to a chromonic aggregate (A_n) is independent to the size of the aggregate (n), which means that the aggregation constant (K) does not depend on the aggregate size. This behavior is known as isodesmic assembly [109], and it is a distinctive characteristic of chromonic systems.



According to the isodesmic model, there is no minimum aggregate size, and the formation of columnar stacks starts with the formation of dimers. This explains why chromonic mesogens aggregate even at very low concentrations ($10^{-7} - 10^{-5}$ M) in water. Despite this, it should be noted that chromonic self-assembly is not completely isodesmic, as the formation n -mers (up to $n = 4 - 5$ molecules) causes a slightly higher decrease in the free energy than larger aggregates [96], as exemplified in the free energy graph of Figure 1.8.

Table 1.1. Comparison between the main characteristics of the amphiphilic self-assembly and the chromonic self-assembly.

Amphiphilic self-assembly	Chromonic self-assembly
Forms micelles	Forms columnar stacks
Cooperative process (non-isodesmic)	Additive process (isodesmic)
Entropically driven	Enthalpically driven
Limited by Krafft point	Limited by ice formation
Starts at the critical micelle concentration	Starts by the formation of dimers (very low concentrations)

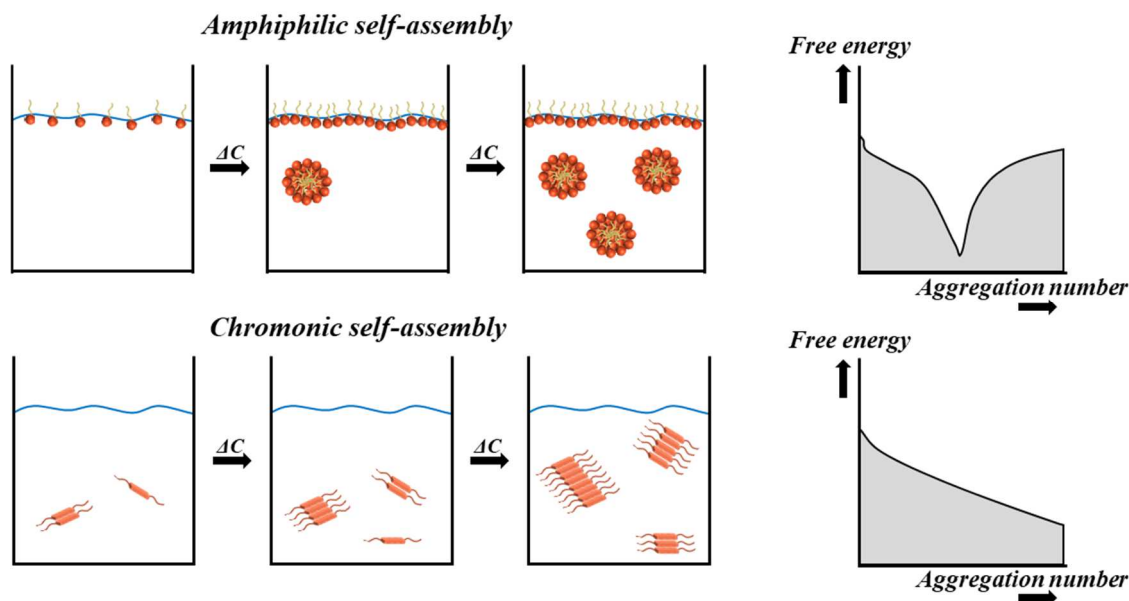


Figure 1.8. Comparison between amphiphilic and chromonic self-assembly. In the left part, a scheme of how the micelles and columnar aggregates grow is depicted. It can be seen how amphiphilic micelles have an optimal size, meanwhile chromonic columnar aggregates gradually elongate. The graphs on the right part represent the variation of the free energy as a function of the aggregation number. The free energy has a clear minimum for amphiphile micellization (non-isodesmic), in contrast to chromonic aggregates, which elongate with a gradual decrease in the free energy (isodesmic). Note that the free energy decrease for chromonic aggregate elongation is not completely linear. This results from the higher free energy reduction of the formation of dimers and small n -mers. ΔC means increment in the concentration.

Furthermore, chromonic molecules can self-assemble in two ways: face-to-face, by stacking the aromatic cores on top of each other (H-aggregates), or tilted (J-aggregates), as shown in Figure 1.9. The tilting angle (θ) classifies an aggregate as H- or J-aggregate, with H-aggregation occurring for $\theta > 54.7^\circ$ and J-aggregation for $\theta < 54.7^\circ$ ^[110,111]. The optical properties of the aggregates change compared to the non-aggregated monomeric species, and such variations highly depend on the kind of aggregate formed.

H-aggregates cause a hypsochromic (blue) shift in the maxima of absorbance as compared to the monomeric species, with hypochromicity (absorbance intensity reduction), and quenched fluorescence. J-aggregates, on the other hand, produce a bathchromic (red) shift. This behavior can be partially explained by Kasha's molecular exciton model for dye dimers ^[112], which defends that upon photon-induced excitation of a dye dimer, the excited state splits in two as a result of the interaction between the transition dipoles moments formed in each molecule of the dimer. Kasha's model states that, of these two levels formed, the highest energy one is forbidden for J-aggregates whereas the lowest energy level is not allowed for H-aggregates, which explains the absorbance shift (Figure 1.10). Note that there is a point in which the two split excited states have the same energy ($\theta = 54.7^\circ$), where the absorbance spectrum is not distinguishable from that of monomeric species, and which is considered the frontier between H- and J-aggregation. Moreover, this model also considers the so-called Kasha's rule ^[113], that states that "the emitting excited level of a given multiplicity is the lowest excited level of that multiplicity", and, since the lowest-energy excited level is forbidden for H-aggregates, fluorescence gets quenched. It is worth noting that Kasha's model may be oversimplified for some systems, since it only considers only the formation of dimers and supposes that there is no overlapping between the π -orbitals of molecules forming the dimer.

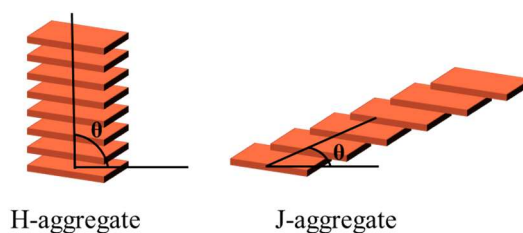


Figure 1.9. Structure of H-aggregates (left) and J-aggregates (right). The angle (θ) defines the kind of aggregate, being H-aggregate for $\theta > 54.7^\circ$ and J-aggregate for $\theta < 54.7^\circ$.

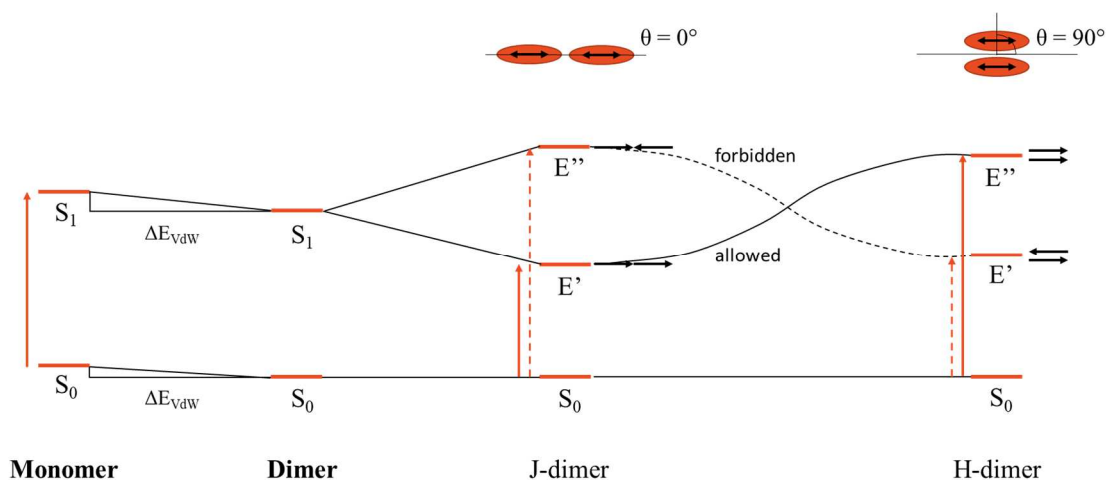


Figure 1.10. Schematic energy level diagram for H- and J-dimers according to the Kasha's model of excitonic coupling. S_0 and S_1 correspond to the ground and excited states, respectively. ΔE_{vdw} indicates the energy reduction of the dimer compared to the monomer, resulting from the attractive intermolecular interactions between the two molecules of the dimer. Note that this value is higher in the excited state, due to the higher polarizability of the excited molecules. E' and E'' correspond to each of the two energy levels in which the excited state of dimers is split, and the arrows next to these levels indicate the direction of the transition dipoles of each molecule. Note that the splitting results from the electrostatic interactions between the transition dipoles.

Normally, chromonic mesophases are formed by H-aggregated species with unimolecular cross-sections^[95], when the interactions between long columnar aggregates trigger orientational and/or positional order within the system at high concentrations, forming lyotropic chromonic liquid crystal phases. However, more complex aggregates with head-to-tail arrays^[114,115], bimolecular cross-sections^[116], and multimolecular hollow-chimney and brick-wall^[117] structures have been described (Figure 1.11). The next section covers the main LCLC phases and their structures, along with the principal characterization techniques for LCLC phase identification.

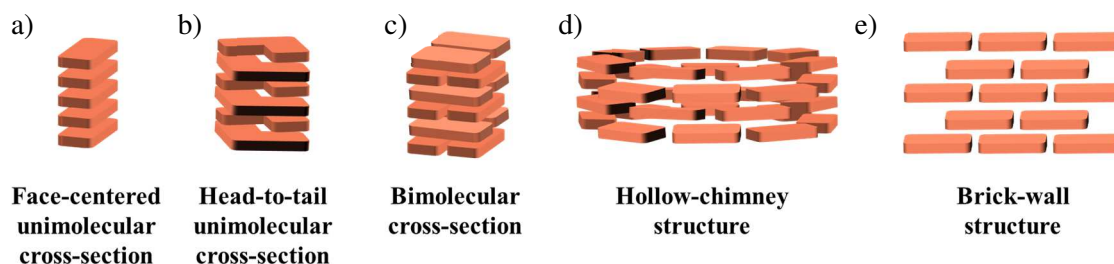


Figure 1.11. Different aggregate structures forming chromonic mesophases. Note that the hydrophilic chains have not been drawn to improve the visualization of the pictures.

1.3.2 Chromonic liquid crystal phases

The first description of chromonic liquid crystals phase came from the studies of Hartshorne, Woodard, Cox and Walter McCrone^[97,98], from the McCrone Research Institute, about the lyotropic mesophases formed by the anti-asthmatic drug disodium cromoglycate (DSCG), during the 1970s. They described two different mesophases for DSCG in water: the nematic (N) phase and the hexagonal or middle (M) phase. The selection of these terms to name the mesophase was due to the similarity between DSCG polarized optical microscopy (POM) textures and those of thermotropic nematic and lyotropic amphiphilic hexagonal phases, respectively. Note that the lyotropic hexagonal phase is also called *middle* phase, as it is usually in the middle between the isotropic solution and the lamellar phases.

Later work by Lydon and Attwood, among others, helped to define the *chromonics* as a particular and differentiated class of lyotropic liquid crystals^[118]. It was demonstrated that the formation of chromonic mesophases was a widespread phenomenon among small anti-asthmatic and anti-allergy drugs, and water-soluble dyes. Furthermore, X-ray experiments of the chromonic mesophases were performed, models explaining the structure of both N and M phases were drawn and new chromonic mesophases were discovered.

Today it is widely accepted that both N and M phases are typically formed by face-centered stacks of chromonic mesogens with a stacking distance of ~ 3.4 Å between adjacent mesogens (note that there are some exceptions, as shown in Figure 1.11). In N phases, these columnar stacks interact triggering orientational order (i.e. all the stacks are oriented towards one direction defined by the director vector, $\vec{n}$, in localized regions of the mesogenic sample). Additionally, in M phases, these columnar stacks also gain positional order, arranging in a hexagonal lattice (Figure 1.12a).

In general, chromonic liquid crystals show multi-peritectic¹ phase diagrams (Figure 1.12b), with N phases forming at lower concentrations than M phases, and with a narrow concentration range in which all the possible mesophases (for a system forming exclusively N and M phases) are observed by increasing the temperature. The phase transitions are $M \rightarrow M + N \rightarrow N \rightarrow N + I \rightarrow M + I \rightarrow I$ (see the red arrow of Figure 1.12b). Note that I means isotropic phase (i.e. no liquid crystal). In contrast, conventional amphiphilic lyotropic liquid crystals do not typically show all the mesophases by changing the temperature for a given concentration and exhibit multi-eutectic¹ phase diagrams.

However, three more mesophases have also been described: the P phase, the rectangular or orthorhombic (O) phase and a lamellar phase. The chromonic P phase (Figure 1.13a) is most prevalent exception to the common N/M behavior represented in Figure 1.12. The P phase consist in an intermediate mesophase that appears during the $M \leftrightarrow N$ transition. Typically, the $M \leftrightarrow N$ transition is an abrupt first-order process. However, chromonic systems with circular column cross-sections, formed either by discotic mesogens or plank-like mesogens stacked in a helical fashion, promote a smoother higher-order phase transition that give rise to the P mesophase. This probably occurs due to the higher rotational freedom within the columnar chromonic aggregate of discotic and helically stacked mesogens^[95].

¹ The term peritectic is commonly used in phase diagrams to refer to a solid and a liquid in equilibrium with a second different solid at a particular temperature and composition. In contrast, the term eutectic is used for liquid systems in equilibrium with two different solid phases. These concepts can be applied to chromonics by changing “solid” for “liquid crystal phase”, and “liquid” for “isotropic phase”. In chromonics, there is typically a point where the $M + I$ phase is in equilibrium with the N phase, and a second point where the solid + I phase is in equilibrium with the M phase, hence showing a multi-peritectic behavior

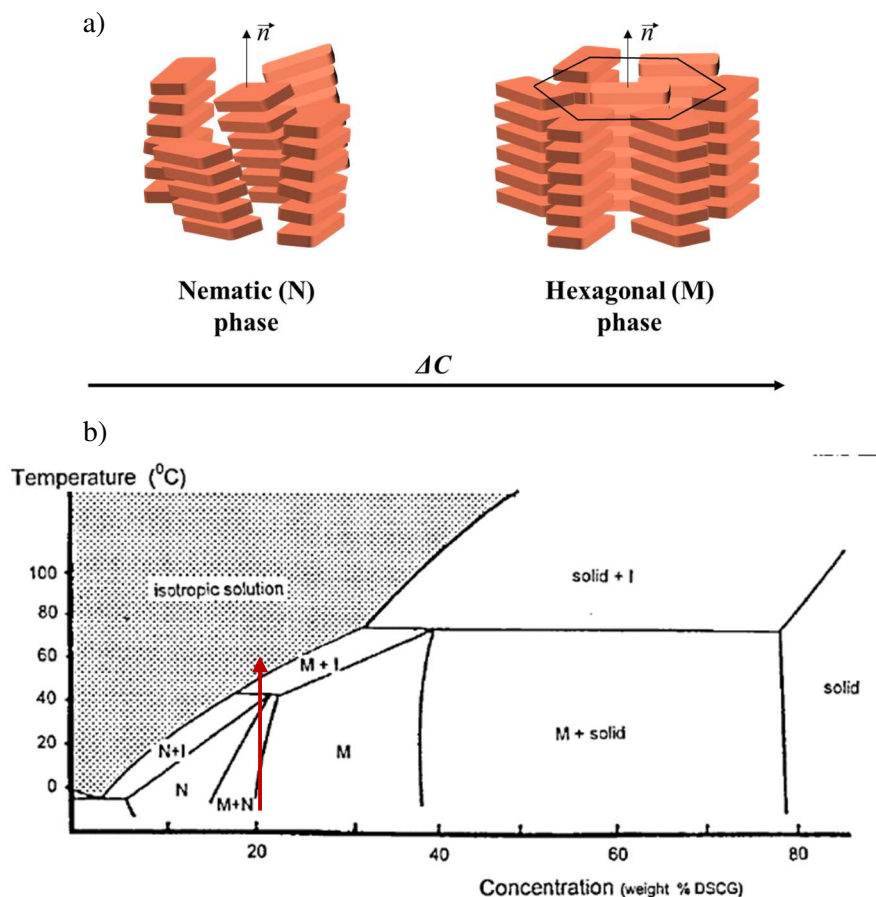


Figure 1.12. a) Structure of the nematic (N) and the hexagonal (M) chromonic mesophases. b) Phase diagram of DSCG. Reproduced with permission from Elsevier ^[93]. The red arrow indicates the narrow concentration range in which all the chromonic mesophases are observed as a function of temperature: $M \rightarrow M + N \rightarrow N \rightarrow N + I \rightarrow M + I \rightarrow I$.

The chromonic O phase consists in a mesophase with orientational and positional order in which the chromonic aggregates self-assemble in a rectangular lattice (Figure 1.13b). This phase is expected to exist at lower temperatures and/or higher concentrations than the M phase ^[95], although there is only few evidence of its existence ^[119–121], which suggests that the formation of O mesophases is not a widespread phenomenon in chromonic systems.

Lamellar mesophases have been scarcely observed in chromonic systems, mainly for cyanine dyes stacked in brick-wall structures (see Figure 1.11) at rather low concentrations ^[117]. However, recently, a hemicyanine dye was reported to form lamellar phases at high concentrations and temperatures, and a model of face-centered columnar aggregates aligned in a lamellar fashion was proposed (Figure 1.13c) ^[120,121].

In addition to these five differentiated mesophases (N, M, P, O and lamellar), chiral mesogens or achiral mesogens with chiral dopants can also form chiral or cholesteric nematic mesophases (N^*). This phase is thermodynamically equivalent to the N phase, but it forms a chiral superstructure in which the director vector is helically twisted along the sample (Figure 1.13d) ^[122–124].

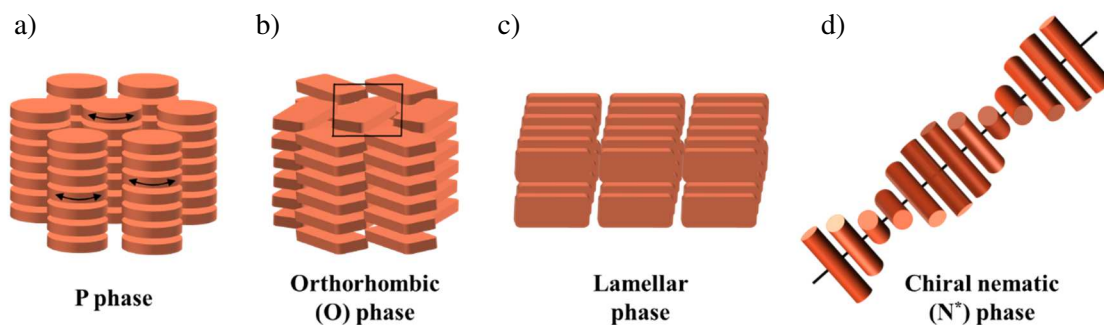


Figure 1.13. Structure of the chromonic a) P phase, b) rectangular or orthorhombic (O) phase, c) lamellar phase and d) cholesteric or chiral nematic (N^*) phase. Note the discotic units of the P phase are not necessarily disk-like mesogens, as they can also be plank-like mesogens stacked in a helical fashion. Also note that the cylinders of the N^* phase do not represent single mesogens but columnar aggregates, which twist helically giving rise to a chiral superstructure.

The characterization of chromonic mesophases is typically done by polarized optical microscopy (POM) and small-angle X-ray scattering (SAXS), although other techniques such as $^1\text{H-NMR}$, differential calorimetry (DSC) and spectrophotometric techniques are also useful.

POM is a technique consisting in observing a sample between crossed polarizers with an optical microscope. This allows to distinguish the presence of birefringent (anisotropic) materials and mesophases. Notably, when observed by POM, the different chromonic mesophases exhibit distinguishable textures, which converts POM in an excellent technique to identify the mesophase formed by a sample.

In particular, N phases show Schlieren textures consisting in more or less uniform bright zones along with dark points and lines (Figure 1.14a), corresponding to particular regions where the director vector is aligned with one of the two crossed polarizers ^[125]. In contrast, M phases show either a grainy or a herringbone texture, depending on the purity of the sample and the method of preparation ^[95,126]. In general, purer samples prepared by partially evaporating the solvent of a nematic phase yield herringbone textures (Figure 1.14b), whereas impure samples or phases prepared directly mixing the mesogen with water at a concentration giving M phases favor grainy textures. Furthermore, POM is a very useful technique to detect the formation of cholesteric nematic mesophases, since the twisting of the director vector gives the mesophase a easily distinguishable fingerprint texture (Figure 1.14c) ^[127], similar to that observed in cholesteric nematic phases of thermotropic liquid crystals ^[128]. Despite this, the identification of P, O and lamellar mesophases by POM is trickier. P phases do not exhibit a distinctive texture as they are an intermediate phase between N and M phases, and generally appears as a grainy M-like texture more fluid than a conventional M phase (this is further discussed in the Results and Discussion chapters 4.1 and 4.2). Furthermore, O phases exhibit mosaic and focal conic textures that can be hard to distinguish from the hexagonal textures ^[119,120], and lamellar phases have been shown to display oily streaks and grainy textures ^[117,120]. Alternatively, orthogonal and lamellar phases can be distinguished from conventional N and M phases by X-ray diffraction techniques.

X-ray diffraction techniques also proved to be very useful to characterize and distinguish the chromonic mesophases, as they allow to get insights about the structure of the columns and their organization. Generally, chromonic materials are characterized by small- and wide-angle X-ray scattering (SAXS and WAXS).

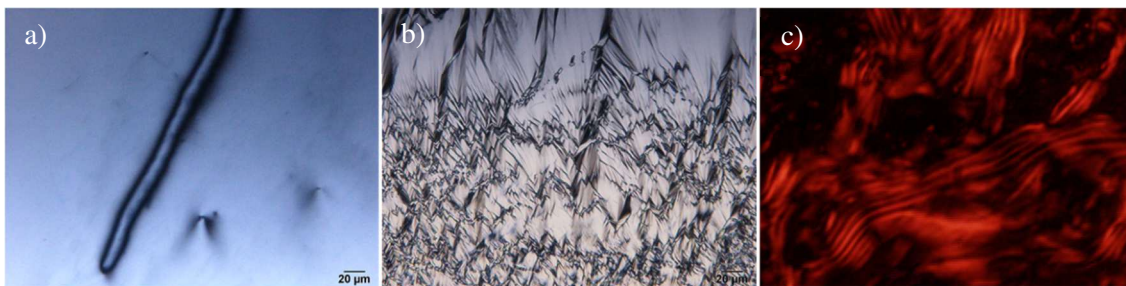


Figure 1.14. POM images of a) Schlieren nematic texture and b) herringbone hexagonal texture of DSCG. Both images were taken of an isotropic DSCG solution in water by lateral evaporation of the solvent. c) POM image of a chiral nematic phase formed by a perylene diimide ^[127].

X-ray diffraction techniques are used to determine the atomic or molecular structure of materials by analyzing the diffraction patterns generated by the interaction of X-rays with the materials. This diffraction occurs in materials with aligned planes such as crystals or other ordered materials (including liquid crystals). By measuring the intensity of the scattered X-rays as a function of the angle of incidence of the X-rays to the material planes for a given X-ray wavelength, it is possible to measure the spacing between the planes, according to the Bragg equation:

$$n \cdot \lambda = 2 \cdot d \cdot \sin(\theta) \quad (\text{Eq. 1})$$

Where n is a natural number that indicates the order of diffraction, λ is the wavelength of the X-rays, d is the spacing between the material planes and θ is the angle of incidence of the X-rays to the material planes. Bragg law says that the constructive scattering will only occur when the phase shift of the scattered X-rays is a multiple of 2π , condition expressed by Eq. 1.

X-ray diffraction permits studying materials with planes of all sizes, from atomic (sub-nanometric) to nanostructures with d -spacings above 100 nm ^[129]. However, different incident angles will be required depending on these d -spacings. According to Eq. 1, the d -spacing and the incident angle are inversely proportional, which means that materials with atomic-sized spacings will require wider angles than materials with nanometric spacings. Consequently, SAXS (operating at angles between 0.1 and 10°) allows studying materials with interplanar spacings in the nanometer scale, whereas WAXS (operating at angles above 10°), permits studying shorter interplanar distances.

SAXS offers a straightforward method to identify the chromonic mesophases of a sample, as the intercolumnar distances of N and M phases are typically of few nanometers. Furthermore, different signals will appear depending on the chromonic mesophase. Notably, SAXS plots are typically expressed as the scattered X-ray intensity as a function of a parameter called scattering vector (q), which is proportional to the sine of the incident angle ($\sin \theta$).

In N phases the columnar stacks are aligned towards one direction defined by the director vector, but lack of positional order and thus do not have a well-established intercolumnar distance (distance between the centers of two adjacent aligned columns, see Figure 1.15a). Consequently, the SAXS spectra for N phases yields a broad band with the scattering vector of maximum X-ray scattering intensity proportional to the inverse of the mean distance between aggregates. The average first order ($n = 1$) d -spacing (average intercolumnar distance or d_0) can be calculated from the q of maximum intensity using Eq. 2, which is derived from the Bragg equation.

$$d = 2\pi/q \quad (\text{Eq. 2})$$

In contrast, the positional order between aligned columns in a hexagonal lattice present in M phases (Figure 1.15b) produces a SAXS pattern with multiple sharp peaks at scattering vector

ratios $1:\sqrt{3}:\sqrt{4}:\sqrt{7}$, corresponding to the planes with (h k l) Miller indices (1 0 0), (1 1 0), (2 0 0) and (2 1 0), respectively. The d -spacing of each reflection can be calculated with Eq. 2, whereas the hexagonal lattice parameter (a , see Figure 1.15b) can be determined using Eq. 3, from the slope resulting of plotting $1/d^2$ as a function of $(h^2 + hk + k^2)$, where d corresponds to the d -spacing of each plane; h , k and l are the Miller indices, and a and c are hexagonal lattice parameters. Notably, the square root of the slope should ideally correspond to the inverse of the d -spacing of the (1 0 0) plane (d_H from Figure 1.15b). It is also worth noting that the Miller index l is always 0, as chromonic M phases are 2D hexagonal lattices, where the unit cell is infinite in the vertical axis, making the term (l^2/c^2) always null.

$$\frac{1}{d^2} = \frac{4}{3 \cdot a^2} \cdot (h^2 + hk + k^2) + \frac{l^2}{c^2} \quad (\text{Eq. 3})$$

In addition to nematic and hexagonal, SAXS also permits the identification and lattice parameter calculation of rectangular and lamellar mesophases. Rectangular or orthorhombic mesophases usually exhibit two reflections corresponding to the (0 2 0) and (1 1 0). Contrary to hexagonal phases, a fixed ratio between the peaks cannot be considered as it depends on the ratio between the lattice parameters a and b (see Figure 1.15c and Eq. 4). As for hexagonal mesophases, l is always 0 and thus the l^2/c^2 term is null (2D rectangular lattice).

$$\frac{1}{d^2} = \frac{h^2}{a^2} + \frac{k^2}{b^2} + \frac{l^2}{c^2} \quad (\text{Eq. 4})$$

Lamellar mesophases consist in stacked lamellas at a d_L distance. Lamellar mesophases exhibit unit cells that are infinite in the horizontal planes (along each lamella) and hence only show X-ray reflections resulting from the repetition of lamellar along one dimension. This means that these reflections come from the planes with Miller indices (1 0 0), (2 0 0), (3 0 0), etc. Consequently, lamellar mesophases show multiple SAXS sharp peaks at ratios 1:2:3:4:... (see Figure 1.15d and Eq. 5).

$$\frac{1}{d^2} = \frac{h^2}{d_L^2} \quad (\text{Eq. 5})$$

Furthermore, if the volume fraction is known, SAXS permits the calculation of parameters such as the cross-section of hexagonally packed chromonic aggregates or the lamellae size in lamellar mesophases. This was used to infer the bimolecular cross-section of Bordeaux dye chromonic aggregates (Figure 1.11c) [116].

WAXS, on the other hand, is a useful technique to study the structure of the mesogens within a column. Most chromonic systems exhibit a WAXS peak at $q \approx 18.2 \text{ nm}^{-1}$, which corresponds to the distance between adjacent face-centered stacked mesogens (3.4 Å). For some cases, such as the chromonic food colorant Sunset Yellow FCF, a second WAXS reflection at $q \approx 9.2 \text{ nm}^{-1}$ also appears, corresponding to a stacking distance of 6.8 Å [96,114,115]. This value doubles the conventional 3.4 Å and results from the particular stacking head-to-tail stacking behavior of this mesogen (Figure 1.11b).

In summary, POM and X-ray diffraction are very effective straightforward and non-destructive techniques to characterize the chromonic (and other thermotropic and lyotropic) mesophases. Among the different mesogens forming chromonic systems, perylene diimides are of particular interest due to their unique properties and technological potential. In the next section, a brief description of perylene diimides (PDIs) and other rylene dyes will be addressed, since this thesis is principally focused on the formation of chromonic molecular segregation systems based on PDIs.

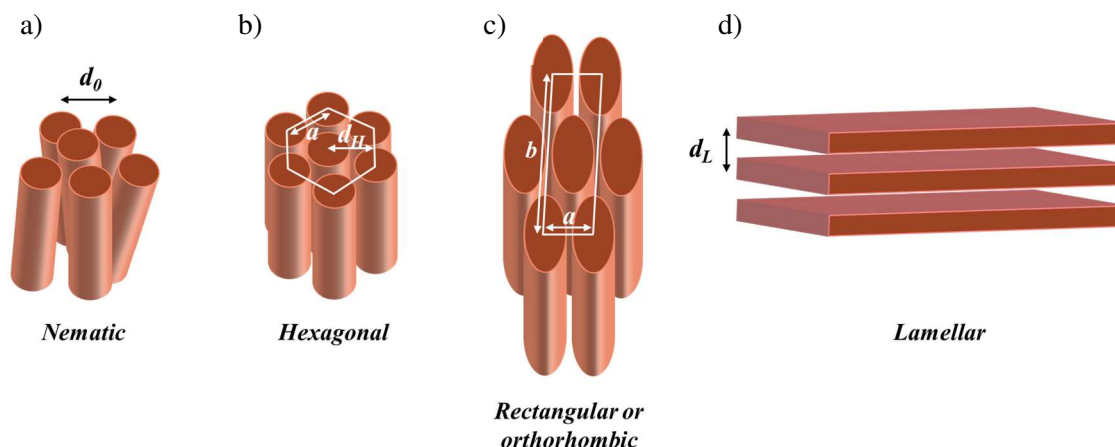


Figure 1.15. Structure of the liquid crystalline mesophase with the main lattice parameters.

1.3.3 Rylene diimides

Rylene diimides (RDI), also called rylene bisimides or RBI, are compounds chemically composed by an extended aromatic core formed by fused naphthalene units functionalized at both sides with an imide group (Figure 1.16). Depending on the amount of naphthalene units (n), rylene dyes are classified in naphthalene ($n = 1$, abbr. NDI), perylene ($n = 2$, abbr. PDI), terrylene ($n = 3$, abbr. TDI) and quaterylene ($n = 4$, abbr. QDI) diimides.

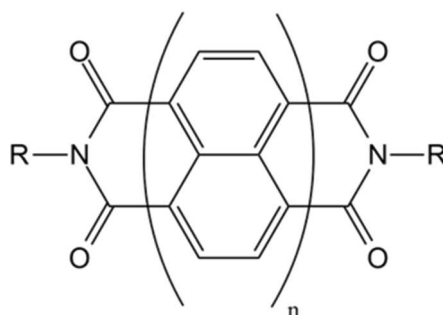


Figure 1.16. Chemical structure of the rylene diimides, that include naphthalene ($n = 1$), perylene ($n = 2$), terrylene ($n = 3$) and quaterylene ($n = 4$).

Rylene diimides have attracted the attention of many researchers from different fields because of their outstanding physicochemical properties. RDIs are robust, show fluorescent quantum yields close to unity, have excellent thermal, chemical and photochemical stabilities, its structure of electron-rich core functionalized with electron-withdrawing groups grant them excellent electron-transfer behavior and air-stability^[130] and are very versatile in terms of organic synthesis^[131], since they can be functionalized both in the imide position and in the aromatic framework. Such combination of unique properties gives applications to RDIs as fluorescent probes, n-type semiconductors for organic electronics^[132] and building blocks in supramolecular chemistry^[133,134]. Furthermore, their relatively small size and planarity converts RDIs in potential candidates to intercalate inside DNA, thus becoming potential anticancer agents^[135].

Among the different rylene diimides, naphthalene and, particularly, perylene diimides are no-doubt the most important and used. The main reason is their ease of synthesis and relative low-cost. Both are prepared from the corresponding rylene tetracarboxylic acid dianhydride (RTCDA) in a single step imidation reaction with a primary amine and a base (sometimes metallic catalyzers such as $\text{Zn}(\text{OAc})_2$ are also used). Both naphthalene (NTCDA) and perylene (PTCDA) tetracarboxylic acid dianhydrides are commercially available for laboratory scale at prices around

1 – 2 €/g and purities above 97 %. Interestingly, NTCDA and PTCDA are easily synthesized from pyrene^[136] and acenaphthene^[137], respectively, which, in turn, are directly obtained from coal. On the other hand, larger rylene diimides syntheses are not straightforward and normally require using perylene cores as precursors in multi-step reactions, which make the final products significantly more expensive.

Among the many self-assembled structures that rylene diimides form, there are both thermotropic and lyotropic liquid crystals. The interest in mesogenic rylene diimides started around 25 years ago when Cormier and Gregg prepared a series of perylene diimides functionalized with branched oligoethylene glycol chains that formed thermotropic mesophases upon heating^[138,139]. Since then, many thermotropic mesogenic rylene diimides, namely perylene diimides, but also naphthalene and terrylene diimides, have been synthesized^[140].

More recently perylene and quaterrylene chromonic mesogens have been prepared and characterized. Differently to the thermotropic mesogens, which are normally functionalized with long flexible chains such as ethylene glycol, alkyl chains, siloxane, dendritic peptides, phenyl and triphenylene derivatives, among others^[140]; rylene-based chromonic mesogens are formed appending small ionic groups to the imide positions. To date, only few chromonic mesogenic rylene diimides have been reported and most of them consist in cationic perylene diimides functionalized with quaternary amines^[100,123,140–148]. Chromonic perylene diimides hold considerable technological promise resulting of their combined mesogenic behavior and outstanding physicochemical properties^[95,150].

1.3.4 Applications of lyotropic chromonic liquid crystals

In the last 20 years, it has been shown that taking advantage of the self-assembly and liquid-crystalline properties of chromonic mesophases permits the preparation of several promising functional materials and devices^[150–152]. In general, the applications of chromonics can be divided into three areas: i) those consisting in drying, polymerizing or gelling chromonic mesophases to obtain anisotropic materials, mainly films; ii) those using the LCLC phases in water, such as sensing devices, and iii) those taking advantage of the self-assembled chromonic structures as templates for the preparation of porous nanofibers, including silica and carbon nanofibers.

Undoubtedly, the preparation of anisotropic films by drying chromonic solutions is the most widely studied application of these mesophases. The high interest in dried films arises from the capacity of the chromonic mesophases to retain their orientationally ordered structures upon solvent evaporation. Typically, the obtention of oriented films is achieved in three steps. First, a chromonic aqueous solution is prepared, and then it is deposited in a substrate and aligned. There are several techniques to align the chromonic mesophase in the substrate, which include using strong magnetic fields, mechanical shearing or modifying the substrate with an alignment layer^[150]. Finally, the anisotropic films are obtained by solvent evaporation.

The anisotropic films obtained by this method exhibit unique properties such as birefringence, polarization-dependent light absorption, the ability to align other materials, semiconductor behavior and direction-dependent charge mobility^[151,152]. This allowed the application of the chromonic dried films for several applications including the preparation of visible and near-IR polarizing films with high dichroic ratios^[142,153], polarizing and aligning plates for thermotropic materials in twisted-nematic liquid crystal displays^[154], micropatterned chromonic films with multiple alignment directions within the same film^[155,156], and materials for organic electronics^[157]. More recently, polarizing and micropatterned films were prepared by polymerizing a chromonic material^[148], and a photoconductive film was obtained by gelling a water-soluble

perylene diimide with chromonic self-assembly ^[158]. These materials showed improved mechanical properties compared to the chromonic films obtained by drying the solvent.

In addition to the preparation of dried, polymerized or gelled films, chromonic liquid crystals also show promising applications in solution. For example, Lavrentovich et al. designed biosensing devices based in the N phase of LCLC, in which the chromonic mesophase was embedded in between crossed polarizers so that the director vector stays parallel to one polarizer, and hence no light crosses between the two polarizers. The transduction mechanism of this sensor was based in the director vector distortions caused by large particles, which generated birefringence and light passing through the crossed polarizers. Such biosensors were effectively used to detect an antibody in the presence of antigen-coated particles, that distort the director vector upon agglomeration ^[159].

Additionally, LCLC mesophases have been used as optical compensators ^[160], and to guide the self-assembly of gold nanorods either in a side-by-side or an end-to-end fashion depending on the charge of the gold nanorods surface ^[161]. By coating the gold nanorods with CTAB (positively charged) and adding them to a DSCG chromonic solution (negatively-charged), side-by-side aggregates were obtained. In contrast, further coating the CTAB-gold nanorods with polyacrylate (negatively charged) resulted in the formation of end-to-end gold nanorod aggregates.

The third area of applications of chromonic mesophases is their use as templates for porous organic and inorganic materials. Rodríguez-Abreu et al. investigated the possibility of using chromonic cyanine dyes ^[162,163] and perylene diimides ^[164] to template hierarchically micro- and mesoporous silica and carbon nanofibers (Figure 1.17). Silica nanofibers were obtained in a sol-gel method using cationic chromonic mesogens as templates and tetraethyl orthosilicate (TEOS) as the silica precursor in basic aqueous solution. The resulting nanofibers consisted in high-aspect ratio materials with aligned stripes of the chromonic template within, which could be removed by calcination, yielding silica fibers with aligned micro- or mesopores with diameters close to the size of the chromonic dye core used as template, indicating that the selection of the template determines the pore size of the silica material. Notably, the silica templating occurred in an isotropic solution of the chromonic dyes, suggesting that hierarchical dye-TEOS aggregates are formed during the process.

Furthermore, they used the cyanine dye – silica composite to prepare micro- and mesoporous carbon nanofibers ^[165]. Their approach consisted in filling the silica pores with a carbon precursor (furfuryl alcohol) and carbonizing the precursor – silica composite in an inert N₂ atmosphere to produce carbon nanofibers and then remove the silica by etching with a strong base (Figure 1.17). This resulted in high-surface area carbon nanofibers that exhibited high performance as electrodes for supercapacitor devices.

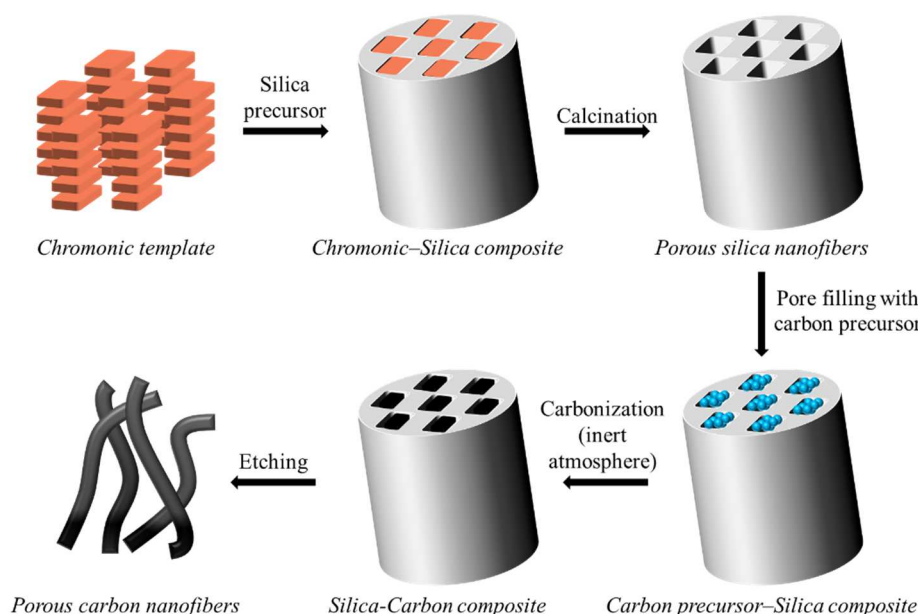


Figure 1.17. Scheme of the preparation of silica and carbon nanofibers using chromonic materials as templates ^[162-165].

Supercapacitors, also called electrical double-layer capacitors (EDLC) or ultracapacitors, are electrochemical energy (EES) storage devices that gained popularity in the recent years for their unique properties such as fastness of charging/discharging, long life cycle, and large power density (i.e. energy expended per unit of time and mass) ^[166-169]. Supercapacitors are formed by two electrodes separated by an electrolyte solution and an insulator. Their working principle consists in the formation of an electric double layer in the electrode surface upon the application of a voltage. When charging a supercapacitor, a voltage between the two electrodes is applied and charge is stored, which induces the polarization of the electrolyte solution by separating the positive and negative ions to opposite electrodes. During the discharging, the electrolyte solution is depolarized and electrical current flow between the two electrodes through a wire (Figure 1.18).

Despite the advantages of supercapacitors described in the previous paragraph, they are still far behind batteries in terms of energy density (i.e. energy stored per unit of mass after one charging), and hence much effort has been put in improving this parameter ^[170]. An effective strategy to achieve this is to induce faradaic pseudocapacitance. Pseudocapacitors are capacitive devices that, besides storing energy with the formation of an electric double-layer, experience fast and reversible electron transfer processes in the electrode surface, without phase changes in the electrode material nor ion penetration into the electrode network, which would reduce their power density and cycle life, as it occurs in battery-like devices. Pseudocapacitance can be induced by doping the carbon surface with nitrogen or other heteroatoms. This increases the overall specific capacitance (C_s)² without affecting the other unique properties of EDLCs ^[171-174]. According to these properties, EDLCs are not intended to substitute batteries but to complement them, being useful as power backups ^[175], for regenerative braking ^[176], and for hybrid vehicles ^[175,177].

Notably, despite commercial supercapacitors being typically composed by two-electrode cells, as shown in Figure 1.18; in laboratory scale three-electrode cells are more commonly used to measure the specific capacitance of an electrode (note that three-electrode cells do not permit the

² Specific capacitance (C_s) is the total amount of charge that can be stored by a supercapacitor per unit of surface area or mass, and it is typically expressed as F/m² or F/g (F refers to Farads). Higher C_s leads to devices with higher energy densities.

calculation of energy and power densities). In general, EES devices are evaluated by a combination of techniques including cyclic voltammetry (CV), galvanostatic charge-discharge (GCD) and electrochemical impedance spectroscopy (EIS) ^[171,178,179].

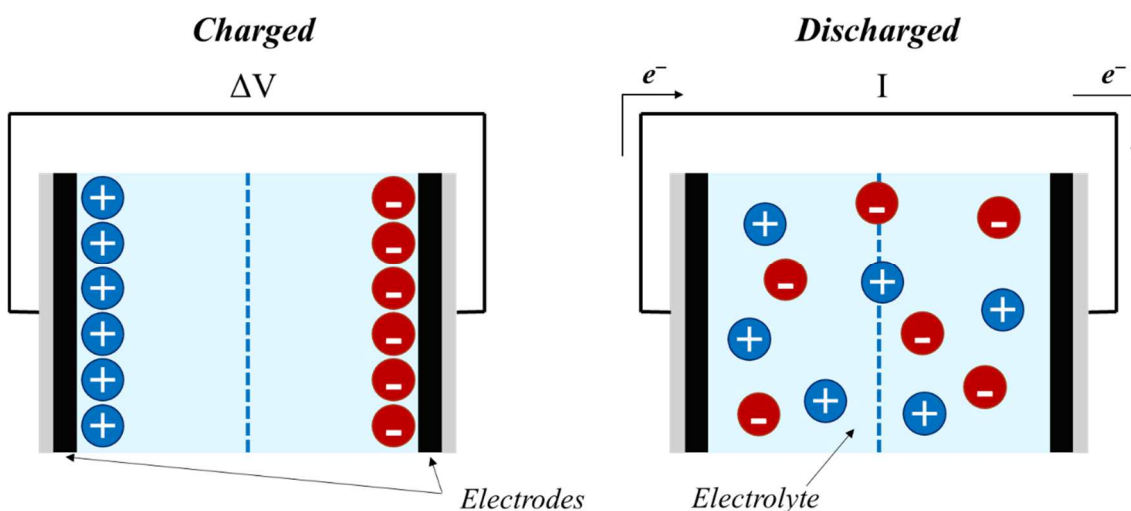


Figure 1.18. Scheme of an electrical double-layer capacitor (EDLC) or supercapacitor. In the charging (left), the electrodes are polarized by the application of a voltage and the energy is stored by the formation of an electric double-layer at the electrode surface. In the discharging process (right), the stored energy is released through a connection between the two electrodes and the ions return to the bulk of the solution. Note that no chemical redox reactions are involved in the charge-discharge processes.

In CV experiments, the EES device is charged and discharged in a potential window (V) at a fixed scan rate (i.e. constant dV/dt), and changes in current as a function of the voltage are recorded. In contrast, GCD measurements are carried out by fixing the current and recording changes in the voltage as a function of time. Differently, EIS studies the variation of impedance as a function of the frequency of a small-amplitude sinusoidal voltage superimposed on a fixed voltage, and it is commonly used to determine the equivalent series resistance (ESR) of a EES system.

CV and GCD curves permit to distinguish between supercapacitor, pseudocapacitor and battery-like devices. Typically, supercapacitor devices (storage charge solely by the formation of an electric double-layer in the electrode surface) exhibit rectangular CV (Figure 1.19a) and triangular GCD curves (Figure 1.19d), as no redox reactions occur in the charging/discharging and hence the variation of current as a function of voltage is constant. In contrast, batteries work converting chemical energy in electric energy through redox reactions. Consequently, batteries exhibit non-linear CV curves with two marked asymmetric bands (Figure 1.19c) and GCD curves with plateaus in both charging and discharging processes (Figure 1.19f). Both the asymmetric bands and the plateau correspond to the redox reactions, where a burst of energy is delivered.

Pseudocapacitors exhibit an intermediate behavior between supercapacitors and batteries ^[171]. For example, pseudocapacitors show rectangular CV curves with symmetric bands (Figure 1.19b) corresponding to the faradaic processes (note that the faradaic bands are symmetric in pseudocapacitors and asymmetric in batteries). Furthermore, pseudocapacitor GCD curves are triangular, but present a small sinusoidal shape (Figure 1.19e), also resulting from the faradaic reactions.

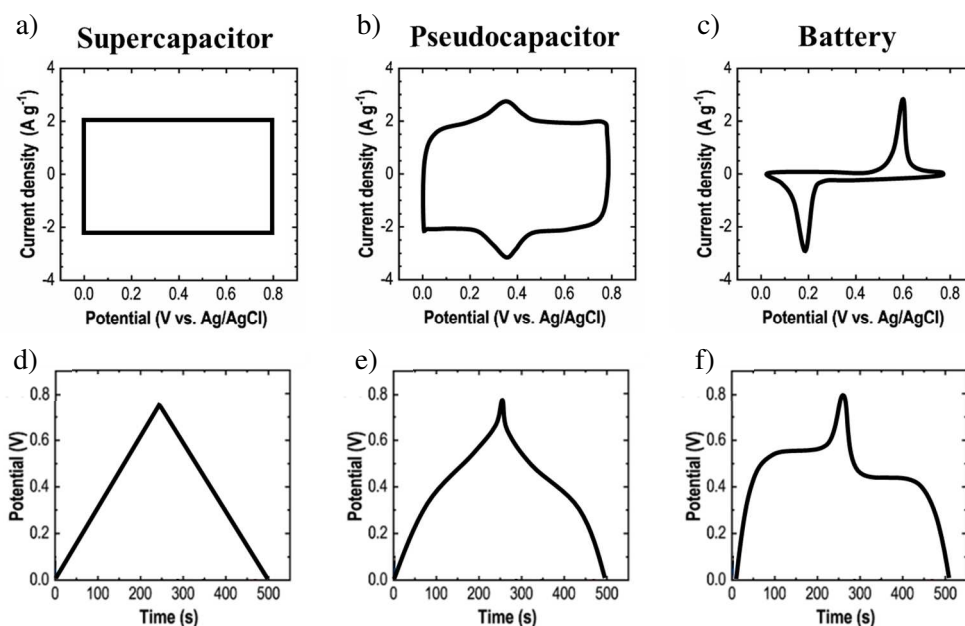


Figure 1.19. CV curves of a) supercapacitor, b) pseudocapacitor and c) battery-like devices and GCD curves of d) supercapacitors, e) pseudocapacitors and f) battery-like devices.

Additionally, there are other reports in the literature about the fabrication of carbon nanomaterials from chromonic materials. For example, Sousa et al.^[180] obtained arrays of carbon nanotubes carbonizing the chromonic mesogen indanthrone disulfonate, and Rodríguez-Abreu et al.^[181] prepared carbon nanofibers with excellent supercapacitor performance using ionic self-assemblies produced from chromonic porphyrins.

1.4 Overview of emulsions

The liquid crystals described in the previous two sections consist in systems showing molecular segregation behavior at the nanoscale, as the different self-assembled structures formed by both thermotropic and lyotropic materials exhibit nanometric sizes. In contrast, emulsions are systems that show molecular segregation at the microscale.

Emulsions are heterogeneous mixtures composed of two immiscible liquids, typically water and an oil. Resulting from the entropically unfavorable interactions between the polar water molecules and the hydrophobic oil, the two components phase separate. However, by applying energy, the two phases can be mixed forming a colloidal dispersion, consisting in a liquid phase dispersed in the form of droplets within a continuous liquid phase. Such dispersions are called emulsions.

Generally, emulsions are classified as oil-in-water (O/W) or water-in-oil (W/O) emulsions depending on which is the dispersed phase. Emulsions are thermodynamically unstable systems, which tend to phase separate in segregated equilibrium phases. As a result of the unfavorable oil-water intermolecular interactions, the interfacial tension between the two phases in absence of surfactants is rather high, which convert emulsions in thermodynamically unstable systems that tend to phase separate in order to reduce the interfacial area between the two immiscible phases.

Various mechanisms have been described to cause this phase separation. These are: sedimentation, creaming, flocculation, coalescence and Ostwald ripening (Figure 1.20)^[182]. Sedimentation and creaming correspond to the downward and upward movement of the dispersed droplets resulting from different densities between phases, respectively. Flocculation is the reversible aggregation with no merging between droplets. Instead, coalescence consists in an

irreversible merging of droplets forming a bigger droplet. Finally, Ostwald ripening occurs when a bigger droplet absorbs part of smaller droplets reducing the total oil-water interfacial area. Normally multiple destabilization mechanisms participate in the emulsion breakdown simultaneously, and their occurrence depends on the emulsion type, the composition, the preparation method, the ionic strength and the droplet size, among other parameters.

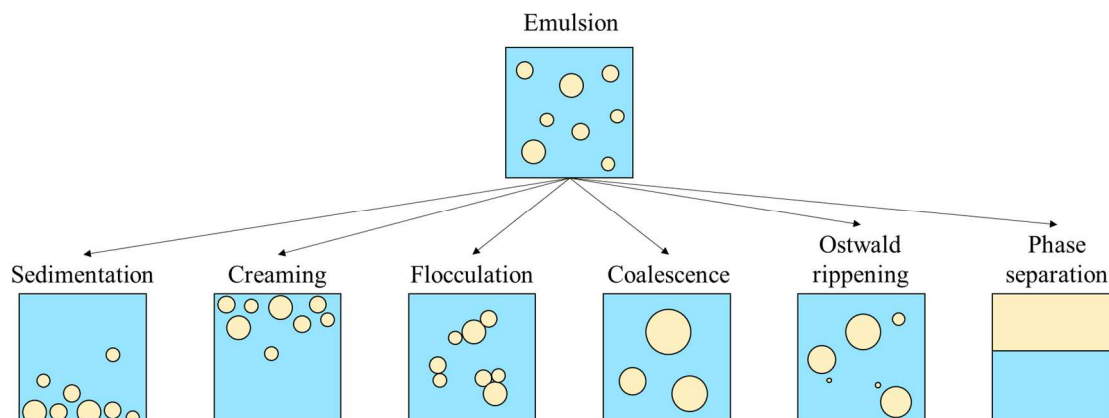


Figure 1.20. Main destabilization mechanism triggering phase separation in O/W and W/O emulsions.

Despite emulsions being thermodynamically unstable, they can be kinetically stabilized by adding some substances that absorb at the oil-water interface, which increase the electrostatic and/or steric repulsion between droplets delaying the destabilization and phase separation. The products used to increase emulsion lifetime are, typically, surfactants, which stay at the oil-water interface and generate electrostatic and steric repulsion between droplets, and particles, that form the so-called Pickering emulsions.

Physically, emulsions appear as milky or cloudy blueish liquids, due to the droplets' capacity to disperse light. The color of the emulsion depends on the concentration and droplet size of the dispersed phase. Conventional emulsions prepared in absence of colorants, with micrometric droplet diameters (0.2 – 100 μm), are normally white suspensions. In contrast, diluted emulsions and emulsions with nanometric droplets (also called nanoemulsions, 50 – 200 nm^[183]) are generally translucent and blueish. Such color differences come from the so-called Tyndall effect, in which the intensity of the scattered light is inversely proportional to the fourth power of the wavelength^[184,185], meaning that blue light is more effectively scattered. Additionally, the small size (< 200 nm) of nanometric droplets limits their capability to scatter high-wavelength visible red light, as light only interacts with particles whose size exceeds about a quarter of its wavelength.

Nowadays, emulsions are widely used in food industry (mayonnaise, salad dressing...), cosmetics (cream, lotions...), pharmaceutical industry, paints, bitumen products, and much more, which demonstrates the high technological and scientific importance of these heterogeneous liquid systems^[182]. However, O/W and W/O emulsions present similar drawbacks as amphiphilic lyotropic liquid crystals, namely the requirement of surfactants, oils or both for their formulation, which are bad for the environment and may be toxic. Alternatively, there is a surfactant- and oil-free variety of emulsions consisting in all-aqueous polymer – polymer or polymer – salt heterogeneous mixtures. These emulsions are called water-in-water (W/W) emulsions, and are very promising in fields such as food and pharmaceutical industries due to their higher biocompatibility and reduced processing limitations (do not form foam).

Notably, oil-in-oil (O/O) emulsions also exist, and consist in heterogeneous mixtures of two immiscible oils, like vegetable and silicon oils. However, these emulsions fall out of the scope of this work and will not be discussed further.

1.5 Water-in-water (W/W) emulsions

Water-in-water (W/W) emulsions are liquid-liquid colloidal dispersions of an aqueous phase into another aqueous phase ^[22,186]. In contrast to conventional O/W and W/O emulsions, phase separation in W/W emulsion does not arise from immiscibility between two solvents (since water is used in both phases), but from thermodynamic incompatibility between two hydrophilic solutes dissolved in water, typically two polymers, two salts or one polymer and one salt ^[187,188].

This thermodynamic incompatibility leads to a positive free energy of mixing two polymers/salts in water that, above a critical concentration of each compound, triggers phase separation forming aqueous two-phase systems (ATPS), in which each phase is enriched with one of the two compounds and saturated with the other. ATPS can be emulsified by applying energy to the system, thus forming W/W emulsions. Notably, this all-aqueous phase separation behavior is typically called segregative phase separation. Alternatively, associative phase separation can occur in water solutions of two components, if the solute1-solute2 interactions are more favorable than the interactions between the solutes and the solvent. In this case, a suspension of a coacervate in a solute-depleted water phase is obtained (Figure 1.21) ^[189].

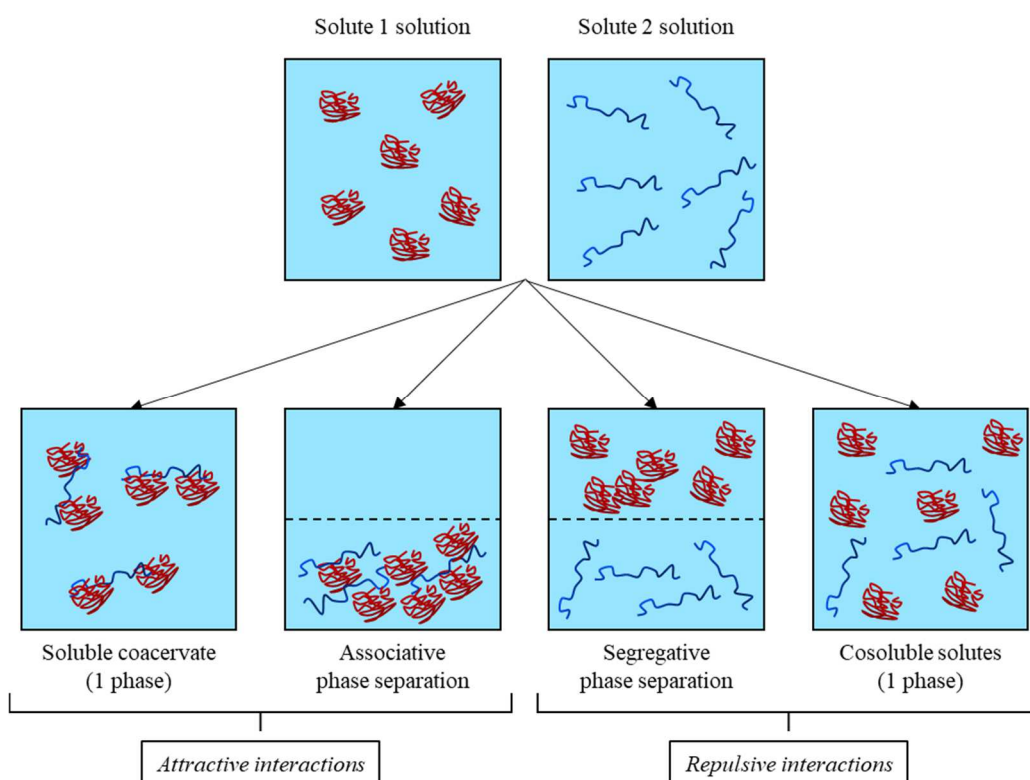


Figure 1.21. Different interactions that can occur in mixtures of polymers and/or salts in water, including attractive (coacervate formation) and repulsive interactions. Adapted from ^[189].

The thermodynamics leading to phase separation are typically explained by the Flory-Huggins interaction parameters ^[22]. The Flory-Huggins interaction parameter (χ_{ij}) was introduced independently by Paul Flory ^[190] and Maurice Huggins ^[191] and indicate the strength of attraction or repulsion between two components i and j . This parameter is directly proportional to $(2E_{ij} - E_{ii} - E_{jj})$, where E_{ij} , E_{ii} and E_{jj} correspond to the energy of interaction between the components i and

j , and between each component with itself. Consequently, a positive χ_{ij} account for high repulsive interactions between i and j , whereas a negative χ_{ij} indicate attractive interactions ^[192].

In three-component systems such ATPS (solute i , solute j , and solvent s), three Flory-Huggins interaction parameters are defined: χ_{ij} , χ_{is} and χ_{js} . In general, due to the higher molecular weight of the solutes compared to the solvent, the interaction parameter χ_{ij} dominates the energy of mixing, and thus large and positive χ_{ij} lead to segregation and phase separation whereas negative χ_{ij} trigger associative coacervation ^[193].

W/W emulsions exhibit several distinctive properties compared to conventional O/W and W/O emulsions. For example:

- The interfaces are relatively thick, ill-defined and the interfacial tensions are extremely low ($10^{-4} - 10^{-2}$ mN/m), which is often much lower than oil/water interfaces ^[186].
- Surfactants cannot adsorb at the water/water interface and thus cannot stabilize W/W emulsions. Alternatively, W/W need to be stabilized with particles adsorbed at the interface (Pickering) ^[194–196] or by forming a membrane at the water/water interface ^[186].

To date, many different types of ATPS have been described to form W/W emulsions, including biopolymer mixtures such as gelatin/maltodextrin or mixtures that include synthetic polymers like polyethylene glycol/dextran. Furthermore, W/W emulsions have been used for several applications. For example, W/W emulsions have been used as delivery systems for probiotic bacteria ^[197] and enzymes ^[198], and templates for microgel ^[199] preparation.

More recently, a new variety of W/W emulsions consisting in polymer/chromonic liquid crystal mixtures has been described ^[200]. These W/W emulsions gained interest among the scientific community both from theoretical (to understand the chromonic behavior in confined domains) and practical (for the preparation of chromonic-based sensors or complex gelled particles of interest as drug delivery systems) points of view.

In the next and last section of this introduction, the emulsification of liquid crystals (with particular focus on lyotropic chromonic liquid crystals) and their behavior in confined domains will be discussed.

1.6 Liquid crystal droplets: confinement effects on chromonic mesophases

The confinement of liquid crystals in emulsion droplets can be achieved either by emulsifying thermotropic liquid crystals in water, forming O/W emulsions ^[201], or by emulsifying lyotropic chromonic liquid crystals in oils or polymer-rich aqueous solution that trigger phase separation upon mixing, forming W/O or W/W emulsions ^[200,202]. The confinement effects on liquid crystal structures is typically studied in nematic mesophases ^[201].

As described in Sections 1.2 and 1.3, bulk nematic liquid crystals are characterized for exhibiting localized orientational order in which the mesogens or stacks of mesogens are oriented towards one direction defined by the director vector. However, confining these liquid crystals in small regions generates elastic deformations in the anisotropic aggregates distorting the director vector in the boundaries of the confined regions (Figure 1.22).

Such elastic deformations can be divided into three: splay, twist and bend deformations (Figure 1.23) ^[203]. Each of these deformations has an associated energy cost, and the interplay between the deformation energy and interfacial tension of emulsified liquid crystal droplets defines the droplet shape. For high interfacial tensions, the droplet will form spherical shapes, assuming the

energy cost of deforming the liquid crystal structure. However, in low interfacial tension emulsions (such as W/W emulsions), the droplets can deform forming undulated and tactoidal structures, which minimize the director vector deformation ^[201].

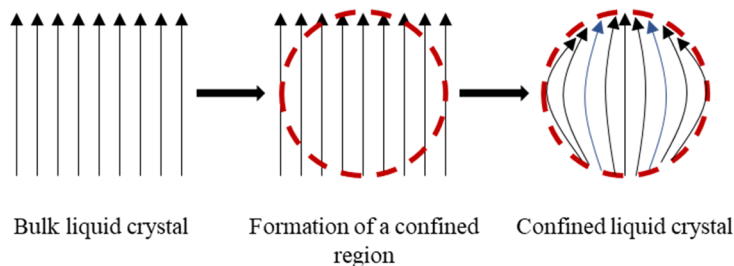


Figure 1.22. Director vector deformation of a bulk liquid crystal in small confined regions.

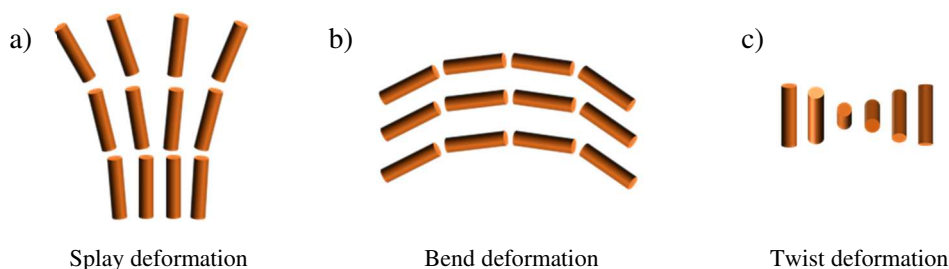


Figure 1.23. Elastic deformations of liquid crystal phases within confined volumes: a) splay, b) bend and c) twist deformations. Note that the cylinders correspond either to aligned mesogens (thermotropic calamitic liquid crystals) or columnar mesogen stacks (thermotropic discotic and lyotropic chromonic liquid crystals).

In addition to elastic deformations, the confinement of liquid crystals also produces point defects within the confined region. The most typical configurations within a liquid crystal droplet are radial and bipolar configurations, which exhibit one and two point defects, respectively ^[204,205]. In radial configuration, the director vector is perpendicular to the interface (which is denoted as homeotropic anchoring) and shows a topological point defect in the middle of the droplet (Figure 1.24a). In contrast, bipolar droplets show two topological point defects in opposite poles of the droplet, also called boojums, and the director vector is parallel to the droplet's interface (which is denoted as planar anchoring) (Figure 1.24b). Notably, more complex configurations can also be formed ^[206].

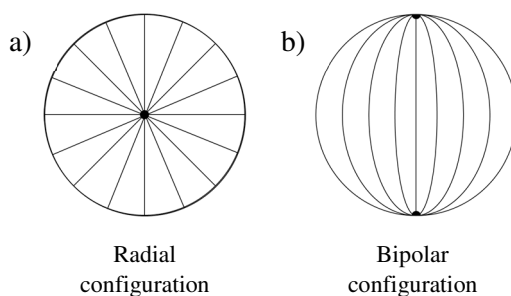


Figure 1.24. a) Radial and b) bipolar configurations of nematic liquid crystal droplets. The lines indicate the director vectors.

Summarizing, the behavior of nematic liquid crystals in confined emulsion droplets is typically defined by the interfacial tension of the emulsion, the energy cost of the splay, bend and twist deformations and the preferred anchoring (homeotropic or planar) ^[206].

For the scope of this thesis, a brief review about the formation of W/W emulsions with confined chromonic structures is very appropriate. The first report of chromonic-based W/W emulsions was published by Simon et al. in 2008^[200]. They described how DSCG formed chromonic droplets in presence of nonionic polymers such as polyacrylamides or polyvinyl alcohols. Notably, depending on the polymer used, spherical radial or tactoidal bipolar droplets were obtained, suggesting a high influence of the polymer in the anchoring of the DSCG mesophase in the droplet interface.

Upon the addition of polyethylene glycol (PEG), Sunset Yellow FCF chromonic nematic mesophases were also shown to phase separate into a N phase with higher mesogen concentration and an I phase enriched with PEG^[207]. Furthermore, such phase separation was enhanced at relatively low Sunset Yellow FCF concentrations by the addition of small amounts of NaCl. More recently, a chromonic nickel (II) phthalocyanine was reported to form stable W/W emulsions with polyvinylpyrrolidone^[208]. Both spherical and tactoidal droplets were obtained.

In summary, the formation of chromonic-based W/W emulsions is a promising but relatively unexplored field, and new research about it is expected to give new insights about chromonic self-assembly in all-aqueous confined regions. Furthermore, promising applications of these systems for templating anisotropic materials and generating complex gelled particles and capsules are envisioned.

2. OBJECTIVES

The main objective of this thesis is the systematic formulation of water-soluble perylene systems with molecular segregation. These include lyotropic chromonic liquid crystals and water-in-water emulsions with confined chromonic droplets.

This thesis also aims to prepare advanced anisotropic materials from these chromonic structures, including silica porous nanomaterials and carbon nanofibers, and evaluate their performance as drug carriers and electrode materials for supercapacitor devices, respectively. Additionally, the DNA-binding properties of various perylene diimides will be evaluated.

Accordingly, the specific objectives of this thesis could be summarized as follows:

- Contribute to a better understanding of the chromonic liquid crystal formation by a systematic study of chromonic phase behavior in water of a series of nonionic, anionic and cationic perylene diimides.
- Discuss how the chemical structure of the mesogens influences the chromonic phase behavior.
- Compare the self-assembly behavior at low concentrations with the chromonic mesophases formed at high concentrations.
- Expand the field of chromonic-based all-aqueous emulsions by systematically formulating W/W and W/W/W emulsions with chromonic perylene diimides and nonionic polymers
- Investigate the potential applications of the perylene-based chromonic mesophase to template inorganic materials such as porous silica and carbon nanofibers.
- Evaluate the capacity of the templated silica materials to carry and release small drugs and the performance of the carbon nanofibers as electrodes for supercapacitor devices.
- Study the DNA-intercalation and cytotoxic properties of the synthesized perylene diimides and compare it to similar naphthalene diimides.

The objectives of each chapter are summarized as follows:

Chapter 1: Chromonic PEGylated perylene diimides for the aqueous synthesis of nanostructured silica materials.

- Synthesize a series of nonionic PEGylated perylene diimides with the formula $(\text{PEG}_n)_2\text{PDI}$ and study their self-assembly and chromonic phase behavior in water.
- Determine the relationship between PEG chain length and chromonic phase behavior.
- Evaluate the capacity of these mesogens to serve as templates for microporous silica fibers.

Chapter 2: Formulation of complex all-aqueous emulsions and gels from ionic chromonic perylene diimides.

- Characterize the chromonic phase behavior of a chiral alanine-functionalized perylene diimide $(\text{Ala})_2\text{PDI}$.
- Formulate W/W, W/W/W and W/W/O emulsions of $(\text{Ala})_2\text{PDI}$ with nonionic high molecular weight polymers and compare them with similar emulsions based on an achiral cationic chromonic perylene diimide.
- Investigate the effects of the polymer and mesogen on the droplets' configuration and morphology by POM.

- Evaluate the gelling properties of (Ala)₂PDI chromonic N phases in the presence of glucono- δ -lactone. Investigate if the nematic structure is retained in the obtained hydrogels.

Chapter 3: Preparation of silica monoliths for the encapsulation and release of ibuprofen

- Prepare porous silica materials with a cationic perylene diimide as template and mixtures of tetraethyl orthosilicate (TEOS) and hexadecyl trimethoxy silane (HDTMS) as silica precursors.
- Evaluate the effects of changing the silica precursor TEOS/HDTMS ratio in the final silica materials, by electron microscopy, Fourier-transform infrared spectroscopy (FTIR) and nitrogen physisorption.
- Design the silica materials as monoliths and characterize them.
- Evaluate the capacity of the silica monoliths to encapsulate ibuprofen and investigate the release kinetics in simulated body fluid (SBF)
- Investigate the hydroxyapatite growth in the silica materials to design robust drug-loaded silica-hydroxyapatite composites.

Chapter 4: Perylene-templated N-doped hierarchically porous carbon nanofibers as efficient supercapacitor electrode materials.

- Prepare nitrogen-doped porous carbon nanofibers (CNFs) from a chromonic perylene diimide, by preparing a chromonic-silica composite, carbonizing and removing the silica by etching.
- Evaluate the effect of carbonizing temperature and etching agents on the physicochemical properties of the CNFs by a combination of techniques including electron microscopy, SAXS, X-ray diffraction (XRD), Raman, FTIR, X-ray photoelectron spectroscopy (XPS), Turbiscan and nitrogen physisorption.
- Prepare electrochemical three-electrode cells doping the working electrode with the CNFs and evaluate their performance for supercapacitor devices by cyclic voltammetry (CV), galvanostatic charge-discharge (GCD) and electrochemical impedance spectroscopy (EIS).
- Discuss and compare the performance of the CNFs as supercapacitor electrodes with other carbon materials described in the literature.

Chapter 5: DNA-intercalation properties of rylene diimides.

- Study the capacity of several perylene and naphthalene diimides as DNA-intercalators by absorbance and fluorescence spectrophotometry, circular dichroism (CD) and viscosity measurements.
- Investigate how the rylene diimides electric charge and core size affect their DNA-intercalation behavior.
- Evaluate the cytotoxicity of the rylene diimides with HeLa cells

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 General reagents

Perylene tetracarboxylic acid dianhydride >98% (PTCDA, TCI Europe), naphthalene tetracarboxylic acid dianhydride >97% (NTCDA, Alfa Aesar), mPEG4–1-amine (TCI Europe), mPEG7-1-amine (Iris Biotech), 500 MW mPEG-1-amine (Sigma), 750 MW mPEG-1-amine (ABCR), 5000 MW mPEG-1-amine (ABCR), L-alanine (TCI Europe), N,N-diethylethylenediamine (TCI Europe), p-methyl toluenesulfonate (Sigma), zinc acetate anhydrous 99.9% (Alfa Aesar), imidazole >98% (TCI Europe), fluorescein isothiocyanate (Sigma), rhodamine isothiocyanate (Sigma), dibutyltin dilaurate (Sigma), glucono- β -lactone (GdL, TCI Europe), tetraethyl orthosilicate (TEOS, Sigma), hexadecyl trimethoxy silane (HDTMS, TCI Europe), ibuprofen (Ibu, Alfa Aesar) and sorbitan monooleate (Span 80, Fluka) were used without further purification.

3.1.2 Solvents

Pyridine 99% (Sigma), hexane (Sigma), anhydrous hexane (TCI Europe), dichloromethane (Carlo-Erba), diethyl ether (Merck), chloroform (Carlo-Erba), ethyl acetate (Panreac), absolute ethanol (Merck), dimethyl sulfoxide (DMSO, Sigma), acetone (J.T. Baker), dimethyl formamide (DMF, Sigma) hydrochloric acid 37 % (HCl, Merck), ammonia 25 % (NH₄OH, Panreac) were used without further purification. Ultrapure water (resistivity = 18.2 M Ω /cm) was used in all the experiments.

3.1.3 Polymers

Dextran (Dex) MW 86000 (Acros Organics), Polyethylene glycol (PEG) MW 10000 (Sigma), MW 6000 (Sigma) and MW 400 (Sigma), and DNA from salmon testes (Sigma) were used without further purification.

Fluorescein- and rhodamine-functionalized Dex were prepared as follows: 5 g of dextran MW 86000, 12.5 mg of fluorescein or rhodamine isothiocyanate and 100 mg of dibutyl dilaurate were dissolved in 50 mL DMSO with 5 droplets of pyridine, and stirred at 65 °C for 2 h. Then, 200 mL of ethanol were added to the mixture at room temperature, and the formed precipitate was recovered by filtration, rinsed with ethanol until the washing solution was completely transparent and dried at 40 °C.

3.1.4 Salts and buffer solutions

Several salts and buffer organic compounds were used to prepare buffer solutions and for other experiments. These include sodium chloride (NaCl, Fluka), potassium chloride (KCl, Panreac), sodium phosphate monobasic (Na₂HPO₄, Merck), potassium phosphate monobasic (KH₂PO₄, Merck), sodium bicarbonate (NaHCO₃, Sigma), potassium phosphate dibasic trihydrate (K₂HPO₄·3H₂O, Calbiochem), magnesium chloride (MgCl₂, Sigma), calcium chloride (CaCl₂, Merck) sodium sulfate (Na₂SO₄, Fluka), calcium nitrate tetrahydrate (Ca(NO₃)₂·4H₂O, Acros Organics) and tris(hydroxymethyl)aminomethane (Tris, Merck).

Phosphate buffered saline (PBS) was prepared by dissolving NaCl (8 g), KCl (0.2 g), Na₂HPO₄ (1.44 g) and KH₂PO₄ (0.24 g) in ~800 mL of water, adjusting the pH to 7.4 with HCl and then adding water to a total volume of 1 L.

Simulated body fluid (SBF) was prepared by dissolving NaCl, (8.04 g), KCl, (0.224 g), NaHCO₃ (0.35 g), K₂HPO₄·3H₂O (0.23 g) and MgCl₂ (0.146 g) in ~500 mL of water with 15 mL of HCl 1M, and then adding CaCl₂ (0.29 g), Na₂SO₄ (0.072 g) and Tris (7.04 g), adjusting the pH to 7.4 with HCl and adding water to a total volume of 1 L.

3.2 Instrumentation

3.2.1 Nuclear magnetic resonance (NMR)

The NMR experiments were conducted at IQAC-CSIC using a Varian Mercury – 400 MHz spectrometer equipped with a VNMRs console. The temperature was regulated with a OneNMR probe and maintained at 25 °C. Proton chemical shifts are denoted in parts per million (δ scale) and calibrated using residual undeuterated solvent peaks as the internal reference (CDCl₃: δ 7.26, DMSO-d₆: δ 2.50, MeOD: δ 3.31, D₂O: δ 4.79). The ¹H-NMR spectra data is presented as follows: chemical shift (δ ppm) (multiplicity, coupling constant (Hz), integration, interpretation). Multiplicities are denoted as follows: s = singlet, d = doublet, t = triplet, m = multiplet.

3.2.2 Fourier-transform infrared spectroscopy (FTIR)

The synthesized rylene diimides were subjected to FTIR analysis in the solid phase at IQAC-CSIC, using a Thermo Nicolet Avatar 360 spectrophotometer equipped with a Thermo Scientific Smart iTR Attenuated Total Reflectance (ATR). The measurements were carried out in the wavenumber range 4000 – 600 cm⁻¹. Intensities are indicated with s = strong, m = medium, w = weak.

For the carbon nanofibers (CNF), FTIR measurements were obtained by a Nicolet Nexus 670 FTIR at NIMS, Japan. The measurements were done in the ATR mode.

3.2.3 Absorption (UV-Vis) spectrophotometry

UV-Vis absorption spectrophotometry spectra were collected with different equipments.

-A double-beam Perkin Elmer Lambda 365 spectrophotometer at IQAC-CSIC, in the range between 190 and 1100 nm. Samples were measured in 1 cm path-length quartz cuvettes. Temperature-controlled experiments were carried out with a Lauda Loop L100 circulation thermostat operating between 4 and 80 °C.

-A single-beam Cary Eclipse 300 Bio UV-Visible spectrophotometer at IQAC-CSIC, with quartz cells of 1 cm path length, operating only at room temperature (ca. 20 °C) and in a range between 200 and 800 nm.

-A single-beam Jasco V-650 spectrometer at IQAC-CSIC, operating only at 800 nm, with quartz cells of 1 cm path length. Temperature-controlled experiments were performed with a Jasco ETC-717 temperature controller.

3.2.4 Fluorescence spectrophotometry

Fluorescent spectra were collected at room temperature (ca. 20 °C) using an Agilent Cary Eclipse at IQAC-CSIC, with quartz cuvettes of 1 cm path length. The detector voltage was set between 400 and 800 V. Temperature-controlled experiments were carried out with a Lauda Loop L100 circulation thermostat operating between 4 and 80 °C.

3.2.5 Microplate reader

Absorbance of the 96 well-plate was measured with a Synergy H1 microplate reader (BioTek) at IQAC-CSIC, at 570 nm.

3.2.6 Circular dichroism (CD)

Circular dichroism spectra were collected at Scientific-technical services, UB, Spain, with a J-815 CD Spectrometer (Jasco) between 200 and 700 nm.

3.2.7 Optical microscopy

Optical microscopy observations were carried out with two different equipments:

-An Olympus BX51TRF-6 microscope at IQAC-CSIC, coupled to an Olympus DP73 camera. Temperature controlled measurements were carried out using a Hot Stage T95 PE Linkam Scientific Instrument Ltd, using microscope slides and cover slips sealed with glue.

-An upright widefield/fluorescence microscope (Nikon Eclipse Ni-E) at INL, Portugal. The microscope was equipped with a SOLA lamp with an output power of 3.5 W and a wavelength range of 380 – 660 nm.

3.2.8 Raman microscopy

Raman microscopy experiments were carried out at INL, Portugal, using an ALPHA300 R Confocal Raman Microscope (WITec). The microscope was equipped with a 532 nm and a 785 nm laser lights for excitation, employing a backscattering geometry. The experiments were conducted at room temperature (ca. 20 °C), with the laser beam focused on the sample by a 100× Zeiss lens. The measurements were performed using a variable laser power ranging from 1 to 30 mW, and an acquisition time of 2 s was employed.

3.2.9 Tensiometer

Surface tension and wettability measurements were carried out at room temperature (ca. 20 °C) by the Wilhelmy plate and the Washburn methods, respectively, using an Attension Sigma 701 force tensiometer (Biolin Scientific) at IQAC-CSIC, equipped with a platinum plate (L = 39.24 mm, for surface tension) or a glass cylinder with a porous membrane (for wettability).

3.2.10 Rheometer

The characterization of rheological properties under deformation and flow regimes was performed at IQAC-CSIC, by an AR-G2 Rheometer (TA instruments) equipped with a stationary stage with concentric cylinders and a gap of 4000 μm . The equipment was set in flow steady state mode, and measurements were taken at shear rates between 1 and 100 s^{-1} .

3.2.11 Small- and wide-angle X-ray scattering (SAXS and WAXS)

SAXS and WAXS measurements were carried out using two different equipments:

-A HECUS X-Ray system GMBH (Graz, Austria), model S3MICRO at IQAC-CSIC. A radiation source with a wavelength (λ) of 0.1542 nm, generated by a $\text{CuK}\alpha$ anode, was employed. Scattering data were recorded using a MBraun PSD-50 M linear detector. The generator was operated at 1 mA and 50 kV. Temperature control was achieved with a Peltier device from Anton Paar (Austria).

-A SAXSess camera (Anton Paar, Austria) at INL, Portugal, was used in conjunction with a PW3830 sealed-tube anode X-ray generator from PANalytical. The monochromatic X-ray beam used had a wavelength (λ) of 0.1542 nm, and was focused with a Göbel mirror and a block (line) collimator.

For liquid samples, wax-sealed glass capillaries with diameters ranging from 0.9 to 1.5 mm were employed., and the analysis was conducted at various temperatures. Solid samples, on the other hand, were placed on a 1 mm thick sample holder, sealed by two Kallebrat films (Kalle Austria GmbH) and analyzed at 25 °C.

The d -spacings and lattice parameters were calculated with Eq. 2 and 3. For nematic and silica and gel samples exhibiting a single broad band in SAXS, the d_0 parameter was determined with Eq. 2. For hexagonal samples, the d_H and a lattice parameters were determined from the slope of Eq. 3.

3.2.12 Powder X-ray diffraction (XRD)

Powder X-Ray diffraction measurements were performed at NIMS, Japan, with a Rigaku X-Ray diffractometer (RINT, Japan) operated at 40 kV and 40 mA with Cu-K α radiation at 25 °C in the range between 10 and 50 °.

3.2.13 Scanning electron microscopy (SEM)

SEM images were taken by different equipments:

- A tabletop Hitachi TM1000 SEM microscope operating at 5 kV at IQAC-CSIC.
- A tabletop Hitachi TM4000Plus SEM microscope operating at 5 kV at IQAC-CSIC.
- A field emission Hitachi S-4800 SEM microscope operating at 10 kV, at NIMS, Japan.
- A Quanta 650FEG ESEM microscope at INL, Portugal, operating at 5 kV and equipped with Energy dispersive X-ray analysis (EDX).

Samples were prepared by drop casting ethanol or water dispersions on a silicon wafer, or by directly depositing solid samples on 12 mm carbon tabs.

3.2.14 Transmission electron microscopy (TEM)

TEM images were taken by different equipments:

- A JEOL JEM-2100 microscope operating at 200 kV, at Scientific-technical services, UB, Spain.
- A JEOL JEM-1210 microscope operating at 120 kV, at ICMAB-CSIC, Spain.
- A JEOL JEM-3100 microscope operating at 300 kV, at NIMS, Japan.

The solid samples were dispersed in solvent (namely ethanol) and deposited on a copper grid covered with a layer of carbon (Carbon support film on copper 200 Square Mesh). Before observation, it was left at room temperature for the solvent to completely evaporate.

3.2.15 Nitrogen sorption

Nitrogen sorption experiments were performed with two equipments:

- A Quantachrome Instrument, Autosorb-IQ at IQAC-CSIC.
- A Quantachrome Instrument, Autosorb-1, at NIMS, Japan.

Before measurements, samples were degassed at high vacuum and temperature:

- Silica samples: 120 °C for 12 h and 125 °C for 2 h.
- Carbon samples: 120 °C for 24 h.

For all samples, the weight after degassing was used for calculations.

The specific surface area (SSA) was estimated from nitrogen adsorption/desorption isotherm analysis (BET model), the micropore area and volume were estimated from the t-plot, the pore size distribution and pore diameter were calculated by using the Barrett-Joyner-Halenda (BJH) and non-local density functional theory (NLDFT) models, and the total pore volume was determined from the last point ($P/P_0 \approx 0.99$) of the isotherms.

3.2.16 Carbon dioxide sorption

Carbon dioxide adsorption isotherms were obtained with a Micrometrics ASAP 2020, degassing the sample at 50°C for 12 h, at ICMAB-CSIC, Spain.

3.2.17 Raman spectroscopy

Raman spectroscopy was measured by a Jasco NRS-3100, at NIMS, Japan.

3.2.18 Thermogravimetric analysis (TGA)

TGA was performed by two instruments:

-A Mettler-Toledo 851e TGA/SDTA instrument at IQAC-CSIC, operating between 25 and 600 °C in oxygen atmosphere.

-A TA Instruments SDT650 DSC/TGA simultaneous instrument at IQAC-CSIC, operating between 25 and 1000 °C in nitrogen atmosphere.

3.2.19 X-ray photoelectron spectroscopy (XPS)

XPS was collected with a Theta Probe spectrometer (Thermo Electron Co.) equipped with a monochromatic Al-K α radiation source with a photon energy of 15 KeV, at NIMS, Japan.

3.2.20 Turbiscan

The stability of solvent-dispersed carbon nanofibers was tested with a Turbiscan Lab Expert at IQAC-CSIC, using a laser of 880 nm wavelength.

3.3 Methods

3.3.1 Synthesis of rylene diimides

3.3.1.1 Synthesis of PEGylated PDI, (PEG_n)₂PDI (n = 4 – 16)

The synthesis of these products was prepared following a previously described method (Figure 3.1) [209]. First, PTCDA (1 eq., see Table 3.1), zinc acetate (~1.3 eq., see Table 3.1) and the corresponding methoxy-PEG_n-amine (mPEG_n-1-amine, ~2.1 eq., see Table 3.1) were dissolved in 5 mL of pyridine and heated to reflux for 20 h. Subsequently, the pyridine was removed by rotary evaporation, resulting in a solid residue. The solid was dissolved in 3 mL of dichloromethane (CH₂Cl₂) and precipitated using hexane. The resulting purple precipitate was collected through filtration, re-dissolved in 3 mL of CH₂Cl₂ and added dropwise to an excess of diethyl ether. After being refrigerated for 12 h, the formed precipitate was collected by filtration and dried under vacuum to give the corresponding (PEG_n)₂PDI, with yields between 47 and 68 %. Note that the *n* values of mPEG_n-1-amine are 4, for mPEG₄-1-amine; 7, for mPEG₇-1-amine; ~10, for 500 MW mPEG-1-amine and ~16, for 750 MW mPEG-1-amine.

Table 3.1. Summary of the specific amounts of reagents and yields for the synthesis of (PEG_n)₂PDI (n = 4 – 16).

Product	PTCDA	Zinc acetate	m(PEG) _n -1-amine	Yield (%)
(PEG ₄) ₂ PDI	78 mg, 0.20 mmol	50 mg, 0.26 mmol	100 mg, 0.48 mmol	-
(PEG ₇) ₂ PDI	238 mg, 0.61 mmol	145 mg, 0.80 mmol	450 mg, 1.33 mmol	68
(PEG ₁₀) ₂ PDI	115 mg, 0.29 mmol	70 mg, 0.36 mmol	340 mg, 0.68 mmol	54
(PEG ₁₆) ₂ PDI	90 mg, 0.23 mmol	55 mg, 0.30 mmol	375 mg, 0.50 mmol	47

All the samples were characterized with ¹H-NMR and FTIR:

(PEG₄)₂PDI. ¹H-NMR (400 MHz, CDCl₃)³ δ = 8.68 (d, J = 8.0 Hz, 4H, Ar-H), 8.61 (d, J = 8.0 Hz, 4H, Ar-H), 4.46 (t, J = 6.0 Hz, 4H, NCH₂H₂O), 3.86 (t, J = 6.0 Hz, 4H, NCH₂CH₂O), 3.60 (m, 12H, OCH₂CH₂O), 3.35 (s, 6H, OCH₃). FTIR (cm⁻¹)⁴: 2877 m, 1688 s, 1655 s, 1591 s, 1578 m, 1507 w, 1435 w, 1403 w, 1371 w, 1336 m, 1301 w, 1261 w, 1243 w, 1178 w, 1105 m, 1064 m, 1044 w, 1020 m, 962 w, 856 w, 809 m, 790 w, 745 w.

³ s = singlet, d = doublet, t = triplet, q = quadruplet, br = broad.

⁴ s = strong, m = medium and w = weak.

(PEG₇)₂PDI. ¹H-NMR (400 MHz, CDCl₃): δ = 8.68 (d, J = 8.0 Hz, 4H, Ar-H), 8.62 (d, J = 8.0 Hz, 4H, Ar-H), 4.46 (t, J = 6.0 Hz, 4H, NCH₂H₂O), 3.86 (t, J = 6.0 Hz, 4H, NCH₂CH₂O), 3.63 (m, 24H, OCH₂CH₂O), 3.37 (s, 6H, OCH₃). FTIR (cm⁻¹): 2867 s, 1690 s, 1656 s, 1592 s, 1578 m, 1508 w, 1437 w, 1404 w, 1336 m, 1301 w, 1244 m, 1179 w, 1098 s, 1063 m, 1022 m, 946 w, 856 w, 810 m, 791 w, 746 w.

(PEG₁₀)₂PDI. ¹H-NMR (400 MHz, CDCl₃): δ = 8.68 (d, J = 8.0 Hz, 4H, Ar-H), 8.63 (d, J = 8.0 Hz, 4H, Ar-H), 4.46 (t, J = 6.0 Hz, 4H, NCH₂H₂O), 3.85 (t, J = 6.0 Hz, 4H, NCH₂CH₂O), 3.65 (m, 72H, OCH₂CH₂O), 3.37 (s, 6H, OCH₃). FTIR (cm⁻¹): 2872 s, 1691 s, 1659 s, 1593 s, 1579 m, 1508 w, 1439 w, 1404 w, 1373 w, 1336 m, 1300 w, 1244 m, 1099 s, 1026 m, 951 w, 854 w, 810 m, 746 w.

(PEG₁₆)₂PDI. ¹H-NMR (400 MHz, CDCl₃): δ = 8.69 (d, J = 8.0 Hz, 4H, Ar-H), 8.65 (d, J = 8.0 Hz, 4H, Ar-H), 4.46 (t, J = 6.0 Hz, 4H, NCH₂H₂O), 3.85 (t, J = 6.0 Hz, 4H, NCH₂CH₂O), 3.64 (m, 120H, OCH₂CH₂O), 3.37 (s, 6H, OCH₃). FTIR (cm⁻¹): 2867 s, 1692 s, 1658 s, 1592 s, 1577 m, 1507 w, 1438 w, 1404 w, 1337 m, 1301 w, 1244 m, 1094 s, 947 w, 856 w, 810 m, 745 w.

3.3.1.2 Synthesis of PEGylated PDIs, (PEG_n)₂PDI (n = 113)

This synthetic procedure was adapted from literature^[210]. PTCDA (5 mg, 0.013 mmol), 5000 MW mPEG-1-amine (140 mg, 0.028 mmol) and imidazole (1.2 g) were mixed in a pressure tube and heated until the imidazole melted (~ 90 °C). Then, the mixture was homogenized by vortex agitation and heated up to 120 °C for 72 h. Afterwards, the sample was cooled to room temperature, and the obtained solid was dissolved in a small quantity of dichloromethane (CH₂Cl₂). The CH₂Cl₂ solution was added dropwise to an excess of diethyl ether under stirring. The precipitate was collected through filtration and dried under vacuum to give (PEG₁₁₃)₂PDI with a 12 % yield.

(PEG₁₁₃)₂PDI. ¹H-NMR (400 MHz, CDCl₃) δ = 8.70 (d, J = 8.0 Hz, 4H, Ar-H), 8.66 (d, J = 8.0 Hz, 4H, Ar-H), 4.46 (t, J = 6.0 Hz, 4H, NCH₂H₂O), 3.84 (t, J = 6.0 Hz, 4H, NCH₂CH₂O), 3.62 (m, ~900H OCH₂CH₂O), 3.36 (s, 6H, OCH₃). FTIR (cm⁻¹): 2883 s, 1695 w, 1659 w, 1595 w, 1466 m, 1455 w, 1360 w, 1341 w, 1279 m, 1241 m, 1146 w, 1099 s, 1060 s, 959 m, 842 m, 810 w, 746 w.

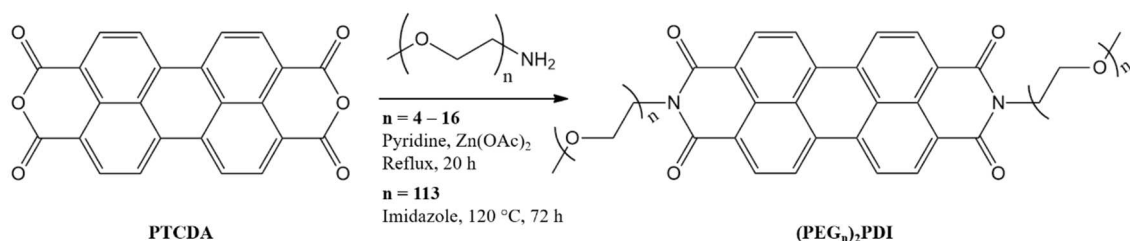


Figure 3.1. Scheme of the synthesis of (PEG_n)₂PDIs (n = 4 – 113).

3.3.1.3 Synthesis of alanine-functionalized PDI, (Ala)₂PDI

This synthesis was adapted from literature (Figure 3.2)^[158]. PTCDA (790 mg, 2 mmol), L-alanine (360 mg, 4 mmol) and imidazole (5.6 g, 40 mmol) were mixed in a round bottom flask and purged with nitrogen for 15 minutes. Then, mixture was heated to 120 °C and stirred for 5 h in a N₂ inert atmosphere. Afterwards, the mixture was cooled to 90 °C and water (6 mL) was added, observing the formation of a red precipitate (unreacted PTCDA). This mixture was stirred for 30 min at 90 °C and then cooled to room temperature and filtrated to remove the remaining PTCDA. Then, 50 mL of HCl 2 M were added to the filtrate to precipitate the synthesized H₂(Ala)₂PDI (the acidic form of (Ala)₂PDI) and the mixture was stirred overnight. Finally, the obtained precipitate was recovered by centrifugation, washed several times with slightly acidic water and dried.

The dibasic (Ala)₂PDI was obtained by grinding the acid compound obtained before and mixing it (0.47 mmol, 250 mg) with Na₂CO₃ (0.47 mmol, 50 mg) in an ethanol/water 5:1 mixture (90 mL) and stirring at 50 °C overnight. Afterwards, the mixture was centrifuged and the precipitate was recovered, washed with the ethanol/water 5:1 mixture several times and dried.

The acid and dibasic forms of (Ala)₂PDI were characterized with ¹H-NMR and FTIR:

H₂(Ala)₂PDI. ¹H-NMR (400 MHz, DMSO-d₆) δ = 12.80 (br s, 2H, C-COOH), 8.58 (br s, 8H, Ar-H), 8.38 (br s, 8H, Ar-H), 5.61 (q, J = 7.0 Hz, 2H, C-H), 1.66 (d, J = 7.0 Hz, 6H, C-CH₃). FTIR (cm⁻¹): 3500 w, 3100 w, 2944 w, 2563 w, 1742 m, 1693 s, 1641 s, 1589 s, 1575 s, 1484 w, 1437 w, 1385 s, 1340 s, 1254 s, 1178 s, 1102 m, 1027 w, 973 m, 959 w, 895 w, 859 m, 837 w, 808 s, 745 s, 732 m, 710 m, 663 m, 596 w.

(Ala)₂PDI. ¹H-NMR (400 MHz, MeOD) δ = 8.72 (d, J = 8.0 Hz, 8H, Ar-H), 8.53 (d, J = 8.0 Hz, 8H, Ar-H), 5.46 (q, J = 7.0 Hz, 2H, C-H), 1.61 (d, J = 7.0 Hz, 6H, C-CH₃). FTIR (cm⁻¹): 3340 w, 1692 m, 1645 s, 1610 m, 1589 s, 1574 s, 1506 w, 1462 w, 1436 w, 1402 w, 1365 m, 1341 s, 1298 w, 1255 m, 1196 w, 1178 w, 1149 w, 1095 w, 1038 w, 971 w, 958 w, 895 w, 852 w, 807 s, 745 s, 713 w, 643 m, 598 w.

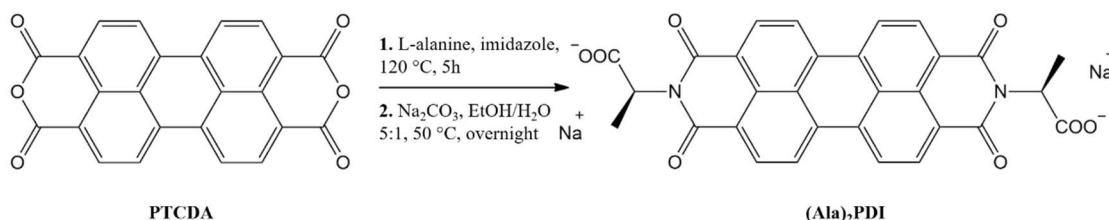


Figure 3.2. Scheme of the synthesis of (Ala)₂PDI.

3.3.1.4 Synthesis of cationic diethylethylenediamine-PDI derivative, MeCatPDI.

The synthesis of the MeCatPDI was adapted from literature (Figure 3.3) ^[100]. It was divided into three steps.

Step 1. First, PTCDA (4.56 g, 11.6 mmol) was dissolved in N,N-diethylethylenediamine (25 mL, 173 mmol) and stirred at 100 °C for 17 h. Afterwards, the so-formed red precipitate was recovered by vacuum filtration, thoroughly washed with ethanol and diethyl ether and dried to yield compound MeCatPDI_d1 of Figure 3.3, with a 87 % yield.

Step 2. Part MeCatPDI_d1 obtained in step 1 (1.5 g, 2.6 mmol) was dissolved in 18 mL of p-methyl toluenesulfonate and stirred at 50 °C overnight. Afterwards, the sample was cooled to room temperature and an excess of methanol was added. Then, the solution was filtered to remove unreacted MeCatPDI_d1 and the filtrate was partially evaporated to reduce the methanol volume. Subsequently, the solution was added dropwise to an excess of diethyl ether, observing the formation of a red precipitate, which was recovered by vacuum filtration and thoroughly washed with an ethanol/diethyl ether mixture and dried to yield MeCatPDI_d2. Yield: 80 %⁵.

Step 3. All the MeCatPDI_d2 obtained in step 2 (~ 2 g, 2.1 mmol) was dissolved in 15 mL of concentrated HCl (180 mmol, ~ 90 eq.) and stirred at 85 °C for 45 min. Then, the solution was cooled and added dropwise to an excess of acetone, observing the formation of a red precipitate, which was recovered by vacuum filtration, thoroughly washed with acetone and diethyl ether and dried to yield MeCatPDI. Yield: 75 %.

⁵ In the original synthesis, Tam-Chang et al. ^[100] further suspended the product in pure ethanol and recovered it by filtration. However, we observed that the product was partially soluble in ethanol, and by applying this step, the yields were decreased almost to 0 %. Consequently, this step was removed.

MeCatPDI. $^1\text{H-NMR}$ (400 MHz, D_2O) δ = 7.79 (br s, 8H, Ar-H), 4.46 (br s, 8H, $\text{NCH}_2\text{CH}_2\text{N}$), 3.60 (br s, 8H, NCH_2CH_3), 3.22 (br s, 6H, NCH_3), 1.51 (br s, 12H, NCH_2CH_3)⁶. FTIR (cm^{-1}): 3228 m, 2983 m, 1688 s, 1651 s, 1591 m, 1575 m, 1510 w, 1480 w, 1437 m, 1400 m, 1360 m, 1340 m, 1251 m, 1164 w, 1127 w, 1099 w, 1053 w, 1023 w, 852 w, 808 m, 792 w, 746 m, 626 w.

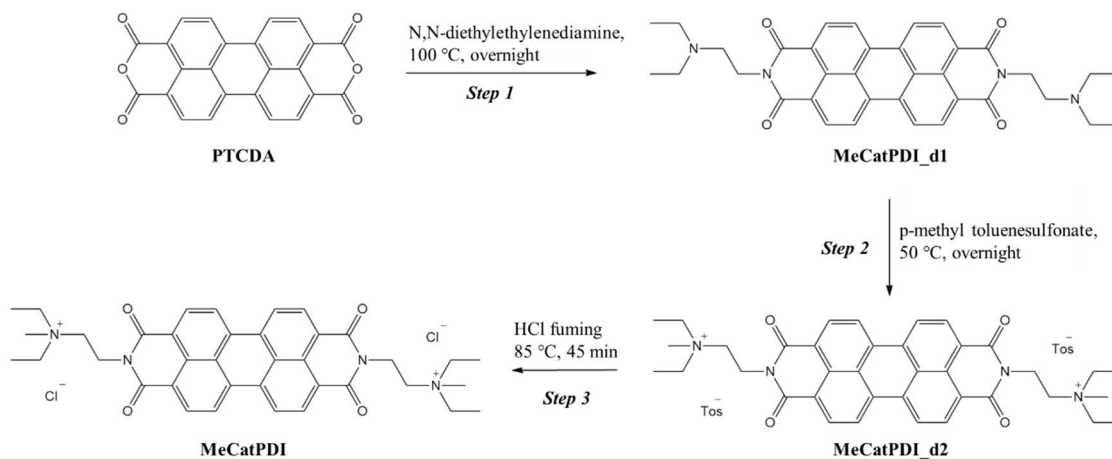


Figure 3.3. Scheme of the synthesis of MeCatPDI.

3.3.1.5 Synthesis of glycine-functionalized NDI. $(\text{Gly})_2\text{NDI}$

The synthesis of $(\text{Gly})_2\text{NDI}$ was reproduced from the literature [211]. NTCDA (268 mg, 1 mmol) and glycine (150 mg, 2 mmol) were dissolved in 15 ml of propanoic acid and heated to reflux for 12 h at 115 °C. The mixture was then cooled down to room temperature and the resulting precipitate was filtered and washed with ethanol three times. The acid $\text{H}_2(\text{Gly})_2\text{NDI}$ was obtained as a pale-yellow solid and dried in a stove at 40 °C. Yield: 69 %.

The dibasic specie was subsequently obtained by the neutralization of $\text{H}_2(\text{Gly})_2\text{NDI}$ with NaHCO_3 . The equivalent amount of NaHCO_3 and the acid product obtained in previous step were dissolved in 17 ml water. After stirring the solution, the water was removed by rotary evaporation. The obtained product was dried in a stove at 40 °C.

The acid $\text{H}_2(\text{Gly})_2\text{NDI}$ was characterized by $^1\text{H-NMR}$ and FTIR.

$^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ 13.22 (br s, 2H, C-COOH), 8.76 (s, 4H, Ar-H), 4.79 (s, 4H, N-CH₂-COOH). FTIR (cm^{-1}): 3082 w, 3043 w, 3006 w, 2962 w, 2672 w, 2578 w, 1723 m, 1666 s, 1583 m, 1456 m, 1447 w, 1387 m, 1359 m, 1324 m, 1244 s, 1189 m, 1008 m, 954 m, 900 m, 879 w, 809 w, 775 m, 725 w, 710 w, 610 w.

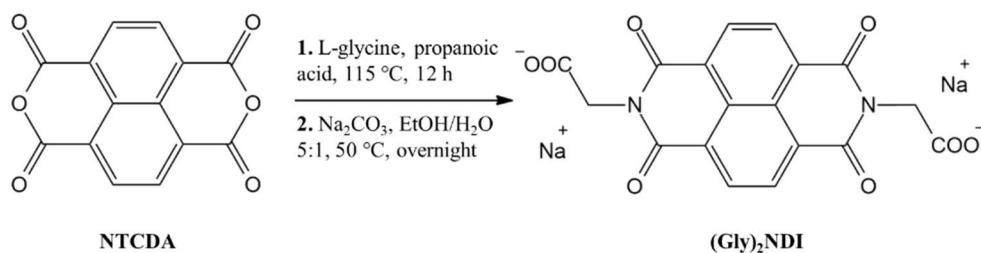


Figure 3.4. Scheme of the synthesis of $(\text{Gly})_2\text{NDI}$.

⁶ All signals are broad singlets due to the aggregation of MeCatPDI in water.

3.3.1.6 Synthesis of cationic diethylethylenediamine-NDI derivative. HCatNDI.

The synthesis of the diethylethylenediamine-NDI derivative was reproduced from literature ^[212]. In a first step, NTCDA (370 mg, 1.38 mmol) and N,N-diethylethylenediamine (493 mg, 5.6 mmol) were mixed with imidazole (14.1 g, 207 mmol) in a round-bottom flask and heated to 130 °C in an inert nitrogen atmosphere for 2 h. Then, the resulting mixture was allowed to cool down to room temperature and the formed precipitate was suspended in methanol to dissolve the imidazole. Finally, a brown solid was collected by filtration using a nylon membrane filter (pore diameter: 0.45 µm). The obtained product was washed with methanol 5 times, mixed using a vortex and centrifuged. Finally, the product was filtered and dried in a stove at 40 °C. Yield: 19 %.

In the second step, the obtained product was acidified by dissolving it in excess HCl 0.5 M and warmed until complete dissolution. Then, the remaining solvent was removed by rotary evaporation obtaining a yellowish solid, which was furtherly dried on a stove at 40 °C.

The product before the acidification was characterized by ¹H-NMR and FTIR.

¹H-NMR (400 MHz, CDCl₃) δ 8.69 (s, 4H, Ar-H), 4.25 (t, 4H), 2.72 (t, 4H), 2.58 (q, 8H), 0.98 (t, 12H). FTIR (cm⁻¹): 3077 w, 2969 m, 2938 w, 2834 w, 2813 w, 1699 m, 1652 s, 1582 m, 1453 m, 1373 m, 1348 s, 1322 m, 1287 m, 1243 s, 1218 w, 1165 m, 1097 m, 1068 m, 1043 m, 986 w, 957 w, 893 m, 769 m, 712 w, 605 w.

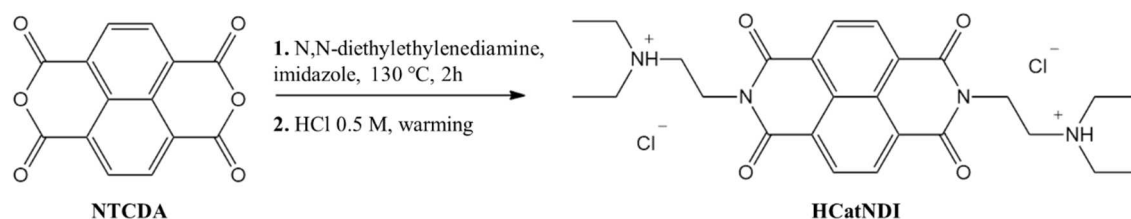


Figure 3.5. Scheme of the synthesis of HCatNDI.

3.3.2 Qualitative determination of the chromonic phase behavior

The formation of chromonic liquid crystal phases was qualitatively tested by contact and evaporation tests. In contact tests, a small solid crystal was placed in between a glass slide and a cover slip and a droplet of water was deposited on the edge of the cover slip so that water diffused through the solid crystal dissolving it and creating a concentration gradient. The sample was subsequently observed by POM.

In evaporation tests, diluted isotropic solutions in water were prepared (1 – 10 wt%) and a droplet was deposited in between a glass slide and a cover slip. It was left for water to evaporate from the edge of the cover slip creating a concentration gradient. The sample was subsequently observed by POM.

3.3.3 Quantitative determination of the chromonic phase behavior: Phase diagrams

Samples at known concentrations were prepared, and studied by POM and SAXS. Highly viscous samples were prepared in a flame-sealed glass tube (diameter = 5 mm) with a constriction in the middle and centrifuged repeatedly at room temperature and stirring with a vortex under heating to 50 °C in between centrifugations.

POM observations at various temperatures were done by placing a droplet of the prepared sample between a glass slide and a cover slip and sealing it with glue. Temperature-controlled

experiments were performed in a range between -10 and 90 °C using a peltier hot stage with a ± 1 °C precision placed below the glass slide. POM was used to determine the chromonic phase at each concentration and temperature studied.

SAXS measurements were performed by introducing the sample in a glass capillary (diameter = 0.9 – 1 mm) and sealing it with wax. The samples were placed in a vacuum chamber with controlled temperature. SAXS was used in selected samples to confirm the POM observations and determine *d*-spacings and lattice parameters.

To draw the phase diagrams, the temperatures at which phase transitions occurred at each concentration were recorded. Additionally, the chromonic phases at the studied temperatures were determined. The average concentration between two studied samples with different chromonic phases at a given temperature was considered to correspond to the phase transition between the two chromonic phases at that temperature. For example, if one sample is isotropic at 10 wt% and 80 °C, and another is nematic + isotropic at 12 wt% at the same temperature, the point at 11 wt% and 80 °C is considered to have a nematic + isotropic to isotropic phase transition.

3.3.4 Lower critical solution temperature (LCST) determination

LCST determinations of (PEG_n)₂PDI compounds were performed preparing a water solution of the (PEG_n)₂PDI at $\sim 10^{-4}$ M with salts at concentrations between 0.5 and 2 M. Then, the samples were heated with a temperature-controlled oil-bath until the solution became turbid (LCST value). Some selected samples were also studied by temperature-controlled absorbance spectrophotometry. The following salts were used: LiCl, NaCl, KCl, CaCl₂, NH₄Cl, NH₄F and (NH₄)₂SO₄.

3.3.5 Surface tension and wettability measurements

Surface tension measurements were performed with the tensiometer Attension Sigma 701 at IQAC-CSIC, by preparing (PEG_n)₂PDI water solutions in glassware thoroughly washed with ethanol and acetone and dried in a stove at 40 °C. The solutions were poured in a crystallizer (diameter = 50 mm) previously immersed in a chromic acid solution for several hours and thoroughly washed with ethanol, and measured with the tensiometer at room temperature (ca. 20 °C) using a Wilhelmy plate also washed with chromic acid solution and ethanol.

Wettability was measured with the Washburn method. 100 mg of the studied sample were loaded into a glass cylinder with a porous membrane. The method consisted in placing the cylinder in the tensiometer (Attension Sigma 701) and putting in contact with a water-filled cuvette. The mass increment as a function of time was measured.

3.3.6 Preparation of two-phase and three-phase emulsions

Water-in-water (W/W) emulsions were prepared by weighting the polymers/mesogens in a glass assay tube (diameter = 10 mm) and adding the corresponding amount of water. Then, mixtures were centrifuged and mixed with a vortex for 10 minutes. More viscous emulsions were prepared similarly, but after the 10 min vortex they were heated to 50 °C and mixed another 5 min with the vortex.

Three-phase water-in-water-in-water (W/W/W) emulsions were prepared by weighing two of the compounds, adding the corresponding amount of water, centrifuging and mixing with vortex for 10 min. Then, the third compound was added, the sample was centrifuged, mixed with vortex for 10 min, heated to 50 °C for 5 min and mixed again with vortex for 10 min.

W/W and W/W/W emulsions were kept in a 25 °C bath for at least 5 min before the observation by optical microscopy.

Three-phase water-in-water-in-oil emulsions (W/W/O) were prepared by first preparing a W/W emulsion as described previously, and then incorporating it in hexadecane with Span 80. The mixture was vortexed for 10 min and immediately observed with optical microscopy. The hexadecane was washed several times with water prior to the preparation of the emulsions to saturate it with water.

3.3.7 Characterization of water-in-water emulsions

Emulsions were characterized by optical microscopy and SAXS. Optical microscope samples were prepared pouring a droplet of the emulsion with a spatula on a glass slide and covering with a cover slip. Samples were observed by bright field microscopy, POM and fluorescence microscopy at room temperature (ca. 20 °C). Temperature-controlled experiments in a range between -10 and 90 °C were performed sealing the cover slip with glue and heating with a peltier hot stage with a ± 1 °C precision placed below the glass slide.

SAXS measurements were performed by introducing the sample in a glass capillary (diameter = 0.9 – 1 mm) and sealing it with wax. The samples were placed in a vacuum chamber with controlled temperature.

3.3.8 Preparation of chromonic liquid crystal gels

Chromonic liquid crystal gelation experiments were performed by first preparing a nematic sample of (Ala)₂PDI in an assay tube (diameter = 10 mm) and adding solid glucono- δ -lactone (GdL), centrifuging and mixing with a vortex for 10 min to ensure the complete dissolution of GdL. Then, the sample was left at room temperature during 24 h for the (Ala)₂PDI to gel.

3.3.9 Characterization of chromonic liquid crystal gels

Gels were characterized by optical microscopy and SAXS. Optical microscopy samples were prepared by placing a piece of the gel (after gelation) in a glass slide and covering with a cover slip. Other samples were prepared by directly gelling them in the glass slide. For that, a droplet of the sample after the addition of GdL and vortex homogenizing was poured in a glass slide, covered with a cover slip and sealed with glue to prevent water evaporation. Samples were observed by bright field microscopy and POM.

SAXS samples were prepared by introducing the sample before gelation (i.e. after GdL addition and vortex homogenization) in a glass capillary (diameter = 0.9 – 1 mm) and sealing it with wax. The samples gelled inside the capillary and then were placed in a vacuum chamber with controlled temperature and analyzed.

3.3.10 Synthesis of nanostructured templated silica materials

3.3.10.1 (PEG_n)₂PDI templated silica

Nanostructured silica synthetic procedure was adapted from the literature [213,214]. First, (PEG_n)₂PDI (where $n = 7, 10, 16$) was dissolved in 5 M HCl aqueous solution. Then, tetraethyl orthosilicate (TEOS) was added to the solution, and the entire mixture was stirred at 60 °C for 24 h. The mass ratio of (PEG_n)₂PDI to aqueous HCl to TEOS was 2/93/5. After that, the sample was kept at 80 °C for an additional 24 h. Ethanol was subsequently added to the mixture, which was stirred for 20 h. Finally, the mixture was filtered, washed with more ethanol, and dried at 80 °C for 5 h.

A portion of the resulting red powder was calcined at 600 °C for 6 h in a Carbolite GHA 12/450 oven, under a heating rate of 1 °C/min, resulting in a white powder. The products were labelled as **Si-x**, where x indicates the mesogen used in the synthesis. Specifically, **Si-2** for (PEG₇)₂PDI, **Si-3** for (PEG₁₀)₂PDI and **Si-4** for (PEG₁₆)₂PDI.

3.3.10.2 MeCatPDI templated silica

Nanostructured silica synthesis was adapted from the literature ^[164]. First MeCatPDI was dissolved in ammonia 25 %. Then, the silica precursor (TEOS or a TEOS/HDTMS 9:1 mixture) was added to the solution and the whole mixture was stirred at 25 °C for 90 minutes and at 50 °C for another 150 minutes, the MeCatPDI/ammonia 25%/silica precursor mass ratio was 1/50/10. After that, the formed precipitate was recovered by vacuum filtration, thoroughly washed with water, dried and calcined at 600 °C for 6 h in a Carbolite GHA 12/450 oven (heating rate: 1 °C/min) to obtain a white powder. The products were labelled as SiHDTMS_*n*, where *n* indicates the mass percentage of HDTMS used for the synthesis.

3.3.10.3 MeCatPDI templated silica monoliths

The preparation of nanostructured silica monoliths was prepared as follows. First, 100 mg of MeCatPDI were dissolved in 5 g of ammonia 25 wt%. Afterwards, 9 Eppendorf tubes were filled with ~510 mg of the prepared solution. Note that each Eppendorf contains ~10 mg of MeCatPDI and ~500 mg of ammonia 25 %. Subsequently, 100 mg of the silica precursor (TEOS or a TEOS/HDTMS 9:1 mixture) were added to each Eppendorf, so that the MeCatPDI/ammonia 25%/silica precursor mass ratio was 1/50/10, and homogenized with vortex immediately after addition. Then, the samples were left with no agitation at 25 °C for 90 min and at 50 °C for 150 min. After that time, red monoliths were formed inside the eppendorf vials. The monoliths were washed inside the eppendorf vials by filling them with water several times. Next, the eppendorf vials were filled again with water, three small holes were made in the cap with a needle and then were frozen and lyophilized to detach the monoliths from the vials, obtaining conical red silica-PDI monoliths. The MeCatPDI template was removed by calcining the monoliths at 600 °C for 6 h in a Carbolite GHA 12/450 oven (heating rate: 1 °C/min) to obtain white nanostructured silica monoliths. The monoliths were labelled as SiMonHDTMS_*n*, where *n* indicates the mass percentage of HDTMS used for the synthesis.

3.3.10.4 Hydroxyapatite growth on nanostructured silica materials

Hydroxyapatite was grown on silica fibers as follows. First, silica was cleaned with a 2% SDS solution overnight, washed and activated with a HCl 37 %/Methanol 1:1 mixture for 30 min at room temperature, aiming to induce the formation of silanol (Si-OH) in the silica surface. Afterwards, 50 mg of the activated silica were suspended in water (5 mL) and 59 mg of Ca(NO₃)₂·4H₂O were added, the pH was adjusted to 10 with ammonia and the mixture was stirred at room temperature for 3 h, to favor the Si-O --- Ca interactions. Then, 21 mg of Na₂HPO₄ were added and the pH was again adjusted to 10 with ammonia. The mixture was stirred for another 24 h at room temperature. Finally, the suspended white solid was recovered by centrifugation, washed with MiliQ water and ethanol and dried.

3.3.11 Encapsulation of ibuprofen in the silica powder samples

Ibuprofen was encapsulated in silica powder samples by two methods: the soaking + drying method and the melting method.

The soaking + drying method (SDM), involved suspending the silica in a 33 mg/mL ibuprofen solution in hexane (66 mg of silica per mL of hexane) and letting it equilibrate for 15 minutes, followed by evaporation of the solvent under a nitrogen flow. Then, the silica was suspended in 1 mL hexane, stirred and recovered by centrifugation. This was done twice (30 s stirring in the first wash and 10 s stirring in the second wash), to remove surface-adsorbed ibuprofen.

The melting method (MM), involved preparing a solid silica-ibuprofen composite at a 2:1 mass proportion and heating it to 85°C for 10 minutes to allow the ibuprofen to melt and penetrate

within the pores. Then, the silica was suspended in 1 mL hexane, stirred and recovered by centrifugation. This was done twice (30 s stirring in the first wash and 10 s stirring in the second wash), to remove surface-adsorbed ibuprofen.

The ibuprofen encapsulation efficiency (*IbuEE*) was calculated by thermogravimetric analysis (TGA) of pure silica materials and the ibuprofen-loaded silica materials. The total loaded ibuprofen was determined as the weight loss between 100 and 700 °C, applying a correction from the weight loss of the unloaded silica materials in this temperature range. *IbuEE* was determined by Eq. 6.

$$IbuEE (\%) = \frac{Ibu_{Load} (mg)}{Ibu_{Total} (mg)} \cdot 100 \quad (Eq. 6)$$

Where *Ibu_{Load}* is the amount of loaded ibuprofen (calculated from the TGA) and *Ibu_{Total}* is the total ibuprofen used in the encapsulation method.

3.3.12 Encapsulation of ibuprofen in the silica monolithic samples

Ibuprofen was encapsulated in silica monolithic samples by the SDM method. For this, 1 mL of ibuprofen dissolved in hexane (16.5 mg/mL) was added to a monolith (average weight = 30 mg) inside an Eppendorf vial and allowed to equilibrate for 15 min before drying in a stove at 30 °C until complete evaporation of the solvent.

The *IbuEE* of ibuprofen in the silica monoliths was determined by weighting the monoliths before and after drug loading, and considering the difference as the total amount of ibuprofen incorporated into the monoliths (Eq. 7).

$$IbuEE (\%) = \frac{Mon_{Load} (mg) - Mon_w (mg)}{Ibu_{Total} (mg)} \cdot 100 \quad (Eq. 7)$$

Where *Mon_{Load}* and *Mon_w* are the weights of the monoliths after and before ibuprofen loading and *Ibu_{Total}* is the total ibuprofen used in the encapsulation method (16.5 mg).

3.3.13 Ibuprofen quantification with absorbance spectrophotometry

Quantification of ibuprofen in solution was performed with absorption spectrophotometry. A calibration curve of ibuprofen was done in Simulated Body Fluid (SBF, pH = 7.4) at room temperature (ca. 20 °C), with UV absorbance at 264 nm. For this, ten solutions of ibuprofen in SBF (0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45 and 0.50 mg/mL) were prepared and absorbance was measured at 264 nm with a quartz cell (path length = 1 cm). The calibration curve was plotted and a regression line was obtained (Figure 3.6). For samples of unknown concentration, absorbance was measured and substituted in the equation of Figure 3.6 (y) to calculate the concentration (x).

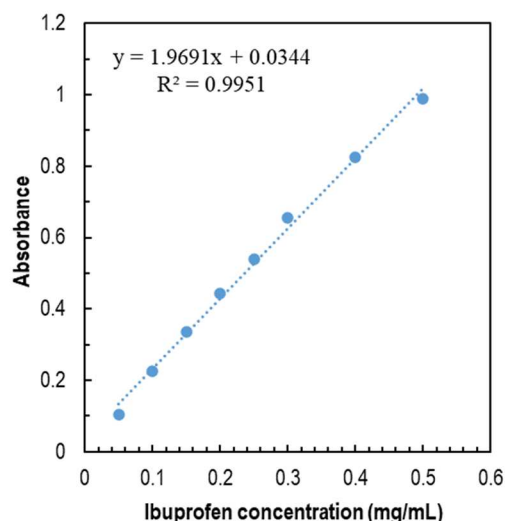


Figure 3.6. *Ibuprofen calibration curve in simulated body fluid (SBF) pH 7.4, at ca. 20 °C.*

3.3.14 Determination of ibuprofen solubility in SBF

An excess of ibuprofen (50 mg) was added to SBF (10 mL, pH = 7.4) and stirred overnight at 37 °C. Then, the mixture was filtered to obtain a saturated solution of ibuprofen in SBF, and its ibuprofen concentration was determined by UV absorbance at 264 nm, using the calibration curve of Figure 3.6. The solubility of ibuprofen in SBF at 37 °C was determined to be 2.02 mg/mL.

3.3.15 *In-vitro* ibuprofen release

Drug release experiments were performed with the ibuprofen-loaded silica monoliths. The release assays were performed in jacketed glasses coupled to a thermostatic bath to maintain the temperature at 37 °C. The jacketed glasses were filled with water and a Falcon tube was filled with 20 mL of the receptor medium (SBF). A dialysis bag (Spectra/Por molecular porous dialysis membranes with molecular weight cut-off (MWCO)⁷ of 3.5 kD) was prewetted inside the Falcon tube for 15 min. Afterwards, one ibuprofen-loaded silica monolith was placed in the dialysis bag and 1 mL SBF was added. Immediately, the bag was closed and introduced again into the Falcon tube and the timer for the release experiment was turned on. Aliquots of 1 mL were withdrawn from the release receptor medium after different time intervals, and it was subsequently refilled with 1 mL of new receptor medium (SBF) to maintain the total volume constant. Before taking every aliquot, the Falcon tube was removed from the jacketed glass and very carefully homogenized to collect the water condensed in the Falcon tube walls and cap.

A total of 19 aliquots were taken (5, 10, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 300, 360, 540 min, and 24, 27, 30 and 48 h). The ibuprofen concentration of each aliquot was determined with UV-Vis absorbance at 264 nm. All the samples were measured in triplicate experiments. The drug release profiles were plotted as the percentage (%) of ibuprofen released as a function of time.

The volume of receptor medium used in the release experiments (20 mL) was determined to ensure that it could solubilize at least three times the amount of ibuprofen present in the dosage form, hence ensuring sink conditions.

Furthermore, the drug release profiles were fitted to several kinetic models ^[215] using the *DDSolver* Excel template ^[216]:

⁷ MWCO refers to the lowest molecular weight solute (in Daltons) in which 90% of the solute is retained by the membrane. In our case, the membrane would retain 90% of a solute with MW of 3.5 kD.

Zero-order model: The zero-order model describes dissolution kinetics in which the dissolution rate is constant. This is expressed by Eq. 8.

$$-\frac{dC_D}{dt} = k \quad (\text{Eq. 8})$$

Where C_D indicates the concentration of drug in the dosage form and t is time. Hence dC_D/dt indicates the drug dissolution rate from the dosage form. By integrating, Eq. 9 is obtained.

$$C_{D0} - C_D = k \cdot t \quad (\text{Eq. 9})$$

Where C_{D0} is the amount of drug loaded in the dosage form and $C_{D0} - C_D$ is the concentration of drug released (C_R). Hence, dividing each side of the equation by C_{D0} and multiplying by 100, Eq. 9 can be rewritten as shown in Eq. 10.

$$\%Rel = k_0 \cdot t \quad (\text{Eq. 10})$$

Where $\%Rel$ is the percentage of released drug, and k_0 is equal to $k \cdot 100/C_{D0}$.

First-order model: The first-order model considers dissolution kinetics in which the drug dissolution rate depends on the drug concentration (Eq. 11)

$$-\frac{dC_D}{dt} = k \cdot C_D \quad (\text{Eq. 11})$$

Where C_D indicates the concentration of drug in the dosage form and t is the time. Hence dC_D/dt indicates the drug dissolution rate from the dosage form. After integration, Eq. 12 is obtained.

$$C_D = C_{D0} \cdot e^{-kt} \quad (\text{Eq. 12})$$

Where C_{D0} is the amount of drug loaded in the dosage form. By subtracting each side of Eq. 12 to C_{D0} , Eq. 13 is obtained.

$$C_{D0} - C_D = C_{D0} \cdot (1 - e^{-kt}) \quad (\text{Eq. 13})$$

Where $C_{D0} - C_D$ is the concentration of drug released (C_R). Finally, by dividing each side of Eq. 13 by C_{D0} and multiplying by 100, Eq. 14 is obtained.

$$\%Rel = 100 \cdot (1 - e^{-kt}) \quad (\text{Eq. 14})$$

Where $\%Rel$ is the percentage of released drug.

Higuchi model: ^[217] This model considers an ideal situation in which the drug is released from a solid matrix saturated with drug to a receptor container in complete sink conditions.

According to this model, the dissolution rate depends on the dissolution of the drug from the saturated solid matrix, which is a diffusion-limited process that follows Fick's Laws of diffusion. Additionally, Higuchi's model considers that other processes leading to drug release such as swelling or erosion do not occur.

The Higuchi model is mathematically described by Eq. 15, that indicates that the amount of drug released ($\%Rel$) is proportional to the square root of time.

$$\%Rel = k_H \cdot t^{0.5} \quad (\text{Eq. 15})$$

Where k_H is the Higuchi's constant.

Korsmeyer-Peppas: ^[218] The Korsmeyer-Peppas model is a semi-empirical model that establishes an exponential relation between the percentage of drug released ($\%Rel$) and time (Eq. 16). Notably, the Korsmeyer-Peppas model can only be applied up to release percentages below 60 % ^[219].

$$\%Rel = k_{KP} \cdot t^n \quad (Eq. 16)$$

Where k_{KP} is the Korsmeyer-Peppas constant, and n is the Korsmeyer-Peppas exponent.

However, in contrast to the Higuchi model, this exponent is not limited to 0.5, but it is variable. If $n = 0.5$, the Korsmeyer-Peppas model is reduced to the Higuchi model.

The determination of the n parameter of drug delivery systems gives insights about the mechanism of drug release. A release exponent $n \leq 0.5$ corresponds to fully Fickian-based transport of the drug from the dosage form to the bulk solution, whereas $0.5 < n < 1$ indicates an anomalous non-Fickian diffusion.

3.3.16 Synthesis of carbon nanofibers

Carbon nanofibers were prepared in a two-step process using MeCatPDI as the carbon precursor and silica as a hard template. In the initial step, nanostructured silica was prepared following the literature: MeCatPDI (400 mg) was dissolved in ammonia 25 % (20 g) and TEOS (1 g) was added dropwise under vigorous agitation. Then, the mixture was homogenized with vortex and stirred at 25 °C for 90 min and at 70 °C for another 90 min. Afterwards, the obtained red precipitate was recovered by filtration, washed with abundant water and dried to obtain a silica-PDI composite.

In the second step, carbon nanofibers (CNFs) were prepared through pyrolysis of the nanostructured SiO₂ materials previously obtained. This was performed under an inert nitrogen atmosphere at high temperatures (900 – 1100 °C) for 2 h (heating at a rate of 5 °C/min), which produced a silica-carbon nanofiber composite. The silica was etched with aqueous solutions of strong bases (aq. 1 M NaOH or 1 M KOH, 90 °C, 17 h), resulting in black precipitates that were washed by successive centrifugations, at 2880 relative centrifugation force (RCF), with Milli-Q water. The washing process was considered complete when the pH of the supernatant was between 7 and 8. The obtained wet product, which consisted in a black ink-like viscous dispersion of CNFs in water, was dried by lyophilization, to obtain the carbon nanofibers (hereinafter CNF-PDIs). Four materials were prepared: CNF-PDI_N900, CNF-PDI_N1000, CNF-PDI_N1100 and CNF-PDI_K900, where the number indicates the carbonization temperature and the letter the base used for etching (N for NaOH and K for KOH).

3.3.17 Determination of carbon nanofiber electrochemical properties

The electrochemical energy storage (EES) performances of the CNF-PDI materials were studied using cyclic voltammetry (CV), chronopotentiometry (galvanostatic charge discharge, or GCD), and electrochemical impedance spectroscopy (EIS) at 25 °C, and in a three-electrode system with an aqueous electrolyte solution (1M H₂SO₄). The working electrode consisted in a glassy carbon electrode (GCE) modified with the CNF-PDI. In contrast, the reference and counter electrodes were an Ag/AgCl electrode and a platinum wire, respectively. The working electrodes were prepared with the carbon nanofiber materials by first grounding the CNF-PDIs on an agate mortar with a pestle and then dispersing them in a solvent mixture consisting in a water/ethanol mixture (4:1, 1 mg/mL), by applying sonication for 60 min in a bath sonicator. 3 µL of the resulting suspensions were deposited onto the clean and dry GCE surface and dried in a hot-air oven at 60 °C for 2 h. 5 µL of a 5 % in ethanol Nafion solution was used as binder. The solution was incorporated and dried under reduced pressure at 80 °C for 6 h. The electrochemical energy storage measurements were conducted at 25 °C with a CHI 660E workstation from CH Instruments, Inc. Austin, TX, USA. The electrochemical impedance spectroscopy (EIS) measurements were performed at an open-circuit potential in the frequency range from 0.01 Hz to 100 kHz, and at an amplitude of 5 mV. The specific capacitance (C_s) of the electrode was

calculated from both the cyclic voltammetry (CV) and galvanostatic charge-discharge (GCD) curves, by Eq. 17 and Eq. 18, respectively.

$$C_s = \frac{\int_{V_1}^{V_2} I(V) dV}{2 \cdot m \cdot \Delta V \cdot \nu} \quad (\text{Eq. 17})$$

Where I is the current intensity, V is the potential, m is the mass of active electrode material (3 μg), ΔV is the potential window and ν is the scan rate. Note that $\int_{V_1}^{V_2} I(V) dV$ is the integrated area under the CV curves (both charge and discharge curves)

$$C_s = \frac{2 \cdot I \int_{t_{D1}}^{t_{D2}} V(t_D) dt_D}{m \cdot \Delta V^2} \quad (\text{Eq. 18})$$

Where I is the intensity, V is the potential, t_D is the discharge time, m is the mass of the active electrode material (3 μg) and ΔV is the potential window. Note that I/m is the current density. Also note that $\int_{t_{D1}}^{t_{D2}} V(t_D) dt_D$ is the integrated area under the discharge GCD curve.

The rate capability was calculated from the CV and GCD curves dividing the C_s at a given scan rate or current density by the C_s at the lowest scan rate or current density studied.

Furthermore, the coulombic efficiency (CE) and the energy efficiency (EE) were calculated from the GCD curves with Eq. 19 and Eq. 20, respectively.

$$CE = \frac{t_D}{t_C} \cdot 100 \quad (\text{Eq. 19})$$

$$EE = \frac{\int_{t_{D1}}^{t_{D2}} V(t_D) dt_D}{\int_{t_{C1}}^{t_{C2}} V(t_C) dt_C} \cdot 100 \quad (\text{Eq. 20})$$

Where t_C and t_D are the charge and discharge times, respectively.

Finally, the cycle life stability was determined from the GCD experiments, as the ratio between the C_s after 10000 charge-discharge cycles and the C_s of the first cycle.

3.3.18 Preparation of carbon nanofiber films

Carbon nanofiber films were prepared by drop casting a concentrated dispersion of CNF-PDIs in water onto a previously cleaned glass substrate framed with adhesive tape and spreading such drop with a glass roller. The black ink-like dispersion obtained during the synthesis, before the lyophilization step, was used. This resulted in a black layer, which was dried on a stove. This procedure was repeated for five times. The resistivity of the prepared CNF-PDI films was measured with a digital multimeter (Silver Electronics, Model 9965).

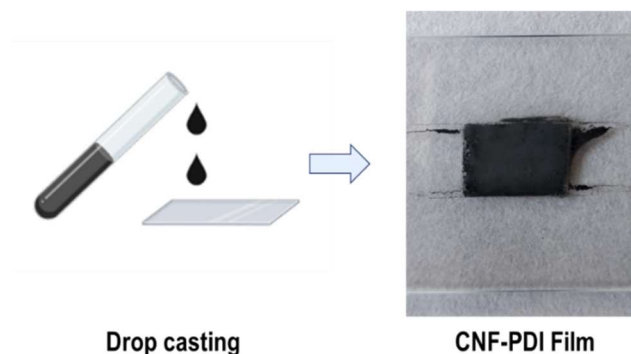


Figure 3.7. Scheme for the preparation of CNF_Films.

3.3.19 Preparation of DNA-RDI solutions

DNA concentrated solutions were prepared by mixing 20 mg of DNA with 10 mL of PBS and stirring for several hours at room temperature (ca. 20 °C). After DNA was completely dissolved, it was stored in the fridge and used for no more than 4 days.

Separately, a RDI 200 mg/L solution in PBS was prepared. DNA-RDI solutions were prepared by diluting the two concentrated solutions together with PBS to obtain a mixed solution with the desired concentration of each component. These dissolutions were stored in the fridge and analyzed within the following 24 h.

3.3.20 Determination of the RDI DNA-intercalation properties

The in-vitro DNA-intercalating behavior of the different synthesized RDIs was assessed by multiple techniques. Absorption and fluorescence spectrophotometry spectra were taken at room temperature (ca. 20 °C) using a quartz cuvette in the range between 300 and 700 nm. Circular dichroism was analyzed in the range between 200 and 700 nm, also using a quartz cuvette. Viscosity measurements were run with a rheometer using the concentric cylinders' geometry, as described in Section 3.2.10. Viscosity measurements were performed in triplicate. The average viscosity of the shear rates between 10 and 30 s⁻¹ was taken as the viscosity of each sample, and the standard error of the measurement was considered from the calculated viscosities of the triplicate measurements.

3.3.21 Cytotoxicity tests

The cytotoxicity of the different RDIs was evaluated in HeLa cells. For that, HeLa cells were cultivated in Dulbecco's Modified Eagle Medium (DMEM) at 37 °C and 5 % CO₂ atmosphere. DMEM was previously supplemented with 10 % fetal bovine serum (FBS). HeLa cells (5000 cells) were placed in a 96-well plate in the presence of DMEM (200 µL) and incubated for 24 hours. After the incubation, DMEM was removed and cells were incubated for 24 h with DMEM modified with RDI at 10 µM (200 µL). Then, the system containing media was removed and replaced with fresh DMEM (200 µL) and incubated for 16 hours. Afterwards, 20 µL of MTT (5 mg/mL) were added and cells were incubated for another 3 hours, observing the formation of formazan crystals. Finally, the DMEM was removed and 200 µL of DMSO were added to dissolve formazan crystals and the absorbance of each well of the 96 well-plate was measured at 570 nm in a microplate reader.

4. RESULTS AND DISCUSSION

4.1 Chromonic PEGylated perylene diimides for the aqueous synthesis of nanostructured silica materials

This chapter describes the synthesis and characterization of five PEGylated perylene diimides formed by attaching PEG substituents of different length at the imide position of the perylene core (Figure 4.1). Hence, these compounds are formed by a hydrophobic perylene core with hydrophilic PEGylated functional groups at the imide positions. These nonionic hydrophilic groups impart solubility and capacity to self-assemble in water to the molecules, whereas the polyaromatic core leads to π -stacking. The diluted solutions of the synthesized PEGylated perylene dyes exhibit interesting optical and fluorescent properties that vary depending on the solvent and concentration. These compounds also show a lower critical solution temperature (LCST) and surface activity. Furthermore, at high concentrations, the PEGylated PDIs produce lyotropic chromonic liquid crystals (LCLC) with nematic and hexagonal structures, formed by aligned molecular stacks. These nonionic compounds are interesting since the formation of chromonic mesophases by nonionic compounds has not been systematically studied, and can be useful in applications where the potential toxicity of the cationic perylene-based chromonic mesogens may be a concern. The lyotropic chromonic liquid crystals reported here were used for the fabrication of composite chromonic-silica materials consisting in a SiO₂ matrix with ordered perylene nanostripes, that formed aligned nanopores (with a diameter of ca. 1.4 nm) upon calcination. Notably, the distance between aligned PDI nanostripes and consequently the pore wall thickness could be finely controlled solely by changing the length of the PEG chain.

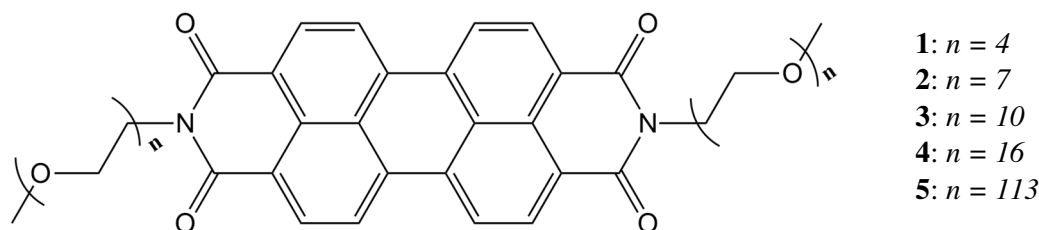


Figure 4.1. Chemical structure of the synthesized PEGylated PDIs. A total of five molecules were prepared.

4.1.1 Synthesis of compounds

The syntheses of the (PEG_n)₂PDIs (named **1** – **5** according to the PEG chain length, see Figure 4.1) were carried out as described in the Sections 3.3.1 and 3.3.2 of the experimental chapter. The products were characterized by ¹H-NMR, FTIR and UV-Vis spectroscopy (see Annexes). The ¹H-NMR spectra matched with those described in the literature for the same products (except **5**, which had not been previously reported, but presented a similar spectra) [209,220,221]. The FTIR spectra (Figure 4.2 and Annexes) showed two bands at ~1690 and ~1655 cm⁻¹ while bands in the range between 1700 and 1800 cm⁻¹ were not detected. This indicates a complete formation of the imide bond and the absence of anhydrides, respectively. Furthermore, the three bands at 1510, 1580 and 1590 cm⁻¹ are typical of the perylene core and a broad band at ~2900 cm⁻¹, which corresponds to C – H stretching, increased with the PEG chain length, as expected. The UV-Vis spectra in chloroform (Figure 4.3) showed a succession of three bands at 459 (0 → 2 transition band), 490 (0 → 1 transition band) and 526 nm (0 → 0 transition band), which are also typical of perylene diimides [222].

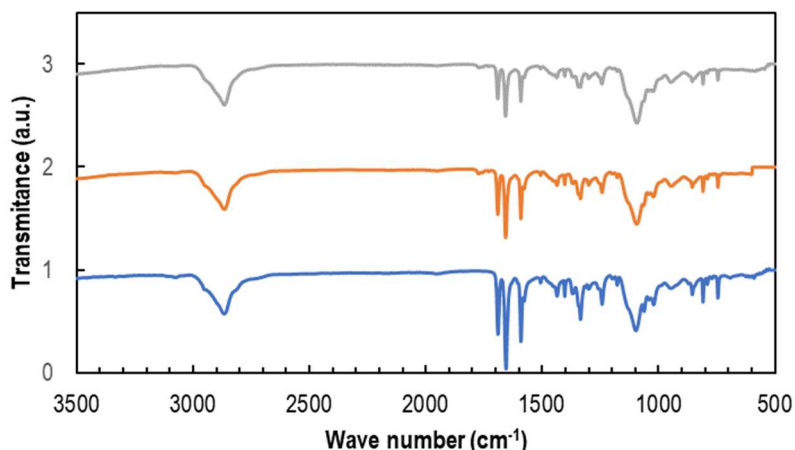


Figure 4.2. Comparison between the FTIR spectra of **2** (blue), **3** (orange) and **4** (grey). Note that **3** and **4** spectra have been shifted vertically for a better visualization.

4.1.2 Solution properties and surface activity

First, the solubility of compounds **1** – **5** in various solvents, including chloroform (CHCl₃), ethyl acetate (EtOAc), dimethyl sulfoxide (DMSO), ethanol (EtOH), and water (H₂O) was tested (Table 4.1). Compounds **4** and **5** exhibited complete solubility in all tested solvents, while **2** and **3** showed low solubility in ethanol. Compound **1** was only soluble in chloroform and completely insoluble in water. Therefore, the shortest PEG chain ($n = 4$) produced a compound that is insoluble in polar solvents (water and ethanol), and long PEG chains led to compounds soluble in all the studied solvents. However, when mixing compounds **1** and **2** in water at a 1:2 molar ratio with a total concentration of $3 \cdot 10^{-4}$ M, the **1** + **2** mixture was completely soluble. This suggests that the initially water-insoluble **1** was intercalated within the columnar aggregates of **2** and solubilized. Conversely, when the ratio was 2:1, the mixture was only partially soluble, indicating that there was not enough **2** to completely intercalate and dissolve **1**. On the other hand, **3** and **4** could not solubilize **1** at any ratio, which means that some intercalation occurs only if the PEG chain lengths (n) of the mixing (PEG) _{n} PDI are similar (see Table 4.1). This is interesting since it postulates PEGylated PDIs as potential carriers for insoluble small aromatic drugs.

Table 4.1. Solubility of the compounds **1** - **5** at room temperature (ca. 20 °C) and ca. 10^{-4} M in different solvents. Green = Soluble; Yellow = Partially Soluble; Red = Insoluble; (-) = Not studied. *Indicates the average n value for **1** + **2** mixtures, being 5 for mixtures at a 2:1 ratio and 6 for mixtures at a 1:2 ratio.

Compound	n	CHCl ₃	EtOAc	DMSO	EtOH	H ₂ O
1	4	Green	Yellow	Yellow	Red	Red
2	7	Green	Green	Green	Yellow	Green
3	10	Green	Green	Green	Yellow	Green
4	16	Green	Green	Green	Green	Green
5	113	Green	Green	Green	Green	Green
1 + 2	5*	-	-	-	-	Yellow
1 + 2	6*	-	-	-	-	Green

Furthermore, the color of the dissolution clearly changed depending on the solvent used (Inset Figure 4.3). This indicates that the PEGylated PDIs present solvatochromism, as other similar compounds [223–226]. To confirm such behavior, the absorbance in the visible range of the compounds **1** – **5** in various solvents was measured, and the resulting spectra are shown in Figure

4.3. Note that Figure 4.3 only shows the spectra of **4**, however, all the compounds presented similar spectra when dissolved in the corresponding solvent (see Annexes). This suggests that, although the PEG chain length determines the solubility of the compound in each solvent, it does not significantly affect the electronic properties of the perylene core. The absorption spectra of **4** in organic solvents is composed by a series of three bands of decreasing intensity at lower wavelengths, corresponding to the $0 \rightarrow \nu$ ($\nu = 0, 1$ and 2) electronic transitions induced by the absorption of a photon ^[134]. Note that all transitions are from the ground state to the excited state, the numbers (ν) indicate the vibronic level of such states, higher ν means a vibronic level at higher energy.

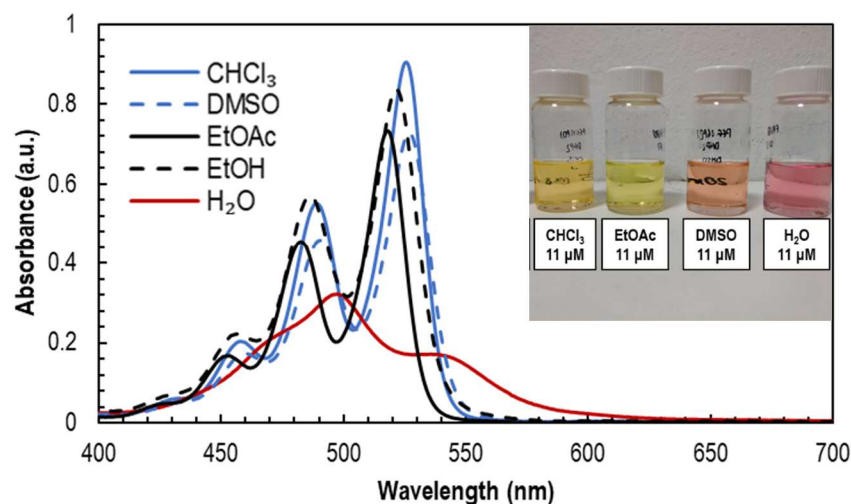


Figure 4.3. Visible absorption spectra of **4** at 11 μM in all the solvents tested at ca. 20 $^{\circ}\text{C}$. Inset: Image of **4** in different solvents at 11 μM . The solvatochromism is clearly observed.

The main difference in the absorption behavior is observed with water as solvent. Figure 4.4 shows how the addition of water to a DMSO solution of compound **3** changed the absorption spectra dramatically. Adding water caused a clear hypochromicity (reduced light absorption) of the largest band (i.e. $0 \rightarrow 0$ transition, at 529 nm) with a concomitant broadening and overlapping of the three bands observed in organic solvents. This behavior indicates the loss of the vibronic fine structure of the single molecules in solution due to the formation of H-aggregates in water, i.e. the $(\text{PEG}_n)_2\text{PDIs}$ form single face-to-face columnar stacks in water ^[112]. This behavior is similar for all the compounds **2** – **5**, which indicates that the $(\text{PEG}_n)_2\text{PDIs}$ aggregate in water even when functionalized with very large PEG chains ($n = 113$), suggesting high aggregation capacity. Such behavior in water suggests that the PDI–PDI interactions do not only result from π -stacking but there is also a significant hydrophobic effect.

A careful comparison of the absorption behavior of **2** – **5** in water also reveals that the PEG chain length slightly affects the aggregation behavior of the $(\text{PEG}_n)_2\text{PDIs}$ in water. In Figure 4.5a, the molar extinction coefficient spectra (ϵ) of **2** – **5** at 11 μM are compared, and it is observed that it increases at larger PEG chain lengths (the only exception is **3** showing a lower ϵ than **2**), which suggests a reduced degree of aggregation for $(\text{PEG}_n)_2\text{PDIs}$ with increasing n . These results were confirmed in Figure 4.5b, where the $0 \rightarrow 0 / 0 \rightarrow 1$ band ratios of **2** – **5** at concentrations between 1.8 and 11 μM are plotted. It is observed that the ratio is higher for **5** and increases as $(\text{PEG}_n)_2\text{PDI}$ concentration decreases, resulting from a decreased aggregation at lower concentrations and longer PEG chain lengths.

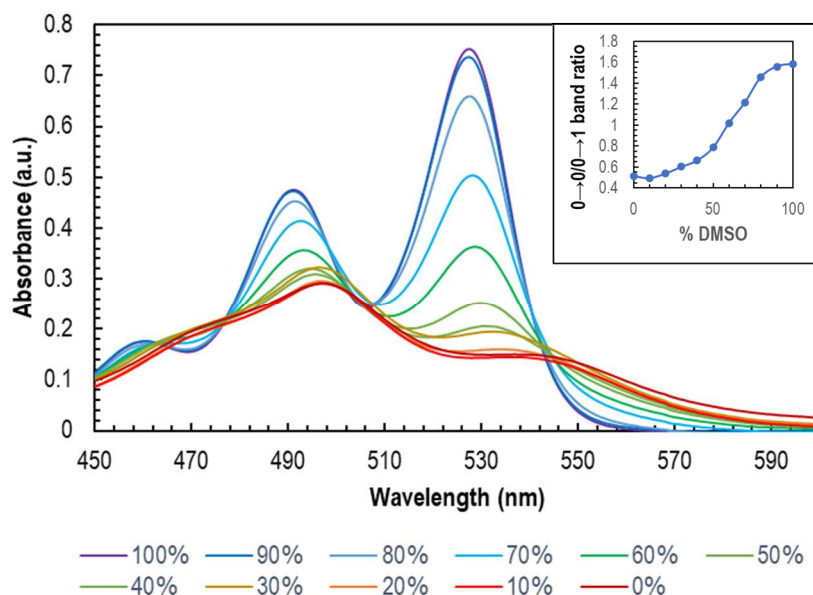


Figure 4.4. Visible absorption spectra of **3** at 11 μM in DMSO + water mixtures at different DMSO concentrations (in wt%) at ca. 20 °C. The band at 529 nm (largest in 100% DMSO) decreases with increasing water content, which indicates increased aggregation of **3** in water. Inset: Ratio between the maxima of absorption of the $0 \rightarrow 0$ and $0 \rightarrow 1$ bands as a function of the DMSO concentration.

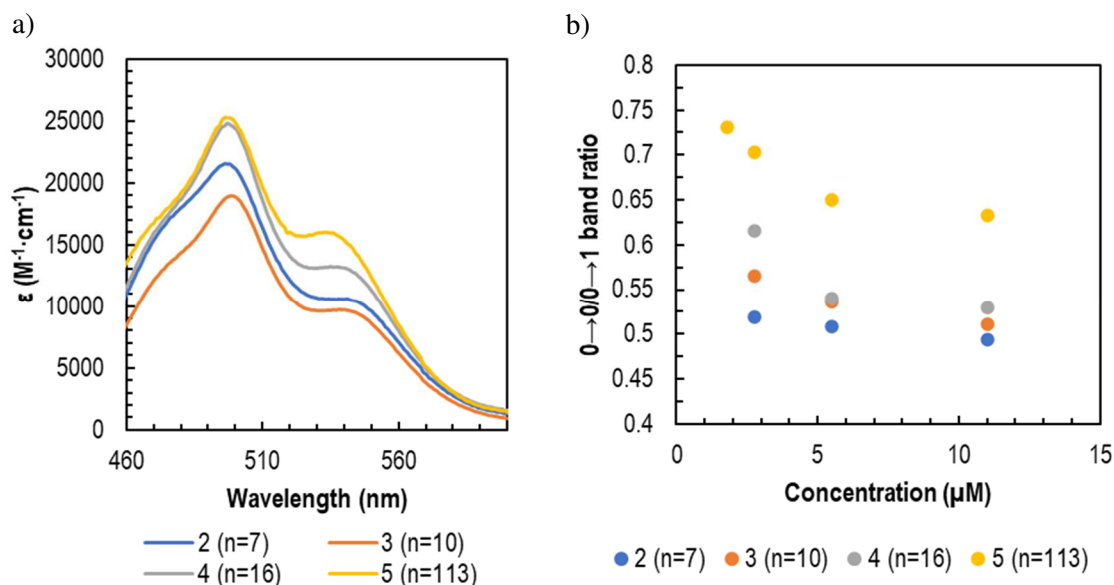


Figure 4.5. a) Extinction molar coefficient spectra of **2** – **5** at 11 μM and b) $0 \rightarrow 0 / 0 \rightarrow 1$ band ratio of **2** – **5** at 1.8, 2.8, 5.5 and 11 μM at ca. 20 °C, and b) $0 \rightarrow 0 / 0 \rightarrow 1$ band ratio as a function of the $(\text{PEG}_n)_2\text{PDI}$ concentration.

The formation of H-aggregates by $(\text{PEG}_n)_2\text{PDI}$ molecules in water was confirmed by comparing the fluorescence emission of the $(\text{PEG}_n)_2\text{PDIs}$ in water and organic solvents. As expected from Kasha's exciton model^[112], H-aggregates should cause a significant fluorescence quenching for dimeric dyes. In Figure 4.6a, such quenching can be clearly observed, as fluorescence decreases from high emission yields in chloroform, ethyl acetate and ethanol, to almost zero in water. Interestingly, there is some quenching in DMSO, where, according to UV-Vis absorbance data, there is no aggregation. This behavior was attributed to some effect of the solvent on the fluorescence emission.

The fluorescence spectra of **4** in all solvents (including water, see Inset Figure 4.6a), are composed by two bands of decreasing intensity, which are the specular image of the $0 \rightarrow 0$ and $0 \rightarrow 1$ absorption bands. Notably, the fact that the spectra in water also shows the same shape as in organic solvents (i.e. the $0 \rightarrow 0$ fluorescence transition is more intense than the $0 \rightarrow 1$ transition) suggests that the fluorescence emission comes from the small non-aggregated fraction of $(\text{PEG}_n)_2\text{PDI}$ and therefore the aggregated species emit zero fluorescence. Fluorescence spectra also manifest solvatochromism in the emission wavelength. Interestingly, plotting the maximum of absorbance and fluorescence as a function of the dielectric constant of the solvent (Figure 4.6b) reveals a hypsochromic (blue) shift on both the absorption and emission maxima from low to mid dielectric constants and a bathochromic (red) shift from mid to high dielectric constants. Such a behavior has previously been observed for similar PDIs^[223].

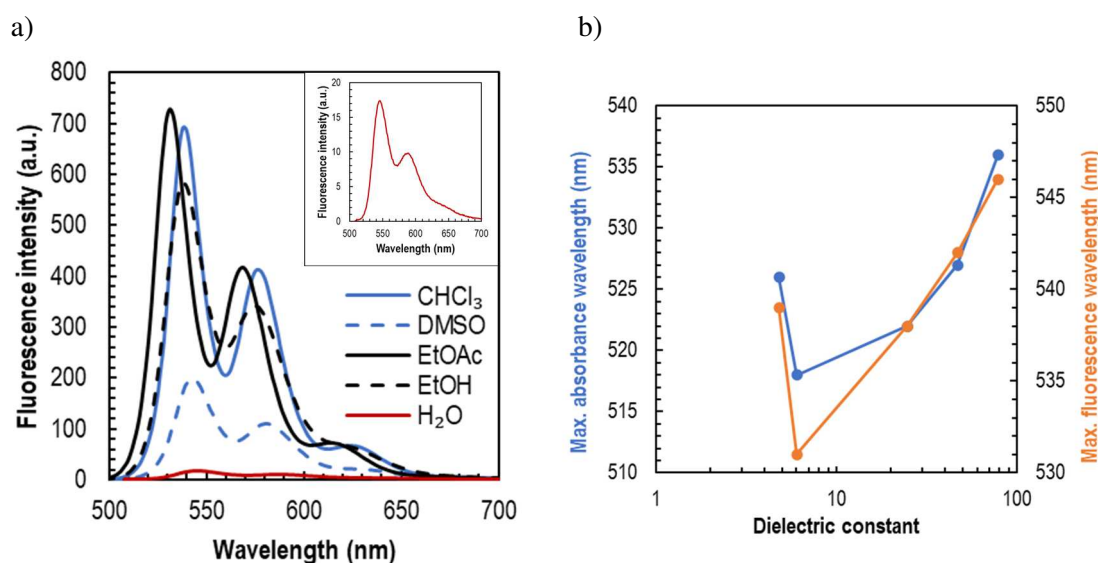


Figure 4.6. a) Fluorescence of **4** in various solvents at 11 μM at ca. 20 $^\circ\text{C}$. Solvatochromism and fluorescent quenching in DMSO and water are clearly observed. Samples were excited at 490 nm. Inset a) Zoom-in of the fluorescence spectra of **4** in water. b) Plot of the wavelength with maximum intensity of absorbance and fluorescence spectra at ca. 20 $^\circ\text{C}$ as a function of the dielectric constant of the solvent. Note that the dielectric constants are: 4.81 for CHCl_3 , 6.02 for EtOAc, 24.6 for EtOH, 46.7 for DMSO and 78.4 for H_2O .

Fluorescence spectra were also obtained at various concentrations of $(\text{PEG}_n)_2\text{PDI}$ using chloroform as the solvent. An intriguing red-shift in the maxima of emission was observed, shifting from 539 to 578 nm, which is translated into a concentration-dependent change in the color of the solution when irradiated in the dark using a UV lamp with a wavelength of 365 nm (see Figure 4.7). This behavior was also observed in a study about a $(\text{HO-PEG}_4)_2\text{PDI}$ with a hydroxyl termination at each PEG chain (note that our compounds end with a methoxy group), which showed that this compound's fluorescence also red-shifts as its concentration in chloroform increases until showing a completely red emission ($\lambda_{\text{Max}} = 674 \text{ nm}$) at 31 mM^[227]. This can only be explained by the Franck-Condon principle, which states that a change in the vibrational energy level in an electronic transition will be more likely to occur if the two vibrational wave-functions overlap^[228,229]. It has been described that the formation PDI aggregates favors the overlapping between wave-functions of different vibrational energy levels of the ground and excited electronic states, hence favoring the $0 \rightarrow \nu$ transitions for $\nu > 0$ over the $0 \rightarrow 0$ transition both in absorbance and fluorescence^[227]. Note that in absorbance the transition is $0_{\text{ground-state}} \rightarrow \nu_{\text{excited-state}}$ and in

fluorescence it is $0_{\text{excited-state}} \rightarrow \nu_{\text{ground-state}}$, which means that aggregation causes a blue-shift of the maximum in absorption and a red-shift in fluorescence.

Accordingly, the concentration-related red-shift in the fluorescence spectra exhibited by **4** solutions in chloroform indicate that this compound also aggregates in organic solvents, by π -stacking rather than by solvophobic effects, at much higher concentrations than in water, and most likely forming self-assemblies of variable size and dynamic nature (i.e. aggregates of variable size that rapidly exchange PDI units with other aggregates and monomeric species) ^[227].

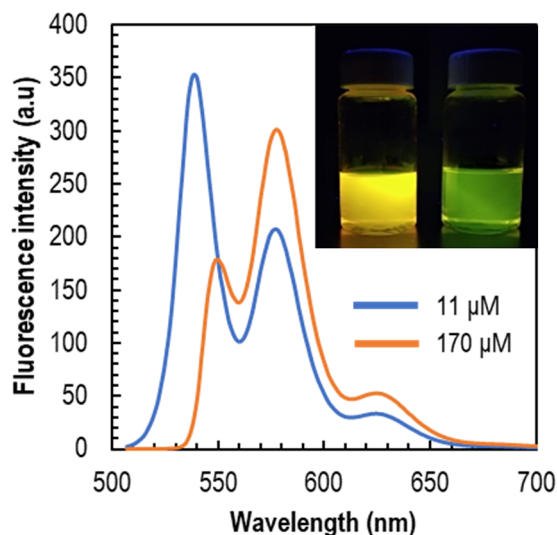


Figure 4.7. Fluorescence of **4** in chloroform at two different concentrations. Samples were excited at 490 nm. Inset: Photograph of the samples when excited with a UV lamp at 365 nm, the green one corresponds to 11 μM and the yellow one to 170 μM .

Further investigation was carried out to examine the behavior of $(\text{PEG}_n)_2\text{PDIs}$ in water. The objective was to determine if such molecules present surface activity and to explore the occurrence of phase separation due to the presence of a lower critical solution temperature (LCST) of the PEG chains. Note that LCST refers to the minimum temperature at which two components of a mixture phase-separate. PEGylated molecules are dissolved through hydration of the PEG chain at low temperature, however, by heating, the H_2O – PEG interactions are unfavored and the compound dehydrates inducing either solid – liquid or liquid – liquid phase separation.

Table 4.2 provides a summary of the surface tension measurements of **2** – **4** at 10^{-4} M. The results indicate that these compounds have surface activity as they reduce the surface tension of water (ca. 72.0 mN/m ^[230]). However, no significant changes are observed with the PEG chain length. A possible explanation to this behavior is that the water surface is already saturated at the tested concentrations. Furthermore, strong π – π interactions could produce a relatively compact adsorbed layer in spite of the steric hindrance of the PEG chains.

Table 4.2. Surface tension measurements for **2** – **4** ($c = 10^{-4}$ M) in water at room temperature (ca. 20 °C).

Compound	Surface tension (mN/m)
2	49.2 ± 0.0
3	46.3 ± 0.0
4	48.2 ± 0.0

LCST measurements were initially conducted heating solutions in a temperature-controlled bath up to 90 °C, and the onset of precipitation or turbidity was visually observed. Compounds **2** – **4**

were studied at concentrations ranging from 10^{-4} M to 10^{-3} M, but no phase separation was observed. This lack of phase separation can be attributed to the relatively long PEG chains, which do not exhibit LCST within the studied temperature range. Interestingly, the **1** + **2** mixture, which can be considered as a $(\text{PEG}_n)_2\text{PDI}$ with $n = 6$ as average, exhibited a LCST at 73°C at a concentration of $1 \cdot 10^{-4}$ M in water. This temperature could be reduced down to 67°C when the concentration was tripled. In order to search for a LCST in the compounds **2** – **4**, a collection of salts at various concentrations were added to the solutions.

Compound **2** was extensively studied with LiCl, NaCl, KCl, CaCl_2 , Na_2SO_4 , NH_4Cl , NH_4F and $(\text{NH}_4)_2\text{SO}_4$ at concentrations between 0.5 and 2 M. Table 4.3 reveals that Na_2SO_4 and $(\text{NH}_4)_2\text{SO}_4$ are the best salts to induce the precipitation of **2**, followed by CaCl_2 , NH_4F and KCl. Compounds **3** and **4** LCSTs in $(\text{NH}_4)_2\text{SO}_4$ 0.5 M were also measured, observing LCSTs of 68°C and 74°C , respectively, values slightly higher than that observed for **2** (52°C). These LCST values were confirmed by temperature-controlled transmission measurements at 800 nm (PDIs do not absorb at this wavelength), as shown in Figure 4.8. The LCST of the **1** + **2** mixture ($n = 6$) in $(\text{NH}_4)_2\text{SO}_4$ 0.5 M was 38°C . The presence of a LCST for the **1** + **2** mixture both in pure water and in $(\text{NH}_4)_2\text{SO}_4$ 0.5 M at lower values than for **2** confirms the intercalation between **1** and **2**.

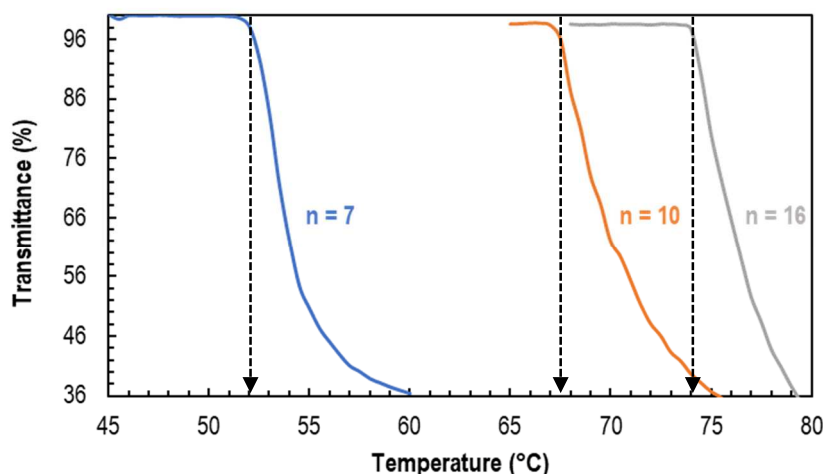


Figure 4.8. Transmittance as a function of temperature for **2** (blue), **3** (orange) and **4** (grey) in aq. $(\text{NH}_4)_2\text{SO}_4$ 0.5 M at 800 nm. The reduction in transmittance indicates the formation of particles in suspension, which increases light scattering. The temperature at which the transmittance started decreasing was taken as the LCST (see black arrows).

Remarkably, even though PEG itself does not exhibit a lower critical solution temperature (LCST) below 90°C even at high molecular weights^[231], the presence of salts can decrease this LCST. The reduction in LCST follows the anionic series: $\text{SO}_4^{2-} > \text{F}^- > \text{Cl}^-$ and the cationic series: $\text{K}^+ > \text{Na}^+ > \text{NH}_4^+ > \text{Li}^+$, for monovalent cations^[232] (as shown in Table 4.3). This trend aligns with the results observed for **2** in Table 4.3, confirming that both pure PEG and **2** follow the Hofmeister series with minor alterations, such as the ammonium ion, which ordinarily should be placed above the potassium ion^[233–236].

Notably, **2** apparently show higher LCST values than PEG MW 20000^[232] in the presence of both monovalent cations (K^+ , Na^+ , NH_4^+ and Li^+) and monovalent and divalent anions (SO_4^{2-} , F^- and Cl^-). However, this trend is reversed in presence of a divalent cation such as Ca^{2+} , which reduced the LCST for $(\text{PEG}_n)_2\text{PDIs}$ even more than K^+ at the same concentration, meanwhile for PEG MW 20000 its effect was minor, similar to that of Na^+ at the same concentration (Table 4.3). This indicates that, contrary to PEG MW 20000, **2** also follows the Schulze-Hardy rule^[237–239], that

states that multivalent ions should destabilize colloidal dispersions more efficiently than monovalent ions. The Schulze-Hardy rule has also failed for the Triton X-100 PEGylated nonionic surfactant, consisting in an octylphenol adduct containing 9-10 ethylene oxide units per molecule ^[240], and it has been attributed to the formation of coordination complexes, in which the ethoxylated chains work as polydentate ligands for the multivalent cations, over the formation of hydrated cations, which should increase the water-solubility of the PEG chain. Differently, for **2**, the shorter PEG chain length and the small PDI-PDI distance within the H-aggregate could prevent the formation of $[2\text{-Ca}]^{2+}$ complexes and hence the Schulze-Hardy rule is not violated.

Besides, it has been previously described that bolamphiphilic PEGylated PDIs (i.e. PDIs PEGylated at both sides, forming a hydrophilic – hydrophobic – hydrophilic structure) exhibit LCST below 100 °C in absence of salts. In the study by Würthner et al. ^[241], the synthesis of two bolaform PDIs was described. These PDIs featured tridentate PEG substituents with 9 and 15 ethoxy units connected to the perylene core by a benzyl moiety. These compounds showed LCSTs at 26 and 69 °C respectively, at a concentration of $4.9 \cdot 10^{-4}$ M in the absence of salts. Additionally, three naphthalene diimides (NDIs) with the same substituent structure as the tridentate PEG-PDI (with a total ethoxy units of 9, 12 and 15 on each side) were synthesized. LCST values of 59, 78 and 88 °C were reported for these NDIs at a concentration of $1 \cdot 10^{-3}$ M in water ^[242]. Notably, these LCST values are significantly lower than those of $(\text{PEG}_n)_2\text{PDIs}$. This difference can be attributed to the presence of the benzyl group, which facilitates the aggregation, as well as the branching of the PEG chains, which may hinder hydration of the inner ethylene glycol units and increase the degree of aggregation.

To provide further insights, it could be informative to compare the LCST behavior of the current $(\text{PEG}_n)_2\text{PDI}$ with that of nonionic (ethoxylated) bolaform surfactants, specifically those with a general formula $(\text{PEG}_n)_2\text{C12}$. Zana et al. reported LCST values of 50 °C and 71 °C for $(\text{PEG}_3)_2\text{C12}$ and $(\text{PEG}_4)_2\text{C12}$, respectively ^[243]. Considering that compound **1** ($n = 4$) is insoluble in water at room temperature, it can be concluded that the PDI moiety is more hydrophobic (and more rigid) than the dodecyl chain.

Despite differences in aggregation mechanism and chemical structure, certain comparisons can also be drawn with other bolaform-like surfactants, such as Pluronics, which feature a hydrophobic moiety of polypropylene glycol (PPO) with more hydrophilic PEG groups on both sides. It is worth noting that Pluronics with similar PEG content to **1** (approximately 50 wt%) are soluble in water and exhibit cloud points between 73 and 91 °C at 1% ^[244] (note that this concentration is much larger than the one used in this work). These cloud points are even higher than the LCST observed for the **1** + **2** mixture with $n = 6$. This suggests that the aggregation is more favored for bolaform PEGylated PDIs compared to Pluronics, likely because of the higher hydrophobicity (and rigidity) of the perylene core. It is important to note that in the aforementioned bolaform-type surfactants, the phase separation above LCST is a liquid-liquid phase separation, while for $(\text{PEG}_n)_2\text{PDI}$ a solid-liquid phase separation occurs.

The presence of a LCST and its reduction upon salt addition and shortening of PEG chain length provide evidence for the significant role of PEG chain hydration in the dissolution and aggregation of $(\text{PEG}_n)_2\text{PDI}$ in water. Furthermore, the results obtained from absorbance and fluorescence experiments suggest that the hydrophobic effect, combined with $\pi - \pi$ interactions, promote the formation of H-aggregates for $(\text{PEG}_n)_2\text{PDI}$ in water, even at extremely low concentrations ($< 2 \mu\text{M}$). In contrast, in chloroform and other organic solvents, the driving force for aggregation is solely based in π -stacking, without significant influence of solvophobic effects.

Table 4.3. LCST values in the presence of salts. Increasing the salt concentration reduces the LCST. Moreover, the ability of the different ions to precipitate (PEG_n)₂PDI follow a pattern similar to the Hofmeister series. (> 90) means that the LCST was not detected in the range of temperatures studied. (Ins.) means that the compound was insoluble at room temperature (ca. 20 °C).

Mesogen (Conc.)	Salt (Conc.)	LCST (°C)
2 (10 ⁻⁴ M)	LiCl (1 M)	> 90
2 (10 ⁻⁴ M)	LiCl (1.5 M)	88
2 (10 ⁻⁴ M)	LiCl (2 M)	77
2 (10 ⁻⁴ M)	NaCl (1 M)	> 90
2 (10 ⁻⁴ M)	NaCl (1.5 M)	73
2 (10 ⁻⁴ M)	NaCl (2 M)	58
2 (10 ⁻⁴ M)	KCl (1 M)	81
2 (10 ⁻⁴ M)	KCl (1.5 M)	70
2 (10 ⁻⁴ M)	KCl (2 M)	61
2 (10 ⁻⁴ M)	CaCl ₂ (1 M)	64
2 (10 ⁻⁴ M)	CaCl ₂ (1.5 M)	54
2 (10 ⁻⁴ M)	CaCl ₂ (2 M)	54
2 (10 ⁻⁴ M)	NH ₄ Cl (1 M)	> 90
2 (10 ⁻⁴ M)	NH ₄ F (1 M)	78
2 (10 ⁻⁴ M)	Na ₂ SO ₄ (1 M)	Ins.
2 (10 ⁻⁴ M)	Na ₂ SO ₄ (0.5 M)	40
2 (10 ⁻⁴ M)	(NH ₄) ₂ SO ₄ (1 M)	Ins.
2 (10 ⁻⁴ M)	(NH ₄) ₂ SO ₄ (0.5 M)	52
3 (10 ⁻⁴ M)	(NH ₄) ₂ SO ₄ (0.5 M)	68
4 (10 ⁻⁴ M)	(NH ₄) ₂ SO ₄ (0.5 M)	74
1+2 (10 ⁻⁴ M)	(NH ₄) ₂ SO ₄ (0.5 M)	38
1+2 (10 ⁻⁴ M)	None	73
1+2 (1.5·10 ⁻⁴ M)	None	69
1+2 (3·10 ⁻⁴ M)	None	67
1+2 (10 ⁻⁴ M)	CaCl ₂ (0.5 M)	54
1+2 (10 ⁻⁴ M)	CaCl ₂ (1 M)	42
1+2 (1.5·10 ⁻⁴ M)	CaCl ₂ (0.5 M)	54
1+2 (1.5·10 ⁻⁴ M)	CaCl ₂ (1 M)	42
1+2 (3·10 ⁻⁴ M)	CaCl ₂ (0.5 M)	53
1+2 (3·10 ⁻⁴ M)	CaCl ₂ (1 M)	42
PEG MW 20000 (1 wt%) ^[232]	LiCl (1 M)	87
PEG MW 20000 (1 wt%) ^[232]	NaCl (1 M)	76
PEG MW 20000 (1 wt%) ^[232]	KCl (1 M)	74
PEG MW 20000 (1 wt%) ^[232]	NH ₄ Cl (1 M)	85
PEG MW 20000 (1 wt%) ^[232]	NaF (1 M)	42
PEG MW 20000 (1 wt%) ^[232]	Na ₂ SO ₄ (0.3 M)	55
PEG MW 20000 (1 wt%) ^[232]	CaCl ₂ (0.5 M)	85
PEG MW 20000 (1 wt%) ^[232]	CaCl ₂ (1 M)	79

Also, several experimental and computational studies suggested that PDI H-aggregates most likely consist in face-to-face stacked molecules twisted ~30 ° around the stacking axis, in organic solvents ^[245–248], aqueous solution ^[249] and thermotropic mesophases ^[250]. Notably, such a twisting between adjacent molecules not necessarily implies the formation of only helical aggregates

(Figure 4.9a), but also of alternating clockwise-anticlockwise stacks (Figure 4.9b). Accordingly, $(\text{PEG}_n)_2\text{PDI}$ s were considered to form both helical and alternating twisted columnar aggregates.

In summary, $(\text{PEG}_n)_2\text{PDI}$ aggregates in aqueous solution form twisted columnar stacks with a clear trend of increasing the degree of aggregation as PEG chain is shortened. Thus, it is expected for the shorter PEG chain length products to form chromonic liquid crystal phases at lower concentrations.

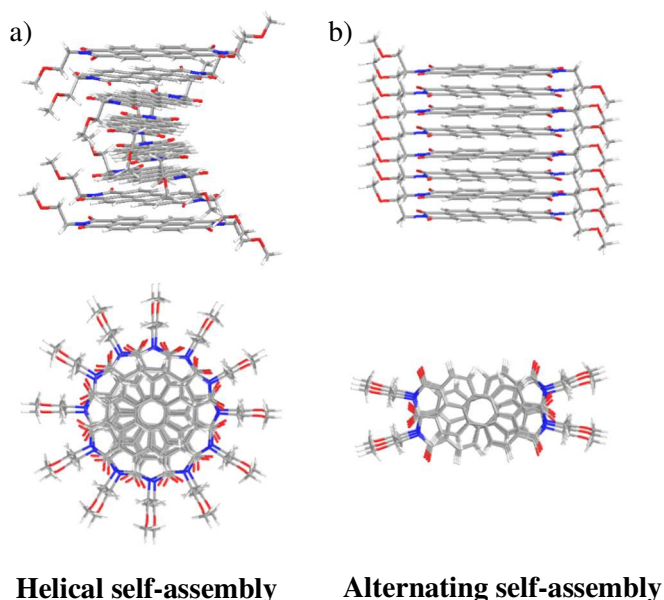


Figure 4.9. Proposed models for $(\text{PEG}_n)_2\text{PDI}$ self-assembly. a) Top: helical model, lateral view. Bottom: helical model, view from above. b) Top: alternating model, lateral view. Bottom: alternating model, view from above. Most likely, $(\text{PEG}_n)_2\text{PDI}$ H-aggregates are a mixture of both models. The molecules were drawn with the Chem3D software.

4.1.3 Chromonic phase behavior at high concentrations

The chromonic phase behavior of **1** – **5** was firstly assessed by contact and evaporation tests observed under polarized light optical microscopy (POM). Since **1** is insoluble in water, no liquid crystalline phases could be obtained, and consequently phase behavior was not studied. In contrast, **2** and **3** contact and evaporation tests showed the existence of both nematic (N) and hexagonal (M) phases (Figures 4.10a,b,i). For **4**, only M phase was observed in both contact and evaporation tests (Figure 4.10c). Finally, **5** did not show any chromonic mesophase despite being water-soluble, indicating that its PEG chain length is too large, and thus the compound is too hydrophilic, to permit the self-assembly in chromonic mesophases.

Further POM characterization of the chromonic behavior of **2** – **4** was assessed by preparing samples at selected concentrations and studying their phase behavior as a function of the temperature (Table 4.4). For **2**, it was observed that N phases start to appear at 15 wt% and 18 °C, meanwhile **3** formed N phases between 20 and 25 wt% at room temperature (ca. 20 °C). Above 25 wt%, M chromonic phases emerged for **3**. Surprisingly, despite **2** showing N mesophases at lower concentrations than **3**, it did not form M chromonic phases up to 35 wt% below 20 °C and 40 wt% at room temperature. Briefly, the N phase is formed in a wide range for **2** (from 15 wt% to 35 wt% at ca. 20 °C), while this nematic range is significantly narrower for **3** (from approximately 20 to 25 wt% at ca. 20 °C).

This trend is supported by the findings for compound **4**, which only formed hexagonal phases, starting at a 25 wt% at ca. 20 °C. Even with increasing temperature, nematic textures could not

be observed, and the sample passed from M phase to a mixed hexagonal-isotropic (M + I) phase before eventually becoming completely isotropic. Additionally, in the range from 20 to 25 wt%, **4** exhibited a M + I phase, but it transitioned to a completely isotropic phase at 17.5 wt%. In contrast, for compounds **2** and **3**, transitions from M phases to mixed hexagonal-nematic (M + N) phases, followed by N phases, mixed nematic-isotropic (N + I) phases and finally isotropic (I) phases were clearly observed upon increasing temperature (see Table 4.4). Therefore, it can be concluded that the range of PEG chain lengths in which (PEG)_nPDIs can form mesophases lies between $n = 7$ and $n = 16$ (note that n represents half the total ethoxy units, as each mesogen is functionalized by two PEG chains), and that the PEG chain length highly influences the chromonic phase behavior.

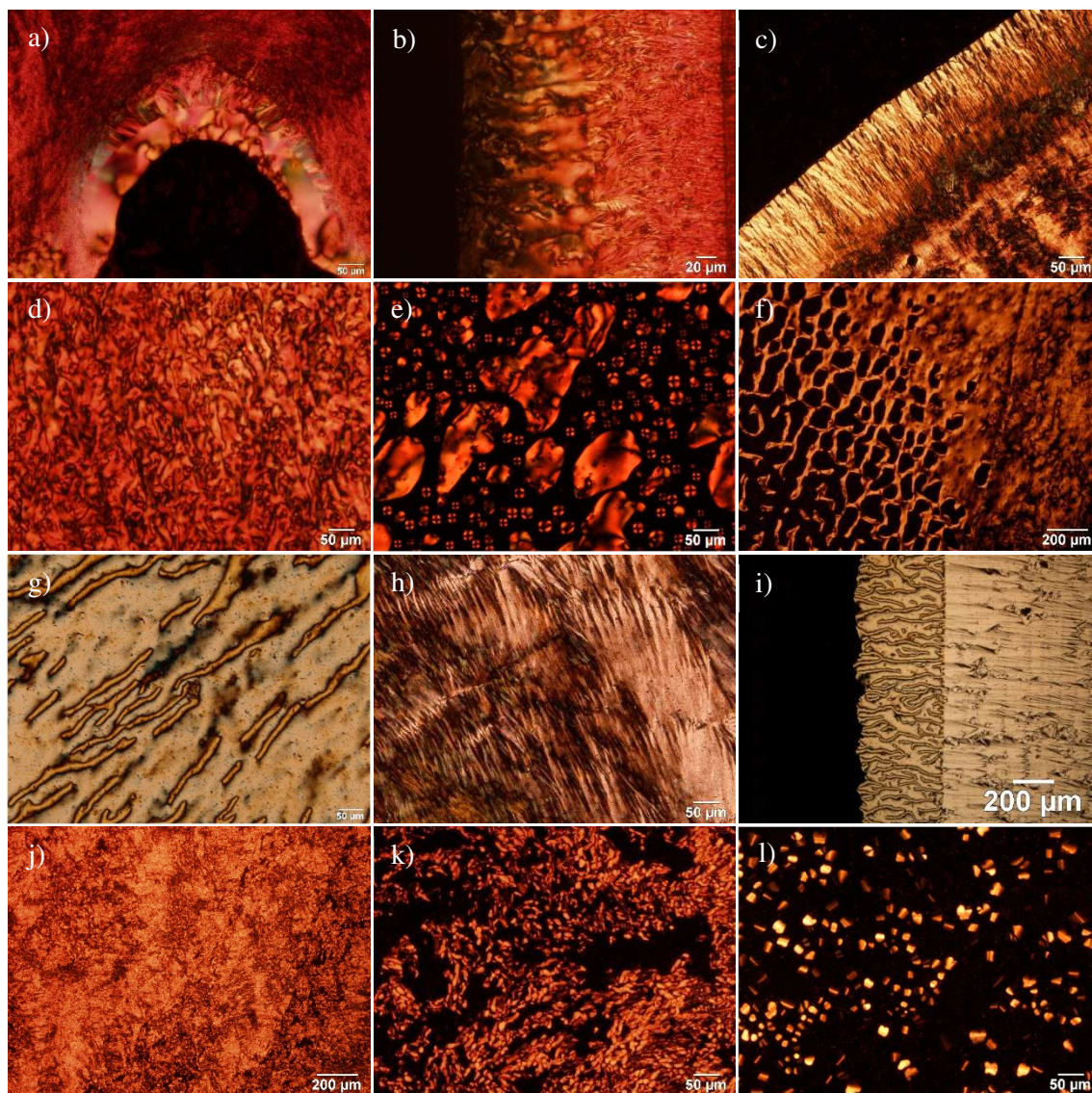


Figure 4.10. POM images of a) Contact test of **3**, b) Evaporation test of **2**, c) Contact test of **4**, d) Schlieren texture formed by the N phase of **2** at ca. 20 °C and 30 wt%, e) N + I phase of **2** at ca. 20 °C and 17.5 wt%, f) N + I phase of **2** at 36 °C and 25 wt%, g) Tiger-skin N phase of **3** at ca. 20 °C and 23 wt%, h) Herringbone texture of M phase of **3** at ca. 20 °C and 25.5 wt%, i) Contact test of **3**, j) Grainy M phase of **4** at ca. 20 °C and 25 wt%, k) M + I phase of **4** at 38 °C and 25 wt% and l) M gems formed by cooling down a 25 wt% solution of **4** from the I phase.

Carefully analyzing the POM images of **2** – **4** at different conditions, a big variety of birefringent textures can be observed (Figure 4.10a-l). For example, **2** formed conventional Schlieren nematic

textures ^[251] between 17.5 and 30 wt% concentration range at room temperature (Figure 4.10d), but showed nematic droplets with radial internal structure dispersed in an isotropic medium at 15 wt% at room temperature, forming a N + I phase (Figure 4.10e), meanwhile isotropic islands in a nematic continuous phase appeared when heating nematic phases (Figure 4.10f). This curvature difference of the N + I phase suggests that the interfacial tension between the N and I phases is rather low ^[96]. Differently, **3** formed nematic phases with a tiger-skin texture (Figure 4.10g) and hexagonal phases with a herringbone pattern (Figure 4.10h) similar to those formed by **4** in the contact test (Figure 4.10c). Notably, the evaporation test of **3** also confirmed both the tiger-skin nematic texture and the herringbone pattern in the hexagonal phase (Figure 4.10i). Instead, **4** only formed hexagonal phases consisting in a grainy structure (Figure 4.10j), which suggests more brittle structure than for **3**. By heating **4** hexagonal phases, M + I bicontinuous phases were obtained (Figure 4.10k), meanwhile cooling **4** isotropic phases induced the formation of M domains in an isotropic continuous medium (Figure 4.10l), suggesting again a low interfacial tension between chromonic and isotropic phases.

Table 4.4. Phase transitions for **2**, **3** and **4** at different concentrations (N: nematic, M: hexagonal, I: Isotropic). *Mesophase at room temperature (ca. 20 °C).

Conc. (wt%)	Mesophase*	M→M+N (°C)	M+N →N (°C)	N→N+I (°C)	N+I→I (°C)
10	I	-	-	-	-
12.5	I	-	-	-	11
15	N+I	-	-	18	32
17.5	N	-	-	44	51
20	N	-	-	53	60
25	N	-	-	64	71
30	N	-	-	67	74
35	N	19	24	-	-
40	M	33	42	-	-
17.5	I	-	-	-	-
20	N	-	-	29	45
23	N	2	2	40	52
25.5	M	31	33	60	65
27	M	49	52	80	85
30	M	52	55	82	86
Conc.	Mesophase*	M→ M+I (°C)		M+I→I (°C)	
17.5	I	-	-	-	-
20	M+I	-	-	42	-
23	M+I	-	-	46	-
25	M	34	-	50	-
27	M	36	-	52	-
30	M	40	-	55	-
5	No liquid crystal phases are formed				

With the data included in Table 4.4, partial phase diagrams of the compounds **2** – **4** were drawn (Figure 4.11a-c). As expected, as PEG chain length and hence polarity and water-solubility increase, the concentration at which the corresponding (PEG_n)₂PDI starts forming mesophases also rises (15 wt% for **2**, 20 wt% for **3** and 25 wt% for **4**). Also, the phase diagrams clearly show

how increasing the PEG chain length expands the regions of hexagonal phase and reduces the nematic range of the phase diagrams until it disappears for **4**. This is in agreement with a computational work by Kuriabova et al. [252], in which they studied the chromonic self-assembly with a coarse-grained model considering the chromonic mesogens as sticky cylinders that experience a favorable binding energy by stacking and a core repulsion caused by the excluded volume effect. They used cylinders with length to diameter ratios (L/D) of 0.5 (see Figure 4.11d). Monte Carlo simulations and analytic theory predicted that the sticky cylinders would stack forming flexible linear aggregates that evolve to N and M phases above critical volume fractions (Figure 4.11d). Notably, this model also predicted that the N phase is favored for mesogens with a high binding energy, whereas direct isotropic-to-hexagonal transitions are preferred for mesogens with a low binding energy. This matches with **2**, that presented a higher degree of aggregation, and hence a higher binding energy, forming N phases in a wide range of concentrations and **4** showing only M and I phases. It is worth noting that the L/D ratio considered by Kuriabova et al. matches with that of PDI considering a length of 0.34 nm (the stacking distance of chromonic self-assemblies) and a diameter of 0.7 nm (only the perylene core, see Figure 4.11e). If the whole perylene diimide core is considered as D , the L/D ratio is notably decreased.

It is particularly interesting to note that the phase behavior of the $(\text{PEG}_n)_2\text{PDI}$ differs from that of conventional ionic LCLC such as DSCG (Figure 1.12b) [93,98], Sunset Yellow [115] or ionic perylene diimides [144], and the theoretical phase diagram predicted by Kuriabova et al. (Figure 4.11d) [252], in several aspects. First, $(\text{PEG}_n)_2\text{PDI}$ chromonic mesophases emerge at higher concentrations than many ionic conventional chromonics ($\sim 1 - 10$ wt%), although for some ionic compounds chromonic phases may appear at much higher concentrations (> 30 wt%) [100]. In fact, a series of chromonic cationic PDIs composed by a perylene core functionalized with quaternary amines with different counter anions reported by Tam-Chang et al. [100] showed that the compounds with small counter anions (Cl^-) formed chromonic phases at rather low concentrations ($5 - 8$ wt%) independently of the quaternary amine used but with bigger counter anions (tosylate) chromonic mesophases did not appear until 30 wt%, suggesting that the steric effect of the counter anions plays an important role in the formation of chromonic mesophases for cationic PDIs. Consequently, the higher required concentration for the appearance of chromonic mesophases for $(\text{PEG}_n)_2\text{PDI}$ with larger n may not only result from the increased water-solubility but also from the steric hindrance between the PEG chains.

Interestingly, another possible effect of the steric hindrance of the PEG chain may be observed by carefully analyzing the M + N phase of **2** and **3**. For **3**, the $\text{M} \rightarrow \text{M} + \text{N} \rightarrow \text{N}$ transitions were abrupt and clearly detected by POM (Figure 4.12b) and occurred in a rather narrow temperature window (~ 2 °C). Differently, for **2**, such transitions were not easily detected (Figure 4.12a), as the changes in the birefringent texture were slow and gradual, and occurred in a wider temperature window (> 5 °C).

This behavior indicates that **2** probably presents the so-called P phase. Normally, the $\text{M} \rightarrow \text{N}$ transition is a first-order process that occurs abruptly by heating/cooling a chromonic system, as observed for **3**. However, for some compounds, such process is apparently second- or higher-order, resulting in a smooth transition between M and N phases. In the latter case, such “transition phase” is called P phase (see Section 1.3.2) [93,95].

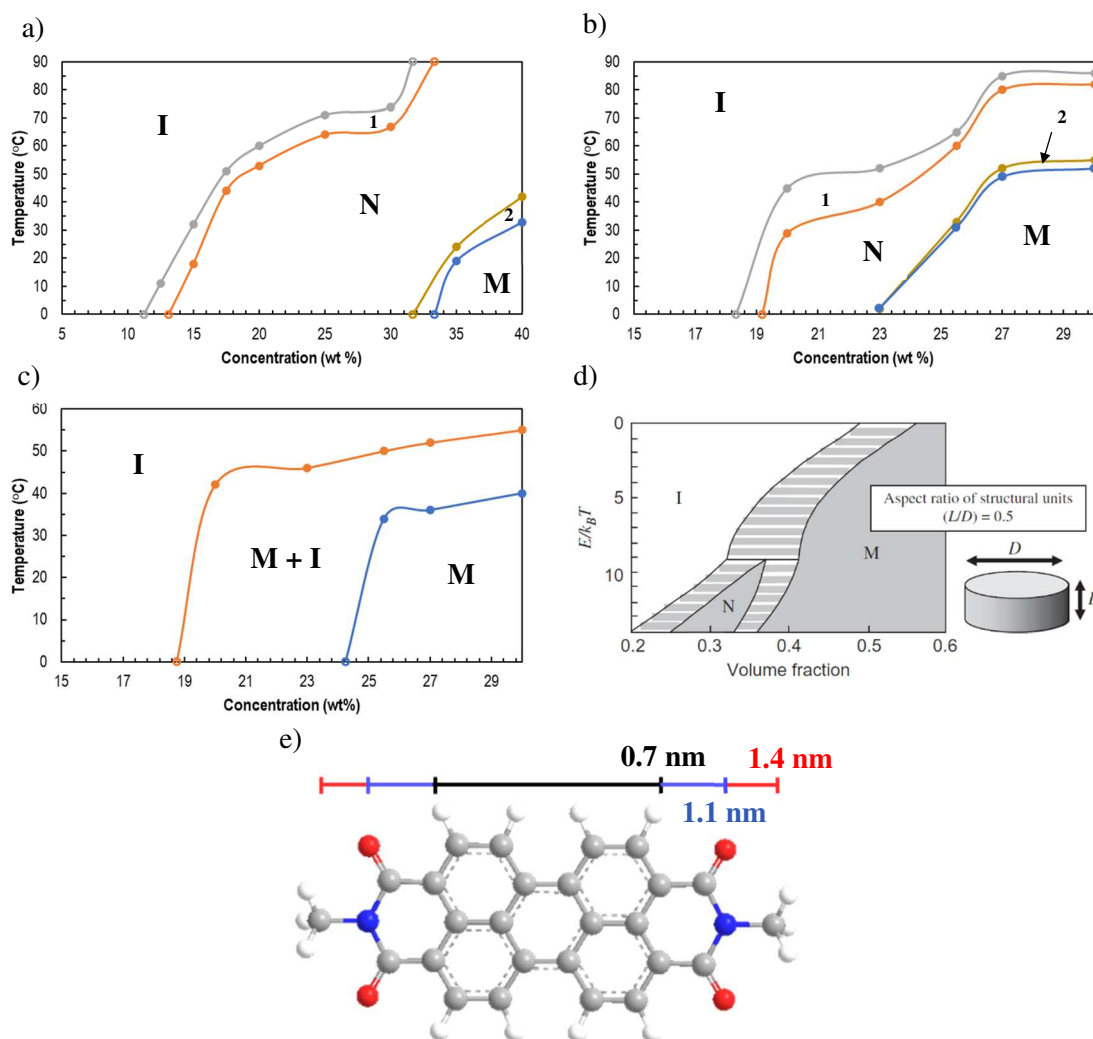


Figure 4.11. Partial phase diagrams of a) **2**, b) **3** and c) **4**. Filled circles refer to experimentally measured temperature-induced phase transitions, whereas empty circles are approximated as the average concentration between two measured samples with a different chromonic phase at a given temperature. ¹ = N + I and ² = M + N. d) Chromonic phase diagram predicted by Kuriabova et al. [252] considering a L/D ratio of 0.5. Redrawn with the Royal Society of Chemistry permission from [252] and reproduced with Taylor and Francis permission from [196]. e) Perylene core size measured by the Chem3D software. Three values are shown: the perylene core (0.7 nm), the perylene diimide core (1.1 nm) and the perylene diimide core with one methyl substituent group (1.4 nm).

It is believed that P phases appear for chromonic systems formed by mesogens that can rotate within the columnar stacks, which typically occurs for molecules with circular cross-section or for plank-like compounds stacked in a helical fashion (Figure 4.12c). This suggests that **2** has more rotational freedom within the columnar aggregates than **3**, which could result from its shorter PEG chains causing less steric hindrance between adjacent PDI molecules and facilitating the rotation. Moreover, the absence of N phase for **4** could be caused by the higher steric hindrance between the long PEG chains, that completely prevents any rotation and impossibilities the M → N transition.

Other remarkable aspects of the $(\text{PEG}_n)_2\text{PDI}$ phase diagrams are the high stability of the chromonic phases at high temperatures (many times water started to evaporate before reaching the complete isotropic phase) and that peritectic points were not detected, although they could appear at higher concentrations. Furthermore, no M + I phase was detected for **2** and **3**. Instead, the M phase evolved to an N phase by heating, which is not very common for conventional ionic

chromonic mesogens, where the $M \rightarrow N$ transition only occurs in a very narrow concentration range (1 – 2 wt%), as demonstrated by DCSG phase diagram (Figure 1.12b), and the simulated phase diagram of Figure 4.11d ^[252].

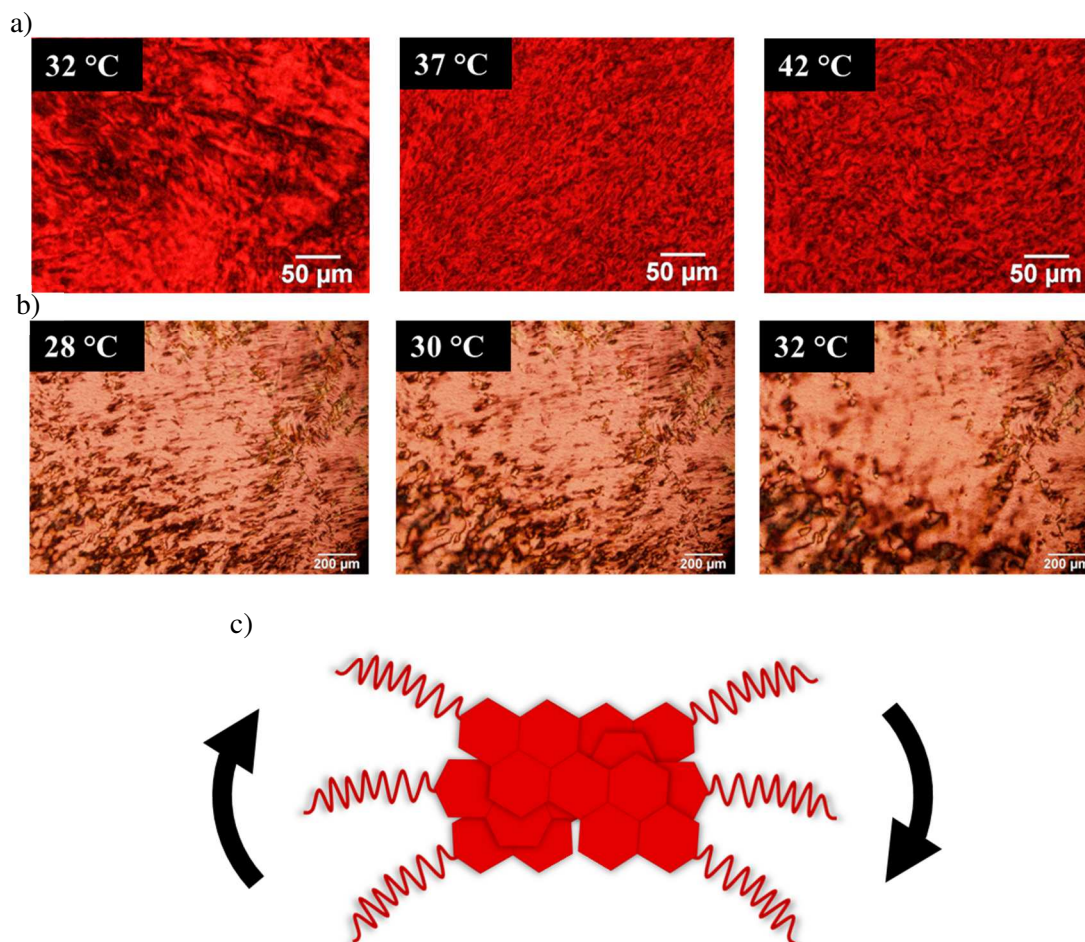


Figure 4.12. $M \rightarrow N$ phase transition of a) **2** (40 wt%), and b) **3** (25 wt%). c) Model of the rotation of the PDI mesogens within the columnar stacks in the *P* phase. Bigger PEG chains may hinder the rotation and prevent the formation of *P* phases.

Further characterization of the phase behavior of **2** – **4** was performed by SAXS. SAXS allowed the confirmation of the formation of *M* phases for **2** at 40 wt% and **3** and **4** at 27 wt% at 25 °C (Figure 4.13), since patterns of three peaks at scattering vector (q) ratios of $1:\sqrt{3}:\sqrt{4}$ were obtained for the three samples. Remarkably, the first peak of **4** was apparently divided into two, which may result from the higher polydispersity of the PEG-amine used for the synthesis.

The d_H (i.e., Bragg distance between (1 0 0) planes) and a parameter of the hexagonal lattice (i.e., distance between the center of two adjacent columns) were determined from Eq. 3 (see Table 4.5 and the Inset of Figure 4.13). Interestingly, the distances increased as the PEG chain length between **3** and **4** increased. At the higher temperature of 60 °C, the three samples exhibited a single broad peak in the SAXS pattern, indicating the presence of a nematic phase. In the case of compound **4**, which does not form *N* phases, it can be considered that heating the hexagonal phase of **4** results in the disruption of this phase leading to the formation of disordered non-birefringent aggregates which maintain an almost identical column-column distance as in the hexagonal phase.

These findings illustrate how minor changes in the aggregation properties of diluted can lead to significant differences in terms of chromonic liquid crystal behavior. If the PEG chain is too short,

the resulting product has a too low water-solubility to form mesophases. Longer PEG chains (~ 16 ethoxy units) favor chromonic hexagonal phases over nematic phases, likely due to a structuring effect induced by higher hydration of the PEG chains, whereas intermediate chains (from 7 to 10 ethoxy units) favor the formation of N phases. However, further increasing the PEG chain length produce too soluble compounds in which the steric hindrance between the PEG substituents is too high. This hinders the formation of ordered mesophases. Consequently, the formation of chromonic mesophases only occurs for $(\text{PEG}_n)_2\text{PDIs}$ between $n = 7$ (for **2**) and $n = 16$ (for **4**).

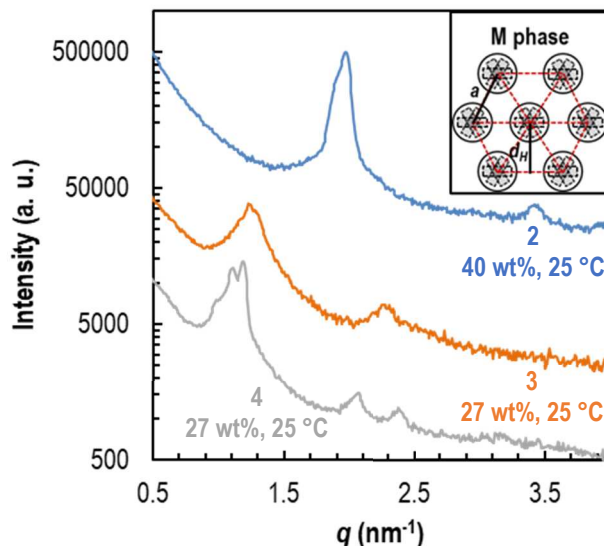


Figure 4.13. Small-angle X-ray scattering (SAXS) patterns of **2** (40 wt%, 25 °C, blue), **3** (27 wt%, 25 °C, orange) and **4** (27 wt%, 25 °C, gray) in hexagonal phases. Note that **2** and **3** spectra have been shifted vertically for a better visualization. Inset: Graphical representation of the hexagonal phase with the definitions of d -spacing (d_H) and the distance between the centers of two adjacent columns (a), each circle represents the cross-section of columnar molecular stacks observed from top. Note that circular cross-sections were considered as the PDI aggregates are expected to present helical and alternating stacking resulting in cylindrical columns.

Table 4.5. Hexagonal lattice parameters of **2**, **3**, and **4** at different concentrations. d_H is the d -spacing (interlayer distance) and a is the distance between the centers of aggregates.

Mesogen	d_H (nm)	a (nm)
2 (40 wt%, 25 °C)	3.2	3.7
3 (27 wt%, 25 °C)	4.9	5.6
4 (27 wt%, 25 °C)	5.4	6.2

In summary, $(\text{PEG}_n)_2\text{PDIs}$ have demonstrated to be excellent nonionic mesogens for the formation of chromonic mesophases. Note that, to date, very few nonionic compounds have been reported to form chromonic mesophases^[21], being TP6EO2M (formed by a triphenylene core substituted with six $-\text{O}(\text{CH}_2\text{CH}_2\text{O})_2\text{CH}_3$ chains) the most widely studied^[106,108,253]. Interestingly, TP6EO2M showed a very rich phase diagram similar to that observed for **2**, in which the N phase appears at concentrations around ~ 15 wt% and the nematic range occupies a large region of the phase diagram (from 14 to 51 wt%), and the concentration interval in which all the $\text{M} \rightarrow \text{N}$ transitions can be seen by heating is rather high^[253], which may suggest that this behavior can be common in nonionic chromonic mesogens functionalized with relatively short PEG chains. However, contrary to $(\text{PEG}_n)_2\text{PDIs}$, TP6EO2M homologues with PEG chain lengths other than 2 did not form any mesophase at all, showing that $(\text{PEG}_n)_2\text{PDIs}$ actually form chromonic phases in a relatively wide range of n values.

Moreover, further comparison with TP6EO2M reveals that $(\text{PEG}_n)_2\text{PDI}$ mesophases are also more stable at high temperatures, since TP6EO2M presents a LCST of $\sim 50\text{ }^\circ\text{C}$ [253], which make $(\text{PEG}_n)_2\text{PDI}$ more versatile mesogens with potential applications in the templating of hard silica materials, where high temperatures are commonly used, as it will be described in the next section.

4.1.4 Preparation of nanostructured silica materials by the sol-gel method

Among the potential applications of chromonic liquid crystals, the templating of hard materials is a topic of growing interest due to the high control over the shape and morphology of the final product using chromonic materials as templates [162,164]. In this context, we proposed the preparation of templated silica nanoporous materials using the chromonic **2** – **4** mesogens as soft templates. Briefly, templated silica was prepared in acid media by first preparing a $(\text{PEG}_n)_2\text{PDI}$ solution in HCl 5 M at a 1 wt% concentration and then adding a silica precursor (TEOS) that polymerized forming the final silica material with the embedded chromonic phase, which was removed by calcining the sample at $600\text{ }^\circ\text{C}$, yielding the final nanoporous silica material. This procedure is described in detail in chapter 3 (Section 3.2.10.1).

The silica samples were named as **Si-2**, **Si-3** and **Si-4**, where the number indicates the mesogen used as template. Before the calcination step, all the obtained silica materials were completely red, even after thoroughly washing them with ethanol, which indicated that these chromonic materials were fully embedded in the silica framework. These non-calcined samples were further characterized by SEM, TEM and SAXS to get new insights about the structure of the silica-*perylene* composites.

SAXS patterns (Figure 4.14) showed a resolved peak for **Si-2** and **Si-3**, indicating the formation of ordered nanostructures with first-order d -spacings (d_0) of 2.8 and 3.5 nm, respectively (calculated with Eq. 2). However, the SAXS profile for **Si-4** did not show any clear peak, which suggested the absence of any ordered nanostructure in this material. Interestingly, the d_0 value increased with PEG chain length, suggesting that longer PEG chain lengths increase the lattice distance of the nanostructured silica materials.

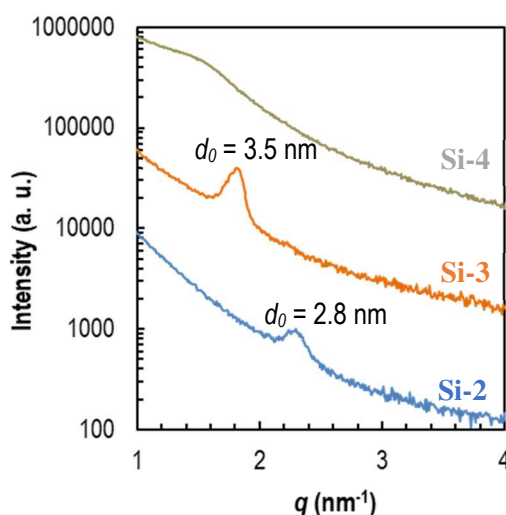


Figure 4.14. SAXS patterns of different non-calcined silica samples. From top to bottom: **Si-4**, **Si-3** and **Si-2**. Peaks indicating the presence of some nanostructure are resolved for all samples except for **Si-4**. d_0 was calculated with Eq. 2, using the scattering vector value at the maximum of the peak.

Further characterization was performed with electron microscopy. Low-resolution tabletop SEM revealed that **Si-2** and **Si-3**, consist in polygonal rods with a width of ca. $1\text{ }\mu\text{m}$ and a length between 1 and $10\text{ }\mu\text{m}$, respectively (see Figure 4.15a,b). In contrast, **Si-4** is an agglomeration of

spherical microparticles with a diameter between 0.5 and 2 μm (Figure 4.15c), similar to what is obtained by the sol–gel method in the absence of templates ^[254], i.e. there is no templating effect for **4**. Moreover, the rods of **Si-2** seem to present a higher aspect ratio than those of **Si-3**.

It is important to note that at the concentrations of $(\text{PEG}_n)_2\text{PDI}$ used in the synthesis of silica, there is no liquid crystal present in the absence of TEOS. This means that the formation of nanostructures takes place via cooperative self-assembly between the hydrolyzed silica species and diluted aggregates, similar to what has previously been described for other chromonic mesogens ^[162,164]. This mechanism potentially induces the formation of elongated particles through hierarchical, large-scale assembly of molecular stacks.

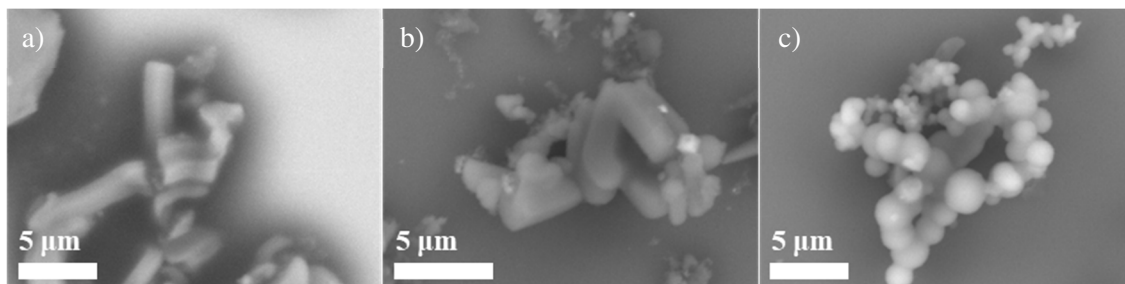


Figure 4.15. Low-resolution tabletop SEM images of non-calcined a) **Si-2**, b) **Si-3** and c) **Si-4**.

TEM images of **Si-(2-4)** confirmed the SAXS results, observing the formation of a periodic inner nanostructure for non-calcined **Si-2** and **Si-3** (Figure 4.16), and the lack thereof for **Si-4** (Figure 4.17). The ordered nanostructure consists in periodic stripes with a width around 1.4 nm (measured from the TEM image). This approximately matches with the length of the perylene diimide core (see Figure 4.11e), which indicates that the stripes are formed by $(\text{PEG}_n)_2\text{PDI}$ face-to-face unimolecular stacks. This suggests that the silica precursor (TEOS) polymerizes around the chromonic PDI stacks, making the PEG chains end up trapped inside the silica walls, as reported for other templating agents, and attributed to the high affinity of PEG for silica ^[255–258]. Accordingly, the varying PEG chain length of the chromonic $(\text{PEG}_n)_2\text{PDI}$ mesogen does not change the pore diameter but instead modifies the silica wall thickness, that increases from ca. 1.4 nm for **Si-2** to 2.1 nm for **Si-3** (estimated from the TEM images). Interestingly, such PEG trapping within the silica walls can lead to small micropores interconnecting the bigger pores, which is promising for the preparation of ordered mesoporous carbons by carbonizing the silica-PDI composite, as also occurs in other systems ^[259,260]. Fast Fourier-Transform (FFT) was applied to the TEM images of **Si-2** and **Si-3** (Inset Figure 4.16) resulting in lattice d -spacings of 2.8 and 3.5 nm, respectively, thus precisely matching the values determined by SAXS.

The calcination of the silica-perylenes composites yielded white materials, indicating that the organic phase was completely removed. Calcined **Si-2** and **Si-3** were characterized with SAXS, SEM and TEM to reveal the effect of the harsh calcination process on their nanoscale properties. Nitrogen-sorption experiments, although interesting, were not performed as not enough amount of sample was obtained.

SAXS measurements (Figure 4.18) revealed that **Si-2** maintained its nanostructure after the removal of the organic phase, since a resolved peak appeared at $q = 2.7 \text{ nm}^{-1}$, indicating a d -spacing (d_0) of 2.3 nm. As expected, calcination at 600 °C produced a shrinkage of the nanostructure from the initial value of 2.8 nm, but it did not disrupt the nanostructure, which remained highly ordered, as evidenced by the very similar shapes of the SAXS peak before and after calcination. SAXS of calcined **Si-3** was not performed due to lack of sample.

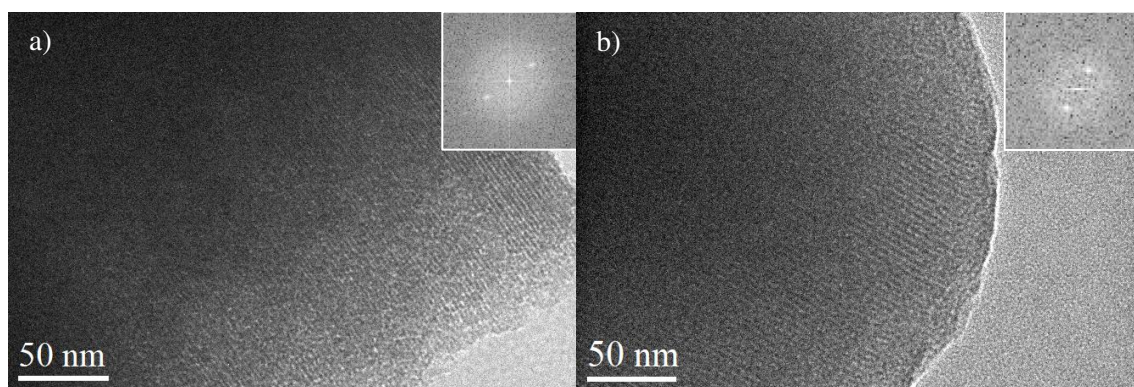


Figure 4.16. TEM images of non-calcined a) **Si-2** and b) **Si-3**. The insets of the images correspond to the FFT of the aligned stripes.

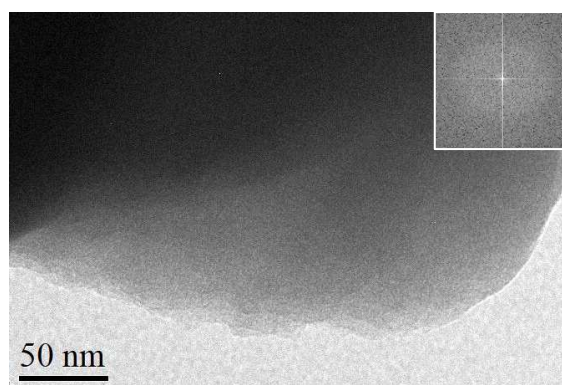


Figure 4.17. TEM image of non-calcined **Si-4**. Inset) FFT of a region of the image. The absence of nanostructure is confirmed.

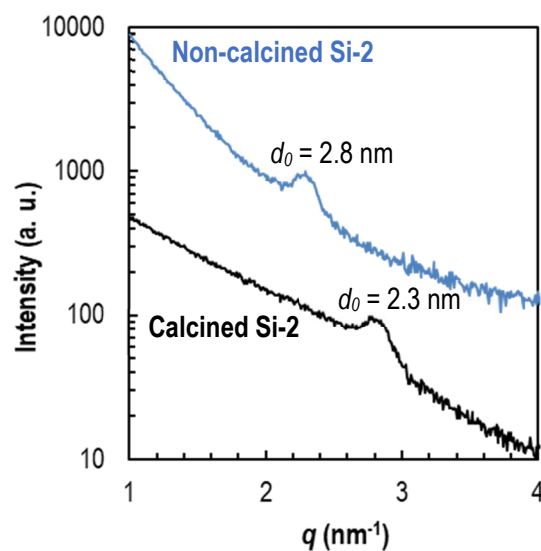


Figure 4.18. SAXS patterns of the non-calcined **Si-2** (blue) and the calcined **Si-2** (black). Peaks indicating the presence of some nanostructure are resolved for all samples. d_0 was calculated with Eq. 2, using the scattering vector value at the maximum of the peak.

SEM images of calcined **Si-2** and **Si-3** (Figure 4.19a,b) confirmed that, after calcination, the morphology of the particles was mostly preserved and **Si-2** rods presented a higher aspect ratio than those of **Si-3**. Moreover, TEM observations (Figure 4.19c,d) revealed that aligned micropores of ca. 1.4 nm in diameter are formed in the place where the perylene stripes were located, confirming the templating effect of the chromonic PEGylated PDIs. Interestingly, the d -

spacing of calcined **Si-2** measured from the FFT of the TEM images (Inset Figure 4.19c) is similar to that obtained by SAXS. Also, the d -spacing determined for calcined **Si-3** from the FFT of the TEM images (Inset Figure 4.19d) was ca. 2.8 nm, confirming that the silica materials shrink upon calcination.

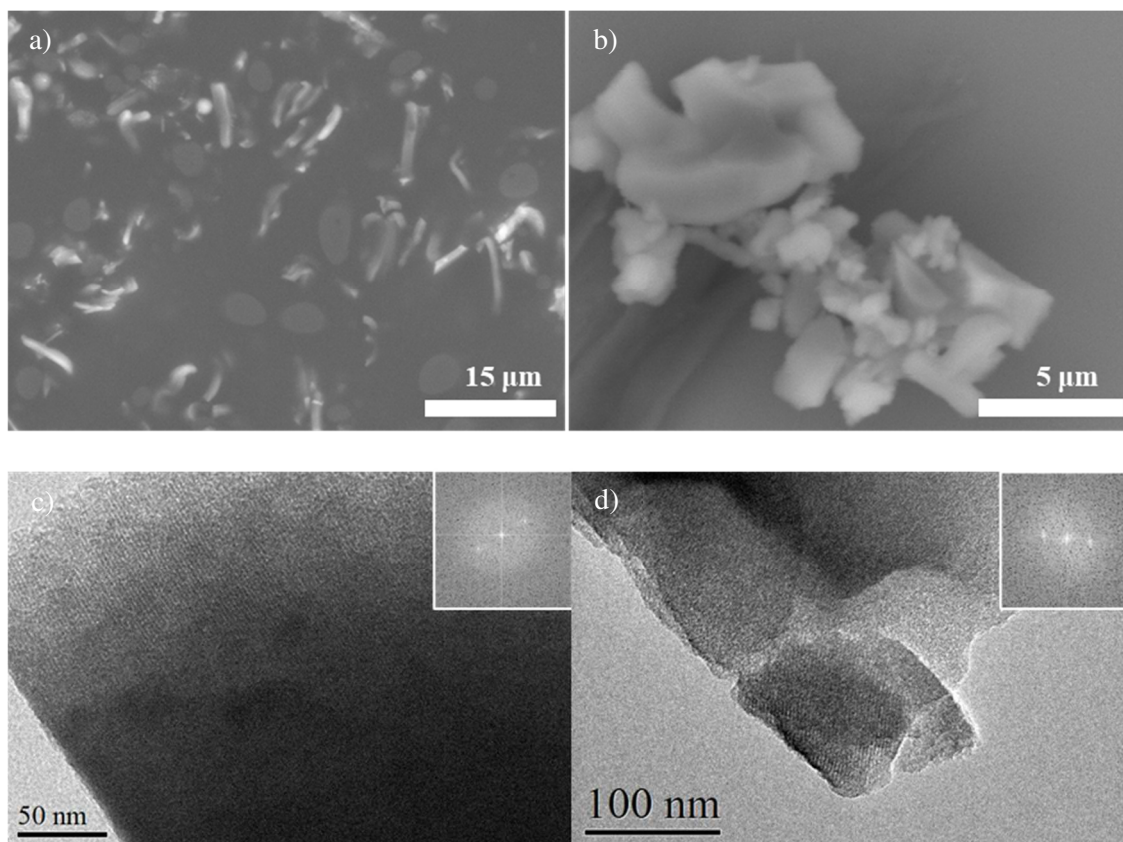


Figure 4.19. Low-resolution tabletop SEM images of a) calcined **Si-2** and b) calcined **Si-3**. TEM images of c) calcined **Si-2** and d) **Si-3**. The insets of the TEM images correspond to the FFT of the aligned micropores.

In summary, chromonic (PEG_n)₂PDI with $n = 7$ and $n = 10$ worked as templates for the preparation of nanostructured porous silica rods. The templating effect seemed to improve as PEG chains become shorter, as **Si-2** rods showed a higher aspect ratio than those of **Si-3**; and **Si-4**, with longer PEG chains, did not form rods at all. This may result either from the richer chromonic behavior of **2** (forms mesophases at lower concentrations) and its higher capacity to form nematic phases or from the higher steric hindrance of the longer PEG chains of **4**. Interestingly, Dai et al. [261] reported the preparation of hexagonal mesoporous silica SBA-3 using different surfactant mixtures as templates. They observed that SBA-3 d -spacing increased from 3.5 nm with the cationic surfactant CTAB template, to 4.5 – 5.5 nm with nonionic PEGylated surfactants with short ethylene oxide chains such as C₁₆EO₁₀ or C₁₈EO₁₀. Furthermore, no templating was observed for nonionic surfactants with longer ethylene oxide chains such as C₁₈EO₂₀. Similarly, nonionic chromonics **2** and **3** template silica materials with longer d -spacing than their ionic homologues reported by Rodríguez-Abreu et al. [164], who prepared nanostructured silica templated with a chromonic cationic PDI, and reported d -spacings of 2.2 and 2.1 nm before and after calcination, respectively. Additionally, the nonionic chromonic **4** with longer ethylene oxide chains did not produce ordered silica nanostructures.

It has been found that the higher d_0 of **Si-3** over **Si-2** does not result from a higher pore size, but from a thicker pore wall, which means that the pore wall thickness can be finely tuned by the PEG

chain length. A similar control over the pore wall thickness has been described for nonionic surfactant-templated silica materials ^[262] which exhibited thicker silica walls than the materials templated with ionic surfactants. This is interesting as it can potentially prevent the collapse of the silica nanostructure under extreme conditions such as very high temperatures, making these materials promising for the preparation of ordered mesoporous carbons through pyrolysis of the silica materials impregnated with a carbon source. Additionally, the pore size of the resulting carbon materials can be ultimately controlled by the silica pore wall thickness, since these ordered carbons are the negative image of the silica parent structure. For example, Lee et al. ^[263] prepared nanostructured hexagonal silica of different pore wall thickness by using mixtures of cationic HTAB and nonionic C₁₆EO₈ surfactants at different proportions which yield porous carbon materials upon pyrolysis and silica etching, with different pore sizes depending on the pore wall thickness of the parent silica. This fine control over the silica wall thickness is also interesting for the preparation of ordered hybrid materials, in which the distance between the perylene stripes can be subnanometrically controlled by varying the PEG chain length.

4.1.5 Conclusions

The effect of the PEG chain length on the solution properties, chromonic phase behavior and silica templating capabilities of compounds **1** – **5** have been determined. In all cases, solvatochromism was observed along with a loss of the vibronic fine structure and a fluorescence quenching using water as the solvent, due to the formation of H-aggregates. The presence of salts, such as ammonium sulphate, resulted in a lower critical solution temperature below 90 °C, for compounds **2** – **4**. The spectrophotometric experiments also indicated a high degree of aggregation of that synthesized PEGylated PDIs in water, primarily driven by the hydrophobic effect and $\pi - \pi$ interactions. Moreover, (PEG_n)₂PDIs showed surface activity and formed dynamic aggregates in organic solvents at high concentrations, as revealed by fluorescence spectra of **4** in chloroform. Compound **5** showed concentration-dependent absorption spectra with a slightly reduced degree of aggregation, but H-aggregate formation was still evident even at very low concentrations. These findings contribute to a better understanding of molecular assembly induced by multiple coexisting interactions, including π -stacking, hydration, and the hydrophobic effect ^[264].

Additionally, the (PEG_n)₂PDIs exhibited chromonic phase behavior and demonstrated silica templating properties. Mesomorphism was observed for PEG chain lengths ranging from $n = 7$ to $n = 16$, with the nematic phase favored for smaller n values and the hexagonal phase favored for larger n values. However, it was found that only PEG chains with lengths between 7 and 10 ethoxy units were suitable for the preparation of nanostructured silica. The resulting silica materials exhibited ordered columns of perylene separated by a silica wall, with a thickness of the wall dependent on the PEG chain length. This finding holds promise for the development of ordered mesoporous carbons. Additionally, upon calcination, nanoporous silica materials, with a pore diameter around 1.5 nm, were generated.

4.2 Formulation of complex all-aqueous emulsions and gels from ionic chromonic perylene diimides

This chapter explores the chromonic behavior of an anionic perylene diimide, (Ala)₂PDI, which is synthesized by adding two L-alanine terminal groups to a perylene core (Figure 4.20). At low concentrations in water, (Ala)₂PDI forms face-to-face columnar aggregates that evolve into nematic and hexagonal mesophases at higher concentrations (above 12.5 wt% and 30 wt%, respectively). Interestingly, the mesogen displays cholesteric fingerprints within the nematic phase, suggesting that it forms chiral nematic self-assemblies due to the presence of chiral groups in the aminoacid functionalization. In addition, this chapter describes the preparation of birefringent water-in-water (W/W) emulsions by combining (Ala)₂PDI with high molecular weight nonionic polymers, such as polyethylene glycol (PEG) MW 10,000 g/mol and dextran (Dex) MW 86,000 g/mol, at high concentrations. An achiral cationic PDI (MeCatPDI, Figure 4.20) is also used to prepare W/W emulsions with both PEG and Dex. These emulsions exhibit nematic-in-isotropic (N/I) and isotropic-in-nematic (I/N) phases depending on the mesogen and polymer concentrations. The nematic droplets exhibit bipolar, radial, or twisted radial configurations, depending on the isotropic phase polymer and droplet size. Furthermore, triphasic W/W/O and W/W/W emulsions are prepared by dispersing the W/W emulsions in oil or a third aqueous phase. These triphasic systems consist of biphasic droplets dispersed in an isotropic continuous phase, with droplets adopting various morphologies such as core-shell, snowman, and Janus. Finally, the chapter discusses the gelation experiments performed on (Ala)₂PDI and MeCatPDI to prepare anisotropic gelled particles. GdL is added to (Ala)₂PDI to form nematic low molecular weight gels that are responsive to temperature and light, while MeCatPDI is intended to gel by adding acrylate to the system. These results are promising for obtaining complex anisotropic gelled particles.

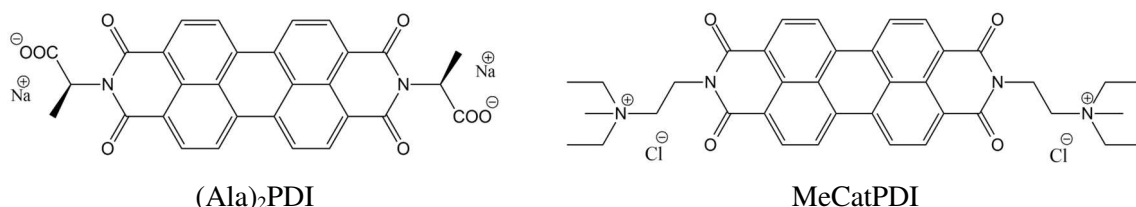


Figure 4.20. Chemical structure of (Ala)₂PDI and MeCatPDI.

4.2.1 Solution properties of (Ala)₂PDI

The self-assembly behavior of (Ala)₂PDI in water was evaluated by absorbance spectrophotometry. A total of eight solutions of increasing concentration from 0.27 to 11 μ M in water were prepared and observed in the visible range (400 – 700 nm). Notably, in the low-concentration range (0.27 – 1.1 μ M, Figure 4.21a), (Ala)₂PDI solutions exhibited three absorption bands of decreasing intensity at 535, 500 and 470 nm, corresponding to the $0 \rightarrow v$ ($v = 0, 1$ and 2) electronic transitions from the ground to the excited states, where v indicates the vibronic level. Differently, at higher (Ala)₂PDI concentrations (2.7 – 11 μ M, Figure 4.21b) the $0 \rightarrow 0$ transition band (535 nm) decreased with respect to the $0 \rightarrow 1$ transition band (500 nm), which resulted in a decrease of the molar extinction coefficient (ϵ) of the $0 \rightarrow 0$ band by increasing concentration (Figure 4.21c,d) with no significant changes in the ϵ of the $0 \rightarrow 1$ band (Figure 4.21e). Such a behavior indicates that (Ala)₂PDI exists mostly in a monomeric state at low concentrations ($< 1 \mu$ M) in water but forms face-to-face H-aggregates with increasing concentration ^[112,265]. Interestingly, a careful comparison of (Ala)₂PDI with the (PEG_n)₂PDI presented in the Chapter

4.1 reveals that the latter have a higher degree of aggregation as the (PEG_n)₂PDI showed a lower $0 \rightarrow 0$ transition band ϵ ($\sim 10000 - 18000 \text{ M}^{-1}\cdot\text{cm}^{-1}$, depending on n) than (Ala)₂PDI ($\sim 24000 \text{ M}^{-1}\cdot\text{cm}^{-1}$) at $11 \mu\text{M}$, and the $0 \rightarrow 0 / 0 \rightarrow 1$ band ratios of the (Ala)₂PDI at all concentration tested (Figure 4.21f) were even higher than that of the most polar (PEG_n)₂PDI at $1.8 \mu\text{M}$. In fact, the band ratio of (Ala)₂PDI at $0.27 \mu\text{M}$ (~ 1.35) was higher than that of (PEG₁₀)₂PDI in a DMSO/H₂O 70:30 mixture (Figure 4.4), confirming that (Ala)₂PDI has a lower degree of aggregation than the (PEG_n)₂PDI in water.

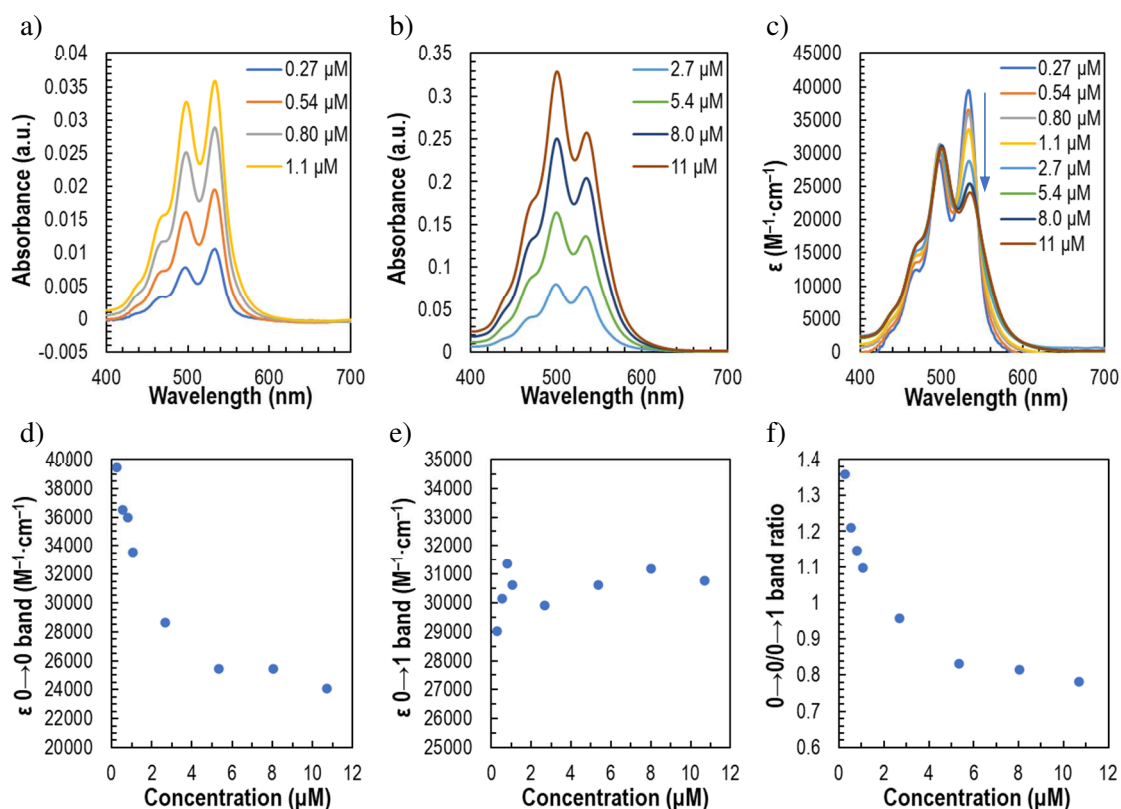


Figure 4.21. Visible absorbance spectra of (Ala)₂PDI at a) lower concentrations (0.27 – 1.1 μM) and b) higher concentrations (2.7 – 11 μM) in water at ca. 20 °C. c) Molar extinction coefficient (ϵ) of (Ala)₂PDI at all concentrations tested. A decrease on the ϵ of the $0 \rightarrow 0$ band can be observed. d) ϵ of the $0 \rightarrow 0$ band and e) of the $0 \rightarrow 1$ band as a function of the (Ala)₂PDI concentration and f) band ratio of the absorbance spectra as a function of the (Ala)₂PDI concentration.

The decreased aggregation for (Ala)₂PDI is most likely due to the presence of charged terminal groups that repel each other hindering the formation of face-to-face dimers. Further comparison between the aggregation behavior of (Ala)₂PDI and a selected (PEG_n)₂PDI ($n = 16$, compound **4** of Chapter 4.1) in water was performed by temperature-dependent absorbance and fluorescence spectrophotometry (Figure 4.22). The absorbance of both (Ala)₂PDI and a selected (PEG_n)₂PDI ($n = 16$) at $11 \mu\text{M}$ in water solution increased with temperature (Figure 4.22a,b). However, for (Ala)₂PDI the $0 \rightarrow 0$ band increased greatly as compared to the $0 \rightarrow 1$ band, which resulted in a significant increase of the $0 \rightarrow 0 / 0 \rightarrow 1$ band ratio (Figure 4.22c). Differently, the absorbance rise of the $0 \rightarrow 0$ band was more subtle for the (PEG_n)₂PDI, and the variation in the band ratio was less pronounced. This confirms the reduced aggregation of (Ala)₂PDI with respect to (PEG_n)₂PDI in water.

Temperature-dependent fluorescence spectra of both (Ala)₂PDI (Figure 4.22d) and the selected (PEG_n)₂PDI (Figure 4.22e) in water at $11 \mu\text{M}$ displayed two bands of decreasing intensity at

higher wavelengths at all the temperatures tested, resulting from the $0 \rightarrow 0$ and $0 \rightarrow 1$ fluorescent relaxations from the excited to the ground state, respectively. Interestingly, the band ratio between the two bands was around 1.8 – 2 independently of the compound and temperature used (Figure 4.22f). Such a similarity between the fluorescence spectra shape and band ratio at all temperatures for both compounds despite existing significant differences in aggregation, as shown by absorbance spectrophotometry, matches with the conclusion of the previous chapter regarding to the fluorescence emission coming only from the non-aggregated fraction of the PDIs. Moreover, the fluorescence intensity of aqueous (Ala)₂PDI solutions were ca. 5 times that of the selected (PEG_n)₂PDI at the same temperature, as (Ala)₂PDI has a higher fraction of monomeric PDI.

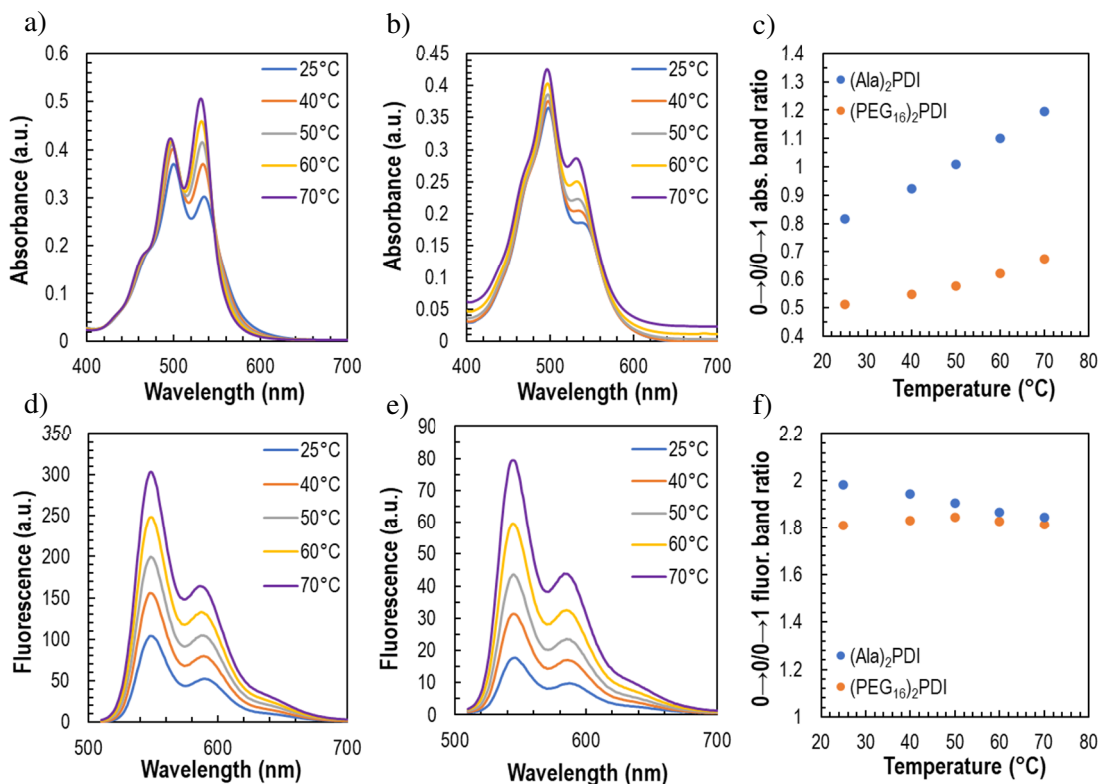


Figure 4.22. Temperature-dependent absorbance spectra of a) (Ala)₂PDI and b) a selected (PEG_n)₂PDI ($n = 16$, compound **4** of Chapter 4.1) and c) $0 \rightarrow 0 / 0 \rightarrow 1$ band ratios at all temperatures tested. Temperature-dependent fluorescence spectra of d) (Ala)₂PDI and e) the selected (PEG_n)₂PDI and f) $0 \rightarrow 0 / 0 \rightarrow 1$ band ratios at all temperatures tested.

4.2.2 Chromonic phase behavior at high concentrations

The chromonic phase behavior of (Ala)₂PDI solutions in water was firstly assessed by POM. Contact tests showed that (Ala)₂PDI forms chromonic mesophases in water (Figure 4.23). Nematic Schlieren textures can be observed in Figure 4.23a, along with a grainy texture at higher concentrations probably corresponding to an hexagonal mesophase. Moreover, a cholesteric fingerprint texture also appeared in some regions within the nematic phase (Figure 4.23b-d). Such texture is typical of chiral nematic liquid crystals, and results from an helicoidal twisting in the direction of the columnar stacks along an axis (Figure 1.13d), caused by the presence of chiral center in the mesogen structure or by adding chiral dopants to a non-chiral mesogen [124,266,267].

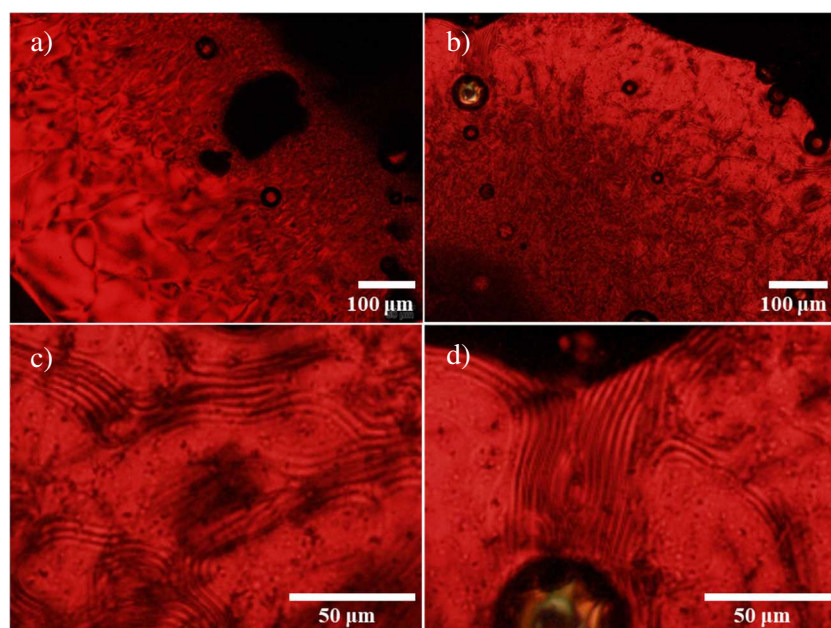


Figure 4.23. a) Contact test of $(Ala)_2PDI$. Both N and M phases appear. c) Contact test of $(Ala)_2PDI$ in a selected region where the cholesteric nematic phase appeared. c) and d) selected regions with the cholesteric nematic phase at higher magnification.

Further POM characterization of the chromonic liquid crystal behavior of $(Ala)_2PDI$ in water was assessed by preparing samples aqueous $(Ala)_2PDI$ solutions at selected concentrations (from 10 to 35 wt%) and studying their phase behavior as a function of the temperature (up to 70 °C) (Table 4.6). It was observed that $(Ala)_2PDI$ started forming nematic + isotropic (N + I) chromonic phases at 11 wt% in water (Figure 4.24a) at ca. 20 °C, and was completely nematic at 12.5 wt% (Figure 4.24b). Interestingly, $(Ala)_2PDI$ forms chromonic N phases in water at lower concentrations than all $(PEG_n)_2PDI$ s tested in Chapter 4.1 (15 – 20 wt%), despite presenting a significantly lower degree of aggregation at diluted concentrations. This suggests that the aggregation of chromonic mesogens at diluted concentrations is not extrapolable to the chromonic behavior at high concentrations. Hexagonal (M) phases appeared above 30 wt% (Figure 4.24c). Such M phases presented a grainy texture, suggesting a high number of defects within the hexagonal structure. Differently, when obtained by concentrating a N phase upon water evaporation, a herringbone texture appeared (Figure 4.24d).

Temperature-dependent POM observations revealed that both N and M phases exist in a wide range of temperatures. For example, samples with concentrations between 20 and 27.5 wt% were completely nematic at the whole temperature range studied, showing a high stability of such phase at high temperatures (70 °C). Samples at lower concentrations (12.5 – 18.75 wt%) confirmed such observations as isotropic regions only appeared at relatively high temperatures (40, 54 and 66 °C at 12.5, 15 and 18.75 wt%, respectively). For the 12.5 wt% sample, the N + I phases obtained by heating appeared as isotropic droplets within a nematic framework (Figure 4.24e), and evolved to nematic droplets in an isotropic background (Figure 4.24f) before becoming completely isotropic. This indicates a low interfacial tension between the N and I phases. A similar behavior was observed by lateral evaporation of an isotropic sample, where nematic droplets appeared at lower concentrations, and evolved to a bicontinuous N + I phase at higher concentrations (Figure 4.24g). Cholesteric fingerprints were clearly observed both in the nematic droplets and nematic continuous phases of Figure 4.24g (see Figure 4.24h,i).

Table 4.6. Phase transitions for (Ala)₂PDI at different concentrations (N: nematic, M: hexagonal, I: Isotropic). *Mesophase at room temperature (ca. 20 °C).

Conc. (wt%)	Mesophase*	M→M+N (°C)	M+N →N (°C)	N→N+I (°C)	N+I→I (°C)
10	I	-	-	-	-
11	N + I	Temperature effect not tested			
11.5	N	Temperature effect not tested			
12	N	Temperature effect not tested			
12.5	N	-	-	40	49
15	N	-	-	54	65
18.75	N	-	-	66	70
20	N	-	-	-	-
22.5	N	-	-	-	-
25	N	-	-	-	-
27.5	N	-	-	-	-
30	M	45	55	-	-
32.5	M	-	-	-	-
35	M	-	-	-	-

Besides, M phases were also very stable at high temperatures. For example, samples at 32.5 and 35 wt% retained the M phase from 25 to 70 °C. Differently, (Ala)₂PDI in water at 30 wt% showed an M phase at ca. 20 °C that smoothly evolved to a N phase in between 40 and 60 °C (Figure 4.25). SAXS was used to determine the structure of chromonic phases at 25 and 60 °C (Figure 4.26). At 25 °C, as a succession of peaks at $1:\sqrt{3} q$ ratio was observed, confirming the formation of an M phase, with a d_H of 2.7 nm and a hexagonal lattice parameter (a) of 3.1 nm (see Inset of Figure 4.13). Notably, these values are lower than those calculated from the SAXS spectra of (PEG)_nPDIs in Chapter 4.1, even for samples at 40 wt%, which suggest a closer packing between the columnar aggregates for (Ala)₂PDI M phases, probably resulting from the reduced size and steric hindrance of the alanine moieties as compared to the PEG chains. In contrast, the SAXS spectra of (Ala)₂PDI 30 wt% in water solution at 60 °C showed a single broad band centered at the same q as the first peak of the hexagonal sample at 25 °C, indicating a transition to an N phase by heating. This behavior hints that (Ala)₂PDI at 30 wt% forms a P phase in between 45 and 55 °C, suggesting that its columnar aggregates consist in helically stacked (Ala)₂PDI mesogens with an overall circular cross-section and rotational freedom at the studied temperatures.

The reduced steric hindrance mentioned in the previous paragraph could also explain why (Ala)₂PDI forms nematic phases at lower concentrations than the (PEG)_nPDIs, despite the latter having a higher tendency to aggregate at low concentrations: we hypothesize that the steric hindrance between the PEG chains may be insignificant at low concentrations, allowing for a higher degree of aggregation for these nonionic compounds in dilute solutions. However, it could impede the packing of long columnar aggregates within a nematic framework at higher concentrations, as suggested in Chapter 4.1. On the other hand, for (Ala)₂PDI, the anion-anion repulsive forces that reduce its aggregation in dilute water solutions may be screened by the Na⁺ ions at higher concentrations, thereby facilitating self-assembly of (Ala)₂PDI into chromonic mesophases.

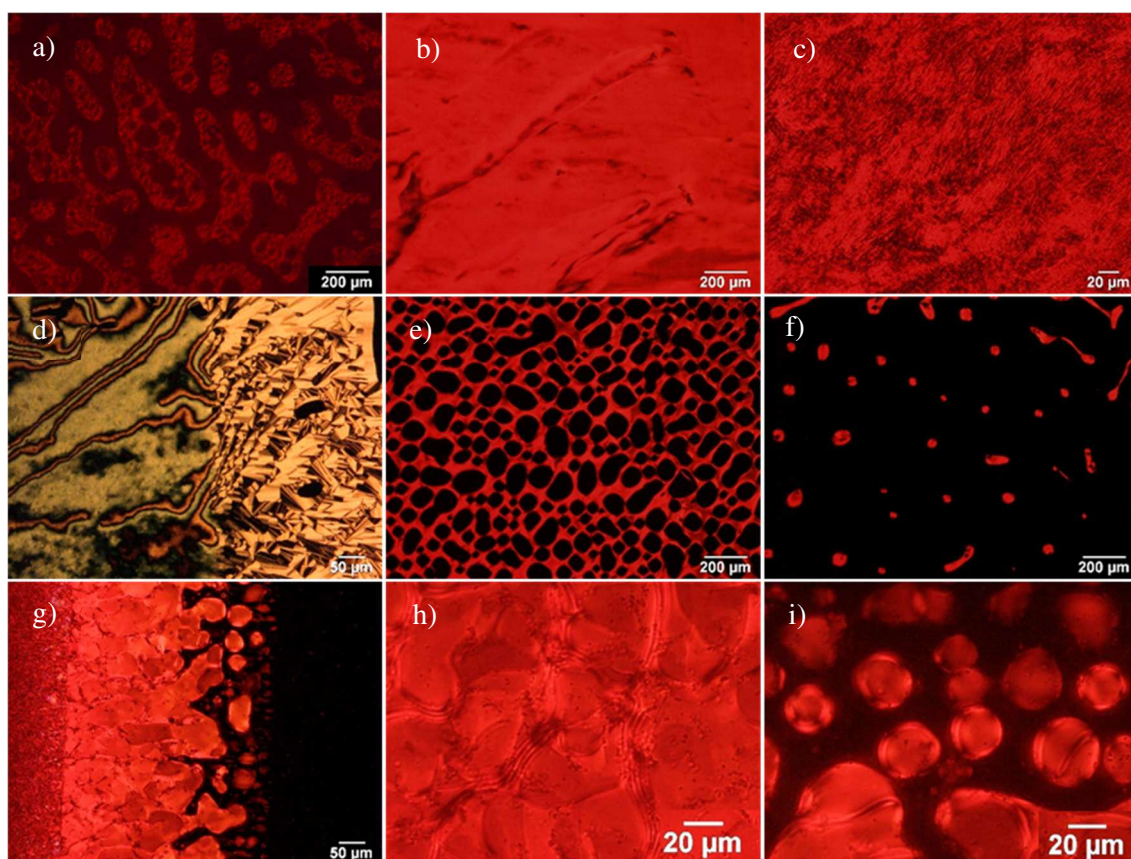


Figure 4.24. POM images of $(Ala)_2PDI$ at different concentrations and temperatures. a) $N+I$ texture at 11 wt% and ca. 20 °C, b) N phase at 12.5 wt% and ca. 20. °C, c) M phase at 32.5 wt% and ca. 20 °C, d) herringbone M texture formed by lateral evaporation of a 20 wt% $(Ala)_2PDI$ water solution at ca. 20 °C, e) isotropic droplets surrounded by a nematic phase formed at 12.5 wt% and 44 °C, f) nematic droplets in an isotropic medium at 12.5 wt% and 47 °C. g) Lateral evaporation of an isotropic 10 wt% sample, h) selected area within the nematic region and i) selected area within the $N+I$ region of the chromonic mesophases formed by the concentrated 10 wt% solution at ca. 20 °C.

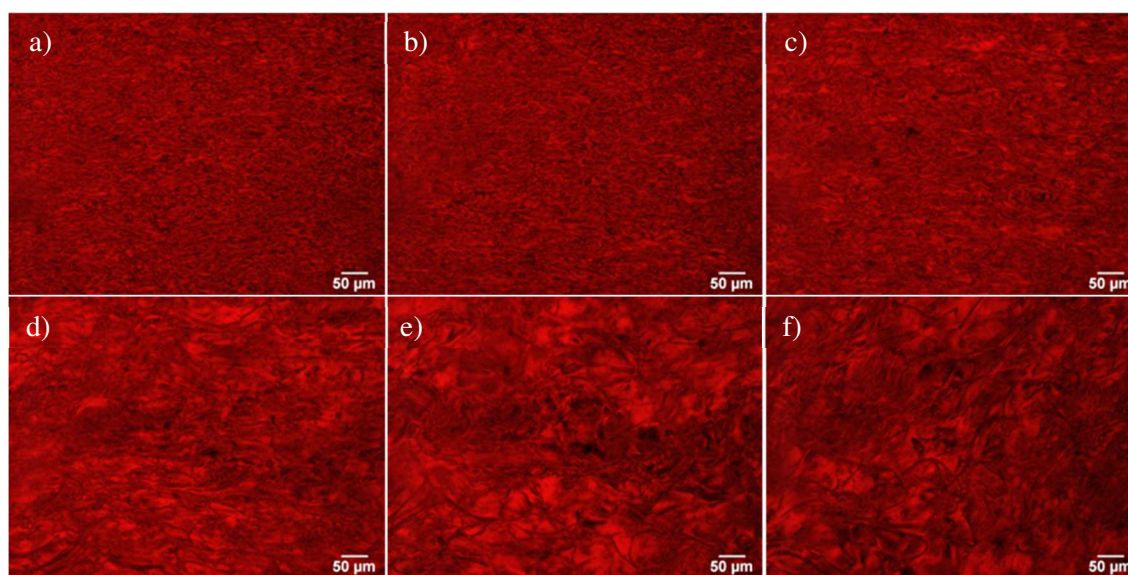


Figure 4.25. POM images of $(Ala)_2PDI$ at 30 wt% and a) 40 °C. b) 45 °C. c) 50 °C d) 55 °C, e) 60 °C and f) 65 °C.

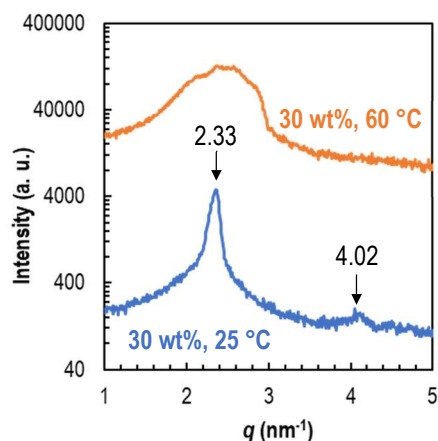


Figure 4.26. SAXS spectra of (Ala)₂PDI in water at a) 30 wt% and 25 °C (blue) and 60 °C (orange).

Further SAXS measurements of (Ala)₂PDI in water at 10, 12.5 and 15 wt% at ca. 20 °C (Figure 4.27a) showed a broad band centered at 0.98, 1.13 and 1.31 nm⁻¹ scattering vectors, corresponding to *d*-spacings (*d*₀) of 6.4, 5.6 and 4.8 nm, respectively. Note that for the nematic 12.5 and 15 wt% samples *d*₀ refers to the average distance between the centers of the aligned nematic columnar aggregates. For the isotropic 10 wt% sample, the broad SAXS band most likely results from non-birefringent aggregates with average inter-assembly distances of 6.4 nm. Notably, the scattering vector (*q*) as a function of the mesogen concentration typically follows a power law with ~0.5 exponent for semidilute nematic rods [268], which is translated in a log-log plot with a ca. ~0.5 slope. However, (Ala)₂PDI showed a higher slope of ~0.8 (Figure 4.27b). Such a deviation from the model has been previously reported for a chromonic PDI and attributed to the 0.5 exponent being derived assuming that a hexagonal packing entropy term dominates the interparticle interactions within the nematic phase, which is not necessarily the case for chromonic PDIs [164,269].

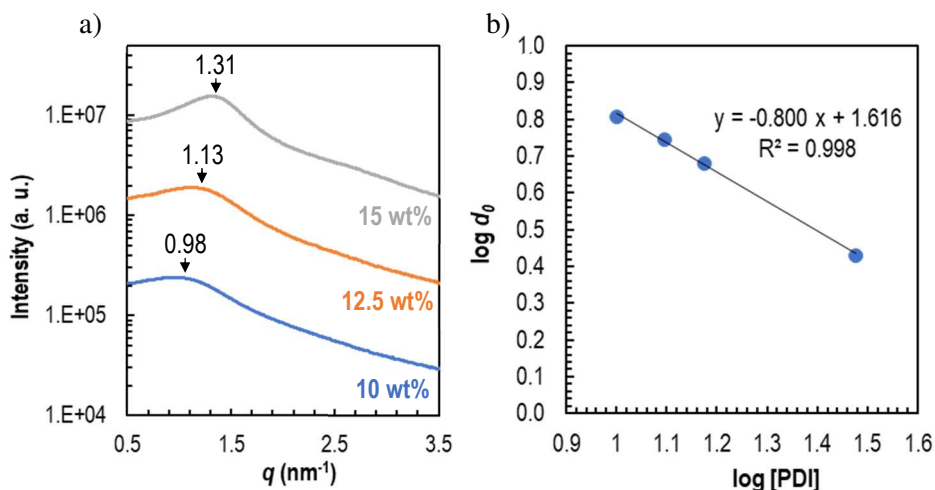


Figure 4.27. a) SAXS spectra of (Ala)₂PDI in water at 10 wt% (blue), 12.5 wt% (orange) and 15 wt% (grey) at 25 °C. b) Log-log plot of the *d*-spacing and the (Ala)₂PDI concentration.

With the data of Table 4.6, a chromonic partial phase diagram of (Ala)₂PDI was drawn (Figure 4.28). Such a phase diagram evidences the high stability of the chromonic phases at high temperatures and that the N phase exists in a wide range of concentrations for (Ala)₂PDI-water systems, as observed for the PEGylated PDI with 7 ethylene oxide units in the previous chapter. However, contrary to (PEG)_nPDI (*n* = 7, 10), the M phase did not typically evolved to an N phase

by heating for (Ala)₂PDI, since such transition was only observed at 30 wt% within the temperature range studied. The reduced $M \rightarrow N$ transitions by heating is also frequent for other ionic chromonic mesogens like DSCG (Figure 1.12b) [93,98,115], and supports the hypothesis proposed in the previous chapter that the prevalence of temperature-induced $M \rightarrow N$ transitions is typical of nonionic chromonic mesogens such as (PEG)_nPDI and TP6EO2M [253]. Furthermore, (Ala)₂PDI phase diagram could potentially show peritectic points at temperatures above the boiling point of water.

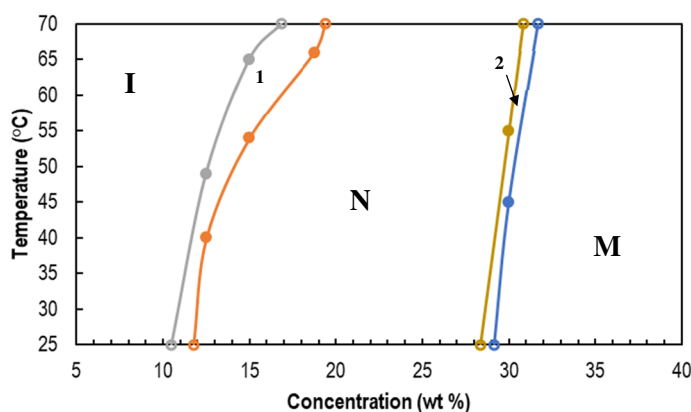


Figure 4.28. Partial chromonic phase diagram of (Ala)₂PDI in water. ¹ $\rightarrow N + I$ phase, ² $\rightarrow M + N$ phase. Filled circles refer to experimentally measured temperature-induced phase transitions, whereas empty circles are approximated as the average concentration between two measured samples with a different chromonic phase at a given temperature.

It may be interesting to compare the chromonic behavior of (Ala)₂PDI with that of other chromonic PDIs besides the (PEG)_nPDIs described before. As discussed in the previous chapter, Tam-Chang et al. [142–144] prepared several achiral water-soluble cationic PDIs that formed chromonic mesophases in a wide range of concentrations. Furthermore, the chromonic behavior of these molecules was almost non-affected by the size of the quaternary amine used, but resulted highly dependent on the counteranion [100]. Remarkably, diluted solutions of these cationic PDIs showed similar absorbance spectra than that of (Ala)₂PDI but with lower $0 \rightarrow 0 / 0 \rightarrow 1$ band ratios at similar concentrations, suggesting that the degree of aggregation of the cationic PDIs is higher than that of (Ala)₂PDI at diluted concentrations. This could explain why the cationic PDIs formed chromonic mesophases at lower concentrations than (Ala)₂PDI (about 6 wt% in water) when small counterions (Cl[−]) were used, although the aggregating behavior at low concentrations is not necessarily extrapolable to the formation of chromonic mesophases, as stated above.

Additionally, other chromonic PDIs have been reported in the literature. For instance, Naidu et al. [146] prepared a cationic chiral PDI with Cl[−] counteranions and observed the formation of cholesteric nematic phases at concentrations between 9 and 15 wt%. Similar to the cationic PDIs prepared by Tam-Chang et al., this compound revealed absorbance spectra with lower $0 \rightarrow 0 / 0 \rightarrow 1$ band ratios as compared to (Ala)₂PDI at similar concentrations and temperature (~ 0.6 band ratio at 1.7 μ M and 25 °C). In fact, the band ratio was as low as for the (PEG)_nPDIs, suggesting a high degree of aggregation at diluted concentrations.

Later on, Naidu et al. prepared bromo- and pyrrolidine-bay-substituted chromonic achiral [147] and chiral [123] mesogens. The obtention of bay-substituted chromonic mesogens is interesting as the electronic properties of the perylene core can be easily tuned depending on the substituent. Adding electron-donating such as pyrrolidine groups highly modifies the electronic properties of the PDIs red-shifting the maxima of absorption [123]. In contrast, the incorporation of electron-withdrawing groups such as halogens, can enhance the air-stability and n-type semiconductor properties of the PDIs, which is very interesting for applications in organic electronics [270–272]. However, obtaining

chromonic bay-substituted PDIs is not a straightforward task due to the synthetic difficulties^[131] and there are deformations of the perylene core resulting from the steric hindrance between the bay-substituents, which prevents the formation of $\pi - \pi$ columnar aggregates^[273–275]. Despite this, the PDIs prepared by Naidu et al.^[123,147] formed both N and M chromonic mesophases in water at very low concentrations (1 wt%) and room temperature, as revealed by POM images. Unfortunately, the X-ray results and the phase diagrams were never published. The formation of M phases at such low concentrations is rather surprising and has not been reported for core-unsubstituted PDIs, suggesting that the bay-substitution plays an important role in the stabilization of the M phase at low concentrations. Indeed, a similar behavior has been observed for thermotropic PDIs, which tend to display columnar discotic mesophases in a wider temperature range if functionalized in the bay position^[276].

In two recent reports^[148,149], a gellable cationic PDI functionalized with two terminal methacrylate groups and with bromide as counteranion was described to form chromonic nematic mesophases in water at concentrations about 7 – 10 wt%. Furthermore, chromonic mesophases have also been observed for PDIs functionalized with methylated pyridine groups^[141], and for other rylene dyes such as cationic quaterylene diimides^[153]. These results indicate that the formation of chromonic mesophases is rather common for cationic rylene dyes.

In contrast, anionic PDIs forming lyotropic chromonic liquid crystals have been surprisingly less studied. To date, the formation of chromonic mesophases by anionic mesogens is limited to a PDI functionalized an aromatic moiety with sulfonate groups^[141], which formed nematic mesophases above 10 wt%; and to L-valine and L-leucine functionalized PDIs with K^+ counterions, which were mentioned to form chromonic mesophases at 11.8 wt% in aq. KOH 0.001 M, in an invited paper by Tam-Chang et al.^[145]. However, these chromonic phases were not further characterized.

4.2.3 Formulation of chromonic liquid crystal W/W emulsions

The formation of birefringent anisotropic droplets by emulsifying liquid crystal phases has drawn the attention of researchers from various fields, from mathematics to applied biology, and including both theoretical and applied perspectives^[277–281]. The emulsification of lyotropic chromonic liquid crystals is of particular interest, as it can be achieved using either oil or polymer aqueous solutions as the continuous phase, resulting in the formation of water-in-oil^[202] or water-in-water emulsions^[200,207,208], respectively, which adds more versatility to the chromonic emulsion systems.

Herein, we investigated the phase behavior of (Ala)₂PDI chromonic liquid crystals in presence of water-soluble nonionic polymers that separate out the nematic phase forming W/W emulsions with a (Ala)₂PDI-rich phase and a polymer-rich phase. We also studied the preparation of three-phase systems by dispersing the as-formed W/W emulsions either in a third water phase or in an oil phase, forming W/W/W and W/W/O emulsions, respectively (see Section 4.2.4). Moreover, the (Ala)₂PDI emulsion behavior was compared to that of an achiral cationic PDI, hereinafter called MeCatPDI (Figure 4.20). This compound was selected due to its rich chromonic behavior^[100]. This planar mesogen is known to form chromonic nematic phases between 6 and 7 wt%. However, we observed that concentrations up to 8 wt% were required for it to form N + I phases. This difference may arise from adsorbed water, as inferred from the FTIR spectra of MeCatPDI, which shows a broad band about 3000 – 3500 cm^{-1} . Notably, this broad band was very hard to remove, even after keeping the material in the stove at 40 °C for various days. Alternatively, the material could have some tosylate impurities, as suggested by previous works^[164]. These impurities did not appear in the NMR spectra, but they could be hidden by the broad band at 7.79 ppm (see Annexes). In any case, such impurities are minor or inexistent.

The phase behavior of (Ala)₂PDI-polymer mixtures in water was first assessed by preparing (Ala)₂PDI aqueous solutions with dextran (Dex, MW 86000 g/mol) and polyethylene glycol

(PEG, MW 10000 g/mol). Multiple (Ala)₂PDI, Dex and PEG concentrations were tested (Table 4.7). It was observed that biphasic systems were obtained for samples with (Ala)₂PDI concentrations of at least 10 wt% and Dex concentrations above 8.4 wt% for (Ala)₂PDI-Dex systems. This indicates that the addition of Dex induces the formation of nematic droplets in concentrated isotropic (Ala)₂PDI water solutions, similar to other chromonic-polymer W/W emulsions such as DSCG-polyol or DSCG-polyamide systems^[200]. However, it is worth noting that very few nematic droplets appeared at 10 and 11.2 wt% (Ala)₂PDI concentrations with 8.8 wt% of Dex (Figure 4.29a), suggesting a reduced capacity of Dex to form (Ala)₂PDI chromonic droplets in isotropic solutions. On the other hand, introducing Dex to a nematic 12.5 wt% (Ala)₂PDI water solution also induced a phase separation into a (Ala)₂PDI-rich chromonic phase and a Dex-rich isotropic phase, but generated isotropic islands in a nematic continuous phase (isotropic-in-nematic or I/N emulsions, Figure 4.29c) rather than nematic droplets in an isotropic medium (nematic-in-isotropic or N/I emulsions). Furthermore, no phase separation was observed for Dex concentrations below 4.2 wt% (Figure 4.29b).

In contrast, (Ala)₂PDI-PEG systems formed biphasic systems for all PEG concentrations tested (4.2 – 17.5 wt%) and 12.5 wt% of (Ala)₂PDI, suggesting a reduced miscibility of (Ala)₂PDI with PEG as compared to Dex. All the (Ala)₂PDI-PEG W/W emulsions with relatively low PEG content (4.2 – 13.1 wt%) consisted in N/I emulsions (Figure 4.29d,e). However, grainy non-spherical birefringent droplets appeared when PEG concentration was increased to 17.5 wt% (Figure 4.29f). The grainy texture suggests that the birefringent phase is not nematic. A plausible hypothesis would be that the birefringent phase becomes highly concentrated in (Ala)₂PDI and forms hexagonal (M) phases or crystalline precipitates. In summary, the formation of (Ala)₂PDI chromonic W/W emulsions highly depends on the concentration and the polymer used.

Table 4.7. Composition of the (Ala)₂PDI water-in-water emulsions with Dex and PEG at ca. 20°C. *None means that no emulsion is formed at these concentrations, I/N means isotropic-in-nematic, N/I means nematic-in-isotropic, and M/I means hexagonal-in-isotropic.

Sample name	[(Ala) ₂ PDI] (wt%)	[Dex] (wt%)	[PEG] (wt%)	[H ₂ O] (wt%)	Emulsion type*
(Ala) ₂ PDI_Em1	10	8.8	-	81.2	N/I
(Ala) ₂ PDI_Em2	11.2	8.8	-	80.0	N/I
(Ala) ₂ PDI_Em3	12.5	4.2	-	83.3	None
(Ala) ₂ PDI_Em4	12.5	8.4	-	79.1	I/N
(Ala) ₂ PDI_Em5	12.5	12.5	-	75.0	I/N
(Ala) ₂ PDI_Em6	12.5	-	4.2	83.3	N/I
(Ala) ₂ PDI_Em7	12.5	-	8.4	79.1	N/I
(Ala) ₂ PDI_Em8	12.5	-	12.5	75.0	N/I
(Ala) ₂ PDI_Em9	12.5	-	13.1	74.4	N/I
(Ala) ₂ PDI_Em10	12.5	-	17.5	70.0	M/I?

SAXS measurements were used for further characterization of the chromonic (Ala)₂PDI water-in-water emulsions, specifically of (Ala)₂PDI_Em4 (I/N emulsion) and (Ala)₂PDI_Em7 (N/I emulsion) (Figure 4.30a). Notably, (Ala)₂PDI_Em4 revealed a SAXS spectra quasi-identical to that of (Ala)₂PDI 12.5 wt% nematic solution in absence of polymers, with only a slight increase in the scattering vector of maximum intensity from 1.13 to 1.16 nm⁻¹. This suggests that the packing between the (Ala)₂PDI columnar aggregates in the chromonic-rich phase is not affected by the presence of Dex-rich isotropic droplets. In contrast, the SAXS spectra of (Ala)₂PDI_Em7 showed significant variations, with a widening of the band and a shift to lower scattering vectors (0.85 – 1.1 nm⁻¹) with no clear maximum. Such a behavior indicates an increased polydispersity of the *d*₀ value probably resulting from the higher number of defects and the director vector

deformations caused by the spherical confinement of the chromonic phase. Furthermore, both emulsions displayed another band in the WAXS region at 18.4 nm^{-1} (Figure 4.30b). This signal was also evident for $(\text{Ala})_2\text{PDI}$ 12.5 wt% without polymers and corresponds to a Bragg spacing of 0.34 nm resulting from the stacking distance between adjacent perylene cores inside the columnar stack. This demonstrate that the PDI–PDI stacking is not affected by the presence of Dex and PEG. SAXS characterization of $(\text{Ala})_2\text{PDI_Em10}$ is necessary to confirm the nature of the dispersed phase, and it will be performed in the future.

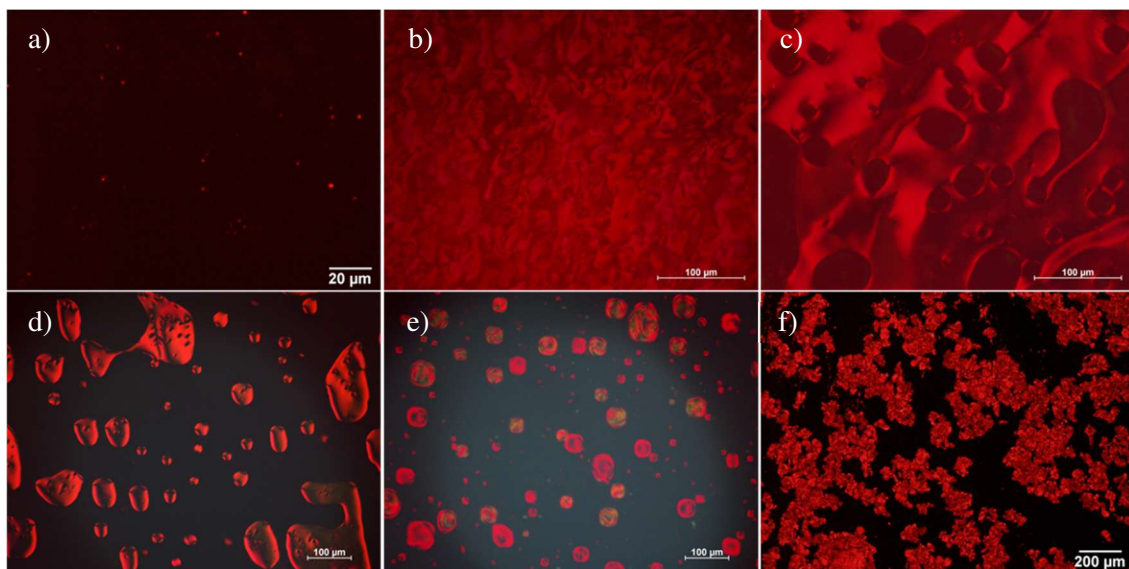


Figure 4.29. POM images of a) $(\text{Ala})_2\text{PDI_Em1}$, b) $(\text{Ala})_2\text{PDI_Em3}$, c) $(\text{Ala})_2\text{PDI_Em4}$, d) $(\text{Ala})_2\text{PDI_Em6}$, e) $(\text{Ala})_2\text{PDI_Em7}$, f) $(\text{Ala})_2\text{PDI_Em10}$ at ca. 20 °C.

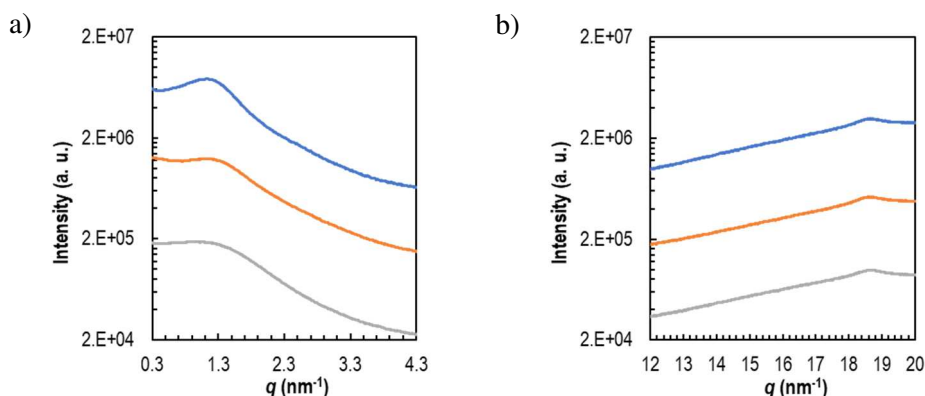


Figure 4.30. a) SAXS and b) WAXS spectra of $(\text{Ala})_2\text{PDI}$ 12.5 wt% (blue), $(\text{Ala})_2\text{PDI_Em4}$ (orange) and $(\text{Ala})_2\text{PDI_Em7}$ (grey).

The phase behavior of MeCatPDI with Dex (Table 4.8) and PEG (Table 4.9) was also studied. MeCatPDI-Dex systems revealed a more complex phase behavior compared to $(\text{Ala})_2\text{PDI}$ -polymer systems, since both N/I (Figure 4.31a) and I/N emulsions (Figure 4.31b) were formed as well as two new types of biphasic systems. These two systems are: bicontinuous emulsions (abbreviated as Bicont.), consisting in a biphasic mixture in which there is no clear dispersed and continuous phases, and the two phases form interconnected networks (Figure 4.31c), and nematic-in-nematic emulsions (abbreviated as N/N), in which both dispersed and continuous phases are birefringent. These two birefringent coexisting phases were observed by POM (Figure 4.31d). These N/N emulsions are very interesting, and our knowledge, this is the first time that chromonic nematic-in-nematic emulsions have been observed. They appear at rather high polymer and mesogen concentrations (see Table 4.8).

Table 4.8. Composition of the *MeCatPDI* water-in-water emulsions with *Dex* at ca. 20°C. *None means that no emulsion is formed at these concentrations, I/N means isotropic-in-nematic, N/I means nematic-in-isotropic, Bicont. means bicontinuous and N/N means nematic-in-nematic.

Sample name	[MeCatPDI] (wt%)	[Dex] (wt%)	[PEG] (wt%)	[H ₂ O] (wt%)	Emulsion type*
MeCatPDI_Em1	8.7	4.4	-	86.9	None
MeCatPDI_Em2	8.8	2.8	-	89.0	None
MeCatPDI_Em3	8.9	6.6	-	84.5	None
MeCatPDI_Em4	9.0	9.0	-	82.0	None
MeCatPDI_Em5	9.1	1.0	-	89.9	None
MeCatPDI_Em6	9.1	1.9	-	89.0	None
MeCatPDI_Em7	9.1	3.1	-	87.8	None
MeCatPDI_Em8	9.2	0.4	-	90.4	None
MeCatPDI_Em9	9.2	2.9	-	87.9	N/I
MeCatPDI_Em10	9.2	12.2	-	78.6	None
MeCatPDI_Em11	9.3	4.7	-	86.0	N/I
MeCatPDI_Em12	9.4	6.9	-	83.7	N/I
MeCatPDI_Em13	9.4	10.1	-	80.5	N/I
MeCatPDI_Em14	9.5	9.5	-	81.0	N/I
MeCatPDI_Em15	9.6	3.0	-	87.4	N/I
MeCatPDI_Em16	9.7	4.9	-	85.4	Bicont.
MeCatPDI_Em17	9.8	1.1	-	89.1	N/I
MeCatPDI_Em18	9.8	2.0	-	88.2	N/I
MeCatPDI_Em19	9.8	7.2	-	83.0	N/I
MeCatPDI_Em20	10.0	0.5	-	89.5	N/I
MeCatPDI_Em21	10.0	6.0	-	84.0	N/I
MeCatPDI_Em22	10.0	8.0	-	82.0	N/I
MeCatPDI_Em23	10.0	10.0	-	80.0	N/I
MeCatPDI_Em24	10.1	3.4	-	86.4	N/I
MeCatPDI_Em25	10.2	2.1	-	87.7	N/I
MeCatPDI_Em26	10.2	5.1	-	84.7	Bicont.
MeCatPDI_Em27	10.4	7.6	-	82.0	Bicont.
MeCatPDI_Em28	10.8	8	-	81.2	N/N
MeCatPDI_Em29	10.9	3.7	-	85.4	Bicont.
MeCatPDI_Em30	11.0	0.5	-	88.5	I/N
MeCatPDI_Em31	11.0	5.5	-	83.5	Bicont.
MeCatPDI_Em32	11.0	11.0	-	78.0	N/N
MeCatPDI_Em33	11.1	8.2	.	80.7	N/N
MeCatPDI_Em34	11.5	1.3	-	87.2	I/N
MeCatPDI_Em35	11.6	3.9	-	84.5	N/N
MeCatPDI_Em36	11.6	5.8	-	82.6	N/N
MeCatPDI_Em37	12.0	8.0	-	80.0	N/N
MeCatPDI_Em38	12.0	12.0	-	76.0	N/N
MeCatPDI_Em39	12.1	1.3	-	86.6	I/N
MeCatPDI_Em40	12.1	4.1	-	83.8	N/N
MeCatPDI_Em41	12.1	6.1	-	81.8	N/N
MeCatPDI_Em42	14.0	14.0	-	72.0	N/N

Temperature-dependent POM observations of the N/N emulsion MeCatPDI_Em42 (14 wt% MeCatPDI, 14 wt% Dex and 72 wt% water) were performed, and it was observed that the N $\rightarrow$ I phase transition occurred at lower temperatures for the dispersed phase (50 °C, Figure 4.31e) than for the continuous phase (65 °C), hence suggesting a higher MeCatPDI concentration in the continuous phase. Additionally, by cooling down to room temperature, small nematic droplets with radial configuration in a continuous nematic phase appeared (Figure 4.31f), and grew until forming a N/N emulsion similar to that before the heating/cooling cycle (Figure 4.31g).

Notably, these N/N emulsions have been detected only by POM, and they should also be characterized by SAXS. However, for those experiments larger amounts of products are required.

The phase behavior MeCatPDI-Dex systems significantly differs from that of (Ala)₂PDI-Dex aqueous mixtures. For example, the formation of N/I emulsions is preferred over I/N emulsions for MeCatPDI-Dex systems, as it occurred in a wide range of concentrations, and I/N emulsions only appeared at relatively high MeCatPDI concentrations (< 11 wt%) and very low Dex concentrations (> 2 wt%). In contrast, mostly I/N emulsions were observed for (Ala)₂PDI-Dex systems. Moreover, MeCatPDI-Dex formed N/N emulsions at high polymer and mesogen concentrations, which was a unique feature among all the W/W emulsions studied herein.

On the other hand, the phase behavior of MeCatPDI and (Ala)₂PDI with PEG were more similar. MeCatPDI-PEG mixtures mostly formed N/I emulsions at the studied concentrations (Figure 4.31h), and I/N emulsions were only formed at 11 wt% of MeCatPDI and 1 wt% of PEG (Figure 4.31i). Furthermore, MeCatPDI-PEG emulsions at high mesogen and polymer concentrations formed complex multiphasic with an isotropic phase (PEG-rich) and two birefringent phases with grainy textures (Figure 4.31j), instead of forming N/N emulsions as with Dex. Notably, these grainy textures indicate that the birefringent phases are not nematic, but consist in either M phases or solid crystals. This behavior suggests a higher immiscibility of MeCatPDI with PEG than with Dex, since high PEG concentrations produce a highly concentrated MeCatPDI phase that forms hexagonal phases or crystalline precipitates, whereas high Dex concentrations allow the formation of nematic phases in the Dex-rich phase.

With the data of Tables 4.8 and 4.9, phase diagrams of MeCatPDI-Dex and MeCatPDI-PEG W/W emulsions were drawn (Figure 4.32). In both cases, phase separation does not occur for MeCatPDI concentrations below 9 wt%, which suggests that adding a polymer to isotropic MeCatPDI solution does not induce the formation of nematic droplets as it occurred for (Ala)₂PDI-Dex systems and other chromonic dyes^[200]. Furthermore, increasing the polymer concentration by maintaining constant the total amount of water induces a I/N $\rightarrow$ Bicont. $\rightarrow$ N/I transition for both MeCatPDI-Dex and MeCatPDI-PEG W/W emulsions. This behavior indicates that increasing the polymer/MeCatPDI ratio increases the volume fraction of the isotropic solution and causes the phase inversion from I/N to N/I. It is widely known that the continuous phase of water-in-water emulsions is usually the phase with larger volume fraction, because there is no any surfactant that controls the interfacial curvature^[22].

It is also worth noting that N/I emulsions exist in a wider range of concentrations for MeCatPDI-PEG emulsions than for MeCatPDI-Dex emulsions, which also happened for W/W emulsions formulated with (Ala)₂PDI.

Furthermore, the binodal curve of both MeCatPDI-Dex and MeCatPDI-PEG phase diagrams is highly asymmetric, as very small amounts of polymers are required to form biphasic mixtures meanwhile the MeCatPDI concentration has to reach ~ 10 wt%. This means that dextran and PEG are less soluble in the MeCatPDI phase, but MeCatPDI is more soluble in both Dex and PEG

solutions. Probably, this lack of symmetry is caused by the unfavorable penetration of polymers into MeCatPDI columnar stacks.

Similarly, (Ala)₂PDI – polymer biphasic aqueous systems require much higher concentrations of the PDI than of the polymer. This behavior is typically observed when the molecular weights of the two components forming the aqueous two-phase systems are very different ^[282], which is indeed the case for the PDIs (MW $\approx$ 600 g/mol) and the polymers (MW > 10000 g/mol). Despite this, it is worth noting that isotropic-in-isotropic W/W emulsions were not formed for any mesogen – polymer system neither in this work nor in the literature ^[200,207,208], which indicates that the formation of liquid crystal mesophases is required for phase separation to occur. This suggests that phase separation is not triggered by interactions between the polymers and single PDI molecules but rather between the polymers with anisotropic chromonic PDI self-assemblies.

Table 4.9. Composition of the (Ala)₂PDI water-in-water emulsions with Dex and PEG at ca. 20°C. *None means that no emulsion is formed at these concentrations, I/N means isotropic-in-nematic, N/I means nematic-in-isotropic, Bicont. means bicontinuous and Ms means multiphasic in the presence of solids.

Sample name	[MeCatPDI] (wt%)	[Dex] (wt%)	[PEG] (wt%)	[H ₂ O] (wt%)	Emulsion type*
MeCatPDI_Em43	8.6	-	10.5	80.9	None
MeCatPDI_Em44	9.0	-	11.0	80.0	N/I
MeCatPDI_Em45	9.1	-	0.8	90.1	None
MeCatPDI_Em46	9.1	-	4.7	86.2	None
MeCatPDI_Em47	9.5	-	0.9	89.6	Bicont.
MeCatPDI_Em48	9.9	-	5.2	84.9	N/I
MeCatPDI_Em49	10.0	-	0.9	89.1	Bicont.
MeCatPDI_Em50	10.0	-	2.0	88.0	N/I
MeCatPDI_Em51	10.0	-	5.2	84.8	N/I
MeCatPDI_Em52	10.0	-	6.0	84.0	N/I
MeCatPDI_Em53	10.0	-	6.6	83.4	N/I
MeCatPDI_Em54	10.0	-	8.3	81.7	N/I
MeCatPDI_Em55	10.0	-	10.0	80.0	N/I
MeCatPDI_Em56	10.0	-	12.0	78.0	M _s
MeCatPDI_Em57	10.9	-	5.7	83.4	N/I
MeCatPDI_Em58	10.9	-	7.3	81.8	N/I
MeCatPDI_Em59	11.1	-	1.0	87.9	I/N
MeCatPDI_Em60	12.0	-	6.0	82.0	Bicont.
MeCatPDI_Em61	12.0	-	8.0	80.0	M _s
MeCatPDI_Em62	12.0	-	10.0	78.0	M _s

Additionally, the phase behavior of MeCatPDI was also tested with PEG of lower molecular weight (400 and 6000 g/mol, note that the PEG used in previous samples had a MW of 10000 g/mol). The addition of the PEG with the smallest molecular weight did not affect the N phase formed by MeCatPDI (Figure 4.31k). However, the PEG with 6000 g/mol molecular weight disrupted the MeCatPDI nematic phase (for a total concentration of 10 wt% MeCatPDI and 10 wt% PEG MW 6000). Furthermore, at higher concentrations, MeCatPDI-PEG MW 6000 mixtures formed fibrous birefringent precipitates (Figure 4.31l). These observations suggest that

polymers with rather high molecular weights are required for the phase separation of chromonic phase into either N/I or I/N water-in-water emulsions to occur.

This positive effect of high-molecular weights for aqueous phase separation has been long known for many aqueous two-phase systems such as dextran-polyethylene glycol ^[283] or sodium sulfate-polyethylene glycol ^[284]. It is known that the increase in polymer chain length decreases miscibility. This is commonly attributed to the conformational restrictions that are imposed on long polymers at high concentrations. Often, long molecules cannot move freely and these conformational restrictions produce an unfavorable entropy of mixing, which promotes phase separation ^[282].

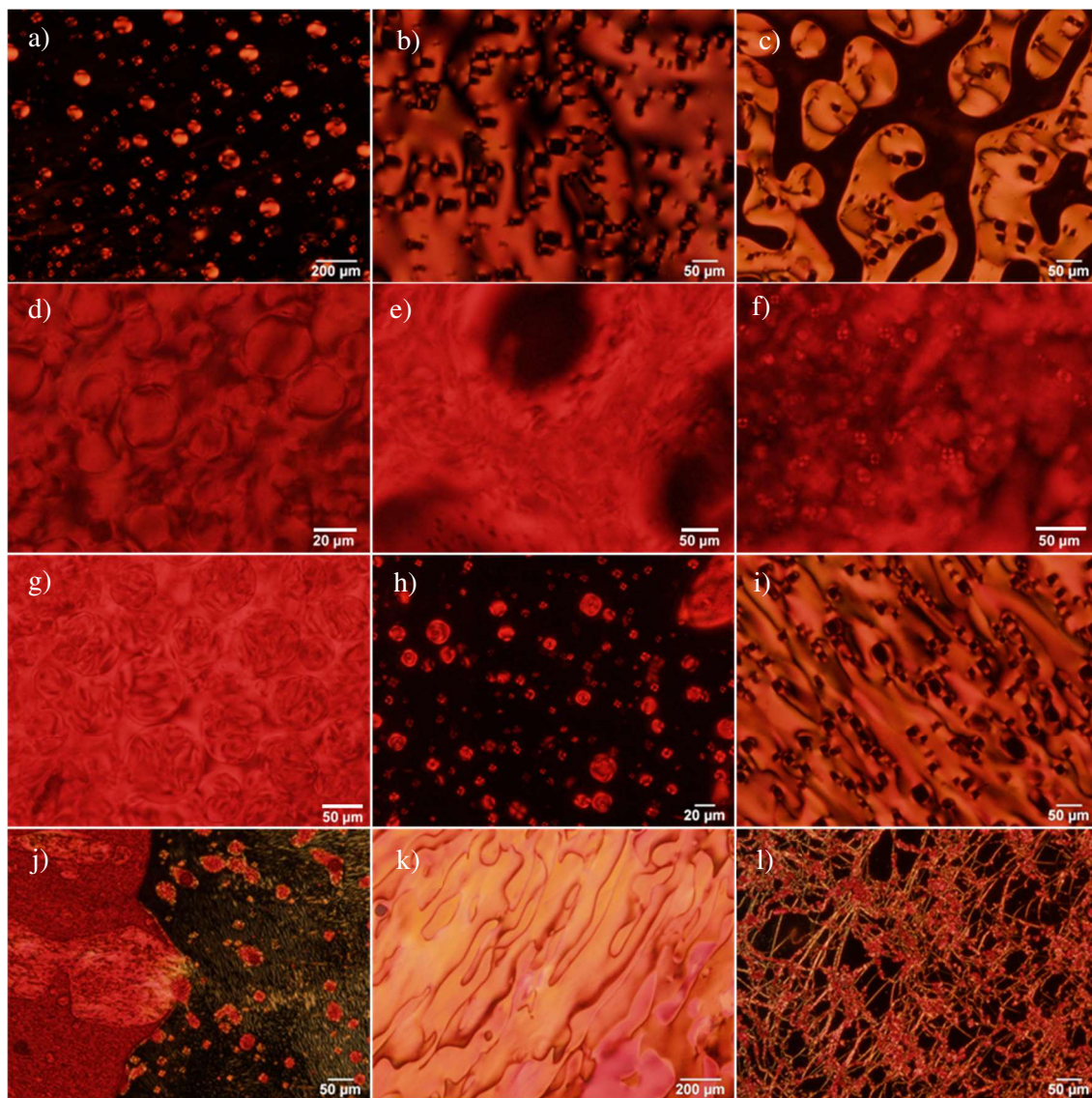


Figure 4.31. POM images at ca. 20 °C of a) MeCatPDI_Em23, b) MeCatPDI_Em30, c) MeCatPDI_Em27, d) MeCatPDI_Em42 at ca. 20 °C, e) 50 °C, f) while cooling down to room temperature and g) at ca. 20 °C after one heating/cooling cycle, h) MeCatPDI_Em54, i) MeCatPDI_Em59, j) MeCatPDI_Em56, k) mixture of MeCatPDI with PEG MW 400 in water, at 10 wt% each, and l) mixture of MeCatPDI with PEG MW 6000 in water, at 10 wt% each.

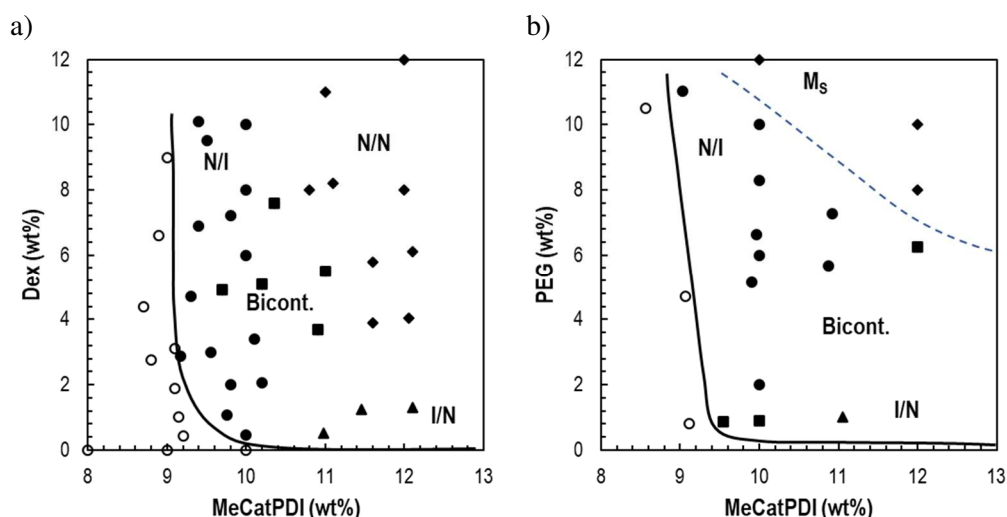


Figure 4.32. Phase diagrams of a) MeCatPDI-Dex and b) MeCatPDI-PEG water-in-water emulsions at ca. 20 °C. White circles (○) represent monophasic samples, black circles (●) are biphasic N/I emulsions, black triangles (▲) are biphasic I/N emulsions, black squares (■) correspond to biphasic bicontinuous emulsions and black diamonds (◆) are N/N or multiphasic in the presence of solids (M_s) emulsions. The black continuous line indicates the separation between monophasic and biphasic regions, meanwhile the dotted line indicates the approximate boundaries between biphasic and multiphasic in presence of solids regions. The boundaries between different biphasic regions (N/I, I/N, N/N and Bicont.) were not drawn because they are prone to experimental error, as sharp transitions from discrete emulsions to bicontinuous emulsions do not exist. These transitions are always gradual and it is difficult to stablish a criterion to differentiate between discrete and bicontinuous emulsions.

Careful observation of the chromonic droplets formed in N/I water-in-water emulsions reveals that the droplet morphology and texture vary depending on the PDI and polymer used, the concentrations of each component and the application of a shearing force. Most droplets adopt spherical shapes in order to minimize the interfacial energy between the two phases of the W/W emulsions. However, applying a shearing force, for example by putting a cover slide for optical microscopy observation, can deform such droplets towards elongated shapes (see Figure 4.29d). In fact, strong shearing forces (i.e. pressing the cover slide with the finger) can disrupt the emulsified nematic droplets making them coalesce and form elongated fibers that slowly return to small spherical droplets after the applied force stops. This indicates a high dependency of the emulsion morphology on the sample preparation and observation, and evidences that the boundaries between discrete emulsions (N/I or I/N) and bicontinuous emulsions are diffuse and hard to define, and there is a significant inherent uncertainty on these boundaries. Such a behavior results from a low interfacial tension, which is typical for W/W emulsions, and a high viscosity of the chromonic nematic phase ^[285,286].

Furthermore, the droplet birefringent textures also varied depending on the polymer and concentrations used. As for bulk liquid crystals, the birefringence within a chromonic droplet results from the formation of anisotropic aggregates within the droplet. However, the droplet's confined volume inside a curved interface causes the formation of defects within the nematic phase, which are manifested as unique chromonic textures that do not appear for bulk liquid crystals ^[287–289]. As described in the Chapter 1 (Section 1.6), chromonic confined droplets typically adopt radial or bipolar configurations, although more complex structures can be formed. The adoption of one or another configuration depends on the interplay between the interfacial tension, the preferred anchoring and the elastic splay, bend and twist deformations.

MeCatPDI-Dex biphasic systems revealed nematic droplets with a dark point in the middle of a Maltese cross birefringent texture (Figure 4.33a), which corresponds to a radial configuration of the director vector. However, as the droplet diameter increased, the topological defect moved

towards the droplet's surface (Figure 4.33b) and finally two topological defects at opposite poles where formed, suggesting a bipolar configuration (Figure 4.33c). This behavior was attributed to the increasing splay deformations that radial configurations have to adapt as the droplet grows, which may be more energetically expensive than moving to a bipolar configuration where the bend deformations are less radius-dependent.

MeCatPDI-PEG biphasic systems also showed small nematic droplets with radial configurations (Figure 4.33d). However, instead of evolving to bipolar configurations as the diameter increased, the droplets showed a birefringent texture with a single topological defect point in the middle with a loop line defect surrounding it (Figure 4.33e) for diameters about 10 – 20 μm . This behavior indicates the formation of twisted nematic self-assemblies retaining the radial configuration^[290]. More complex structures were formed for droplets with diameters above 20 μm , in which spiraling disclinations were observed (Figure 4.33f). This suggests that the PEG not only separates out the nematic chromonic phases forming W/W emulsions but also favors a homeotropic anchoring over a planar anchoring even for bigger droplets and induces the formation of chiral nematic phases for undoped achiral mesogens. We hypothesize that the PEG adsorbs on the nematic droplet's interface and favors both homeotropic anchoring and twisting. The formation of chiral twisted structures for achiral mesogens in confined spherical volumes has been previously described^[291], and is considered to result from a lower twisting elastic deformation constant as compared to bend and splay deformation constants, which is typical of chromonic mesophases^[292–294]. Therefore, the twisting deformations appear to relax the energetically expensive splay and bend deformations.

Furthermore, (Ala)₂PDI-PEG N/I biphasic systems revealed a very rich variety of droplet configurations which depended on the PEG concentration used. For instance, the emulsion with lower PEG content (4.2 wt%) mostly showed bipolar configuration even for the smaller droplets (Figure 4.33g). In contrast, increasing the PEG content to 8.4 wt% generated droplets with a topological single centered defect and a loop line defect, suggesting the formation of twisted nematic self-assemblies (Figure 4.33h), and bigger droplets with spiraling disclinations (Figure 4.33i), similar to those obtained in MeCatPDI-PEG N/I emulsions. At even higher PEG concentrations (12.5 wt%), mostly small achiral radially oriented nematic droplets were formed (Figure 4.33j), but some chiral droplets with bigger diameters also appeared (Figure 4.33k). These results confirm that the PEG tends to stabilize radial configurations over bipolar configurations. In fact, PEG has already been described to favor homeotropic anchoring for discotic thermotropic liquid crystals^[295]. Additionally, PEG seems to facilitate the formation of twisted chiral structures within droplets with medium diameters (5 – 20 μm) for both chiral and achiral mesogens, but not for smaller and bigger droplets. This was attributed to the fact that too small droplets probably forbid the formation of helical structures as the droplet diameter is smaller than the pitch distance (i.e. the distance required for the director vector perform a 2π rotation). In contrast, too big diameters tend to yield droplets with random non-defined textures (Figure 4.33l), as the confining effects are less significant.

In summary, both (Ala)₂PDI and MeCatPDI chromonic perylene diimides phase separate in presence of nonionic polymers such as Dex and PEG. However, the type of W/W emulsion formed (either N/I or I/N) and the droplet director vector configuration (radial, bipolar, twisted) highly depend both in the PDI and polymers used, and the concentration of each component. We observed that increasing the PEG concentration favors the homeotropic anchoring of the nematic assemblies at the droplet interface forming radially structured droplets, and it can induce the formation of twisted chiral assemblies even for the achiral MeCatPDI-rich nematic droplets. Besides, only I/N emulsions were obtained for (Ala)₂PDI with Dex, meanwhile a big variety of

W/W emulsions (including nematic-in-nematic or N/N) were observed for MeCatPDI-Dex biphasic systems.

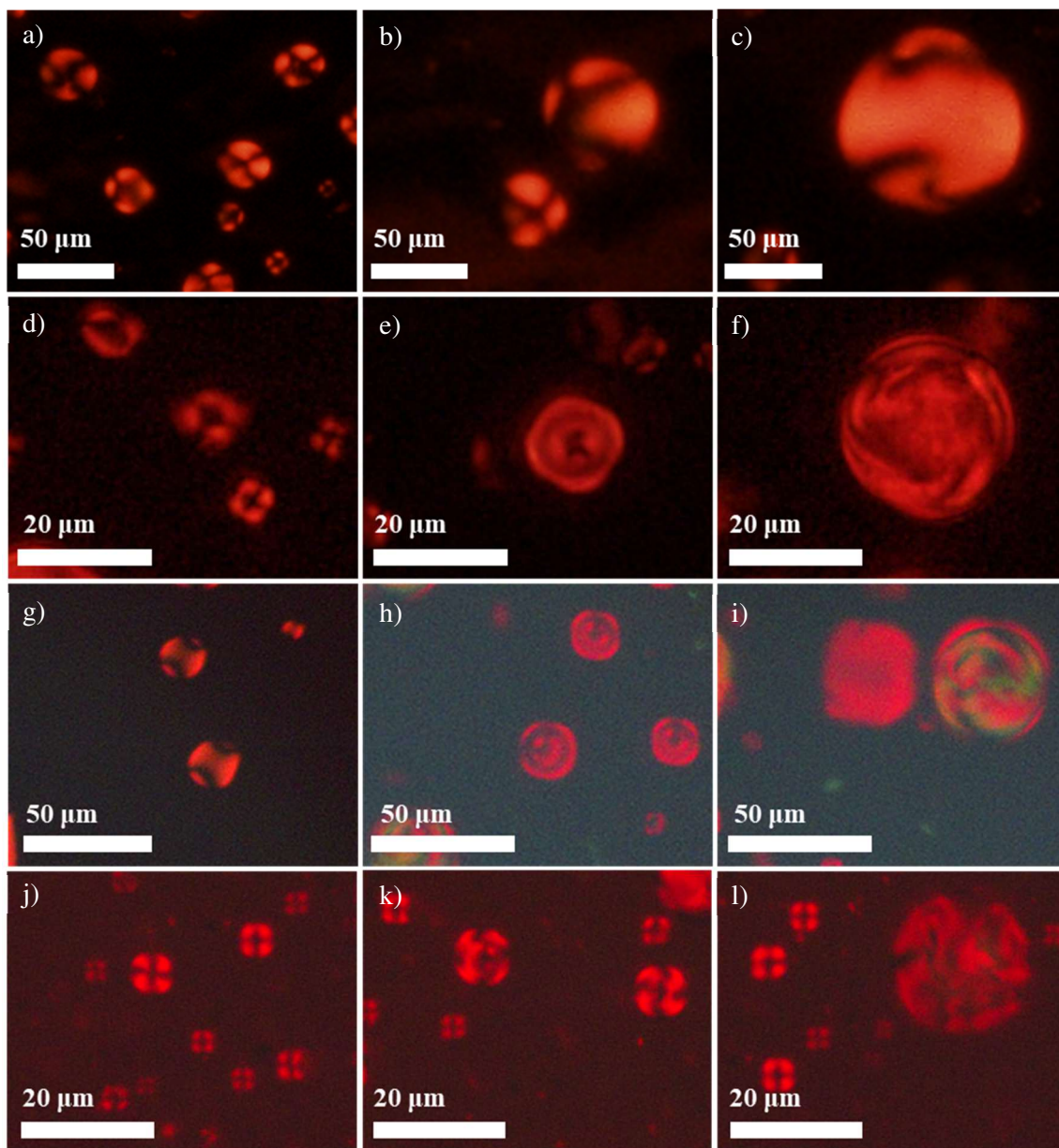


Figure 4.33. POM images of nematic droplets for a – c) MeCatPDI_Em23 (10 wt% MeCatPDI, 10 wt% Dex and 80 wt% H₂O), d – f) MeCatPDI_Em54 (10 wt% MeCatPDI, 8.3 wt% PEG and 81.7 wt% H₂O), g) (Ala)₂PDI_Em6 (12.5 wt% (Ala)₂PDI, 4.2 wt% PEG and 83.3 wt% H₂O), h,i) (Ala)₂PDI_Em7 (12.5 wt% (Ala)₂PDI, 8.4 wt% PEG and 79.1 wt% H₂O), and j – l) (Ala)₂PDI_Em8 (12.5 wt% (Ala)₂PDI, 12.5 wt% PEG and 75.0 wt% H₂O).

4.2.4 Formulation of chromonic liquid crystal triphasic emulsions

Water-in-water emulsions offer a straightforward method for creating triphasic systems by combining these emulsions with a third phase that is immiscible with both aqueous phases. This can be accomplished by introducing either an oil ^[296], an aqueous polymer solution that are mutually immiscible (at least partially) and thus two liquid phases separate and form W/W emulsion ^[196], or a polyelectrolyte aqueous solution that gels the W/W emulsion ^[297].

Triphasic systems are often formed by a continuous phase (P_A) with two dispersed phases (P_B and P_C). These triphasic systems can display a wide range of different droplet morphologies, that can

be classified into complete engulfing, partial engulfing and non-engulfing (Figure 4.34a) [298,299]. The formation of one or another morphology depends on an interplay between the volume fraction of each phase and the interfacial tension between the three phases (γ_{AB} , γ_{AC} and γ_{BC} , where γ_{AB} and γ_{AC} are the interfacial tensions between the dispersed phases P_B and P_C with the continuous phase P_A , respectively, and γ_{BC} is the interfacial tension between the two dispersed phases). Higher interfacial tension means that the formation of an interface between the two phases is more energetically expensive. The composite droplets will adopt the morphology with minimum energy, which implies that the area of higher energy interfaces will decrease until the overall interfacial energy reaches the minimum. Thus, the relation between γ_{AB} , γ_{AC} and γ_{BC} can help to predict the morphology of the composite droplets.

For example, if $\gamma_{BC} \gg \gamma_{AB}$ and $\gamma_{BC} \gg \gamma_{AC}$, no engulfing will take place, as the formation of interface between the two dispersed phase is energetically unfavorable. In contrast, if $\gamma_{AC} \gg \gamma_{BC}$ and $\gamma_{BC} > \gamma_{AB}$, the formation of an interface between the dispersed phase P_C and the continuous phase P_A will be much less favored than the formation of an interface between P_B and P_A , and a complete engulfing of P_C into P_B will take place, forming a P_C -in- P_B -in- P_A emulsion and giving rise to core-shell droplets. Partially engulfed systems will appear in particular cases where either $\gamma_{AB} \approx \gamma_{AC} \approx \gamma_{BC}$ or $\gamma_{AB} \gg \gamma_{BC}$ and $\gamma_{AC} \gg \gamma_{BC}$. In cases where the three interfacial tensions are similar, the formation of snowman-like morphologies will be favored, and the contact angles θ_B and θ_C (Figure 4.34b) will vary as a function of small changes in the interfacial tensions. In contrast, if $\gamma_{AB} \gg \gamma_{BC}$ and $\gamma_{AC} \gg \gamma_{BC}$, the system will form Janus droplets, in which the two dispersed phases ideally occupy equal volumes of a sphere, and with $\theta_B \approx \theta_C \approx 90^\circ$. The formation of Janus droplets is of particular interest for the formation of Janus particles, which are typically hard to obtain [300,301] but result very useful in many applications such as emulsion stabilization [302], drug-delivery [303], nanorobotics [304] or SERS sensing [305].

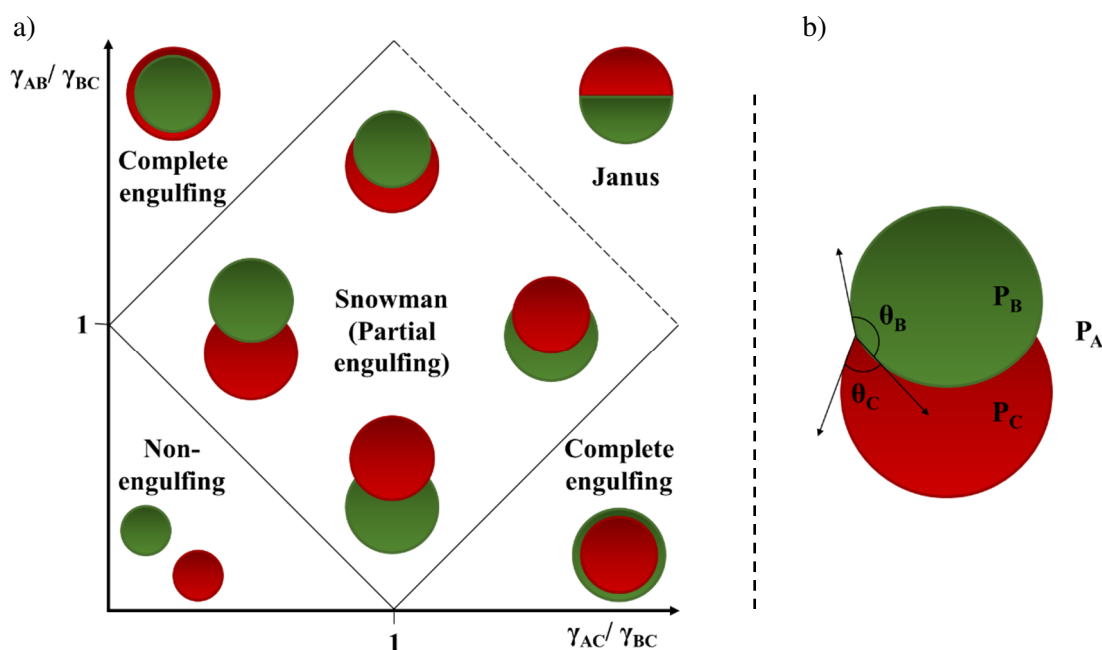


Figure 4.34. a) Diagram of the most favorable drop morphology depending on the ratios between the interfacial tension of each dispersed phase with the continuous phase (γ_{AB} and γ_{AC}) with the interfacial tension between the two dispersed phases (γ_{BC}). b) Scheme of a partial engulfing droplet with the contact angles θ_B and θ_C . Figure adapted from Guzowski et al. [298]

To achieve the required interfacial tensions to form Janus droplets, a practical method is to emulsify a W/W emulsion in an oil phase, as the interfacial tensions between aqueous phases are

much lower than in oil-water interfaces ^[186,306]. Therefore, it is expected for $\gamma_{AB} \gg \gamma_{BC}$ and $\gamma_{AC} \gg \gamma_{BC}$, being P_A the oil phase and P_B and P_C the two aqueous phases. Accordingly, we prepared a three-phase W/W/O emulsion dispersing a (Ala)₂PDI 12.5 wt% – PEG MW 10,000 8.4 wt% W/W emulsion in hexadecane with Span 80 as the surfactant, to a total concentration of 85.5:4.5:10 for hexadecane / Span 80 / W/W emulsion, as described in Section 3.3.6. The obtained aqueous dispersed droplets had spherical shapes and were composed by two phases with partial engulfing (Figure 4.35a,b), resulting in Janus droplets.

However, in several droplets, the birefringent phase seemed to partially engulf the isotropic PEG-rich phase. For example, in Figure 4.35c, two droplets in which the birefringent phase partially engulfs the isotropic phase, showing a moon-like shape with high curvature, are shown. It is important to note that the droplets observed by optical microscopy and 2D projections of spherical tridimensional structures, and hence the interfacial curvature of the two phases forming the droplet can vary depending on the orientation of the droplet (see Figure 4.35d,e). Despite this, the high curvature exhibited by multiple droplets of the W/W/O emulsion can only be explained by a partial engulfing of the isotropic phase by the birefringent phase (see Figure 4.35e).

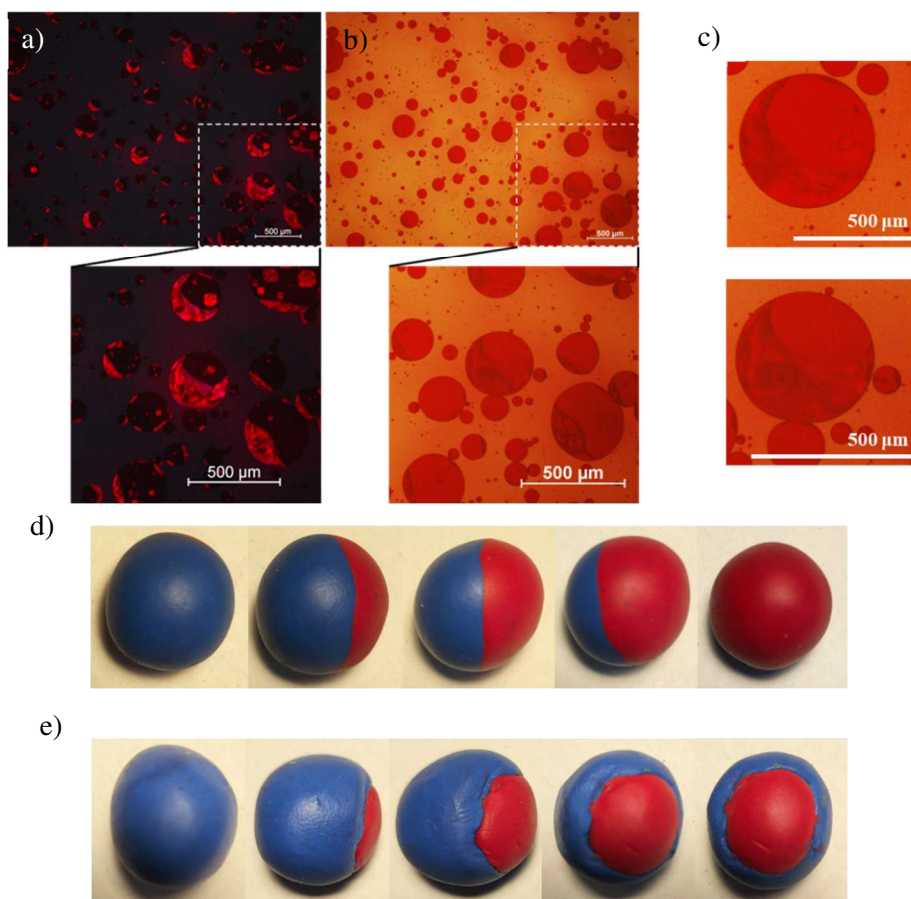


Figure 4.35. Janus droplets formed in aq. (Ala)₂PDI – aq. PEG MW 10,000 – hexadecane W/W/O emulsions at ca. 20 °C a) with crossed polarizers and b) without crossed polarizers. c) Two selected Janus droplets. d) Different views of a model 3D perfect Janus droplet depending on the orientation. e) Different views of a model 3D Janus droplet with a phase (blue) partially engulfing the other (red), depending on the orientation. These models were designed with polymer clay.

This behavior is surprising considering that the birefringent (Ala)₂PDI-rich phase is the dispersed phase in W/W emulsions formed at the (Ala)₂PDI and PEG concentrations used herein, and hence has a lower volume fraction than the PEG-rich phase. This suggests a lower interfacial tension between the (Ala)₂PDI-rich aqueous phase with the oil phase than for the PEG-rich phase with

the oil phase. Despite the success in obtaining Janus droplets, these W/W/O emulsions were highly unstable, and phase separated within few minutes.

Interestingly, applying a shearing force to the W/W/O emulsion showed in Figure 4.35 induced the formation of smaller aqueous droplets with an increased engulfing of the PEG-rich phase into the (Ala)₂PDI-rich phase, forming droplets that appear in POM as isotropic cores with birefringent shells (Figure 4.36). Such droplets could either be Janus (with partial engulfing, see Figure 4.35e) or core-shell, in which there is a complete engulfing of the isotropic phase into the birefringent one. This confirms that the interfacial tension between the (Ala)₂PDI-rich aqueous phase and the oil phase is lower than between the PEG-rich aqueous phase and the oil. Furthermore, the formation of core-shell droplets is valuable for applications such as drug delivery ^[307].

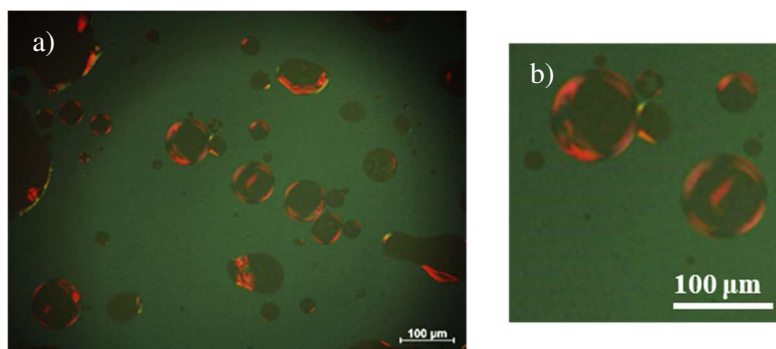


Figure 4.36. a) POM image of the core-shell (or Janus) droplets formed in aq. (Ala)₂PDI – aq. PEG MW 10000 – hexadecane W/W/O emulsions after applying a shearing force to the microscope slide. b) Zoom-in of two selected core-shell (or Janus) droplets.

The formation of three-phase emulsions was further assessed by preparing W/W/W emulsions composed by a PDI-rich nematic, a Dex-rich and a PEG-rich phase. For this, first a biphasic W/W emulsion with the selected PDI (either (Ala)₂PDI or MeCatPDI) and Dex was prepared. Afterwards, PEG was added to the system and homogenized as described in Section 3.3.6. W/W/W emulsions were kept at 25 °C and studied by optical microscopy.

Aqueous-three phase systems composed by (Ala)₂PDI, Dex and PEG were studied at different concentrations of each component. All the mixtures prepared with (Ala)₂PDI at 12.5 wt% did not show triphasic systems; rather, two immiscible phases (one birefringent and one isotropic) forming a biphasic system were observed. Furthermore, these two-phase systems were highly unstable, as the birefringent phase did not appear as small droplets but as liquid-crystalline big droplets resulting from an increased coalescence (Figure 4.37a). In contrast, W/W/W could be obtained using (Ala)₂PDI at 10 wt%. Note that (Ala)₂PDI forms isotropic solutions at this concentration. For example, three-phase systems were obtained by mixing (Ala)₂PDI 10 wt%, Dex 4 wt% and PEG 6 wt% in water (Figure 4.37b,c). This W/W/W triphasic emulsion consisted in spherical Janus biphasic droplets dispersed in an isotropic continuous medium, suggesting that the interfacial tensions between the isotropic continuous and the two phases of the droplets are rather high. By careful observation of the Janus droplets of Figures 4.37b,c, it can be seen how the birefringent phase occupies most of the volume, but the isotropic phase is partially engulfing it (Figure 4.37d). This behavior is opposed to that of the W/W/O emulsions, where the (Ala)₂PDI partially engulfed the polymer-rich phase. Additionally, the stability of this W/W/W emulsion was studied. We observed phase separation after 1 day at 25 °C, since the droplet structure was lost and a bicontinuous isotropic emulsion with chromonic droplets dispersed in one of the isotropic phases was formed (Figure 4.37e,f).

These results indicate that the formation of W/W/W multiple emulsions is possible for (Ala)₂PDI–Dex–PEG systems but limited to only few compositions, as increasing the (Ala)₂PDI

concentration disrupts the formation of three-phase composite droplets, forming unstable biphasic W/W emulsions instead. We hypothesize that the continuous phase for the W/W/W emulsion is PEG-rich and the small dispersed isotropic phase is Dex-rich, and that the dextran distributes in both the PEG-rich and the (Ala)₂PDI-rich phases, as it is partially miscible with both (Ala)₂PDI (Table 4.7) and PEG^[283] at concentrations about 5 wt%.

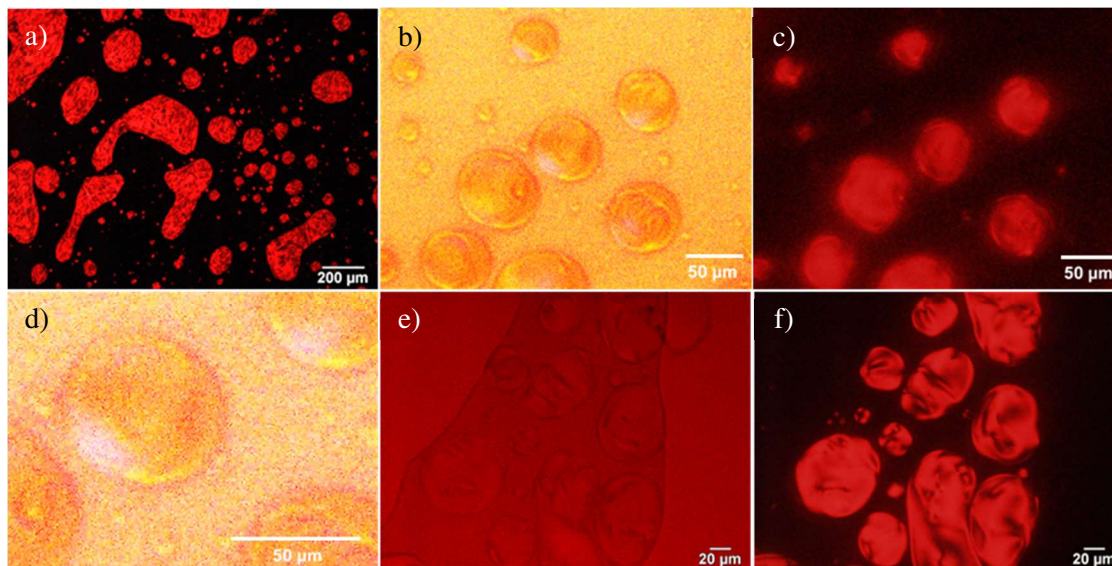


Figure 4.37. a) POM image of a (Ala)₂PDI 12.5 wt%, Dex 4 wt% and PEG 6 wt% mixture in water. b) Image without and c) with crossed polarizers of a (Ala)₂PDI 10 wt%, Dex 4 wt% and PEG 6 wt% W/W/W emulsion, d) Zoom-in of a selected droplet of the emulsion. e) Image without and f) with crossed polarizers of a (Ala)₂PDI 10 wt%, Dex 4 wt% and PEG 6 wt% W/W/W emulsion after 24 h. All images were taken at ca. 20 °C.

Determining which is the Dex-rich and the PEG-rich isotropic phase in W/W/W would be highly desirable to get a clearer picture of the prepared W/W/W emulsions. For this, we tried to functionalize the dextran with fluorescein isothiocyanate (FITC), and detect its location with fluorescence optical microscopy. However, despite such functionalization being useful to discern between Dex and PEG in the case of biphasic Dex/PEG W/W emulsions, it failed in our triphasic systems, as no fluorescence could be observed. Probably, the high absorbance of the (Ala)₂PDI within the visible range causes an almost complete reabsorption of FITC fluorescence (green emission at 519 nm of wavelength) making the functionalized polymer invisible in fluorescence microscopy. Rhodamine (red emission at approximately 595 nm) was also used, but no fluorescence could be detected.

Alternatively, we used Raman microscopy to try to discern between Dex-rich and PEG-rich phases. Raman microscopy is a technique in which a laser beam is focused on a sample, and the scattered light is collected and analyzed to produce a Raman spectrum. This spectrum provides information on the chemical composition of the analyzed area, allowing to characterize the chemical composition in micrometric regions of a sample.

Raman microscopy experiments started with the analysis of (Ala)₂PDI, Dex and PEG in the solid state (Figure 4.38a-c), the laser with 532 nm wavelength was chosen, and the power was set at 2 mW. (Ala)₂PDI showed three intense peaks at 1294, 1377 and 1577 cm⁻¹ (Figure 4.38a). On the other hand, Dex and PEG only showed small peaks at low Raman shifts and a broad band at ~2900 cm⁻¹ (Figure 4.38b,c). Note that the graphs are normalized so the maximum stays at “1” of

intensity, in the raw data, the most intense peaks of Dex and PEG are ca. 1/3 of the most intense peak of (Ala)₂PDI.

The differences between PEG and Dex in Raman microscopy were small due to their similar C–C, C–H and C–O bonds. Despite this, they could be distinguished in the range between 850 and 900 cm⁻¹ and between 1300 and 1500 cm⁻¹, where Dex showed a doublet of peaks meanwhile PEG presented a single peak instead. Also, there were some differences in the Raman shift for the band around 2900 cm⁻¹, where PEG showed a band that could be identified as three overlapped peaks meanwhile Dex presented a single broad band.

The Raman microscopy spectra of each compound dissolved in water at concentrations around 10 wt% were also obtained. As expected, the spectra were significantly different to those observed in the solid state (Figure 4.38d-f). First, the background signal (coming mostly from the cover glass slide) was much more intense in relation to the material peaks, probably due to the lower concentration of the samples when dissolved in water (~10 wt% vs the 100 wt% in the solid state). Because of this background, both PEG and Dex were invisible using the laser at 532 nm at 2 mW, meanwhile for (Ala)₂PDI only the three major peaks appear (Figure 4.38d). Besides, (Ala)₂PDI presented a broad intense band between 2500 and 3500 cm⁻¹, attributed to the perylene photoluminescence.

For the Dex and PEG signals to be detectable in 10 wt% water solutions by Raman microscopy, the laser power had to be increased to 20 mW (Figure 4.38e,f). Even at such high power, the polymer Raman peaks were very small and hard to detect. Dex and PEG 10 wt% also showed a broad band at > 3000 cm⁻¹, which was attributed to glass photoluminescence^[308].

After obtaining the Raman spectra of (Ala)₂PDI, PEG and Dex in water, Raman microscopy was tested as a technique to identify each phase in W/W emulsions formed by the three mentioned compounds. In this context, the Raman microscopy of (Ala)₂PDI_Em4 (12.5 wt% (Ala)₂PDI and 8.4 wt% Dex) and (Ala)₂PDI_Em7 (12.5 wt% (Ala)₂PDI and 8.4 wt% PEG) of Table 4.7 and an emulsion formed PEG and Dex 10 wt% each, were determined (Figure 4.38g-i). Remarkably, the phases in the Dex/PEG emulsion could be easily identified with the laser at 10 mW or more, observing that the continuous phase is PEG-rich and the dispersed one is Dex-rich (Figure 4.38i), which was confirmed by optical microscopy using fluorescently-labelled Dex. This is interesting since it allows the identification of phases in all-aqueous emulsions without labelling one of the phases with fluorescent compounds, which could be useful in systems where labelling is not possible. Similarly, gelatin-maltodextrin aqueous two-phase system phases have already been identified by Raman spectroscopy^[198].

In contrast, W/W emulsions formed with the (Ala)₂PDI could not be analyzed by Raman microscopy since the photoluminescence and high intensity of the perylene Raman peaks eclipsed the Dex and/or PEG signals; and hence only (Ala)₂PDI was observed in both the birefringent and the isotropic phases (Figure 4.38g,h). Note that the phases are identified as dispersed/continuous for the Dex-PEG system and as birefringent/isotropic for the emulsions with (Ala)₂PDI, because the (Ala)₂PDI-rich birefringent phase can be either the dispersed or the continuous phase depending on the polymer used (see Table 4.7). Moreover, the laser power used in the (Ala)₂PDI-containing emulsions was much lower than that used for Dex-in-PEG emulsions, since laser power higher than 2 mW for (Ala)₂PDI surpassed the limits of the detection system, because of the photoluminescence. Note that at 2 mW the Dex and PEG in water signals are not visible (Figure 4.38e,f). In conclusion, neither fluorescence labelling nor Raman microscopy are good approaches to identify each phase in PDI-based chromonic W/W and W/W/W emulsions.

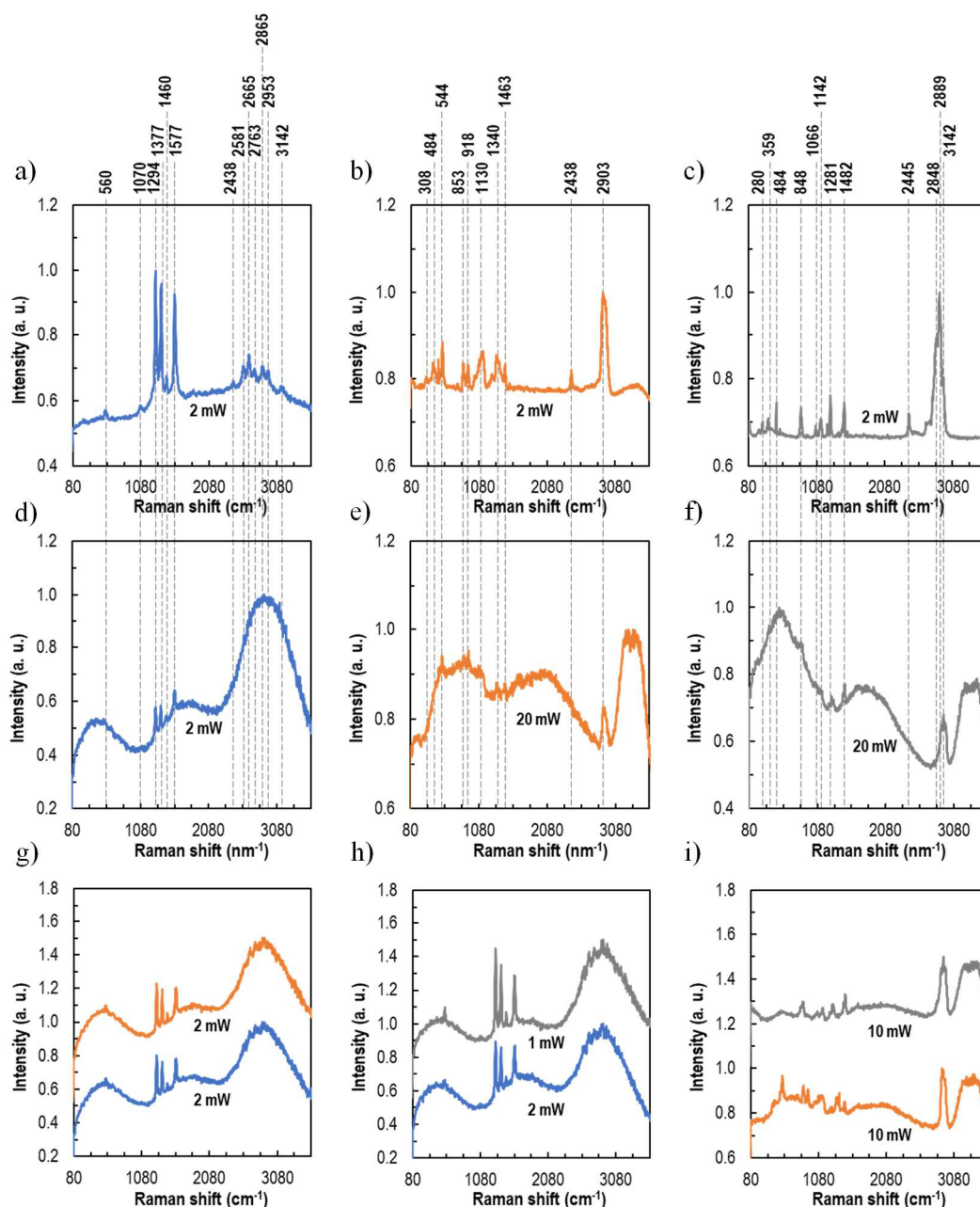


Figure 4.38. Raman microscopy of a) solid $(\text{Ala})_2\text{PDI}$, b) solid Dex, c) solid PEG, d) $(\text{Ala})_2\text{PDI}$ 12.5 wt% water solution, e) Dex 10 wt% water solution, f) PEG 10 wt% water solution, g) $(\text{Ala})_2\text{PDI}$ 12.5 wt% and Dex 8.4 wt% W/W emulsion. The orange curve corresponds to the isotropic Dex-rich phase and the blue to the nematic $(\text{Ala})_2\text{PDI}$ -rich phase. h) $(\text{Ala})_2\text{PDI}$ 12.5 wt% and PEG 8.4 wt% W/W emulsion. The grey curve corresponds to the isotropic PEG-rich phase and the blue to the nematic $(\text{Ala})_2\text{PDI}$ -rich phase. i) Dex 10 wt% and PEG 10 wt% W/W emulsion. The orange curve corresponds to the dispersed Dex-rich phase and the blue to the continuous PEG-rich phase.

In contrast to $(\text{Ala})_2\text{PDI}$, MeCatPDI showed a much better capacity to form W/W/W emulsions with Dex and PEG, as several triphasic systems were obtained by fixing the MeCatPDI concentration at ~ 10 wt% and varying the polymer concentrations (Table 4.10). This may result from the reduced hydrophilicity of the cationic MeCatPDI compared to the anionic $(\text{Ala})_2\text{PDI}$, which makes the former less soluble in the polymer-rich phases and favors phase separation. It was observed that aqueous three-phase systems were obtained for Dex and PEG concentrations above 3 wt% each, and up to 8 wt% each (higher polymer concentrations were not tested as the solution became too viscous). Furthermore, several droplet morphologies were formed, including

snowman, Janus and core-shell (Figure 4.39), depending on polymers' concentration. The emulsion with the lowest polymer concentrations (3.8 wt% of both Dex and PEG) was composed by almost spherical nematic droplets with various isotropic droplets adsorbed at their surface (Figure 4.39a). Notably, by increasing the PEG concentration to 5.7 wt% (Figure 4.39b), the isotropic dispersed phase swelled and partially engulfed the nematic droplets, forming a snowman drop disposition in which $\theta_C > \theta_B$, considering the PDI-rich phase as P_C and the isotropic phase as P_B . In contrast, adding Dex up to 5.8 wt% to the emulsion of Figure 4.39a induced the partial engulfing of the isotropic droplets within the nematic phase, forming snowman droplets with $\theta_B > \theta_C$ (Figure 4.39c). This suggests that the continuous phase is Dex-rich and the isotropic dispersed phase is PEG-rich at these compositions. However, this could not be confirmed experimentally. Further increasing the Dex concentration up to 7.7 wt% favored the engulfing of the isotropic droplet forming droplets appearing in POM as isotropic cores with nematic shells (Figure 4.39d), which can either indicate the formation of core-shell or Janus with partial engulfing droplets.

Furthermore, Janus droplets were formed by increasing simultaneously the Dex and PEG concentrations to 4.7 wt% each (Figure 4.39e) and 5.7 wt% each (Figure 4.39f). Notably, the Janus droplets became more spherical at higher polymer concentrations, suggesting that the interfacial tension between the continuous phase and the two dispersed phases increase by increasing the amount of Dex and PEG.

Table 4.10. Composition of the water-in-water-in-water (W/W/W) emulsions formed by nematic MeCatPDI solutions with Dex and PEG. *These emulsions consist in in systems similar to those of Figure 4.39g,h.

Sample name	[MeCatPDI] (wt%)	[Dex] (wt%)	[PEG] (wt%)	[H ₂ O] (wt%)	Phases	Droplet morphology
MeCatPDI_Em63	9.8	5.9	2.0	82.3	2	-
MeCatPDI_Em64	9.8	7.8	2.0	80.4	2	-
MeCatPDI_Em65	9.6	1.9	3.8	84.7	2	-
MeCatPDI_Em66	9.6	3.8	3.8	82.8	3	Snowman
MeCatPDI_Em67	9.6	4.8	3.8	81.8	3	Snowman
MeCatPDI_Em68	9.6	5.8	3.8	80.8	3	Snowman
MeCatPDI_Em69	9.6	6.7	3.8	79.9	3	Snowman
MeCatPDI_Em70	9.6	7.7	3.8	78.9	3	Janus
MeCatPDI_Em71	9.5	2.9	4.8	82.8	3	Snowman
MeCatPDI_Em72	9.5	3.8	4.8	81.9	3	Snowman
MeCatPDI_Em73	9.5	4.8	4.8	80.9	3	Janus
MeCatPDI_Em74	9.5	5.7	4.8	80.0	3	Janus
MeCatPDI_Em75	9.5	6.7	4.8	79.0	3	Janus
MeCatPDI_Em76	9.5	7.6	4.8	78.1	3	Janus
MeCatPDI_Em77	9.5	8.6	4.8	77.2	3	Janus
MeCatPDI_Em78	9.4	3.8	5.7	81.1	3	Snowman
MeCatPDI_Em79	9.4	5.7	5.7	79.2	3	Janus
MeCatPDI_Em80	9.4	7.5	5.7	77.4	3	Other*
MeCatPDI_Em81	9.3	3.7	7.4	79.6	3	Other*
MeCatPDI_Em82	9.3	5.6	7.4	77.7	3	Other*
MeCatPDI_Em83	9.3	7.4	7.4	75.9	3	Other*

Further increasing the concentration of both polymers significantly reduces the emulsion stability, forming complex multiple W/W/W emulsions that rapidly coalesce and phase separate (Figure 4.39g,h). Notably, these multiple emulsions apparently consist in nematic-in-nematic-in-isotropic emulsions, which could indicate that N/N dispersed phases appear at high Dex or PEG concentrations.

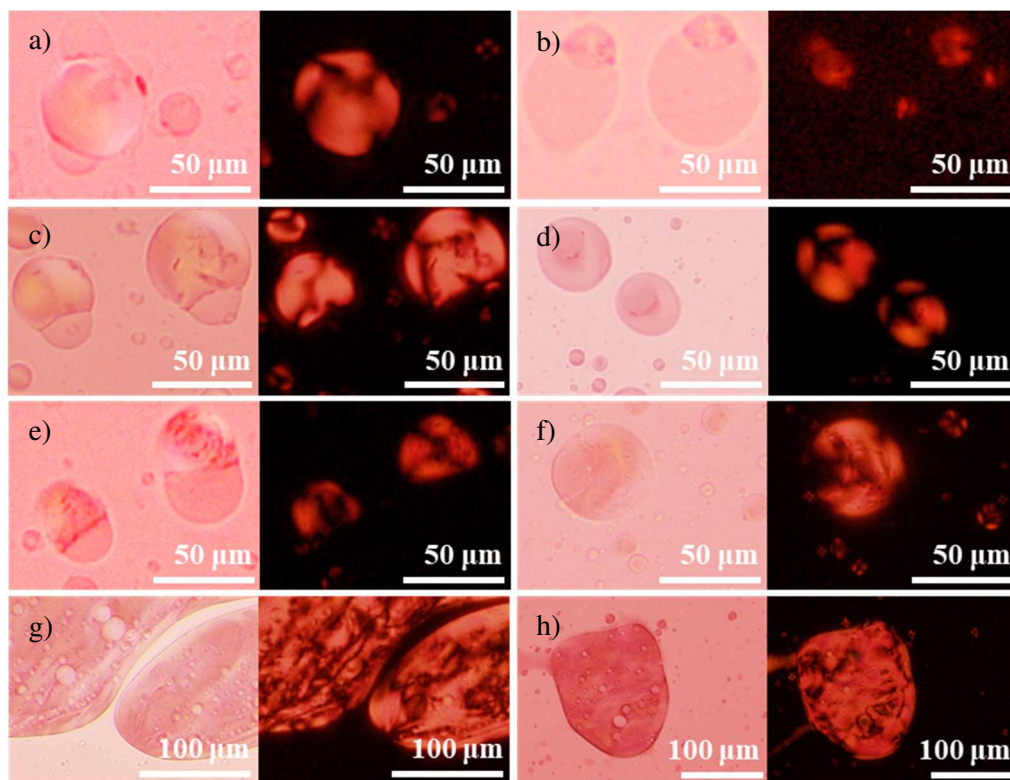


Figure 4.39. Bright-field (left) and POM (right) images of the droplets formed in the following W/W/W emulsions: a) MeCatPDI_Em66, b) MeCatPDI_Em78, c) MeCatPDI_Em68, d) MeCatPDI_Em70, e) MeCatPDI_Em73, f) MeCatPDI_Em79, g) MeCatPDI_Em80 and h) MeCatPDI_Em81. Different morphologies depending on the polymer concentration can be observed, including snowman and Janus droplets.

With the data in Table 4.10, a partial ternary phase diagram of the W/W/W emulsions was drawn (Figure 4.40). Note that the upper vertex corresponds to the sum of the MeCatPDI and water concentrations and that, considering that the MeCatPDI was almost constant for all emulsions tested, changes in the two upper axis imply mean variations in the water content of the emulsion. The phase diagram shows the typical behavior of a segregative system in which a quasi-symmetric binodal curve separates the two- and the three-phase regions ^[22].

In summary, both (Ala)₂PDI and MeCatPDI exhibited a complex biphasic and triphasic behavior in presence of polymers in aqueous solution and oils. Interestingly, gelling or polymerizing these complex droplets can potentially provide an effective method to produce complex microparticles with multiple compartments in all-aqueous environment. In the following section, the gelation of (Ala)₂PDI chromonic mesophases will be discussed.

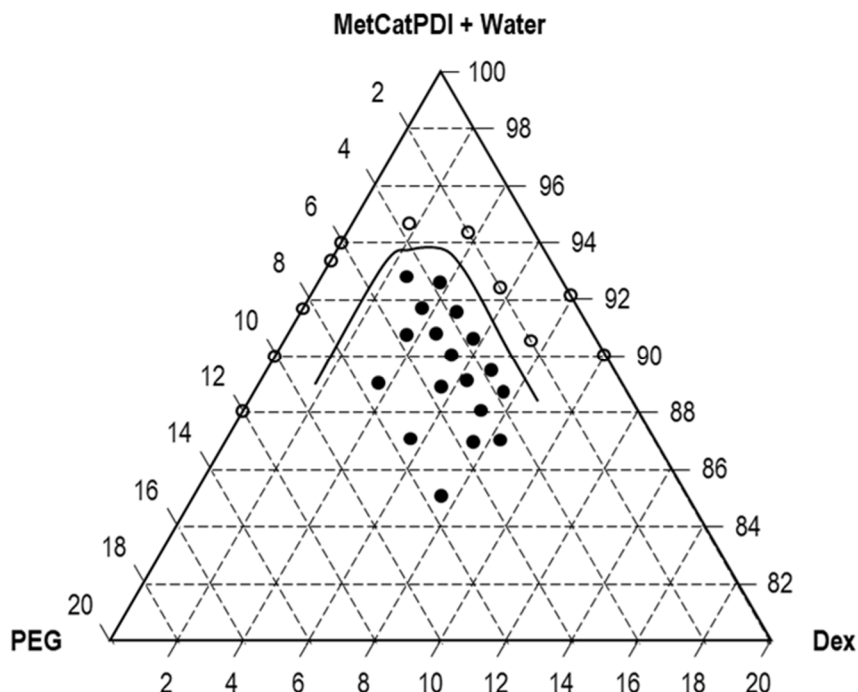


Figure 4.40. Triangular phase diagram of the MeCatPDI-Dex-PEG W/W/W emulsions. Note that one vertex corresponds to the sum of the MeCatPDI and water concentrations. Considering that the MeCatPDI concentration was maintained almost constant, different points of this axis mean different water content. White circles (○) represent monophasic or biphasic systems and black circles (●) are triphasic systems. The black curve corresponds to the approximate binodal curve, indicating the separation between the triphasic and the mono and biphasic systems.

4.2.5 Preparation of chromonic low molecular weight hydrogels

Motivated not only by the rich chromonic behavior of (Ala)₂PDI, but also by its excellent capacity to form biphasic W/W and triphasic W/W/W and W/W/O emulsions, gelation experiments were carried out aiming to form anisotropic PDI-based hydrogels. In the literature, the formation of chromonic PDI-based hydrogels has already been described using a methacrylate functionalized PDI [148,149]. However, (Ala)₂PDI has no polymerizable groups, hence alternative gelation pathways should be explored.

Despite not being polymerizable, (Ala)₂PDI has already been reported to gel at 1 wt% in water by adding glucono- δ -lactone (GdL), which slowly hydrolyzes to gluconic acid reducing the pH of the solution and gelling the (Ala)₂PDI solution [158], hence forming fibrous agglomerates that retain the solvent (water) within their structure, forming the gel⁸. These kind of gels are denoted as low molecular weight gels (LMWG). LMWG are materials composed of small molecules that can self-assemble into a gel-like network. Unlike traditional gels, which are often composed of large crosslinked polymers or proteins, LMWGs are made up of relatively small molecules with molecular weights below 10000 g/mol [309]. Moreover, LMWGs are typically stimuli-responsive, as the self-assembled molecules are not covalently bound and hence are more sensitive to external stimuli such as changes in temperature, pH, ionic strength, the presence of specific additives or

⁸ It is important to note that alanine ($pK_a \approx 2.4$) is more acidic than gluconic acid ($pK_a \approx 3.6$). Consequently, gelation is produced by a lowering on the solution pH and partial protonation of the carboxylic acids of (Ala)₂PDI, rather than by a complete protonation of (Ala)₂PDI.

light exposure ^[310,311]. This makes LMWG promising materials for drug delivery or cell encapsulation and release ^[312].

LMWG are physical gels, formed by weak non-covalent crosslinks. These gels can be based a wide variety of different intermolecular interactions (electrostatic, π - π , hydrogen bonds, dispersion, etc.) ^[309]. There are some general features that are often present in low molecular weight gelators. Namely, they should feature a balance between hydrophobic and hydrophilic groups that facilitates self-assembly, resulting from interactions with both the solvent (usually water) and also with other molecules in the gel network. In particular, the presence of functional groups forming hydrogen bonds or aromatic moieties participating in non-covalent π - π interactions facilitate the gelation ^[313].

Herein, we report the gelation of nematic (Ala)₂PDI solutions and characterize such gels by optical microscopy and SAXS. Gelling lyotropic mesophases is interesting as it permits the preparation of gels that combine the anisotropic structure of the liquid crystal phase with the mechanical properties of a gel, which is interesting for multiple applications from cell culture to silica templating ^[314]. For example, Wang et al. prepared anisotropic polyacrylamide porous hydrogels using DSCG as template which showed an excellent behavior as substrata for cell culture after being functionalized with unpolymerized type I collagen ^[104]. Differently, Dieterich et al. were able to obtain nematic gels from surfactant lyotropic mesophases with promising applications as sensors and actuators ^[315].

The gelation of (Ala)₂PDI chromonic mesophases was achieved by preparing a nematic 12.5 wt% solution in water and adding different amounts of GdL (0.5, 1 and 1.5 eq.). Note that the equivalents (eq.) are determined in terms of proton exchange. For example, the sample with 0.5 eq. of GdL has the same molarity of GdL and (Ala)₂PDI, as (Ala)₂PDI has two basic sites per molecule meanwhile the gluconic acid resulting from the GdL hydrolysis has only one acid site. Also note that (Ala)₂PDI is more acid than GdL, which indicates that putting 1 eq. GdL or more does not mean that all the (Ala)₂PDI is fully protonated. The samples were left to stand overnight for the gelation to occur. The samples were named as (Ala)₂PDI_nGdL, where *n* indicates the equivalents of GdL.

The three (Ala)₂PDI_nGdL samples gelled (Figure 4.41). Interestingly, we compared the gelling properties of the (Ala)₂PDI solutions at 12.5 wt% with those at 1 wt% by preparing (Ala)₂PDI water solutions at 1 wt% and adding 0.5, 1 and 1.5 eq. of GdL. After standing for 24 h, only the sample with 1.5 eq. of GdL gelled, suggesting that the gelation of (Ala)₂PDI water solutions does not only depend on the pH but also on the (Ala)₂PDI concentration.

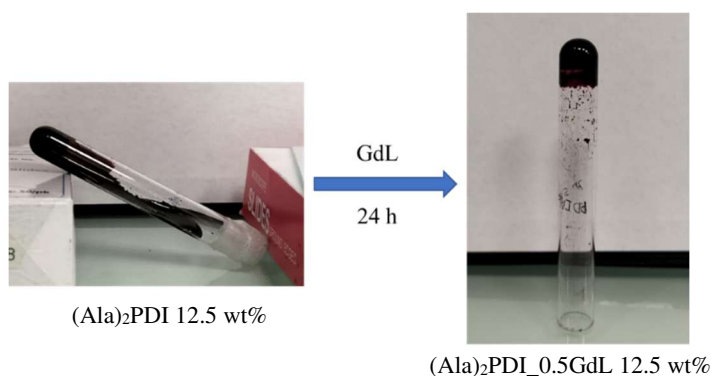


Figure 4.41. Scheme of the preparation of (Ala)₂PDI LMWGs. The right picture is from the gel (Ala)₂PDI_0.5GdL, which does not flow when it is turned upside down.

The $(\text{Ala})_2\text{PDI}_n\text{GdL}$ gels were further characterized by optical microscopy (Figure 4.42). Bright-field microscopy revealed that $(\text{Ala})_2\text{PDI}_{0.5}\text{GdL}$ was composed of micro-sized fibrous aggregates (Figure 4.42a), meanwhile $(\text{Ala})_2\text{PDI}_{1.0}\text{GdL}$ (Figure 4.42b) and $(\text{Ala})_2\text{PDI}_{1.5}\text{GdL}$ (Figure 4.42c) formed amorphous aggregates. Furthermore, POM analysis revealed that the fibrous aggregates of $(\text{Ala})_2\text{PDI}_{0.5}\text{GdL}$ exhibited birefringence (Figure 4.42d), while the empty spaces between them did not. However, both $(\text{Ala})_2\text{PDI}_{1.0}\text{GdL}$ and $(\text{Ala})_2\text{PDI}_{1.5}\text{GdL}$ were practically non-birefringent (Figure 4.42e,f), as the big amorphous aggregates were completely black between crossed polarizers. Therefore, the formation of anisotropic birefringent gels requires concentrations of GdL lower than the stoichiometric ratio.

To further characterize the $(\text{Ala})_2\text{PDI}_{0.5}\text{GdL}$ sample optically, a gel was directly prepared on a microscope slide. A droplet of the $(\text{Ala})_2\text{PDI}$ 12.5 wt% solution in water with 0.5 eq. of GdL was placed onto a microscope slide, sealed with glue, and left to stand for 24 hours to allow the sample to gel within the slide. Afterwards, the sample was observed under the microscope. Bright-field (BF) images showed similar fibrous aggregates as in Figure 4.42a, but with a higher alignment in one direction (Figure 4.43a,b). Additionally, the gel displayed improved birefringence (Figure 4.43c), with uniform birefringence throughout the sample, and small non-birefringent solid regions that likely resulted from the formation of amorphous aggregates during gelation. In fact, POM images of the sample before gelation revealed the formation of non-birefringent precipitates immediately after the addition of GdL (Figure 4.43d), likely in regions with remaining undissolved GdL crystals that generated high gluconic acid concentrations and thus formed non-birefringent precipitates. Also, high-magnification POM images of the $(\text{Ala})_2\text{PDI}_{0.5}\text{GdL}$ gel prepared within the microscope slide showed the presence of single birefringent fibers (Figure 4.43e). This suggests that the improved birefringence of this sample results from the alignment of the fibrous aggregates. Furthermore, the $(\text{Ala})_2\text{PDI}_{0.5}\text{GdL}$ gel retained the high birefringence after 72 h (Figure 4.43f), indicating a high stability of the as-formed hydrogel.

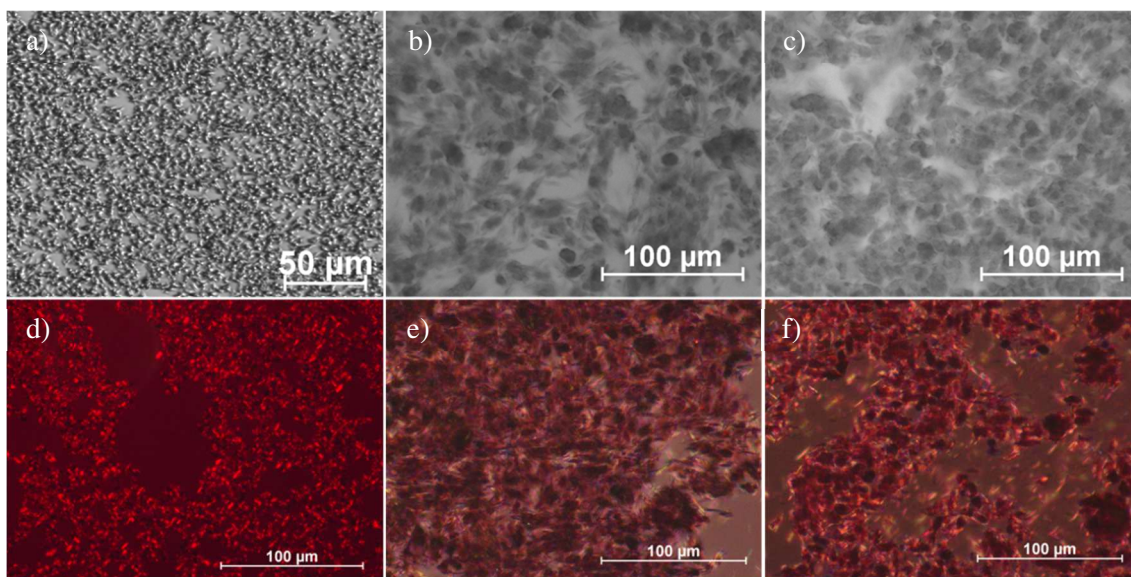


Figure 4.42. Bright field images of a) $(\text{Ala})_2\text{PDI}_{0.5}\text{GdL}$, b) $(\text{Ala})_2\text{PDI}_{1.0}\text{GdL}$ and c) $(\text{Ala})_2\text{PDI}_{1.5}\text{GdL}$. Polarized optical microscopy images of d) $(\text{Ala})_2\text{PDI}_{0.5}\text{GdL}$, e) $(\text{Ala})_2\text{PDI}_{1.0}\text{GdL}$ and f) $(\text{Ala})_2\text{PDI}_{1.5}\text{GdL}$. All pictures were taken at ca. 20 °C.

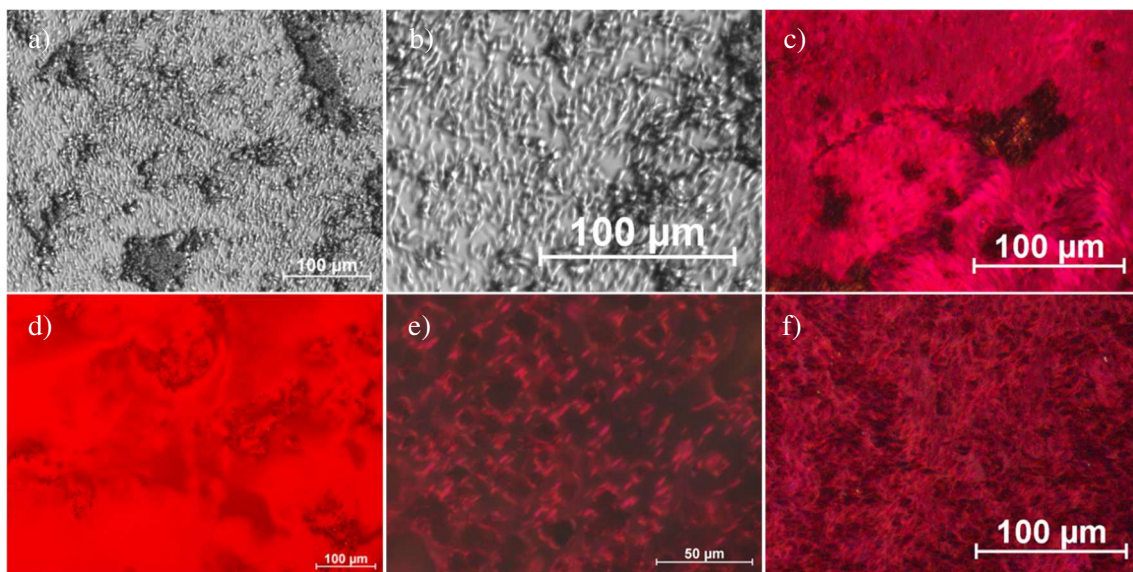


Figure 4.43. Optical microscopy images of $(\text{Ala})_2\text{PDI}_{0.5}\text{GdL}$ prepared in the microscope slide (in-situ). a) Bright-field (BF) image after 24 h gelation, b) Zoom-in of a selected region of the BF image after 24 h gelation, c) POM image after 24 h gelation, d) POM image after 0 h gelation, e) POM image after 24 h gelation at higher magnification and f) POM image after 72 h gelation. Pictures were taken at ca. 20 °C.

To better understand the structure of $(\text{Ala})_2\text{PDI}_n\text{GdL}$ hydrogels, we performed SAXS measurements on $(\text{Ala})_2\text{PDI}_{0.5}\text{GdL}$ and $(\text{Ala})_2\text{PDI}_{1.0}\text{GdL}$ samples (Figure 4.44a). Interestingly, the $(\text{Ala})_2\text{PDI}_{0.5}\text{GdL}$ gel exhibited a broad band in the SAXS spectra, similar to that of the non-gelled $(\text{Ala})_2\text{PDI}$ 12.5 wt% nematic sample at 25 °C, but centered at a lower scattering vector ($\sim 0.85 \text{ nm}^{-1}$ compared to 1.13 nm^{-1} for the non-gelled nematic mesophase). This indicates an increase in the inter-assembly distance from 5.6 to 7.4 nm, probably due to the presence of gluconic acid and gluconate ions. Additionally, a second broad peak not present in the non-gelled sample appeared at $q \approx 4.0 \text{ nm}^{-1}$, which was attributed to the formation of crystalline precipitates within the gel. In contrast, the $(\text{Ala})_2\text{PDI}_{1.0}\text{GdL}$ gel did not show any broad band at low q values, suggesting a loss of nematic structure during gelation. This is consistent with the formation of amorphous non-birefringent aggregates observed by microscopy. In addition, we tested the $(\text{Ala})_2\text{PDI}_{0.5}\text{GdL}$ responsivity to changes in temperature and light exposure. SAXS measurements showed that the broad band at $\sim 0.85 \text{ nm}^{-1}$ disappeared upon heating to 60 °C (Figure 4.44b), indicating a loss of the nematic structure due to heating.

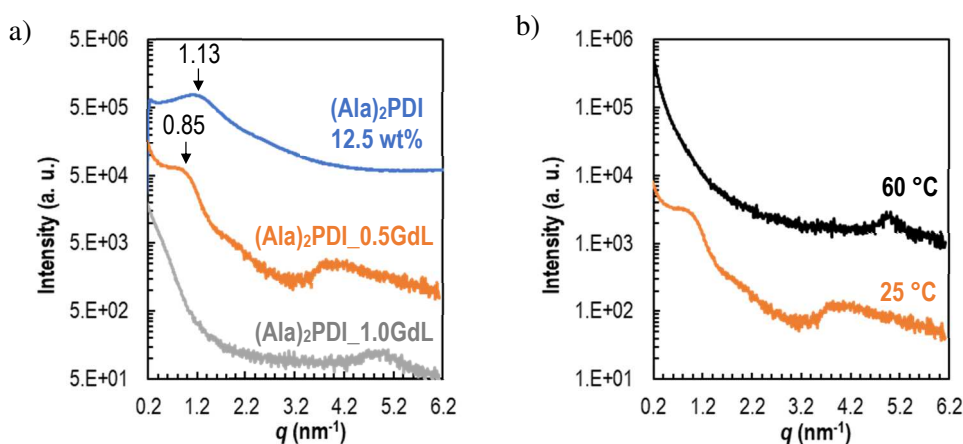


Figure 4.44. a) SAXS spectra of $(\text{Ala})_2\text{PDI}$ 12.5 wt% (blue), $(\text{Ala})_2\text{PDI}_{0.5}\text{GdL}$ (orange) and $(\text{Ala})_2\text{PDI}_{1.0}\text{GdL}$ (grey) at 25 °C. b) SAXS spectra of $(\text{Ala})_2\text{PDI}_{0.5}\text{GdL}$ at 25 °C (orange) and 60 °C (black).

We also conducted light exposure tests using a SOLA lamp with an output power of 3.5 W and a wavelength range of 380 – 660 nm as the light source. It is known that perylene diimides undergo visible light-induced reduction in the presence of electron donors, which leads to the formation of a radical anion within the aromatic core of the PDI (Figure 4.45) ^[316]. The resulting negative charge could prevent the face-to-face aggregation of and disrupt its assembly into chromonic mesophases. Furthermore, the irradiation of perylene diimides with visible light also induce the formation of electronically excited species, which can reduce the hydrophobic interactions between stacked PDIs and thus break the aggregates ^[317].

We observed this effect by exposing a (Ala)₂PDI 12.5 wt% in water solution (i.e. a (Ala)₂PDI non-gelled chromonic nematic phase) to the radiation of the SOLA lamp and observing the sample by POM during the light exposure (Figure 4.46a). The (Ala)₂PDI nematic phase started fading after 30 s of light exposure and was almost completely disrupted after 60 s. Additionally, the nematic phase did not recover even 15 min after turning off the light source (Figure 4.46b), which contrasts with the temperature-induced $N \rightarrow I$ transition, which is rapidly reversed by cooling. Notably, Nastishin et al. ^[317] reported a similar behavior for disodium cromoglycate (DSCG) chromonic mesophases. They observed that DSCG nematic phases faded upon exposure to UV-light and that the regeneration of the nematic phase after the light exposure was a very slow process compared with the temperature-induced transition. Furthermore, they demonstrated that light absorption was essential for the disruption of the nematic phase, as it occurred faster when the sample was illuminated perpendicularly to the director vector (where the absorbance is higher). (Ala)₂PDI exhibited a similar behavior to DSCG, but with visible light instead than UV-light. The mechanism behind this is unknown, but most likely results from a several processes occurring at the same time, such as formation of excited species, formation of perylene anions or dianions by reduction, and temperature-changes caused by light absorption.

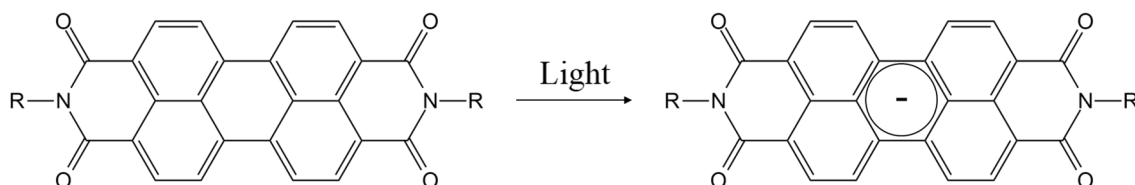


Figure 4.45. General scheme of the formation of a PDI radical anion.

The effect of light exposure on the (Ala)₂PDI_0.5GdL gel was subtler than for the nematic solution (Figure 4.46c), as only a slight reduction in the birefringence was observed after 5 min of light exposure. This behavior may result from the strongest binding between PDI units within the gel due to the partial protonation of the carboxylate terminal groups and the higher viscosity of the gelled sample.

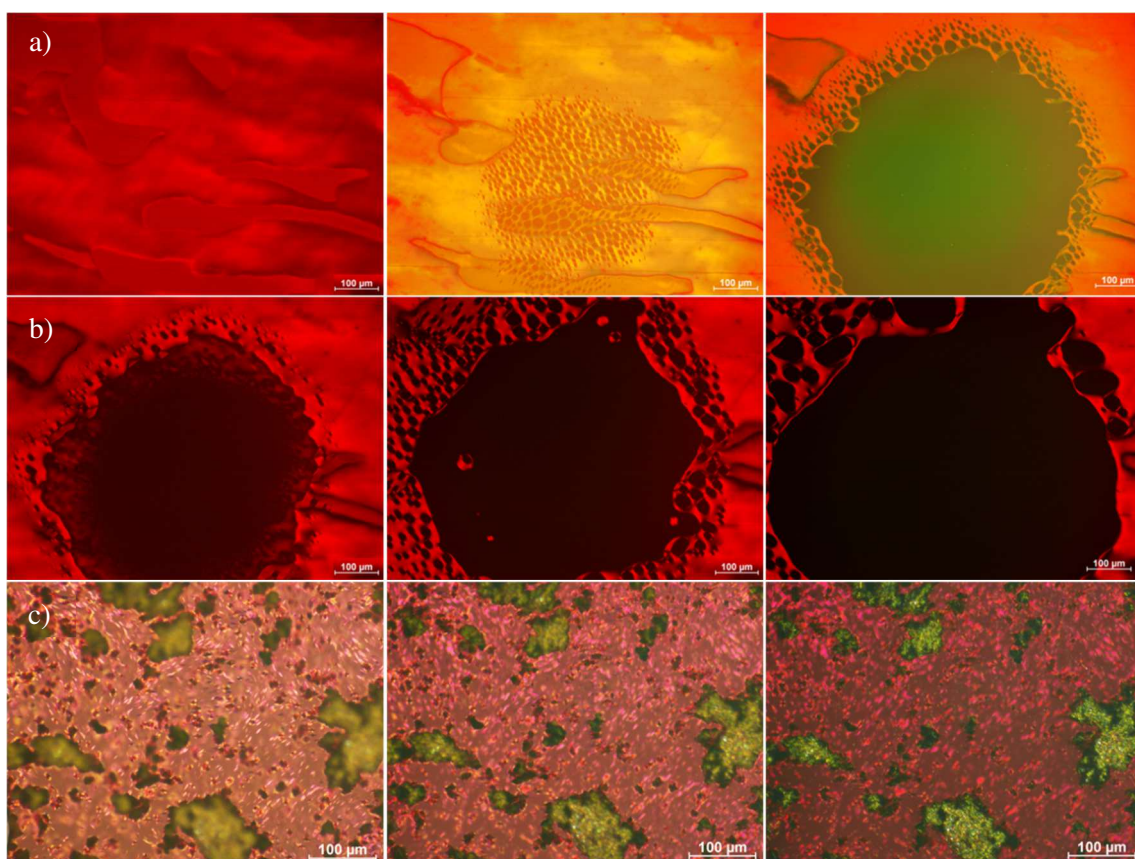


Figure 4.46. a) Change in the nematic texture of $(\text{Ala})_2\text{PDI}$ a 12.5 wt% as it is exposed to light (SOLA lamp, 100 %). From left to right: after 0 s, 30 s and 60 s. b) Sample after the light exposure. From left to right: after 0 s, 5 min and 15 min. c) $(\text{Ala})_2\text{PDI}_{0.5\text{GdL}}$ gel exposed to light (SOLA lamp, 100 %). From left to right: after 0 s, 1 min and 5 min.

Furthermore, we intended to gel a $(\text{Ala})_2\text{PDI}$ -PEG water-in-water emulsion by incorporating 0.5 eq. of GdL to a $(\text{Ala})_2\text{PDI}$ 12.5 wt% with PEG 8.4 wt% N/I emulsion. After 24 h at room temperature, the sample gelled. However, optical microscope observations revealed that as-formed gel was quite similar to that formed by $(\text{Ala})_2\text{PDI}$ 12.5 wt% with no polymer (Figure 4.47), as it was composed of small micrometric birefringent fibers. This suggests that the spherical droplets of the $(\text{Ala})_2\text{PDI}$ -PEG emulsions were disrupted upon the addition of the GdL.

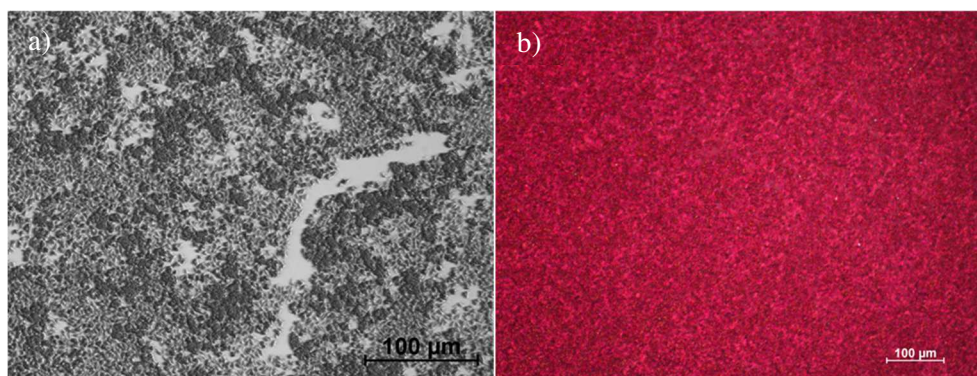


Figure 4.47. a) Bright-field and b) POM optical microscopy images of a gelled $(\text{Ala})_2\text{PDI}$ 12.5 wt% - PEG 8.4 wt% W/W emulsion.

4.2.6 Conclusions

The chromonic and emulsion phase behavior of a chiral alanine-functionalized PDI ((Ala)₂PDI) and an achiral cationic PDI (MeCatPDI) have been investigated. (Ala)₂PDI revealed a rich chromonic phase behavior forming both cholesteric nematic and hexagonal mesophases at ca. 20 °C at 12.5 and 30 wt%, respectively. Furthermore, both (Ala)₂PDI and MeCatPDI formed water-in-water emulsions in presence of the nonionic polymers Dex and PEG, either composed by nematic droplets dispersed in an isotropic continuous phase or vice versa. Notably, the W/W emulsions formed by the PDI – PEG mixtures were composed by nematic droplets with twist deformations and homeotropic anchoring, aligned perpendicularly to the interfaces, for both (Ala)₂PDI and MeCatPDI, suggesting that PEG favors the formation of chiral droplets using either chiral or achiral PDIs. Additionally, triphasic W/W/W and W/W/O emulsions were successfully formulated, and several drop morphologies were observed, including snowman, Janus and core-shell. It was observed that increasing the Dex and PEG concentrations in MeCatPDI-Dex-PEG triphasic W/W/W emulsions transforms the geometry of the droplets from snowman at low concentrations (~ 4 wt%) to core-shell at higher concentrations (~ 8 wt%). Furthermore, temperature- and light-responsive low molecular weight gels were prepared by incorporating GdL to a nematic (Ala)₂PDI solution in water. It was observed that nematic birefringent hydrogels were obtained using low amounts of GdL (< 0.5 eq.), which holds promise for the preparation of anisotropic hydrogels from chromonics mesophases. In contrast, using higher amounts of GdL yielded hydrogels that did not retain the nematic structure. In the future, the rheological properties of these gels will be studied to further understand their behavior.

4.3 Preparation of silica monoliths for the encapsulation and release of ibuprofen

This chapter describes the preparation of micro- and mesoporous silica nanostructured materials in both powder and monolith forms. We used a cationic chromonic perylene diimide (MeCatPDI) as a template for silica preparation, and utilized tetraethyl orthosilicate (TEOS) and hexadecyltrimethoxysilane (HDTMS) as silica precursor and porogen, respectively. We evaluated how varying the proportion of TEOS and HDTMS influenced the physicochemical properties of the final silica materials. Additionally, we investigated the ability of the nanostructured silica materials to deliver ibuprofen, using different drug loading techniques such as soaking + drying or incorporating melted ibuprofen into the silica. Encapsulation efficiency was determined using a combination of techniques that include Fourier-transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), and nitrogen physisorption. We also conducted *in vitro* tests on drug-loaded silica powder and monoliths to evaluate ibuprofen drug release, using a simulated body fluid (SBF, pH 7.4) as the receptor medium and fitting the results to several mathematical drug release models. Finally, we explored the preparation of silica-hydroxyapatite composite materials, which show potential as scaffolds for bone tissue repair and replacement.

4.3.1 Preparation and characterization of the silica materials

Chromonic liquid crystals are soft materials with promising applications in several fields^[96,150,152]. For example, they have demonstrated to be unique materials to prepare oriented films^[142,153], polarizing micropatterns^[155,156] or biosensors^[159]. Furthermore, there is a growing interest in using chromonic self-assemblies as templates to produce nanostructured silica^[162–164] and carbon materials^[165,180,181].

For instance, Rodríguez-Abreu et al. described the preparation of chromonic-templated nanostructured silica from cyanine^[162,163] and perylene diimide^[164] cationic dyes through a sol-gel reaction of a silica precursor at high pH. This process allows the anionic species formed from the hydrolysis of the chosen silica precursor (typically TEOS) to interact with the positively charged dye chromonic aggregates, guiding the formation of nanostructured silica-dye composites. Removal of the templating dyes by calcination produces micro- and mesoporous nanostructured silica.

Herein, we prepare nanostructured silica materials using a cationic PDI (MeCatPDI) as the chromonic template (Figure 4.48). As discussed in the previous chapter, MeCatPDI has a very rich chromonic behavior^[100], and can also be easily emulsified in W/W and multiple W/W/W emulsions with a wide variety of different drop morphologies. Noteworthy, the preparation of nanostructured silica from chromonic MeCatPDI assemblies has already been discussed in the literature^[164]. It was reported that high aspect ratio (from ~ 3 to 20) silica fibers with aligned micropores (~ 1.5 nm diameter) were formed. In this work, we continue this research line by studying the effect of adding a second silica precursor with porogen properties (HDTMS), that contains hexadecyl chains which are removed during the calcination step generating mesopores, and obtaining the silica materials not only as a powder but also as monoliths. Furthermore, we evaluate the capacity of these silica materials to encapsulate and release ibuprofen and prepare silica – hydroxyapatite composites with potential applications for bone tissue repair and replacement.

The silica templated materials were prepared as described in Section 3.3.10.2. Noteworthy, the synthetic conditions were slightly different to those reported in the literature^[164]. Instead of aging the sample at 70 °C for 90 minutes, it was aged at 50 °C for 150 minutes, since the former

conditions yielded too fragile monoliths (see discussion below). We used mixtures at different ratios of TEOS and HDTMS as the silica precursor material, (i.e. 0, 10 and 20 wt% of HDTMS). The obtained silica materials were named as SiHDTMS_*n*, where *n* indicates the weight percentage of HDTMS, for powder samples, and SiMonHDTMS_*n*, for monoliths.

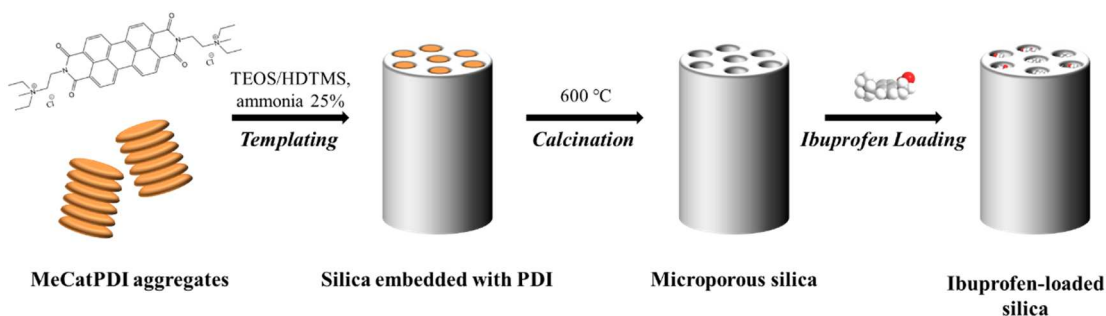


Figure 4.48. Scheme of the preparation of ibuprofen-loaded microporous silica.

The templated powder silica materials before the calcination step were completely red for SiHDTMS_0 and SiHDTMS_10, but white spheres appeared for SiHDTMS_20, suggesting that part of the HDTMS does not adsorb on the MeCatPDI assemblies and hence polymerizes without chromonic templating. This inhomogeneity is not desirable as it can make the SiHDTMS_20 synthesis not reproducible. For this reason, the SiHDTMS_20 sample was not further studied. Moreover, during the washing step prior to calcination no perylene diimide was released from the solid product, indicating that the PDI was completely embedded in the silica framework.

After calcination, a fine white powder was obtained for both SiHDTMS_0 and SiHDTMS_10 samples. The morphology of these materials was studied by field-emission scanning electron microscopy (FESEM). FESEM images revealed that both SiHDTMS_0 (Figure 4.49a,b) and SiHDTMS_10 (Figure 4.49c,d) are composed of fibers with lengths up to 15 μm and widths about 300 nm, which supposes an increased aspect ratio (up to 50) as compared to that of the fibers obtained by curing at 70 °C ^[164]. Considering that the fibers reported in the literature also have a width about 300 nm, such an increase in the aspect ratio by reducing the curing temperature indicate that lower temperatures produce longer fibers.

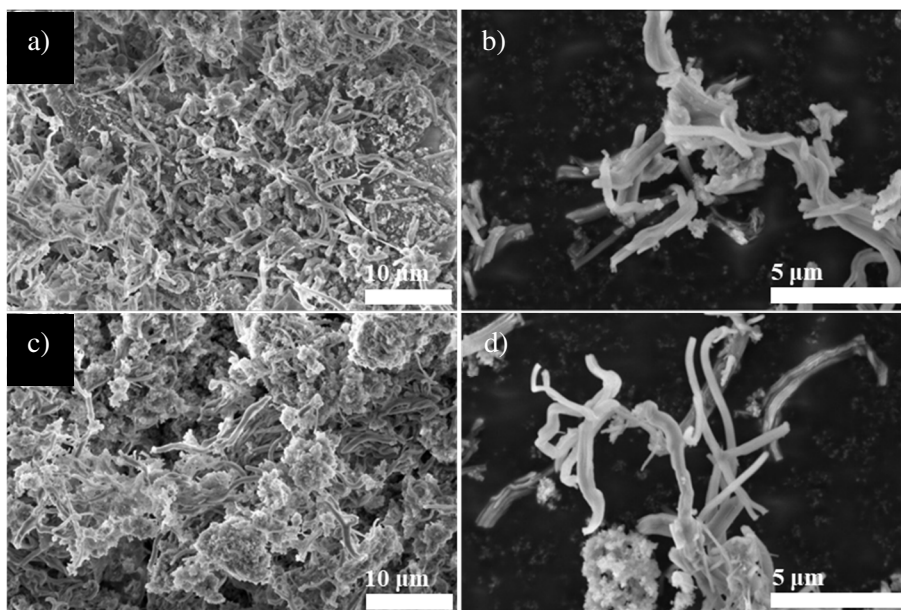


Figure 4.49. FESEM images of a,b) SiHDTMS_0, and c,d) SiHDTMS_10.

SAXS was used to further characterize SiHDTMS_0 (Figure 4.50a) and SiHDTMS_10 (Figure 4.50b) before and after calcination. SiHDTMS_0 before calcination revealed two peaks at $1:\sqrt{3}$ ratio, centered at scattering vectors (q) of ~ 2.9 and $\sim 5.1 \text{ nm}^{-1}$. This suggests that SiHDTMS_0 PDI stripes are hexagonally arranged within the silica framework. The d_H spacing (see Figure 1.15b and Eq. 3) for non-calcined SiHDTMS_0 is 2.1 nm, and the hexagonal lattice parameter a is 2.4 nm. In contrast, upon calcination, the high order reflection at 5.1 nm^{-1} disappears and the first SAXS peak is shifted to a higher q value of $\sim 3.1 \text{ nm}^{-1}$, which corresponds to a d_0 (calculated with Eq. 2) of 2.0 nm. This suggests that the silica material shrinks upon calcination, as it occurred for the $(\text{PEG}_n)_2\text{PDI}$ templated silica. Note that d_H and a cannot be calculated for the calcined sample as the hexagonal lattice is lost during calcination, and d_0 has to be calculated instead. Despite this, d_H and d_0 are comparable as both of them cause the first-order reflection.

In contrast to SiHDTMS_0, the SAXS spectra of SiHDTMS_10 showed broader and less-intense peaks both before and after calcination, which suggests a more polydisperse structure. Before calcination, a SAXS peak at $q \approx 3.1 \text{ nm}^{-1}$ appeared, alongside with a very broad and diffuse high order reflection at a $1:\sqrt{3}$ ratio ($q \approx 5.4 \text{ nm}^{-1}$). This indicates that the hexagonal structure present in SiHDTMS_0 before calcination is partially retained after adding HDTMS in the synthesis. The d_H and a hexagonal lattice parameter for non-calcined SiHDTMS_10 are 2.0 and 2.3 nm, respectively. After calcination, a single broad diffuse band centered at $q \approx 3.5 \text{ nm}^{-1}$ could be hinted, which corresponds to a d_0 of 1.8 nm. Noteworthy, the Bragg spacing of SiHDTMS_10 is shorter than for SiHDTMS_0 both before and after calcination. This is counterintuitive as HDTMS is expected to form bigger pores and increase the distance between the centers of such pores, hence increasing the Bragg spacing. We hypothesize that during the formation of cooperative self-assemblies between the PDI aggregates and the silica species, the hydrolyzed HDTMS molecules self-assemble forming micelles that push the PDI-TEOS aggregates together reducing the Bragg spacing.

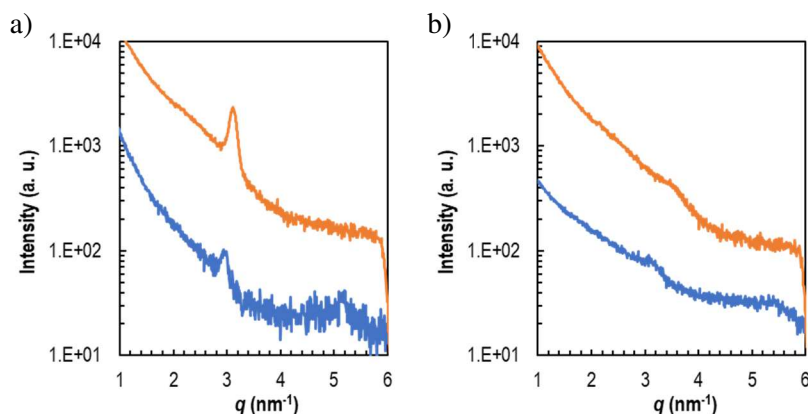


Figure 4.50. SAXS spectra of a) SiHDTMS_0 before (blue) and after (orange) calcination, and b) SiHDTMS_10 before (blue) and after (orange) calcination. The high noise-to-signal ratio of the non-calcined SiHDTMS_0 results from a shorter acquisition time caused by sample loss during the SAXS measurement.

The pore properties of the calcined SiHDTMS_0 and SiHDTMS_10 samples were evaluated using nitrogen physisorption. Prior to measurement, both materials were degassed at 120°C for 12 hours. The nitrogen adsorption-desorption isotherms of both samples indicated a significant uptake of nitrogen at low pressures (Figure 4.51a), suggesting a high degree of microporosity within the samples, followed by a gradual uptake at higher pressures, which was clearly increased for SiHDTMS_10, indicating a higher meso- and macroporosity for this sample and higher mesopore size. Both SiHDTMS_0 and SiHDTMS_10 exhibited Type-IV adsorption isotherms with a distinct hysteresis loop at higher relative pressures, which is associated with interconnected

micro- and mesopores. Notably, SiHDTMS_0 showed a type H4 hysteresis loop, as the two curves (adsorption and desorption) stay nearly horizontal and parallel within the hysteresis loop. On the other hand, SiHDTMS_10 exhibited a hysteresis loop with the two branches tilted more than 30° with respect to the horizontal axis, indicating an H3-like hysteresis loop (Figure 4.51b). Both H4 and H3 hysteresis loops are associated with plate-like particles that create slit-like pores^[318]. However, the H4-type hysteresis loop indicates narrower slit pores^[319], suggesting that the presence of the porogen favors the formation of bigger pores. This was confirmed by analyzing the pore diameter distribution of each material using the Barrett-Joyner-Halenda (BJH) method (Figure 4.51c), which suggested bigger mesopores for SiHDTMS_10.

The specific surface area (SSA), micropore area (S_{Micro}), total pore volume (V_P) and micropore volume (V_{Micro}) were determined for both samples (Table 4.11). The SSA were calculated with the BET model from the adsorption isotherm at low pressures. All BET linear fits had coefficients of determination (R^2) above 0.9999, confirming the reliability of the results. The BET specific surface area increased from 343.1 to 385.2 $\text{m}^2\cdot\text{g}^{-1}$ by using the HDTMS in the synthesis. Similarly, the total pore volumes, calculated from the last point of the adsorption isotherm ($P/P_0 = 0.995$), were 0.301 and 0.585 $\text{cm}^3\cdot\text{g}^{-1}$ for SiHDTMS_0 and SiHDTMS_10, respectively. In contrast, the micropore area and micropore volume calculated by the t-plot method ($R^2 \approx 0.99$) were significantly higher for SiHDTMS_0 as compared to SiHDTMS_10. These results indicate that the incorporation HDTMS favors the formation of meso- and macropores, and slightly reduces the total amount of micropores within the silica material.

Furthermore, the median pore width was calculated by the NLDFT model (Figure 4.51d), being about 1.3 – 1.4 nm for both SiHDTMS_0 and SiHDTMS_10, thus matching with the length of the perylene diimide core ($\sim 1.1 - 1.4$ nm, see Figure 4.11e). Note that these values are the median pore width and not the average pore width. Additionally, it should be noted that the equipment used for these measurements does not collect many points at low relative pressures, which makes the NLDFT calculations incapable of determining pores with diameters below 1.3 nm.

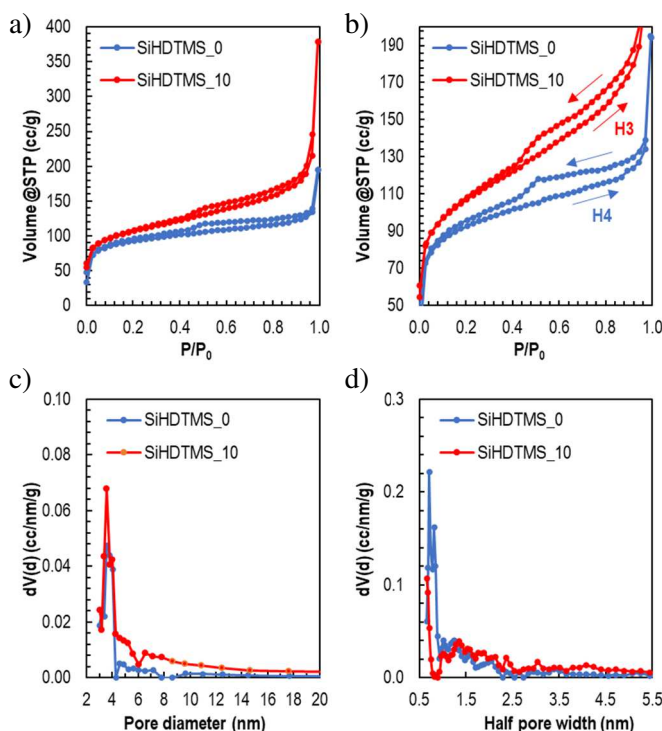


Figure 4.51. a) Nitrogen physisorption isotherms of the powder silica materials, b) detail of the hysteresis loops of the nitrogen physisorption isotherms at low adsorbed volumes, c) pore size distribution calculated from the BJH method, d) micropore width distribution calculated by NLDFT model.

Table 4.11. Porosity properties of powder SiHDTMS_*n* samples. SSA = specific surface area, V_p = total pore volume, S_{Micro} = micropore surface area, V_{Micro} = micropore volume, W_p = median pore width calculated by the NLDFT model.

Sample	SSA ($m^2\ g^{-1}$)	V_p ($cm^3\ g^{-1}$)	S_{Micro} ($m^2\ g^{-1}$)	V_{Micro} ($cm^3\ g^{-1}$)	W_p (nm)
SiHDTMS_0	343	0.30	249	0.10	1.4
SiHDTMS_10	385	0.59	206	0.09	1.3

Fourier-transform infrared spectroscopy was used to characterize the surface functional groups of the silica nanostructured fibers. Both SiHDTMS_0 and SiHDTMS_10 exhibited similar spectra (Figure 4.52a), with a small band at $806\ cm^{-1}$ and an intense band at $1059\ cm^{-1}$, attributed to Si–O–Si bonds [320,321]. However, SiHDTMS_10 also showed a broad band at $\sim 3400\ cm^{-1}$, which corresponds to hydroxyl groups, and small band at $964\ cm^{-1}$ (Figure 4.52b), attributed to Si–OH (silanol) bonds [322]. Furthermore, the absence of bands at $\sim 2900\ cm^{-1}$ indicates the absence of C–H groups in the silica materials, suggesting that calcination completely removed the hexadecyl chains. The formation of silanol moieties for SiHDTMS_10 was attributed to a steric effect of the hexadecyl chain, which prevented the polymerization of the three Si–O[−] groups of the hydrolyzed HDTMS leaving more unpolymerized free silanol groups in the final material.

The complete removal of the hexadecyl chains was further confirmed by studying the water-absorption properties of both SiHDTMS_0 and SiHDTMS_10, with the Washburn method. This was achieved by employing a tensiometer equipped with a glass cylinder with a porous membrane. The samples were loaded into the glass cylinder, placed in contact with Milli-Q water, and the adsorbed water mass as a function of time was measured. It was observed that both silica materials became water-saturated in about 5 s (Figure 4.52c), suggesting a high hydrophilicity and a complete removal of the hydrophobic hexadecyl chain for SiHDTMS_10. Apparently, SiHDTMS_0 saturated slightly before SiHDTMS_10, which could suggest higher hydrophilicity for the sample without the porogen. However, it is important to note that differences in porosity and the degree of compaction of the samples inside the cylinder may significantly impact the permeability profiles. As a result, we concluded that the differences between the two materials in terms of hydrophilicity were not significant.

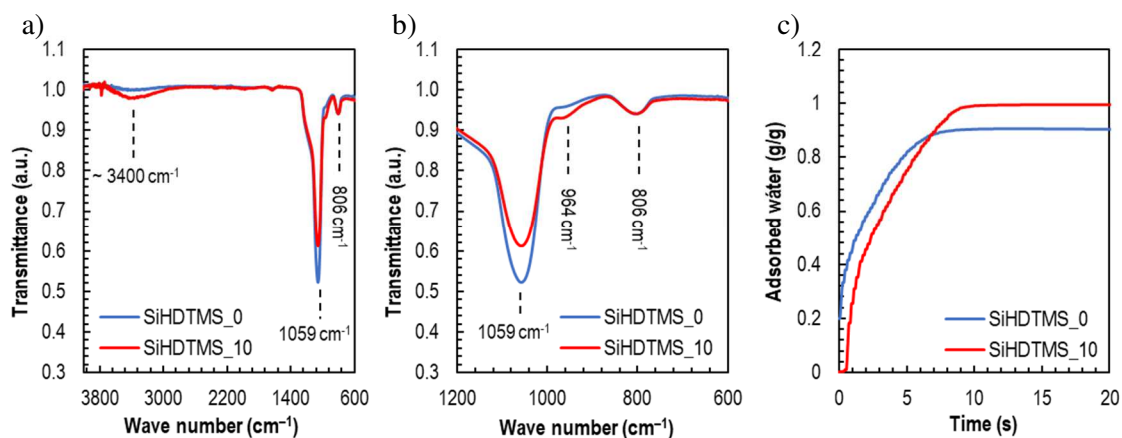


Figure 4.52. a) FTIR spectra of the silica materials. b) FTIR of a selected region between 600 and 1200 cm^{-1} . c) Water-permeability profiles of the silica materials. Note that the adsorbed water is the mass of water adsorbed per gram of the silica material.

Summarizing, the incorporation of HDTMS in the synthesis of MeCatPDI-templated silica generates meso- and macropores and reduces the pore ordering within the silica framework (Figure 4.53), without significantly affecting the morphology, chemical composition and

hydrophilicity of the material. Therefore, these results confirm that HDTMS is indeed playing the role of porogen.

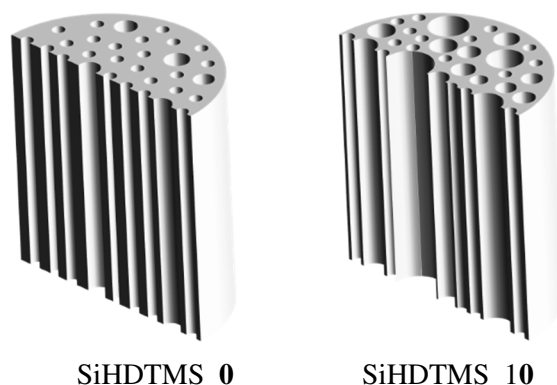


Figure 4.53. Model of the pore size and morphology of the SiHDTMS_n samples. Note that the longitudinal cross-section of the fibers is represented.

The silica materials were also prepared as disk and conic monoliths, as described in Section 3.3.10.3. Silica monoliths with controlled shape and continuous porous structures have received much attention in several fields. For instance, they have been used as bioactive regenerative grafts for bone tissue repairment^[323–325], drug delivery systems^[326], random lasers^[327], microreactors for selective catalysis^[328], or monolithic columns for chromatography^[329].

Herein, we formulate the MeCatPDI-templated porous silica monoliths in different shapes (discotic and conic monoliths). Briefly, the MeCatPDI, ammonia 25 % and the silica precursors were mixed at the desired proportions in a plastic mold (a 20 mL cylindric vial or a 1.5 mL Eppendorf vial) and left standing at 25 °C for 90 min and aged at various temperatures (25 – 70 °C). Afterwards, several water aliquots were carefully added to the as-formed red monoliths to remove remnant ammonia and then the samples were lyophilized and calcined at 600 °C.

Disk-like monoliths were prepared in a plastic cylindric vial at different curing conditions (25 °C/3 days, 50 °C/150 min and 70 °C/90 min) using 100 % TEOS as silica precursor. We observed that white light silica disks were obtained at 25 and 50 °C (Figure 4.54a,b), but it was impossible to obtain monoliths at 70 °C, as the sample became too fragile, most likely due to the bubbles formed during the curing process. Noteworthy, the sample at 25 °C presented a smoother surface than at 50 °C, confirming that lower aging temperatures yield more resistant and uniform materials. Despite this, 50 °C curation was selected for further experiments over 25 °C to maintain similar conditions to those previously reported^[164].

In contrast, conic monoliths were prepared in an Eppendorf vial rather than in a cylindric vial (Figure 4.54c,d), with curation at 50 °C for 150 min. Conic monoliths were significantly more robust than the discotic ones, due to their increased thickness, and hence could be more easily handled for ibuprofen drug encapsulation and release. For this reason, conic monoliths cured at 50 °C were chosen over disk-like monoliths for further characterization and experiments.

The porosities of the conic monoliths prepared with 100 wt% TEOS (SiMonHDTMS_0) and 90 wt% TEOS + 10 wt% HDTMS (SiMonHDTMS_10) as silica precursors were evaluated by nitrogen physisorption (Figure 4.55). As for the powder samples, the isotherms of both monolithic silicas exhibited a nitrogen high uptake at low pressures, and the one with HDTMS revealed a significant uptake at higher pressures (Figure 4.55a), suggesting that both monoliths are microporous and SiMonHDTMS_10 has increased meso- and macroporosity.

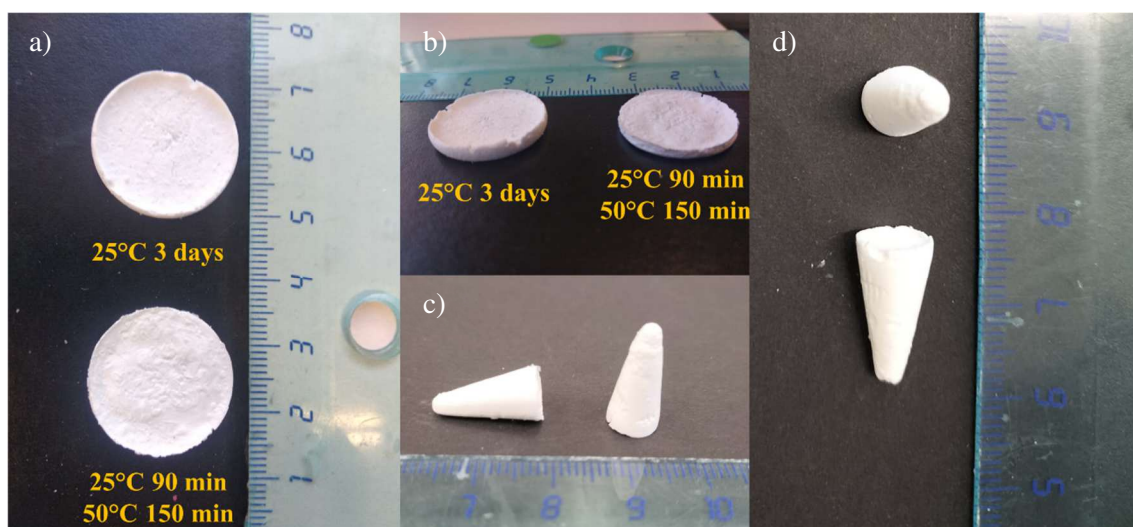


Figure 4.54. a,b) Disk-like monoliths obtained at different curing conditions. c,d) Conic monoliths obtained curing at 50 °C for 150 min. For all the monoliths shown herein 100 % TEOS was used as silica precursor. The conic monoliths prepared with 90 % TEOS + 10 % HDTMS were visually identical to those prepared with 100 % TEOS.

Pore size distributions determined by the BJH method (Figure 4.55b) and the NLDFT model (Figure 4.55c) revealed that SiMonHDTMS_10 has bigger pores than SiMonHDTMS_0, as it was observed for powder samples, indicating that the presence of the porogen favors the formation of bigger pores also in monolithic silica materials.

The SSA of the monoliths were calculated with the BET model from the adsorption isotherms at low pressures (Table 4.12). All BET linear fits had coefficients of determination (R^2) above 0.9997, confirming the reliability of the results. The calculated SSA were 335.6 and 383.4 m² g⁻¹ for the samples without and with HDTMS, respectively. These values are very similar to those obtained for the powder samples, suggesting that the monoliths are similar to their powder analogues. The total pore volume, micropore surface area, micropore volume and median micropore width were also determined (Table 4.12). As for the powder samples, incorporating HDTMS reduces the micropore surface area and volume, hence increasing the meso- and macroporosity within the silica. Noteworthy, the total pore volume decreased for the monoliths as compared to the powder samples. This is rather surprising considering that the monoliths clearly have a lower density, since the same mass forming a monolith reduced to powder would barely occupy 1/3–1/4 of the total volume of the monolith. Such an inconsistency probably arises from very big macropores within the monoliths not detected by nitrogen physisorption. The median micropore width was 1.3 nm for both samples.

SiMonHDTMS_n materials are, to our knowledge, the first chromonic-templated silica monoliths described in the literature. Similarly, Chu et al. [330] prepared monolithic silica aerogels with fire-resistant properties using liquid-crystalline cellulose nanocrystals as templates and tetramethyl orthosilicate (TMOS) as silica precursor. The resulting materials showed aligned micropores and a high BET specific surface area of ~ 300 m²·g⁻¹. Furthermore, other templates such as lyotropic surfactant liquid crystals [13,331], emulsions [332], ionic liquids [333] or polymer monoliths [334,335] have been used to produce porous silica monoliths through the nanocasting approach.

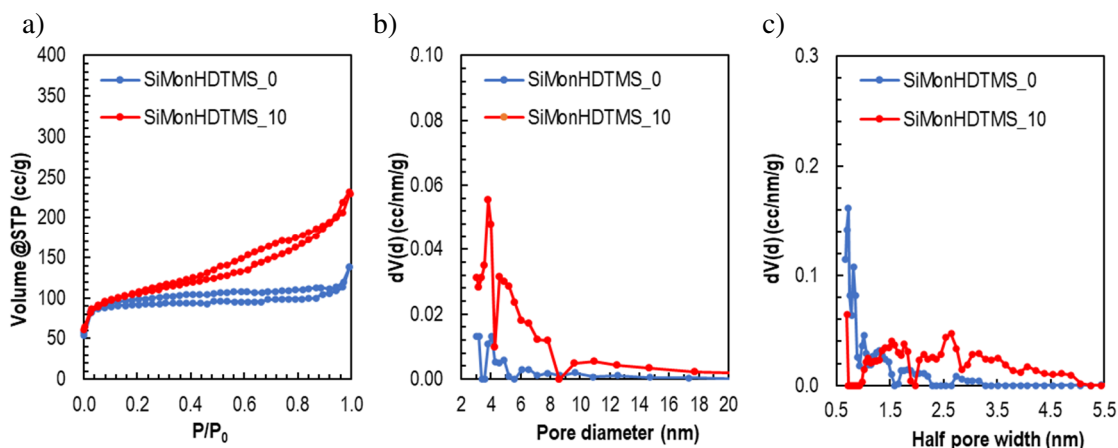


Figure 4.55. a) Nitrogen physisorption isotherms of the silica monoliths, b) pore size distribution calculated from the BJH method, c) micropore width distribution calculated by NLDFT model.

Table 4.12. Porosity properties of SiHDTMS_*n* monolithic samples. SSA = specific surface area, V_p = total pore volume, S_{Micro} = micropore surface area, V_{Micro} = micropore volume, W_p = Pore width (from NLDFT).

Sample	SSA (m ² g ⁻¹)	V_p (cm ³ g ⁻¹)	S_{Micro} (m ² g ⁻¹)	V_{Micro} (cm ³ g ⁻¹)	W_p (nm)
SiMonHDTMS_0	336	0.21	316	0.13	1.3
SiMonHDTMS_10	383	0.36	231	0.10	1.3

4.3.2 Ibuprofen encapsulation and release

Micro- and mesoporous silica materials have shown great potential as drug delivery systems due to their unique physicochemical properties^[336–338], such as high surface area, large pore volume, tunable pore size, and excellent biocompatibility. Moreover, they are resistant to heat^[339], pH^[340], and can be easily functionalized with targeting moieties, such as antibodies, peptides, or aptamers, which can selectively bind to specific cells or tissues, enabling targeted drug delivery^[341–343].

In general, mesoporous silica materials are preferred over their microporous analogues as the larger mesopore size (2 – 50 nm) enables the encapsulation of bigger molecules that do not fit within the small micropores (< 2 nm)^[344]. However, the smaller pore sizes of microporous silica materials can also provide some advantages for certain drug delivery applications. For example, they can be used to encapsulate small molecules. Additionally, their smaller pore sizes can result in slower drug release rates^[345]. Interestingly, the combination of micro- and mesoporosity of SiHDTMS_*n* materials could favor the encapsulation of small drugs within the micropores and facilitate the drug encapsulation and release due to the interconnections between the micro- and mesopores.

Herein, we evaluated the drug encapsulation and release properties of SiHDTMS_0 and SiHDTMS_10 using ibuprofen as model drug (Figure 4.56a). Ibuprofen is a non-steroidal anti-inflammatory drug very commonly used to relieve pain, fever and inflammation. In terms of chemical structure, ibuprofen is a 2-aryl propionate with an isobutyl moiety attached to the carbon number 5 of the aryl group. The dimensions of the ibuprofen were estimated with Chem3D software running a molecular mechanical MM2 energy minimization. The obtained values are: ca. 1.20 nm length, 0.72 nm height and 0.65 nm width (Figure 4.56b,c).

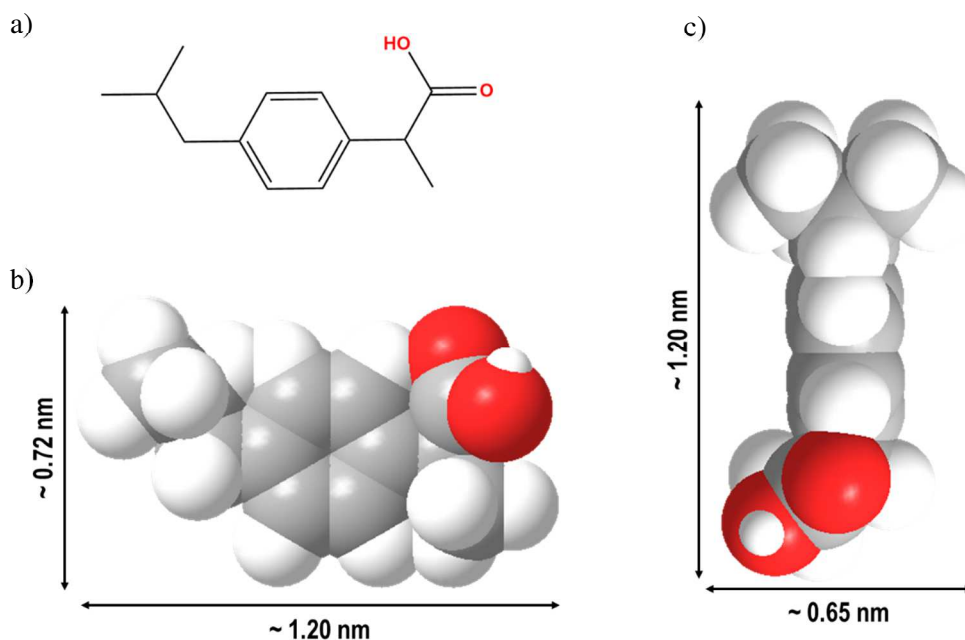


Figure 4.56. a) Chemical structure of ibuprofen. b,c) Ibuprofen molecular dimensions calculated using the Chem3D software

SiHDTMS_0 and SiHDTMS_10 materials are expected to have micropore diameters about ~ 1.5 nm, as inferred from the literature ^[164] and the NLDFT models (Figures 4.51 and 4.53). Accordingly, ibuprofen should fit within the silica micropores.

Two different methods were used to evaluate the encapsulation of ibuprofen in MeCatPDI-templated silica materials. The first method, known as the soaking + drying method (SDM), involved suspending the silica in a 33 mg/mL ibuprofen solution in hexane (66 mg of silica per mL of hexane) and letting it equilibrate for 15 minutes, followed by evaporation of the solvent under a nitrogen flow. The second method, the melting method (MM), involved preparing a solid silica-ibuprofen composite at a 2:1 mass proportion and heating it to 85°C for 10 minutes to allow the ibuprofen to melt and penetrate within the pores. In both methods, the drug-loaded silica materials were washed with pure hexane to remove the ibuprofen that remained adsorbed at the external surface of the materials, and thus, did not penetrate into the pores (see Section 3.3.11).

The drug encapsulation efficiency, i.e. percentage of ibuprofen loaded into the silica material (*IbuEE*, see Eq. 6 in Section 3.3.11) was determined by performing thermogravimetric analysis (TGA) of unloaded SiHDTMS_0 (Figure 4.57a) and the ibuprofen-loaded silica materials (Figure 4.57b-d). The total loaded ibuprofen was determined as the weight loss between 100 and 700 °C, applying a correction from the weight loss of the unloaded silica materials in this temperature range. As shown in the Table 4.13, the encapsulation efficiencies of the SDM and MM were quite similar, without significant differences. In contrast, significant differences were observed depending on the silica material used, as SiHDTMS_10 showed a much higher encapsulation efficiency by the SDM as compared to SiHDTMS_0, which was attributed to the improved specific surface area of the silica with HDTMS.

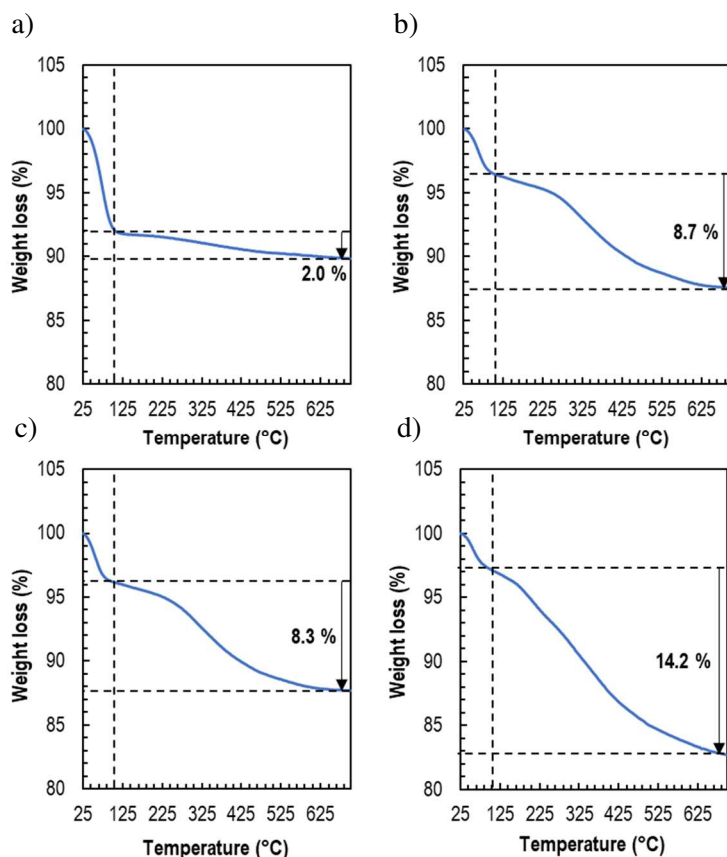


Figure 4.57. TGA of a) unloaded SiHDTMS_0, b) SDM and c) MM ibuprofen-loaded SiHDTMS_0 and d) SDM ibuprofen-loaded SiHDTMS_10.

Table 4.13. Ibuprofen encapsulation efficiency (IbuEE) of SiHDTMS_n materials depending on the drug loading method. m_0 is the amount of ibuprofen used in the encapsulation process, m_{loaded} represents the amount of encapsulated ibuprofen, calculated from the TGA data (see Section 3.3.11 for more details).

Sample	Loading method	m_0 (mg)	m_{loaded} (mg)	IbuEE (%)
SiHDTMS_0	SDM	33.0	4.8	14.4
	MM	33.0	4.4	13.3
SiHDTMS_10	SDM	33.0	9.2	27.8

The location of the ibuprofen in the loaded silica materials was characterized by FTIR and nitrogen physisorption. FTIR measurements were taken of the SiHDTMS_0 and SiHDTMS_10 samples loaded with ibuprofen using both the SDM and MM before and after washing with hexane (Figure 4.58). Before loading with ibuprofen, a single small band appeared at 1600 cm^{-1} for both SiHDTMS_0 and SiHDTMS_10 silica samples, corresponding to surface adsorbed water [346]. After loading with both methods, a succession of bands in the range between $1300 - 1600\text{ cm}^{-1}$ and a large band at $\sim 1715\text{ cm}^{-1}$ were observed, corresponding to ibuprofen (pure ibuprofen was measured to confirm this, see Annexes). Notably, the intensity of the ibuprofen signals decreased significantly after washing with hexane, indicating that most of the surface-adsorbed ibuprofen was effectively removed. Furthermore, the ibuprofen signals prior to washing were much more intense for the MM loaded samples (Figure 4.58b,d) than for the SDM (Figure 4.58a,c), suggesting that the latter method is more effective in facilitating drug penetration within the micropores.

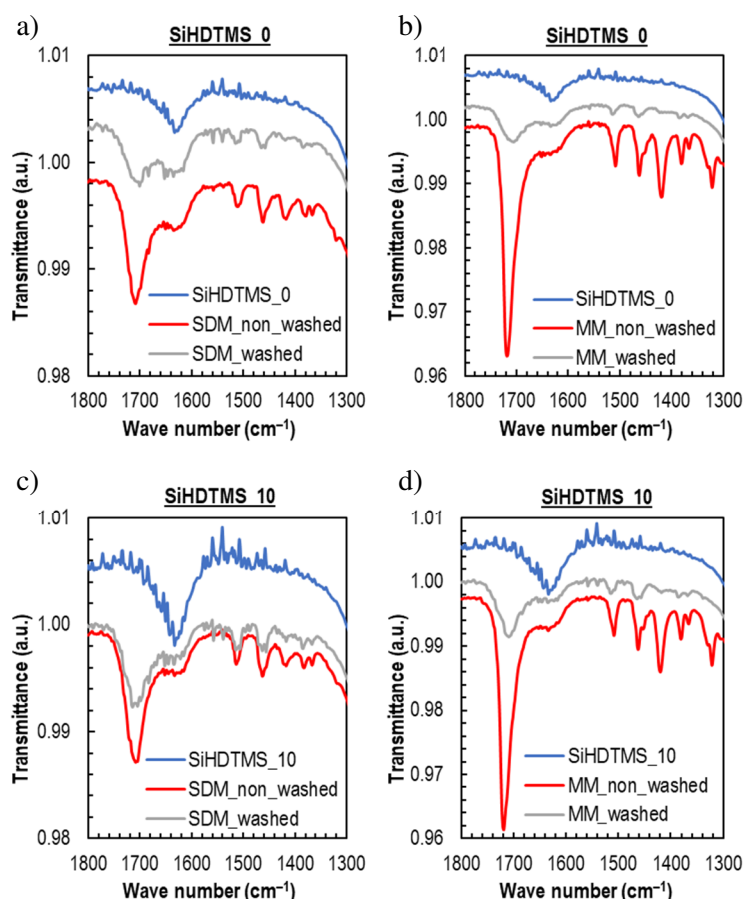


Figure 4.58. FTIR spectra of a) SiHDTMS_0 loaded by the SDM, b) SiHDTMS_0 loaded by the MM, c) SiHDTMS_10 loaded by the SDM and d) SiHDTMS_10 loaded by the MM.

Ibuprofen-loaded materials were characterized by nitrogen physisorption to investigate how the introduced drug altered the porosity. In contrast to the unloaded materials, isotherms of the ibuprofen-loaded samples showed almost zero nitrogen uptake at low pressures (Figure 4.59a,c), suggesting an absence of open microporosity available to nitrogen, confirming that these micropores were filled with ibuprofen. At higher pressures, a small increase in the adsorbed volume could be observed for all the loaded samples alongside with a big nitrogen uptake when $P/P_0 \approx 1$, indicating that the macropores remained mostly empty. Careful observation of the adsorption-desorption isotherms at low pressures (Figure 4.59b,d) revealed hysteresis loops similar to those observed for unloaded SiHDTMS_0 and SiHDTMS_10. However, these hysteresis loops did not close until $P/P_0 \approx 0$. The presence of hysteresis at low relative pressures indicates that adsorbed species (ibuprofen) have blocked the micropores, thus impeding the desorption of nitrogen from the mesopores as it would require crossing the blocked micropore channels to reach the exterior^[318]. Notably, the hysteresis loops were minor for samples loaded by the MM, suggesting that ibuprofen has a poorer micropore penetration by this method, as inferred from the FTIR measurements.

The SSA of the ibuprofen-loaded samples were about $20 - 30 \text{ m}^2 \cdot \text{g}^{-1}$, independently of the silica material and loading method used, and the detected micropore surface area and volume were virtually zero (Table 4.14), confirming that the micropore volume was completely filled with the loaded drug. These results are rather surprising as it was expected for the MM to show higher microporosity, as both the TGA and FTIR analysis suggested a lower encapsulation efficiency for this method. We hypothesize that the ibuprofen fills evenly the micropores upon encapsulation by the SDM, meanwhile it accumulates in the entrance of the micropores for the MM (Figure

4.60), as it has been proposed for SBA-15 silica materials loaded with itraconazole [347]. The absence of microporosity observed by nitrogen physisorption for the melting method loaded silicas could be explained considering that the air imprisoned within the micropores is not removed during the outgassing. Note that the outgassing temperature used for ibuprofen-loaded silica materials was much lower (~ 40 °C) than for the unloaded materials (~ 120 °C), to avoid ibuprofen melting. This can lead to incomplete degassing, potentially leaving water or hexane trapped within the pores. This presence of residual substances could affect the accuracy of nitrogen sorption measurements, leading to underestimated surface areas and pore volumes.

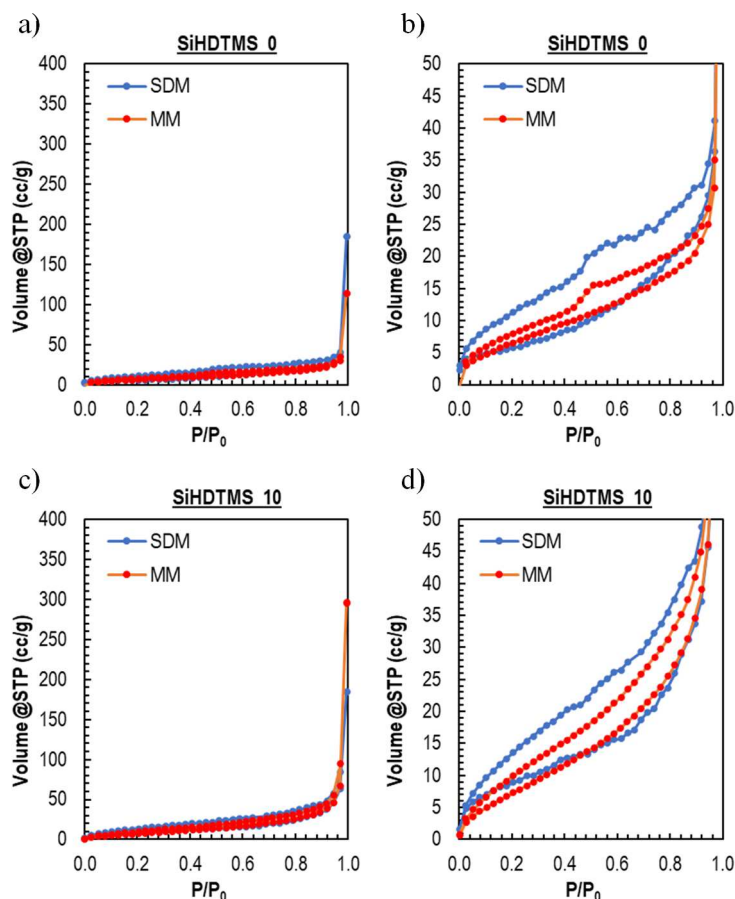


Figure 4.59. Nitrogen physisorption isotherms of a,b) ibuprofen-loaded SiHDTMS_0, and c,d) ibuprofen-loaded SiHDTMS_10.

Table 4.14. Porosity properties of ibuprofen-loaded powder SiHDTMS_n samples. SSA = specific surface area, V_p = total pore volume, S_{Micro} = micropore surface area, V_{Micro} = micropore volume. Note that these values are much lower compared to those prior to ibuprofen loading (Table 4.11).

Sample	Loading method	SSA ($\text{m}^2 \cdot \text{g}^{-1}$)	V_p ($\text{cm}^3 \cdot \text{g}^{-1}$)	S_{Micro} ($\text{m}^2 \cdot \text{g}^{-1}$)	V_{Micro} ($\text{cm}^3 \cdot \text{g}^{-1}$)
SiHDTMS_0	SDM	21	0.29	0	0.00
	MM	28	0.18	0	0.00
SiHDTMS_10	SDM	33	0.29	0	0.00
	MM	28	0.46	0	0.00

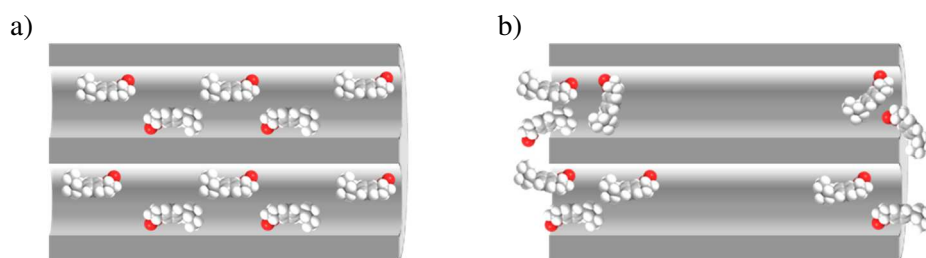


Figure 4.60. Model of the ibuprofen location within the silica micropores in a) solvent + drying method loaded and b) melting method loaded silica materials.

Ibuprofen was also encapsulated in the monolithic samples with conic shapes SiMonHDTMS_0 and SiMonHDTMS_10 by the SDM (see Section 3.3.13). For that, 1 mL of ibuprofen dissolved in hexane (16.5 mg/mL) was added to a monolith inside an Eppendorf vial and allowed to equilibrate for 15 min before drying in a stove at 30 °C until complete evaporation of the solvent. Since monoliths weigh an average of 25 – 30 mg each, the total silica-ibuprofen mass ratio was maintained close to 2:1. As for powder samples, the monoliths were washed with pure hexane to remove surface-adsorbed ibuprofen. The melting method was not tested for monolithic samples as it yielded poorer encapsulation efficiencies for powder samples and is not easily adapted to monolithic materials since they cannot be milled to prepare solid homogeneous mixtures.

The encapsulation efficiency of ibuprofen in the silica monoliths was determined by weighting the monoliths before and after drug loading, and considering the difference as the total amount of ibuprofen incorporated into the monoliths. The encapsulation efficiencies were 74 ± 11 % and 77 ± 6 % for SiMonHDTMS_0 and SiMonHDTMS_10, respectively. In contrast to powder samples, the encapsulation efficiencies of the silica monoliths were about 3 to 4 times higher and showed no significant differences between the monolith with and without porogen. This was attributed to the monolithic shape, which reduced the external surface area in contact with the hexane bulk solution as compared to the powder samples, hence reducing the washing efficiency.

The release profiles of ibuprofen from the loaded silica monoliths in simulated body fluid (SBF, pH = 7.4, see Section 3.1.4) at 37 °C were measured (Figure 4.61). For this, the ibuprofen-loaded monoliths were placed in a dialysis membrane and 1 mL of SBF was added. Immediately, it was incorporated to a flask with 20 mL of SBF, and 1 mL aliquots were taken as a function of time, adding 1 mL of SBF after each aliquot to maintain constant the total SBF volume. To ensure sink conditions, which require the dissolution media to dissolve more than 3 times the total amount of drug present in the dosage form, the ibuprofen solubility in SBF was determined preparing a saturated ibuprofen-in-SBF solution at 37 °C and measuring its concentration using UV-Vis spectrophotometry. It was determined that ibuprofen had a solubility of 2.02 mg/mL in SBF at pH = 7.4, which is superior to the solubility of ibuprofen in pure water (0.021 mg/mL)^[348] and in PBS pH = 7.2 (~ 1 mg/mL)^[349] at ca. 20 °C. Therefore, the release experiments were performed with sufficient SBF to dissolve between 3 and 3.5 times the ibuprofen encapsulated into the monoliths (see Sections 3.3.14 and 3.3.15).

The ibuprofen-loaded monoliths exhibited an initial burst release followed by a prolonged release up to ~ 24 h. Both samples reached a 100 % release after approximately 24 h, indicating that no drug was retained within the micropores, and no significant differences were observed between the two release profiles, as the experimental error (calculated as the standard deviation) overlapped at all measured times. This is noteworthy as the higher microporosity and lower average pore size of SiMonHDTMS_0 should yield slower release profiles^[350]. However, as most of ibuprofen is expected to be in the macropores, the effect of the different microporosities may be not significant.

Furthermore, it is worth noting that the error bars are rather high, especially for SiMonHDTMS_0. This high variability may arise from factors not related to the sample itself, but to sample processing and handling. For instance, some monoliths broke during the release and others not, and the contact between the dissolution media and the monoliths inside the dialysis membrane where the release was performed was not very reproducible between samples.

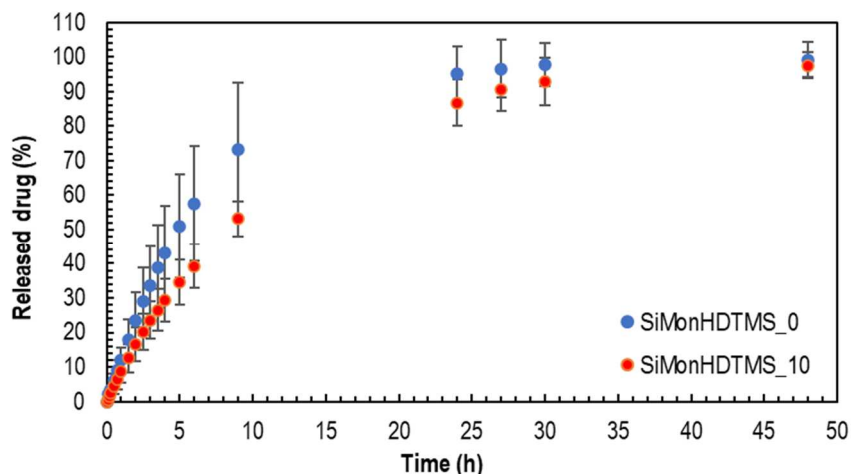


Figure 4.61. Drug release profiles of SiMonHDTMS_*n* samples. The error bars indicate the standard deviation, calculated using 3 replicas (see Section 3.3.15).

The ibuprofen release profiles of SiMonHDTMS_*n* dosage forms were fitted to different kinetic models including zero-order, first-order, Higuchi [217], and Korsmeyer-Peppas [218] models using the DDSolver software [216] (see Section 3.3.15). Notably, for the Korsmeyer-Peppas model, data was fitted only up to 60 % release, as the model is not valid for later-stage release conditions [219]. The equations, parameters and R^2 coefficients of determination for each model are summarized in Table 4.15. It was observed that both SiMonHDTMS_*n* samples follow first-order kinetics ($R^2 > 0.999$), which produced the best coefficient of determination. This is typical of porous non-swelling solids loaded with water-soluble drugs [351,352]. The fitting to the Korsmeyer-Peppas model was also good ($R^2 > 0.99$), although this model was only applied to the initial steps of release.

Notably, such good fitting to the first-order model suggests a Fickian diffusion-based drug transport mechanism [353,354]. However, Korsmeyer-Peppas release exponents above 0.5, which is the case for ibuprofen-loaded SiMonHDTMS_*n* dosage forms (see Table 4.15), are associated to anomalous non-Fickian diffusion mechanism, whereas exponents of 0.5 indicate a purely Fickian drug diffusion. Note that the Higuchi model is equivalent to the Korsmeyer-Peppas with a release exponent n of 0.5. This suggests a complex drug transport behavior for SiMonHDTMS_*n* materials.

It is interesting to compare the SiMonHDTMS_*n* Korsmeyer-Peppas n release exponents with those of other drug-loaded mesoporous silica materials. For example, MCM-48 (cubic), LP-*la3d* (cubic, big mesopores) [355] and SBA-15 (hexagonal, big mesopores) [356], fitted to the Higuchi model, indicating a purely Fickian drug transport. However, release exponents above 0.5 suggesting a non-Fickian diffusion have been observed for ibuprofen-loaded MCM-41 [357], which corresponds to a hexagonal silica material with narrower mesopores. This anomalous behavior for MCM-41 was attributed to the sterically hindered free diffusion of the ibuprofen from the narrow mesopores. Similarly, the thin aligned micro- and mesopores of SiMonHDTMS_*n* materials could cause the higher Korsmeyer-Peppas release exponents. In fact, SiMonHDTMS_0, that has narrower pores, having a higher release exponent (0.848) than SiMonHDTMS_10

(0.793). Moreover, SiHDTMS_n materials most likely have lower pore interconnections than MCM-41, which would favor the anomalous diffusion as well.

Table 4.15. Drug release parameters for ibuprofen-loaded silica monoliths according to zero-order, first-order, Higuchi and Korsmeyer-Peppas kinetic models.

Model	Equation	Parameters		R ² coefficient	
		SiMon-HDTMS_0	SiMon-HDTMS_10	SiMon-HDTMS_0	SiMon-HDTMS_10
Zero-order	$\%Rel = k_0 \cdot t$	$k_0 = 0.051$	$k_0 = 0.047$	$R^2 = 0.5327$	$R^2 = 0.7672$
First-order	$\%Rel = 100 \cdot (1 - e^{k_1 \cdot t})$	$k_1 = 0.002$	$k_1 = 0.001$	$R^2 = 0.9994$	$R^2 = 0.9997$
Higuchi	$\%Rel = k_H \cdot t^{0.5}$	$k_H = 2.309$	$k_H = 2.034$	$R^2 = 0.9312$	$R^2 = 0.9664$
Korsmeyer-Peppas	$\%Rel = k_{KP} \cdot t^n$	$k_{KP} = 0.404$ $n = 0.848$	$k_{KP} = 0.370$ $n = 0.793$	$R^2 = 0.9977$	$R^2 = 0.9975$

4.3.3 Preparation of silica-hydroxyapatite composites

One drawback of the silica monoliths described above is their poor mechanical properties. The unloaded monoliths had to be handled carefully as they broke rather easily. This can hinder the application of the as-prepared silica scaffolds in applications such as bone tissue engineering. One way to improve the mechanical properties of the silica materials is to prepare composites with other ceramic materials, as ceramics composite materials are known to improve the mechanical properties of their individual components [358–360].

In particular, silica-hydroxyapatite composites are promising materials for several biomedical applications. Hydroxyapatite is a naturally occurring biomineral consisting in calcium hydroxyphosphate ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), and is well known for being the main inorganic component of the bone. Therefore, it is fully biocompatible and has the ability to support bone growth [361]. However, pure hydroxyapatite also presents some drawbacks that limit its application for bone tissue engineering, such as poor mechanical properties, reduced osteogenesis and low solubility that slows down its bone bonding rate after implantation [362].

The incorporation of silicon atoms within the hydroxyapatite matrix is known to increase its solubility, osteogenic ability and angiogenesis [363–365]. Furthermore, the Si–OH groups present in the silica surface promote the nucleation of hydroxyapatite [366], converting porous silica materials in valuable molds for the preparation of drug-loaded composite scaffolds [367,368]. In this context, we evaluated the possibility of growing hydroxyapatite in SiHDTMS_0 powder materials.

Hydroxyapatite was grown in SiHDTMS_0 by first activating the silica material with HCl/MeOH to increase the amount of silanol surface groups and then incorporating it to a calcium solution, favoring the adsorption of Ca^{2+} ions in the silica surface. Finally, hydroxyapatite was formed by adding phosphates in the Ca^{2+} -silica aqueous suspension. In particular, calcium nitrate tetrahydrate and disodium hydrogenphosphate were used as calcium and phosphate sources, respectively (see Section 3.3.10.4).

The formation of silanol surface groups upon activation was evaluated by FTIR (Figure 4.62). The FTIR spectra of SiHDTMS_0 before and after activation exhibited similar bands at 806 and 1059 cm^{-1} , corresponding to Si–O–Si groups, and the activated sample also revealed a small band at 964 cm^{-1} , which is related to the stretching vibration of the Si–O bond in silanol groups. This confirms that the activation process produced Si–OH groups in the silica surface.

The silica-hydroxyapatite composite was characterized by field-emission scanning electron microscopy equipped with energy-dispersive X-ray analysis (FESEM-EDX). SEM images

revealed a composite of entangled silica fibers in an amorphous matrix (Figure 4.63), and almost no single fibers or single amorphous matrix agglomerates were found. Moreover, EDX measurements confirmed the presence of Si, P and Ca in the composite structure (Table 4.16). EDX was measured in three regions with different morphology within the sample (see Figure 4.63b): one concentrated in fibers (1), one concentrated in the amorphous matrix (2) and one with both fibers and amorphous matrix (3), observing Si, O and Ca in all regions and P in regions 2 and 3. This indicates that the composite consists in silica fibers entangled in a hydroxyapatite matrix, which is promising for bone tissue implants, since ceramic matrices doped with fibers are known to exhibit excellent mechanical properties [369].

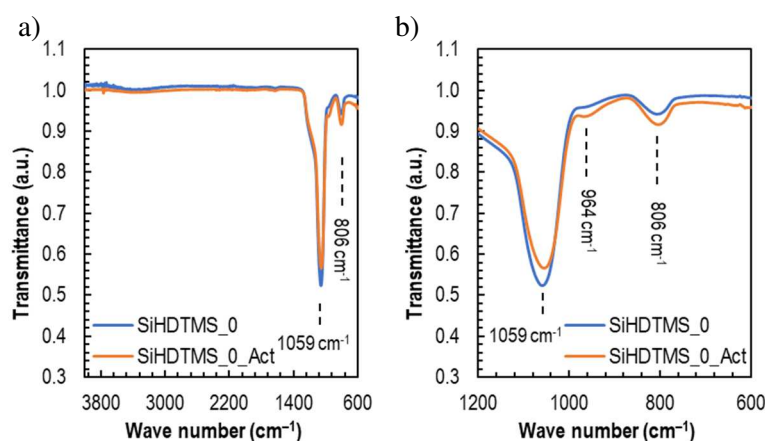


Figure 4.62. a) FTIR of SiHDTMS_0 before (blue) and after (orange) activation with HCl/MeOH. b) Selected region of the FTIR of SiHDTMS_0 before (blue) and after (orange) activation.

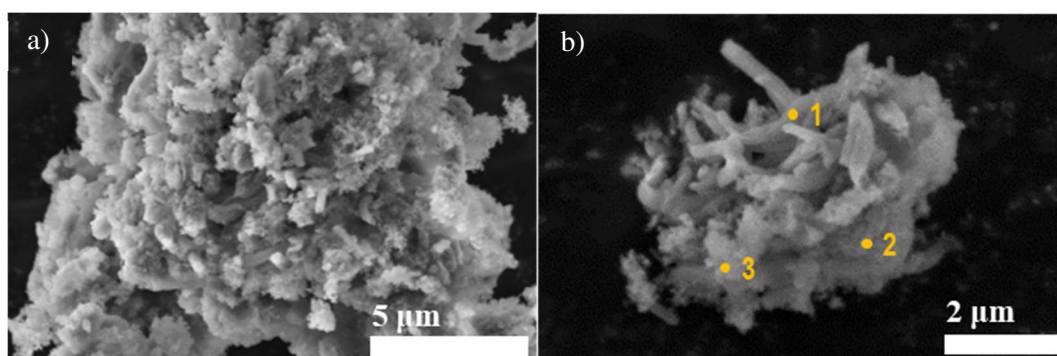


Figure 4.63. FESEM images of different regions of the SiHDTMS_0-Hydroxyapatite composite. (1) (2) and (3) indicated the three regions where EDX analysis were performed, selected because its different morphology.

Table 4.16. Atomic composition of each region of the SiHDTMS_0-Hydroxyapatite composite analyzed by EDX.

Sample region	Si (atomic %)	O (atomic %)	Ca (atomic %)	P (atomic %)
1	15.8	82.5	1.7	-
2	7.9	77.6	9.0	5.5
3	11.8	73.5	9.2	5.5

Further characterization of the porosity, mechanical properties, drug loading and release behavior, and the osteogenic properties of this composite material will be performed in the future to evaluate its applications for bone tissue engineering.

4.3.4 Conclusions

Chromonic-derived hierarchical micro- and mesoporous silica fibers were prepared using a cationic perylene diimide as template and TEOS and HDTMS as silica precursors. It was observed that the HDTMS plays the role of porogen and favors the formation of bigger pores, reducing the microporosity and pore ordering of the silica materials without affecting their morphology and chemical composition. Furthermore, the porous silica materials were prepared as monolithic structures, that take the shape of the mold where they are prepared. Both powder and monolithic silica materials were evaluated as drug delivery systems, using ibuprofen as a model drug. It was observed the encapsulation method and the material used affected the encapsulation efficiency of the system, determining higher encapsulation capacity for monoliths and powder samples loaded incorporating the ibuprofen dissolved in hexane and evaporating the solvent (soaking + drying method). The ibuprofen release profiles of the monolithic samples in simulated body fluid (SBF, pH = 7.4) were studied. It was determined that the ibuprofen release followed first-order kinetics with non-Fickian diffusion, and that it was independent of the type of monolith used. Finally, the SiHDTMS_0 powder silica fibers demonstrated to favor the growth of hydroxyapatite in presence of Ca^{2+} ions and phosphates, forming composite materials consisting in silica fibers embedded in a hydroxyapatite matrix, which are promising for bone tissue repair and replacement.

4.4 Perylene-templated N-doped hierarchically porous carbon nanofibers as efficient supercapacitor electrode materials⁹

This chapter describes the synthesis of nitrogen-doped hierarchical micro/mesoporous carbon nanofibers (CNFs) using chromonic perylene diimide (PDI) self-assemblies as templates. The method involves the formation of a PDI-templated silica similar to the one described in the Chapter 4.3 followed by carbonization and etching (Figure 4.64). This strategy does not require any additional carbon or nitrogen precursor and therefore omits the associated silica-impregnation step, since the precursor is the PDI itself. The obtained carbon nanofibers showed a high surface area (695.2 m²/g) and a well-developed porosity with a hierarchical micro- and mesopore structure, resulting from the high pore size and structure control of the templating method, along with a high sp² carbon content and heteroatom (N and O) doping, coming from the PDI precursor. This improves the CNFs' electrical conductivity and wettability, and creates redox active sites in the CNF surface which can promote fast reversible redox reactions and improve the electrochemical properties of the material. Owing to these unique properties, the carbon nanofibers were evaluated as electrode materials for supercapacitor applications, and exhibited an excellent performance as electrical double-layer capacitors with faradaic pseudocapacitance, in a three-electrode cell system with 1 M H₂SO₄ as aqueous electrolyte solution. The electrochemical system prepared with the optimal sample achieved a high specific capacitance of 251 F·g⁻¹ at a current density of 1 A·g⁻¹, with excellent capacitance retention of ~90% at a high current density of 50 A·g⁻¹. This indicates an efficient and fast electrolyte ion diffusion at the electrode surface. Additionally, the system showed an outstanding cycle stability of 99% after 10000 successive charge-discharge cycles at a current density of 50 A·g⁻¹. These results surpass those of many commercial activated carbons such as Maxsorb, highlighting the high potential of chromonic-derived hierarchically porous carbon fibers as electrode materials for high-performance supercapacitors. Furthermore, the CNFs' high microporosity and heteroatom doping prompted their evaluation as CO₂ adsorbents, revealing a CO₂ uptake of 2.02 mmol·g⁻¹ at 0 °C. The unique properties and performance of these CNFs make them promising candidates for various applications, while their preparation method offers advantages over electrospinning and catalytic fabrication techniques by avoiding the use of heavy metals and organic solvents.

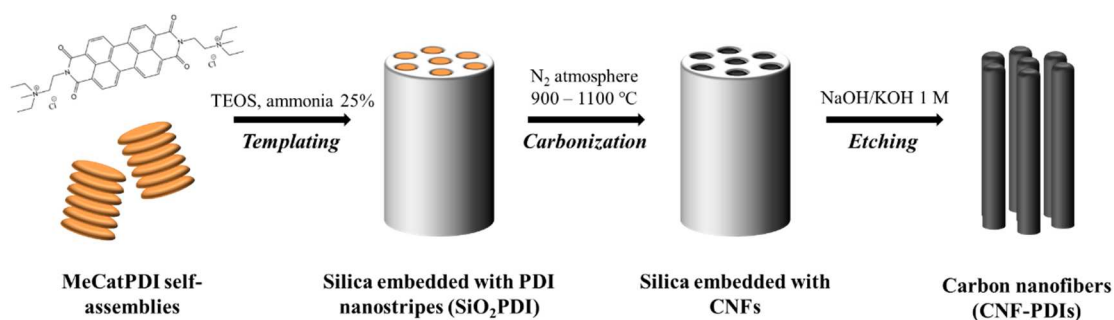


Figure 4.64. Scheme of the preparation of carbon nanofibers (CNFs) using PDI-silica composite as both carbon precursor and template.

4.4.1 Synthesis and characterization of the carbon nanofibers (CNFs)

As in Chapter 4.3, MeCatPDI (see Figure 4.64) was chosen as the chromonic template for the preparation of templated silica fibers embedded with PDI aligned nanostripes. The silica-PDI

⁹ Part of the work described in this chapter was done in collaboration with the *Supramolecules* group, directed by K. Ariga and L.K. Shrestha, in the National Institute for Materials Science (NIMS), in Japan.

composite, hereinafter called SiO₂PDI, was prepared following a procedure already described in the literature [164]. Briefly, this method consisted in dissolving MeCatPDI in aqueous ammonia 25 %, adding TEOS for a final PDI/ammonia/TEOS concentration ratio of 1:50:10, and stirring this mixture for 90 min at 25 °C and another 90 min at 70 °C, to finally recover the as-formed precipitate with vacuum filtration and washing it with a large amount of water. Similar to the PDI-silica composites obtained in Chapters 4.1 and 4.3, the precipitate was completely red and the washing water was transparent, suggesting that the amount of lost MeCatPDI is negligible, and thus, approximately all MeCatPDI was captured inside the silica framework.

In contrast to the previous chapters, where the silica-PDI composites were calcined to remove the organic phase and obtain microporous silica, here the composite was carbonized in an inert nitrogen atmosphere to convert the organic PDI in carbon nanofibers, which were recovered by removing the silica template with a basic etching solution that dissolved SiO₂. A total of four CNF samples were prepared varying the carbonization temperature (900 – 1100 °C) and the etching agent (NaOH 1 M or KOH 1 M). These samples are named as CNF-PDI_N900, CNF-PDI_N1000, CNF-PDI_N1100 and CNF-PDI_K900, where the letter and the number indicate the etching agent (N for NaOH and K for KOH) and the carbonization temperature, respectively.

Besides the already discussed properties of MeCatPDI associated to its rich chromonic behavior [100,370], relatively low-cost and superior templating capacity to form silica fibers with high aspect-ratio [164], this PDI also has a high amount of sp² carbon atoms within its structure (63 % of the total carbon) and presents a significant number of heteroatoms (8 wt% nitrogen and 9 wt% oxygen), that can increase the conductivity, the wettability and the heteroatom doping of the final CNFs, which is beneficial for electrochemical energy storage applications [172,371–374]. Moreover, a thermogravimetric analysis (TGA) of MeCatPDI in a nitrogen atmosphere demonstrated that the selected PDI is a good carbon precursor that provides a 33 % carbon yield (Figure 4.65) after carbonization. Additionally, it simultaneously serves as a structure-directing agent and a carbon source, thus avoiding an extra impregnation step of the silica with a carbon source, such as furfuryl alcohol or sucrose, in the synthesis. Furthermore, there is no need to consume a sacrificial surfactant, which is calcined to form pores, to be subsequently filled with a carbon precursor [375].

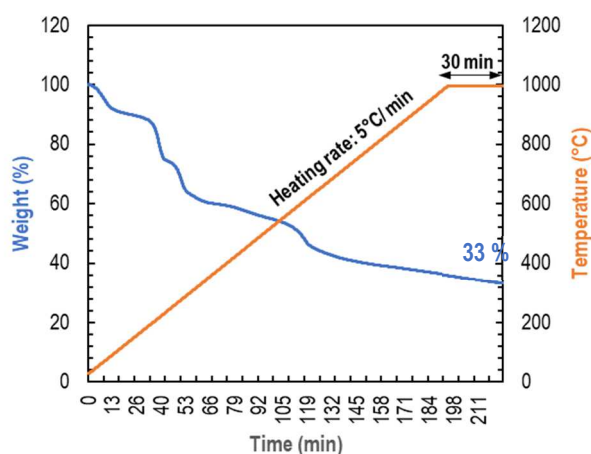


Figure 4.65. TGA of MeCatPDI in a nitrogen inert atmosphere. Protocol: heating from 20 to 1000 °C at 5 °C/min and leaving the sample 30 min at the maximum temperature.

Both SiO₂PDI (the material before carbonization) and the CNF-PDIs were characterized by SAXS (Figure 4.66). SiO₂PDI showed two broad peaks at ~ 2.8 and ~ 4.8 nm⁻¹, which are at a 1: $\sqrt{3}$ ratio, suggesting a hexagonal structure, as evidenced by previous TEM observations [164]. Interestingly, the repetition distance of the SiO₂PDI material calculated with Eq. 2 is around 2.2 nm, equivalent

to a pore wall thickness of 0.8 nm, significantly thinner than those of the $(\text{PEG}_n)_2\text{PDI}$ templated silica described in the Chapter 4.1. This was attributed to the effect of the PEG chains in widening the silica pore walls. Differently, none of the prepared CNF-PDIs (materials after carbonization and etching) showed any resolved peak in the SAXS spectra, which suggests the loss of the hexagonal structure. This may result from a lack of interconnections between the perylene aligned nanostripes in the silica-perylen composite material that prevented the formation of carbon bridges between the fibers, thus making the hexagonal structure collapse during the etching step. In fact, the importance of the pore interconnections in the silica host material for the preservation of the template structure has been widely assessed in the literature^[259,376]. For example, Ryoo et al.^[377] described the formation of a cubically ordered mesoporous carbon material (CMK-1) obtained using the $Ia3d$ cubic aluminosilicate MCM-48 as the template and sucrose as the carbon source. Interestingly, CMK-1 cubic structure was not exactly the same as that of MCM-48, as it consisted in a $I4_1/a$ structure rather than a $Ia3d$. Instead, when using the MCM-48 as template but impregnating with the carbon source by chemical vapor deposition, the $Ia3d$ structure was retained^[378]. Another revealing example is the synthesis of carbon materials from MCM-41^[379] and SBA-15^[260] silica templates. Both silica materials are formed by hexagonally arranged, one-dimensional cylindrical pores. However, meanwhile there is no pore interconnections for MCM-41, small micropores connect the aligned pores in SBA-15^[380]. Consequently, only the carbon replica materials obtained from SBA-15 (known as CMK-3) retained the hexagonal parent structure. The reason why SBA-15 presented more pore interconnections than MCM-41 results from SBA-15 being prepared using a PEO-PPO-PEO triblock copolymer (Pluronic P123), whose polyethylene oxide (PEO) chains penetrate within the silica walls during the synthesis, forming bridging micropores upon calcination^[255,256].

Interestingly, despite the CNF-PDIs described herein do not maintain the parent silica structure, the $(\text{PEG}_n)_2\text{PDI}$ templated silica described in Chapter 4.1 could allow the preparation of ordered carbon materials that preserve the initial structure. Note that MCM-41 and SBA-15 structures are hexagonal, i.e. similar to that of the chromonic PDI templated silica. Still, the absence of a hexagonal structure in CNF-PDIs does not necessarily mean that these materials are not porous, hence further characterization with electron microscopy and nitrogen-sorption is required to determine if CNF-PDIs are promising for electrochemical applications. Actually, Magana et al. recently reported silica-templated carbon materials, using furfuryl alcohol as carbon source which showed high surface area and superior electrochemical energy storage behavior despite not preserving the parent silica structure^[165].

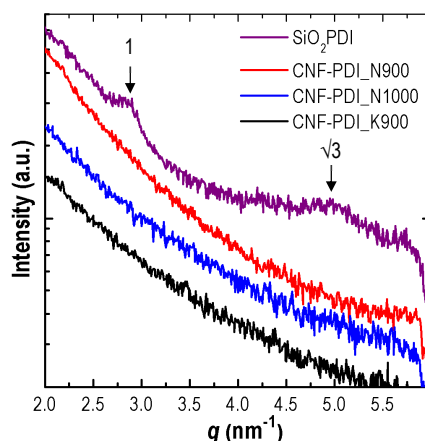


Figure 4.66. SAXS patterns of the silica and CNF-PDI materials. Two broad peaks with relative positions $1:\sqrt{3}$ appear for SiO_2PDI , indicating a hexagonally ordered structure in the non-carbonized sample.

The four prepared CNF-PDIs and SiO₂PDI samples were further characterized by electron microscopy. Low-resolution SEM images showed that SiO₂PDI samples are constituted by long silica fibers (Figure 4.67a), as it has been described in the literature ^[164]. Also, low-resolution SEM demonstrated the absence of fibers for CNF-PDI_N1100 (Figure 4.67b,c). Instead, an amorphous material (Figure 4.67c) was obtained. This can be attributed to a collapse of the fibrous structure at the very high temperature used (1100 °C) during the carbonization. Note that the low-resolution SEM image of the carbonized silica-carbon composite material before etching only showed few fibers, being most of them broken into small pieces (Figure 4.67b). Consequently, it was concluded that 1100 °C is a too high temperature. Thus, CNF-PDI_N1100 was not further studied, since the main objective of the experiments was to produce carbon nanofibers.

Field-emission SEM observations with higher resolution confirmed the fibrillar structure of CNF-PDI_N900, CNF-PDI_N1000 and CNF-PDI_K900 (Figure 4.67d-f). The three samples consisted in long entangled carbon nanofibers with high aspect ratio and diameters between 100 and 500 nm. Besides, HR-TEM images showed the presence of disordered pores in the CNF-PDI fibers (Figure 4.67g-i), with an absence of stacked graphene layers, pointing to a lack of crystalline graphite in the structure of CNF-PDIs.

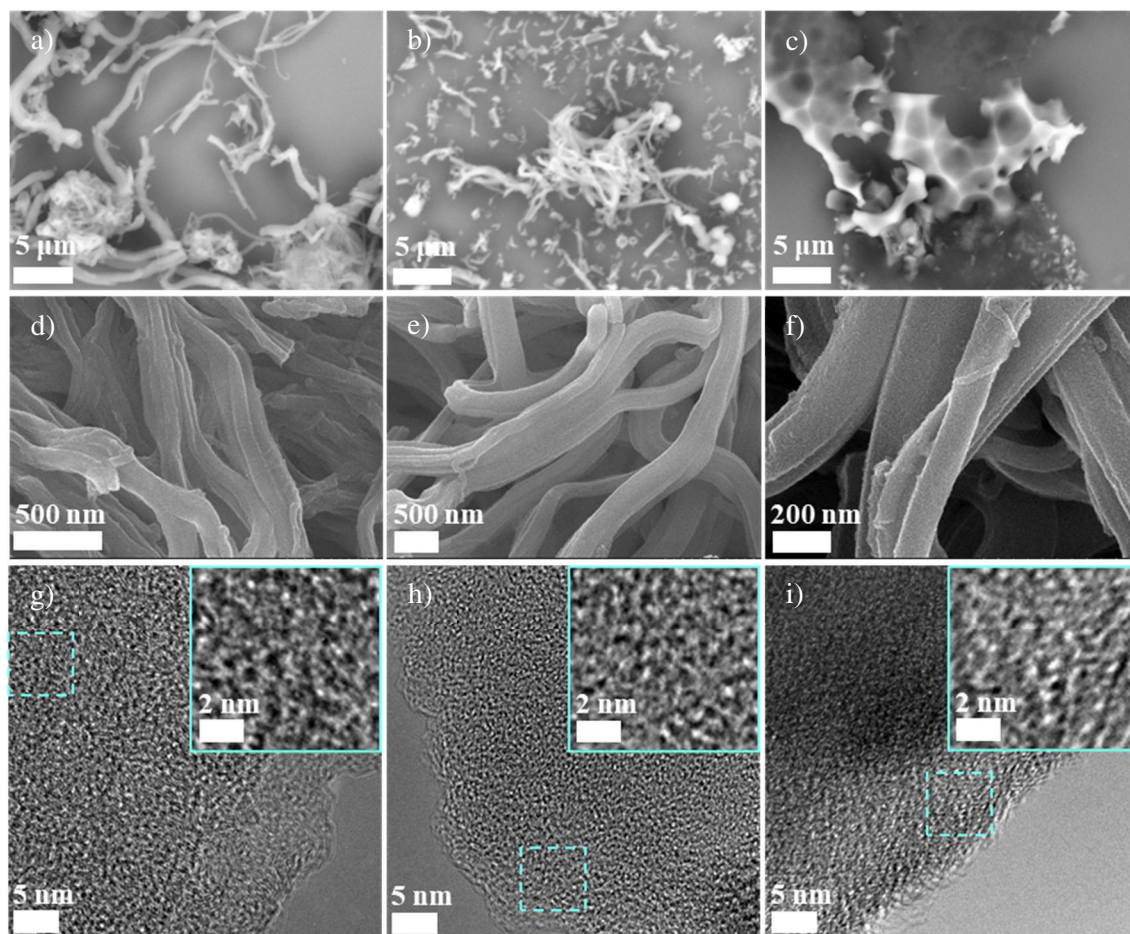


Figure 4.67. Low-resolution tabletop SEM images of a) SiO₂PDI, b) Silica–CNF-PDI_N1100 composite before etching, and c) CNF-PDI_N1100 after etching. A loss of the fibrillar structure can be clearly observed. High-resolution FESEM images of d) CNF-PDI_N900, e) CNF-PDI_N1000 and f) CNF-PDI_K900. HR-TEM images of g) CNF-PDI_N900, h) CNF-PDI_N1000 and i) CNF-PDI_K900. Inset g – i) Zoom-in of selected regions, showing small disordered pores.

X-ray diffraction (XRD) and Raman scattering were used to confirm the absence of crystalline graphite in the prepared CNFs. XRD (Figure 4.68a) showed two broad bands at ~26 ° and ~44 °,

corresponding to the (002) and the (100) graphite planes, respectively. However, no sharp peaks expected from graphite with high crystallinity were observed, which suggests that the CNF-PDI materials are composed of amorphous disordered graphene layers^[381]. Notably, the XRD (002) band, which corresponds to stacked graphite layers, gets sharper at lower carbonization temperatures, which suggests a reduced number of defects at lower carbonization temperatures. The (002) XRD signal seems to contrast with the absence of stacked graphite layers inferred by HR-TEM. A possible explanation for this is that the CNF-PDI samples are disordered conglomerates of small nanocrystallites. This structure, denoted as turbostratic^[382], is an intermediate structure between crystalline graphite and amorphous carbon. It is caused by a high number of defects within the graphitic carbon structure, which produces misalignment between the stacked graphene nanosheets, resulting in a broadening of the XRD graphite peaks^[382].

The Raman scattering spectra (Figure 4.68b) exhibited the usual graphite band at 1582 cm^{-1} , also known as G-band, and another prominent band at around 1350 cm^{-1} , also called D-band, which is assigned to a disordered graphite structure with a high number of defects. The presence of both G and D bands along with the absence of a third band at $\sim 2700\text{ cm}^{-1}$, corresponding to the so-called 2D or G' band, which commonly appear for highly crystalline materials, confirm the graphitic nature of the synthesized CNFs with a disordered structure and a high defect concentration (turbostratic). Moreover, analyzing the ratio between the G and D bands (I_G/I_D), values above 1 are determined for the three CNF-PDIs (1.10 for CNF-PDI_K900, 1.07 for CNF-PDI_N900 and 1.04 for CNF-PDI_N1000). Such a ratio is typically used to quantify the defect concentration present in carbon materials such as carbon nanofibers or carbon nanotubes^[383]. Interestingly, ratios above 1 indicate a graphitic nature of the materials, meanwhile below 1 suggest a very high number of defects^[384,385]. Herein, the I_G/I_D values are close to this critical value and decrease as carbonization temperature increases, hence confirming the results inferred from HR-TEM and XRD. A similar behavior has been reported for biomass derived carbon materials, whose ratio decreased, and hence the defect concentration rose, as carbonization temperature was increased from $600\text{ }^{\circ}\text{C}$ to $1000\text{ }^{\circ}\text{C}$ ^[386].

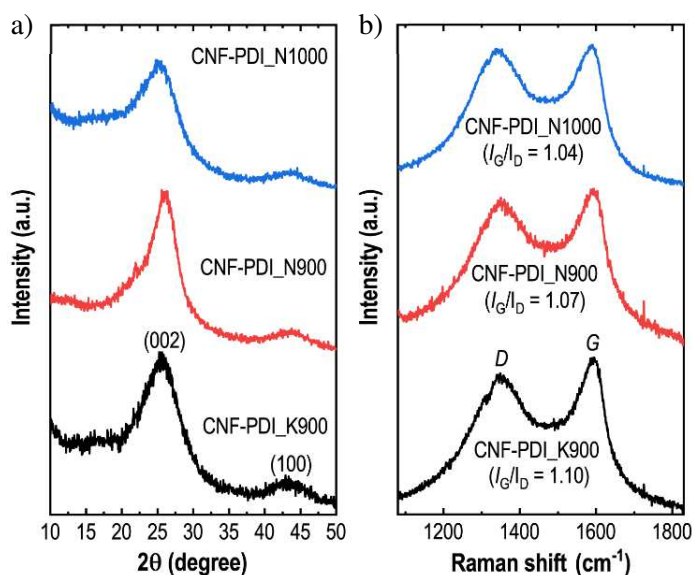


Figure 4.68. a) XRD patterns, and b) Raman scattering spectra of CNF-PDI_K900, CNF-PDI_N900, and CNF-PDI_N1000 samples. I_G/I_D represents the ratio between the Raman G and D bands.

Notably, the porous amorphous carbon nanofibers described herein are obtained by an organic solvent-free method and in the absence of heavy metals, contrary to other typical methods for the fabrication of CNFs such as electrospinning^[387–389] and chemical vapor deposition (CVD)^[390]. Consequently, the templating method appears as a greener alternative to those conventional techniques.

Besides the aqueous synthesis and the absence of toxic heavy metals, one of the main advantages of the templating method is the high control over the CNF pore size and structure^[391,392]. Physisorption measurements were performed (Figure 4.69) to obtain new insights of the surface area, pore dimensions and pore volumes of the CNF-PDI materials. The nitrogen adsorption-desorption isotherms of all the samples displayed a significant nitrogen uptake at low relative pressures and gradual increase in nitrogen uptake at higher relative pressures. Particularly, CNF-PDI_N900 sample exhibited a characteristic Type-IV adsorption isotherm (Figure 4.69a), with a distinct hysteresis loop at relatively high pressures, indicating the presence of hierarchically micro- and mesopore structures within the sample. This observation is further supported by the pore size distribution profiles through the density functional theory, or DFT (Figure 4.69b), and BJH methods (Figure 4.69c). The three CNF-PDI materials showed similar micropore widths between 0.55 and 0.6 nm, and mesopore diameters between 3.1 and 3.7 nm (Table 4.17). The micropore diameter (~0.6 nm) is slightly thinner than the parent silica pore wall thickness (~0.8 nm, as revealed by SAXS, Figure 4.66), suggesting a shrinking of the SiO₂PDI structure during the heat treatment. Note that CNF-PDI micropores result from the silica etching and hence should present similar dimensions to the parent silica pore wall thickness. Moreover, the total pore volumes, calculated from the last point of the isotherms ($P/P_0 \approx 0.99$) were of 0.455 (CNF-PDI_K900), 0.671 (CNF-PDI_N900) and 0.453 cm³·g⁻¹ (CNF-PDI_N1000).

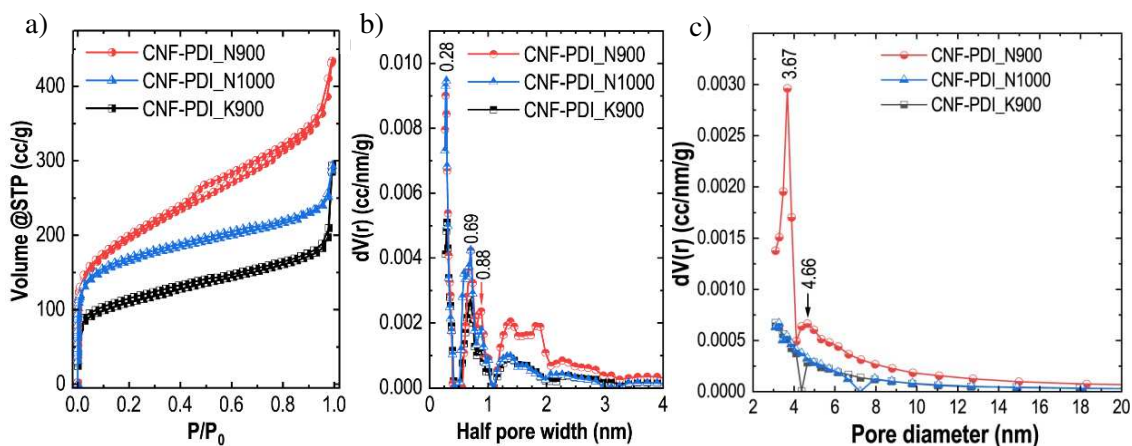


Figure 4.69. a) Nitrogen sorption isotherms of CNF-PDI_K900, CNF-PDI_N900, and CNF-PDI_N1000, and b) the corresponding pore size distribution profiles obtained from the DFT method and c) pore size distribution from the BJH method.

The specific surface areas (SSA) of the different CNF-PDIs (as shown in Table 4.17) were calculated with the BET model, from the adsorption isotherm at low pressures. All BET linear fits had coefficients of determination (R^2) above 0.9999, confirming the reliability of the results. The BET surface areas of CNF-PDIs demonstrated a considerable increase depending on the etching agent, from 392 m²·g⁻¹ for CNF-PDI_K900 to 695 m²·g⁻¹ for CNF-PDI_N900, and a slight decrease to 602 m²·g⁻¹ for CNF-PDI_N1000, when the temperature was increased. This slight reduction in the surface area at higher temperatures is generally attributed to a pore disruption. The unexpectedly low surface area of CNF-PDI_K900 was attributed to sintering and

blocking of the micropores by remaining non-etched silica or salt. Note that CNF-PDI_K900 has a similar total pore volume to CNF-PDI_N1000 but a much lower surface area, indicating a reduced microporosity for this sample. Additionally, it is worth noting that KOH and NaOH, used as silica etching agents in the CNF synthesis, are commonly employed for the chemical activation of carbon, which not only generates additional microporosity but also introduces various chemical species on the carbon surface [393].

Table 4.17. Porosity properties of PDI-CNFs samples. SSA = specific surface area, V_p = total pore volume, W_p = pore width obtained from the DFT method, D_p = average mesopore diameter obtained from the BJH method.

Sample	SSA ($\text{m}^2 \text{g}^{-1}$)	V_p ($\text{cm}^3 \text{g}^{-1}$)	W_p (nm)	D_p (nm)
CNF-PDI_K900	392	0.46	0.60	3.1
CNF-PDI_N900	695	0.67	0.55	3.7
CNF-PDI_N1000	602	0.45	0.57	3.3

TGA in oxygen atmosphere (Figure 4.70) showed the formation residue corresponding to the 7 wt% that persisted even at 600 °C. This finding suggests that the silica is not completely etched and/or there are some remanent inorganic species adsorbed on the carbon material, which may cause micropore blocking and a decrease in the accessible surface area. The presence of residual silica after the etching has been also reported in previous works [165,392,394]. Additionally, TGAs showed a slight weight loss at ~80 °C, which could result from adsorbed water. Notably, the pH of resuspended CNF-PDIs in water was almost neutral (approximately 7.5), which indicates that any remanent of the etching agents (NaOH and KOH) should be in form of COONa or COOK on the carbon surface, but not as free basic hydroxide remaining in the sample, which would cause a much more basic pH. All these results confirmed that the highest porosity is achieved by carbonizing at 900 °C and etching with NaOH.

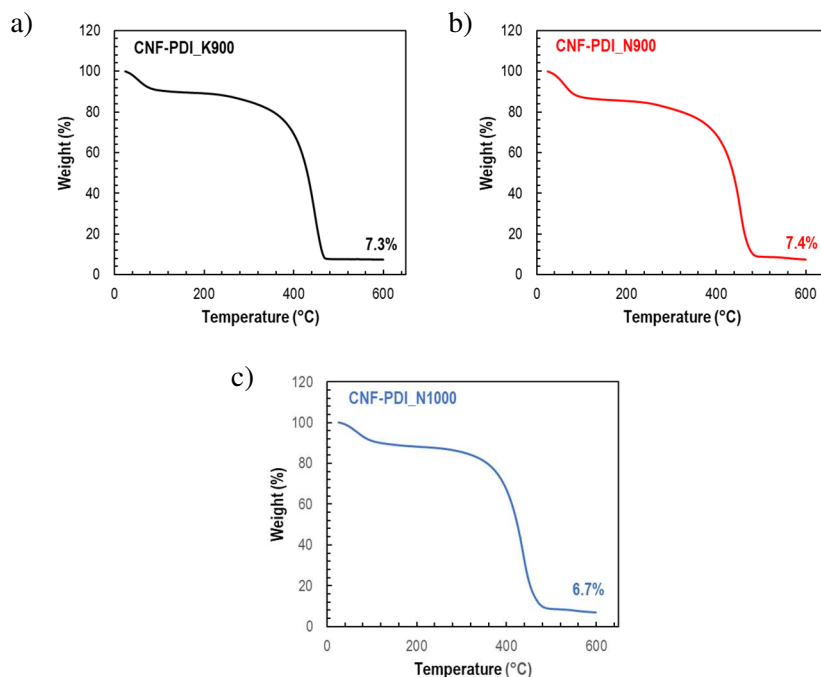


Figure 4.70. TGA of a) CNF-PDI_K900, b) CNF-PDI_N900 and c) CNF-PDI_N1000 in oxygen atmosphere.

It can be expected that the CNF-PDI materials have a relatively high heteroatom, namely nitrogen (N) and oxygen (O) doping, because of the high heteroatom (N and O) content of the PDI and the etching with NaOH/KOH. XPS and FTIR analysis were used to reveal the heteroatom concentration and composition of the synthesized carbon nanofibers.

XPS analysis revealed the presence of nitrogen and oxygen on the surface of the CNF-PDIs, with concentrations of approximately 2.5 % and 16 %, respectively (Figure 4.71). Upon careful examination, it was observed that the sample carbonized at higher temperature (CNF-PDI_N1000) exhibited a lower N-doping (Figure 4.71a). These relatively high concentrations of heteroatoms are expected to enhance the wettability and dispersibility of CNF-PDIs in water^[395–397], and contribute to an increased the electrochemical energy storage capacity of the materials in supercapacitor devices^[398–400].

The high-resolution XPS C 1s spectra of the CNF-PDIs were deconvoluted into four different peaks centered at approximately 284.1, 284.5, 285.7, and 289.1 eV that correspond to C=C (sp^2), C–C (sp^3), C–N (sp^3) and C=O bonds, respectively (Figure 4.71b). The XPS N 1s spectra were deconvoluted into three peaks at 398.1, 400.2, and 401.8 eV, representing pyridinic, pyrrolic and graphitic nitrogen, respectively (Figure 4.71c). Interestingly, while the intensity of the pyridinic-N peak is similar for all CNF-PDIs, the ratio between pyrrolic-N and pyridinic-N peaks was the highest for CNF-PDI_N900 (~1.35) compared to CNF-PDI_N1000 and CNF-PDI_K900 (~1.20), suggesting a higher formation of pyrrolic moieties for this sample. The graphitic-N peak intensity was the lowest for all samples. It is important to note that N-doping of carbon fibers in this case is endogenous and occurs in-situ during the carbonization of MeCatPDI. Similarly, the XPS O 1s spectra of the carbon fibers were deconvoluted into three peaks centered at 530.3, 531.9, and 533.2 eV corresponding to the C=O, O–(C=O)–C and O–C–O bonding states of oxygen, respectively (Figure 4.71d). Briefly, the three CNF-PDI materials showed a rich heteroatom content, coming from the nitrogen and oxygen moieties of MeCatPDI, and from the basic etching agents.

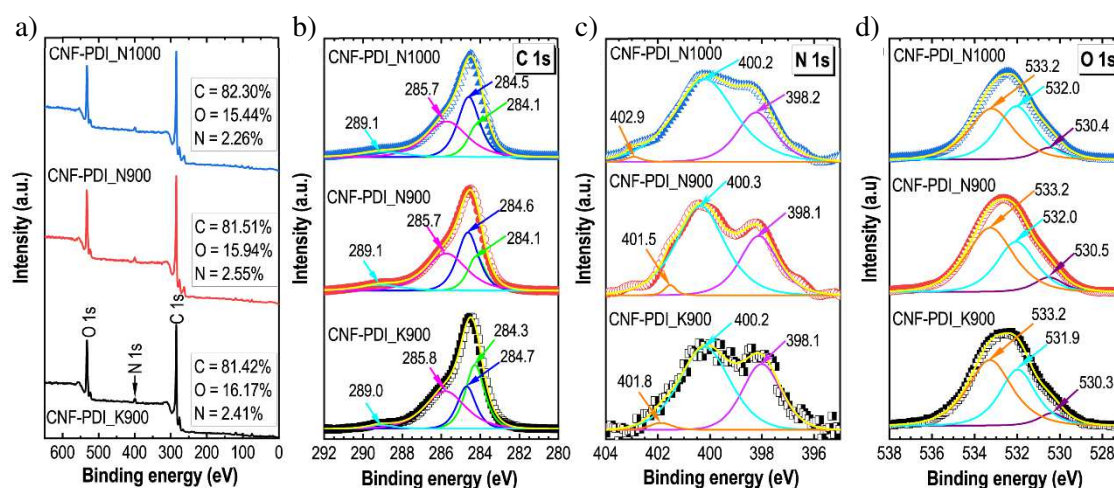


Figure 4.71. a) XPS survey spectra of CNF-PDI nanofibers, b) corresponding XPS C 1s spectra with the deconvoluted peaks, c) XPS N 1s spectra with the deconvoluted peaks and d) XPS O 1s spectra with the deconvoluted peaks.

Baseline-corrected FTIR spectra (Figure 4.72) provided further confirmation of the presence of C–X (X = C, N, O) bonds^[401]. Several characteristic bands were observed in the spectra. The wide band around 3300 cm^{-1} was attributed to the stretching modes of O–H, which is typical of water, and N–H bonds. Two small sharp bands at 2915 and 2870 cm^{-1} were assigned to C–H

stretching modes. Additionally, a small and broad band appeared at 1800 cm^{-1} , suggesting the presence of C=O stretching modes of carboxylic groups. Two overlapped strong bands appeared at approximately 1550 and 1450 cm^{-1} , suggesting the presence of C=X (X = C, pyridinic-N) and C–C stretching modes, respectively. Another group of overlapped prominent bands in the range from 1100 to 1250 cm^{-1} indicated the existence of C–X (X = N, O) groups. Two weak bands at 950 and 850 cm^{-1} were attributed to O–H and N–H out-of-plane deformation vibrations, respectively ^[402].

Notably, for CNF-PDI_N900, the overlapped bands between 1050 and 1250 cm^{-1} were stronger compared to the other carbons, particularly at 1100 cm^{-1} . This was attributed to C–O–C groups and, especially, pyrrolic-N moieties on the carbon surface ^[403]. It is worth noting that pyridinic-N produces a FTIR signal at 1550 cm^{-1} , and its intensity is very similar for all CNF-PDIs, and the graphitic-N FTIR fingerprint (1330 cm^{-1}) is absent in the three studied materials. This supports the conclusions drawn from XPS regarding N-doping, and confirms the higher amount of pyrrolic-N for CNF-PDI_N900. Interestingly, some reports have suggested that pyrrolic-N may have more beneficial effect than pyridinic-N for high-performance supercapacitors ^[404].

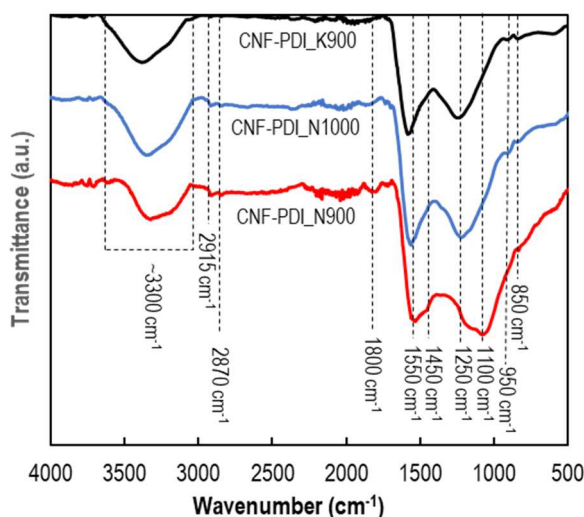


Figure 4.72. FTIR of CNF-PDI_K900, CNF-PDI_N1000 and CNF-PDI_N900. The main bands are labelled. Note that the spectra of CNF-PDI_K900 and CNF-PDI_N1000 have been vertically shifted to facilitate visualization.

The high concentration of heteroatoms (N and O) and polar functional groups such as C=O, O–(C=O)–C or pyrrolic- and pyridinic-N facilitated the dispersion of CNF-PDIs in water. In fact, this high water-dispersibility permitted the straightforward fabrication of carbon films by drop-casting a dispersion of these materials in water onto a glass substrate, and flattening it with a roller. The resulting films were prepared with the CNF-PDIs before the lyophilization step (after the washing, see a more detailed synthetic method in Section 3.3.18), where a viscous black ink-like dispersion of the CNFs was obtained. The resistivity of the films was measured with a digital multimeter. It was found that the samples carbonized at higher temperatures (1000 and 1100 °C) were significantly more electrically conductive than those prepared at 900 °C , which displayed a quite poor electrical conductivity (Table 4.18). In order to ensure that the prepared CNFs are electrically conductive materials, which is required for supercapacitor applications, carbonizations temperatures below 900 °C were not used. The results obtained by carbonizing at 900 and 1000 °C are promising for the formulation of colloidal CNF aqueous dispersions, such as conductive inks for printed electrodes ^[405] or CNF-polymer composites for solar cells ^[406].

Table 4.18. Resistivity of the CNF-PDI films. The measurements were performed with the multimeter set at the 200 M resistivity mode. The distance between the two electrodes was approximately 1 cm.

Sample	Resistivity ($M\Omega$)
CNF-PDI_K900	16.1
CNF-PDI_N900	32.4
CNF-PDI_N1000	4.5
CNF-PDI_N1100	1.4

To further evaluate the CNF-PDIs dispersibility, diluted dispersions of CNF-PDI_N900 were prepared in several solvents, including water, aqueous 100 mM NaCl solution, dimethyl formamide (DMF), chloroform and toluene. The dispersion process involved stirring the samples overnight followed by ultrasonication using an ultrasound bath for 15 min and with an ultrasonic tip (with 5-second pulses) for 1 min. The ability of carbon-based materials to disperse well, particularly in water, is crucial for their bio-applications, as it allows the preparation of films and coatings under mild conditions and using non-toxic solvents ^[395,407].

Visual observation of the dispersions (Figure 4.73), indicated that DMF exhibited the best dispersibility for CNF-PDIs, which is consistent with previous findings about carbon nanotubes ^[408]. Chloroform also showed relatively good capacity to disperse CNF-PDI_N900. Interestingly, the sample displayed good dispersibility in water, as confirmed by Turbiscan analysis (Figure 4.74). The Turbiscan Stability Index (TSI), which enables the comparison of the stability of different samples, was rather low during the first hours. Additionally, the increase on the transmittance was moderate. This suggests a slow and progressive sedimentation of the carbon sample, indicating that CNF-PDI_N900 can remain dispersed in water for at least few hours. In contrast, the 100 mM NaCl solution and toluene could not disperse CNF-PDI_N900, which was attributed to the presence of polar groups on the carbon surface.

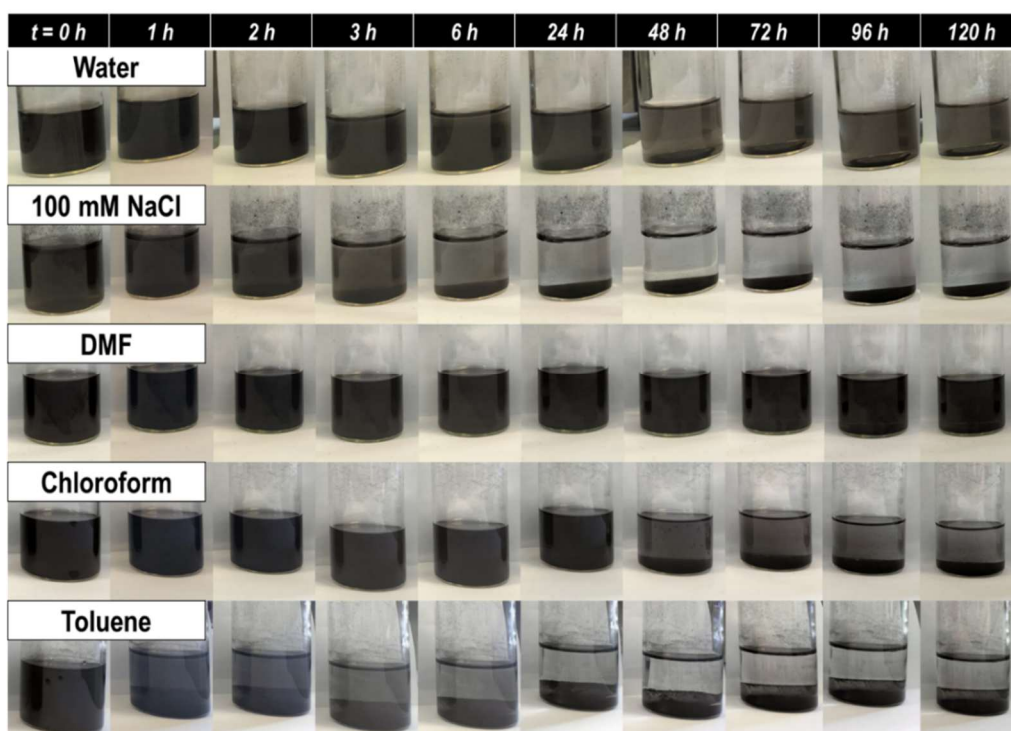


Figure 4.73. Colloidal stability of CNF-PDI_N900 dispersed in different solvents.

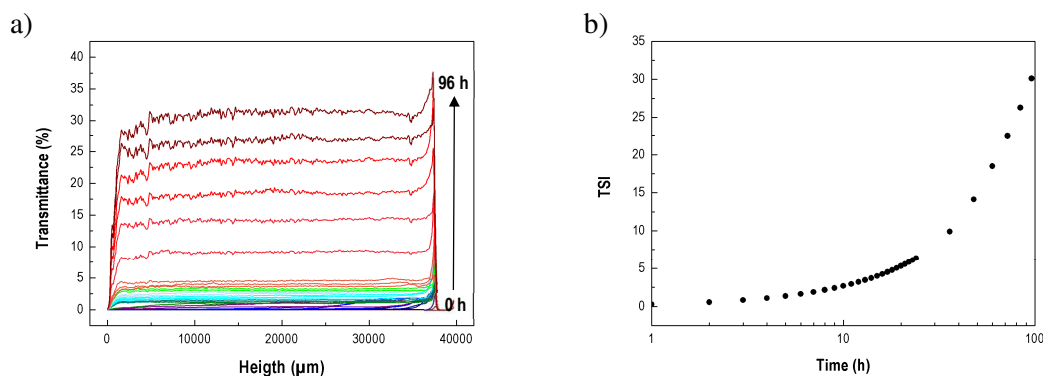


Figure 4.74. Variation of a) transmittance and b) TSI values with time for CNF-PDI_N900 suspended in water. The sample was measured every hour during the first 24 hours and every 12 hours for the following three days.

In summary, CNF-PDI materials consist in disordered graphitic high aspect-ratio porous carbon nanofibers with high surface area and hierarchical micro- and mesopores. Furthermore, the CNFs show a high amount of sp^2 carbon and are doped with nitrogen (~2.5 %) and oxygen (~16 %), which gives them a high wettability and dispersibility in water and other solvents such as DMF and chloroform.

This combination of properties makes CNF-PDIs promising candidates as electrode materials for supercapacitors [166,409,410]. As described in Section 1.3.4, supercapacitors are high-capacity devices with low internal resistance capable of storing energy by forming an electric double layer on the electrode-electrolyte interface upon the application of a voltage. Since the energy storage occurs at the electrode-electrolyte interface, high surface area materials are required to improve the total specific capacitance of the device. Also, supercapacitors are intended to work as ultrafast charging/discharging devices, hence having hierarchical micro- and mesopores and a high wettability is vital for the electrolyte to penetrate to the electrode surface rapidly and facilitate rapid charge/discharge of the device in a short time [411].

Moreover, the presence of N-doping within the carbon framework induces the formation of electrochemically active sites that promote fast and reversible redox reactions, thus increasing the total specific capacitance of the material without affecting other unique properties of supercapacitors such as the long-life cycle or the fastness on charging/discharging [412,413]. Additionally, nitrogen functionalities have a free electron-pair that can work as a Lewis base, which along with the large surface area and microporosity, convert CNF-PDIs in promising materials for the capture of Lewis acids such as carbon dioxide [414]. Both the electrochemical properties and CO_2 capture capacity of CNF-PDIs are addressed in the following sections.

4.4.2 Electrochemical energy storage evaluation

The electrochemical energy storage (EES) properties of the prepared CNF-PDIs were evaluated in a three-electrode system with aqueous 1 M H_2SO_4 as electrolyte solution. CNF-PDIs were deposited onto a clean glassy carbon electrode, which was used as the working electrode. Ag/AgCl and a Pt wire were used as reference and counter electrode, respectively. Cyclic voltammetry (CV), chronopotentiometry (galvanostatic charge discharge or GCD) and electrochemical impedance spectroscopy (EIS) were measured (see Section 3.3.17).

CV curves of CNF-PDI_K900, CNF-PDI_N900, and CNF-PDI_N1000 samples were obtained at a constant scan rate of $5 \text{ mV} \cdot \text{s}^{-1}$ (Figure 4.75a). These curves exhibited quasi-rectangular shapes, which are indicative of an electrical double-layer charge storage mechanism. However, there was

a deviation from the ideal rectangular shape due to the presence of broad symmetric peaks in the range of 0.3 to 0.45 V. These peaks were attributed to surface redox reactions caused by the presence of heteroatoms (N and O) in the carbon fibers [171]. Significantly, these electrochemical processes occurring in the surface of the carbon electrode may be either of capacitive or battery-like nature depending on the kinetics of the process, which is diffusion-limited for battery-like materials but it is not for capacitive materials. Making a proper differentiation between faradaic capacitive materials (pseudocapacitors) and battery-like materials is vital as the calculation of electrochemical parameters such as capacitance or capacity highly depend on a proper classification of the materials, i.e., determining the capacitance is only correct for capacitive (and pseudocapacitive) devices, meanwhile capacity should be calculated for batteries [178,179].

The CV curves of CNF-PDIs at higher scan rates (Figures 4.75b-d) can help to properly discriminate between batteries and pseudocapacitors. Such curves revealed that the quasi-rectangular shape was maintained even at high scan rates ($500 \text{ mV} \cdot \text{s}^{-1}$) for the three materials, which should occur for capacitive-like materials. Nevertheless, there are some significant differences in the CV curves depending on the CNF-PDI used. For CNF-PDI_N900, the CV curve shape was retained with almost no alterations from 5 to $500 \text{ mV} \cdot \text{s}^{-1}$, which is ideal for capacitive materials (Figure 4.75c). However, both CNF-PDI_K900 and CNF-PDI_N1000 (Figure 4.75b and d, respectively) showed rounding of the CV curves at the beginning of the charging and discharging, which is non-ideal and results from an increase on the internal resistance of the system and suggests a low capacitance retention at high scan rates [415,416].

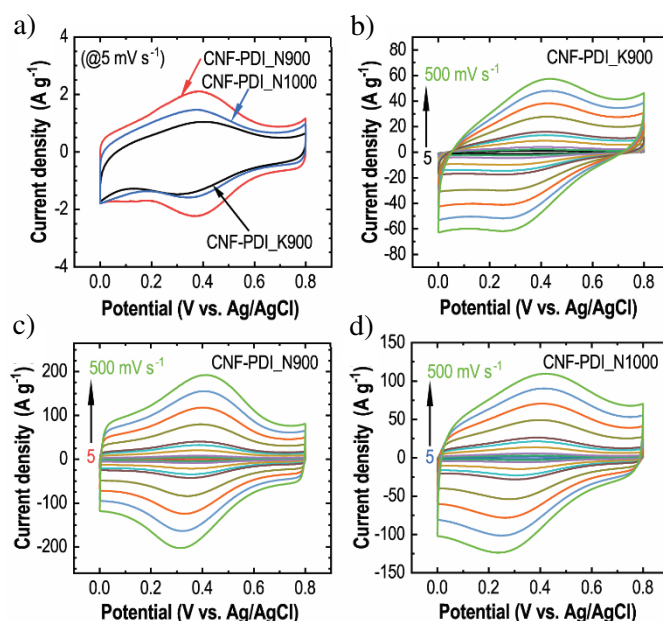


Figure 4.75. a) Comparison of the CV profiles of CNF-PDIs carbon materials at fixed scan rate of 5 mV s^{-1} , and the corresponding CV profiles of (b) CNF-PDI_K900, (c) CNF-PDI_N900, and (d) CNF-PDI_N1000, at different scan rates from $5 - 500 \text{ mV s}^{-1}$ at 25°C .

Furthermore, broad symmetric faradaic peaks in the CV curves suggest a pseudocapacitive behavior, meanwhile for batteries the peaks should be sharper and asymmetric. Notably, the three materials tested herein manifested broad symmetric peaks at low and high scan rates, hence suggesting a capacitive behavior. In fact, in Table 4.19, the values of the voltage at the center of the faradaic peaks for the three samples at all the scan rates tested are shown, observing that there

is almost no displacement in such voltage (ΔV) for CNF-PDI_N900 between its charging and discharging curves, confirming its highest capacitive behavior among the prepared CNFs.

Studying the kinetics of the charge storage mechanism is the key for the differentiation between pseudocapacitive and battery materials. This is accomplished by studying the relation between the current density and the scan rate, that is generally expressed as ^[179,417,418]:

$$i = a \cdot v^b \quad (\text{Eq. 21})$$

Where i is the current density ($\text{A} \cdot \text{g}^{-1}$), v is the scan rate ($\text{mV} \cdot \text{s}^{-1}$) and a and b are parameters that define the kinetics of the systems. Particularly, the kinetics are generally described by b . For pseudocapacitor materials, b should be close to 1, hence showing a linear relationship between the current density and the scan rate; meanwhile batteries show b values of 0.5, making the current density proportional to the square root of the scan rate.

In Table 4.19 the values of current density at the maximum (and minimum) of the discharging (and charging) CV curves at all scan rates tested are shown. Note that this maximum corresponds to the center of the broad peak caused by the redox reactions. By plotting such current density values as a function of the scan rate (Figure 4.76), b values very close to 1 are obtained for CNF-PDI_N900 (0.98 charging, 0.98 discharging) and CNF-PDI_N1000 (0.94 charging, 0.95 discharging) in both the charging and discharging processes, meanwhile CNF-PDI_K900 exhibited a lower b value of 0.86 and 0.81 from the charging and discharging curves, respectively.

Summarizing, the CV curves shape, symmetry and the determined kinetics demonstrate the pseudocapacitor behavior of CNF-PDIs, specially for CNF-PDI_N900 ($b = 0.98$). On the other hand, CNF-PDI_K900 show the highest deviations from an ideal pseudocapacitor material and its b values suggest partially diffusion-limited processes, which may result from its decreased surface area, reduced microporosity and partial pore blocking. Still, the prepared CNF-PDIs-based three-electrode systems behave as pseudocapacitors rather than batteries, and will be treated from now on as capacitive devices.

Specific capacitances (C_s) of the CNF-PDI materials were calculated from the CV curves with Eq. 17, as explained in Section 3.3.17. Accordingly, samples with a higher total integral current (I_{faradaic} , from Table 4.19) should show higher specific capacitances. Consequently, CNF-PDI_N900 is expected to give the highest specific capacitance among the samples studied, meanwhile CNF-PDI_K900 should provide the lowest specific capacitance.

Using Eq. 17 with the CV curves at $5 \text{ mV} \cdot \text{s}^{-1}$ (Figure 4.75a), the calculated C_s are ca. $280 \text{ F} \cdot \text{g}^{-1}$ for CNF-PDI_N900, $206 \text{ F} \cdot \text{g}^{-1}$ for CNF-PDI_N1000 and $167 \text{ F} \cdot \text{g}^{-1}$ for CNF-PDI_K900. Notably, the specific capacitance of the optimal sample (CNF-PDI_N900) is better than many commercial activated carbons ^[413,419,420] (see Table 4.22, at the end of the chapter) and is close to that of commercial Maxsorb ($334 \text{ F} \cdot \text{g}^{-1}$) ^[419], which is commonly used as reference.

Specific capacitances (C_s) at higher scan rates were also calculated with Eq. 17 (Table 4.20). As expected, C_s decreased with an increase in the scan rate (Figure 4.77a). This behavior can be attributed to the influence of micropores on energy storage. Typically, lower scan rates allow sufficient time for mass transfer and ion penetration into the micropores, resulting in higher specific capacitances. However, with an increase in the scan rate, the time for electrolyte ion transfer to the micropores decreases due to a rapid change in the potential, and hence the ion penetration decreases as well. Consequently, C_s is reduced. Despite this, CNF-PDI_N900 shows an excellent rate capability, retaining about 90 % of capacitance ($252 \text{ F} \cdot \text{g}^{-1}$) at the high scan rate

of $500 \text{ mV}\cdot\text{s}^{-1}$, considering that the maximum capacitance is that obtained at the slowest scan rate ($5 \text{ mV}\cdot\text{s}^{-1}$). In contrast, the rate capabilities of CNF-PDI_K900 and CNF-PDI_N1000 are 47 % (78 F g^{-1}) and 77 % (159 F g^{-1}) at 500 mV s^{-1} , respectively (Figure 4.77b). Noteworthy, commercial Maxsorb presents a much lower rate capability of 22 % (73 F g^{-1}) at $50 \text{ mV}\cdot\text{s}^{-1}$ [419], which demonstrates the high potential of CNF-PDIs for supercapacitor devices.

Table 4.19. Data extracted from the CV curves of the CNF-PDI based electrode systems. V_{redox} represents the voltage of the charging and discharging curves at which the redox reaction takes place. ΔV is the increment between the charging and discharging redox potentials. Values close to 0 indicate highly symmetric CV curves. i is the maximum (or minimum) current density of the charging (or discharging) CV curves, i.e. the current density at the center of the broad band caused by the redox reactions.

Material	Scan rate ($\text{mV}\cdot\text{s}^{-1}$)	V_{redox} (charging) (V)	V_{redox} (discharging) (V)	ΔV (V)	i (charging) ($\text{A}\cdot\text{g}^{-1}$)	i (discharging) ($\text{A}\cdot\text{g}^{-1}$)
CNF-PDI_K900	5	0.41	0.31	-0.10	1.1	-1.5
	10	0.41	0.30	-0.11	2.2	-2.6
	20	0.41	0.29	-0.12	4.1	-4.7
	50	0.41	0.26	-0.15	9.0	-10.9
	80	0.41	0.25	-0.16	13.3	-14.6
	100	0.41	0.25	-0.16	16.0	-17.5
	200	0.41	0.25	-0.16	27.7	-30.0
	300	0.42	0.25	-0.17	38.2	-41.3
	400	0.43	0.25	-0.18	48.0	-51.8
	500	0.44	0.25	-0.19	57.4	-61.8
CNF-PDI_N900	5	0.38	0.38	0.00	2.1	-2.2
	10	0.38	0.37	-0.01	4.2	-4.4
	20	0.38	0.36	-0.02	8.3	-8.7
	50	0.38	0.36	-0.02	20.5	-21.5
	80	0.39	0.35	-0.04	32.5	-34.3
	100	0.39	0.35	-0.04	40.5	-42.3
	200	0.39	0.34	-0.05	79.7	-83.9
	300	0.40	0.33	-0.07	117.9	-124.3
	400	0.41	0.32	-0.09	155.5	-163.9
	500	0.41	0.31	-0.10	192.3	-202.8
CNF-PDI_N1000	5	0.38	0.34	-0.04	1.5	-1.6
	10	0.38	0.34	-0.04	2.9	-3.1
	20	0.38	0.33	-0.05	5.7	-6.1
	50	0.38	0.32	-0.06	13.7	-14.6
	80	0.38	0.30	-0.08	21.3	-22.9
	100	0.39	0.30	-0.09	26.2	-28.4
	200	0.40	0.28	-0.12	49.2	-54.1
	300	0.40	0.26	-0.14	70.7	-78.3
	400	0.42	0.25	-0.17	90.5	-101.6
	500	0.42	0.24	-0.18	109.6	-123.9

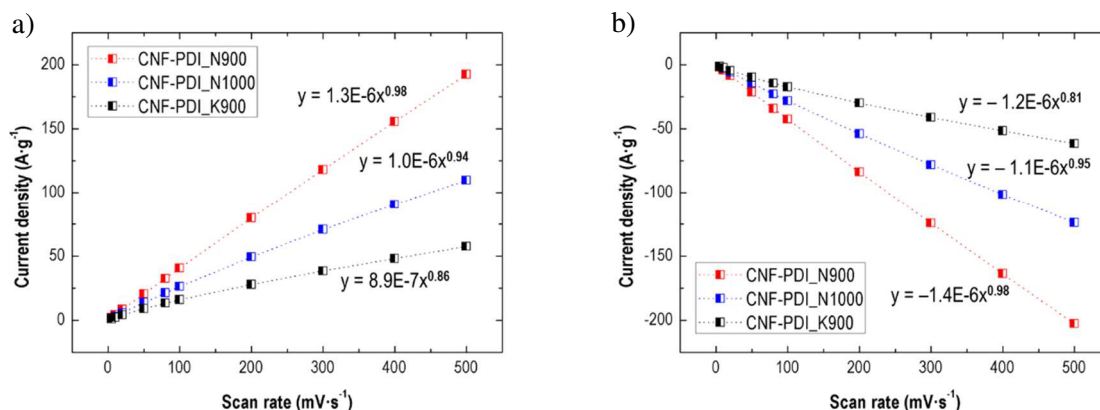


Figure 4.76. Plot of the current density (i) as a function of the scan rate at the maximum intensity of the redox peak of the a) charging and b) discharging CV curve. The data were fitted to power laws. The exponents (marked in bold) represent the b value, which are 0.98 (charging) and 0.98 (discharging) for CNF-PDI_N900 (red), 0.94 (charging) and 0.95 (discharging) for CNF-PDI_1000 (blue) and 0.86 (charging) and 0.81 (discharging) for CNF-PDI_K900 (black).

The exceptional rate capability of CNF-PDI_N900 can be attributed to two key factors. Firstly, the hierarchical structure of micro- and mesopores that facilitates electrolyte diffusion, and secondly, the presence of heteroatoms, which give a high wettability to the carbon material and promote the formation of fast and reversible pseudocapacitive redox reactions.

CNF-PDI_N1000 also exhibits a good rate capability, although slightly lower compared to CNF-PDI_N900. This was attributed to the decreased surface area and lower heteroatom doping caused by the higher carbonization temperature, which suggests that these two parameters are relatively more important than electrical conductivity for a high supercapacitor performance, as proposed previously [165].

In contrast, the low rate capability of CNF-PDI_K900 is attributed to the absence of hierarchical a micro- and mesopores structure and to the reduced microporosity. Furthermore, the electrolyte mass transport to the electrode surface may be slower due to the presence of K^+ ions on the carbon surface in the form of COOK and residual silica, which may block some pores and cause a sluggish mass transport and hinder charge storage performance. Overall, there is a strong correlation between the charge storage performance of the carbon fibers and their surface textural properties, including surface area, heteroatom doping, and pore structure.

Additionally, it is important to note that C_s were calculated from the total area of the CV curves, which include both charging and discharging processes. In the non-ideal situation in which the discharging capacitance is lower than the charging capacitance (energy efficiency < 100 %), the values shown in Table 4.20 could be overestimating the real specific capacitance of the system [179,421].

Table 4.20. Specific capacitances (C_s) of the CNF-PDIs calculated from the CV curves (charging and discharging) at different scan rates (5 to 500 $\text{mV}\cdot\text{s}^{-1}$). The values between parenthesis correspond to the rate capability.

Scan rate ($\text{mV}\cdot\text{s}^{-1}$)	Specific capacitance ($\text{F}\cdot\text{g}^{-1}$)		
	CNF-PDI_K900	CNF-PDI_N900	CNF-PDI_N1000
5	167 (100 %)	280 (100 %)	206 (100 %)
10	155 (93 %)	275 (98 %)	199 (97 %)
20	143 (86 %)	270 (97 %)	194 (94 %)
50	126 (75 %)	265 (95 %)	187 (91 %)
80	116 (69 %)	263 (94 %)	183 (89 %)
100	111 (67 %)	262 (94 %)	181 (88 %)
200	96 (58 %)	258 (92 %)	173 (84 %)
300	88 (53 %)	255 (91 %)	168 (82 %)
400	82 (49 %)	253 (91 %)	163 (79 %)
500	78 (47 %)	252 (90 %)	159 (77 %)

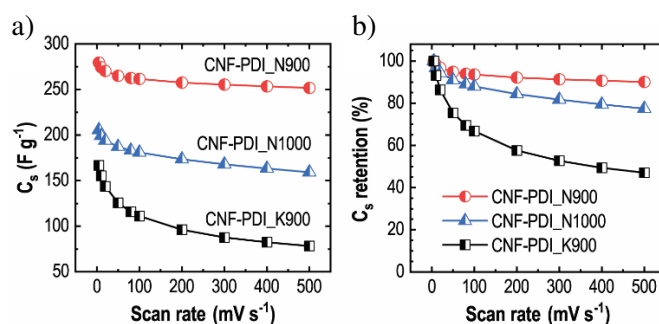


Figure 4.77. a) The calculated C_s from the CV curves at different scan rates, and b) the corresponding C_s retentions.

Galvanostatic charge-discharge (GCD) curves of the CNF-PDIs were also recorded at current densities between 1 and 50 $\text{A}\cdot\text{g}^{-1}$ (Figure 4.78). The GCD curves at 1 $\text{A}\cdot\text{g}^{-1}$ exhibited a quasi-triangular shape (Figure 4.78a), which is characteristic of an electrical double-layer energy storage mechanism. Slight deviations from the ideal triangular shape appear as both the charge and discharge curves show a sinusoidal-like rather than an ideal supercapacitor linear shape, which is common for N-doped carbon materials with a pseudocapacitor behavior ^[171]. Noteworthy, the discharge time for CNF-PDI_N900 is significantly larger than for CNF-PDI_N1000 and CNF-PDI_K900, indicating improved charge storage properties for this sample.

GCD curves at higher current densities (Figure 4.78b-g) revealed that the quasi-triangular shape is maintained even at high current densities (50 $\text{A}\cdot\text{g}^{-1}$), hence suggesting a good rate capability and the absence of diffusion-limited processes. However, CNF-PDI_K900 GCD curves showed a slight asymmetry between the charge and discharge curves, which is clearly manifested at higher current densities (Figure 4.78e), where the charge curve showed a concave shape meanwhile the discharge curve was deviated towards a convex shape. This behavior is indicative of lower energy efficiency and higher internal resistance ^[422,423].

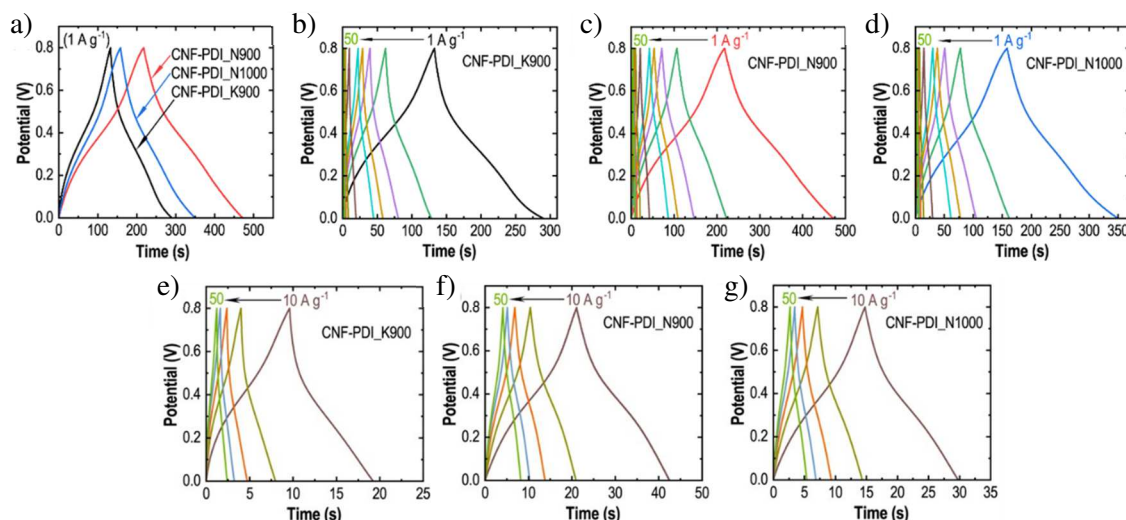


Figure 4.78. Galvanostatic charge-discharge (GCD) curves of a) CNF-PDIs at $1 \text{ A} \cdot \text{g}^{-1}$, b) CNF-PDI_K900, c) CNF-PDI_N900 and d) CNF-PDI_N1000 from 1 to $50 \text{ A} \cdot \text{g}^{-1}$, and a zoom of the $10 - 50 \text{ A} \cdot \text{g}^{-1}$ region for e) CNF-PDI_K900, f) CNF-PDI_N900 and g) CNF-PDI_N1000.

Specific capacitances (C_s) were calculated from the discharge curve of the GCD plots, with Eq. 18, as described in Section 3.3.17. Accordingly, the calculated specific capacitances for the three carbon samples at $1 \text{ A} \cdot \text{g}^{-1}$ are ca. $251 \text{ F} \cdot \text{g}^{-1}$ for CNF-PDI_N900, $174 \text{ F} \cdot \text{g}^{-1}$ for CNF-PDI_N1000 and $136 \text{ F} \cdot \text{g}^{-1}$ for CNF-PDI_K900, which confirmed conclusions from the CV data, i.e. CNF-PDI_N900 is the optimal sample in terms of energy storage behavior.

Specific capacitances at higher current densities were also determined (Table 4.21), along with other parameters such as the rate capability, the coulombic efficiency (CE) and the energy efficiency (EE) (Eq. 19 and Eq. 20, in Section 3.3.17). Furthermore, the average scan rate was determined by dividing the potential window (0.8 V) by the discharge time.

The GCD calculated specific capacitances depicted in Table 4.21 are lower than those calculated from CV curves at comparable scan rates. This may result from i) the scan rates of the CV curves and those calculated from the GCD curves are not completely comparable as the former is fixed during the experiment and the latter varies during the discharge process, because the discharge curves are not linear, but have a sinusoidal-like shape, ii) the capacitance values determined from the CV curves result from the average of both charging and discharging processes, while those from the GCD curves only consider the discharge curve and iii) CV curves may overestimate specific capacitance for carbon pseudocapacitor materials^[424,425], as the current density is highly increased during the faradaic reaction. Moreover, real supercapacitor applications are more likely to demand constant currents rather than constant scan rates, making GCD-based capacitance a more reliable and realistic value. Consequently, the C_s values shown in Table 4.21 will be considered as the real capacitances and rate capabilities for the CNF-PDIs. Nonetheless, knowing the values of C_s calculated from the CV curves (Table 4.20) is interesting to compare our materials with others from the literature that only reported C_s calculated from the CV curves.

Rate capabilities calculated from the GCD curves are similar to those determined from the CV curves (Figure 4.79), being 89 % for CNF-PDI_N900, 76 % for CNF-PDI_N1000 and 38 % for CNF-PDI_K900 at $50 \text{ A} \cdot \text{g}^{-1}$, which confirms the conclusions extracted from the CV curves. More interestingly, GCD curves also permit the calculation of the coulombic and energy efficiencies. Coulombic efficiency (CE) and energy efficiency (EE) are two parameters introduced for the

evaluation of the capacity of an energy storage system to release all the loaded charge without losses ^[179]. In other words, they evaluate the efficiency of an energy storage system to convert stored to released energy without losing it. *CE* results from the ratio between the discharge time and charge time (Eq. 19), meanwhile *EE* is calculated as the ratio between the integrated area of the charge and discharge GCD curves (Eq. 20). *CE* and *EE* are equivalent if the charge and discharge curves are linear and symmetric, however, only *EE* considers different charging and discharging profiles. Consequently, and considering that CNF-PDIs are pseudocapacitor materials and their curves are not completely symmetric, *EE* should be considered more precise and reliable, whereas *CE* values are highly inaccurate.

Table 4.21. Different electrochemical energy storage (EES) parameters calculated from the GCD curves, including the specific capacitance (C_s), the rate capability, and the coulombic (*CE*) and energy efficiencies (*EE*). Note that the coulombic efficiency shows values above 100 %, which has no physical sense. These high values are due to the non-symmetric charge and discharge curves.

	Current density ($A \cdot g^{-1}$)	Specific capacitance ($F \cdot g^{-1}$)	Rate capability (%)	Average Scan Rate ($mV \cdot s^{-1}$)	Coulombic efficiency (%)	Energy efficiency (%)
CNF-PDI_K900	1	136	100	5	118.6	78.7
	2	122	90	12	105.4	75.1
	3	113	83	19	104.6	72.4
	4	107	79	27	101.0	70.5
	5	101	74	36	101.1	68.3
	10	84	62	83	100.2	64.1
	20	68	50	201	99.0	61.1
	30	60	44	342	97.9	60.0
	40	55	41	500	98.2	60.2
	50	51	38	672	97.5	60.2
CNF-PDI_N900	1	251	100	3	116.8	90.8
	2	244	97	7	108.5	92.3
	3	240	95	11	104.9	91.9
	4	238	95	14	103.6	92.1
	5	236	94	18	102.8	91.7
	10	232	92	37	101.3	91.4
	20	229	91	76	100.8	91.6
	30	227	90	116	100.7	91.3
	40	225	89	155	101.0	91.1
	50	224	89	195	100.2	90.9
CNF-PDI_N1000	1	174	100	4	120.6	85.9
	2	168	96	10	108.8	87.3
	3	164	95	15	106.5	88.0
	4	162	93	20	104.2	87.5
	5	160	92	26	101.0	87.3
	10	154	88	53	101.4	85.6
	20	146	84	111	100.4	82.9
	30	140	80	165	103.6	80.6
	40	135	78	234	99.4	78.9
	50	132	76	299	100.0	77.6

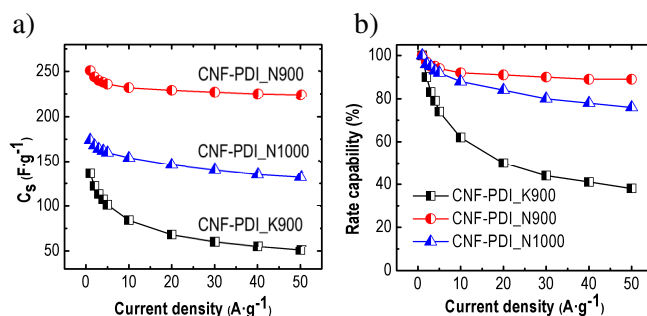


Figure 4.79. a) The calculated C_s from the GCD curves at different current densities, and b) the corresponding C_s retentions (rate capability).

As shown in Table 4.21, the *EE* of the optimal CNF-PDI_N900 sample is above 90 % for all the current densities tested, which indicates that this sample has a low internal resistance and almost does not lose energy in the charge/discharge process. Otherwise, the lower *EE* of CNF-PDI_N1000 and, especially, CNF-PDI_K900 suggest a higher internal resistance and charge loss, resulting in less ideal pseudocapacitor devices.

Besides this, the cyclic stability after 10000 charge/discharge cycles of the CNF-PDIs was tested at 50 $A \cdot g^{-1}$, observing that the three prepared electrodes retained almost all the capacitance after those 10000 cycles (Figure 4.80a). Particularly, the C_s retentions were ca. 99.8 % (CNF-PDI_N900), 99.2 % (CNF-PDI_N1000) and 97.4 % (CNF-PDI_K900), confirming the potential of the CNF-PDIs samples for high-performance supercapacitor electrodes. Moreover, these values are similar to those reported in the literature for similar materials (~ 95 %, as shown in Table 4.22), which benefits the long-term performance of CNF-PDIs in supercapacitor devices. Notably, the cyclic stability was measured at a very high current density (50 $A \cdot g^{-1}$), which demonstrates the utility of CNF-PDI in applications where high power is required. Furthermore, this high cycle life also confirms the reversibility of the surface redox reactions for all CNF-PDIs, and the pseudocapacitive nature of the redox processes. It is important to note that in batteries the cycle stability is typically low, due to the phase changes of the solid electrode material during the redox processes (resulting from the diffusion-limited penetration of electrolyte ions into the solid electrode material).

The charge storage mechanism based on diffusion kinetics of the electrolyte ions at the electrode-electrolyte interface was further investigated using electrochemical impedance spectroscopy (EIS). The Nyquist plots of the CNF-PDIs samples (Figure 4.80b) exhibited the typical characteristics of supercapacitor carbon materials. Specifically, the CNF-PDI_N900 sample, which displayed the highest specific capacitance and rate capability among the studied materials, showed a nearly vertical line at low frequency, indicating lower ion diffusion resistance. The presence of heteroatoms (N and O) in the carbon fibers contributes to the weak semicircular behavior at higher frequency (Inset of Figure 4.80b). The equivalent series resistance (ESR) values, estimated from the intersection point on the real axis, are approximately 4.28 Ω (CNF-PDI_K900), 6.48 Ω (CNF-PDI_N900), and 3.97 Ω (CNF-PDI_N1000). The slightly higher ESR value of the best performing sample suggests that differences in the energy storage supercapacitance performances of the carbon fibers can be attributed to variations in their porosity and surface chemistry resulting from the heat treatment and etching processes.

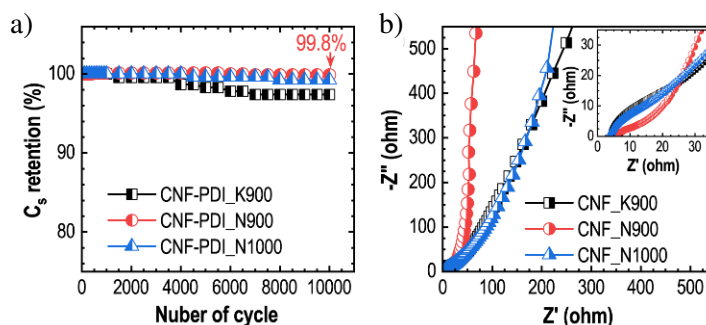


Figure 4.80. a) Cycling stability of CNF-PDIs electrodes and b) The Nyquist plots obtained from the EIS measurements for all the samples. Z' and Z'' represent the real and imaginary impedance, respectively.

Summarizing, CNF-PDIs demonstrated to be suitable carbon materials for the preparation of electrodes for pseudocapacitor energy storage devices. The best sample (CNF-PDI_N900) showed a high specific capacitance ($251 \text{ F} \cdot \text{g}^{-1}$) at a current density of $1 \text{ A} \cdot \text{g}^{-1}$, an outstanding rate capability of 89 % at $50 \text{ A} \cdot \text{g}^{-1}$, high energy efficiencies at all the current densities tested and an excellent cyclic stability of almost 100 % after 10000 cycles at $50 \text{ A} \cdot \text{g}^{-1}$. CNF-PDI_N1000 electrochemical energy storage behavior was fairly similar to that of CNF-PDI_N900 but with decreased capacitance and rate capability values, which result from its lower surface area and heteroatom doping caused by the higher carbonization temperature. Differently, CNF-PDI_K900 deviated significantly from an ideal capacitive behavior showing diffusion-limited kinetics, asymmetric GCD curves at high current densities and significantly lower specific capacitances and rate capabilities. This behavior was attributed to its decreased microporosity.

The high potential of CNF-PDIs as carbon electrode active materials for high-performance supercapacitor devices is further demonstrated by comparing their EES behavior with other super- and pseudocapacitive carbon-based materials, already reported in the literature (see Table 4.22). With the objective of making a fair comparative study, only porous carbon materials without doping with metallic elements, studied in three-electrode systems with aqueous electrolytes will be considered, as changing these parameters may cause significant variations not only in the specific capacitance and rate capability but also in the intrinsic EES behavior of the system.

As previously discussed, commercial activated carbons such as Calgon Carbon^[420] or YP-50^[413] typically show specific capacitances lower than CNF-PDI_N900, with the exception of Maxsorb, which shows a C_s up to $334 \text{ F} \cdot \text{g}^{-1}$ ($5 \text{ mV} \cdot \text{s}^{-1}$) but with a much lower rate capability^[419]. Similarly, biomass-derived activated carbons, produced by carbonizing biomass from wood, coconut, bamboo, prawn shells, etc. and further activating the as-obtained char with chemical agents such as acids, bases or gases, yield porous carbon materials with C_s in the order of $150 - 300 \text{ F} \cdot \text{g}^{-1}$ ^[424,426,427] (despite some of them showing specific capacitances above $300 \text{ F} \cdot \text{g}^{-1}$ at low current densities^[425,428,429]) but with lower rate capabilities than CNF-PDIs (typically between 50 and 70 % at current densities between 10 and $50 \text{ A} \cdot \text{g}^{-1}$) that prevent using both commercial and activated carbons in applications that demand working at high powers (i.e. working at high current densities), such as regenerative braking for electric vehicles, where the supercapacitor should be able to rapidly transform and store the kinetic energy of moving vehicles to electrical energy and then promptly release these energy to restart and accelerate the vehicle^[176,430,431].

Noteworthy, activated carbons typically show ultrahigh specific surface areas (SSA), ca. 2–4 times those of CNF-PDIs, but surprisingly manifest similar C_s and lower rate capabilities. Such a behavior occurs because activated carbons have very small micropores that cannot be accessed

by electrolyte ions. Also, the absence of a hierarchical interconnected micro- and mesopore structure prevents the proper function of these materials at high current densities as there is no sufficient time for the electrolyte ions to penetrate into the narrow carbon micropores. This reveals that not only a high SSA is required for high-performance supercapacitor carbon materials, but pore size and morphology are also important, to favor the accessibility to the pores by the electrolyte ions, as illustrated by Figure 4.81.

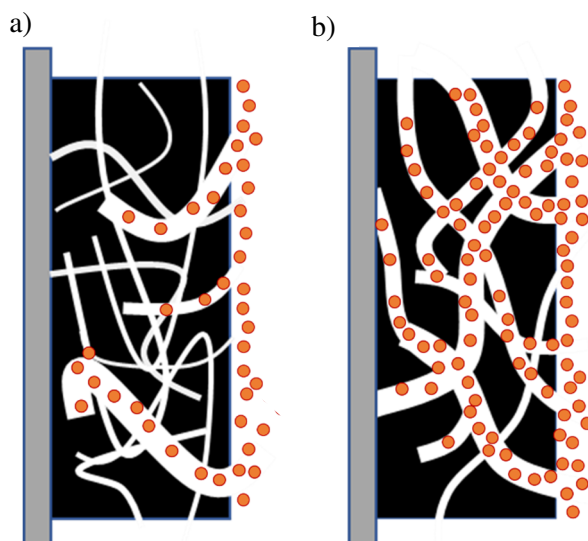


Figure 4.81. Comparison between a) highly microporous activated carbons with high surface area and small micropores with low pore connectivity, which is typical of activated carbons and b) micro/mesoporous carbon materials with a hierarchical structure with polydisperse and interconnected pores, like CNF-PDIs and other templated carbons. The orange circles represent the electrolyte ions.

The importance of a hierarchical micro- and mesopore structure is clearly observed in a study by Lee et al. in which they prepared polyacrylonitrile (PAN)-derived carbon nanofibers by electrospinning the polymer dissolved in DMF and further carbonizing and activating with KOH the electrospun fiber to obtain CNFs [432]. The prepared carbon nanofibers revealed a C_s of 181 $\text{F}\cdot\text{g}^{-1}$ at 5 $\text{mV}\cdot\text{s}^{-1}$ and an average rate capability of 61 % at 50 $\text{mV}\cdot\text{s}^{-1}$, values similar to those obtained for activated carbons. However, by incorporating silica nanoparticles into the polymer solution before electrospinning and etching the silica after preparing the carbon nanofibers, they obtained CNFs with a much higher mesopore volume that showed slightly higher specific capacitances (up to 197 $\text{F}\cdot\text{g}^{-1}$) but dramatically improved rate capabilities (up to 87 % at 50 $\text{mV}\cdot\text{s}^{-1}$).

Interestingly, electrospinning has demonstrated to be a cost-effective and scalable technique to prepare porous CNFs from either synthetic polymers such as PAN [433] or from biomass, being cellulose and lignin the most common sources [434,435]. Particularly interesting is the work carried on by Zhu et al. about lignin-based CNFs [435], in which they prepared PAN-lignin composite electrospun carbon nanofibers by first chemically treating the lignin with isophorone diisocyanate to bind different lignin units and form a high-molecular weight polymer. Notably, the final CNFs revealed an outstanding C_s of 429 $\text{F}\cdot\text{g}^{-1}$ at 1 $\text{A}\cdot\text{g}^{-1}$.

However, electrospinning still has a low control over the pore size and structure and presents the drawback of requiring specific equipments and typically organic solvents, which is not environmentally-friendly and can be toxic. Alternatively, templated carbon nanofibers such as the CNF-PDIs prepared herein emerge as a method to prepare porous carbon nanofibers with tailored

pore size and structure, which allow the preparation of promising customized materials with hierarchical micro- and mesopore structure for supercapacitor applications [387]. Additionally, multiple studies have revealed that carbon materials displaying a pore diameter similar to the size of the non-solvated electrolyte ions can dramatically increase the specific capacitance of the supercapacitor devices, as it forces the desolvation of the electrolyte and reduces the distance between the charged ion and the carbon surface, which significantly enhances the specific capacitance, thus demonstrating the importance of having a subtle control over the pore size and morphology [436].

Despite this, the number of reports about templated porous CNFs is surprisingly low. One of the most studied templated CNFs for supercapacitor applications is the CMK-3 family. CMK-3 is a type of ordered mesoporous carbons formed by hexagonally aligned carbon nanofibers using SBA-15 silica as template, which is impregnated with a carbon precursor, carbonized and etched with NaOH or HF to remove the SBA-15 template [259,260]. Xia et al. [437] evaluated the EES behavior of a series of CO₂-activated sucrose-based CMK-3 materials. The materials revealed high specific surface areas of $\sim 1000 \text{ m}^2 \cdot \text{g}^{-1}$ for non-activated CMK-3 and up to $2749 \text{ m}^2 \cdot \text{g}^{-1}$ for CMK-3 activated with CO₂, and C_s of 115 and $223 \text{ F} \cdot \text{g}^{-1}$ at $2 \text{ mV} \cdot \text{s}^{-1}$ for the non-activated and the CO₂-activated CMK-3, respectively. In another example, He et al. [438] prepared HNO₃-treated CMK-3 using again sucrose as carbon source, observing a decrease in the surface area upon the acid treatment but an improvement of the C_s from $150 \text{ F} \cdot \text{g}^{-1}$ up to $203 \text{ F} \cdot \text{g}^{-1}$, at $1 \text{ A} \cdot \text{g}^{-1}$. In a more recent paper, Yan et al. [439] analyzed the EES behavior of a CMK-3/CNT composite and observed that the presence of the carbon nanotubes increased the C_s from $172 \text{ A} \cdot \text{g}^{-1}$ for pure CMK-3 to $316 \text{ A} \cdot \text{g}^{-1}$ for the CMK-3/CNT composite, which was attributed to an increase on the electrical conductivity and pore connectivity resulting from the CNT-doping.

Besides CMK-3 family, other templated CNFs with good EES properties have been described. In particular, Magana et al. [165] reported the formation of hollow carbon nanofibers using a silica template prepared from a chromonic pinacyanol acetate. Interestingly, they also used a chromonic material for the preparation of the silica template, but in contrast to CNF-PDIs the chromonic material was removed to obtain a micro/mesoporous silica that was subsequently impregnated with a carbon precursor (furfuryl alcohol), carbonized and etched. They determined an outstanding C_s of $327 \text{ F} \cdot \text{g}^{-1}$ at $5 \text{ mV} \cdot \text{s}^{-1}$, and a 79 % capacitance retention at $500 \text{ mV} \cdot \text{s}^{-1}$. However, the specific capacitance calculated from the GCD curves revealed a significantly lower value of $\sim 195 \text{ F} \cdot \text{g}^{-1}$ at $1 \text{ A} \cdot \text{g}^{-1}$, which may suggest a low energy efficiency. Moreover, they also treated the CNFs at a very high temperature (2000°C) to graphitize the material, by sacrificing specific surface area, and observed a huge decrease of the C_s to $\sim 100 \text{ F} \cdot \text{g}^{-1}$ at $5 \text{ mV} \cdot \text{s}^{-1}$, which indicates that a high electrochemically-available specific surface area is more important than a high electrical conductivity for a good supercapacitor behavior.

CNFs can also be produced by carbonizing self-assembled fibrous aggregates [440]. For example, Liu et al. [441] prepared perylene diimide hydrogels using a PDI functionalized with 5-aminoisophthalic acid with melamine and glucono- β -lactone in aqueous triethylamine solution, and carbonized it to obtain CNFs. They determined a C_s of $80 \text{ F} \cdot \text{g}^{-1}$ at $1 \text{ mV} \cdot \text{s}^{-1}$, which could be improved up to $346 \text{ F} \cdot \text{g}^{-1}$ at $1 \text{ mV} \cdot \text{s}^{-1}$ incorporating F-127 surfactant into the hydrogel, as it formed mesopores during the carbonization process, hence confirming the importance of having mesoporosity to allow the diffusion of ions. Differently, Rodríguez-Abreu et al. [181] produced porous CNFs by carbonizing an ionic fibrous assembly formed by mixing cationic and anionic chromonic porphyrins. The obtained CNFs showed a high C_s of $205 \text{ F} \cdot \text{g}^{-1}$ at $5 \text{ mV} \cdot \text{s}^{-1}$ and a rate

capability of 45 % at $300 \text{ mV} \cdot \text{s}^{-1}$. These results demonstrate that both chromonic materials and perylene diimides are promising materials for high-performance supercapacitor electrodes.

Notably, the CNF-PDIs (namely the CNF-PDI_N900) showed C_s above the average templated carbons and higher rate capabilities at very high current densities ($50 \text{ A} \cdot \text{g}^{-1}$). Such a behavior may result from the pseudocapacitance contribution and high wettability resulting from the heteroatom (N and O) doping. It is interesting to cite and discuss a study published by Tian et al. [442] in 2020 in which they evaluate the EES behavior of single-site pyrrolic-N-doped carbons (i.e. carbon materials only doped with pyrrolic N moieties) prepared from the co-condensation of 3-aminophenol (N source), 3-halogenated(F,Cl,Br)-phenol and formaldehyde, its carbonization at relatively low temperature (500°C) and further activation with KOH also at 500°C . The resulting materials manifested heteroatom doping in the form of pyrrolic-N and different O species (including carbonyl and hydroxyl groups), and the optimal sample showed an outstanding C_s of $618 \text{ F} \cdot \text{g}^{-1}$ at $1 \text{ A} \cdot \text{g}^{-1}$, and a rate capability of 72 % at $20 \text{ A} \cdot \text{g}^{-1}$. Also, the shape of the CV and GCD curves was very similar to that of CNF-PDI_N900 and CNF-PDI_N1000, showing rectangular CV profiles with symmetric faradaic peaks around 0.3 – 0.4 V and symmetric sinusoidal-like charge-discharge GCD curves. Tian et al. attributed this behavior to faradaic pseudocapacitance resulting from the surface pyrrolic/pyrrolic nitrogen redox pair (Figure 4.82a) and quinone/hydroquinone redox pair (Figure 4.82b). The results suggest that similar redox reactions could be occurring for CNF-PDIs.

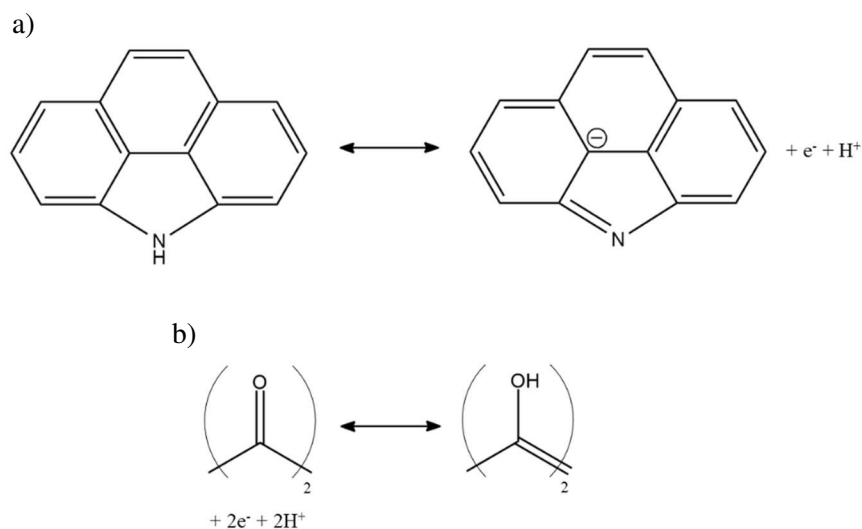


Figure 4.82. Redox reactions of a) pyrrolic nitrogen and b) quinone/hydroquinone pair.

Other examples of the beneficial effect of N-doping in carbon materials for EES applications are observed for N-doped 3D graphene materials [371]. For instance, an N-doped graphene oxide evaluated by H. Zhang et al. [443] displayed a high C_s of $295 \text{ F} \cdot \text{g}^{-1}$ at a current density of $5 \text{ A} \cdot \text{g}^{-1}$, meanwhile a KOH-activated activated graphene material showed an ultrahigh C_s of $383 \text{ F} \cdot \text{g}^{-1}$ at $1 \text{ A} \cdot \text{g}^{-1}$ [444]. Also, a N-doped graphene material synthesized by chemical vapor deposition revealed a C_s of $380 \text{ F} \cdot \text{g}^{-1}$ but at a lower current density of $0.6 \text{ A} \cdot \text{g}^{-1}$ [445], and a rate capability of 63 % at $80 \text{ A} \cdot \text{g}^{-1}$. These values better than for non-doped reduced graphene oxides (rGO) [446,447]. This behavior is confirmed by carbon nanotubes (CNTs) coated with nitrogen-rich conductive polymers, which revealed increased C_s and rate capabilities compared to pristine CNTs [448,449] (Table 4.22).

Summarizing, we can extract some general conclusions about the relation between the carbon materials and their supercapacitor performance: i) a proper balance between surface area and electrical conductivity is needed, ii) it is important for the surface area to be accessible to ions during the charge-discharge process, which is favored by a well interconnected micro- and mesopores, iii) tuning the micropore size to make it similar to the electrolyte ion radius can enhance the specific capacitance significantly and iv) the heteroatom doping (N and O) is beneficial to increase the wettability and produce non-diffusion-limited surface faradaic reactions (pseudocapacitance). Consequently, the high C_s and outstanding rate capability of CNF-PDI_N900 can be explained by its high surface area, hierarchical micro- and mesopore structure and heteroatom doping that gives high wettability and pseudocapacitance properties.

Also, it is important to note that the specific capacitance and rate capability values can vary significantly with many factors such as the electrolyte used, the preparation of the working electrodes (the use of a binder or the amount of active material mass used), the curves used to calculate the C_s (CV or GCD) or using an linear equations to calculate C_s from non-linear curves (for example, the C_s of CNF-PDI_N900 calculated from the GCD curves with the linear equation gives a value of $317 \text{ F}\cdot\text{g}^{-1}$ at $1 \text{ A}\cdot\text{g}^{-1}$, which is a $\sim 25\%$ higher than the $251 \text{ F}\cdot\text{g}^{-1}$ determined by integration), which is not uncommon in the literature. Consequently, comparisons must be done carefully.

Table 4.22. Reported carbon materials for supercapacitors. C_s = Specific capacitance, PAN = Polyacrylonitrile, c. = cycles.

Electrode material	Electrolyte	Surface area ($\text{m}^2 \cdot \text{g}^{-1}$)	C_s ($\text{F} \cdot \text{g}^{-1}$)	Rate capability (%)	Cyclic stability (%)	Ref.
<i>Activated carbon (AC)</i>						
Commercial Maxsorb	30 wt% aq. KOH	3300	334 ($5 \text{ mV} \cdot \text{s}^{-1}$)	22 ($5\text{-}50 \text{ mV} \cdot \text{s}^{-1}$)	-	[419]
Commercial AC, Calgon Carbon	2 M aq. NaOH	880	78 ($2 \text{ mA} \cdot \text{cm}^{-2}$)	-	-	[420]
Commercial AC, YP-50	20 mol/kg aq. LiTFSI	1724	73 ($0.2 \text{ A} \cdot \text{g}^{-1}$)	-	~61 (10000 c.)	[413]
H ₃ PO ₄ -act. bamboo cane	1 M aq. H ₂ SO ₄	1431	206 ($1 \text{ A} \cdot \text{g}^{-1}$)	33 ($1\text{-}10 \text{ A} \cdot \text{g}^{-1}$)	~93 (1000 c.)	[424]
K ₂ FeO ₄ -act. bamboo stalk	6 M aq. KOH	1732	171 ($1 \text{ A} \cdot \text{g}^{-1}$)	69 ($1\text{-}10 \text{ A} \cdot \text{g}^{-1}$)	-	[429]
N-doped bamboo shoots AC	6 M aq. KOH	972	~310 ($1 \text{ A} \cdot \text{g}^{-1}$)	~65 ($1\text{-}10 \text{ A} \cdot \text{g}^{-1}$)	-	[176]
N-doped prawn shells AC	1 M aq. H ₂ SO ₄	1606	~380 ($1 \text{ A} \cdot \text{g}^{-1}$)	~65 ($1\text{-}10 \text{ A} \cdot \text{g}^{-1}$)	95 (5000 c.)	[430]
N-doped prawn shells AC	6 M aq. KOH	1606	~310 ($1 \text{ A} \cdot \text{g}^{-1}$)	~90 ($1\text{-}10 \text{ A} \cdot \text{g}^{-1}$)	95 (5000 c.)	[430]
N-doped nelum-bo nucifera AC	1 M aq. H ₂ SO ₄	2330	379 ($1 \text{ A} \cdot \text{g}^{-1}$)	66 ($1\text{-}50 \text{ A} \cdot \text{g}^{-1}$)	99 % (10000 c.)	[425]
N-doped poultry waste AC	1 M aq. H ₂ SO ₄	444	520 ($2 \text{ A} \cdot \text{g}^{-1}$)	70 ($1\text{-}10 \text{ A} \cdot \text{g}^{-1}$)	-	[431]
<i>Electrospun carbon nanofibers (CNF)</i>						
Electrospun PAN	1 M aq. H ₂ SO ₄	1697	181 ($5 \text{ mV} \cdot \text{s}^{-1}$)	61 ($5\text{-}50 \text{ mV} \cdot \text{s}^{-1}$)	-	[432]
Electrospun PAN/SiO ₂ 90:10	1 M aq. H ₂ SO ₄	1625	189 ($5 \text{ mV} \cdot \text{s}^{-1}$)	65 ($5\text{-}50 \text{ mV} \cdot \text{s}^{-1}$)	-	[432]
Electrospun PAN/SiO ₂ 70:30	1 M aq. H ₂ SO ₄	1796	195 ($5 \text{ mV} \cdot \text{s}^{-1}$)	83 ($5\text{-}50 \text{ mV} \cdot \text{s}^{-1}$)	-	[432]
Electrospun PAN/SiO ₂ 50:50	1 M aq. H ₂ SO ₄	1632	197 ($5 \text{ mV} \cdot \text{s}^{-1}$)	87 ($5\text{-}50 \text{ mV} \cdot \text{s}^{-1}$)	-	[432]
Electrospun PAN	3 M aq. KOH	-	94 ($1 \text{ A} \cdot \text{g}^{-1}$)	38 ($1\text{-}20 \text{ A} \cdot \text{g}^{-1}$)	-	[433]

Table 4.22 continuation. PEI = polyethylene imine, ML = modified lignin, MS = magnetic stirring, US = ultrasound, CNT = carbon nanotube, FA = furfuryl alcohol.

Electrode material	Electrolyte	Surface area (m ² ·g ⁻¹)	C _s (F·g ⁻¹)	Rate capability (%)	Cyclic stability (%)	Ref.
<i>Electrospun carbon nanofibers (CNF)</i>						
N-doped electrospun PAN/PEI	3 M aq. KOH	-	193 (1 A·g ⁻¹)	43 (1-20 A·g ⁻¹)	-	[433]
Electrospun cellulose	6 M aq. KOH	> 290	105 (0.2 A·g ⁻¹)	~50 (1-10 A·g ⁻¹)	-	[434]
N-doped electrospun cellulose	6 M aq. KOH	282	203 (1 A·g ⁻¹)	84 (1-10 A·g ⁻¹)	98 (10000 c.)	[434]
Electrospun non-ML/PAN	6 M aq. KOH	873	139 (1 A·g ⁻¹)	~75 (1-10 A·g ⁻¹)	-	[435]
Electrospun ML/PAN	6 M aq. KOH	2043	429 (1 A·g ⁻¹)	~80 (1-10 A·g ⁻¹)	-	[435]
<i>Templated carbon nanofibers (CNF)</i>						
Sucrose-based CMK-3	6 M aq. KOH	984	115 (2 mV·s ⁻¹)	80 (2-50 mV·s ⁻¹)	-	[437]
CO ₂ -act. sucrose-based CMK-3	6 M aq. KOH	2749	223 (2 mV·s ⁻¹)	72 (2-50 mV·s ⁻¹)	-	[437]
Sucrose-based CMK-3	3 M aq. KOH	976	150 (1 A·g ⁻¹)	87 (1-2 A·g ⁻¹)	95 (500 c.)	[438]
MS-HNO ₃ -act. CMK-3	3 M aq. KOH	773	180 (1 A·g ⁻¹)	92 (1-2 A·g ⁻¹)	91 (500 c.)	[438]
US-HNO ₃ -act. CMK-3	3 M aq. KOH	789	203 (1 A·g ⁻¹)	90 (1-2 A·g ⁻¹)	94 (500 c.)	[438]
Commercial CMK-3, XF Nano	1 M aq. H ₂ SO ₄	1109	172 (1 A·g ⁻¹)	~50 (1-50 A·g ⁻¹)	-	[439]
CMK-3/CNT composite	1 M aq. H ₂ SO ₄	801	316 (1 A·g ⁻¹)	69 (1-50 A·g ⁻¹)	-	[439]
SiO ₂ -templated FA	1 M aq. H ₂ SO ₄	826	327 (5 mV·s ⁻¹)	79 (5-50 mV·s ⁻¹)	93 (10000 c.)	[165]
SiO ₂ -template FA (2000 °C)	1 M aq. H ₂ SO ₄	271	~100 (5 mV·s ⁻¹)	~35 (5-50 mV·s ⁻¹)	-	[165]

Table 4.22 continuation. PDI = perylene diimide, rGO = reduced graphene oxide, MWCNT = multi-walled carbon nanotubes, PANI = polyaniline, PEDOT = poly(3,4-ethylenedioxythiophene) polystyrene sulfonate.

Electrode material	Electrolyte	Surface area (m ² ·g ⁻¹)	C _s (F·g ⁻¹)	Rate capability (%)	Cyclic stability (%)	Ref.
<i>Templated carbon nanofibers (CNF)</i>						
Carbonized PDI-hydrogel	2 M aq. H ₂ SO ₄	124	80 (1 mV·s ⁻¹)	-	-	[441]
Carbonized F127 + PDI-hydrogel	2 M aq. H ₂ SO ₄	520	346 (1 mV·s ⁻¹)	34 (1-100 mV·s ⁻¹)	~150 (1000 c.)	[441]
Ionic porphyrin 1D carbon	1 M aq. H ₂ SO ₄	194	205 (5 mV·s ⁻¹)	45 (5-300 mV·s ⁻¹)	-	[181]
CNF-PDI_N900	1 M aq. H ₂ SO ₄	695	251 (1 A·g ⁻¹)	89 (1-50 A·g ⁻¹)	100 (10000 c.)	<i>This work</i>
CNF-PDI_N1000	1 M aq. H ₂ SO ₄	602	174 (1 A·g ⁻¹)	76 (1-50 A·g ⁻¹)	99 (10000 c.)	<i>This work</i>
CNF-PDI_K900	1 M aq. H ₂ SO ₄	392	136 (1 A·g ⁻¹)	38 (1-50 A·g ⁻¹)	97 (10000 c.)	<i>This work</i>
<i>Other carbon materials</i>						
Single site pyrrolic-doped	1 M aq. H ₂ SO ₄	450	618 (1 A·g ⁻¹)	72 (1-20 A·g ⁻¹)	100 (30000 c.)	[442]
Hydrazine-rGO	6 M aq. KOH	705	135 (0.01 A·g ⁻¹)	-	-	[446]
Urea-rGO	6 M aq. KOH	590	255 (0.5 A·g ⁻¹)	43 (1-30 A·g ⁻¹)	93 (1200 c.)	[447]
Annealed urea-rGO	6 M aq. KOH	630	172 (0.5 A·g ⁻¹)	61 (1-30 A·g ⁻¹)	94 (1200 c.)	[447]
N-doped hydrothermal GO	6 M aq. KOH	412	295 (5 A·g ⁻¹)	-	92 (4500 c.)	[443]
KOH-act. N-doped graphene	6 M aq. KOH	1816	383 (1 A·g ⁻¹)	83 (1-20 A·g ⁻¹)	-	[444]
CVD N-doped graphene	6 M aq. KOH	1816	380 (0.6 A·g ⁻¹)	63 (1-80 A·g ⁻¹)	94 (4600 c.)	[445]
MWCNT /PEDOT composite	1 M aq. H ₂ SO ₄	-	95 (5 mV·s ⁻¹)	74 (2-10 mV·s ⁻¹)	-	[448]
MWCNTs /PANI composite	1 M aq. H ₂ SO ₄	200	328 (5 mV·s ⁻¹)	77 (5-50 mV·s ⁻¹)	-	[449]

4.4.3 Carbon dioxide capture properties

Developing materials for an efficient carbon dioxide (CO₂) capture is of utmost importance in a world in which the high emissions of greenhouse gases has become a serious threat for the climate. In this context, new materials such as activated carbons (AC) ^[450] or carbon nanofibers ^[451] have been developed for an efficient and selective CO₂ adsorption. Interestingly, a recent study about AC revealed that having a high microporosity, especially in the form of small micropores (< 1 nm) improves the CO₂ uptake ^[452]. Also, the presence of N-doping seems to be beneficial for better CO₂ adsorption properties ^[453], although some studies suggested that the N-content may not be very important in some cases ^[454].

Consequently, and motivated by the high microporous surface area and heteroatom-doping of the CNF-PDIs, the CO₂ adsorption properties of CNF-PDI_N900 were also investigated. Figure 4.83a shows the CO₂ adsorption isotherm at 0 °C. A rapid increase in the CO₂ adsorbed volume at low pressures was observed, suggesting the presence of micropores that can adsorb CO₂ within the CNF-PDI_N900 structure. Notably, the total adsorbed volume of CO₂ at 100 kPa (atmospheric pressure) reached 45 cm³·g⁻¹, which corresponds to an adsorption of 2.02 mmol of CO₂ per gram of carbon material. This value is comparable to other high-performance materials like electrospun ^[451] and templated ^[455] carbon nanofibers, highlighting the potential of CNF-PDIs for carbon dioxide retention and storage.

The pore size distribution was calculated by the Horvath-Kawazoe method (Figure 4.83b). The analysis reveals that the majority of CNF-PDI_N900 CO₂ adsorbing pores are micropores with diameters between 0.5 and 0.9 nm, which is consistent with the micropore widths described by the nitrogen sorption isotherms (Table 4.17). The excellent CO₂ capture performance of CNF-PDI_N900 may be attributed to its high microporosity and heteroatom functionality, particularly N-doping ^[414].

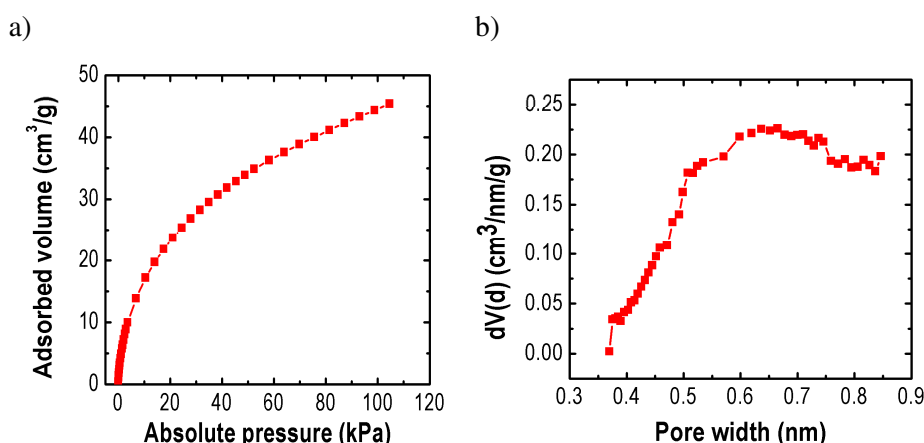


Figure 4.83. a) CO₂ adsorption isotherm and b) pore size distribution of CNF-PDI_N900 calculated from the Horvath-Kawazoe (HK) method.

4.4.4 Conclusions

Three different carbon nanofiber samples (CNF-PDIs) were successfully prepared using a simple method that utilized a cationic chromonic perylene diimide both as template for SiO₂ and carbon source. This demonstrates the potential of the silica nanocasting approach for synthesis of carbon nanofibers. The resulting CNF-PDIs presented remarkable properties, including high surface areas, hierarchically structured meso- and microporosity and a significant heteroatom doping (~16 % O and ~2.5 % N).

Electrochemical analysis of the best performing sample (CNF-PDI_N900) revealed quasi-rectangular cyclic voltammetry curves with a slight deviation between 0.3 and 0.45 V. This deviation was attributed to pseudocapacitance induced by the fast and reversible redox reactions occurring due to the heteroatom doping, as confirmed by investigating the kinetics of the charge and discharge processes. The sample displayed a specific capacitance of $251 \text{ F} \cdot \text{g}^{-1}$ at a current density of $1 \text{ A} \cdot \text{g}^{-1}$, along with an impressive rate capability of almost 90 % at $50 \text{ A} \cdot \text{g}^{-1}$. These results surpassed many commercial and biomass-derived activated carbon materials. Furthermore, the CNF-PDI_N900 exhibited excellent cyclic stability, retaining 99.8% of its initial capacitance after 10000 cycles at $50 \text{ A} \cdot \text{g}^{-1}$. This highlights the potential use of perylene diimides and other lyotropic chromonic liquid crystals for the fabrication of high-performance electrical double-layer supercapacitors and pseudocapacitors.

Moreover, the CNF-PDIs samples demonstrated excellent dispersibility in various solvents, including DMF, chloroform, and notably, water. This was exploited to prepare conductive carbon films under mild conditions, which hold promise for the formulation of conductive inks for printed electrodes.

In addition to the outstanding electrochemical properties of the CNF-PDIs, the sample with the highest microporosity (CNF-PDI_N900) exhibited a significant CO_2 adsorption of approximately $2 \text{ mmol} \cdot \text{g}^{-1}$ at atmospheric pressure and 0°C . This good performance in carbon dioxide retention and storage positions CNF-PDIs as highly valuable carbon materials in this application, comparable to similar carbon-based materials.

In summary, the prepared CNF-PDI samples demonstrated a wide range of unique properties, including excellent electrochemical performance, dispersibility in various solvents, potential for CO_2 capture, and the ability to fabricate conductive carbon films and inks. These findings demonstrate the high potential of chromonic-derived carbon nanofibers for the development of high-performance energy storage devices and for other energy and environmental applications.

4.5 DNA-intercalation properties of rylene diimides

This chapter describes the DNA-binding properties of three perylene (PDI) and two naphthalene (NDI) diimides (Figure 4.84) with different side chain charge (cationic, anionic and nonionic). Multiple physicochemical techniques including absorbance and fluorescence spectrophotometry, circular dichroism and viscosity measurements were used to determine the capacity of these dyes to interact with DNA and define the binding mode (intercalation, groove-binding or electrostatic aggregation). Furthermore, *in vitro* cytotoxicity tests with HeLa cells were performed to evaluate the biocompatibility of the rylene diimides. It was observed that the diimide side charge plays a fundamental role for the dye interaction with DNA, and the rylene core size (naphthalene or perylene) influences the rylene dye cytotoxicity.

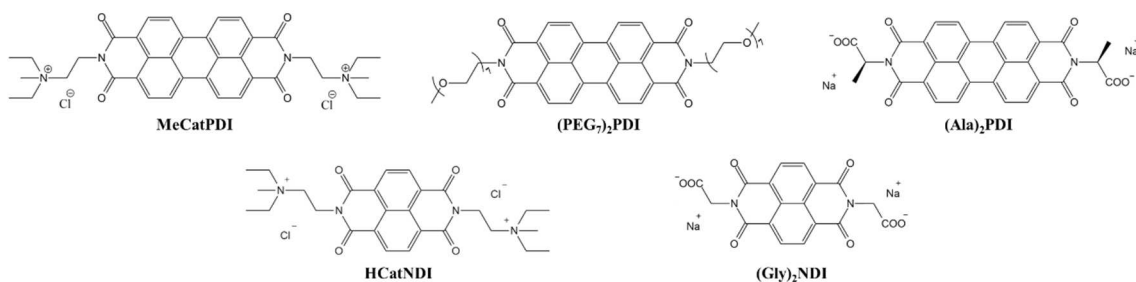


Figure 4.84. Chemical structures of three perylene dyes (MeCatPDI, (PEG₇)₂PDI and (Ala)₂PDI) and the two naphthalene diimides (HCatNDI and (Gly)₂NDI) evaluated as DNA-intercalators.

4.5.1 Study of the DNA-intercalation behavior of rylene dyes by physicochemical tests

Deoxyribonucleic acid (DNA) is a complex biomolecule that contains all the biological information of a living organism. It is formed by two negatively-charged strands winded in a twisted ladder, forming a double-helix structure^[456–458]. Each strand is composed of alternating sugars (deoxyribose) and phosphate groups, with a specific nitrogenous base (adenine, thymine, cytosine or guanine) attached to each sugar unit, which not only contain the information for building and maintaining a living organism, but also direct the formation of the double-stranded structure by H-bonding and π -stacking^[459].

DNA provides multiple binding sites for various guest molecules, including intercalators that bind in between stacked base pairs and groove-binding agents that interact with the minor and major grooves of DNA strands^[460,461]. In particular, intercalating binding agents are interesting as they tend to modify the DNA structure, influencing its physiological functions. Thus, DNA-intercalators exhibit a high potential as chemotherapeutic drugs^[462]. Besides intercalation and groove-binding, molecules can also associate with DNA by electrostatic binding, like positively charged big molecules or aggregates that do not fit in any of the DNA binding sites. All the binding modes are depicted in Figure 4.85.

The chemical structure of the DNA-binding agents greatly influences the binding mode. For instance, groove-binding agents typically consist in banana-shaped molecules with at least two aromatic groups coupled by a rigid moiety. Furthermore, the presence of positively charged groups capable of forming hydrogen bonds and a molecular size fitting either the minor or major DNA grooves are vital for the groove-binding to occur^[461]. Examples of groove-binding agents are imidazole-pyrrole polyamides^[463], netropsin and distamycin derivatives^[464,465] and furamidines^[466].

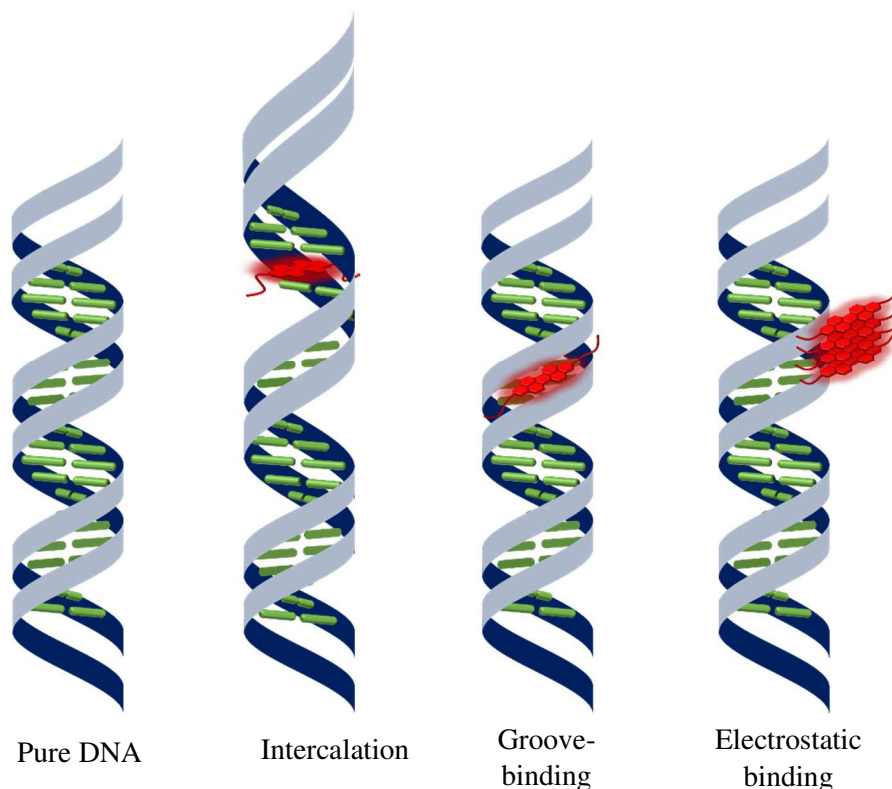


Figure 4.85. Different binding modes with DNA. From left to right: pure DNA, Intercalation, Groove-binding (minor Groove) and Electrostatic binding. Note the DNA double-helix enlargement in the intercalation binding mechanism.

In contrast, DNA-intercalating agents are small planar polyaromatic compounds which are typically protonated, and hence positively charged, at physiological pH^[461]. Some examples are acridine^[467,468], anthraquinones^[469], cyanine dyes^[470,471], azine dyes^[472], quinolizinium ions^[473,474], phenanthridinium ions^[475], porphyrins^[476] and also rylene dyes such as naphthalene^[477–481] and perylene^[249,482–484] diimides. Notably, many of these compounds also form lyotropic chromonic liquid crystals, which suggests that positively charged chromonics have potential ability to intercalate into DNA helices.

Herein, we evaluate the DNA-binding properties of the perylene diimides used in the previous chapters, i.e., MeCatPDI, (PEG)₇PDI and (Ala)₂PDI, and compare them with two naphthalene diimides (one cationic, HCatNDI, and one anionic, (Gly)₂NDI). All the chemical structures are shown in Figure 4.84.

To evaluate whether a molecule intercalates or not within double-stranded DNA, a combination of selected physicochemical methods is required^[461]. According to Long and Barton^[485], these methods are divided in three categories:

- i) Methods involving the study of changes in the DNA helix, such as solution viscosity, sedimentation coefficient, electrophoresis and ³¹P-NMR.
- ii) Methods that show the electronic interactions between the intercalating agent and the DNA, including absorbance and fluorescence spectrophotometry and ¹H-NMR.
- iii) Methods that evaluate the molecular orientation or rigidity. These include linear flow and electric dichroism spectroscopy, and luminescence polarization experiments. Furthermore, circular dichroism spectroscopy can also provide some insights about the binding mode of drugs with DNA^[486].

In this work, the DNA-binding behavior of the selected rylene dyes was evaluated by a combination of absorbance and fluorescence spectrophotometry, circular dichroism spectroscopy and solution viscosity measurements.

Absorbance spectrophotometry experiments were conducted on the rylene dyes in PBS aqueous solution at 29 μM (in the case of (Ala)₂PDI, the concentration was 22 μM because of preparation error) with varying concentrations of DNA (Figure 4.86). Note that the DNA concentration corresponds to the base-pair concentration, considering a base-pair molecular weight of 660 g/mol.

For MeCatPDI, the absorbance spectra of an aqueous solution in PBS at 29 μM without DNA exhibited two overlapped bands indicating the formation of H-aggregates. Upon adding DNA, the absorbance of MeCatPDI initially decreased by increasing DNA concentration up to 27 μM (Figure 4.86a), alongside with a slight red-shift of the absorbance bands and the appearance of an isosbestic point at ~ 560 nm. In contrast, at higher DNA concentrations (Figure 4.86b), this trend was reversed, resulting in an increase in the absorbance of the perylene dye and a change in the spectra shape, leading to the formation of three well-differentiated bands corresponding to the $0 \rightarrow 0$, $0 \rightarrow 1$ and $0 \rightarrow 2$ vibronic transitions of monomeric perylene dyes (see Chapter 4.1).

This spectral behavior has been previously observed for other aromatic dyes with DNA, including cyanines [487], phthalocyanines [488], acenaphto[1,2b]pyrrole [489], and other cationic perylene diimides [490]. The mechanism behind this unusual behavior is attributed to an initial hypochromicity caused by the agglomeration of positively charged dye aggregates around the polyanionic DNA chains for high dye/DNA concentration ratios, followed by the dilution of the dye over the DNA binding sites at higher DNA concentrations, which leads to the breaking of the dye aggregates, allowing single dye units to bind within the biomolecule [490].

In contrast, (PEG₇)₂PDI (Figure 4.86c) and (Ala)₂PDI (Figure 4.86d) absorbance spectra exhibited two overlapped broad bands with one shoulder, indicating the formation of H-aggregates, and no changes in their absorbance spectra were observed upon adding increasing amounts of DNA, suggesting an absence of interaction between these perylene dyes and the DNA. Additionally, we conducted absorbance measurements of the naphthalene diimides HCatNDI (Figure 4.86e) and (Gly)₂NDI (Figure 4.86f). Non-aggregated NDIs typically exhibit a succession of three bands of decreasing intensity corresponding to the $0 \rightarrow v$ vibronic transitions, but at lower wavelengths (300 – 400 nm) compared to their perylene counterparts (450 – 550 nm) [491]. As suggested by spectra in Figures 4.86e and 4.86f, both studied NDIs were present in the monomeric state at the concentrations tested in PBS, indicating a much lower degree of aggregation than the PDIs, due to the smaller naphthalene hydrophobic core as compared to the perylene core.

The addition of DNA to a 29 μM HCatNDI aqueous PBS solution led to a gradual hypochromicity and the formation of an isosbestic point at ~ 390 nm, without any shift in the absorbance maxima (Figure 4.86e), hence suggesting a classical intercalation binding between the HCatNDI and the DNA [492–494]. In contrast, (Gly)₂NDI – DNA solutions did not exhibit any spectral changes by varying the DNA concentration (Figure 4.86f), suggesting no interactions between the NDI and the DNA.

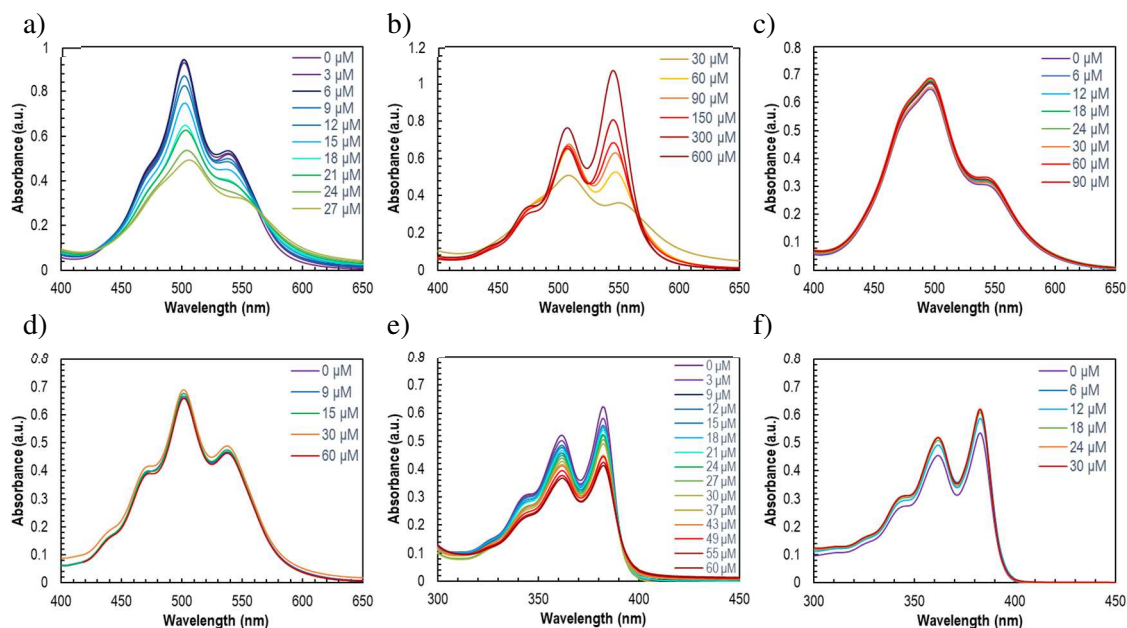


Figure 4.86. Absorbance spectrophotometry of a) MeCatPDI 29 μM in PBS with DNA at different concentrations (0 – 27 μM), b) MeCatPDI 29 μM in PBS with DNA at different concentrations (30 – 600 μM), c) (PEG₇)₂PDI 29 μM in PBS with DNA at different concentrations (0 – 90 μM), d) (Ala)₂PDI 22 μM in PBS with DNA at different concentrations (0 – 60 μM), e) HCatNDI 29 μM in PBS with DNA at different concentrations (0 – 60 μM), and f) (Gly)₂NDI 29 μM in PBS with DNA at different concentrations (0 – 30 μM).

To further understand the interaction between the rylene dyes and DNA, fluorescence spectrophotometry experiments were performed (Figure 4.87). The aqueous PBS solution of MeCatPDI at 29 μM exhibited fluorescence quenching upon addition of DNA (Figure 4.87a), which occurred in three different steps (Figure 4.87d). At low DNA concentrations (0 – 18 μM), almost no quenching was observed, which contrasted with the hypochromic absorbance behavior depicted in Figure 4.86a at these concentrations. When DNA concentration was increased up to 30 μM , the PDI fluorescence was dramatically quenched until almost no fluorescence could be detected. Finally, from 30 to 600 μM , the MeCatPDI – DNA solutions exhibited no fluorescence at all. Similar behaviors were obtained using lower MeCatPDI concentrations (1.5 and 15 μM), where a significant fluorescence quenching was observed for DNA concentrations similar to those of the cationic dye, i.e. dye/DNA ratio $\approx$ 1. This postulates MeCatPDI as a potential probe for DNA qualitative and quantitative sensing.

The fluorescence of DNA-binding agents is typically either enhanced^[495,496] or quenched^[497,498] upon adding DNA, depending on the fluorescence of both the dissolved binding agent and the binding agent-DNA complex^[499]. For example, several DNA-binding cationic PDIs exhibited a gradually decreasing fluorescence intensity by increasing DNA concentration^[482,483]. However, the three-step fluorescence quenching exhibited by MeCatPDI is rather atypical, as normally, fluorescence titration experiments consisting in gradually adding DNA aliquots to a solution with a fixed concentration of binding agent result in an also gradual enhancement/quenching of the fluorescence. The atypical behavior of MeCatPDI suggests a complex binding mechanism in which several processes occur at the same time (electrostatic attraction, PDI aggregate disruption and intercalation).

Unlike MeCatPDI, the fluorescence intensity of both (PEG₇)₂PDI (Figure 4.87b,e) and (Ala)₂PDI (Figure 4.87c,f) did not change when incorporating DNA, which matched with the DNA-

independent absorbance spectra and confirmed the absence of interactions between the nonionic and anionic PDIs with the DNA.

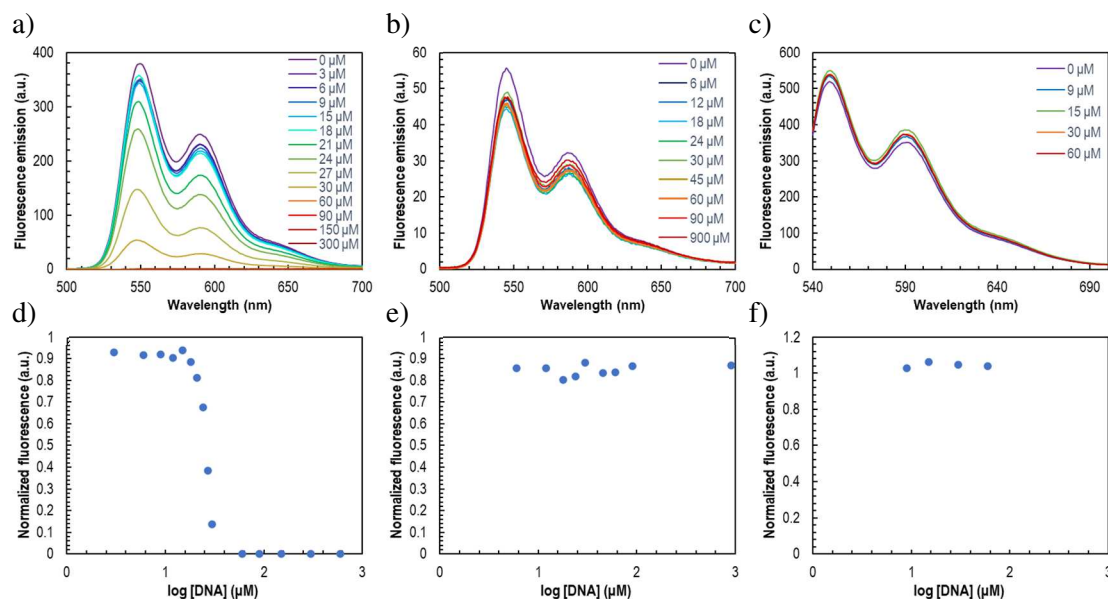


Figure 4.87. Fluorescence spectra of a) MeCatPDI 29 μM in PBS with DNA at different concentrations (0 – 600 μM), b) (PEG₇)₂PDI 29 μM in PBS with DNA at different concentrations (0 – 900 μM), c) (Ala)₂PDI 22 μM in PBS with DNA at different concentrations (0 – 60 μM). Normalized fluorescence intensity at 550 nm as a function of the DNA concentration for d) MeCatPDI 29 μM , e) (PEG₇)₂PDI 29 μM and f) (Ala)₂PDI 22 μM . Excitation was fixed at 490 nm.

Additionally, the fluorescence spectrophotometry of the naphthalene dyes was also studied. The anionic (Gly)₂NDI did not exhibit any fluorescence neither with or without DNA (data not shown). In contrast, the cationic HCatNDI showed a gradual fluorescence quenching upon addition of DNA (Figure 4.88), indicating that the fluorescence of the naphthalene diimide is quenched by binding with DNA, and suggesting a single binding mode, in contrast to MeCatPDI.

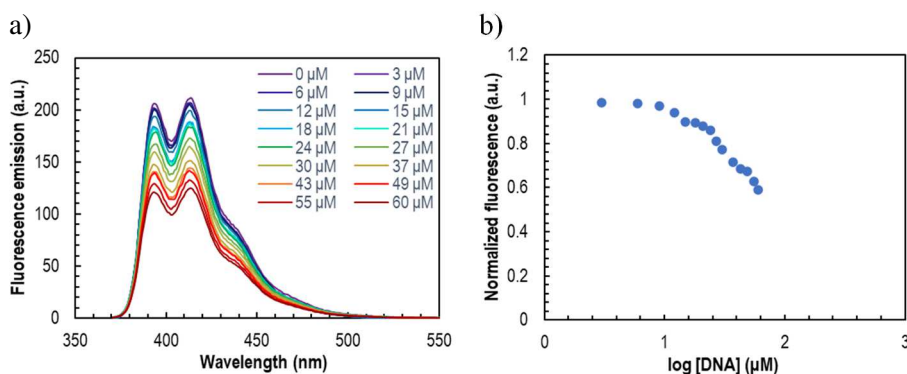


Figure 4.88. a) Fluorescence spectra of HCatNDI 29 μM at different DNA concentrations (0 – 60 μM). b) Normalized fluorescence intensity of HCatNDI 29 μM as a function of DNA concentration.

Circular dichroism (CD) spectroscopy experiments were also performed to gain further insights of the DNA-binding properties of the studied PDIs (Figure 4.89a) and NDIs (Figure 4.89b). The CD spectra of DNA dissolved in aqueous PBS at 50 μM exhibited a negative band at ~ 245 nm, induced by the polynucleotide helicity, and a positive band at ~ 273 nm, owing to the base-pair stacking. This is typical of DNA in the right-hand B-form^[500,501]. Like for absorbance and fluorescence spectrophotometry, none of the nonionic and anionic rylene dyes at 3 μM affected the CD spectra of the DNA, indicating the absence of interactions. In contrast, both MeCatPDI

and HCatNDI at 3 μM increased the positive band at 273 nm in about 1 – 2 millidegrees. The enlargement of the base-pair stacking band indicates an enlargement of the distance between adjacent base-pairs, which suggest an intercalation binding mode for the two cationic rylene dyes^[502]. Furthermore, a careful analysis of the negative CD band revealed that it is completely unaffected by HCatNDI, but MeCatPDI slightly reduces its intensity. This was confirmed performing a CD spectrum of a MeCatPDI-DNA solution at higher PDI concentration and lower DNA concentration (Figure 4.90). These results suggest a partial unwinding of the DNA double helix upon MeCatPDI binding and a transition from B-DNA to A-DNA^[503].

Additionally, a second weak positive band at the region where the perylene dye absorbs light (~ 500 nm) appeared. This band is commonly called induced circular dichroism (ICD) band and emerges for achiral molecules surrounded by chiral environments (such as DNA). Notably, a weak positive ICD band is generally induced by a chromophore with the transition dipole moment perpendicular to the long-axis of the binding site (DNA long axis) and parallel to the pseudo-dyad axis (base-pair axis)^[504]. This suggests that MeCatPDI intercalates between two adjacent base-pairs with the long-axis of the PDI parallel to the base-pair axis. Despite this, it should be noted that groove-binders can also produce positive ICD bands, but typically with higher intensity^[504].

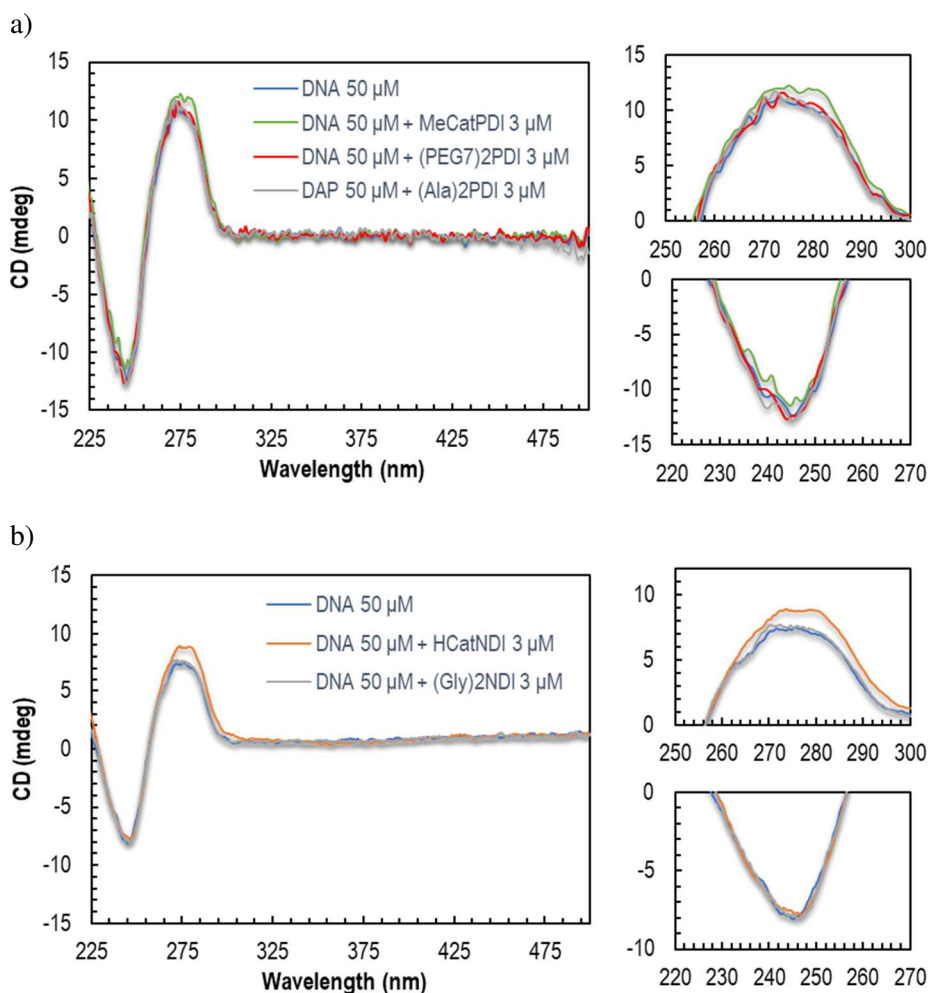


Figure 4.89. a) Left: Circular dichroism spectra of DNA 50 μM with the different perylene diimides tested at 3 μM . Right: Zoom-in of the positive (top) and negative (bottom) bands. b) Left: Circular dichroism spectra of DNA 50 μM with the different naphthalene diimides tested at 3 μM . Right: Zoom-in of the positive (top) and negative (bottom) bands.

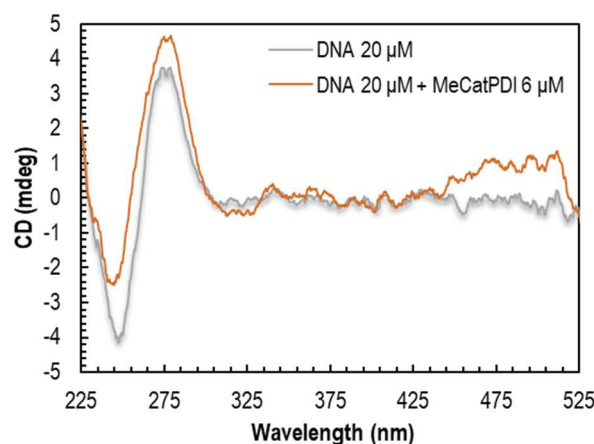


Figure 4.90. Circular dichroism spectra of DNA 20 μM with the different perylene diimides tested at 6 μM .

In addition to these spectrophotometric measurements, viscosity changes of DNA aqueous solutions upon the addition of the rylene dyes were monitored. Viscometry, and other hydrodynamic techniques such as sedimentation and gel electrophoresis, offer reliable methods to determine the DNA binding-mode when more complex techniques such as X-ray diffraction or NMR are not available. Typically, DNA-intercalators increase the viscosity of DNA aqueous solutions, due to the enlargement and unwinding of the DNA double-helix (see Figure 4.85). In contrast, groove-binders and electrostatic binders do not produce such viscosity increment ^[461].

In this context, we measured the viscosity of a DNA solution in aqueous PBS at 50 μM as a function of the added rylene dyes concentration. Viscosity was determined using a rheometer with concentric cylinders geometry (see Section 3.3.20 for more details), and the ratio between the viscosity of the DNA + rylene samples and the viscosity of pure DNA was plotted as a function of the rylene dye concentration (Figure 4.91). Notably, the cationic MeCatPDI and HCatNDI dyes increased the viscosity of the DNA solution, indicating an intercalation binding mode. As expected, the nonionic (PEG₇)₂PDI and the anionic (Ala)₂PDI and (Gly)₂NDI did not significantly modify the viscosity of the DNA aqueous solution, confirming that these dyes do not interact with DNA.

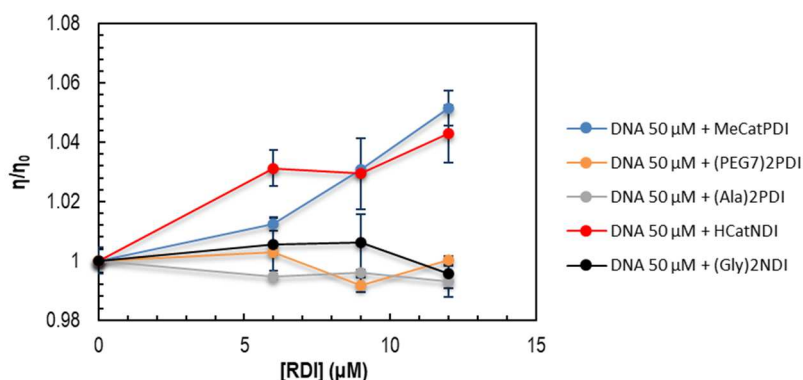


Figure 4.91. Effect of increasing rylene dye concentration [RDI] on the viscosity of a DNA 50 μM solution in aqueous PBS. The y-axis corresponds to the ratio between the viscosity of the DNA solution with a rylene dye at a selected concentration (η) and the viscosity of the DNA solution with no rylene dye (η_0).

4.5.2 *In vitro* cytotoxicity tests

The DNA-binding properties of the cationic dyes MeCatPDI and HCatNDI could lead to DNA-damage and induce cell death. In this context, evaluating the cytotoxic properties of the prepared perylene and naphthalene diimides is of foremost importance for several reasons. For instance, a high cytotoxicity against cancer cells implies anticarcinogen activity. However, at the same time,

it can limit their application in other fields, such as carriers for drug delivery, since cytotoxic drug delivery systems are normally undesired.

To examine the inhibitory effect of the five rylene dyes studied herein in human cancer cell lines, a MTT assay with HeLa cells was performed [505–507]. For this, HeLa cells were incubated in DMEM solution at 37 °C with 10 μ M of the selected rylene dye. It was observed that only HCatNDI had strong inhibitory effect after 24 h incubation, with roughly a 10 % cell survival. In contrast, none of the other four rylene dyes significantly affected the cell survival ratio, indicating no cytotoxic properties (Figure 4.92).

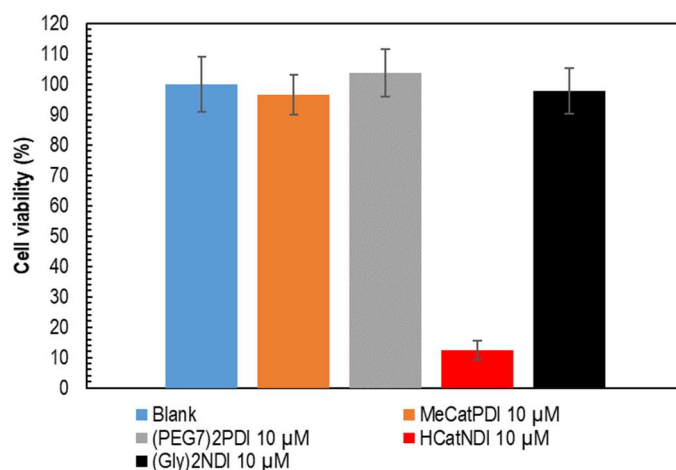


Figure 4.92. MTT assay of HeLa cells incubated for 24 h in presence of the rylene dyes MeCatPDI, (PEG₇)₂PDI, HCatNDI and (Gly)₂NDI at 10 μ M. Tests with (Ala)₂PDI have not been performed yet.

The absence of cytotoxic properties for MeCatPDI is rather surprising, as the physicochemical spectrophotometric tests and the viscosity measurements suggested that this compound intercalates in DNA double-helix. In the literature, it has been reported that the terminal imide group size plays a major role in the cell growth inhibitory properties of cationic perylene dyes [483], since bulky imide groups exhibited poor cytotoxic properties despite indicating DNA-binding behavior in the absorbance, fluorescence and circular dichroism tests, at similar concentrations, which could explain the lower cytotoxicity of MeCatPDI as compared to other perylene diimides with less bulky imide groups [482]. In any case, the cationic MeCatPDI might not display cytotoxic properties at show times on HeLa cells, but induce other toxicity effects. Therefore, the biological activity of MeCatPDI should be reevaluated in the future, possibly using higher concentrations.

4.5.3 Conclusions

The DNA-binding properties of three perylene diimides and two naphthalene diimides with different side charge were investigated. Absorbance and fluorescence spectrophotometry measurements indicated that the cationic rylene dyes, MeCatPDI and HCatNDI, interact with DNA. However, only HCatNDI exhibited a classical intercalator behavior, whereas MeCatPDI showed a more complex binding mechanism with multiple steps. Neither the nonionic (PEG₇)₂PDI nor the anionic (Ala)₂PDI and (Gly)₂NDI compounds bound to DNA, indicating that the positive charge is required for rylene dye-DNA binding to occur. CD spectra and viscometry data suggested that the cationic MeCatPDI and HCatNDI dyes were bound to DNA, most likely through intercalation, although the CD spectra suggested that MeCatPDI also unwinds the DNA double-helix changing it from the B-form to the A-form, as reported for other reported cationic perylene-based DNA-intercalators [482,483].

Furthermore, the cytotoxic properties of the rylene dyes were evaluated in human cancer HeLa cells, and it was found that only HCatNDI presented cytotoxic properties, suppressing HeLa cell growth by about 90% after 24 h incubation at 10 μ M. Surprisingly, MeCatPDI did not cause cell death, despite its inferred intercalation into the DNA double helix. We hypothesize that this compound may be cytotoxic at higher concentrations, which will be evaluated in future studies.

In conclusion, our findings demonstrate that the charge of rylene diimides is crucial for binding to the DNA double helix. Moreover, the rylene dye core plays a significant role in defining the DNA-binding mechanism and cytotoxicity of the compound. The cationic naphthalene diimide HCatNDI was identified as a potential anticancer agent due to its high cytotoxicity against HeLa cells.

5. MAIN CONCLUSIONS

In this thesis, several nonionic, cationic and anionic perylene diimides have been synthesized and characterized. Their chromonic behavior in water at high concentrations and in the presence of high molecular weight nonionic polymers has been evaluated and compared with the results previously reported in the literature. Furthermore, complex emulsions, advanced gels, and silica and carbon nanomaterials have been obtained using the chromonic mesophases as templates. The interactions of perylene diimides with DNA have also been studied. Herein, the main conclusions for each topic covered by this thesis are summarized.

Self-assembly of perylene diimides

- All the perylene diimides prepared in this thesis produced H-aggregates in aqueous solutions. These aggregates consisted in long face-centered columnar aggregates with unimolecular cross-section.
- The solubility in water of perylene diimides functionalized with linear polyethylene glycol (PEG) substituents, i.e. (PEG_n)₂PDI, increased with n . Notably, the (PEG_n)₂PDI concentration needed for aggregation is much lower for water than for organic solvents, suggesting a combined effect of π -stacking and hydrophobic effects for (PEG_n)₂PDI in water.
- The (PEG_n)₂PDI showed surface activity and a lower critical solution temperature (LCST) in the presence of salts. Notably, the LCST decreased at lower n values and in the presence of kosmotropic salts, following the Hofmeister series and the Schulze-Hardy rule.
- The anionic alanine-functionalized perylene diimide, i.e. (Ala)₂PDI, was only soluble in water, and presented a significantly lower degree of aggregation compared to the (PEG_n)₂PDIs, which is caused by its ionic character.

Chromonic phase behavior

- (PEG_n)₂PDI exhibit a rich chromonic phase behavior in water for PEG chains with intermediate length ($7 \leq n \leq 16$), but result too soluble for higher n PEG chain length and insoluble for lower n PEG chain length.
- The chromonic phase behavior of the (PEG_n)₂PDI is highly dependent on the value of n , as shorter PEG chains favored nematic phases whereas longer PEG chains produced mostly hexagonal phases. This suggests a higher binding energy for shorter (PEG_n)₂PDI, as predicted by Kuriabova et al. ^[252] in a computational model for chromonic systems, which is in agreement with the higher degree of aggregation in water of (PEG_n)₂PDI with shorter PEG chains inferred from absorbance, fluorescence and LCST studies at low concentrations.
- The phase diagrams of (PEG_n)₂PDI with shorter PEG chains differed from those of conventional chromonic mesogens. The most significant differences are the absence of peritectic points and the extended concentration range in which the M $\rightarrow$ N transition occurs by heating. This is consistent to results previously reported in literature for another nonionic PEGylated chromonic mesogen (TP6EO2M). This indicates that this phase behavior may be a distinctive feature of nonionic mesogens.
- In contrast, the anionic L-alanine-functionalized PDI, i.e. (Ala)₂PDI showed a more conventional chromonic phase diagram, indicating that the distinctive features of (PEG_n)₂PDI likely result from the nonionic nature of the mesogen rather than the perylene core. Additionally, the chirality of the alanine functionalization favored the formation of chiral nematic phases. It is interesting to remark that aminoacid-functionalized PDIs have been barely studied as chromonic mesogens, and the rich chromonic phase behavior of

(Ala)₂PDI presented herein suggests that studying other aminoacid-derived PDIs can be interesting to further comprehend the structure-phase behavior relationship in chromonics.

Formation of chromonic emulsions

- Chromonic water-in-water (W/W) emulsions are formed by the anionic (Ala)₂PDI and the cationic MeCatPDI perylene diimides in presence of nonionic polymers such as dextran (Dex, MW = 86000 g/mol) and polyethylene glycol (PEG, MW = 10000 g/mol). It was demonstrated that the phase behavior varied depending on the mesogen and polymer used.
- Several emulsion morphologies, including high-curvature nematic-in-isotropic (N/I) and isotropic-in-nematic (I/N) and low-curvature bicontinuous emulsions were found. More complex morphologies were also obtained, including nematic-in-nematic (N/N) or systems with hexagonal (M) and/or solid phases. Notably, N/N phases were only obtained in MeCatPDI-Dex systems, whereas the formation of birefringent phases with hexagonal and/or solid phases was typical of systems with PEG at high polymer concentrations, suggesting a higher immiscibility of PEG with the chromonic mesogens.
- The phase diagrams of the MeCatPDI-Dex and MeCatPDI-PEG biphasic systems were constructed. An asymmetric binodal line was obtained, indicating that small polymer concentrations (~1 – 2 wt%) are enough for phase separation whereas higher mesogen amounts (~ 10 wt%) are required to induce phase separation. This results from a low polymer solubility in the chromonic mesophases.
- Various nematic droplet configurations of the director vector (namely radial, bipolar and twisted) were obtained. It was observed that PEG induced the formation of twisted (chiral) configurations in both the chiral (Ala)₂PDI and the achiral MeCatPDI chromonic droplets. The formation of twisted structures was also attributed to the low twisting deformation constants of chromonic systems.
- Multiple W/W/W and W/W/O emulsions were obtained. W/W/O emulsions prepared in (Ala)₂PDI-PEG-Hexadecane triphasic systems consisted in Janus aqueous biphasic droplets where the nematic phase partially engulfed the polymer-rich phase, suggesting a lower interfacial tension between the (Ala)₂PDI-rich phase and the oil compared to that of the PEG-rich phase with the same oil.
- W/W/W multiple emulsions consisted in a chromonic-rich phase, a PEG-rich phase and a Dex-rich phase. MeCatPDI exhibited an improved capacity to form W/W/W emulsions compared to (Ala)₂PDI, which was attributed to the higher water-solubility of the latter.
- MeCatPDI showed a very rich all-aqueous triphasic behavior with PEG and Dex, and several complex morphologies such as Janus and snowman droplets were obtained. It was observed that snowman droplets were favored at lower polymer concentrations whereas Janus droplets required higher polymer concentrations. This is promising to fabricate complex particles in all-aqueous systems.

Preparation of gels from chromonic mesophases

- The incorporation of glucono- δ -lactone (GdL) to (Ala)₂PDI nematic phases produced the gelation of the chromonics material, hence forming a low molecular weight gel, because of the gradual decrease of pH induced by GdL. The nematic structure was only retained at moderate amounts of GdL (0.5 eq.).

- The nematic gels were responsive to temperature and light, which is promising for drug delivery applications.

Templating of silica nanostructured fibers/rods

- Porous silica rods were prepared using chromonic (PEG₇)₂PDI and (PEG₁₀)₂PDI as templates, although no ordered materials could be obtained using (PEG₁₆)₂PDI. Notably, the silica rods presented higher aspect ratio using (PEG₇)₂PDI, and the pore wall thickness increased with PEG chain length.
- MeCatPDI also demonstrated to be an excellent template to produce silica fibers with aligned nanopores, as had been previously reported ^[164]. The effect of changing the silica precursor from pure TEOS to TEOS/HDTMS mixtures was studied, observing that the incorporation of HDTMS generates porous silica fibers with similar specific surface area but larger pore sizes and volume and a less ordered pore structure.
- The MeCatPDI-templated silica was also formulated in the form of monoliths, and both conic and discotic monoliths were prepared. The obtention of robust monoliths is of high interest to prepare biocompatible implants for bone tissue repair and replacement.

Ibuprofen loading and release from the porous silica fibers

- The capacity of MeCatPDI-templated silica to carry ibuprofen for drug delivery applications was evaluated. Different encapsulation methods (soaking + drying and melting) were tested. The powder silica templated with TEOS/HDTMS 90:10 mixture and loaded by the soaking + drying method showed the highest encapsulation efficiency.
- The ibuprofen encapsulation efficiencies of the monolithic MeCatPDI-templated silica samples by the soaking + drying method were also studied. Silica monoliths exhibited much higher encapsulation efficiencies than the powder samples, but there were no significant differences between the monoliths templated with pure TEOS as silica precursor to those using a TEOS/HDTMS mixture.
- The ibuprofen release from the monolithic samples was studied in simulated body fluid (SBF) media. The release profiles exhibited an initial burst release followed by a gradual release until reaching the 100 % after approximately 24 h. The release profiles were suitably fitted to first-order models and to the Korsmeyer-Peppas model with exponents about 0.7 – 0.8, which may indicate an anomalous non-Fickian diffusion of the drug. This could be attributed to the aligned and non-interconnected pores of the silica materials.
- There were not significant differences between the release kinetics of the silica monoliths prepared with TEOS and those prepared with TEOS/HDTMS mixtures.

Hydroxyapatite growth in the silica materials

- The growth of hydroxyapatite on the porous silica materials was tested. It was observed that silica-hydroxyapatite composites can be obtained. These materials consisted in silica fibers embedded in a hydroxyapatite matrix, and are promising to prepare monoliths with improved mechanical properties to be used as bone implants. Further characterization of the porosity and the mechanical properties of the composites is required.

Carbon nanofibers for electrochemical energy storage applications

- N-doped carbon nanofibers with high surface area, hierarchical micro- and mesopores, and high wettability were prepared using the chromonic MeCatPDI as template. For this,

a chromonic-silica composite was prepared, carbonized and the silica phase was removed with strong bases to obtain the carbon nanofibers.

- The electrochemical energy storage properties of these fibers were tested in a three-electrode cell system, by CV, GCD and EIS experiments. The highest-performance carbon nanofibers were obtained by utilizing a carbonization temperature of 900 °C and removing silica with NaOH 1M. This sample exhibited a pseudocapacitive behavior and presented a high specific capacitance of $\sim 250 \text{ F} \cdot \text{g}^{-1}$ at a current density of $1 \text{ A} \cdot \text{g}^{-1}$ and an outstanding rate capability of almost 90 % at $50 \text{ A} \cdot \text{g}^{-1}$, outperforming commercial carbon materials such as Maxsorb® [419].
- The best-performing carbon nanofiber material also showed an outstanding cycle life stability with almost 100 % of capacitance retention after 10000 charge-discharge cycles at $50 \text{ A} \cdot \text{g}^{-1}$. This excellent electrochemical energy storage behavior was attributed to the presence of both micro- and mesopores, the high wettability of the carbon surface and the faradaic pseudocapacitance from the nitrogen-doping within the carbon nanofibers.
- Notably, the combination of high cycle life and outstanding capacitance retention at high current densities convert the obtained carbon nanofibers in very promising materials for applications where high-power densities are required, such as regenerative braking.
- A selected sample showed a good carbon dioxide capture capacity, adsorbing 2 mmol of CO_2 per gram of carbon material, at 0 °C.

DNA-intercalation and cytotoxic properties of rylene diimides

- The capacity of the nonionic $(\text{PEG}_7)_2\text{PDI}$, the anionic $(\text{Ala})_2\text{PDI}$ and the cationic MeCatPDI to interact with DNA was evaluated and compared to those of an anionic and a cationic naphthalene diimide.
- Only the cationic compounds (both the perylene and the naphthalene diimide) interacted with DNA. The cationic naphthalene diimide showed a conventional DNA-intercalator behavior, producing absorbance and fluorescence quenching of the diimide at higher DNA concentrations, alterations in the positive circular dichroism DNA band and increasing the viscosity of DNA aqueous solutions, as a function of the concentration of the naphthalene diimide.
- In contrast, the cationic MeCatPDI presented a more complex interaction that probably combines various mechanisms at the same time, including intercalation, aggregate disruption and electrostatic agglomeration, as indicated by the complex spectrophotometric behavior of the MeCatPDI-DNA mixtures.
- Cytotoxicity tests with HeLa cells were performed and the results indicated a high cytotoxicity for the cationic naphthalene diimide and almost zero cell death for the other compounds. With these results it is possible to conclude that the cationic naphthalene diimide is a potent DNA intercalator, whereas all the nonionic and anionic compounds are biocompatible. For MeCatPDI, the results are not conclusive, and more cytotoxicity tests are required.
- The low cytotoxicity of the $(\text{PEG}_7)_2\text{PDI}$ and $(\text{Ala})_2\text{PDI}$ demonstrate the potential of nonionic and anionic chromonic systems for bioapplications where biocompatible mesogens are required.

6. BIBLIOGRAPHY

1. Iqbal, P., Preece, J. A., & Mendes, P. M. (2012). Nanotechnology: The “Top-Down” and “Bottom-Up” Approaches. In *Supramolecular Chemistry: From Molecules to Nanomaterials*. John Wiley & Sons, Inc. <https://doi.org/10.1002/9780470661345.smc195>
2. Thiruvengadathan, R., Korampally, V., Ghosh, A., Chanda, N., Gangopadhyay, K., & Gangopadhyay, S. (2013). Nanomaterial processing using self-assembly-bottom-up chemical and biological approaches. *Reports on Progress in Physics*, 76(6), 066501. <https://doi.org/10.1088/0034-4885/76/6/066501>
3. Lombardo, D., Calandra, P., Pasqua, L., & Magazù, S. (2020). Self-assembly of organic nanomaterials and biomaterials: The bottom-up approach for functional nanostructures formation and advanced applications. *Materials*, 13(5), 1–43. <https://doi.org/10.3390/ma13051048>
4. Alavi, M., Karimi, N., & Safaei, M. (2017). Application of various types of liposomes in drug delivery systems. *Advanced Pharmaceutical Bulletin*, 7(1), 3–9. <https://doi.org/10.15171/apb.2017.002>
5. Guimarães, D., Cavaco-Paulo, A., & Nogueira, E. (2021). Design of liposomes as drug delivery system for therapeutic applications. *International Journal of Pharmaceutics*, 601, 120571. <https://doi.org/10.1016/j.ijpharm.2021.120571>
6. Azmi, I. D. M., Moghimi, S. M., & Yaghmur, A. (2015). Cubosomes and hexosomes as versatile platforms for drug delivery. *Therapeutic Delivery*, 6(12), 1347–1364. <https://doi.org/10.4155/tde.15.81>
7. Magana, J. R., Homs, M., Esquena, J., Freilich, I., Kesselman, E., Danino, D., Rodríguez-Abreu, C., & Solans, C. (2019). Formulating stable hexosome dispersions with a technical grade diglycerol-based surfactant. *Journal of Colloid and Interface Science*, 550, 73–80. <https://doi.org/10.1016/j.jcis.2019.04.084>
8. Magana, J. R., Esquena, J., Solans, C., & Rodríguez-Abreu, C. (2021). Deconstruction of technical grade diglycerol isostearate enables the controlled preparation of hexosomes and liposomes. *Journal of Molecular Liquids*, 343, 117594. <https://doi.org/10.1016/j.molliq.2021.117594>
9. Wadhwa, J., Nair, A., & Kumria, R. (2012). Emulsion forming drug delivery system for lipophilic drugs. *Acta Poloniae Pharmaceutica - Drug Research*, 69(2), 179–191.
10. Yukuyama, M. N., Ghisleni, D. D. M., Pinto, T. J. A., & Bou-Chacra, N. A. (2016). Nanoemulsion: Process selection and application in cosmetics - A review. *International Journal of Cosmetic Science*, 38(1), 13–24. <https://doi.org/10.1111/ics.12260>
11. Espitia, P., Fuenmayor, C. A., & Otoni, C. G. (2018). Nanoemulsions: Synthesis, Characterization, and Application in Bio-Based Active Food Packaging. *Comprehensive Reviews in Food Science and Food Safety*, 18(1), 264–285. <https://doi.org/10.1111/1541-4337.12405>
12. Feng, J., Rodríguez-Abreu, C., Esquena, J., & Solans, C. (2020). A Concise Review on Nano-emulsion Formation by the Phase Inversion Composition (PIC) Method. *Journal of Surfactants and Detergents*, 23(4), 677–685. <https://doi.org/10.1002/jsde.12414>
13. Attard, G. S., Glyde, J. C., & Göltner, C. G. (1995). Liquid-crystalline phases as templates for the synthesis of mesoporous silica. *Nature*, 378, 366–368. <https://doi.org/10.1038/378366a0>
14. Rodríguez-Abreu, C., & Lazzari, M. (2008). Emulsions with structured continuous phases. *Current Opinion in Colloid and Interface Science*, 13(4), 198–205. <https://doi.org/10.1016/j.cocis.2007.09.004>
15. Rodríguez-Abreu, C., Esquena, J., Aramaki, K., & López-Quintela, M. A. (2009). Mesoporous silica from reverse lyotropic liquid crystals: A novel approach. *Microporous and Mesoporous Materials*, 119(1–3), 338–343. <https://doi.org/10.1016/j.micromeso.2008.11.003>
16. Esquena, J., Nestor, J., Vílchez, A., Aramaki, K., & Solans, C. (2012). Preparation of mesoporous/macroporous materials in highly concentrated emulsions based on cubic phases by a single-step method. *Langmuir*, 28(33), 12334–12340. <https://doi.org/10.1021/la302120f>
17. Nestor, J., Vílchez, A., Solans, C., & Esquena, J. (2013). Facile synthesis of meso/macroporous dual materials with ordered mesopores using highly concentrated emulsions based on a cubic liquid

- crystal. *Langmuir*, 29(1), 432–440. <https://doi.org/10.1021/la303801b>
18. Randolph, T. W., & Jones, L. S. (2002). Surfactant-Protein Interactions. In J. F. Carpenter & M. C. Manning (Eds.), *Rational Design of Stable Protein Formulations* (pp. 159–175). Springer New York. https://doi.org/10.1007/978-1-4615-0557-0_7
19. Otzen, D. E. (2002). Protein unfolding in detergents: Effect of micelle structure, ionic strength, pH, and temperature. *Biophysical Journal*, 83(4), 2219–2230. [https://doi.org/10.1016/S0006-3495\(02\)73982-9](https://doi.org/10.1016/S0006-3495(02)73982-9)
20. Otzen, D. E. (2011). Protein-surfactant interactions: A tale of many states. *Biochimica et Biophysica Acta - Proteins and Proteomics*, 1814(5), 562–591. <https://doi.org/10.1016/j.bbapap.2011.03.003>
21. Masters, A. (2016). Chromonic liquid crystals: more questions than answers. *Liquid Crystals Today*, 25(2), 30–37. <https://doi.org/10.1080/1358314X.2016.1149925>
22. Esquena, J. (2016). Water-in-water (W/W) emulsions. *Current Opinion in Colloid and Interface Science*, 25, 109–119. <https://doi.org/10.1016/j.cocis.2016.09.010>
23. Mateescu, M. A., Ispas-Szabo, P., & Assaad, E. (2015). The concept of self-assembling and the interactions involved. In *Controlled Drug Delivery* (pp. 1–20). Woodhead Publishing. <https://doi.org/10.1016/b978-1-907568-45-9.00001-9>
24. McNaught, A. D., Wilkinson, A., & Chalk, S. J. (2019). *Ionic Bond*. The IUPAC Compendium of Chemical Terminology, 2nd Ed. (the “Gold Book”). <https://doi.org/10.1351/goldbook.it07058>
25. Faul, C. F. J., & Antonietti, M. (2003). Ionic self-assembly: Facile synthesis of supramolecular materials. *Advanced Materials*, 15(9), 673–683. <https://doi.org/10.1002/adma.200300379>
26. Faul, C. F. J. (2014). Ionic self-assembly for functional hierarchical nanostructured materials. *Accounts of Chemical Research*, 47(12), 3428–3438. <https://doi.org/10.1021/ar500162a>
27. McNaught, A. D., Wilkinson, A., & Chalk, S. J. (2019). *Dipole–Dipole Interaction*. The IUPAC Compendium of Chemical Terminology, 2nd Ed. (the “Gold Book”). <https://doi.org/10.1351/goldbook.d01758>
28. McNaught, A. D., Wilkinson, A., & Chalk, S. J. (2019). *Hydrogen Bond*. The IUPAC Compendium of Chemical Terminology, 2nd Ed. (the “Gold Book”). <https://doi.org/10.1351/goldbook.h02899>
29. McNaught, A. D., Wilkinson, A., & Chalk, S. J. (2019). *Hydrogen Bond in Theoretical Organic Chemistry*. The IUPAC Compendium of Chemical Terminology, 2nd Ed. (the “Gold Book”). <https://doi.org/10.1351/goldbook.ht07050>
30. Bondi, A. (1964). Van der waals volumes and radii. *Journal of Physical Chemistry*, 68(3), 441–451. <https://doi.org/10.1021/j100785a001>
31. Parthasarathi, R., Subramanian, V., & Sathyamurthy, N. (2006). Hydrogen bonding without borders: An atoms-in-molecules perspective. *Journal of Physical Chemistry A*, 110(10), 3349–3351. <https://doi.org/10.1021/jp060571z>
32. Steiner, T. (2002). The Hydrogen Bond in the Solid State. *Angewandte Chemie-International Edition*, 41, 48–76. [https://doi.org/10.1002/1521-3773\(20020104\)41:1<48::AID-ANIE48>3.0.CO;2-U](https://doi.org/10.1002/1521-3773(20020104)41:1<48::AID-ANIE48>3.0.CO;2-U)
33. London, F. (1936). The general theory of molecular forces. *Transactions of the Faraday Society*, 8(8), 8–26. <https://doi.org/10.1039/TF937330008B>
34. Wagner, J. P., & Schreiner, P. R. (2015). London Dispersion in Molecular Chemistry - Reconsidering Steric Effects. *Angewandte Chemie - International Edition*, 54(42), 12274–12296. <https://doi.org/10.1002/anie.201503476>
35. Klapetek, P. (2018). Force and Mechanical Properties. In *Quantitative Data Processing in Scanning Probe Microscopy: SPM Applications for Nanometrology: Second Edition* (pp. 151–201). Elsevier. <https://doi.org/10.1016/B978-0-12-813347-7.00006-6>
36. Sinnokrot, M. O., & Sherrill, C. D. (2006). High-accuracy quantum mechanical studies of π - π interactions in benzene dimers. *Journal of Physical Chemistry A*, 110(37), 10656–10668.

- <https://doi.org/10.1021/jp0610416>
37. Williams, J. H., Cockcroft, J. K., & Fitch, A. N. (1992). Structure of the Lowest Temperature Phase of the Solid Benzene–Hexafluorobenzene Adduct. *Angewandte Chemie International Edition in English*, 31(12), 1655–1657. <https://doi.org/10.1002/anie.199216551>
 38. Reczek, J. J., Villazor, K. R., Lynch, V., Swager, T. M., & Iverson, B. L. (2006). Tunable columnar mesophases utilizing C2 symmetric aromatic donor-acceptor complexes. *Journal of the American Chemical Society*, 128(24), 7995–8002. <https://doi.org/10.1021/ja061649s>
 39. Hunter, C. A., & Sanders, J. K. M. (1990). The Nature of π - π Interactions. *Journal of the American Chemical Society*, 112(14), 5525–5534. <https://doi.org/10.1021/ja00170a016>
 40. Wheeler, S. E., & Houk, K. N. (2008). Substituent effects in the benzene dimer are due to direct interactions of the substituents with the unsubstituted benzene. *Journal of the American Chemical Society*, 130(33), 10854–10855. <https://doi.org/10.1021/ja802849j>
 41. Watt, M., Hardebeck, L. K. E., Kirkpatrick, C. C., & Lewis, M. (2011). Face-to-face arene-arene binding energies: Dominated by dispersion but predicted by electrostatic and dispersion/polarizability substituent constants. *Journal of the American Chemical Society*, 133(11), 3854–3862. <https://doi.org/10.1021/ja105975a>
 42. Wheeler, S. E. (2011). Local nature of substituent effects in stacking interactions. *Journal of the American Chemical Society*, 133(26), 10262–10274. <https://doi.org/10.1021/ja202932e>
 43. Thakuria, R., Nath, N. K., & Saha, B. K. (2019). The Nature and Applications of π - π Interactions: A Perspective. *Crystal Growth and Design*, 19(2), 523–528. <https://doi.org/10.1021/acs.cgd.8b01630>
 44. Grimme, S. (2008). Do special noncovalent π - π stacking interactions really exist? *Angewandte Chemie - International Edition*, 47(18), 3430–3434. <https://doi.org/10.1002/anie.200705157>
 45. Martinez, C. R., & Iverson, B. L. (2012). Rethinking the term “pi-stacking.” *Chemical Science*, 3(7), 2191–2201. <https://doi.org/10.1039/c2sc20045g>
 46. Watson, M. D., Fechtenkötter, A., & Müllen, K. (2001). Big is beautiful - “Aromaticity” revisited from the viewpoint of macromolecular and supramolecular benzene chemistry. *Chemical Reviews*, 101(5), 1267–1300. <https://doi.org/10.1021/cr990322p>
 47. Cubberley, M. S., & Iverson, B. L. (2001). ¹H NMR investigation of solvent effects in aromatic stacking interactions. *Journal of the American Chemical Society*, 123(31), 7560–7563. <https://doi.org/10.1021/ja015817m>
 48. Carroll, W. R., Pellechia, P., & Shimizu, K. D. (2008). A rigid molecular balance for measuring face-to-face arene-arene interactions. *Organic Letters*, 10(16), 3547–3550. <https://doi.org/10.1021/ol801286k>
 49. Ikkanda, B. A., & Iverson, B. L. (2016). Exploiting the interactions of aromatic units for folding and assembly in aqueous environments. *Chemical Communications*, 52(50), 7752–7759. <https://doi.org/10.1039/c6cc01861k>
 50. McNaught, A. D., Wilkinson, A., & Chalk, S. J. (2019). *Hydrophobic Interaction*. The IUPAC Compendium of Chemical Terminology, 2nd Ed. (the “Gold Book”). <https://doi.org/10.1351/goldbook.h02907>
 51. McNaught, A. D., Wilkinson, A., & Chalk, S. J. (2019). *Hydrophobicity*. The IUPAC Compendium of Chemical Terminology, 2nd Ed. (the “Gold Book”). <https://doi.org/10.1351/goldbook.ht06964>
 52. Frank, H. S., & Evans, M. W. (1945). Free volume and entropy in condensed systems III. Entropy in binary liquid mixtures; Partial molal entropy in dilute solutions; Structure and thermodynamics in aqueous electrolytes. *The Journal of Chemical Physics*, 13(11), 507–532. <https://doi.org/10.1063/1.1723985>
 53. Buchanan, P., Aldiwan, N., Soper, A. K., Creek, J. L., & Koh, C. A. (2005). Decreased structure on dissolving methane in water. *Chemical Physics Letters*, 415(1–3), 89–93. <https://doi.org/10.1016/j.cplett.2005.08.064>

54. Snyder, P. W., Lockett, M. R., Moustakas, D. T., & Whitesides, G. M. (2014). Is it the shape of the cavity, or the shape of the water in the cavity? *European Physical Journal: Special Topics*, 223(5), 853–891. <https://doi.org/10.1140/epjst/e2013-01818-y>
55. Sun, Q. (2022). The Hydrophobic Effects: Our Current Understanding. *Molecules*, 27(20), 7009. <https://doi.org/10.3390/molecules27207009>
56. Smithrud, D. B., Wyman, T. B., & Diederich, F. (1991). Enthalpically Driven Cyclophane-Arene Inclusion Complexation: Solvent-Dependent Calorimetric Studies. *Journal of the American Chemical Society*, 113(14), 5420–5426. <https://doi.org/10.1021/ja00014a038>
57. Biedermann, F., Nau, W. M., & Schneider, H. J. (2014). The Hydrophobic Effect Revisited - Studies with Supramolecular Complexes Imply High-Energy Water as a Noncovalent Driving Force. *Angewandte Chemie - International Edition*, 53(42), 11158–11171. <https://doi.org/10.1002/anie.201310958>
58. Plyasunov, A. V., & Shock, E. L. (2000). Thermodynamic functions of hydration of hydrocarbons at 298.15 K and 0.1 MPa. *Geochimica et Cosmochimica Acta*, 64(3), 439–468. [https://doi.org/10.1016/S0016-7037\(99\)00330-0](https://doi.org/10.1016/S0016-7037(99)00330-0)
59. Hillyer, M. B., & Gibb, B. C. (2016). Molecular Shape and the Hydrophobic Effect. *Annual Review of Physical Chemistry*, 67, 307–329. <https://doi.org/10.1146/annurev-physchem-040215-112316>
60. Sunner, J., Nishizawa, K., & Kebarle, P. (1981). Ion-solvent molecule interactions in the gas phase. The potassium ion and benzene. *Journal of Physical Chemistry*, 85(13), 1814–1820. <https://doi.org/10.1021/j150613a011>
61. Xiu, X., Puskar, N. L., Shanata, J. A. P., Lester, H. A., & Dougherty, D. A. (2009). Nicotine binding to brain receptors requires a strong cation- interaction. *Nature*, 458(7237), 534–537. <https://doi.org/10.1038/nature07768>
62. Ma, J. C., & Dougherty, D. A. (1997). The Cation- π Interaction. *Chem. Rev.*, 97, 1303–1324. <https://doi.org/10.1021/cr9603744>
63. Quiñónero, D., Garau, C., Rotger, C., Frontera, A., Ballester, P., Costa, A., & Deyà, P. M. (2002). Anion- π Interactions: Do They Exist? *Angewandte Chemie - International Edition*, 114, 3539–3542. [https://doi.org/10.1002/1521-3773\(20020916\)41:18<3389::AID-ANIE3389>3.0.CO;2-S](https://doi.org/10.1002/1521-3773(20020916)41:18<3389::AID-ANIE3389>3.0.CO;2-S)
64. Martínez, D., Pérez, A., Cañellas, S., Silió, I., Lancho, A., García-Raso, A., Fiol, J. J., Terrón, A., Barceló-Oliver, M., Ortega-Castro, J., Molins, E., & Frontera, A. (2020). Synthesis, reactivity, X-ray characterization and docking studies of N7/N9-(2-pyrimidyl)-adenine derivatives. *Journal of Inorganic Biochemistry*, 203, 110879. <https://doi.org/10.1016/j.jinorgbio.2019.110879>
65. Berryman, O. B., Bryantsev, V. S., Stay, D. P., Johnson, D. W., & Hay, B. P. (2007). Structural criteria for the design of anion receptors: The interaction of halides with electron-deficient arenes. *Journal of the American Chemical Society*, 129(1), 48–58. <https://doi.org/10.1021/ja063460m>
66. Kelker, H. (1988). Survey of the Early History of Liquid Crystals. *Molecular Crystals and Liquid Crystals Incorporating Nonlinear Optics*, 165(1), 1–43. <https://doi.org/10.1080/00268948808082195>
67. Heintz, W. (1855). Ueber die Fette. *Journal Für Praktische Chemie*, 66(1), 1–51. <https://doi.org/10.1002/prac.18550660101>
68. Virchow, R. (1853). No Title. *Virchow Archive for Pathological Anatomy and Physiology*, 6, 571.
69. Kelker, H., & Knoll, P. M. (1989). Plenary lecture: Some pictures of the history of liquid crystals. *Liquid Crystals*, 5(1), 19–42. <https://doi.org/10.1080/02678298908026350>
70. Mettenheimer, C. (1857). Mikroskopische beobachtungen mit polarisiertem licht. *Corr. Blatt d. Vereins Gem. Arb. z. Förderung d. Wissenschatl. Heilkunde*, 24, 331.
71. Reinitzer, F. (1888). Beiträge zur kenntniss des cholesterins (Contributions to the understanding of cholesterol). *Monatshefte Für Chemie*, 9, 421–441. <https://link.springer.com/content/pdf/10.1007%2FBF01516710.pdf>
72. Lehmann, O. (1889). Über fließende krystalle. *Zeitschrift Für Physikalische Chemie*, 4(1), 462–472.

-
73. Mitov, M. (2014). Liquid-crystal science from 1888 to 1922: Building a revolution. *ChemPhysChem*, 15(7), 1245–1250. <https://doi.org/10.1002/cphc.201301064>
74. Lehmann, O. (1904). *Flüssige Kristalle sowie Plastizität von Kristallen im allgemeinen, molekulare Umlagerungen und Aggregatzustandsänderungen*. Verlag von Wilhelm Engelmann.
75. Gattermann, L., & Ritschke, A. (1890). Ueber Azoxyphenoläther. *Berichte Der Deutschen Chemischen Gesellschaft*, 23(1), 1738–1750. <https://doi.org/10.1002/cber.189002301285>
76. Gray, G. W., Harrison, K. J., & Nash, J. A. (1973). New family of nematic liquid crystals for displays. *Electronics Letters*, 9(6), 130–131. <https://doi.org/10.1049/el:19730096>
77. Shao, Y., & Zerda, T. W. (1998). Phase transitions of liquid crystal PAA in confined geometries. *Journal of Physical Chemistry B*, 102(18), 3387–3394. <https://doi.org/10.1021/jp9734437>
78. Mallia, V. A., & Tamaoki, N. (2004). Design of chiral dimesogens containing cholesteryl groups; formation of new molecular organizations and their application to molecular photonics. *Chemical Society Reviews*, 33(2), 76–84. <https://doi.org/10.1039/b106617j>
79. Wöhrle, T., Wurzbach, I., Kirres, J., Kostidou, A., Kapernaum, N., Litterscheidt, J., Haenle, J. C., Staffeld, P., Baro, A., Giesselmann, F., & Laschat, S. (2016). Discotic Liquid Crystals. *Chemical Reviews*, 116(3), 1139–1241. <https://doi.org/10.1021/acs.chemrev.5b00190>
80. Chandrasekhar, S., Sadashiva, B. K., & Suresh, K. A. (1977). Liquid crystals of disc-like molecules. *Pramana*, 9(5), 471–480. <https://doi.org/10.1007/BF02846252>
81. Tinh, N. H., Destrade, C., & Gasparoux, H. (1979). Nematic disc-like liquid crystals. *Physics Letters A*, 72(3), 251–254. [https://doi.org/10.1016/0375-9601\(79\)90019-7](https://doi.org/10.1016/0375-9601(79)90019-7)
82. Pal, S. K., Setia, S., Avinash, B. S., & Kumar, S. (2013). Triphenylene-based discotic liquid crystals : recent advances. *Liquid Crystals*, 40(12), 1769–1816. <https://doi.org/10.1080/02678292.2013.854418>
83. Wicklein, A., Lang, A., Muth, M., & Thelakkat, M. (2009). Swallow-tail substituted liquid crystalline perylene bisimides: Synthesis and thermotropic properties. *Journal of the American Chemical Society*, 131(40), 14442–14453. <https://doi.org/10.1021/ja905260c>
84. Li, L. L., Hu, P., Wang, B. Q., Yu, W. H., Shimizu, Y., & Zhao, K. Q. (2010). Synthesis and mesomorphism of ether-ester mixed tail C3-symmetrical truxene discotic liquid crystals. *Liquid Crystals*, 37(5), 499–506. <https://doi.org/10.1080/02678290903215337>
85. Piechocki, C., Simon, J., Skoulios, A., Guillon, D., & Weber, P. (1982). Discotic Mesophases Obtained from Substituted Metallophthalocyanines. Toward Liquid Crystalline One-Dimensional Conductors. *Journal of the American Chemical Society*, 104(19), 5245–5247. <https://doi.org/10.1021/ja00383a050>
86. Fechtenkötter, A., Saalwächter, K., Harbison, M. A., Müllen, K., & Spiess, H. W. (1999). Highly ordered columnar structures from Hexa-peri-hexabenzocoronenes- synthesis, X-ray diffraction, and solid-state heteronuclear multiple-quantum NMR investigations. *Angewandte Chemie - International Edition*, 38(20), 3039–3042. [https://doi.org/10.1002/\(SICI\)1521-3773\(19991018\)38:20<3039::AID-ANIE3039>3.0.CO;2-5](https://doi.org/10.1002/(SICI)1521-3773(19991018)38:20<3039::AID-ANIE3039>3.0.CO;2-5)
87. Petrov, A. G. (2000). *The Lyotropic State of Matter: Molecular Physics and Living Matter Physics*. Gordon and Breach Science Publishers.
88. Friedel, G. (1922). Les états mésomorphes de la matière. *Annales de Physique*, 9(18), 273–474. <https://doi.org/10.1051/anphys/192209180273>
89. Tiddy, G. J. T. (1980). Surfactant-water liquid crystal phases. *Physics Reports*, 57(1), 1–46. [https://doi.org/10.1016/0370-1573\(80\)90041-1](https://doi.org/10.1016/0370-1573(80)90041-1)
90. Fontell, K. (1978). Liquid crystalline behavior in lipid-water systems. *Progress in the Chemistry of Fats and Other Lipids*, 16, 145–162. [https://doi.org/10.1016/0079-6832\(78\)90040-X](https://doi.org/10.1016/0079-6832(78)90040-X)
91. Israelachvili, J. (2011). *Intermolecular and surface forces*, 3rd Ed. Academic Press, Elsevier. <https://doi.org/10.1016/C2009-0-21560-1>

92. Dierking, I., & Neto, A. M. F. (2020). Novel trends in lyotropic liquid crystals. *Crystals*, 10(7), 604. <https://doi.org/10.3390/cryst10070604>
93. Lydon, J. (1998). Chromonic liquid crystal phases. *Current Opinion in Colloid and Interface Science*, 3(5), 458–466. [https://doi.org/10.1016/S1359-0294\(98\)80019-8](https://doi.org/10.1016/S1359-0294(98)80019-8)
94. Lydon, J. (2004). Chromonic mesophases. *Current Opinion in Colloid and Interface Science*, 8(6), 480–490. <https://doi.org/10.1016/j.cocis.2004.01.006>
95. Lydon, J. (2010). Chromonic review. *Journal of Materials Chemistry*, 20(45), 10071–10099. <https://doi.org/10.1039/b926374h>
96. Lydon, J. (2011). Chromonic liquid crystalline phases. *Liquid Crystals*, 38(11–12), 1663–1681. <https://doi.org/10.1080/02678292.2011.614720>
97. Cox, J. S. G., Woodard, G. D., & McCrone, W. C. (1971). Solid-state chemistry of Chromolyn Sodium (Disodium Cromoglycate). *Journal of Pharmaceutical Sciences*, 60(10), 1458–1465. <https://doi.org/10.1002/jps.2600601003>
98. Hartshorne, N. H., & Woodard, G. D. (1973). Mesomorphism in the System Disodium Chromoglycate-Water. *Molecular Crystals and Liquid Crystals*, 23(3–4), 343–368. <https://doi.org/10.1080/15421407308083381>
99. Attwood, T. K., & Lydon, J. E. (1984). Lyotropic Mesophase Formation by Anti-Asthmatic Drugs. *Molecular Crystals and Liquid Crystals*, 108(3–4), 349–357. <https://doi.org/10.1080/00268948408078686>
100. Tam-Chang, S. W., Helbley, J., & Iverson, I. K. (2008). A study of the structural effects on the liquid-crystalline properties of ionic perylenebis(dicarboximide)s using UV-Vis spectroscopy, polarized light microscopy, and NMR spectroscopy. *Langmuir*, 24(5), 2133–2139. <https://doi.org/10.1021/la7027324>
101. Smith, G. P., Fraccia, T. P., Todisco, M., Zanchetta, G., Zhu, C., Hayden, E., Bellini, T., & Clark, N. A. (2018). Backbone-free duplex-stacked monomer nucleic acids exhibiting Watson – Crick selectivity. *Proceedings of the National Academy of Sciences*, 115(33), 7658–7664. <https://doi.org/10.1073/pnas.1721369115>
102. Lydon, J. (1998). Chromonics. In D. Demus, J. Goodby, G. W. Gray, H. W. Spiess, & V. Vill (Eds.), *Handbook of Liquid Crystals: Low Molecular Weight Liquid Crystals II* (pp. 982–1007). Wiley-VCH Verlag. <https://doi.org/10.1002/9783527620623.ch15>
103. Lee, H., & Labes, M. M. (1983). Phase Diagram and Thermodynamic Properties of Disodium Cromoglycate-Water Lyomesophases. *Molecular Crystals and Liquid Crystals*, 91, 53–58.
104. Wang, S., Maruri, D. P., Boothby, J. M., Lu, X., Rivera-Tarazona, L. K., Varner, V. D., & Ware, T. H. (2020). Anisotropic, porous hydrogels templated by lyotropic chromonic liquid crystals. *Journal of Materials Chemistry B*, 8(31), 6988–6998. <https://doi.org/10.1039/d0tb00904k>
105. Ichikawa, T., Kuwana, M., & Suda, K. (2022). Chromonic Ionic Liquid Crystals Forming Nematic and Hexagonal Columnar Phases. *Crystals*, 12(11), 1548. <https://doi.org/10.3390/cryst12111548>
106. Walker, M., Masters, A. J., & Wilson, M. R. (2014). Self-assembly and mesophase formation in a non-ionic chromonic liquid crystal system: Insights from dissipative particle dynamics simulations. *Physical Chemistry Chemical Physics*, 16(42), 23074–23081. <https://doi.org/10.1039/c4cp03092c>
107. Walker, M., & Wilson, M. R. (2015). A Simple Model for Chromonic Aggregation. *Molecular Crystals and Liquid Crystals*, 612(1), 117–125. <https://doi.org/10.1080/15421406.2015.1030580>
108. Akinshina, A., Walker, M., Wilson, M. R., Tiddy, G. J. T., Masters, A. J., & Carbone, P. (2015). Thermodynamics of the self-assembly of non-ionic chromonic molecules using atomistic simulations. the case of TP6EO2M in aqueous solution. *Soft Matter*, 11(4), 680–691. <https://doi.org/10.1039/c4sm02275k>
109. Collings, P. J., Goldstein, J. N., Hamilton, E. J., Mercado, B. R., Nieser, K. J., & Regan, M. H. (2015). The nature of the assembly process in chromonic liquid crystals. *Liquid Crystals Reviews*, 3(1), 1–27. <https://doi.org/10.1080/21680396.2015.1025305>

-
110. Mcrae, E. G., & Kasha, M. (1958). Enhancement of phosphorescence ability upon aggregation of dye molecules. *The Journal of Chemical Physics*, 28(4), 721–722. <https://doi.org/10.1063/1.1744225>
111. Würthner, F., Kaiser, T. E., & Saha-Möller, C. R. (2011). J-aggregates: From serendipitous discovery to supramolecular engineering of functional dye materials. *Angewandte Chemie - International Edition*, 50(15), 3376–3410. <https://doi.org/10.1002/anie.201002307>
112. Kasha, M., Rawls, H. R., & El-Bayoumi, M. A. (1965). The Exciton Model In Molecular Spectroscopy. *Pure and Applied Chemistry*, 11(3–4), 371–392. <https://doi.org/10.1351/pac196511030371>
113. Kasha, M. (1950). Characterization of electronic transitions in complex molecules. *Discussions of the Faraday Society*, 9, 14–19. <https://doi.org/10.1039/DF9500900014>
114. Horowitz, V. R., Janowitz, L. A., Modic, A. L., Heiney, P. A., & Collings, P. J. (2005). Aggregation behavior and chromonic liquid crystal properties of an anionic monoazo dye. *Physical Review E - Statistical, Nonlinear, and Soft Matter Physics*, 72(4), 041710. <https://doi.org/10.1103/PhysRevE.72.041710>
115. Edwards, D. J., Jones, J. W., Lozman, O., Ormerod, A. P., Sentyureva, M., & Tiddy, G. J. T. (2008). Chromonic liquid crystal formation by edicol sunset yellow. *Journal of Physical Chemistry B*, 112(46), 14628–14636. <https://doi.org/10.1021/jp802758m>
116. Tomasik, M. R., & Collings, P. J. (2008). Aggregation Behavior and Chromonic Liquid Crystal Phase of a Dye Derived from Naphthalenecarboxylic Acid. *Journal of Physical Chemistry B*, 112(32), 9883–9889. <https://doi.org/10.1021/jp803648g>
117. Tiddy, G. J. T., Mateer, D. L., Ormerod, A. P., Harrison, W. J., & Edwards, D. J. (1995). Highly Ordered Aggregates in Dilute Dye-Water Systems. *Langmuir*, 11(2), 390–393. <https://doi.org/10.1021/la00002a002>
118. Lydon, J. (2007). A personal history of the early days of chromonics. *Liquid Crystals Today*, 16(2), 13–27. <https://doi.org/10.1080/14645180701876080>
119. Yadav, Y. J., Heinrich, B., De Luca, G., Talarico, A. M., Mastropietro, T. F., Ghedini, M., Donnio, B., & Szerb, E. I. (2013). Chromonic-like physical luminescent gels formed by ionic octahedral iridium(iii) complexes in diluted water solutions. *Advanced Optical Materials*, 1(11), 844–854. <https://doi.org/10.1002/adom.201300236>
120. Magana, J. R., Homs, M., Solans, C., Obiols-Rabasa, M., Salonen, L. M., & Rodríguez-Abreu, C. (2016). Self-Assembly and Formation of Chromonic Liquid Crystals from the Dyes Quinaldine Red Acetate and Pyronin y. *Journal of Physical Chemistry B*, 120(1), 250–258. <https://doi.org/10.1021/acs.jpcb.5b10567>
121. Magana, J. R. (2017). *Molecular Self-Assembly for the preparation of novel nanostructured materials* [Doctoral dissertation, Universitat de Barcelona]. <https://www.tdx.cat/handle/10803/400826#page=1>
122. Lee, H., & Labes, M. M. (1982). Lyotropic Cholesteric and Nematic Phases of Disodium Cromoglycate in Magnetic Fields. *Molecular Crystals and Liquid Crystals*, 84(1–4), 137–157. <https://doi.org/10.1080/00268948208072137>
123. Naidu, J. J., Shin, S., Jeong, K. U., & Lee, M. H. (2010). Optically active lyotropic chromonic liquid crystal based on green perylene bisimide. *Bulletin of the Korean Chemical Society*, 31(9), 2705–2708. <https://doi.org/10.5012/bkcs.2010.31.9.2705>
124. Shirai, T., Shuai, M., Nakamura, K., Yamaguchi, A., Naka, Y., Sasaki, T., Clark, N. A., & Le, K. V. (2018). Chiral lyotropic chromonic liquid crystals composed of disodium cromoglycate doped with water-soluble chiral additives. *Soft Matter*, 14(9), 1511–1516. <https://doi.org/10.1039/c7sm02262j>
125. Goodby, J. W. (2012). Introduction to Defect Textures in Liquid Crystals. In *Handbook of Visual Display Technology* (pp. 1289–1314). https://doi.org/10.1007/978-3-540-79567-4_82
126. Attwood, T. K., Lydon, J., & Jones, F. (1986). The chromonic phases of dyes. *Liquid Crystals*, 1(6), 499–507. <https://doi.org/10.1080/02678298608086274>
127. Pérez-Calm, A. (2019). *Synthesis and characterization of perylene-based building blocks for the*

- fabrication of optically-active nanostructured materials*. Master dissertation, University of Barcelona.
128. Figueiredo Neto, A. M., & Salinas, S. R. A. (2003). The Nematic And Cholesteric Phases. In *Textures of Liquid Crystals* (pp. 190–218). <https://doi.org/https://doi.org/10.1002/3527602054.ch5>
 129. Schnablegger, H., & Singh, Y. (2017). *The SAXS Guide, 4th Edition*. Anton Paar.
 130. Zhan, X., Facchetti, A., Barlow, S., Marks, T. J., Ratner, M. A., Wasielewski, M. R., & Marder, S. R. (2011). Rylene and Related Diimides for Organic Electronics. *Advanced Materials*, 23(2), 268–284. <https://doi.org/10.1002/adma.201001402>
 131. Nowak-Król, A., & Würthner, F. (2019). Progress in the synthesis of perylene bisimide dyes. *Organic Chemistry Frontiers*, 6(8), 1272–1318. <https://doi.org/10.1039/c8qo01368c>
 132. Pron, A., Gawrys, P., Zagorska, M., Djurado, D., & Demadrille, R. (2010). Electroactive materials for organic electronics: Preparation strategies, structural aspects and characterization techniques. *Chemical Society Reviews*, 39(7), 2577–2632. <https://doi.org/10.1039/b907999h>
 133. Kobaisi, M. Al, Bhosale, S. V., Latham, K., Raynor, A. M., & Bhosale, S. V. (2016). Functional Naphthalene Diimides: Synthesis, Properties, and Applications. *Chemical Reviews*, 116, 11685–11796. <https://doi.org/10.1021/acs.chemrev.6b00160>
 134. Würthner, F., Saha-Möller, C. R., Fimmel, B., Ogi, S., Leowanawat, P., & Schmidt, D. (2016). Perylene Bisimide Dye Assemblies as Archetype Functional Supramolecular Materials. *Chemical Reviews*, 116(3), 962–1052. <https://doi.org/10.1021/acs.chemrev.5b00188>
 135. Smith, A. M. Y. R., & Iverson, B. (2017). NDI as a DNA Intercalator. In *Naphthalenediimide and its Congeners: From Molecules to Materials* (pp. 37–71). <https://doi.org/10.1039/9781782621386-00037>
 136. Schiessler, S., & Spietschka, E. (1986). *Process for the preparation of naphthalene-1,4,5,8-tetracarboxylic acid and its 1,8-monoanhydride in a high degree of purity* (Patent No. US4599431). United States Patent. <https://patents.google.com/patent/US4599431A/en>
 137. Hunger, K., & Herbst, W. (2000). Pigments, Organic. In *Ullmann's Encyclopedia of Color Science and Technology* (pp. 380–422). https://doi.org/10.1002/14356007.a20_371
 138. Cormier, R. A., & Gregg, B. A. (1997). Self-Organization in Thin Films of Liquid Crystalline Perylene Diimides. *Journal of Physical Chemistry B*, 101(51), 11004–11006. <https://doi.org/10.1021/jp9732064>
 139. Cormier, R. A., & Gregg, B. A. (1998). Synthesis and Characterization of Liquid Crystalline Perylene Diimides. *Chemistry of Materials*, 10(5), 1309–1319. <https://doi.org/10.1021/cm970695b>
 140. Gupta, R. K., & Sudhakar, A. A. (2019). Perylene-Based Liquid Crystals as Materials for Organic Electronics Applications. *Langmuir*, 35(7), 2455–2479. <https://doi.org/10.1021/acs.langmuir.8b01081>
 141. Boiko, O. P., Lenyk, B. Y., Posudievskiy, O. Y., Slominskiy, Y. L., Tsybulia, S. A., Nastishin, Y. A., & Nazarenko, V. G. (2019). Electronic energy levels in lyotropic chromonic liquid crystals formed by ionic perylene diimide derivatives. *Synthetic Metals*, 257, 116147. <https://doi.org/10.1016/j.synthmet.2019.116147>
 142. Iverson, I. K., & Tam-Chang, S. W. (1999). Cascade of Molecular Order by Sequential Self-Organization, Induced Orientation, and Order Transfer Processes. *Journal of the American Chemical Society*, 121(24), 5801–5802. <https://doi.org/10.1021/ja983803j>
 143. Iverson, I. K., Casey, S. M., Seo, W., Tam-Chang, S. W., & Pindzola, B. A. (2002). Controlling Molecular Orientation in Solid Films via Self-Organization in the Liquid-Crystalline Phase. *Langmuir*, 18(9), 3510–3516. <https://doi.org/10.1021/la011499t>
 144. Tam-Chang, S. W., Iverson, I. K., & Helbley, J. (2004). Study of the Chromonic Liquid-Crystalline Phases of Diimide Dihydrochloride by Polarized Optical Microscopy and ²H NMR Spectroscopy. *Langmuir*, 20(2), 342–347. <https://doi.org/10.1021/la030256t>

145. Tam-Chang, S. W., Huang, L., Gyan, A., Seo, W., Mahinay, D., & Iverson, I. K. (2008). Designing Chromonic Mesogens for the Fabrication of Anisotropic Optical Materials. *Emerging Liquid Crystal Technologies III*, 6911. <https://doi.org/10.1117/12.761012>
146. Naidu, J. J., Bae, Y., Jeong, K., Lee, S. H., Kang, S., & Lee, M. (2009). Synthesis of New Chiral Chromonic Lyotropic Liquid Crystal Based on Perylenebis(dicarboximide). *Bulletin of the Korean Chemical Society*, 30(4), 935–937. <https://doi.org/10.5012/bkcs.2009.30.4.935>
147. Naidu, J. J., Bae, Y., Jeong, K., Shin, S.-H., & Lee, M. (2009). Color Tuning of Perylene Based Lyotropic Chromonic Liquid Crystal. *Bulletin of the Korean Chemical Society*, 30(1), 224–226. <https://doi.org/https://doi.org/10.5012/bkcs.2009.30.1.224>
148. Im, P., Kang, D., Kim, D., Choi, Y., Yoon, W., Lee, M., Lee, I., Lee, C., & Jeong, K. (2016). Flexible and Patterned Thin Film Polarizer: Photopolymerization of Perylene-based Lyotropic Chromonic Reactive Mesogens. *ACS Applied Materials & Interfaces*, 8(1), 762–771. <https://doi.org/10.1021/acsami.5b09995>
149. Kularatne, R. S., Kim, H., Ammanamanchi, M., Hayenga, H. N., & Ware, T. H. (2016). Shape-Morphing Chromonic Liquid Crystal Hydrogels. *Chemistry of Materials*, 28(23), 8489–8492. <https://doi.org/10.1021/acs.chemmater.6b04553>
150. Tam-Chang, S. W., & Huang, L. (2008). Chromonic liquid crystals: Properties and applications as functional materials. *Chemical Communications*, 17, 1957–1967. <https://doi.org/10.1039/b714319b>
151. Park, H. S., & Lavrentovich, O. D. (2012). Lyotropic Chromonic Liquid Crystals: Emerging Applications. In Q. Li (Ed.), *Liquid Crystals Beyond Displays: Chemistry, Physics, and Applications* (pp. 449–484). John Wiley & Sons, Inc. <https://doi.org/10.1002/9781118259993.ch14>
152. Bosire, R., Ndaya, D., & Kasi, R. M. (2021). Recent progress in functional materials from lyotropic chromonic liquid crystals. *Polymer International*, 70(7), 938–943. <https://doi.org/10.1002/pi.6113>
153. Tam-Chang, S. W., Seo, W., Iverson, I. K., & Casey, S. M. (2003). Ionic Quaterrylenebis dicarboximide A Novel Mesogen and Long-Wavelength Polarizing. *Angewandte Chemie - International Edition*, 42(5), 897–900. <https://doi.org/10.1002/anie.200390236>
154. Sergan, T., Schneider, T., Kelly, J., Lavrentovich, O. D., Sergan, T., & Kelly, J. (2000). Polarizing-alignment layers for twisted nematic cells. *Liquid Crystals*, 27(5), 37–41. <https://doi.org/10.1080/026782900202390>
155. Matsunaga, D., Tamaki, T., Aiyama, H., & Ichimura, K. (2002). Photofabrication of Micro-Patterned Polarizing Elements for Stereoscopic Displays. *Advanced Materials*, 20(14), 1477–1480. [https://doi.org/10.1002/1521-4095\(20021016\)14:20<1477::AID-ADMA1477>3.0.CO;2-L](https://doi.org/10.1002/1521-4095(20021016)14:20<1477::AID-ADMA1477>3.0.CO;2-L)
156. Tam-Chang, S. W., Helbley, J., Carson, T. D., Seo, W., & Iverson, I. K. (2006). Template-guided organization of chromonic liquid crystals into micropatterned anisotropic organic solids. *Chemical Communications*, 5, 503–505. <https://doi.org/10.1039/b508220j>
157. Nazarenko, V. G., Boiko, O. P., Anisimov, M. I., Kadashchuk, A. K., & Nastishin, Y. A. (2010). Lyotropic chromonic liquid crystal semiconductors for water-solution processable organic electronics. *Applied Physics Letters*, 97, 263305. <https://doi.org/10.1063/1.3533814>
158. Draper, E. R., Walsh, J. J., McDonald, T. O., Zwijnenburg, M. A., Cameron, P. J., Cowan, J., & Adams, D. J. (2014). Air-stable photoconductive films formed from perylene bisimide gelators. *Journal of Materials Chemistry C*, 2, 5570–5575. <https://doi.org/10.1039/c4tc00744a>
159. Shiyonovskii, S. V., Schneider, T., Smalyukh, I. I., Ishikawa, T., Niehaus, G. D., Doane, K. J., Woolverton, C. J., & Lavrentovich, O. D. (2005). Real-time microbe detection based on director distortions around growing immune complexes in lyotropic chromonic liquid crystals. *Physical Review E - Statistical, Nonlinear, and Soft Matter Physics*, 71(2), 020702. <https://doi.org/10.1103/PhysRevE.71.020702>
160. Lavrentovich, M., Sergan, T., & Kelly, J. (2010). Planar and twisted lyotropic chromonic liquid crystal cells as optical compensators for twisted nematic displays. *Liquid Crystals*, 30(7), 851–859. <https://doi.org/10.1080/0267829031000121017>

161. Park, H., Agarwal, A., Kotov, N. A., & Lavrentovich, O. D. (2008). Controllable Side-by-Side and End-to-End Assembly of Au Nanorods by Lyotropic Chromonic Materials. *Langmuir*, 24(24), 13833–13837. <https://doi.org/10.1021/la803363m>
162. Rodríguez Abreu, C., Aubery Torres, C., & J.T. Tiddy, G. (2011). Chromonic Liquid Crystalline Phases of Pinacyanol Acetate: Characterization and Use as Templates for the Preparation of Mesoporous Silica Nanofibers. *Langmuir*, 27, 3067–3073. <https://doi.org/10.1021/la1048024>
163. Magana, J. R., Solans, C., Salonen, L. M., Carbó-Argibay, E., Gallo, J., Tiddy, G. J. T., & Rodríguez-Abreu, C. (2018). Chromonic self-assemblies in a series of dialkyl-thiacarbocyanine dyes and generalization of a facile route for the synthesis of fluorescent nanostructured silica fibers. *Journal of the Taiwan Institute of Chemical Engineers*, 92, 134–142. <https://doi.org/10.1016/j.jtice.2018.03.027>
164. Rodríguez-Abreu, C., Aubery-Torres, C., Solans, C., López-Quintela, A., & Tiddy, G. J. T. (2011). Characterization of perylene diimide dye self-assemblies and their use as templates for the synthesis of hybrid and supermicroporous nanotubules. *ACS Applied Materials and Interfaces*, 3(10), 4133–4141. <https://doi.org/10.1021/am201016m>
165. Magana, J. R., Kolen'Ko, Y. V., Deepak, F. L., Solans, C., Shrestha, R. G., Hill, J. P., Ariga, K., Shrestha, L. K., & Rodríguez-Abreu, C. (2016). From Chromonic Self-Assembly to Hollow Carbon Nanofibers: Efficient Materials in Supercapacitor and Vapor-Sensing Applications. *ACS Applied Materials and Interfaces*, 8(45), 31231–31238. <https://doi.org/10.1021/acsami.6b09819>
166. Scibioh, M. A., & Viswanathan, B. (2020). *Materials for supercapacitor applications*. Elsevier. <https://doi.org/10.1016/c2019-0-00454-2>
167. Winter, M., & Brodd, R. J. (2004). What are batteries, fuel cells, and supercapacitors? *Chemical Reviews*, 104(10), 4245–4269. <https://doi.org/10.1021/cr020730k>
168. Sharma, P., & Bhatti, T. S. (2010). A review on electrochemical double-layer capacitors. *Energy Conversion and Management*, 51(12), 2901–2912. <https://doi.org/10.1016/j.enconman.2010.06.031>
169. Béguin, F., Presser, V., Balducci, A., & Frackowiak, E. (2014). Carbons and electrolytes for advanced supercapacitors. *Advanced Materials*, 26(14), 2219–2251. <https://doi.org/10.1002/adma.201304137>
170. Wu, Y., & Cao, C. (2018). The way to improve the energy density of supercapacitors: Progress and perspective. *Science China Materials*, 61(12), 1517–1526. <https://doi.org/10.1007/s40843-018-9290-y>
171. Fleischmann, S., Mitchell, J. B., Wang, R., Zhan, C., Jiang, D. E., Presser, V., & Augustyn, V. (2020). Pseudocapacitance: From Fundamental Understanding to High Power Energy Storage Materials. *Chemical Reviews*, 120(14), 6738–6782. <https://doi.org/10.1021/acs.chemrev.0c00170>
172. Hu, Y., Liu, H., Ke, Q., & Wang, J. (2014). Effects of nitrogen doping on supercapacitor performance of a mesoporous carbon electrode produced by a hydrothermal soft-templating process. *Journal of Materials Chemistry A*, 2(30), 11753–11758. <https://doi.org/10.1039/c4ta01269k>
173. Huang, G., Geng, Q., Kang, W., Liu, Y., Li, Y., Xing, B., Liu, Q., & Zhang, C. (2019). Hierarchical porous carbon with optimized mesopore structure and nitrogen doping for supercapacitor electrodes. *Microporous and Mesoporous Materials*, 288, 109576. <https://doi.org/10.1016/j.micromeso.2019.109576>
174. Ilnicka, A., Skorupska, M., Szkoda, M., Zarach, Z., Kamedulski, P., Zielinski, W., & Lukaszewicz, J. P. (2021). Combined effect of nitrogen-doped functional groups and porosity of porous carbons on electrochemical performance of supercapacitors. *Scientific Reports*, 11(1), 18387. <https://doi.org/10.1038/s41598-021-97932-x>
175. Conte, M. (2010). Supercapacitors technical requirements for new applications. *Fuel Cells*, 10(5), 806–818. <https://doi.org/10.1002/fuce.201000087>
176. Zou, Z., Cao, J., Cao, B., & Chen, W. (2015). Evaluation strategy of regenerative braking energy for supercapacitor vehicle. *ISA Transactions*, 55, 234–240. <https://doi.org/10.1016/j.isatra.2014.09.011>
177. Burke, A. F. (2007). Batteries and ultracapacitors for electric, hybrid, and fuel cell vehicles.

- Proceedings of the IEEE*, 95(4), 806–820. <https://doi.org/10.1109/JPROC.2007.892490>
178. Mathis, T. S., Kurra, N., Wang, X., Pinto, D., Simon, P., & Gogotsi, Y. (2019). Energy Storage Data Reporting in Perspective—Guidelines for Interpreting the Performance of Electrochemical Energy Storage Systems. *Advanced Energy Materials*, 9(39), 1902007. <https://doi.org/10.1002/aenm.201902007>
 179. Noori, A., El-Kady, M., Rahmanifar, M., Kaner, R., & Mousavi, M. (2019). Towards establishing standard performance metrics for batteries, supercapacitors and beyond. *Chemical Society Reviews*, 48(5), 1272–1341. <https://doi.org/10.1039/C8CS00581H>
 180. Sousa, M. E., Cloutier, S. G., Jian, K. Q., Weissman, B. S., Hurt, R. H., & Crawford, G. P. (2005). Patterning lyotropic liquid crystals as precursors for carbon nanotube arrays. *Applied Physics Letters*, 87(17), 1–3. <https://doi.org/10.1063/1.2108111>
 181. Rodriguez-Abreu, C., Kolen'ko, Y. V., Kovnir, K., Sanchez-Dominguez, M., Shrestha, R. G., Bairi, P., Ariga, K., & Shrestha, L. K. (2020). 1D materials from ionic self-assembly in mixtures containing chromonic liquid crystal mesogens. *Physical Chemistry Chemical Physics*, 22(40), 23276–23285. <https://doi.org/10.1039/D0CP04348F>
 182. Tadros, T. (2009). *Emulsion science and technology: a general introduction*. Wiley-VCH Verlag. <https://doi.org/10.1002/9783527626564>
 183. Tadros, T., Izquierdo, P., Esquena, J., & Solans, C. (2004). Formation and stability of nano-emulsions. *Advances in Colloid and Interface Science*, 108–109, 303–318. <https://doi.org/10.1016/j.cis.2003.10.023>
 184. Fratter, A. (2016). *Nanoemulsion, method for its preparation and use* (Patent No. US9289738 B2). United States Patent. <https://patents.google.com/patent/US9289738B2/en>
 185. Helmenstine, A. M. (2020). *Tyndall Effect: Definition and Examples*. <https://www.thoughtco.com/definition-of-tyndall-effect-605756>
 186. Esquena, J. (2023). Recent advances on water-in-water emulsions in segregative systems of two water-soluble polymers. *Current Opinion in Food Science*, 51, 101010. <https://doi.org/10.1016/j.cofs.2023.101010>
 187. Grinberg, V. Y., & Tolstoguzov, V. B. (1997). Thermodynamic incompatibility of proteins and polysaccharides in solutions. *Food Hydrocolloids*, 11(2), 145–158. [https://doi.org/10.1016/S0268-005X\(97\)80024-0](https://doi.org/10.1016/S0268-005X(97)80024-0)
 188. Asenjo, J. A., & Andrews, B. A. (2011). Aqueous two-phase systems for protein separation: A perspective. *Journal of Chromatography A*, 1218(49), 8826–8835. <https://doi.org/10.1016/j.chroma.2011.06.051>
 189. Matalanis, A., Jones, O. G., & McClements, D. J. (2011). Structured biopolymer-based delivery systems for encapsulation, protection, and release of lipophilic compounds. *Food Hydrocolloids*, 25(8), 1865–1880. <https://doi.org/10.1016/j.foodhyd.2011.04.014>
 190. Flory, P. J. (1942). Thermodynamics of high polymer solutions. *The Journal of Chemical Physics*, 10(1), 51–61. <https://doi.org/10.1063/1.1723621>
 191. Huggins, M. L. (1941). Solutions of long chain compounds. *The Journal of Chemical Physics*, 9(5), 440. <https://doi.org/10.1063/1.1750930>
 192. Young, N. P., & Balsara, N. P. (2020). Flory-Huggins Equation. In S. Kobayashi & K. Müllen (Eds.), *Encyclopedia of Polymeric Nanomaterials*. Springer Berlin, Heidelberg. <https://doi.org/10.1007/978-3-642-36199-9>
 193. Schmitt, C., Sanchez, C., Desobry-Banon, S., & Hardy, J. (1998). Structure and Technofunctional Properties of Protein-Polysaccharide Complexes : A Review. *Critical Reviews in Food Science and Nutrition*, 38(8), 689–753. <https://doi.org/10.1080/10408699891274354>
 194. Dickinson, E. (2019). Particle-based stabilization of water-in-water emulsions containing mixed biopolymers. *Trends in Food Science and Technology*, 83, 31–40.

195. Binks, B. P., & Shi, H. (2019). Phase Inversion of Silica Particle-Stabilized Water-in-Water Emulsions. *Langmuir*, 35(11), 4046–4057. <https://doi.org/10.1021/acs.langmuir.8b04151>
196. Beldengrün, Y., Dallarís, V., Jaén, C., Protat, R., Miras, J., Calvo, M., García-Celma, M. J., & Esquena, J. (2020). Formation and stabilization of multiple water-in-water-in-water (W/W/W) emulsions. *Food Hydrocolloids*, 102, 105588. <https://doi.org/10.1016/j.foodhyd.2019.105588>
197. Singh, P., Medronho, B., Miguel, M. G., & Esquena, J. (2018). On the encapsulation and viability of probiotic bacteria in edible carboxymethyl cellulose-gelatin water-in-water emulsions. *Food Hydrocolloids*, 75, 41–50. <https://doi.org/10.1016/j.foodhyd.2017.09.014>
198. Beldengrün, Y. (2018). *Water-in-Water Emulsions for Obtaining Enzyme-Loaded Microgels and Encapsulated Emulsions* [Doctoral dissertation, University of Barcelona]. <https://www.tdx.cat/handle/10803/662970#page=1>
199. Beldengrün, Y., Aragon, J., Prazeres, S. F., Montalvo, G., Miras, J., & Esquena, J. (2018). Gelatin/Maltodextrin Water-in-Water (W/W) Emulsions for the Preparation of Cross-Linked Enzyme-Loaded Microgels. *Langmuir*, 34(33), 9731–9743. <https://doi.org/10.1021/acs.langmuir.8b01599>
200. Simon, K. A., Sejwal, P., Gerecht, R. B., & Luk, Y. Y. (2007). Water-in-water emulsions stabilized by non-amphiphilic interactions: Polymer-dispersed lyotropic liquid crystals. *Langmuir*, 23(3), 1453–1458. <https://doi.org/10.1021/la062203s>
201. Posnjak, G. (2018). Liquid Crystal Droplets. In *Topological Formations in Chiral Nematic Droplets* (pp. 29–39). https://doi.org/10.1007/978-3-319-98261-8_3
202. Ignés-Mullol, J., Mora, M., Martínez-Prat, B., Vélez-Cerón, I., Herrera, R. S., & Sagués, F. (2020). Stable and metastable patterns in chromonic nematic liquid crystal droplets forced with static and dynamic magnetic fields. *Crystals*, 10(2), 138. <https://doi.org/10.3390/cryst10020138>
203. Yang, L., Khan, M., & Park, S. Y. (2015). Liquid crystal droplets functionalized with charged surfactant and polyelectrolyte for non-specific protein detection. *RSC Advances*, 5(118), 97264–97271. <https://doi.org/10.1039/c5ra15647e>
204. Brimicombe, P. D., Kischka, C., Elston, S. J., & Raynes, E. P. (2007). Measurement of the twist elastic constant of nematic liquid crystals using pi-cell devices. *Journal of Applied Physics*, 101(4), 043108. <https://doi.org/10.1063/1.2432311>
205. Suwannasopon, S., Meyer, F., Schlickriede, C., Chaisakul, P., T-Thienprasert, J., Limtrakul, J., Zentgraf, T., & Chattham, N. (2019). Miniaturized Metalens Based Optical Tweezers on Optical Motors. *Crystals*, 9(10), 515. <https://doi.org/10.3390/cryst9100515>
206. Prishchepa, O. O., Zyryanov, V. Y., Gardymova, A. P., & Shabanov, V. F. (2008). Optical textures and orientational structures of nematic and cholesteric droplets with heterogeneous boundary conditions. *Molecular Crystals and Liquid Crystals*, 489(1), 84–93. <https://doi.org/10.1080/15421400802219817>
207. Park, H. S., Kang, S. W., Tortora, L., Kumar, S., & Lavrentovich, O. D. (2011). Condensation of self-assembled lyotropic chromonic liquid crystal sunset yellow in aqueous solutions crowded with polyethylene glycol and doped with salt. *Langmuir*, 27(7), 4164–4175. <https://doi.org/10.1021/la200505y>
208. Herrera, R. S. (2019). *Formation of chromonic liquid crystals from dyes and their confinement in water-in-oil and water-in-water emulsions* [Bachelor dissertation, University of Barcelona]. <http://hdl.handle.net/2445/138504>
209. Jalilov, A. S., Nilewski, L. G., Berka, V., Zhang, C., Yakovenko, A. A., Wu, G., Kent, T. A., Tsai, A. L., & Tour, J. M. (2017). Perylene Diimide as a Precise Graphene-like Superoxide Dismutase Mimetic. *ACS Nano*, 11(2), 2024–2032. <https://doi.org/10.1021/acs.nano.6b08211>
210. Shirman, E., Ustinov, A., Ben-shitrit, N., Weissman, H., Iron, M. A., Cohen, R., & Rybtchinski, B. (2008). Stable Aromatic Dianion in Water. *The Journal of Physical Chemistry B*, 112(30), 8855–8858. <https://doi.org/10.1021/jp8029743>

211. Wang, G., Qiu, Z., Liu, T., Chen, Y., Wang, S., Xin, F., & Yu, Y. (2021). Ionic self-assembly for naphthalenediimides-based materials with designable opto-electrochemical properties. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 610, 125704. <https://doi.org/10.1016/j.colsurfa.2020.125704>
212. Wu, G., Zhang, Z. G., Li, Y., Gao, C., Wang, X., & Chen, G. (2017). Exploring High-Performance n-Type Thermoelectric Composites Using Amino-Substituted Rylene Dimides and Carbon Nanotubes. *ACS Nano*, 11(6), 5746–5752. <https://doi.org/10.1021/acsnano.7b01279>
213. Kresge, C. T., Leonowicz, M. E., Roth, W. J., Vartuli, J. C., & Beck, J. S. (1992). Ordered mesoporous molecular sieves synthesized by a liquid-crystal template mechanism. *Nature*, 359, 710–712. <https://doi.org/10.1038/359710a0>
214. Esquena, J., Rodríguez, C., Solans, C., & Kunieda, H. (2006). Formation of mesostructured silica in nonionic fluorinated surfactant systems. *Microporous and Mesoporous Materials*, 92(1–3), 212–219. <https://doi.org/10.1016/j.micromeso.2006.01.003>
215. Luciano Bruschi, M. (2015). Mathematical models of drug release. In *Strategies to Modify the Drug Release from Pharmaceutical Systems* (pp. 63–86). Woodhead Publishing. <https://doi.org/10.1016/b978-0-08-100092-2.00005-9>
216. Zhang, Y., Huo, M., Zhou, J., Zou, A., Li, W., Yao, C., & Xie, S. (2010). DDSolver: An add-in program for modeling and comparison of drug dissolution profiles. *AAPS Journal*, 12(3), 263–271. <https://doi.org/10.1208/s12248-010-9185-1>
217. Higuchi, T. (1963). Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. *Journal of Pharmaceutical Sciences*, 52(12), 1145–1149. <https://doi.org/10.1002/jps.2600521210>
218. Korsmeyer, R. W., & Peppas, N. A. (1981). Effect of the morphology of hydrophilic polymeric matrices on the diffusion and release of water soluble drugs. *Journal of Membrane Science*, 9(3), 211–227. [https://doi.org/10.1016/S0376-7388\(00\)80265-3](https://doi.org/10.1016/S0376-7388(00)80265-3)
219. Laracuent, M. L., Yu, M. H., & McHugh, K. J. (2020). Zero-order drug delivery: State of the art and future prospects. *Journal of Controlled Release*, 327, 834–856. <https://doi.org/10.1016/j.jconrel.2020.09.020>
220. Shirman, E., Ustinov, A., Ben-shitrit, N., Weissman, H., Iron, M. A., Cohen, R., Rybtchinski, B., & Reho, V. (2008). Stable Aromatic Dianion in Water. *Physical Chemistry B*, 112(30), 8855–8858. <https://doi.org/10.1021/jp8029743>
221. Jouault, N., Xiang, Y., Moulin, E., Fuks, G., Giuseppone, N., & Buhler, E. (2012). Hierarchical supramolecular structuring and dynamical properties of water soluble polyethylene glycol-perylen self-assemblies. *Physical Chemistry Chemical Physics*, 14(16), 5718–5728. <https://doi.org/10.1039/c2cp23786e>
222. Langhals, H. (1995). Cyclic carboxylic imide structures as structure elements of high stability. Novel developments in perylene dye chemistry. *Heterocycles*, 40(1), 477–500. <https://doi.org/10.3987/REV-94-SR2>
223. Fuller, C. A., & Finlayson, C. E. (2017). Solvatochromism in perylene diimides; Experiment and theory. *Physical Chemistry Chemical Physics*, 19(47), 31781–31787. <https://doi.org/10.1039/c7cp05039a>
224. Pramanik, B., Mondal, H., Singha, N., Ahmed, S., Mohanty, J., & Das, D. (2016). A Viologen–Perylenediimide Conjugate as an Efficient Base Sensor with Solvatochromic Property. *ChemPhysChem*, 18(2), 245–252. <https://doi.org/10.1002/cphc.201601053>
225. Sharma, P., Kaur, S., Kaur, S., & Singh, P. (2020). Near-IR oxime-based solvatochromic perylene diimide probe as a chemosensor for Pd species and Cu²⁺ ions in water and live cells. *Photochemical and Photobiological Sciences*, 19, 504–514. <https://doi.org/10.1039/c9pp00487d>
226. Farooqi, M., Penick, M., Burch, J., Negrete, G., & Brancalion, L. (2016). Characterization of novel perylene diimides containing aromatic amino acid side chains. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 153, 124–131. <https://doi.org/10.1016/j.saa.2015.08.013>

227. Wang, W., Han, J. J., Wang, L. Q., Li, L. S., Shaw, W. J., & Li, A. D. Q. (2003). Dynamic π - π stacked molecular assemblies emit from green to red colors. *Nano Letters*, 3(4), 455–458. <https://doi.org/10.1021/nl025976j>
228. Franck, J., & Dymond, E. G. (1926). Elementary processes of photochemical reactions. *Transactions of the Faraday Society*, 21, 536–542. <https://doi.org/10.1039/TF9262100536>
229. Hennessy, M. H., Soos, Z. G., Pascal Jr., R. A., & Girlando, A. (1999). Vibronic structure of PTCDA stacks: the exciton–phonon–charge-transfer dimer. *Chemical Physics*, 245(1–3), 199–212. [https://doi.org/10.1016/S0301-0104\(99\)00082-8](https://doi.org/10.1016/S0301-0104(99)00082-8)
230. Kalova, J., & Mares, R. (2015). Reference Values of Surface Tension of Water. *International Journal of Thermophysics*, 36, 1396–1404. <https://doi.org/10.1007/s10765-015-1907-2>
231. Saraiva, A., Persson, O., & Fredenslund, A. (1993). An experimental investigation of cloud-point curves for the poly(ethylene glycol)/water system at varying molecular weight distributions. *Fluid Phase Equilibria*, 91(2), 291–311. [https://doi.org/10.1016/0378-3812\(93\)85105-U](https://doi.org/10.1016/0378-3812(93)85105-U)
232. Han, S. K., & Jhun, B. H. (1984). Effect of additives on the cloud point of polyethylene glycols. *Archives of Pharmacal Research*, 7, 1–9. <https://doi.org/10.1007/BF02856915>
233. Hofmeister, F. (1888). Zur Lehre von der Wirkung der Salze. *Archiv Für Experimentelle Pathologie Und Pharmakologie*, 24, 247–260. <https://doi.org/10.1007/BF01918191>
234. Kunz, W., Henle, J., & Ninham, B. W. (2004). ‘Zur Lehre von der Wirkung der Salze’ (about the science of the effect of salts): Franz Hofmeister’s historical papers. *Current Opinion in Colloid & Interface Science*, 9(1.2), 19–37. <https://doi.org/10.1016/j.cocis.2004.05.005>
235. Hyde, A. M., Zultanski, S. L., Waldman, J. H., Zhong, Y. L., Shevlin, M., & Peng, F. (2017). General Principles and Strategies for Salting-Out Informed by the Hofmeister Series. *Organic Process Research & Development*, 21(9), 1355–1370. <https://doi.org/10.1021/acs.oprd.7b00197>
236. Gregory, K. P., Elliot, G. R., Robertson, H., Kumar, A., Wanless, E. J., Webber, G. B., Craig, V. S., Andersson, G. G., & Page, A. J. (2022). Understanding specific ion effects and the Hofmeister series. *Physical Chemistry Chemical Physics*, 24(21), 12682–12718. <https://doi.org/10.1039/D2CP00847E>
237. Schulze, H. (1882). Schwefelarsen in wässriger Lösung. *Journal Für Praktische Chemie*, 25, 431–452. <https://doi.org/10.1002/prac.18820250142>
238. Hardy, W. B. (1900). A preliminary investigation of the conditions which determine the stability of irreversible hydrosols. *Proceedings of the Royal Society of London*, 66, 110–125. <https://doi.org/10.1098/rspl.1899.0081>
239. Trefalt, G., Szialgyi, I., & Borkovec, M. (2020). Schulze-Hardy rule revisited. *Colloid and Polymer Science*, 298, 961–967. <https://doi.org/10.1007/s00396-020-04665-w>
240. Schott, H. (1973). Salting In of Nonionic Surfactants by Complexation with Inorganic Salts. *Journal of Colloid and Interface Science*, 43(1), 150–155. [https://doi.org/10.1016/0021-9797\(73\)90358-5](https://doi.org/10.1016/0021-9797(73)90358-5)
241. Görl, D., & Würthner, F. (2016). Entropically Driven Self-Assembly of Bolaamphiphilic Perylene Dyes in water. *Angewandte Chemie - International Edition*, 55(39), 12094–12098. <https://doi.org/10.1002/anie.201606917>
242. Syamala, P. P. N., Soberats, B., Görl, D., Gekle, S., & Würthner, F. (2019). Thermodynamic insights into the entropically driven self-assembly of amphiphilic dyes in water. *Chemical Science*, 10(40), 9358–9366. <https://doi.org/10.1039/c9sc03103k>
243. Ishikawa, M., Matsumura, K. I., Esumi, K., Meguro, K., Binana-Limbele, W., & Zana, R. (1992). Aggregation behavior of α,ω -type nonionic surfactant in aqueous solution. *Journal of Colloid And Interface Science*, 151(1), 70–78. [https://doi.org/10.1016/0021-9797\(92\)90238-H](https://doi.org/10.1016/0021-9797(92)90238-H)
244. Alexandridis, P., & Alan Hatton, T. (1995). Poly(ethylene oxide)poly(propylene oxide)poly(ethylene oxide) block copolymer surfactants in aqueous solutions and at interfaces: thermodynamics, structure, dynamics, and modeling. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 96(1–2), 1–46. [https://doi.org/10.1016/0927-7757\(94\)03028-X](https://doi.org/10.1016/0927-7757(94)03028-X)

-
245. Seibt, J., Marquetand, P., Engel, V., Chen, Z., Dehm, V., & Wurthner, F. (2006). On the geometry dependence of molecular dimer spectra with an application to aggregates of perylene bisimide. *Chemical Physics*, 328(1–3), 354–362. <https://doi.org/10.1016/j.chemphys.2006.07.023>
246. Fink, R., Seibt, J., Engel, V., Renz, M., Kaupp, M., Lochbrunner, S., Zhao, H. M., Pfister, J., Wurthner, F., & Engels, B. (2008). Exciton Trapping in π -Conjugated Materials: A Quantum-Chemistry-Based Protocol Applied to Perylene Bisimide Dye Aggregates. *Journal of the American Chemical Society*, 130(39), 12858–12859. <https://doi.org/10.1021/ja804331b>
247. Zhao, H. M., Pfister, J., Settels, V., Renz, M., Kaupp, M., Dehm, V., Wurthner, F., Fink, R. F., & Engels, B. (2009). Understanding Ground- and Excited-State Properties of Perylene Tetracarboxylic Acid Bisimide Crystals by Means of Quantum Chemical Computations. *Journal of the American Chemical Society*, 131(43), 15660–15668. <https://doi.org/10.1021/ja902512e>
248. Chen, Z., Fimmel, B., & Wurthner, F. (2012). Solvent and substituent effects on aggregation constants of perylene bisimide π -stacks – a linear free energy relationship analysis. *Organic and Biomolecular Chemistry*, 10(30), 5845–5855. <https://doi.org/10.1039/C2OB07131B>
249. Görl, D., Zhang, X., & Wurthner, F. (2012). Molecular Assemblies of Perylene Bisimide Dyes in Water. *Angewandte Chemie - International Edition*, 51(26), 6328–6348. <https://doi.org/10.1002/anie.201108690>
250. Chen, Z., Stepanenko, V., Dehm, V., Prins, P., Siebbeles, L. D. A., Seibt, J., Marquetand, P., Engel, V., & Wurthner, F. (2007). Photoluminescence and Conductivity of Self-Assembled π - π Stacks of Perylene Bisimide Dyes. *Chemistry - A European Journal*, 13(2), 436–449. <https://doi.org/10.1002/chem.200600889>
251. Nehring, J., & Saupe, A. (1972). On the schlieren texture in nematic and smectic liquid crystals. *Journal of the Chemical Society, Faraday Transactions 2: Molecular and Chemical Physics*, 68(0), 1–15. <https://doi.org/10.1039/F29726800001>
252. Kuriabova, T., Betterton, M. D., & Glaser, M. A. (2010). Linear aggregation and liquid-crystalline order: Comparison of Monte Carlo simulation and analytic theory. *Journal of Materials Chemistry*, 20(46), 10366–10383. <https://doi.org/10.1039/c0jm02355h>
253. Boden, N., Bushby, R. J., Ferris, L., Hardy, C., & Sixl, F. (1986). Designing new lyotropic amphiphilic mesogens to optimize the stability of nematic phases. *Liquid Crystals*, 1(2), 109–125. <https://doi.org/10.1080/02678298608086498>
254. Stöber, W., Fink, A., & Bohn, E. (1968). Controlled growth of monodisperse silica spheres in the micron size range. *Journal of Colloid and Interface Science*, 26(1), 62–69. [https://doi.org/10.1016/0021-9797\(68\)90272-5](https://doi.org/10.1016/0021-9797(68)90272-5)
255. Zhao, D. Y., Feng, J. L., Huo, Q. S., Melosh, N., Fredrickson, G. H., Chmelka, B. F., & Stucky, G. D. (1998). Triblock copolymer syntheses of mesoporous silica with periodic 50 to 300 angstrom pores. *Science*, 279(5350), 548–552. <https://doi.org/10.1126/science.279.5350.548>
256. Zhao, D., Huo, Q., Feng, J., Fredrickson, G., Chmelka, B., & GD., S. (1998). Nonionic Triblock and Star Diblock Copolymer and Oligomeric Surfactant Syntheses of Highly Ordered, Hydrothermally Stable, Mesoporous Silica Structures. *Journal of the American Chemical Society*, 120(24), 6024–6036. <https://doi.org/10.1021/ja974025i>
257. Ruthstein, S., Frydman, V., Kababya, S., Landau, M., & Godfarb, D. (2003). Study of the Formation of the Mesoporous Material SBA-15 by EPR Spectroscopy. *Journal of Physical Chemistry B*, 107(8), 1739–1748. <https://doi.org/10.1021/jp021964a>
258. Bathfield, M., Rebout, J., Cacciaguerra, T., Lacroix-Desmazes, P., & Gérardin, C. (2016). Thermosensitive and Drug-Loaded Ordered Mesoporous Silica: A Direct and Effective Synthesis Using PEO-b-PNIPAM Block Copolymers. *Chemistry of Materials*, 28(10), 3374–3384. <https://doi.org/10.1021/acs.chemmater.6b00595>
259. Liang, C., Li, Z., & Dai, S. (2008). Mesoporous carbon materials: Synthesis and modification. *Angewandte Chemie - International Edition*, 47(20), 3696–3717. <https://doi.org/10.1002/anie.200702046>

260. Jun, S., Joo, S., Ryoo, R., Kruk, M., Jaroniec, M., Liu, Z., Oshuna, T., & Terasaki, O. (2000). Synthesis of new, nanoporous carbon with hexagonally ordered mesostructured. *Journal of the American Chemical Society*, 122(43), 10712–10713. <https://doi.org/10.1021/ja002261e>
261. Dai, L. R., Wang, T. W., Bu, L. T., & Chen, G. (2001). Mixed surfactant templating route for mesoporous silica. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 181(1–3), 151–157. [https://doi.org/10.1016/S0927-7757\(00\)00791-3](https://doi.org/10.1016/S0927-7757(00)00791-3)
262. Miyata, H., Noma, T., Watanabe, M., & Kuroda, K. (2002). Preparation of mesoporous silica films with fully aligned large mesochannels using nonionic surfactants. *Chemistry of Materials*, 14(2), 766–772. <https://doi.org/10.1021/cm010412m>
263. Lee, J. S., Joo, S. H., & Ryoo, R. (2002). Synthesis of mesoporous silicas of controlled pore wall thickness and their replication to ordered nanoporous carbons with various pore diameters. *Journal of the American Chemical Society*, 124(7), 1156–1157. <https://doi.org/10.1021/ja012333h>
264. Ariga, K., Nishikawa, M., Mori, T., Takeya, J., Shrestha, L. K., & Hill, J. P. (2019). Self-assembly as a key player for materials nanoarchitectonics. *Science and Technology of Advanced Materials*, 20(1), 51–95. <https://doi.org/10.1080/14686996.2018.1553108>
265. Kozma, E., Grisci, G., Mróz, W., Catellani, M., Eckstein-Andicsovà, A., Pagano, K., & Galeotti, F. (2016). Water-soluble aminoacid functionalized perylene diimides: The effect of aggregation on the optical properties in organic and aqueous media. *Dyes and Pigments*, 125, 201–209. <https://doi.org/10.1016/j.dyepig.2015.10.019>
266. Yang, S., Wang, B., Cui, D., Kerwood, D., Wilkens, S., Han, J., & Luk, Y. Y. (2013). Stereochemical Control of Nonamphiphilic Lyotropic Liquid Crystals: Chiral Nematic Phase of Assemblies Separated by Six Nanometers of Aqueous Solvents. *Journal of Physical Chemistry B*, 117(23), 7133–7143. <https://doi.org/10.1021/jp401382h>
267. Lee, H., & Labes, M. M. (1984). Helical Twisting Power of Amino Acids in a Nematic Lyophase. *Molecular Crystals and Liquid Crystals*, 108(1–2), 125–132. <https://doi.org/10.1080/00268948408072102>
268. Maier, E., Krause, R., Deggelmann, M., Hagenbuchle, M., Weber, R., & Fraden, S. (1992). Liquidlike order of charged rodlike particle solutions. *Macromolecules*, 25(3), 1125–1133. <https://doi.org/10.1021/ma00029a019>
269. Odjik, T. (1986). Theory of lyotropic polymer liquid crystals. *Macromolecules*, 19(9), 2313–2329. <https://doi.org/10.1021/ma00163a001>
270. Nowak-Król, A., Shoyama, K., Stolte, M., & Würthner, F. (2018). Naphthalene and perylene diimides—better alternatives to fullerenes for organic electronics? *Chemical Communications*, 54(98), 13763–13772. <https://doi.org/10.1039/c8cc07640e>
271. Shang, X., Ahn, J., Lee, J. H., Kim, J. C., Ohtsu, H., Choi, W., Song, I., Kwak, S. K., & Oh, J. H. (2021). Bay-Substitution Effect of Perylene Diimides on Supramolecular Chirality and Optoelectronic Properties of Their Self-Assembled Nanostructures. *ACS Applied Materials & Interfaces*, 13(10), 12278–12285. <https://doi.org/10.1021/acsami.0c23138>
272. Park, I. E., Brix, S., Martell, M., Ocheje, M. U., Pettipas, R. D., Harris, D. H., Gelfand, B. S., Rondeau-Gagné, S., Lessard, B. H., & Weich, G. C. (2021). An air-stable n-type bay-and-headland substituted bis-cyano N–H functionalized perylene diimide for printed electronics. *Journal of Materials Chemistry C*, 9(39), 13630–13634. <https://doi.org/10.1039/D1TC03873G>
273. Würthner, F., Saha-Möller, C. R., Fimmel, B., Ogi, S., Leowanawat, P., & Schmidt, D. (2016). Perylene Bisimide Dye Assemblies as Archetype Functional Supramolecular Materials. *Chemical Reviews*, 116(3), 962–1052. <https://doi.org/10.1021/acs.chemrev.5b00188>
274. Würthner, F. (2006). Bay-substituted perylene bisimides: Twisted fluorophores for supramolecular chemistry. *Pure and Applied Chemistry*, 78(12), 2341–2349. <https://doi.org/10.1351/pac200678122341>
275. Ganesamoorthy, R., Vijayaraghavan, R., Ramki, K., & Sakthivel, P. (2018). Synthesis, characterization of bay-substituted perylene diimide based D-A-D type small molecules and their

- applications as a non-fullerene electron acceptor in polymer solar cells. *Journal of Science: Advanced Materials and Devices*, 3(1), 99–106. <https://doi.org/10.1016/j.jsamd.2017.11.005>
276. Zhu, M., Guo, H., Yang, F., & Wang, Z. (2016). Novel perylene liquid crystals: Investigation of the influence of bay-substituents on mesomorphism and photophysical property. *Dyes and Pigments*, 133, 387–394. <https://doi.org/10.1016/j.dyepig.2016.06.021>
 277. Drzaic, P. (1995). *Liquid Crystal Dispersions*. World Scientific Publishing. <https://doi.org/10.1142/2337>
 278. Lavrentovich, O. D. (1998). Topological defects in dispersed words and worlds around liquid crystals, or liquid crystal drops. *Liquid Crystals*, 24(1), 117–126. <https://doi.org/10.1080/026782998207640>
 279. López-Leon, T., & Fernández-Nieves, A. (2011). Drops and shells of liquid crystals. *Colloid and Polymer Science*, 289, 345–359. <https://doi.org/10.1007/s00396-010-2367-7>
 280. Carlton, R. J., Hunter, J. T., Miller, D. S., Abbasi, R., Mushenheim, P. C., Tan, L. N., & Abbott, N. (2013). Chemical and biological sensing using liquid crystals. *Liquid Crystals Reviews*, 1(1), 29–51. <https://doi.org/10.1080/21680396.2013.769310>
 281. Xie, R., Li, N., Li, Z., Chen, J., Li, K., He, Q., Liu, L., & Zhang, S. (2022). Liquid Crystal Droplet-Based Biosensors: Promising for Point-of-Care Testing. *Biosensors*, 12(9), 758. <https://doi.org/10.3390/bios12090758>
 282. Iqbal, M., Tao, Y., Xie, S., Chen, D., Wang, X., Huang, L., Peng, D., Sattar, A., Bakr Shabbir, M.A., Hussain, H. I., Ahmed, S., & Yuan, Z. (2016). Aqueous two-phase system (ATPS): an overview and advances in its applications. *Biological Procedures Online*, 18, 18. <https://doi.org/10.1186/s12575-016-0048-8>
 283. Diamond, A. D., & Tsu, J. T. (1989). Phase diagrams for dextran-PEG aqueous two-phase systems at 22°C. *Biotechnology Techniques*, 3, 119–124. <https://doi.org/10.1007/BF01875564>
 284. Sampaio, D. A., Mafra, L. I., Yamamoto, C. I., de Andrade, E. F., de Souza, M. O., Mafra, M. R., & de Castilhos, F. (2016). Aqueous two-phase (polyethylene glycol + sodium sulfate) system for caffeine extraction: Equilibrium diagrams and partitioning study. *The Journal of Chemical Thermodynamics*, 98, 86–94. <https://doi.org/10.1016/j.jct.2016.03.004>
 285. Hans Tromp, R., & De Hoog, H. A. (2008). Band formation on shearing in phase-separated polymer solutions. *Physical Review E*, 77(3), 031503. <https://doi.org/10.1103/PhysRevE.77.031503>
 286. Tea, L., Nicolai, T., Benyahia, L., & Renou, F. (2020). Viscosity and Morphology of Water-in-Water Emulsions: The Effect of Different Biopolymer Stabilizers. *Macromolecules*, 53(10), 3914–3922. <https://doi.org/10.1021/acs.macromol.0c00204>
 287. Lavrentovich, O. D. (1996). Topological defects in dispersed liquid crystals, or words and worlds around liquid crystal drops. *Liquid Crystals*, 24(1), 117–125. <https://doi.org/10.1080/026782998207640>
 288. Lavrentovich, O. D. (2015). *Liquid crystals: Lecture 2. Topological defects, Droplets and Lamellar phases [Lecture]*. Soft Matter In and Out of Equilibrium, Boulder School for Condensed Matter and Materials Physics, Kent State University.
 289. Posnjak, G. (2018). *Topological Formations in Chiral Nematic Droplets* [Doctoral dissertation, University of Ljubljana]. <https://repozitorij.uni-lj.si/IzpisGradiva.php?id=97427&lang=eng>
 290. Orlova, T., Abhoff, S. J., Yamaguchi, T., Katsonis, N., & Brasselet, E. (2015). Creation and manipulation of topological states in chiral nematic microspheres. *Nature Communications*, 6, 7603. <https://doi.org/10.1038/ncomms8603>
 291. Pellegrino, C., De Santo, M. P., Spina, L., & Ciuchi, F. (2021). Induced Chiral Chromonics Confined in Micrometric Droplets. *Advanced Functional Materials*, 31(11), 2010394. <https://doi.org/10.1002/adfm.202010394>
 292. Jeong, J., Davidson, Z. S., Collings, P. J., Lubensky, T. C., & Yodh, A. G. (2014). Chiral symmetry breaking and surface faceting in chromonic liquid crystal droplets with giant elastic anisotropy. *Proceedings of the National Academy of Sciences*, 111(5), 1742–1747.

<https://doi.org/10.1073/pnas.1315121111>

293. Davidson, Z. S., Kang, L., Jeong, J., Still, P., Collings, P. J., Lubensky, T. C., & Yodh, A. D. (2015). Chiral structures and defects of lyotropic chromonic liquid crystals induced by saddle-splay elasticity. *Physical Review E*, 91(5), 019905. <https://doi.org/10.1103/PhysRevE.91.050501>
294. Paparini, S., & Virga, E. G. (2022). Paradoxes for chromonic liquid crystals. *Physical Review E*, 106(4), 044703. <https://doi.org/10.1103/PhysRevE.106.044703>
295. Lugger, J. A. M., Mulder, D. J., Bhattacharjee, S., & Sijbesma, R. P. (2018). Homeotropic Self-Alignment of Discotic Liquid Crystals for Nanoporous Polymer Films. *ACS Nano*, 12(7), 6714–6724. <https://doi.org/10.1021/acsnano.8b01822>
296. Sun, H., Zheng, H., Tang, Q., Dong, Y., Qu, F., Wang, Y., Yang, G., & Meng, T. (2019). Monodisperse Alginate Microcapsules with Spatially Confined Bioactive Molecules via Microfluid-Generated W/W/O Emulsions. *ACS Applied Materials & Interfaces*, 11(40), 37313–37321. <https://doi.org/10.1021/acsnano.9b12479>
297. Michaux, M., Salinas, N., Miras, J., Vílchez, S., González-Azón, C., & Esquena, J. (2021). Encapsulation of BSA/alginate water-in-water emulsions by polyelectrolyte complexation. *Food Hydrocolloids*, 113, 106406. <https://doi.org/10.1016/j.foodhyd.2020.106406>
298. Guzowski, J., Korczyk, P. M., Jakielaa, S., & Garstecki, P. (2012). The structure and stability of multiple micro-droplets. *Soft Matter*, 8(27), 7269–7278. <https://doi.org/10.1039/C2SM25838B>
299. Hervés, C. (2018). *Phase behavior and formulation of structured drops in triphasic systems* [Master dissertation, Universitat de Barcelona]. <https://doi.org/http://hdl.handle.net/2445/126219>
300. Nisisako, T. (2016). Recent advances in microfluidic production of Janus droplets and particles. *Current Opinion in Colloid & Interface Science*, 25, 1–12. <https://doi.org/10.1016/j.cocis.2016.05.003>
301. Walther, A., & Müller, A. H. E. (2013). Janus Particles: Synthesis, Self-Assembly, Physical Properties, and Applications. *Chemical Reviews*, 113(7), 5194–5261. <https://doi.org/10.1021/cr300089t>
302. Lan, Y., Choi, J., Li, H., Jia, Y., Huang, R., Stebe, K. J., & Lee, D. (2019). Janus Particles with Varying Configurations for Emulsion Stabilization. *Industrial & Engineering Chemistry Research*, 58(46), 20961–20968. <https://doi.org/10.1021/acs.iecr.9b02697>
303. Tran, L. T. C., Pharm, D., Lesieur, S., Faivre, V., & Pharm, D. (2014). Janus nanoparticles: materials, preparation and recent advances in drug delivery. *Expert Opinion on Drug Delivery*, 11(7), 1061–1074. <https://doi.org/10.1517/17425247.2014.915806>
304. Su, H., Li, S., Yang, G. Z., & Qian, K. (2022). Janus Micro/Nanorobots in Biomedical Applications. *Advanced Healthcare Materials*, 2202391. <https://doi.org/10.1002/adhm.202202391>
305. Yi, Y., Sánchez, L., Gao, Y., & Yu, Y. (2016). Janus particles for biological imaging and sensing. *Analyst*, 141(12), 3526–3539. <https://doi.org/10.1039/C6AN00325G>
306. Wang, Y., Yuan, J., Zhao, Y., Wang, L., Guo, L., Feng, L., Cui, J., Dong, S., Wan, S., Liu, W., Hoffmann, H., Tieu, K., & Hao, J. (2022). Water-in-Water Emulsions, Ultralow Interfacial Tension, and Biolubrication. *Chinese Chemical Society*, 6(4), 2102–2114. <https://doi.org/10.31635/ccschem.021.202101028>
307. Galogahi, F. M., Zhu, Y., An, H., & Nguyen, N. T. (2021). Formation of core-shell droplets for the encapsulation of liquid contents. *Microfluidics and Nanofluidics*, 25(82). <https://doi.org/10.1007/s10404-021-02483-2>
308. Tuschel, D. (2016). Selecting and Excitation Wavelength for Raman Spectroscopy. *Spectroscopy*, 31(3), 14–23.
309. Draper, E. R., & Adams, D. J. (2017). Low-Molecular-Weight Gels: The State of the Art. *Chem*, 3(3), 390–410. <https://doi.org/10.1016/j.chempr.2017.07.012>
310. Yang, X., Zhang, G., & Zhang, D. (2012). Stimuli responsive gels based on low molecular weight gelators. *Journal of Materials Chemistry*, 22(1), 38–50. <https://doi.org/10.1039/C1JM13205A>

311. Draper, E. R., Eden, E. G. B., McDonald, T. O., & Adams, D. J. (2015). Spatially resolved multicomponent gels. *Nature Communications*, 7, 848–852. <https://doi.org/10.1038/nchem.2347>
312. Skilling, K. J., Citossi, F., Bradshaw, T. D., Ashford, M., Kellam, B., & Marlow, M. (2013). Insights into low molecular mass organic gelators: a focus on drug delivery and tissue engineering applications. *Soft Matter*, 10(2), 237–256. <https://doi.org/10.1039/C3SM52244J>
313. Conte, M. P., Singh, N., Sasselli, I. R., Escuder, B., & Ulijn, R. V. (2016). Metastable hydrogels from aromatic dipeptides. *Chemical Communications*, 52(96), 13889–13892. <https://doi.org/10.1039/C6CC05821C>
314. Steck, K., Dieterich, S., Stubenrauch, C., & Giesselmann, F. (2020). Surfactant-based lyotropic liquid crystal gels – the interplay between anisotropic order and gel formation. *Journal of Materials Chemistry C*, 8(16), 5335–5348. <https://doi.org/10.1039/D0TC00561D>
315. Dieterich, S., Stemmler, F., Preisig, N., & Giesselmann, F. (2021). Micellar Lyotropic Nematic Gels. *Advanced Materials*, 33(8), 2007340. <https://doi.org/10.1002/adma.202007340>
316. Gregg, B. A., & Cormier, R. A. (1998). Liquid crystal perylene diimide films characterized by electrochemical, spectroelectrochemical, and conductivity versus potential measurements. *Journal of Physical Chemistry B*, 102(49), 9952–9957. <https://doi.org/10.1021/jp982842e>
317. Nastishin, Y. A., Savaryn, V. I., Lychkovskyy, E., Yakolev, M. Y., Vankevych, P. I., Krupych, O. M., Hrabchak, V. I., Boiko, O. P., Nazarenko, V. G., & Lavrentovich, O. D. (2018). Effect of UV-light irradiation on phase diagram of lyotropic chromonic liquid crystal. *Journal of Molecular Liquids*, 267, 96–99. <https://doi.org/10.1016/j.molliq.2017.12.079>
318. Yurdakal, S., Garlisi, C., Ozcan, L., Bellardita, M., & Palmisano, G. (2019). Chapter 4 - (Photo)catalyst Characterization Techniques: Adsorption Isotherms and BET, SEM, FTIR, UV–Vis, Photoluminescence, and Electrochemical Characterizations. In G. Marci & L. Palmisano (Eds.), *Heterogeneous Photocatalysis (1st Ed.)* (pp. 87–152). Elsevier. <https://doi.org/10.1016/B978-0-444-64015-4.00004-3>
319. Wang, P., Jiang, Z., Chen, L., Yin, L., Li, Z., Zhang, C., Tang, X., & Wang, G. (2016). Pore structure characterization for the Longmaxi and Niutitang shales in the Upper Yangtze Platform, South China: Evidence from focused ion beam–He ion microscopy, nano-computerized tomography and gas adsorption analysis. *Marine and Petroleum Geology*, 77, 1323–1337. <https://doi.org/10.1016/j.marpetgeo.2016.09.001>
320. Loganathan, S., Tikmani, M., & Ghoshal, A. K. (2013). Novel Pore-Expanded MCM-41 for CO₂ Capture: Synthesis and Characterization. *Langmuir*, 29(10), 3491–3499. <https://doi.org/10.1021/la400109j>
321. Li, K. M., Jiang, J. G., Tian, S. C., Chen, X. J., & Yan, F. (2014). Influence of Silica Types on Synthesis and Performance of Amine–Silica Hybrid Materials Used for CO₂ Capture. *Journal of Physical Chemistry C*, 118(5), 2454–2462. <https://doi.org/10.1021/jp408354r>
322. Cui, S., Cheng, W., Shen, X., Fan, M., Russell, A., Wu, Z., & Yi, X. (2011). Mesoporous amine-modified SiO₂ aerogel: a potential CO₂ sorbent. *Energy & Environmental Science*, 4(6), 2070–2074. <https://doi.org/10.1039/C0EE00442A>
323. Arcos, D., & Vallet-Regí, M. (2010). Sol–gel silica-based biomaterials and bone tissue regeneration. *Acta Biomaterialia*, 6(8), 2874–2888. <https://doi.org/10.1016/j.actbio.2010.02.012>
324. Mieszawska, A. J., Fourligas, N., Georgakoundi, I., Ouhib, N. M., Belton, D. J., Perry, C. C., & Kaplan, D. L. (2010). Osteoinductive silk–silica composite biomaterials for bone regeneration. *Biomaterials*, 31(34), 8902–8910. <https://doi.org/10.1016/j.biomaterials.2010.07.109>
325. Hertz, A., FitzGerald, V., Pignotti, E., Knowles, J. C., Sen, T., & Bruce, I. J. (2012). Preparation and characterisation of porous silica and silica/titania monoliths for potential use in bone replacement. *Microporous and Mesoporous Materials*, 156, 51–61. <https://doi.org/10.1016/j.micromeso.2012.02.004>
326. Pudlo, W., Borys, P., Huszcza, G., Staniak, A., & Zakrzewska, R. (2019). Hierarchical silica monolithic tablets as novel carriers for drug delivery. *European Journal of Pharmaceutics and*

Biopharmaceutics, 141, 12–20. <https://doi.org/10.1016/j.ejpb.2019.05.002>

327. Santos, M. V., Pecoraro, E., Santagneli, S. H., Moura, A. L., Cavicchioli, M., Jerez, V., Rocha, A. L., De Oliveira, L. F., Gomes, A. S. L., De Araujo, C. B., & Ribeiro, S. J. L. (2018). Silk fibroin as a biotemplate for hierarchical porous silica monoliths for random laser applications. *Journal of Materials Chemistry C*, 6(11), 2712–2723. <https://doi.org/10.1039/C7TC03560H>
328. Alotabi, M. T., Taylor, M. J., Liu, D., Beaumont, S. K., & Kyriakou, G. (2016). Selective oxidation of cyclohexene through gold functionalized silica monolith microreactors. *Surface Science*, 646, 179–185. <https://doi.org/10.1016/j.susc.2015.10.039>
329. Staniak, M., Wojciak, M., Sowa, I., Tyszczyk-Rotko, K., Strzemiński, M., Dresler, S., & Mysliński, W. (2020). Silica-Based Monolithic Columns as a Tool in HPLC—An Overview of Application in Analysis of Active Compounds in Biological Samples. *Molecules*, 25(14), 3149. <https://doi.org/10.3390/molecules25143149>
330. Chu, G., Qu, D., Zussman, E., & Xu, Y. (2017). Ice-Assisted Assembly of Liquid Crystalline Cellulose Nanocrystals for Preparing Anisotropic Aerogels with Ordered Structures. *Chemistry of Materials*, 29(9), 3980–3988. <https://doi.org/10.1021/acs.chemmater.7b00361>
331. El-Safty, S. A., & Hanaoka, T. (2003). Monolithic Nanostructured Silicate Family Templated by Lyotropic Liquid-Crystalline Nonionic Surfactant Mesophases. *Chemistry of Materials*, 15(15), 2892–2902. <https://doi.org/10.1021/cm0204829>
332. El-Safty, S. A., & Hanaoka, T. (2004). Microemulsion Liquid Crystal Templates for Highly Ordered Three-Dimensional Mesoporous Silica Monoliths with Controllable Mesopore Structures. *Chemistry of Materials*, 16(3), 384–400. <https://doi.org/10.1021/cm034282o>
333. Zhou, Y., Schattka, J. H., & Antonietti, M. (2004). Room-Temperature Ionic Liquids as Template to Monolithic Mesoporous Silica with Wormlike Pores via a Sol–Gel Nanocasting Technique. *Nano Letters*, 4(3), 384–400. <https://doi.org/10.1021/nl025861f>
334. Álvarez, S., & Fuertes, A. B. (2007). Synthesis of macro/mesoporous silica and carbon monoliths by using a commercial polyurethane foam as sacrificial template. *Materials Letters*, 61(11–12), 2378–2381. <https://doi.org/10.1016/j.matlet.2006.09.017>
335. Lyu, Y., Asoh, T. A., & Uyama, H. (2020). Hierarchical silica monolith prepared using cellulose monolith as template. *Polymer Degradation and Stability*, 117, 109164. <https://doi.org/10.1016/j.polymdegradstab.2020.109164>
336. Simovic, S., Ghouchi-Eskandar, N., Sinn, A. M., Losic, D., & Prestidge, C. A. (2011). Silica Materials in Drug Delivery Applications. *Current Drug Discovery Technology*, 8(3), 250–268. <https://doi.org/10.2174/157016311796799026>
337. Trofimov, A. D., Ivanova, A. A., Zyuzin, M. V., & Timin, A. S. (2018). Porous Inorganic Carriers Based on Silica, Calcium Carbonate and Calcium Phosphate for Controlled/Modulated Drug Delivery: Fresh Outlook and Future Perspectives. *Pharmaceutics*, 10(4), 167. <https://doi.org/10.3390/pharmaceutics10040167>
338. Vallet-Regi, M., Colilla, M., Izquierdo-Barba, I., & Manzano, M. (2018). Mesoporous Silica Nanoparticles for Drug Delivery: Current Insights. *Molecules*, 23(1), 47. <https://doi.org/10.3390/molecules23010047>
339. Sharafian, A., Fayazmanesh, K., McCague, C., & Bahrami, M. (2014). Thermal conductivity and contact resistance of mesoporous silica gel adsorbents bound with polyvinylpyrrolidone in contact with a metallic substrate for adsorption cooling system applications. *International Journal of Heat and Mass Transfer*, 79, 64–71. <https://doi.org/10.1016/j.ijheatmasstransfer.2014.07.086>
340. Chang, D., Gai, Y., Wang, L., Liu, G., Chen, Y., Wang, T., Tao, W., Mei, L., Huang, L., & Zeng, X. (2016). Polydopamine-based surface modification of mesoporous silica nanoparticles as pH-sensitive drug delivery vehicles for cancer therapy. *Journal of Colloid And Interface Science*, 463, 279–287. <https://doi.org/10.1016/j.jcis.2015.11.001>
341. Vinu, A., Hossain, K. Z., & Ariga, K. (2005). Recent Advances in Functionalization of Mesoporous Silica.2005.089. *Journal of Nanoscience and Nanotechnology*, 5(3), 347–371.

- <https://doi.org/10.1166/jnn.2005.089>
342. Bharti, C., Nagaich, U., Pal, A. K., & Gulati, N. (2015). Mesoporous silica nanoparticles in target drug delivery system: A review. *International Journal of Pharmaceutical Investigation*, 5(3), 124–133. <https://doi.org/10.4103/2230-973X.160844>
 343. Ulker, Z., & Erkey, C. (2014). An emerging platform for drug delivery: Aerogel based systems. *Journal of Controlled Release*, 177, 51–63. <https://doi.org/10.1016/j.jconrel.2013.12.033>
 344. Li, J., Shen, S., Kong, F., Jiang, T., Tang, C., & Yin, C. (2018). Effects of pore size on in vitro and in vivo anticancer efficacies of mesoporous silica nanoparticles. *RSC Advances*, 8(43), 24633–24640. <https://doi.org/10.1039/C8RA03914C>
 345. Vilça, N., Machado, A. F., Morais-Santos, F., Amorim, R., Neto, A. P., Logodin, E., Pereira, M. F. R., Sardo, M., Rocha, J., Parpot, P., Fonseca, A. M., Baltazar, F., & Neves, I. C. (2017). Comparison of different silica microporous structures as drug delivery systems for in vitro models of solid tumors. *RSC Advances*, 7(22), 13104–13111. <https://doi.org/10.1039/C7RA01028A>
 346. Srikanth, C., & Chuang, S. S. C. (2013). Infrared Study of Strongly and Weakly Adsorbed CO₂ on Fresh and Oxidatively Degraded Amine Sorbents. *Journal of Physical Chemistry C*, 117(18), 9196–9205. <https://doi.org/10.1021/jp311232f>
 347. Mellaerts, R., Jammaer, J. A. G., Van Speybroeck, M., Chen, H., Van Humbeeck, J., Augustijns, P., Van den Mooter, G., & Martens, J. A. (2008). Physical State of Poorly Water Soluble Therapeutic Molecules Loaded into SBA-15 Ordered Mesoporous Silica Carriers: A Case Study with Itraconazole and Ibuprofen. *Langmuir*, 24(16), 8651–8659. <https://doi.org/10.1021/la801161g>
 348. *Ibuprofen*. (n.d.). Drugbank. <https://doi.org/https://go.drugbank.com/drugs/DB01050>
 349. Cayman Chemicals. (2023). (*S*)-*ibuprofen*. Item n° 16793.
 350. Numpilai, T., Muenmee, S., & Witoon, T. (2016). Impact of pore characteristics of silica materials on loading capacity and release behavior of ibuprofen. *Materials Science and Engineering: C*, 59(1), 43–52. <https://doi.org/10.1016/j.msec.2015.09.095>
 351. Mulye, N. V., & Turco, S. J. (1995). A simple model based on first order kinetics to explain release of highly water-soluble drugs from porous dicalcium phosphate dihydrate matrices. *Drug Development and Industrial Pharmacy*, 21(8), 943–953. <https://doi.org/10.3109/03639049509026658>
 352. Paarakh, M. P., Jose, P. A., Setty, C. M., & Christopher, G. V. P. (2018). Release Kinetics – Concepts and Applications. *International Journal of Pharmacy Research & Technology*, 8, 12–20. <https://doi.org/10.31838/ijprt/08.01.02>
 353. Sriamornsak, P., Nunthanid, J., Luangtana-anan, M., & Puttipatkhachorn, S. (2007). Alginate-based pellets prepared by extrusion/spheronization: A preliminary study on the effect of additive in granulating liquid. *European Journal of Pharmaceutics and Biopharmaceutics*, 67(1), 227–235. <https://doi.org/10.1016/j.ejpb.2006.11.007>
 354. Garcia-Melero, J., Lopez-Mitjalva, J. J., Garcia-Celma, M. J., Rodriguez-Abreu, C., & Grijalvo, S. (2022). Rosmarinic Acid-Loaded Polymeric Nanoparticles Prepared by Low-Energy Nano-Emulsion Templating: Formulation, Biophysical Characterization, and In Vitro Studies. *Materials*, 15(13), 4572. <https://doi.org/10.3390/ma15134572>
 355. Izquierdo-Barba, I., Martinez, A., Doadrio, A. L., Perez-Pariente, J., & Vallet-Regi, M. (2005). Release evaluation of drugs from ordered three-dimensional silica structures. *European Journal of Pharmaceutical Sciences*, 26(5), 365–373. <https://doi.org/10.1016/j.ejps.2005.06.009>
 356. Albayati, T. M., & Jassam, A. A. A. (2019). Synthesis and characterization of mesoporous materials as a carrier and release of prednisolone in drug delivery system. *Journal of Drug Delivery Science and Technology*, 53, 101176. <https://doi.org/10.1016/j.jddst.2019.101176>
 357. Heikkilä, T., Salonen, J., Tuura, J., Hamdy, M. S., Mul, G., Kumar, N., Salmi, T., Murzin, D. Y., Laitinen, L., Kaukonen, A. M., Hirvonen, J., & Lehto, V. P. (2007). Mesoporous silica material TUD-1 as a drug delivery system. *International Journal of Pharmaceutics*, 331(1), 133–138. <https://doi.org/10.1016/j.ijpharm.2006.09.019>

358. Baldacim, S. A., Cairo, C. A. A., & Silva, C. R. M. (2001). Mechanical properties of ceramic composites. *Journal of Materials Processing Technology*, 119(1–3), 273–276. [https://doi.org/10.1016/S0924-0136\(01\)00931-1](https://doi.org/10.1016/S0924-0136(01)00931-1)
359. Zok, F. W., & Levi, C. G. (2001). Mechanical properties of Porous-Matrix Ceramic Composites. *Advanced Engineering Materials*, 3(1–2), 15–23. [https://doi.org/10.1002/1527-2648\(200101\)3:1/2<15::AID-ADEM15>3.0.CO;2-A](https://doi.org/10.1002/1527-2648(200101)3:1/2<15::AID-ADEM15>3.0.CO;2-A)
360. Kaur, G., Kumar, V., Bains, F., Mauro, J. C., Pickrell, G., Evans, I., & Bretcanu, O. (2019). Mechanical properties of bioactive glasses, ceramics, glass-ceramics and composites: State-of-the-art review and future challenges. *Materials Science and Engineering: C*, 104, 109895. <https://doi.org/10.1016/j.msec.2019.109895>
361. Yoshikawa, H., & Myoui, A. (2005). Bone tissue engineering with porous hydroxyapatite ceramics. *Journal of Artificial Organs*, 8, 131–136. <https://doi.org/10.1007/s10047-005-0292-1>
362. Herradi, S., Adouar, I., Bouhazma, S., Chajri, S., Khaldi, M., El Bali, B., & Lachkar, M. (2022). Sol-gel synthesis of a nanometric hydroxyapatite-silica composite. *Materials Today Proceedings*, 53(3), 413–419. <https://doi.org/10.1016/j.matpr.2022.01.393>
363. Porter, A. E., Botelho, C. M., Lopes, M. A., Santos, J. D., Best, S. M., & Bonfield, W. (2004). Ultrastructural comparison of dissolution and apatite precipitation on hydroxyapatite and silicon-substituted hydroxyapatite in vitro and in vivo. *Journal of Biomedical Materials Research*, 69(4), 670–679. <https://doi.org/10.1002/jbm.a.30035>
364. Zhai, W., Lu, H., Chen, L., Lin, X., Huang, Y., Dai, K., Naoki, K., Chen, G., & Chang, J. (2012). Silicate bioceramics induce angiogenesis during bone regeneration. *Acta Biomaterialia*, 8(1), 341–349. <https://doi.org/10.1016/j.actbio.2011.09.008>
365. Lei, T., Zhang, W., Qian, H., Nian Lim, P., San Thian, E., Lei, P., Hu, Y., & Wang, Z. (2020). Silicon-incorporated nanohydroxyapatite-reinforced poly(ϵ -caprolactone) film to enhance osteogenesis for bone tissue engineering applications. *Colloids and Surfaces B: Biointerfaces*, 187, 110714. <https://doi.org/10.1016/j.colsurfb.2019.110714>
366. Soares, P., Laurindo, C. A. H., Torres, R. D., Kuromoto, N. K., Peitl, O., & Zanotto, E. D. (2012). Effect of a bioactive glass-ceramic on the apatite nucleation on titanium surface modified by micro-arc oxidation. *Surface and Coatings Technology*, 206(22), 4601–4605. <https://doi.org/10.1016/j.surfcoat.2012.05.019>
367. Díaz, A., López, T., Manjárez, J., Basaldella, E., Martínez-Blanes, J. M., & Odriozola, M. A. (2006). Growth of hydroxyapatite in a biocompatible mesoporous ordered silica. *Acta Biomaterialia*, 2(2), 173–179. <https://doi.org/10.1016/j.actbio.2005.12.006>
368. Herrera-Alonso, A., Ibarra-Alonso, M., Esparza-González, S., Estrada-Flores, S., García-Cerda, L., & Martínez-Luevanos, A. (2021). Biomimetic Growth of Hydroxyapatite on SiO₂ Microspheres to Improve Its Biocompatibility and Gentamicin Loading Capacity. *Materials*, 14(22), 6941. <https://doi.org/10.3390/ma14226941>
369. Joseph, K., Oksman, K., Goerge, G., Wilson, R., & Appukuttan, S. (2021). *Fiber Reinforced Composites: Constituents, Compatibility, Perspectives, and Applications*. Elsevier. <https://doi.org/10.1016/C2019-0-01926-7>
370. Magana, J. R., Pérez-Calm, A., & Rodríguez-Abreu, C. (2022). Chromonic nematic liquid crystals in room-temperature ionic liquid. *Chemical Communications*, 58(11), 1724–1727. <https://doi.org/10.1039/D1CC05800B>
371. Li, Z., Lin, J., Li, B., Yu, C., Wang, H., & Li, Q. (2021). Construction of heteroatom-doped and three-dimensional graphene materials for the applications in supercapacitors: A review. *Journal of Energy Storage*, 44(B), 103437. <https://doi.org/10.1016/j.est.2021.103437>
372. Ferrero, G. A., Fuertes, A. B., & Sevilla, M. (2015). N-doped porous carbon capsules with tunable porosity for high-performance supercapacitors. *Journal of Materials Chemistry A*, 3(6), 2914–2923. <https://doi.org/10.1039/D1EE00166C>
373. Feng, X., Bai, Y., Liu, M., Li, Y., Yang, H., Wang, H., & Wu, C. (2021). Untangling the respective

- effects of heteroatom-doped carbon materials in batteries, supercapacitors and the ORR to design high performance materials. *Energy & Environmental Science*, 14(4), 2036–2089. <https://doi.org/10.1039/D1EE00166C>
374. Chen, D., Yang, L., Li, Y., & Wu, Q. (2019). Effect of self-doped heteroatoms in biomass-derived activated carbon for supercapacitor applications. *ChemistrySelect*, 4(5), 1586–1595. <https://doi.org/10.1002/slct.201803413>
 375. Alvarez, S., Esquena, J., Solans, C., & Fuertes, A. B. (2004). Meso/Macroporous carbon monoliths from polymeric foams. *Advanced Engineering Materials*, 6(11), 897–899. <https://doi.org/10.1002/adem.200400109>
 376. Ma, T., Liu, L., & Yuan, Z. Y. (2013). Direct synthesis of ordered mesoporous carbons. *Chemical Society Reviews*, 42(9), 3977–4003. <https://doi.org/10.1039/C2CS35301F>
 377. Ryoo, R., Joo, S. H., & Jun, S. (1999). Synthesis of highly ordered carbon molecular sieves via template-mediated structural transformation. *Journal of Physical Chemistry B*, 103(37), 7743–7746. <https://doi.org/10.1021/jp991673a>
 378. Kaneda, M., Tsubakiyama, O., Carlsson, T., Sakamoto, A., Ohsuna, T., Terasaki, O., Joo, H., & Ryoo, R. (2002). Structural study of mesoporous MCM-48 and carbon networks synthesized in the spaces of MCM-48 by electron crystallography. *Journal of Physical Chemistry B*, 106(6), 1256–1266. <https://doi.org/10.1021/jp0131875>
 379. Lee, J., Yoon, S., Oh, S. M., Shin, C. H., & Hyeon, T. (2000). Development of a new mesoporous carbon using an HMS aluminosilicate template. *Advanced Materials*, 12(5), 359–362. [https://doi.org/10.1002/\(SICI\)1521-4095\(200003\)12:5<359::AID-ADMA359>3.0.CO;2-1](https://doi.org/10.1002/(SICI)1521-4095(200003)12:5<359::AID-ADMA359>3.0.CO;2-1)
 380. Ryoo, R., Ko, C. H., Kruk, M., Antochshuk, V., & Janoriec, M. (2000). Block-copolymer-templated ordered mesoporous silica: array of uniform mesopores or mesopore-micropore network. *Journal of Physical Chemistry B*, 108(48), 11465–11471. <https://doi.org/10.1021/jp002597a>
 381. Bonino, F., Brutti, S., Reale, P., Scrosati, B., Gherghel, L., Wu, J., & Mullen, K. (2005). Disordered Carbon as a Novel Anode Material in Lithium-Ion Cells. *Advanced Materials*, 17(6), 743–746. <https://doi.org/10.1002/adma.200401006>
 382. Li, Z. Q., Lu, C. J., Xia, Z. P., Zhou, Y., & Luo, Z. (2007). X-ray diffraction patterns of graphite and turbostratic carbon. *Carbon*, 45(8), 1686–1695. <https://doi.org/10.1016/j.carbon.2007.03.038>
 383. Pimenta, M. A., Dresselhaus, G., Dresselhaus, M. S., Cançado, L. G., Jorio, A., & Saito, R. (2007). Studying disorder in graphite-based systems by Raman spectroscopy. *Physical Chemistry Chemical Physics*, 9(11), 1276–1290. <https://doi.org/10.1039/B613962K>
 384. Lota, G., Krawczyk, P., Lota, K., Sierczynska, A., Kolanowski, L., Baraniak, M., & Buchwald, T. (2016). The application of activated carbon modified by ozone treatment for energy storage. *Journal of Solid State Electrochemistry*, 20, 2857–2864. <https://doi.org/10.1007/s10008-016-3293-5>
 385. Nyak Mazlan, N. S., Kushairi, M. S., Khairunnisa-Atiqah, M. K., Ainul Hafiza, A. H., Mostapha, M., Ellis, A. V., & Zakaria, S. (2023). Macro-Size Regenerated Cellulose Fibre Embedded with Graphene Oxide with Antibacterial Properties. *Polymers*, 15(1), 230. <https://doi.org/10.3390/polym15010230>
 386. Ren, Y., Zhang, J., Xu, Q., Chen, Z., Yang, D., Wang, B., & Jiang, Z. (2014). Biomass-derived three-dimensional porous N-doped carbonaceous aerogel for efficient supercapacitor electrodes. *RSC Advances*, 4(45), 23412–23419. <https://doi.org/10.1039/C4RA02109F>
 387. Zhou, X., Wang, Y., Gong, C., Liu, B., & Gang, W. (2020). Production, structural design, functional control, and broad applications of carbon nanofiber-based nanomaterials: A comprehensive review. *Chemical Engineering Journal*, 402, 126189. <https://doi.org/10.1016/j.cej.2020.126189>
 388. Barranco, V., Lillo-Rodenas, M. A., Linares-Solano, A., Oya, A., Pico, F., Ibañez, J., Agullo-Rueda, F., Amarilla, J. M., & Rojo, J. M. (2010). Amorphous Carbon Nanofibers and Their Activated Carbon Nanofibers as Supercapacitor Electrodes. *Journal of Physical Chemistry C*, 114(22), 10302–10307. <https://doi.org/10.1021/jp1021278>
 389. Nie, G., Zhao, X., Luan, Y., Jiang, J., Kou, Z., & Wang, J. (2020). Key issues facing electrospun

- carbon nanofibers in energy applications: on-going approaches and challenges. *Nanoscale*, 12(25), 13225–13248. <https://doi.org/10.1039/D0NR03425H>
390. Cinke, M., Li, J., Bauschlicher, C. W., Ricca, A., & Meyvappan, M. (2003). CO₂ adsorption in single-walled carbon nanotubes. *Chemical Physics Letters*, 376(5–6), 761–766. [https://doi.org/10.1016/S0009-2614\(03\)01124-2](https://doi.org/10.1016/S0009-2614(03)01124-2)
 391. Shi, Z. G., Feng, Y. Q., Xu, L., Da, S. L., & Zhang, M. (2004). A template method to control the shape and porosity of carbon materials. *Carbon*, 42(8–9), 1677–1682. <https://doi.org/10.1016/j.carbon.2004.02.023>
 392. Liu, X., Liu, X., Sun, B., Zhou, H., Fu, A., Wang, Y., Guo, Y. G., Guo, P., & Li, H. (2018). Carbon materials with hierarchical porosity: Effect of template removal strategy and study on their electrochemical properties. *Carbon*, 130, 680–691. <https://doi.org/10.1016/j.carbon.2018.01.046>
 393. Yang, I., Jung, M., Kim, M. S., Choi, D., & Jung, J. C. (2021). Physical and chemical activation mechanisms of carbon materials based on the microdomain model. *Journal of Materials Chemistry A*, 9(15), 9815–9825. <https://doi.org/10.1039/D1TA00765C>
 394. Brun, N., Sakaushi, K., Yu, L., Giebler, L., Eckert, J., & Titrici, M. M. (2013). Hydrothermal carbon-based nanostructured hollow spheres as electrode materials for high-power lithium–sulfur batteries. *Physical Chemistry Chemical Physics*, 15, 6080–6087. <https://doi.org/10.1039/C3CP50653C>
 395. Lee, K. J., Yoon, S. H., & Jang, J. (2007). Carbon Nanofibers: A Novel Nanofiller for Nanofluid Applications. *Small*, 3(7), 1209–1213. <https://doi.org/10.1002/sml.200700066>
 396. Soll, S., Fellingner, T. P., Wang, X., Zhao, Q., Antonietti, M., & Yuan, J. (2013). Water Dispersible, Highly Graphitic and Nitrogen-Doped Carbon Nanobubbles. *Small*, 9(24), 4135–4141. <https://doi.org/10.1002/sml.201300680>
 397. Das, P., Ganguly, S., Mondal, S., Bose, M., Das, A. K., Banerjee, S., & Das, N. C. (2018). Heteroatom doped photoluminescent carbon dots for sensitive detection of acetone in human fluids. *Sensors and Actuators, B: Chemical*, 266, 583–593. <https://doi.org/10.1016/j.snb.2018.03.183>
 398. Ghosh, S., Barg, S., Jeong, S. M., & Ostrikov, K. (2020). Heteroatom-Doped and Oxygen-Functionalized Nanocarbons for High-Performance Supercapacitors. *Advanced Energy Materials*, 10(32), 2001239. <https://doi.org/10.1002/aenm.202001239>
 399. Lu, S., Yang, W., Zhou, M., Qiu, L., Tao, B., Zhao, Q., Wang, X., Zhang, L., Xie, Q., & Ruan, Y. (2022). Nitrogen- and oxygen-doped carbon with abundant micropores derived from biomass waste for all-solid-state flexible supercapacitors. *Journal of Colloid And Interface Science*, 610, 1088–1099. <https://doi.org/10.1016/j.jcis.2021.11.164>
 400. Kariper, I. A., Korkmaz, S., Karaman, C., & Karaman, O. (2022). High energy supercapacitors based on functionalized carbon nanotubes: Effect of atomic oxygen doping via various radiation sources. *Fuel*, 324(A), 124497. <https://doi.org/10.1016/j.fuel.2022.124497>
 401. Yang, W., Du, Z., Ma, Z., Wang, G., Bai, H., & Shao, G. (2016). Facile synthesis of nitrogen-doped hierarchical porous lamellar carbon for high-performance supercapacitors. *RSC Advances*, 6(5), 3942–3950. <https://doi.org/10.1039/C5RA21431A>
 402. Kumar, A., Singh, R. K., Singh, H. K., Srivastava, P., & Singh, R. (2014). Enhanced capacitance and stability of p-toluenesulfonate doped polypyrrole/carbon composite for electrode application in electrochemical capacitors. *Journal of Power Sources*, 246, 800–807. <https://doi.org/10.1016/j.jpowsour.2013.07.121>
 403. Lazar, P., Mach, R., & Otyepka, M. (2019). Spectroscopic Fingerprints of Graphitic, Pyrrolic, Pyridinic, and Chemisorbed Nitrogen in N-Doped Graphene. *Journal of Physical Chemistry C*, 123(16), 10695–10702. <https://doi.org/10.1021/acs.jpcc.9b02163>
 404. Lee, M. S., Whang, D. R., Song, Y. H., Kim, J. T., Yang, M. H., Choi, U. H., & Chang, D. W. (2019). Effects of pyridine and pyrrole moieties on supercapacitive properties of imine-rich nitrogen-doped graphene. *Carbon*, 152, 915–923. <https://doi.org/10.1016/j.carbon.2019.06.082>
 405. Vita Damasceno, J. P., & Kubota, L. T. (2021). Colloidal chemistry as a guide to design intended

- dispersions of carbon nanomaterials. *Materials Today Chemistry*, 21, 100526. <https://doi.org/10.1016/j.mtchem.2021.100526>
406. Ji, T., Tan, L., Hu, X., Dai, Y., & Chen, Y. (2015). A comprehensive study of sulfonated carbon materials as conductive composites for polymer solar cells. *Physical Chemistry Chemical Physics*, 17(6), 4137–4145. <https://doi.org/10.1039/C4CP04965A>
 407. Hong, C. Y., You, H. Z., & Pan, C. Y. (2005). Synthesis of Water-Soluble Multiwalled Carbon Nanotubes with Grafted Temperature-Responsive Shells by Surface RAFT Polymerization. *Chemistry of Materials*, 17(9), 2247–2254. <https://doi.org/10.1021/cm048054l>
 408. Inam, F., Yan, H., Reece, M. J., & Pejic, T. (2008). Dimethylformamide: an effective dispersant for making ceramic–carbon nanotube composites. *Nanotechnology*, 19, 195710. <https://doi.org/10.1088/0957-4484/19/19/195710>
 409. Shrestha, R. G., Maji, S., Shrestha, L. K., & Ariga, K. (2020). Nanoarchitectonics of nanoporous carbon materials in supercapacitors applications. *Nanomaterials*, 10(4), 1–27. <https://doi.org/10.3390/nano10040639>
 410. Chen, X., Paul, R., & Dai, L. (2017). Carbon-based supercapacitors for efficient energy storage. *National Science Review*, 4(3), 453–489. <https://doi.org/10.1093/nsr/nwx009>
 411. Dujearic-Stephane, K., Gupta, M., Kumar, A., Sharma, V., Pandit, S., Bocchetta, P., & Kumar, Y. (2021). The Effect of Modifications of Activated Carbon Materials on the Capacitive Performance: Surface, Microstructure, and Wettability. *Journal of Composites Science*, 5(3), 66. <https://doi.org/10.3390/jcs5030066>
 412. Lin, T., Chen, W., Liu, F., Yang, C., Bi, H., Xu, F., & Huang, F. (2015). Nitrogen-doped mesoporous carbon of extraordinary capacitance for electrochemical energy storage. *Science*, 350(6257), 1508–1513. <https://doi.org/10.1126/science.aab3798>
 413. Liu, X., Mi, R., Yuan, L., Yang, F., Fu, Z., Wang, C., & Tang, Y. (2018). Nitrogen-Doped Multi-Scale Porous Carbon for High Voltage Aqueous Supercapacitors. *Frontiers in Chemistry*, 6, 475. <https://doi.org/10.3389/fchem.2018.00475>
 414. Saha, D., & Kienbaum, M. J. (2019). Role of oxygen, nitrogen and sulfur functionalities on the surface of nanoporous carbons in CO₂ adsorption: A critical review. *Microporous and Mesoporous Materials*, 287, 29–55. <https://doi.org/10.1016/j.micromeso.2019.05.051>
 415. Liu, H. Y., Wang, K. P., & Teng, H. (2005). A simplified preparation of mesoporous carbon and the examination of the carbon accessibility for electric double layer formation. *Carbon*, 43(3), 559–566. <https://doi.org/10.1016/j.carbon.2004.10.020>
 416. Lim, Y. ., Lai, C. W., & Abd Hammid, S. B. (2017). Porous 3D carbon decorated Fe₃O₄ nanocomposite electrode for highly symmetrical supercapacitor performance. *RSC Advances*, 7(37), 23030–23040. <https://doi.org/10.1039/C7RA00572E>
 417. Lindstrom, H., Sodergren, S., Solbrand, A., Rensmo, H., Hjelm, J., Hagfeldt, A., & Lindquist, S. E. (1997). Li⁺ Ion Insertion in TiO₂ (Anatase). 2. Voltammetry on Nanoporous Films. *Journal of Physical Chemistry B*, 101(39), 7717–7722. <https://doi.org/10.1021/jp970490q>
 418. Augustyn, V., Come, J., Lowe, M. A., Kim, J. W., Taberna, P. L., Tolbert, S. H., Abruña, H. D., Simon, P., & Dunn, B. (2013). High-rate electrochemical energy storage through Li⁺ intercalation pseudocapacitance. *Nature Materials*, 12, 518–522. <https://doi.org/10.1038/nmat3601>
 419. Xing, W., Qiao, S. Z., Ding, R. G., Li, F., Lu, G. Q., Yan, Z. F., & Cheng, H. M. (2006). Superior electric double layer capacitors using ordered mesoporous carbons. *Carbon*, 44(2), 216–224. <https://doi.org/10.1016/j.carbon.2005.07.029>
 420. Wickramaarachchi, W. A. M., Minakshi, M., Gao, X., Dabare, R., & Wong, K. W. (2021). Hierarchical porous carbon from mango seed husk for electro-chemical energy storage. *Chemical Engineering Journal Advances*, 8(15), 100158. <https://doi.org/10.1016/j.cej.2021.100158>
 421. Yu, A., Chabot, V., & Zhang, J. (2013). *Electrochemical Supercapacitors for Energy Storage and Delivery*. CRC Press, Taylor and Francis Group. <https://doi.org/10.1201/b14671>

422. Laheaar, A., Przygocki, P., Abbas, Q., & Beguin, F. (2015). Appropriate methods for evaluating the efficiency and capacitive behavior of different types of supercapacitors. *Electrochemistry Communications*, 60, 21–25. <https://doi.org/10.1016/j.elecom.2015.07.022>
423. Yan, D., Lu, A. H., Chen, Z. Y., He, L., & Li, W. C. (2021). Pseudocapacitance-Dominated Li-Ion Capacitors Showing Remarkable Energy Efficiency by Introducing Amorphous LiFePO₄ in the Cathode. *ACS Applied Energy Materials*, 4(2), 1824–1832. <https://doi.org/10.1021/acsaem.0c02943>
424. Shrestha, L. K., Adhikari, L., Shrestha, R. G., Adhikari, M. P., Adhikari, R., Hill, J. P., Pradhananga, R. R., & Ariga, K. (2016). Nanoporous carbon materials with enhanced supercapacitance performance and non-aromatic chemical sensing with C1/C2 alcohol discrimination. *Science and Technology of Advanced Materials*, 17(1), 483–492. <https://doi.org/10.1080/14686996.2016.1219971>
425. Shrestha, L. K., Shrestha, R. G., Chaudhary, R., Pradhananga, R. R., Tamrakar, B. M., Shrestha, T., Maji, S., Shrestha, R. L., & Ariga, K. (2021). Nelumbo nucifera Seed-Derived Nitrogen-Doped Hierarchically Porous Carbons as Electrode Materials for High-Performance Supercapacitors. *Nanomaterials*, 11(12), 3175. <https://doi.org/10.3390/nano11123175>
426. Gong, Y., Li, D., Luo, C., Fu, Q., & Pan, C. (2017). Highly porous graphitic biomass carbon as advanced electrode materials for supercapacitors. *Green Chemistry*, 19(17), 4132–4140. <https://doi.org/10.1039/C7GC01681F>
427. Chen, X., Zhang, J., Zhang, B., Dong, S., Guo, Z., Mu, X., & Fei, B. (2017). A novel hierarchical porous nitrogen-doped carbon derived from bamboo shoot for high performance supercapacitor. *Scientific Reports*, 7, 7362. <https://doi.org/10.1038/s41598-017-06730-x>
428. Gao, F., Qu, J., Zhao, Z., Wang, Z., & Qiu, J. (2016). Nitrogen-doped activated carbon derived from prawn shells for high-performance supercapacitors. *Electrochimica Acta*, 190(1), 1134–1141. <https://doi.org/10.1016/j.electacta.2016.01.005>
429. Sandhiya, M., Nadira, M. P., & Sathish, M. (2021). Fabrication of Flexible Supercapacitor Using N-Doped Porous Activated Carbon Derived from Poultry Waste. *Energy & Fuels*, 35(18), 15095–15100. <https://doi.org/10.1021/acs.energyfuels.1c01713>
430. Partridge, J., & Ibrahim Abouelamaimen, D. (2019). The Role of Supercapacitors in Regenerative Braking Systems. *Energies*, 12(14), 2683. <https://doi.org/10.3390/en12142683>
431. Hamada, A. T., & Orhan, M. F. (2021). An overview of regenerative braking systems. *Journal of Energy Storage*, 52(C), 105033. <https://doi.org/10.1016/j.est.2022.105033>
432. Lee, D., Jung, J. Y., Jung, M. J., & Lee, Y. S. (2015). Hierarchical porous carbon fibers prepared using a SiO₂ template for high-performance EDLCs. *Chemical Engineering Journal*, 263, 62–70. <https://doi.org/10.1016/j.cej.2014.10.070>
433. Zhao, X., Nie, G., Luan, Y., Wang, X., Yan, S., & Long, Y. Z. (2019). Nitrogen-doped carbon networks derived from the electrospun polyacrylonitrile@branched polyethylenimine nanofibers as flexible supercapacitor electrodes. *Journal of Alloys and Compounds*, 808, 151737. <https://doi.org/10.1016/j.jallcom.2019.151737>
434. Cai, J., Niu, H., Li, Z., Du, Y., Cizek, P., Xie, Z., Xiong, H., & Lin, T. (2015). High-Performance Supercapacitor Electrode Materials from Cellulose-Derived Carbon Nanofibers. *ACS Applied Materials & Interfaces*, 7(27), 14946–14953. <https://doi.org/10.1021/acsaami.5b03757>
435. Zhu, M., Liu, H., Cao, Q., Zheng, H., Xu, D., Gao, H., Wang, S., Li, Y., & Zhou, J. (2020). Electrospun Lignin-Based Carbon Nanofibers as Supercapacitor Electrodes. *ACS Sustainable Chemistry & Engineering*, 8(34), 12831–12841. <https://doi.org/10.1021/acssuschemeng.0c03062>
436. Huang, J., Sumpter, B., & Meunier, V. (2008). Theoretical Model for Nanoporous Carbon Supercapacitors. *Angewandte Chemie - International Edition*, 120(3), 530–534. <https://doi.org/10.1002/ange.200703864>
437. Xia, K., Gao, Q., Jiang, J., & Hu, J. (2008). Hierarchical porous carbons with controlled micropores and mesopores for supercapacitor electrode materials. *Carbon*, 46(13), 1718–1726. <https://doi.org/10.1016/j.carbon.2008.07.018>

-
438. He, Z., Zhang, G., Chen, Y., Xie, Y., Zhu, T., Guo, H., & Chen, Y. (2016). The effect of activation methods on the electrochemical performance of ordered mesoporous carbon for supercapacitor applications. *Journal of Materials Science*, 52, 2422–2434. <https://doi.org/10.1007/s10853-016-0536-x>
439. Yan, K., Sun, X., Ying, S., Cheng, W., Deng, Y., Ma, Z., Zhao, Y., Wang, X., Pan, L., & Shi, Y. (2020). Ultrafast microwave synthesis of rambutan-like CMK-3/carbon nanotubes nanocomposites for high-performance supercapacitor electrode materials. *Scientific Reports*, 10, 6227. <https://doi.org/10.1038/s41598-020-63204-3>
440. Zhang, W., Cheng, R., Bi, H., Lu, Y., Ma, L., & He, X. (2021). A review of porous carbons produced by template methods for supercapacitor applications. *New Carbon Materials*, 36(1), 69–81. [https://doi.org/10.1016/S1872-5805\(21\)60005-7](https://doi.org/10.1016/S1872-5805(21)60005-7)
441. Liu, X., Roberts, A., Ahmed, A., Wang, Z., & Zhang, H. (2015). Carbon nanofibers by pyrolysis of self-assembled perylene diimide derivative gels as supercapacitor electrode materials. *Journal of Materials Chemistry A*, 3(30), 15513–15522. <https://doi.org/10.1039/C5TA03546E>
442. Tian, K., Wang, J., Cao, L., Yang, W., Guo, W., Liu, S., Li, W., Wang, F., Li, X., Xu, Z., Wang, Z., Wang, H., & Hou, Y. (2020). Single-site pyrrolic-nitrogen-doped sp²-hybridized carbon materials and their pseudocapacitance. *Nature Communications*, 11, 3884. <https://doi.org/10.1038/s41467-020-17727-y>
443. Zhang, H., Kuila, T., Kim, N. H., Yu, D. S., & Lee, J. H. (2014). Simultaneous reduction, exfoliation, and nitrogen doping of graphene oxide via a hydrothermal reaction for energy storage electrode materials. *Carbon*, 69, 66–78. <https://doi.org/10.1016/j.carbon.2013.11.059>
444. Li, Z., Li, B., Liu, Z., Li, D., Wang, H., & Li, Q. (2016). One-pot construction of 3-D nitrogen-doped activated graphene-like nanosheets for high-performance supercapacitors. *Electrochimica Acta*, 190, 378–387. <https://doi.org/10.1016/j.electacta.2015.12.210>
445. Zhang, W., Xu, C., Ma, C., Li, G., Wang, Y., Zhang, K., Li, F., Liu, C., Cheng, H. M., Du, Y., Tang, N., & Ren, W. (2017). Nitrogen-Superdoped 3D Graphene Networks for High-Performance Supercapacitors. *Advanced Materials*, 29(36), 1701677. <https://doi.org/10.1002/adma.201701677>
446. Stoller, M. D., Park, S., Zhu, Y., An, J., & Ruoff, R. S. (2008). Graphene-Based Ultracapacitors. *Nano Letters*, 8(10), 3498–3502. <https://doi.org/10.1021/nl802558y>
447. Lei, Z., Lu, L., & Zhao, X. S. (2012). The electrocapacitive properties of graphene oxide reduced by urea. *Energy & Environmental Science*, 5(4), 6391–6399. <https://doi.org/10.1039/C1EE02478G>
448. Yoon, S. B., Yoon, E. H., & Kim, K. B. (2011). Electrochemical properties of leucoemeraldine, emeraldine, and pernigraniline forms of polyaniline/multi-wall carbon nanotube nanocomposites for supercapacitor applications. *Journal of Power Sources*, 196(24), 10791–10797. <https://doi.org/10.1016/j.jpowsour.2011.08.107>
449. Lota, K., Khomenko, B., & Franckowiak, E. (2004). Capacitance properties of poly(3,4-ethylenedioxythiophene)/carbon nanotubes composites. *Journal of Physics and Chemistry of Solids*, 65(2–3), 295–301. <https://doi.org/10.1016/j.jpcs.2003.10.051>
450. Zhang, X., Li, W., & Lu, A. (2015). Designed porous carbon materials for efficient CO₂ adsorption and separation. *New Carbon Materials*, 30(6), 481–501. [https://doi.org/10.1016/S1872-5805\(15\)60203-7](https://doi.org/10.1016/S1872-5805(15)60203-7)
451. Kretzschmar, A., Selmert, V., Weinrich, H., Kungl, H., Tappel, H., & Eichel, R. (2020). Tailored Gas Adsorption Properties of Electrospun Carbon Nanofibers for Gas Separation and Storage. *ChemSusChem*, 13(12), 3180–3191. <https://doi.org/10.1002/cssc.202000520>
452. Sevilla, M., & Fuertes, A. B. (2011). Sustainable porous carbons with a superior performance for CO₂ capture. *Energy & Environmental Science*, 4(5), 1765–1771. <https://doi.org/10.1039/C0EE00784F>
453. Xiao, J., Yuan, X., Zhang, T. C., Ouyang, L., & Yuan, S. (2022). Nitrogen-doped porous carbon for excellent CO₂ capture: A novel method for preparation and performance evaluation. *Separation and Purification Technology*, 298, 121602. <https://doi.org/10.1016/j.seppur.2022.121602>

454. Sevilla, M., Falco, C., Titrici, M. M., & Fuertes, A. B. (2012). High-performance CO₂ sorbents from algae. *RSC Advances*, 2(33), 12792–12797. <https://doi.org/10.1039/C2RA22552B>
455. Sánchez-Sánchez, A., Suarez-García, F., Martínez-Alonso, A., & Tascon, J. M. D. (2014). Influence of Porous Texture and Surface Chemistry on the CO₂ Adsorption Capacity of Porous Carbons: Acidic and Basic Site Interactions. *ACS Applied Materials & Interfaces*, 6(23), 21237–21247. <https://doi.org/10.1021/am506176e>
456. Watson, J. D., & Crick, F. H. C. (1953). Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid. *Nature*, 171, 737–738. <https://doi.org/10.1038/171737a0>
457. Wilkins, M. H. F., Stokes, A. R., & Wilson, H. R. (1953). Molecular Structure of Nucleic Acids: Molecular Structure of Deoxypentose Nucleic Acids. *Nature*, 171, 738–740. <https://doi.org/10.1038/171738a0>
458. Franklin, R. E., & Gosling, R. G. (1953). Molecular Configuration in Sodium Thymonucleate. *Nature*, 171, 740–741. <https://doi.org/10.1038/171740a0>
459. Poater, J., Swart, M., Bickelhaupt, F. M., & Fonseca-Guerra, C. (2014). B-DNA structure and stability: the role of hydrogen bonding, π - π stacking interactions, twist-angle, and solvation. *Organic and Biomolecular Chemistry*, 12(26), 4961–4700. <https://doi.org/10.1039/C4OB00427B>
460. Demeunynck, M., Bailly, C., & Wilson, W. D. (2002). *DNA and RNA Binders: From Synthesis to Nucleic Acid Complexes*. Wiley-VCH Verlag. <https://doi.org/10.1002/3527601783>
461. Ihmels, H., & Otto, D. (2005). Intercalation of Organic Dye Molecules into Double-Stranded DNA – General Principles and Recent Developments. In F. Würthner (Ed.), *Supramolecular Dye Chemistry (1st Ed.)* (pp. 161–204). Springer. <https://doi.org/10.1007/b135804>
462. Venugopal, S., Sharma, V., Mehra, A., Singh, I., & Singh, G. (2022). DNA intercalators as anticancer agents. *Chemical Biology & Drug Discovery*, 100(4), 580–598. <https://doi.org/10.1111/cbdd.14116>
463. Gottesfeld, J. M., Neely, L., Trauger, J. W., Baird, E. E., & Dervan, P. B. (1997). Regulation of gene expression by small molecules. *Nature*, 387, 202–205. <https://doi.org/10.1038/387202a0>
464. Berman, H. M., Neidle, S., Zimmer, C., & Thrum, H. (1979). Netropsin, a DNA-binding oligopeptide structural and binding studies. *Biochimica et Biophysica Acta - Nucleic Acids and Protein Synthesis*, 561(1), 124–131. [https://doi.org/10.1016/0005-2787\(79\)90496-9](https://doi.org/10.1016/0005-2787(79)90496-9)
465. Bailly, C., & Chaires, J. B. (1998). Sequence-Specific DNA Minor Groove Binders. Design and Synthesis of Netropsin and Distamycin Analogues. *Bioconjugate*, 9(5), 513–538. <https://doi.org/10.1021/bc980008m>
466. Farahat, A. A., Kumar, A., Wenzler, T., Brun, R., Paul, A., Gao, P., Wilson, W. D., & Boykin, D. W. (2023). Investigation of the effect of structure modification of furamidine on the DNA minor groove binding and antiprotozoal activity. *European Journal of Medicinal Chemistry*, 252, 115287. <https://doi.org/10.1016/j.ejmech.2023.115287>
467. Lyles, M. B., & Cameron, I. L. (2002). Interactions of the DNA intercalator acridine orange, with itself, with caffeine, and with double stranded DNA. *Biophysical Chemistry*, 96(1), 53–76. [https://doi.org/10.1016/S0301-4622\(02\)00036-4](https://doi.org/10.1016/S0301-4622(02)00036-4)
468. Nafisi, S., Saboury, A. A., Keramat, N., Neault, J. F., & Tajmir-Riahi, H. A. (2007). Stability and structural features of DNA intercalation with ethidium bromide, acridine orange and methylene blue. *Journal of Molecular Structure*, 827(1–3), 35–43. <https://doi.org/10.1016/j.molstruc.2006.05.004>
469. Foye, W. O., Vajragupta, O., & Sengupta, S. (1982). DNA-Binding Specificity and RNA Polymerase Inhibitory Activity of Bis(aminoalkyl)anthraquinones and Bis(methylthio)vinylquinolinium Iodides. *Journal of Pharmaceutical Sciences*, 71(2), 253–257. <https://doi.org/10.1002/jps.2600710228>
470. Yarmoluk, S., Lukashov, S. S., Ogul'chansky, T. Y., Losytskyy, M. Y., & Korniyushyna, O. S. (2001). Interaction of Cyanine Dyes with Nucleic Acids. XXI. Arguments for Half-Intercalation Model of Interaction. *Biopolymers*, 62(4), 219–227. <https://doi.org/10.1002/bip.1016>
471. Biver, T., De Biasi, A., Secco, F., Venturini, M., & Yarmoluk, S. (2005). Cyanine Dyes as Intercalating Agents: Kinetic and Thermodynamic Studies on the DNA/Cyan40 and DNA/CCyan2 Systems.

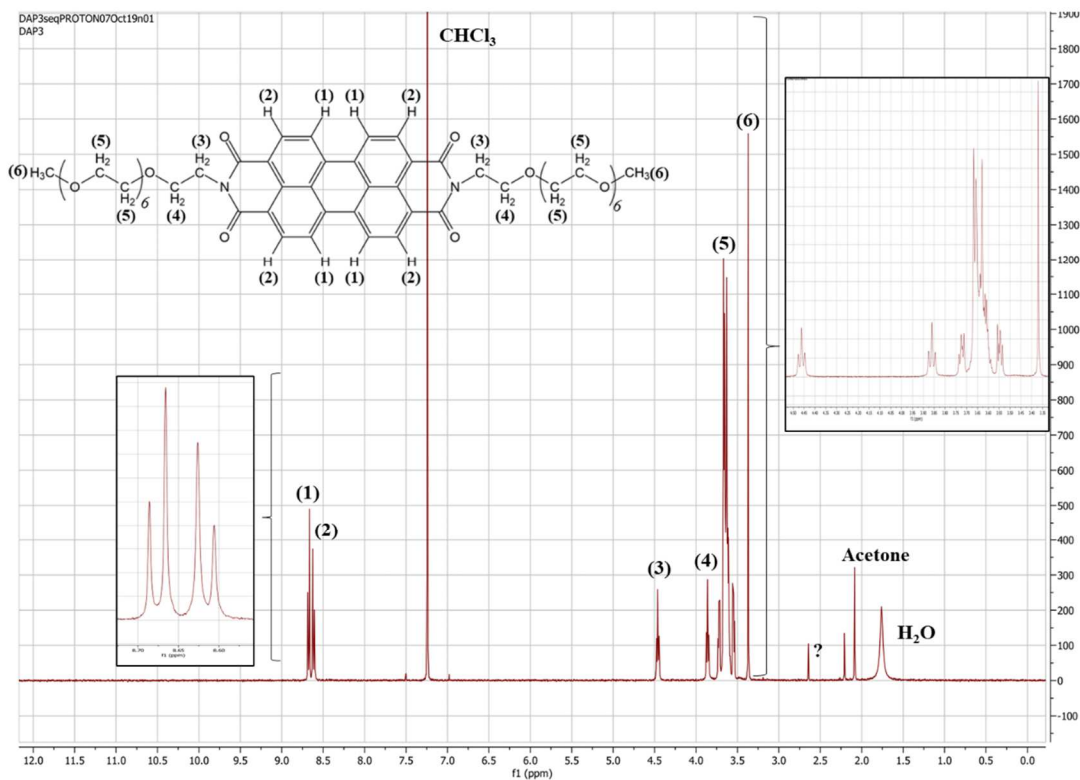
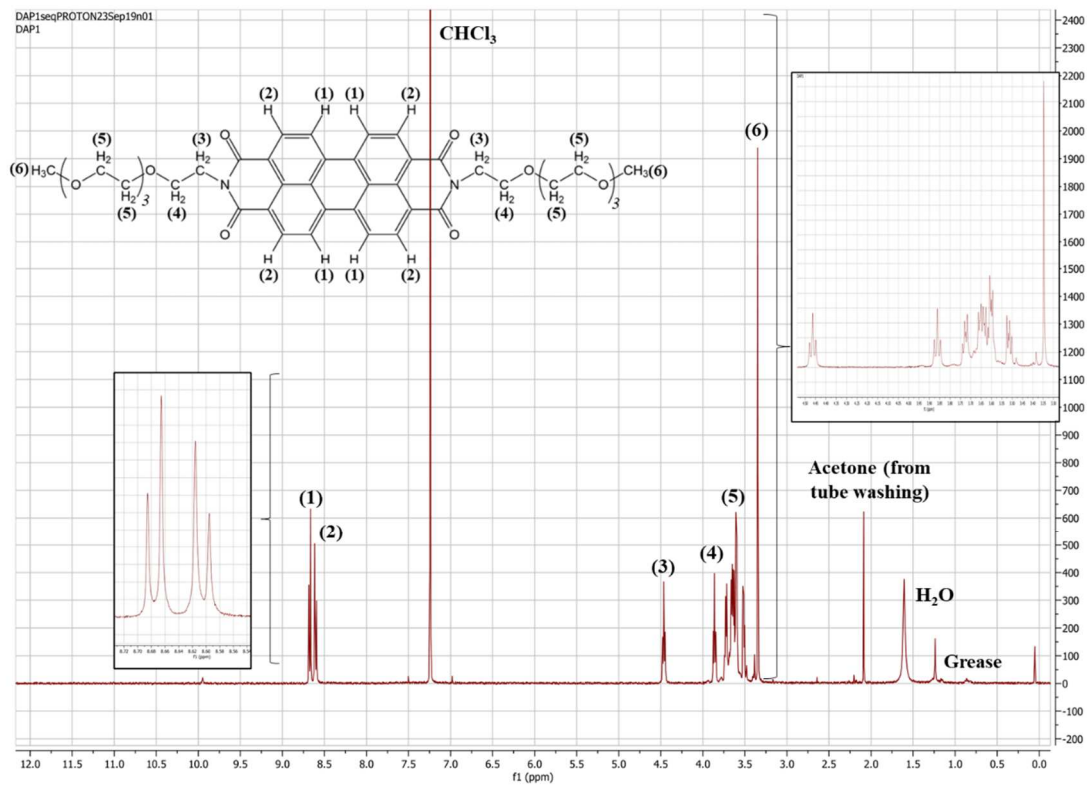
- Biophysical Journal*, 89(1), 374–383. <https://doi.org/10.1529/biophysj.105.059790>
472. Tutte, E., & Norden, B. (1994). Sequence-Specific Interactions of Methylene Blue with Polynucleotides and DNA: A Spectroscopic Study. *Journal of the American Chemical Society*, 116(17), 7548–7556. <https://doi.org/10.1021/ja00096a011>
 473. Wilson, W. D., Gough, A. N., Doyle, J. J., & Davidson, M. W. (1976). Coralyne. Intercalation with DNA as a possible mechanism of antileukemic action. *Journal of Medicinal Chemistry*, 19, 1261–1263. <https://doi.org/10.1021/jm00232a020>
 474. Ihmels, H., Faulhaber, K., Vedaldi, D., Dall'Acqua, F., & Viola, G. (2007). Intercalation of Organic Dye Molecules into Double-stranded DNA. Part 2: The Annelated Quinolizinium Ion as a Structural Motif in DNA Intercalators. *Photochemical and Photobiological Sciences*, 8(5), 1107–1115. <https://doi.org/10.1562/2005-01-25-IR-427>
 475. Waring, M. (1965). Complex formation between ethidium bromide and nucleic acids. *Journal of Molecular Biology*, 13(1), 269–282. [https://doi.org/10.1016/S0022-2836\(65\)80096-1](https://doi.org/10.1016/S0022-2836(65)80096-1)
 476. Sari, M. A., Battioni, J. P., Dupre, D., Mansuy, D., & Le Pecq, J. B. (1990). Interaction of Cationic Porphyrins with DNA: Importance of the Number and Position of the Charges and Minimum Structural Requirements for Intercalation. *Biochemistry*, 29(17), 4205–4215. <https://doi.org/10.1021/bi00469a025>
 477. Sato, S., Tsueda, M., & Takenaka, S. (2010). Electrochemical detection of aberrant methylated gene using naphthalene diimide derivative carrying four ferrocene moieties. *Journal of Organometallic Chemistry*, 695(15–16), 1858–1862. <https://doi.org/10.1016/j.jorganchem.2010.04.034>
 478. McKnight, R. E., Reisenauer, E., Pintado, M. V., Polasani, S. R., & Dixon, D. W. (2011). Substituent effect on the preferred DNA binding mode and affinity of a homologous series of naphthalene diimides. *Bioorganic and Medicinal Chemistry*, 21(14), 4288–4291. <https://doi.org/10.1016/j.bmcl.2011.05.069>
 479. Rhoden Smith, A., Ikkanda, B. A., Holman, G. G., & Iverson, B. L. (2012). Subtle Recognition of 14-Base Pair DNA Sequences via Threading Polyintercalation. *Biochemistry*, 51(22), 4445–4452. <https://doi.org/10.1021/bi300317n>
 480. Minarini, A., Milelli, A., Tumiatti, V., Feruzzi, L., Marton, M., Turrini, E., Hrelia, P., & Fimognari, C. (2012). Design, synthesis and biological evaluation of new naphthalene diimides bearing isothiocyanate functionality. *Journal of Medicinal Chemistry*, 48, 124–131. <https://doi.org/10.1016/j.ejmech.2011.12.003>
 481. Milelli, A., Tumiatti, V., Micco, M., Rosini, M., Zuccari, G., Raffaghello, L., Bianchi, G., Pistoia, V., Fernando-Diaz, J., Pera, B., Trigili, C., Barasoain, I., Musetti, C., Toniolo, M., Sissi, C., Alcaro, S., Moraca, F., Zini, M., Stefanelli, C., & Minarini, A. (2012). Structure–activity relationships of novel substituted naphthalene diimides as anticancer agents. *European Journal of Medicinal Chemistry*, 57, 417–428. <https://doi.org/10.1016/j.ejmech.2012.06.045>
 482. Xu, Z., Guo, K., Yu, J., Sun, H., Tang, J., Shen, J., Mullen, K., Yang, W., & Yin, M. (2014). A Unique Perylene-Based DNA Intercalator: Localization in Cell Nuclei and Inhibition of Cancer Cells and Tumors. *Small*, 10(20), 4087–4092. <https://doi.org/10.1002/sml.201401262>
 483. Xu, Z., Cheng, W., Guo, K., Yu, J., Shen, J., Tang, J., Yang, W., & Yin, M. (2015). Molecular Size, Shape, and Electric Charges: Essential for Perylene Bisimide-Based DNA Intercalator to Localize in Cell Nuclei and Inhibit Cancer Cell Growth. *ACS Applied Materials & Interfaces*, 7(18), 9784–9791. <https://doi.org/10.1021/acsami.5b01665>
 484. Wu, J., Peng, M., Mu, M., Li, J., & Yin, M. (2023). Perylene diimide supramolecular aggregates: Constructions and sensing applications. *Supramolecular Materials*, 2, 100031. <https://doi.org/10.1016/j.supmat.2023.100031>
 485. Long, E. C., & Barton, J. K. (1990). On demonstrating DNA intercalation. *Accounts of Chemical Research*, 23(9), 273–279. <https://doi.org/10.1021/ar00177a001>
 486. Norden, B., & Kurucsev, T. (1994). Analysing DNA complexes by Circular and Linear Dichroism. *Journal of Molecular Recognition*, 7(2), 141–156. <https://doi.org/10.1002/jmr.300070211>

487. Biver, T., Boggioni, A., Secco, F., Turriani, E., Venturini, M., & Yarmoluk, S. (2007). Influence of cyanine dye structure on self-aggregation and interaction with nucleic acids: A kinetic approach to TO and BO binding. *Biochemistry and Biophysics*, 465, 90–100. <https://doi.org/10.1016/j.abb.2007.04.034>
488. Macii, F., Perez-Arnaiz, F., Arrico, L., Busto, N., Garcia, B., & Biver, T. (2020). Alcian blue pyridine variant interaction with DNA and RNA polynucleotides and G-quadruplexes: changes in the binding features for different biosubstrates. *Journal of Inorganic Biochemistry*, 212, 111199. <https://doi.org/10.1016/j.jinorgbio.2020.111199>
489. Zhang, Z., Yang, Y., Zhang, D., Wang, Y., Qian, X., & Liu, F. (2006). Acenaphtho[1,2-b]pyrrole derivatives as new family of intercalators: Various DNA binding geometry and interesting antitumor capacity. *Bioorganic and Medicinal Chemistry*, 14(20), 6962–6970. <https://doi.org/10.1016/j.bmc.2006.06.029>
490. Macii, F., Cupellini, L., Stifano, M., Santolaya, J., Perez-Arnaiz, C., Pucci, A., Barone, G., Garcia, B., Busto, N., & Biver, T. (2021). Combined spectroscopic and theoretical analysis of the binding of a water-soluble perylene diimide to DNA/RNA polynucleotides and G-quadruplexes. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 260, 119914. <https://doi.org/10.1016/j.saa.2021.119914>
491. Bhosale, S. V., Jani, C. H., & Langford, S. J. (2008). Chemistry of naphthalene diimides. *Chemical Society Reviews*, 37(2), 331–342. <https://doi.org/10.1039/B615857A>
492. Selvi, P. T., & Palaniandavar, M. (2002). Spectral, viscometric and electrochemical studies on mixed ligand cobalt(III) complexes of certain diimine ligands bound to calf thymus DNA. *Inorganica Chimica Acta*, 337, 420–428. [https://doi.org/10.1016/S0020-1693\(02\)01112-X](https://doi.org/10.1016/S0020-1693(02)01112-X)
493. Hormann, A., Chaudhuri, B., & Fretz, H. (2001). DNA Binding Properties of the Marine Sponge Pigment Fascaplysin. *Bioorganic and Medicinal Chemistry*, 9(4), 917–921. [https://doi.org/10.1016/s0968-0896\(00\)00313-8](https://doi.org/10.1016/s0968-0896(00)00313-8)
494. Islam, M. M., Fujii, S., Sato, S., Okauchi, T., & Takenaka, S. (2015). Thermodynamics and kinetic studies in the binding interaction of cyclic naphthalene diimide derivatives with double stranded DNAs. *Bioorganic and Medicinal Chemistry*, 23(15), 4769–4776. <https://doi.org/10.1016/j.bmc.2015.05.046>
495. Lepecq, J. B., & Paoletti, C. (1967). A fluorescent complex between ethidium bromide and nucleic acids: Physical—Chemical characterization. *Journal of Molecular Biology*, 27(1), 87–106. [https://doi.org/10.1016/0022-2836\(67\)90353-1](https://doi.org/10.1016/0022-2836(67)90353-1)
496. Olmsted, J., & Kearns, D. R. (1977). Mechanism of ethidium bromide fluorescence enhancement on binding to nucleic acids. *Biochemistry*, 16(16), 3647–3654. <https://doi.org/10.1021/bi00635a022>
497. Plumbridge, T. W., & Brown, J. R. (1978). Studies on the mode of interaction of 4'-epi-adriamycin and 4-demethoxy-daunomycin with DNA. *Biochemical Pharmacology*, 27(14), 1881–1882. [https://doi.org/10.1016/0006-2952\(78\)90037-0](https://doi.org/10.1016/0006-2952(78)90037-0)
498. Plsikova, J., Janovec, L., Koval, J., Ungvarsky, J., Mikes, J., Jendzelovsky, R., Fedorocko, P., Imrich, J., Kristan, P., Kasparkova, J., Brabec, V., & Kozurkova, M. (2012). 3,6-Bis(3-alkylguanidino)acridines as DNA-intercalating antitumor agents. *European Journal of Medicinal Chemistry*, 57, 283–285. <https://doi.org/10.1016/j.ejmech.2012.09.020>
499. Jenkins, T. (1997). Optical absorbance and fluorescence techniques for measuring DNA-drug interactions. In K. Fox (Ed.), *Drug-DNA Interaction Protocols (1st Ed.)* (pp. 195–218). Human Press. <https://doi.org/10.1385/0-89603-447-X:195>
500. Rodger, A., Blagbrough, I. S., Adlam, G., & Carpenter, M. L. (1994). DNA binding of a spermine derivative: Spectroscopic study of anthracene-9-carbonyl-n1-spermine with poly[d(G-C)·(d(G-C))] and poly[d(A-T)·d(A-T)]. *Biopolymers*, 34(12), 1583–1593. <https://doi.org/10.1002/bip.360341203>
501. Maheswari, P. U., & Palaniandavar, M. (2004). DNA binding and cleavage activity of [Ru(NH₃)₄(diimine)]Cl₂ complexes. *Inorganica Chimica Acta*, 357(4), 901–912. <https://doi.org/10.1016/j.ica.2003.07.010>

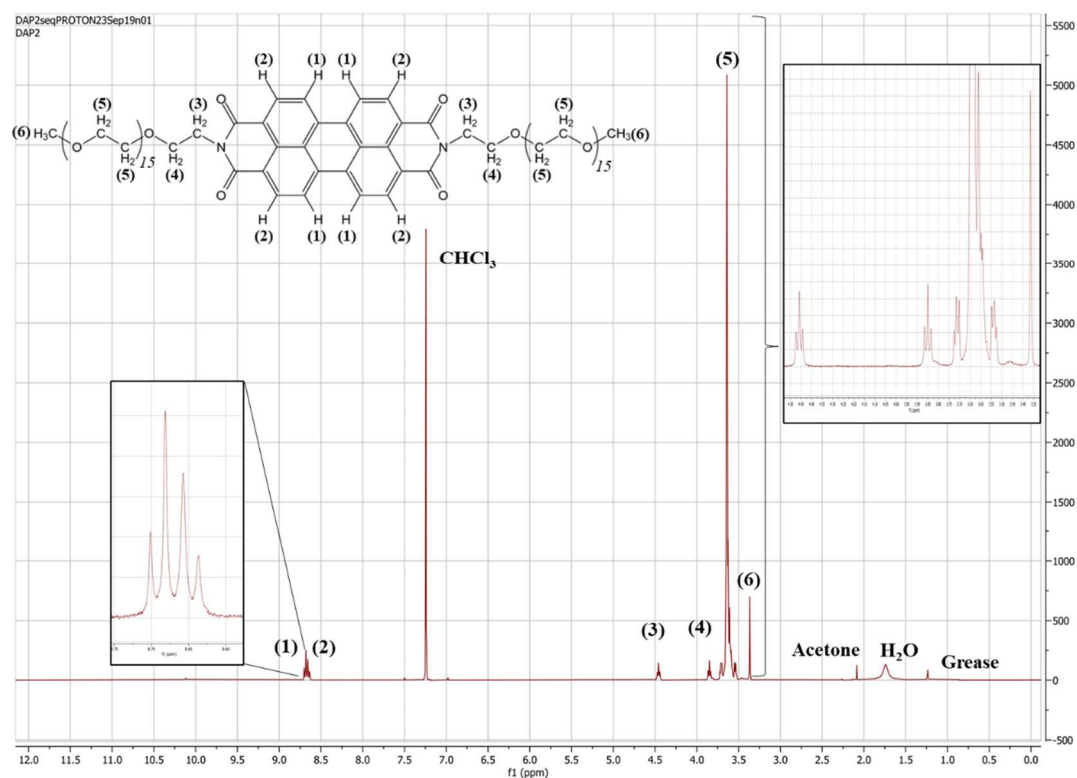
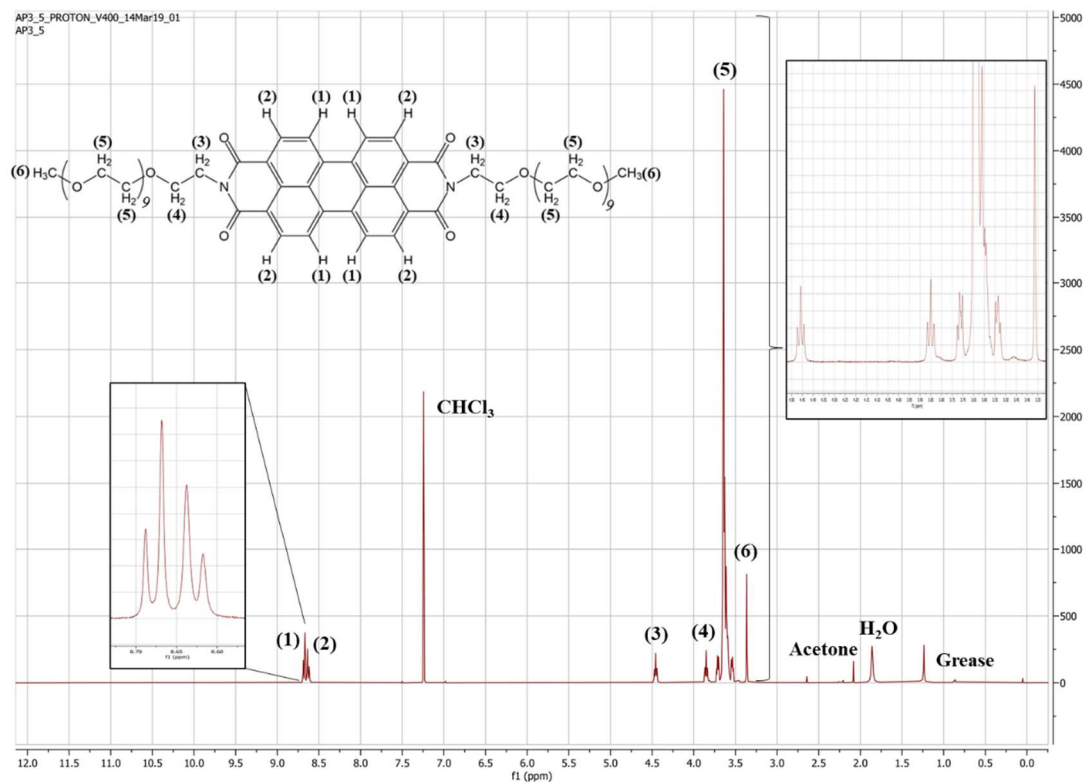
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502. Li, X., Lin, Y., Wang, Q., Yuan, Y., Zhang, H., & Qian, X. (2011). The novel anti-tumor agents of 4-triazol-1,8-naphthalimides: Synthesis, cytotoxicity, DNA intercalation and photocleavage. *European Journal of Medicinal Chemistry*, 46(6), 1274–1279. <https://doi.org/10.1016/j.ejmech.2011.01.050>
503. Maheswari, P. U., & Palaniandavar, M. (2004). DNA binding and cleavage properties of certain tetrammine ruthenium(II) complexes of modified 1,10-phenanthrolines – effect of hydrogen-bonding on DNA-binding affinity. *Journal of Inorganic Biochemistry*, 98(2), 219–230. <https://doi.org/10.1016/j.jinorgbio.2003.09.003>
504. Eriksson, M., & Nordén, B. (2001). Linear and circular dichroism of drug-nucleic acid complexes. *Methods in Enzymology*, 340, 68–98. [https://doi.org/10.1016/S0076-6879\(01\)40418-6](https://doi.org/10.1016/S0076-6879(01)40418-6)
505. Stockert, J. C., Horobin, R. W., Colombo, L. L., & Blazquez-Castro, A. (2018). Tetrazolium salts and formazan products in Cell Biology: Viability assessment, fluorescence imaging, and labeling perspectives. *Acta Histochemica*, 120(3), 159–167. <https://doi.org/10.1016/j.acthis.2018.02.005>
506. Do, T. T. A., Grijalvo, S., Imae, T., Garcia-Celma, M. J., & Rodriguez-Abreu, C. (2021). A nanocellulose-based platform towards targeted chemo-photodynamic/photothermal cancer therapy. *Carbohydrate Polymers*, 270, 118366. <https://doi.org/10.1016/j.carbpol.2021.118366>
507. Clua, A., Grijalvo, S., Erande, N., Gupta, S., Yucius, K., Gargallo, R., Mazzini, S., Manoharan, M., & Eritja, R. (2022). Properties of Parallel Tetramolecular G-Quadruplex Carrying N-Acetylgalactosamine as Potential Enhancer for Oligonucleotide Delivery to Hepatocytes. *Molecules*, 27(12), 3944. <https://doi.org/10.3390/molecules27123944>

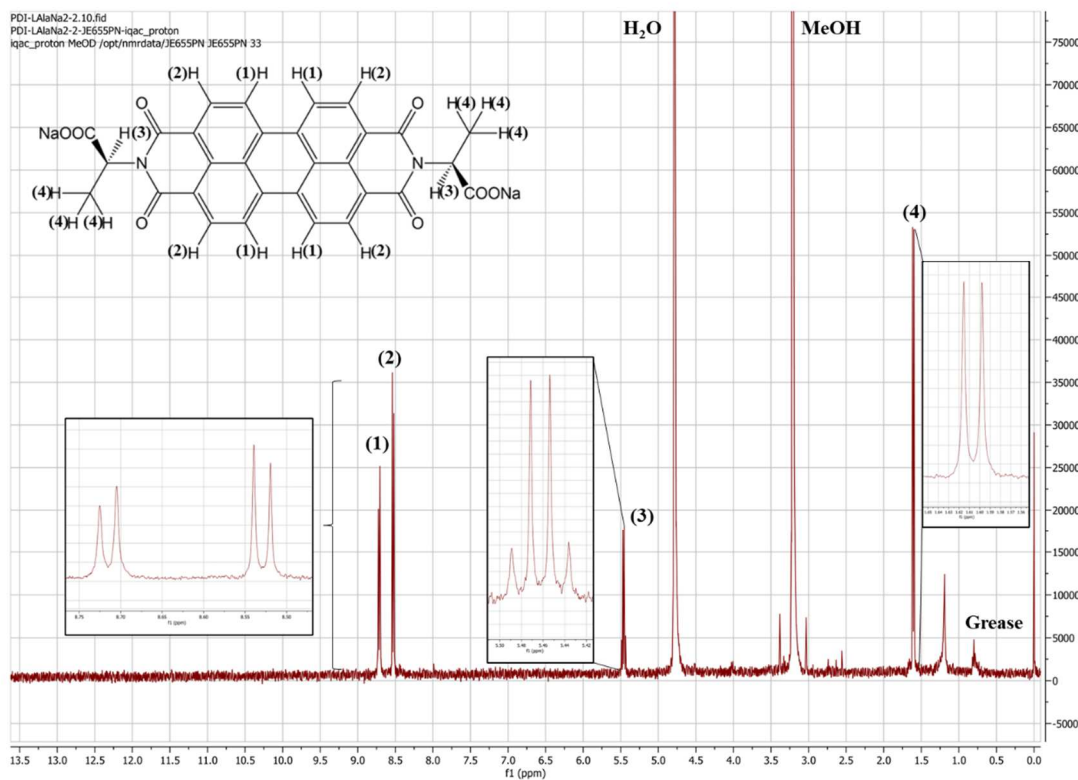
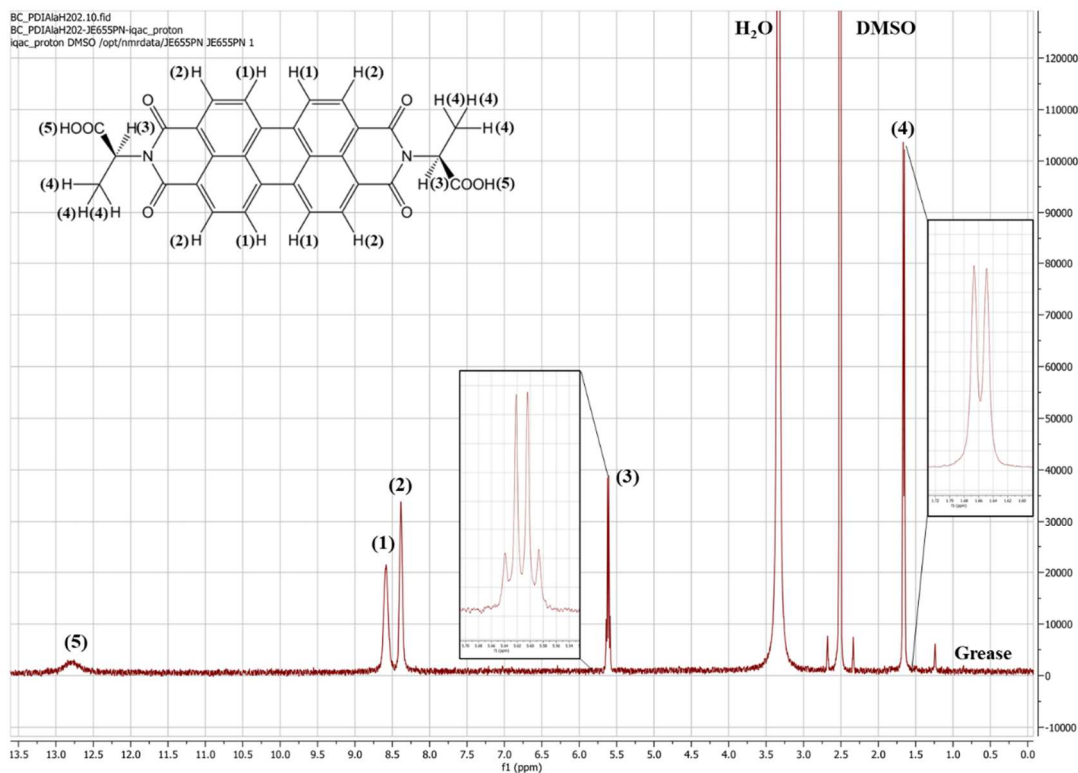
ANNEXES

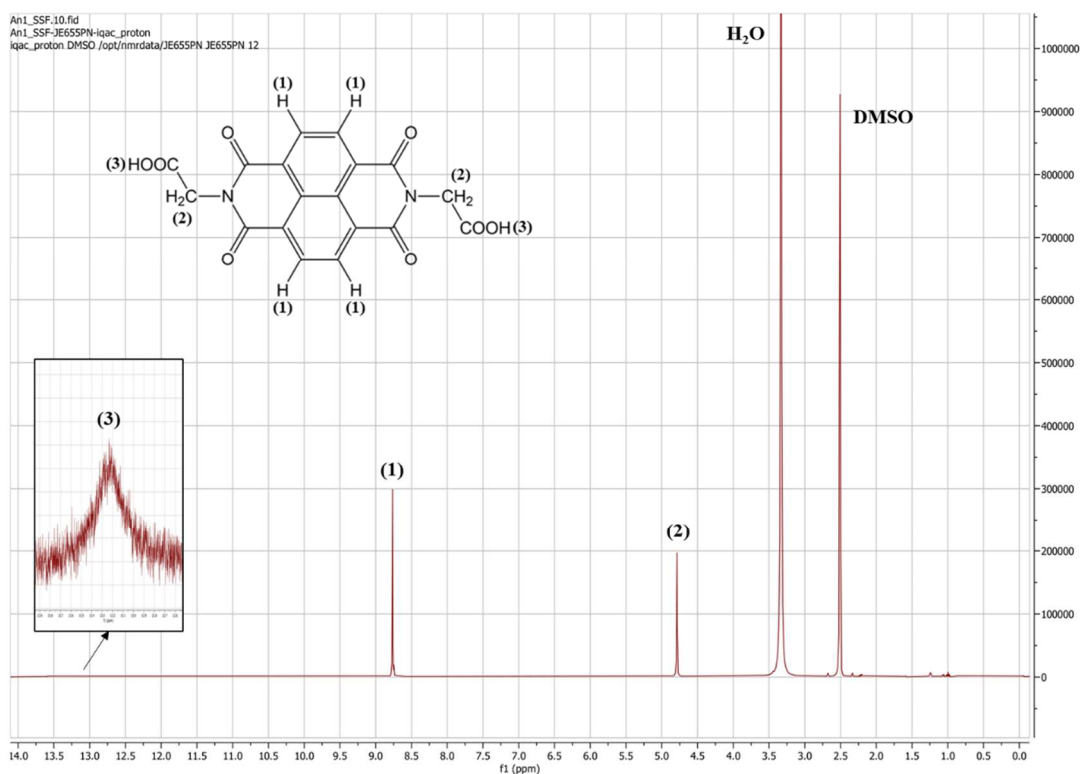
^1H -Nuclear Magnetic Resonance (NMR)

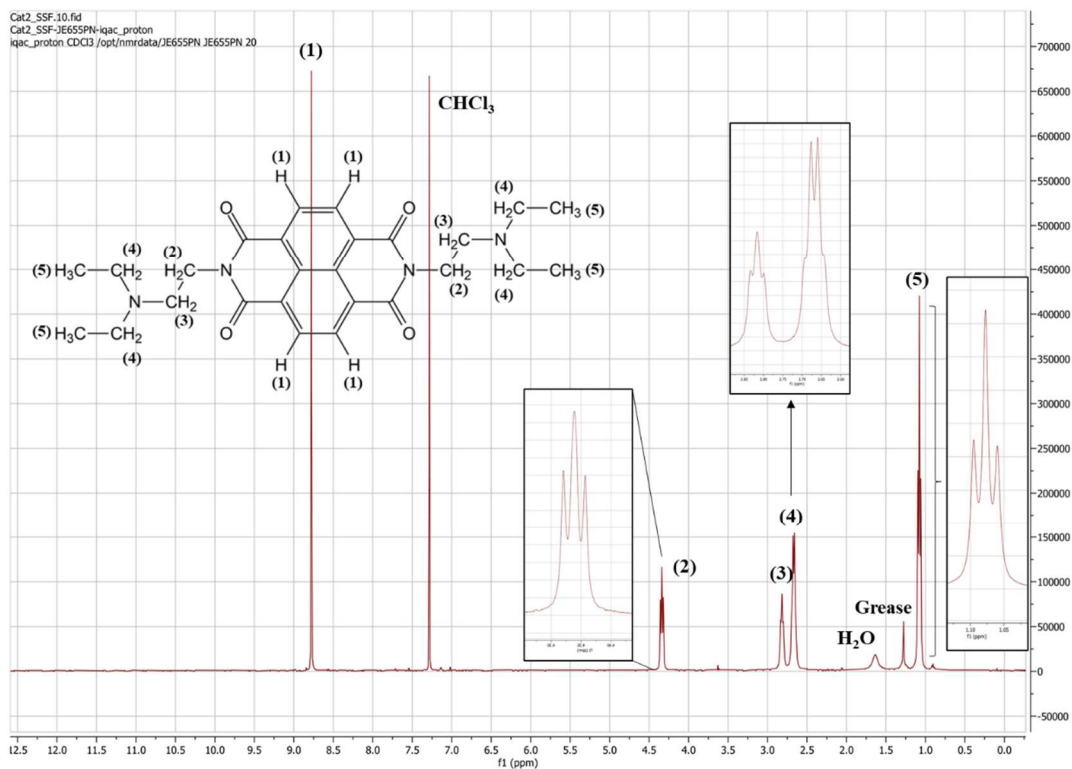


MOLECULAR SEGREGATION AT MULTIPLES SCALES IN PERYLENE-BASED SYSTEMS FOR THE PREPARATION OF ADVANCED MATERIALS

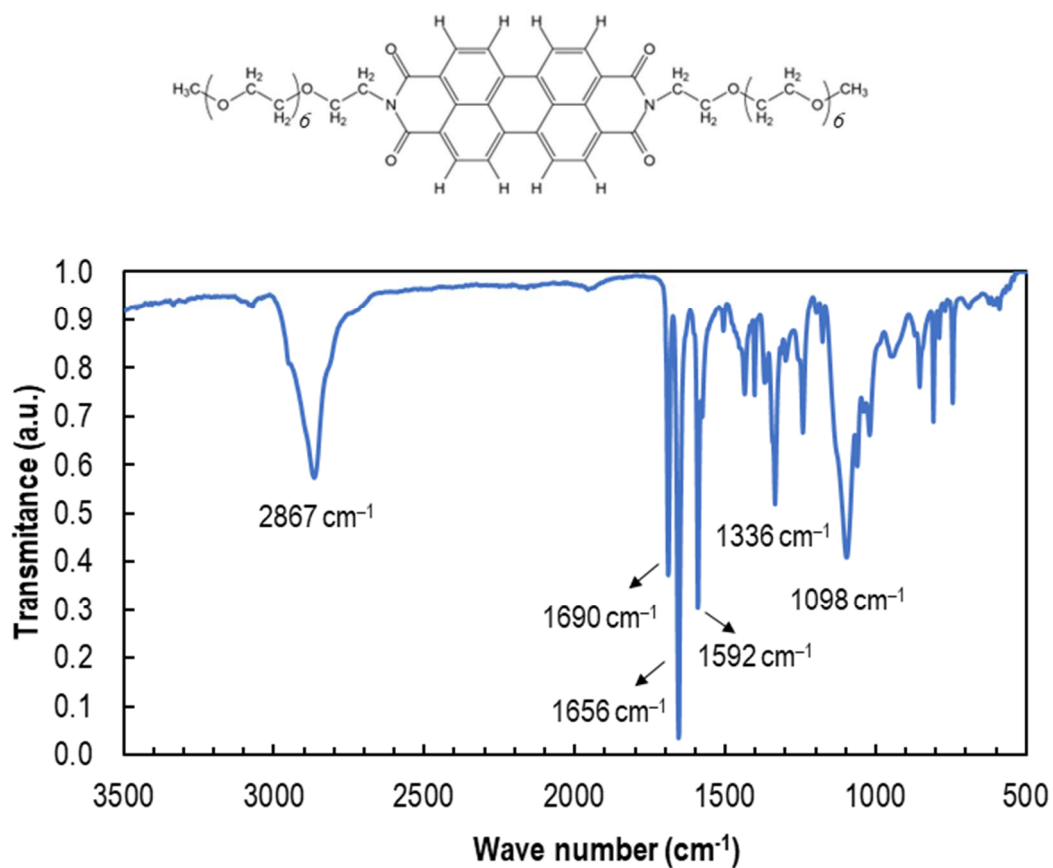


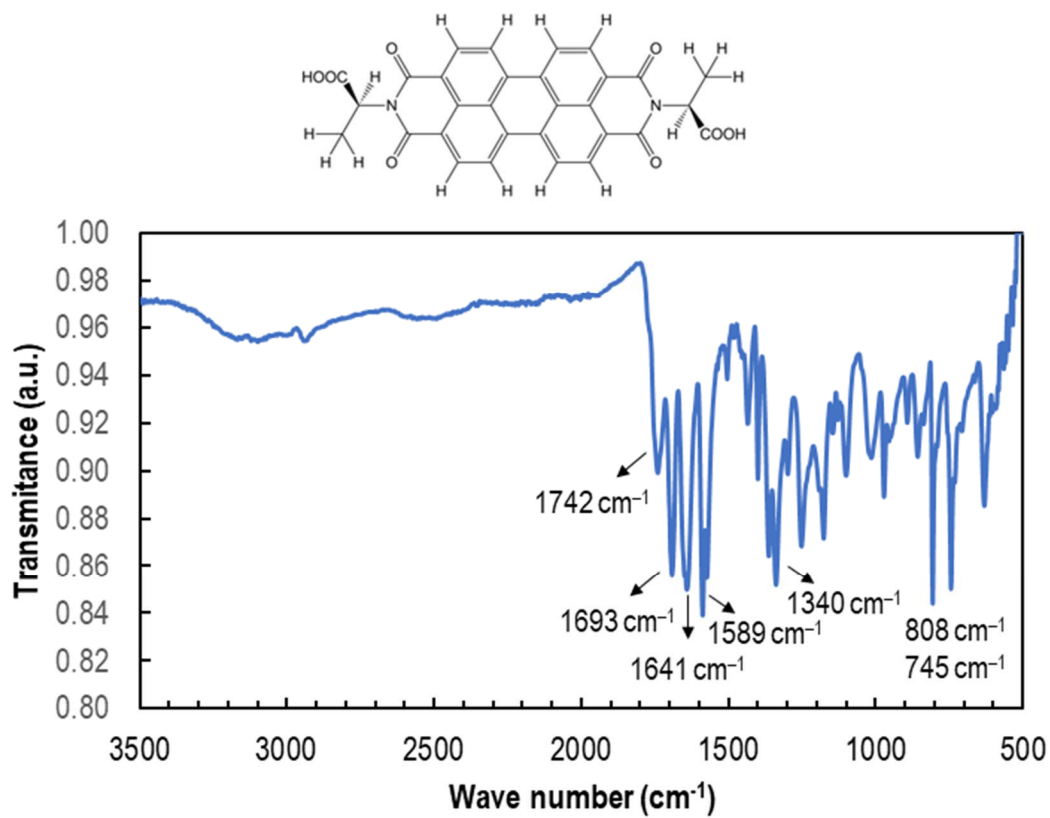
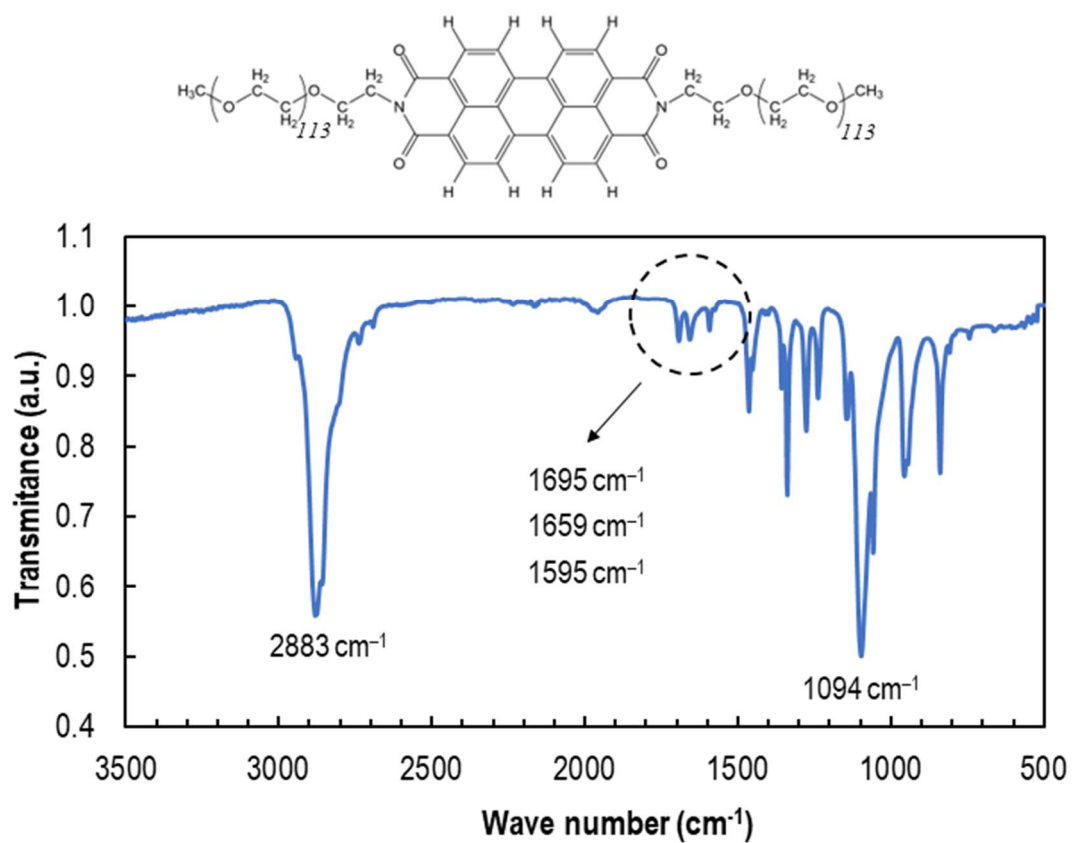


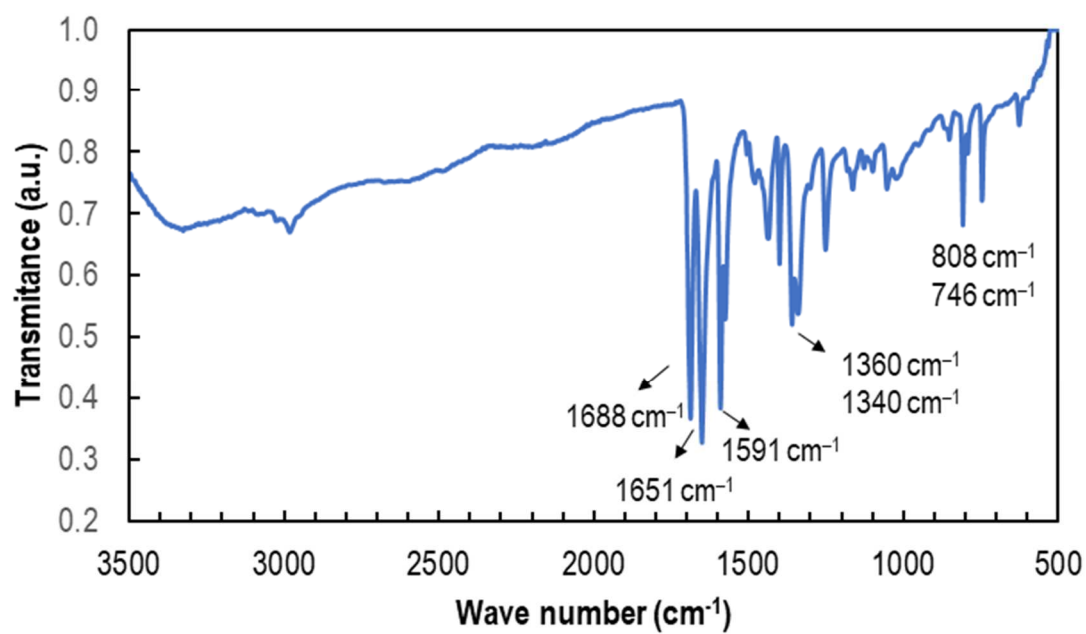
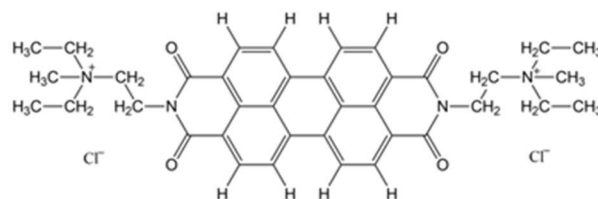
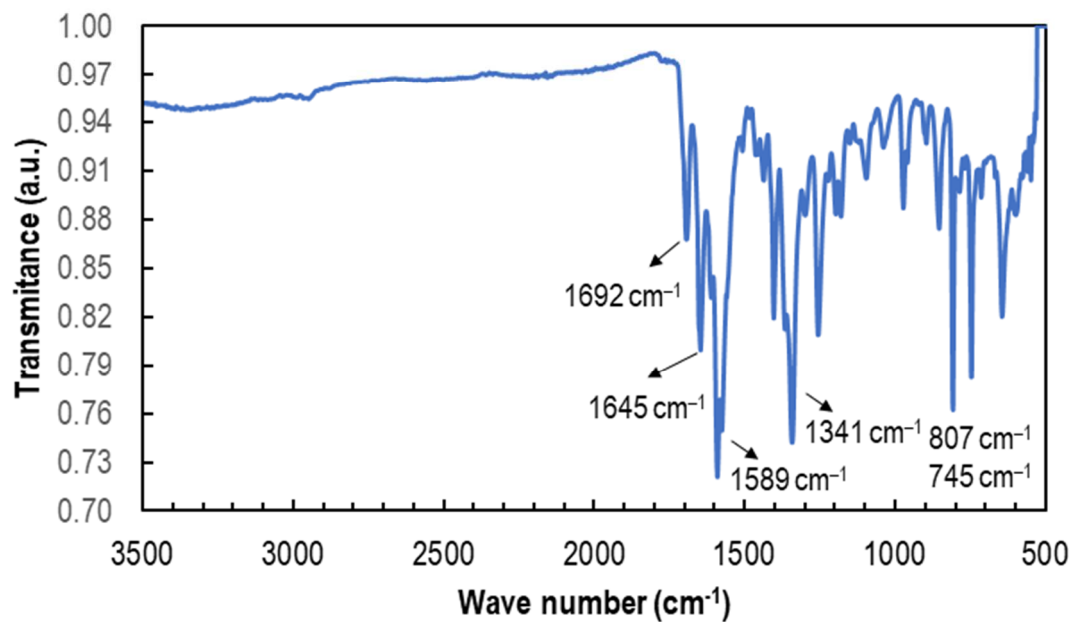
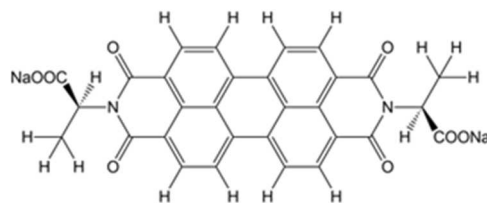


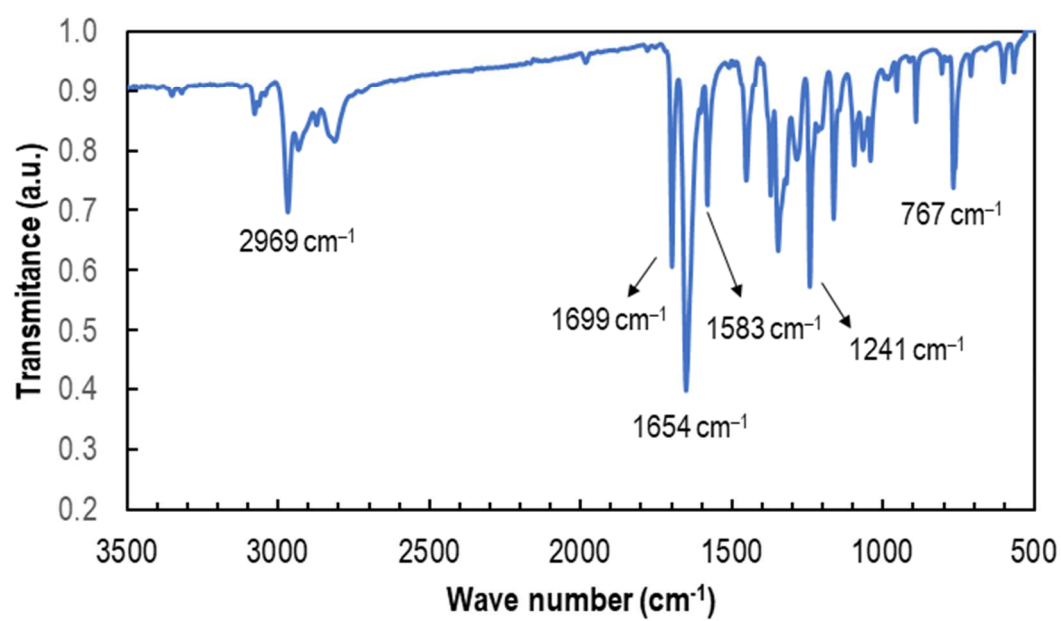
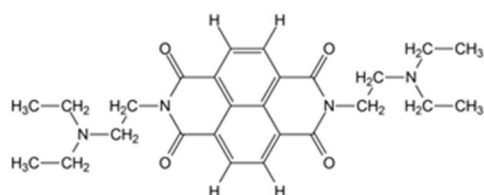
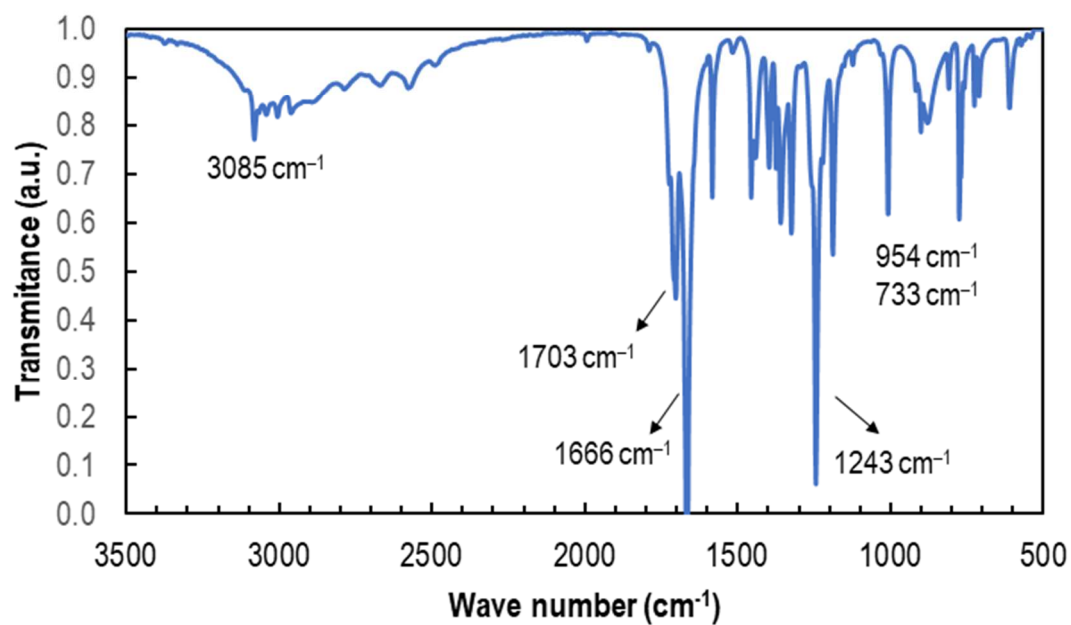
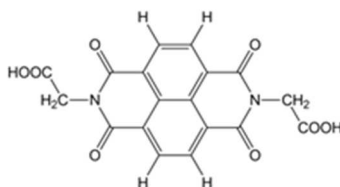


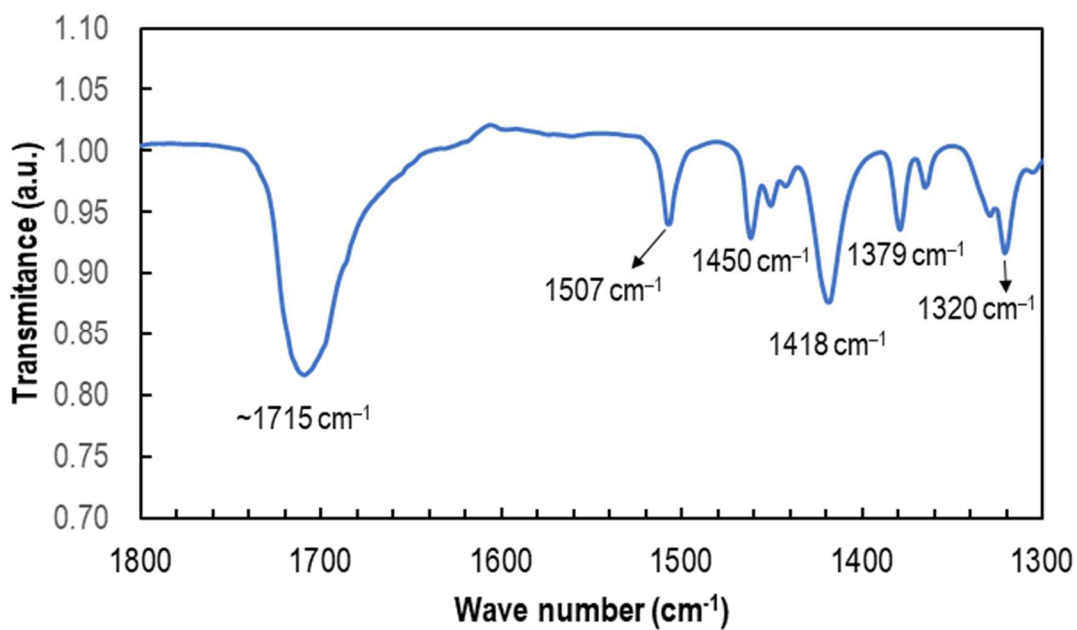
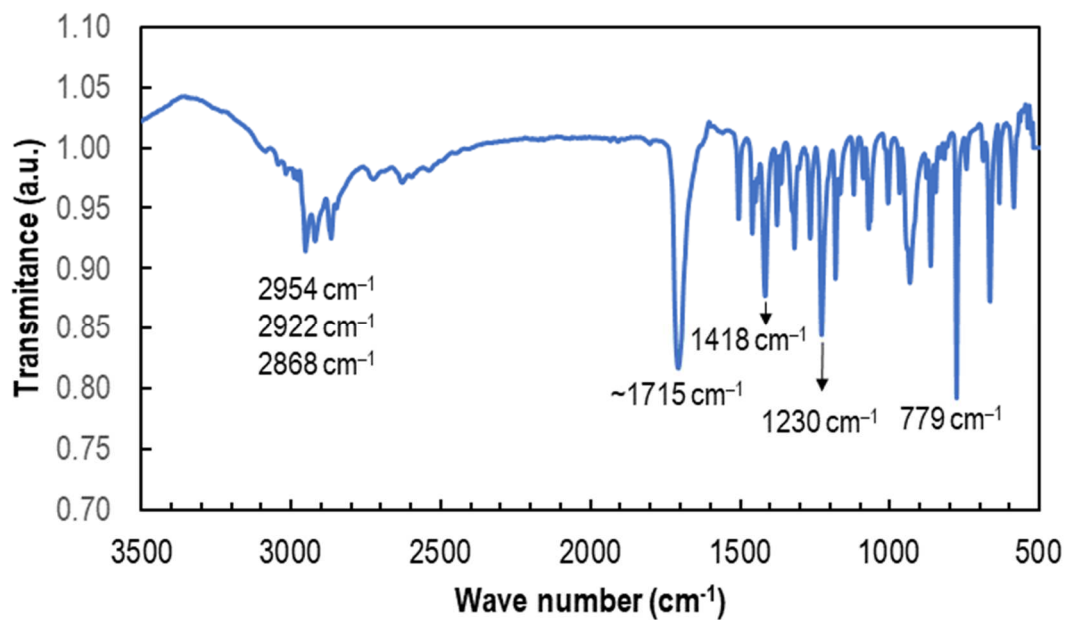
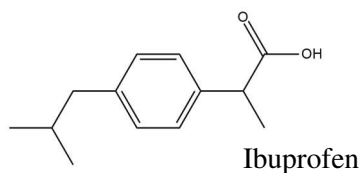
Fourier-Transform Infrared Spectroscopy (FTIR)

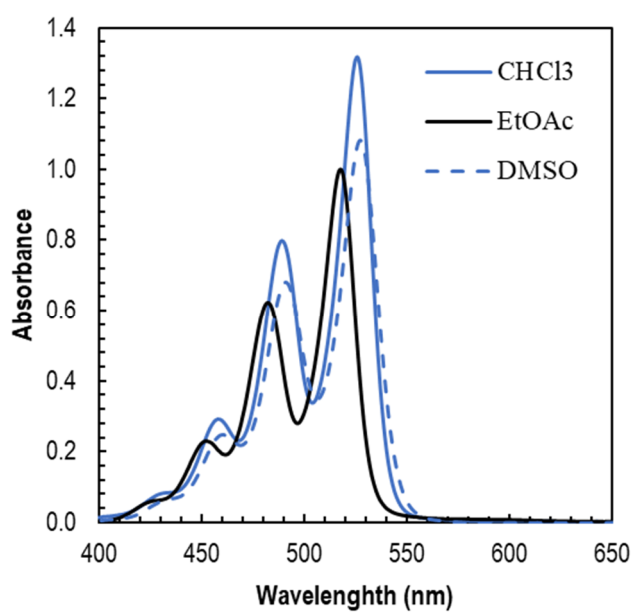




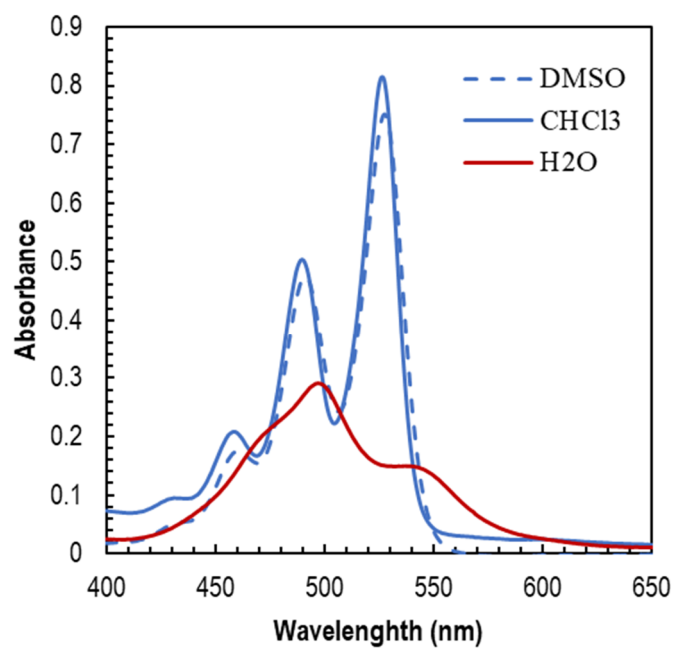






Ultraviolet-Visible spectrophotometry (UV-Vis)

Absorbance spectrophotometry of $(\text{PEG}_4)_2\text{PDI}$ $20\ \mu\text{M}$ in different solvents. Note that this compound is only partially soluble in ethyl acetate and DMSO .



Absorbance spectrophotometry of $(\text{PEG}_{10})_2\text{PDI}$ $11\ \mu\text{M}$ in different solvents.