Historical Perspective

Lipidic lyotropic liquid crystals: Insights on biomedical applications

Guillermo Blanco-Fernández [a,b,g], Bárbara Blanco-Fernandez [c,d,*], Anxo Fernández-Ferreiro [e,f,**], Francisco J. Otero-Espinar [a,b,g,*]

[a] Pharmacology, Pharmacy and Pharmaceutical Technology Department, Faculty of Pharmacy, University of Santiago de Compostela (USC), Santiago de Compostela, Spain
[b] Paraquasil Group, Health Research Institute of Santiago de Compostela (FIDIS), Santiago de Compostela, Spain
[c] CIBER in Bioengineering, Biomaterials and Nanomedicine, CIBER-BBN, Madrid, Spain
[d] Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology, Baldiri Reixac 10–12, Barcelona 08028, Spain
[e] Pharmacology Group, Health Research Institute of Santiago de Compostela (FIDIS), Santiago de Compostela, Spain
[f] Pharmacy Department, University Clinical Hospital of Santiago de Compostela (SERGAS), Santiago de Compostela, Spain
[g] Institute of Materials (iMATUS), University of Santiago de Compostela (USC), Santiago de Compostela, Spain

ARTICLE INFO

ABSTRACT

Liquid crystals (LCs) possess unique physicochemical properties, translatable into a wide range of applications. To date, lipidic lyotropic LCs (LLCs) have been extensively explored in drug delivery and imaging owing to the capability to encapsulate and release payloads with different characteristics. The current landscape of lipidic LLCs in biomedical applications is provided in this review. Initially, the main properties, types, methods of fabrication and applications of LCs are showcased. Then, a comprehensive discussion of the main biomedical applications of lipidic LLCs accordingly to the application (drug and biomacromolecule delivery, tissue engineering and molecular imaging) and route of administration is examined. Further discussion of the main limitations and perspectives of lipidic LLCs in biomedical applications are also provided.

Statement of significance: Liquid crystals (LCs) are those systems between a solid and liquid state that possess unique morphological and physicochemical properties, translatable into a wide range of biomedical applications. A short description of the properties of LCs, their types and manufacturing procedures is given to serve as a background to the topic. Then, the latest and most innovative research in the field of biomedicine is examined, specifically the areas of drug and biomacromolecule delivery, tissue engineering and molecular imaging. Finally, prospects of LCs in biomedicine are discussed to show future trends and perspectives that might be utilized.

This article is an ampliation, improvement and actualization of our previous short forum article "Bringing lipidic lyotropic liquid crystal technology into biomedicine" published in TIPS.

1. Introduction

Liquid crystals (LCs) are those systems between a solid and liquid state [1,2]. They were discovered by the Austrian botanic Friedrich Reinitzer in 1888, who found out cholesterol intermedium transformations [3] and they were lately named liquid crystals by the German physicist Otto Lehmann [4], who also stablished their optic anisotropy and birefringence [2,5]. Jacques Friedel, a pioneer in the field of LCs, defined the liquid-crystalline phase as a mesophase, which comes from the Greek prefix intermediate [6,7]. According to the IUPAC, this mesomorphic state is a molecular arrangement halfway between a liquid/gas or amorphous solid (without any long-range order) and solid crystals (with a long-range orientational and positional 3D order) [8,9]. LCs have a long-range orientational order with a partial or complete positional disorder (Fig. 1A) [7,10]. Indeed, LCs are materials which combine liquids and crystalline solid properties. LCs can flow like a liquid as well as to diffract X-rays, a characteristic shared by crystalline solids [7]. LCs are anisotropic materials, varying their properties depending on the measurement direction. For example, LCs viscosity is lower when it is measured in the same direction that the molecule

* Corresponding authors at: Facultad de Farmacia s/n, Campus Vida, 15705 Santiago de Compostela, A Coruña, Spain.
** Corresponding author at: INSTITUTO DE INVESTIGACIÓN SANITARIA DE SANTIAGO, IDIS Travesía da Choupana S/N 15706, Santiago de Compostela, Galicia, Spain

E-mail addresses: barbara.blanco.fernandez@usc.es (B. Blanco-Fernandez), anxordes@gmail.com (A. Fernández-Ferreiro), francisco.otero@usc.es (F.J. Otero-Espinar).

orientation. Above certain temperatures, LCs anisotropy switch to isotropy behavior, being their properties independent of the measurement direction [1].

These materials have been in nature since the origin of the cell, as cell membranes display a 1-D flat lamellar structure [11]. LCs can be observed in the wall/plasmalemma interface during cell wall growth, that seems to be provoked by the membrane self-assembly [12]. Moreover, spiders store the silk coat protein as LCs [13]. All lipid systems can form LCs under appropriate water content and temperature [14]. But there are many other molecules in nature that form LCs. For instance, mucilage's celluloses can self-assemble in LCs [15], collagen I forms a twisted liquid crystalline phase after several hours post-sonication [16], and even some nucleic acids can form LC phases [17], such as sodium thymonucleate [18] or the six base pairs long complementary RNA oligomers forming chiral and columnar nematics [19]. Another example is the double-stranded DNA of bacteriophage T5, which undergoes a crystalline liquid transition to provide the required genome fluidity to leave the capsid [20]. Lipids secreted by alveolar epithelial cells also form LCs bilayers containing hydrophobic proteins embedded in it in aqueous solutions, which seems to be key for its secretion [21]. This has been proved *in vitro* at physiological temperature with extracts from bovine and porcine lung surfactants which form laminar LCs in aqueous solutions. Interestingly, LCs can recapitulate some biological features. For instance, nanometric dispersions of lyotropic LCs have a thermic biomimetic behavior, mimicking the skin inter-cellular lipids. Indeed, this property gives LCs good permeability and skin retention properties [22]. The most widespread use of LCs is liquid crystal displays (LCD), used in mobile phones, tablets, or laptops, among others [17]. However, in the last years LCs have proved their appropriateness as active substances delivery platforms.

The aim of this review is to highlight the main uses of lipid LCs in biomedicine. Lipid Lyotropic LCs (lipid LLCs) have evidenced their potential in this field by controlling the release of active substances [23], their biodegradation products biocompatibility [24,25], their easiness in tuning and tailoring their properties [26], thermodynamic stability [24], economic profitability [27], scalability [28], *etc.* Therefore, they are a suitable platform for biomedical research. A detailed description of the types, characteristics and manufacturing methods of LCs is provided as well as the most recent research of LCs in biomedical research is discussed, covering the fields of drug and biomacromolecule delivery, molecular imaging, and tissue engineering in the last decade. Finally, the prospects and trends of LCs in biomedicine are discussed.

2. LCs classification and manufacturing

2.1. LCs classification

LCs are classified according to their bond type (ionic and molecular) [29], molecular geometry (discotics, calamitics) [5,29] and, most importantly, their appearance (lyotropics, thermotropics) [1,7,10,29]. Thermotropic LCs are made up of molecules that react to changes in temperature, while lyotropic LCs (LLCs) are made up of molecules that, at a given temperature range and solvent, are formed by changes in the concentration of the molecules. Lyotropics and thermotropics have different mesophases which can be identified by measuring their optical isotropy by cryofracturing electron microscopy, polarized light microscopy, low angle X-ray scattering (SAXS), neutron diffraction and low angle neutron scattering (SANS) [30]. Mesophases can be differentiated between a normal (convex) and inverse (concave) depending on the interface formed by the surfactant and water (Fig. 1B) [31,32]. However, there is a mesophase without curvature (the intermediate point), which is known as the lamellar phase (Lα) (Fig. 1B) [32].

2.1.1. Thermotropic LCs

Thermotropic LCs (TLCs), such as the ones formed by cholesteryl benzoate [10], are formed by some organic molecules through temperature changes, without the requirement of a solvent [29]. They can be subclassified in monotropics – when LCs are only formed by temperature reduction – or enantiotropics – when LCs are formed by temperature increases or reductions [5]. Thermotropic LCs can also be subclassified according to their structure (molecule's disposition) in smectics (molecules with various structure types are aligned forming layers), nematics (molecules follow the direction of a vector and are orientated in the same direction except for some deviated) and cholesterics (molecules have only one structure forming layers disposed with diverse angles with a helical pattern) [1,5,29,33], having the nematic and cholesteric LCs only orientational and not spatial order [6]. In nematics forming films, we can distinguish between planar nematic (parallel to the support) and nematic homeotrope (perpendicular to the support) [2]. Interestingly, cholesterics LCs have their origin in cholesterol, although cholesterol cannot form LCs, almost all its derivatives form cholesteric LCs [2].

2.1.2. Lyotropic LCs

LLCs are formed through changes in the material concentration, over a temperature range when certain substances are dispersed in a liquid.
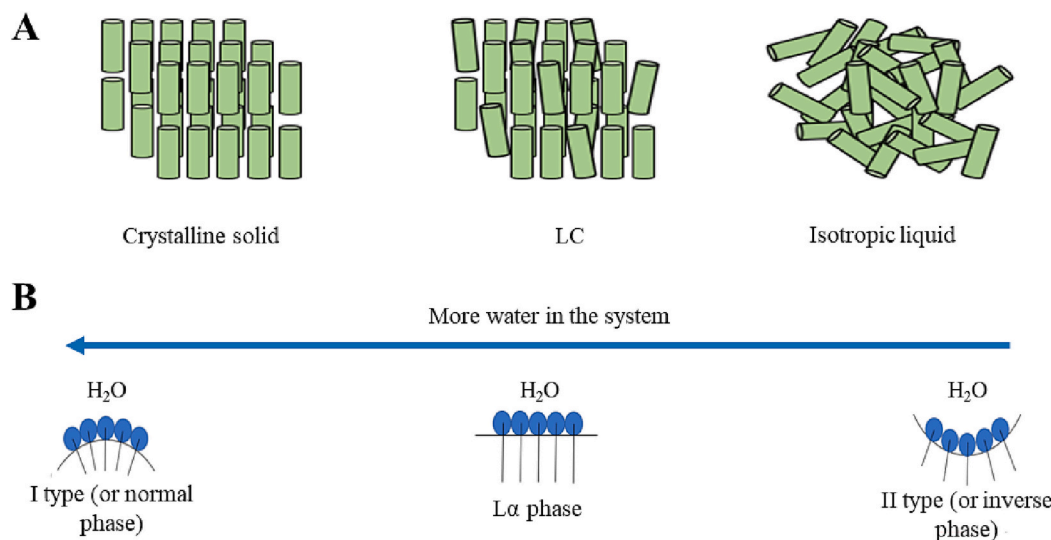

Fig. 1. Internal structure of liquid crystals. (A) Liquid crystals possess an internal structure between solids and liquids/gas and are therefore considered to be an intermediate state or mesophase. (B) Mesophases can experiment different types of curvatures in presence of water: convex (normal), concave (inverse) or without curvature (lamellar phase) depending on the amount of water in the system.

At fixed temperatures, they will appear in a concentration interval [7]. Increasing concentrations of the solute, which it is usually a surfactant, allows the formation of LLCs [29,33]. The most common LLCs used in the biomedical field are lamellar, hexagonal, and cubic phases [7].

Lamellar LLCs, also called the neat soap phase, is the most common LLC and is highly used in the detergent industry [33]. Its lamellar structure [33] is observed as lamellar phase Lα (Fig. 2) or the uncommon lamellar phase L$_\beta$ [31]. Lα is formed by layers of amphiphilic molecules and water [7,31,32,34] and they occur at the interface of the emulsions, since they increase their stability [7].

Hexagonal LLCs have a hexagonal shape and can be subclassified in normal or reverse (Fig. 2). Normal hexagonal (H$_I$) LLCs have a two-dimensional hexagonal grid where cylindrical micelles are arranged and the water is dispersed between the cylinders as a continuous phase [7,31,32,34] whereas in reverse hexagonal (H$_{II}$) LLCs their volume is separated in two, one occupied by hydrocarbon chains and the other occupied by a delimited water nucleus. This delimitation is given by the polar head of the molecules or their ions of the amphiphilic substances [7,31,32,34].

Cubic LLCs are classified in bicontinuous or discontinuous (Fig. 2). Bicontinuous LLCs are based on periodic minimum surfaces, and they correspond to the area between the lamellar and hexagonal phase in phase diagrams [7]. They can have an inverse bilayer with water inside (type I or V$_I$) or polar domains separated by a normal bilayer (type II or V$_{II}$) [7]. On the other hand, discontinuous LLCs are integrated by complex accommodations and the micellar ones, by discrete micellar aggregates. When LLCs are dispersed in water, lamellar phase LLCs form liposomes, whereas cubic phase and hexagonal phase maintain their integrity forming cubosomes or hexosomes respectively [11,35,36]. Within LLCs, there is also a difference between normal and inverted, being inverted LLCs optimal for preparing stable LC nanoparticles (LCNPs) suspensions under excess water conditions [37]. Interestingly hexosomes can cross the cell membrane thanks to a distortion of the cell membrane [38]. Indeed, hexosomes do not experiment fusion or endocytosis, but rather an exhaustion of the regulatory proteins which leads to a distortion of the cell membrane by decreasing its tension, thus crossing the bilayer [38].

LLCs can present other mesostructures, sometimes referred to as illdefined LLCs. Some examples are the sponge phase (L3) and the ribbon phase (R1). L3 are bicontinuous and disordered cubic phase formed by a bicontinuous network of surfactant bilayers highly interconnected. R1 has in general rhombohedral symmetry, although monoclinic and tetragonal symmetries can also be found formed by water and surfactant [34].

2.1.3. Advantages, disadvantages and other uses of TLCs and LLCs

TLCs and LLCs possess advantageous characteristics, such as their stability and stimuli-sensitivity, to be used in biomedical and industrial applications [39]. TLCs are easier to fabricate than LLCs [40], and they possess temperature-sensitivity, making them good thermo-responsive drug delivery systems [41,42]. However, their simplicity and ease of processing is not always compatible with the encapsulation of therapeutics, due to the need of adding a solvent to solubilize the active ingredient. On the other hand, LLCs are easy and economic manufacture, biodegradable, biocompatible, stimuli-responsive, they can encapsulate hydrophobic and hydrophilic drugs, sustain, and control drug release, and it can enhance cellular internalization due to specific interactions [43–48]. However, LLCs possess some drawbacks, such as lower long-term stability and larger complexity when compared to TLCs. Some drugs such as Nafoxidine hydrochloride or Palmitoyl propranolol hydrochloride have thermotropic and lyotropic forms. Despite being a minority of drugs with this property, it can be used to improve the solubility of drugs. For example, Fenoprofen cacium can form TLCs form to achieve greater solubility, to then form LLCs when in contact with water [49].

LCs have a great number of applications, due to their unique characteristics. For example, LCs can form pores of specific diameters, making them suitable materials for the creation of filtration membranes, such as filter membranes for water treatment [50]. However, most of the

Table 1
Main applications of LLCs and TLCs.

| TYPE	USES	REF
LLC	Filtration membranes	[57]
	Optical materials	[58]
	Reduce the self-discharge in supercapacitors	[59]
	Creation of electrodes	[60]
	Gel electrolyte for a solar cell	[61]
TLC	Diagnostic Kit for SARS-CoV-2	[62]
	Report virulence and bacterial quorum sensing	[63]
	Actuators for soft robotics, bionic manufacturing and micro/nano devices with load, ambient temperature, and strain sensing capabilities	[64]
	3D-printing of composites	[65]
	DNA LCs for bioelectronic devices, biocatalysts and biosensors	[66]
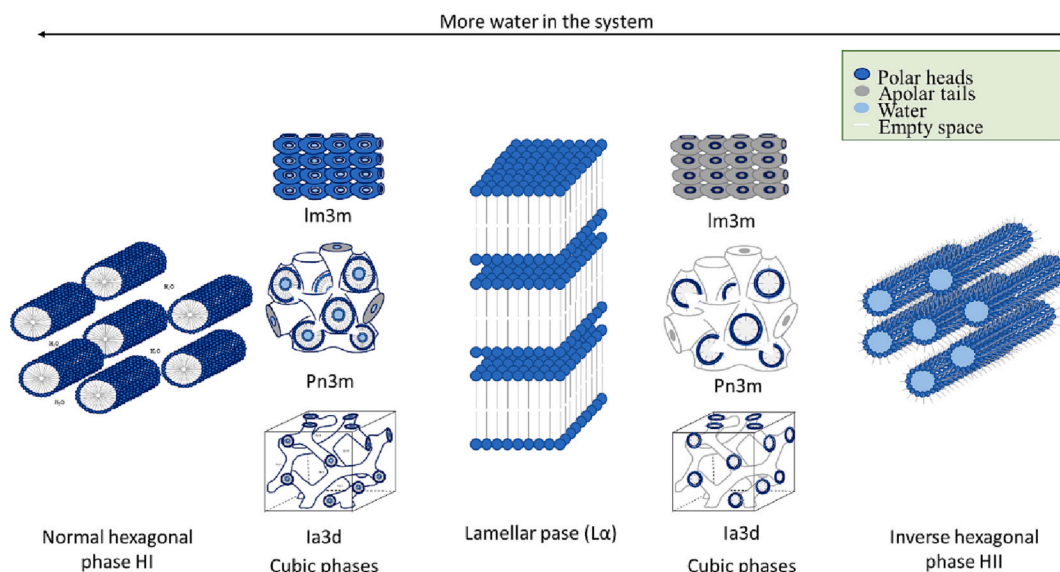
Fig. 2. LLCs classification according to the internal structures, LLCs can show different structures depending on the amount of water in the system.

uses revolve around electronics and detection of biomolecules (Table 1). LCs can manifest changes in birefringence when they interact with biomolecules or even amplify the signal, making them ideal candidates for biodetection [51,52]. They can even be functionalised to react only to selected pathogens [53]. The field of biosensing in LLCs is dominated by chromonic LCs containing aromatic groups with more rigid molecular shapes (blade-shaped or plank-shaped) [54]. Therefore, these properties as well as their low cost, easy manipulation, and fast response [51], make them very attractive detection system and are under constant research.

TLCs are mainly used in optical and electronic devices (flat panel displays, field effect transistors, optical compensating films, hole-transporting material in photovoltaics, electret in nonvolatile memory, mirrorless lasers). For example, nematic TLCs are used for the fabrication of flat panel displays, due to their capability of forming monodomains, fast response to electric fields and low viscosity [55]. Another possible application of TLCs is the possibility of creating fluorescent TLCs encapsulating DNA, with possible applications in the field of bioimaging in solvent-free conditions [56].

2.2. LLCs manufacturing

LLCs are formed by two main components, the material forming the LLCs [35] and the solvent, being water the most used for preparing them. The materials forming LLCs have, in general, amphiphilic molecules with a discoidal or elongated shape, and a random gravity center with a long-range orientation order [29]. The anisotropic form and the weak interactions between the molecules (dipole-dipole interactions, hydrogen bonding and/or dispersion forces) explain the behavior of LLCs [1]. Altogether lead to interactions between the molecules that force them into a preferential orientation. However, molecules can still experiment movements alongside their axes, as they are not under the effect of stronger bonds, their elongated dimensions, and their parallel packing [1].

2.2.1. Materials used for lipid LLCs fabrication

Glyceryl monooleate (GMO)/water is one of the most well-known LLCs´ systems [7] because they can form different types of LLCs such as lamellar (Lα), reversed micellar (L2 phases), cubic (Q) or hexagonal (H) phases. GMO is a lipid characterized by being polar and poorly soluble in water [36]. Nevertheless, there are many other amphiphilic materials that can be used to fabricate LLCs, such as surfactants like amphiphilic block copolymers (Poloxamer 407 [37]) or many other lipids [10,38]. This review will focus on lipid LLCS, as non-lipid LLCs have been previously addressed by other authors [67–72]. Triglycerides and sterols are the most common lipids used for fabricating thermotropic LCs [24], whereas fatty acids such as phospholipids, monoglycerides or galactolipids are the most used lipids in lipid LLCs [24].

LLCs used in biomedicine, usually combine lipids, water, and surfactants to increase their stability [37,73]. GMO and phytantriol are the most used lipids for the formation of non-lamellar LLCs in drug delivery systems [11,74]. These two molecules are surfactant-like lipids [75,76] that can form LLCs in excess of water or other solvents [77]. However, there are many other lipids that can be used, depending on the required properties of the platform. For instance, platforms that need high chemical stability will use molecules without unsaturation or ester bonds like phytantriol, as phospholipids or GMO might be degraded by acid and enzymatic hydrolysis and lipids with unsaturations are susceptible to oxidative degradation. Another crucial factor is the lipid bioactive loading capacity [11], which depends on factors such as its polymorphic state, its chemical structure, or how soluble the drug in question is in the lipid [78,79]. Interestingly, natural products are increasingly being used to obtain delivery platforms. Plant components have been incorporated into LLCs due to their low molecular weight and low viscosity. For instance, essential oils can reduce the occlusion, improve the skin penetration, and increase the loading of active

substances in LLCs in comparison with mineral oils [30]. Some examples of essential oils are andiroba [73], peach [80] or oils obtained from various plants (avocado, passion fruit…) [81]. LLCs can be also combined with metals (metallogenic LLCs), obtaining new materials with magnetic, electrical and/or luminescent properties as well as new geometries [82]. This is due to the variability in coordination geometries of this type of ions [83]. Lipid LLCs are also generally combined with copolymers, such as P407, to stabilize cubosomes and hexosomes, and appears to contribute beyond adsorption on membranes [38].

2.2.2. Lipid LLCs fabrication

LLCs are composed of water and lipidic amphiphilic molecules, surfactants, as well as, in some cases, co-surfactants. They always occur in certain proportions of these components spontaneously or with a low energy input [84,85]. LLC bulk can be formed by simply mixing the lipid phase with the aqueous phase, for example by ultrasonication or vortexing. However, cubosomes and hexosomes require more complicated methods for their fabrication, being the top-down and bottom-up approaches the most common methods (Fig. 3A) [86]. Nevertheless, other strategies like heat treatment [86] or spray drying [86] can be used. Interestingly, several attempts have been done to fabricate LLCs with automated processes [35].

In top-down techniques, the stabilizer(s) and lipid(s) are firstly mixed to obtain a bulk cubic/hexagonal phase [87,88]. Then, they are dispersed by sonication, shearing or high-pressure homogenization in an aqueous medium [86,89–92]. Temperature must be controlled during the fabrication process [86], as it will impact the type obtained or the formation or not of LLCs. For instance, cubosomes can be prepared by homogenization at temperatures ranging from 40 to 60 °C [88,92], whereas hexosomes fabricated by shearing require temperatures of 100 °C [93]. Because of the requirement of high temperatures for LLCs, this method is not suitable for proteins and thermolabile substances [90,94].

In bottom-up methods, a hydrotrope (an amphiphilic molecule without surfactant properties) is used to dissolve the lipids at room temperature avoiding the formation of LLCs at high lipids concentrations [87,89,92]. This precursor is then diluted into the solvent, reducing the lipids solubility, and forming the cubosomes/hexosomes. Bottom-up approaches require lower energies to produce the LLCs, and they are in general more stable and smaller [86,87,93]. Therefore, it is a suitable method for encapsulating proteins and thermolabile substances [90,94].

3. Morphological varieties and physicochemical properties of the LLCs

Hydrophobic forces drive the self-assembly of LLCs to minimize the interactions between the aqueous environment and their hydrocarbon tails. Small variations at the head or tail level in the amphiphilic molecule result in the formation of thermodynamically stable phases with increased interfacial curvature, often resulting in increased dimensionality. Therefore, the structure that these molecules form in water depends on their geometrical shape [11]. In the Table 2 the properties of the different types of LCs are described.

Their structural differences from other drug delivery systems and from each other lead to different properties or variations in certain parameters. In general, we can describe lipid LLCs with the following physicochemical properties: critical packing parameter, curvature, order parameter, small-angle X-ray diffraction, birefringence and other optical properties, and transition temperature. Polarized light microscopy [99], Small-angle X-ray scattering [100], NMR [101] or Cryogenic Transmission Electron Microscopy [100] can be used to characterize LLCs´ physicochemical properties.
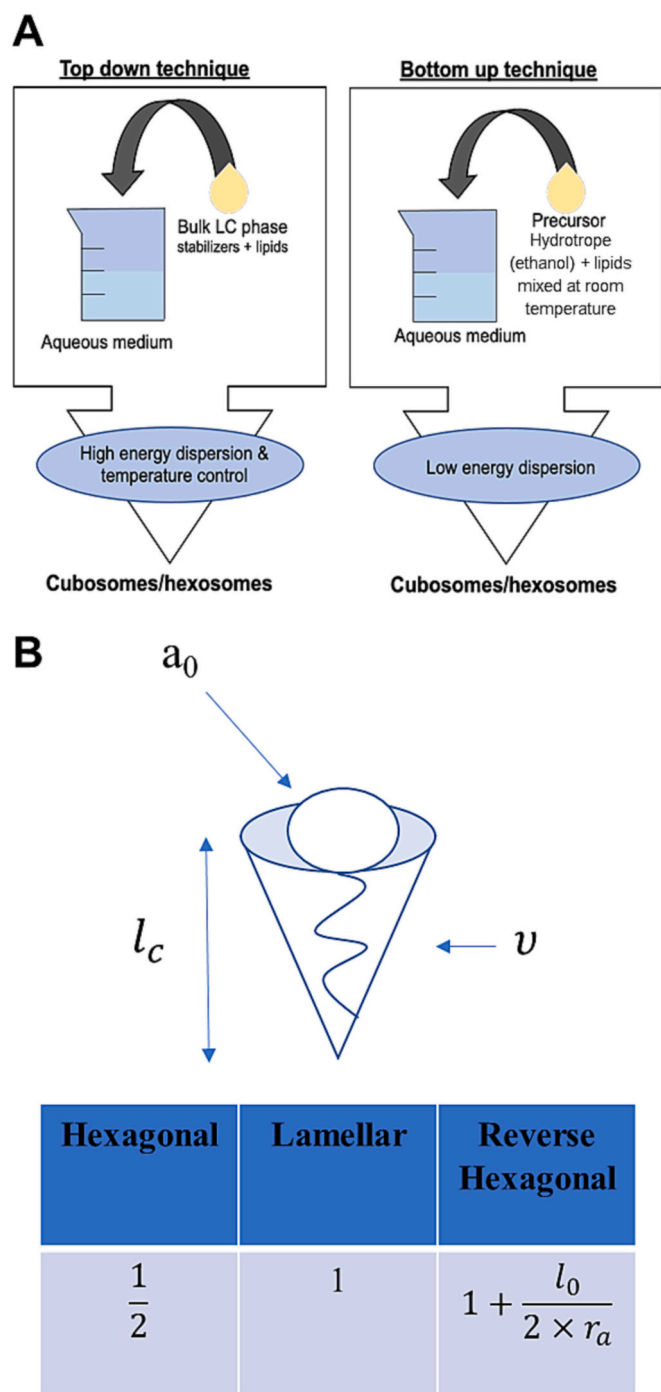
Table 2
Types of LLCs and their physicochemical properties. G (gyroid), D (diamond) and P (Schwarz) refer to the minimum periodic infinite surfaces (IPMS) of cubic LLCs, being IPMS a three-dimensional periodic intersection free surface with an average curvature that is everywhere zero [95].

LLC TYPE		PROPERTIES	REF
Lamellar	Lα	- Fluid - One optical axis	[7,96]
Hexagonal	Normal hexagonal (H_I)	-Do not flow under gravity -Plastic behavior -Lower proportion of surfactant than laminar phase	[7,96]
	Reverse hexagonal (H_{II})	- It is typical of phospholipids and mixtures of fatty acids and phosphatidylcholine.	[7]
Cubic	Cubical phases	- High viscosity - No birefringence -High complexity, difficult to characterize	[7,27,97]
	Bicontinuous	- In a mathematical perspective, G, D and P can be distinguished.	[7,34,97,98]

between the polar and apolar zones in an amphiphile [103]. CCP is defined in eq. 1, being ν the lipid hydrophobic volume, a_0 the hydrophilic group interfacial area and l_c the maximum length of the hydrophobic chains [104]. This parameter allows to decipher which is the most effective molecular shape to use the LLCs as drug carriers [105], by predicting the type of phases most likely to be formed by a particular lipid (Fig. 3B) [99,106].

$$CPP = \frac{\upsilon}{a_0 \times l_c} \tag{1}$$

3.2. Curvature

The curvature of lipid molecules, which is related to the biological functions of lipids [107], also influences the LLCs morphology [108]. The mean curvature allows the differentiation between normal phases (with positive mean curvature) and inverse phases (with negative mean curvature) [108]. For example, a system consisting of water and GMO and resulting in either a bicontinuous cubic phase or a lamellar phase, due to symmetry, will have an average curvature of 0 [109]. This value is defined by eq. 2, being the c_1 and c_2 the two curvatures and R_1 and R_2 the radii of each curvature [24].

$$H = \frac{1}{2} \times \left(\frac{1}{R_1} + \frac{1}{R_2} \right) = \frac{1}{2} \times (c_1 + c_2) \tag{2}$$

The Gaussian curvature (K) is also used to define the LLCs curvature. It enables the determination of the surface topology, being 0 for a cylinder, less than 0 for a hyperboloid and greater than 0 for a sphere [110]. More conclusive studies are needed on how it influences the transitions between non-lamellar and lamellar phases, as theories on this issue have not been able to perfectly match the reality of the transitions, but it seems to be an important parameter [111]. K is defined by (Eq. 3), being c_1 and c_2 the two main curvatures. K values of 0 are obtained in the inversed cylindrical micelles 2D packed such as inverse hexagonal H_{II} phase, whereas K values above 0 are observed in spheres/ellipsoids 3D packed such as inverse ordered micelles and values below 0 are achieved in saddle surfaces 3D packed like inverse bicontinuous phases [24].

$$K = c_1 \times c_2 \tag{3}$$

3.3. Order parameter

The term order parameter ($<P2>$) arises from the average orientation of the molecules around an axis. $<P2>$ measures the order of the molecules with respect to this axis [7,112,113], and it is represented in Eq. 4, where β is the angle formed between the molecule and the

Fig. 3. Methods to produce LLCs and critical packaging parameter (CPP). (A) Diagram of the two main methods of production for cubosomes and hexosomes. In top-down techniques, high dispersion forces are required to mix the lipids with the stabilizers in an aqueous medium. However, bottom-up techniques pre-dissolve the lipids in a hydrotrope and mix it with the solvent, requiring low dispersion forces. (B) CPP description. This value indicates the type of LLCs that will be obtained, and it depends on the lipid hydrophobic volume (ν), the hydrophilic group interfacial area (a_0) and the maximum length of the hydrophobic chains (l_c).

3.1. Critical packing parameter

Amphiphilic molecules shapes favor the formation of different types of LLCs as well as impact the drug entrapment and release. The shape factor effect can be evaluated through the critical packing parameter (*CCP*) [102], which refers to the equilibrium of interaction that occurs

symmetry axis.

$$<P2> = \frac{3 \times (cos^2 \beta) - 1}{2} \tag{4}$$

3.4. Small-angle X-ray diffraction

As each phase has a different structure, the small-angle X-ray diffraction provides us with information about it. Between layers, there are specific repeating distances, d, allowing us to characterize the structures based on this value. The long-range order is measured with small-angle X-ray diffraction using eq. 5 [114], where λ is the X-ray wavelength, n nominates the interference order, and γ is the angle at the interference [114].

$$sen(\gamma) = \frac{n \times \lambda}{2 \times d} \tag{5}$$

3.5. Birefringence

When light strikes the LLCs there is a division into two polarized and perpendicular rays, except the cubic LLCs [7]. There are a few optical properties that allow the distinguishment between different types of LLCs [114] having lamellar LLCs more birefringence with a mosaic texture and crosses [7,114] and hexagonal LLCs less birefringence and a fan texture [7,114].

4. LLCs biomedical applications

4.1. Drug delivery

The use of LLCs in drug delivery has gained popularity in recent years [115]. LLCs are a highly versatile platforms allowing the encapsulation of water-soluble drugs in the polar zone and liposoluble drugs between the hydrocarbon chains. The high viscosity of LLCs allows the sustained release of the payloads. Therapeutic molecules can be released from aqueous channels in the LCs, being its release dependent on the characteristics of the drug [116].

LLCs can also possess stimuli-sensitivity to release the payloads under certain stimuli. LLCs thermodynamic stability enables a release of the encapsulated drug triggered by stimuli that revert the thermodynamic stable structure towards an unstable structure, promoting the release of the payloads at any given moment [24]. Another possibility is to incorporate agents or materials that react to changes in the environment, such as magnetic fields or pH, obtaining stimuli-sensitive LLCs which can experience reversible transformation based on this stimulus and releasing the cargo. For example, Salentinig et al. [117] developed a pH-reactive cubic LLCs at neutral pH that transitioned to inverse micelles at higher pH and to vesicles at lower pH and protected the pH sensitive drug nicergoline. Sun et al. [118] developed LLCs doped in their surface with iron oxide nanoparticles to create a system sensitive to external magnetic fields (Fig. 4). This platform possessed reversibility between an inverted bicontinuous cubic and a H_{II} phases in response to a magnetic force and *in vitro* studies demonstrated the ability to manipulate the release of a drug, alternating between slow and fast release depending on the phase in which it was found.

The most used lipid to make LLCs for drug delivery is GMO, which at room temperature has a bicontinuous cubic phase and a laminar phase [7]. Other lipids highly used to form LLCs are phytantriol to form cubic phases, and andoleyl glycerate or phytanyl glycerate to fabricate hexagonal phases [119].

In addition, the materials and methodologies used for fabricating LLCs are unexpensive. Altogether, lipid LLC systems render suitable drug delivery systems that can be administered by different routes which will be discussed below [120].

In this review, we will highlight the use of LLCs as delivery systems for drugs and biomacromolecules, classifying them according to their
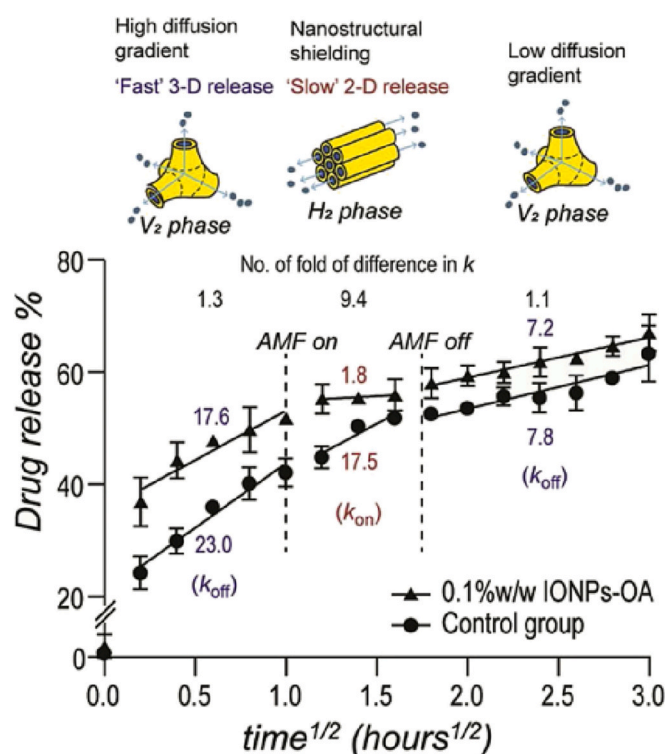

Fig. 4. Drug release of magnetic LLCs under magnetic fields. The drug release velocity depends on the presence of magnetic fields. The interaction of the platform and an external magnetic field provokes that the cubic system changes to HII for the duration of the stimuli, to finally return to cubic. Thus, it is observed that the release slows down with the action of this magnetic field and returns to fast release after the end of the exposure. Reprinted from Ref. [118] with the permission from Elsevier.

routes of administration. Recent examples of lipid LLCs for such use are summarized in Table 3.

4.1.1. Transdermal/topical administration

LLCs have been largely used for topical administration [140–148] due to the similarities of the cubic conformation of LLCs and the cubical architecture of stratum corneum [149]. Additionally, stratum corneum lipids have hexagonal and orthorhombic conformations and even form LLCs [150,151]. Several drugs like antifungals [146,151–154], antimicrobials [146,148], NSAIDs [145,152,155–158], anxiolytics [152] and anticancer drugs [146], among others, have been encapsulated in LLCs and proved their efficacy. For instance, a gel encapsulating hexagonal LLCs carrying zaltoprofen has evidenced its anti-inflammatory efficacy reducing the skin inflammation in a rat model [159]. Another interesting application was reported by Thorn et al. who fabricated a monoolein LLCs sensitive to a bacteria enzyme which showed a promising suitability for the treatment of topic infections of `Pseudomonas aeruginosa` and `Staphylococcus aureus` [160]. The antibiotic rifampicin or the enzyme alginate lyase were encapsulated into the LLCs, and their release was triggered by the presence of bacterial enzyme, being an 82-fold and a 7-fold release in comparison with the enzyme absence, respectively. The release was promoted by the lipase activity which transformed the cubic lm3m LLCs to a lamellar construct (Fig. 5A and B), from where the molecules can diffuse [160].

LLCs can also form supra-amphiphile for the treatment of skin disorders. De Souza et al. developed LLCs supra-amphiphiles incorporating oleic or stearic acids and meglumine. Oleic acid LLCs formed hexagonal LLCs whereas stearic acid formed lamellar LLCs. Stearic acid LLCs rendered highly viscous formulations due to the presence of more stable hydrogen bonds suitable for topical administration. On the other side,

Table 3

Recent examples of the use of LLCs for drug delivery by different routes of administration and applications.

| Structure	Administration route	Lipid	Drug	Pharmacological effect	Ref
Cubosomes	Oral	Phytantriol	Cefpodoxime	Bitter taste model drug	[121]
Lamellar, cubic phase	Topical	Monooleic glyceride	Pirfenidone	Healing promotion, scar prophylaxis	[122]
LCNPS	No studied	Monoolein	Berberine	MCF 7 human breast cancer	[123]
Cubosomes	Intravenous	GMO	Copper acetylacetonate	LS174T colorectal cancer cells	[124]
Cubosomes	Topical ocular	Phytantriol	Natamycin	Ocular fungal infection	[125]
Hexagonal phase	Nasal	Oleic acid	Donepezil	Alzheimer	[126]
QII	Transdermal	Phytantriol	Sinomenine hydrochloride, cinnamaldehyde		[127]
Hexagonal phase	Topical vaginal	Phosphatidylcholine; GMO	Amphotericin B, Miltefosine	Vaginal Candidiasis	[128]
LCNPs	No disclosed	GMO	Resveratrol	lysosomal dysfunctions	[129]
Cubosomes	Oral	GMO	Astaxanthin	Antioxidant	[47]
LCNPs	No-disclosed	GMO, oleic acid	SN-38	Anti-cancer	[130]
In situ LLCs system	Vaginal	GMO	Sertaconazole nitrate	Candidiasis	[131]
Cubosomes	Oral	GMO	Gliclazide	Antidiabetic therapy	[132]
hexagonal mesophase depot system	Intratumoral	GMO	Doxorubicin	Unresectable solid tumours	[133]
Cubosomes, hexosomes	Intravenous	Phytantriol	Phenytoin	Anti-seizure	[134]
Cubosomes, hexosomes	No disclosed	Monoolein, 2-morpholinoethyl oleate	Fluconazole	Antifungal therapy	[135]
LCNPs	Topical inner ear	GMO	Dexamethasone	Drug-induced ototoxicity	[136]
Hexosomes, Lα	Intravenous	docosahexaenoic acid	Docosahexaenoic acid	Brain cancer	[137]
Inverse hexagonal and Bicontinuous cubic phase	Inhalation	GMO, Phytantriol	Tobramycin	Pulmonari anti-pseudomonal antibiotic	[138]
Cubosomes	Subconjuntival	Phytantriol	Latanoprost	Glaucoma	[139]
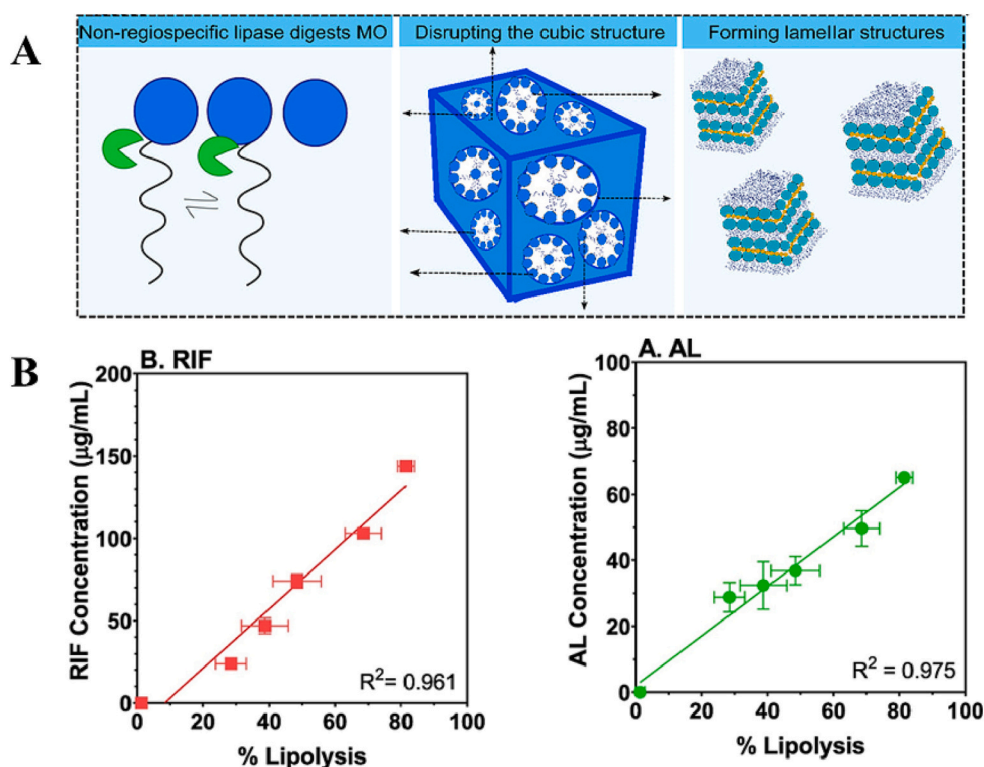
Fig. 5. Drug release from LLCs sensitive to enzymes. (A) Non-regioselective action of a bacterial lipase promote the transformation of the LLCs cubic structure into lamellar LLC phases, which result in the release of the drug or biomacromolecule inside. (B) The graphs show how the release of rifampicin and alginate lyase increases as the sensitive LLC structure is digested. Reprinted from Ref. [160] with the permission from Elsevier.

oleic acid lamellar LLCs showed a higher bioadhesion than the hexagonal ones, with the same amount of water. It is believed that the bioadhesion is caused by the formation of a film at a specific water percentage that interacts with the skin and the water's skin. This is believed to be due to the formation of a non-frozen bound water layer that cooperates through hydrogen bonding, increasing bioadhesion. The *in vivo* results in rabbit skin demonstrated that both LLCs types

containing 60% of water are well tolerated, with no edema or erythema observed on rabbits, both at the primary level, with a patch for seven days, and at the cumulative level, with an application for 5 days every day and then leaving the area occluded for 7 days [161].

Lipid LLCs have also been used to enable the transdermal absorption of several therapeutics as they can modify the skin permeability. For instance, GMO LLCs exerts a lipid disorder at the intercellular skin level

independently of their structure or even whether or not they have penetration enhancers [116,162]. Musa et al. developed a gel made of reversed hexagonal GMO LLCs for the transdermal administration of the anticancer drug exemestane. This formulation reduced the drug side effects in comparison with the oral route, and *in vivo*, this gel increased the drug absorption due to an increase of the skin's permeability the hydration of the skin, resulting in an increase in pore size facilitating the drug absorption. In this regard, it is believed that there is an intact transfer of the drug. This, coupled with the formulations' ability to kill MDA-MB231 cells and a consistent lack of inflammatory infiltrates or visible reaction *in vivo*, demonstrates the effectiveness of this system [163]. Further penetration can be acquired with the combination of penetration enhancers, such as did Cohen-Avrahami et al., who fabricated H_{II} mesophases from GMOs and the triglyceride trioctanoin encapsulating diclofenac sodium. To increase the drug's cellular penetration, they incorporated the peptide penethrin, which produces structural changes on subcutaneous lipids favouring the drug diffusion. Pigskin trials showed that this platform increased the permeability coefficient 2.2 times thanks to the peptide, compared to systems that do not contain this enhancer [164].

4.1.2. Mucosal administration

LLCs' mucoadhesivity is given by their composition and their liquid-crystalline structure. For example, GMO LLCs possess mucoadhesion properties due to its ability to absorb water from the environment. Indeed, the longer the exposure time, the greater the mucoadhesion. Interestingly, the lamellar phase of GMO had a higher mucoadhesivity than the cubic phase, caused by the capture of water of this structure to transition to a cubic phase, whereas cubic phase has a constant amount of water in its interior [165]. Therefore, these results indicate that their mucoadhesivity is promoted by dehydrating the medium as well as from Van der Waals bonds and hydrogen bonds [166]. GMO mucoadhesive properties and its biodegradability and biocompatibility has expanded its use to treat buccal infections [167].

Many other LLCs types have shown mucoadhesive properties and have shown its efficacy targeting oral mucosa. Vesicles of phytantriol and oleic acid could transition to hexosomes at the pH of the oral mucosa (pH 7) due to the presence of oleic acid (Fig. 6A). *Ex vivo* studies carried out on porcine buccal mucosa showed a better hexosomes's bioadhesion and mucosal permanence than control vesicles. This shows the promising utility of drug delivery by this route [168], as it avoids the first pass hepatic effect and the gastrointestinal degradation [169]. Another lipid
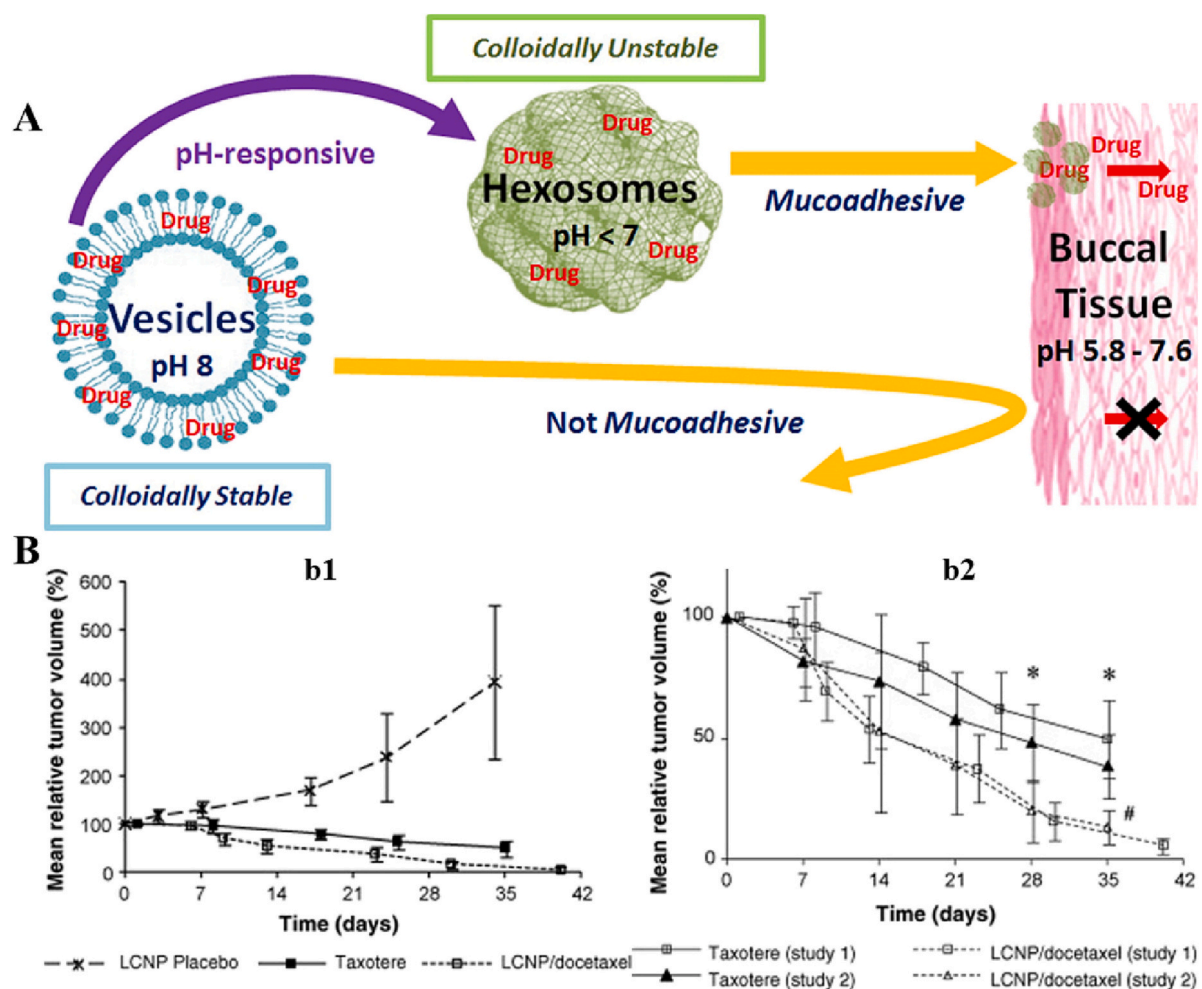

Fig. 6. (A) This diagram shows how the vesicles formed by the lipids phytantriol and oleic acid go on to form liquid crystals when the pH changes. The pH of the oral mucosa transforms the vesicles into hexosomes. Reprinted from Ref. [168] with the permission from Elsevier. (B) b1) A comparative study compared the empty LCNP system, docetaxel-loaded LCNP and the commercial taxotere system. For these, a tumour was induced in mice and the formulations were injected after measuring the tumours. Over time, tumour growth/decline was observed for each treatment group. The drug-treated groups showed a decrease in tumour size, demonstrating that the LCNP-loaded formulation possessed anti-tumour capacity. b2) The next study pitted the commercial formulation Taxotere against the loaded LCNP system and followed the same procedure of measuring tumour size after intravenous injection of the two formulations. The results of the first study were also used for the scheme. A greater decrease in mean tumour size was observed with the docetaxel LCNP formulation than with the commercial formulation, thus proving to be more effective. Reprinted from Ref. [172] with the permission from Elsevier.

with mucoadhesive properties is glyceryl monolinoleate [170]. Jie et al. developed a delivery system encapsulating sinomenine hydrochloride, a drug with anti-cancer properties. The system was administered as *in situ* LLC gel, which, on contact with the vaginal fluid, is transformed into a gel with cubic LLCs, which sustained the release for 144 h *in vitro*. Histopathological studies performed on rats evidenced its safety for the vaginal mucosa. All this, together with a mucosae permanence time of more than 12 **h** *in vivo*, makes this a promising system for administering drugs at the vaginal level [171].

LLCs have also been used for topical-ocular administration [173,174]. Li and co-workers manufactured GMO LLC nanoparticles, with reverse hexagonal phases, loaded with the anti-glaucoma pilocarpine nitrate, a molecule with a low ocular penetration and retention. A comparative study was made with commercial eye drops and showed an improvement in eye penetration with the use of the LLCs. Moreover, this platform showed *in vivo* an extended reduction in intraocular pressure compared to the commercial drug. Low eye irritation was also observed, making it a promising platform for eye administration [173].

The nasal mucosa is another possible route of administration of active substances. Carvalho et al. developed a LLC precursor formulation loaded with the antiretroviral zidovudine that transitioned to a lamellar phase in the nasal mucosa, promoting nasal absorption of the payloads. This transition tripled the mucoadhesivity of the system, as demonstrated *ex vivo* in porcine nasal mucosa and increased the permeability of the drug in comparison with a solution of zidovudine. *In vivo*, in a rat model, a rapid absorption by the nasal system was observed [175].

4.1.3. Parental administration

This type of administration encompasses intravenous, subcutaneous, intramuscular, intra-articular, intravitreal and intralesional [176]. LLCs can be administered by themselves or in formulations that form implants of LLCs. Streck et al. developed benznidazole formulations for Chagas disease based on soy phosphatidylcholine and medium chain triglycerides, and it was found out that at certain proportions of both lipid components lamellar LLCs were formed instead of nanoemulsion. These LLCs formulation increased the drug's loading compared to conventional emulsions and cyclodextrin complexes. It was also observed that when LLCs were formed at a low surfactant-to-oil ratio were not cytotoxic whereas formulations at higher ratio were cytotoxic. Nevertheless, future studies are needed to determine their effectiveness *in vivo* and the most suitable route of administration, whether oral or parenteral [177]. LLCs have also been intravenous administered for the treatment of cancer. LLC nanoparticles (LCNPs) carrying docetaxel showed their efficacy against prostate cancer *in vivo*. The soy phosphatidylcholine-based system showed a greater tumour regression than the commercial formulation Taxotere (Fig. 6B). Therefore, the inclusion of this drug within the carrier enables a higher penetration into the tumour [172]. However, some authors have suggested the possible ineffectiveness of GMO-based LCNPs for parenteral administration, due to massive haemolysis observed in *in vitro* assays due to mixing with the phospholipid membrane by the GMO [178]. This problem is not reported in more recent studies [179], so it is not known whether its haemolytic effects have not been considered or whether haemolysis has not manifested itself. Therefore, a more thorough review would be appropriate to avoid any confusion in the future.

The intra-articular route is also one possibility for administering LLCs. This is the case of cubic LLCs precursors which will absorb water progressively once it enters intramuscular or subcutaneous into the human body. This water comes from peripheral tissues or from body fluids and it is the responsible for the transformation of the precursor in LLC which will act as a reservoir and release the drug. Xia et al. tested this system with sinomenine hydrochloride, as a model drug, and GMO/water for the treatment of rheumatoid arthritis in a rat model. It was injected into the rat's leg joint cavity and a comparative study was made with an injection of aqueous sinomenine hydrochloride solution. Plasma levels showed prolonged release from the developed formulation as opposed to the aqueous solution. Indeed, the maximum concentration was reached after 15 h in the LLCs in the joint cavity, demonstrating the local effect of the drug and proving the appropriateness of the formulation to treat any joint disease [180].

Fong et al. developed a phytantriol and gold-based photothermal system capable of responding to near-infrared light. *Ex vivo* studies showed that depending on skin thickness, and the presence or absence of hair and pigmentation, the effectiveness of near infrared light would vary, being the abdominal skin the best choice for their administration despite the latter being thinner, perhaps due to its lipid composition. *In vivo* studies were then carried out using ^{14}C-glucose as a model drug. A comparative study was carried out between different phytantriol-based formulations, one of them containing gold, and compared with an aqueous glucose solution (control). After administration, they were subjected to 2 h of near-infrared light, followed by ambient light. LLCs containing gold showed were able to increase the plasma concentrations after near-infrared light exposure due to a transition from H_{II} to V_{II}. This results evidence that some LLCs can respond to stimuli under *in vivo* conditions [181]. Similar systems have been developed for parenteral application, such as those capable of reacting to changes in temperature [182].

Fang et al. developed an isotropic solution that converted to a liquid-crystalline gel *in situ* to release 5-Fluorouracil while acting as an embolus, thereby stopping the blood supply to provoke the cell apoptosis. The formulation was formed by phytantriol, and it was observed that higher proportion of phytantriol achieved the desired embolization. *In vitro* cytotoxicity tests showed that the formulations were cytocompatible. *In vivo* experiments in rabbits confirmed the embolization of the middle ear artery and a longer mean residence time compared to the drug solution. These findings indicate that phytantriol-based system could be a promising system for the treatment of hepatocellular carcinoma, specifically as a transcatheter arterial chemoembolization agent [183].

4.1.4. Oral administration

LLCs have also been formulated for the oral administration of drugs [166,184–189]. Lipid vehicles keep the drug solubilized even during digestion. In addition, the preservation of the unchanged liquid crystalline structure of phytantriol and GMO in the gastrointestinal model fluids suggests a slow gastrointestinal release capacity. All these characteristics make lipidic LLCs interesting platforms for oral administration [185]. For example, Nguyen et al. developed hexosomes for the oral administration of cinnarizine, based on glyceryl monooleyl ether, which is not digestible in the stomach, thus protecting the active substance. This result was confirmed with an *in vitro* enzymatic digestion that showed no drug degradation. An *in vivo* study conducted on rats comparing hexosomes of cinnarizine, a bulk formulation of glyceryl monooleyl ether and the active substance administered orally showed that the hexosomes achieved more consistent absorption and plasma concentrations in the first 28 h. This study demonstrated the usefulness of liquid-crystalline materials, such as glyceryl monooleyl ether, which are not digested, for the sustained oral administration of active substances [166].

Cubic LLCs have also been used for improving the efficiency of hydrophobic/hydrophilic therapeutics [190–192]. For instance, Pham and co-workers developed an *in situ* cubic LLCs formulation to increase the gastric retention and sustain the release of hydrophobic drugs. They encapsulate a model drug, cinnarizine, in a phytantriol and tributyrin mixture, which formed cubic LLCs during digestion. These findings open a new window for the sustained oral release of hydrophobic drugs [193].

The inclusion of linoleic acid into the LLCs enable the development of pH-sensitive LLCs [194]. Linoleic acid has a pK_a of approximately 5, being protonated at acid pH and with negative charge at neutral pH, modifying the LLCs critical packing parameter depending on the pH. For instance, pH-sensitive LLCs made of bymonolinolein and linoleic acid developed by Negrini and colleagues can change from a cubic to

hexagonal phase when switching the pH from neutral to acid (pH 2), returning to a cubic phase again when returning to neutral pH, recapitulating the intestine and stomach pH respectively. A model hydrophilic drug, phloroglucinol, was encapsulated inside this platform to test the effect of the pH in the release. It was founded out that the release was 4 times faster for cubic conformation than the hexagonal one, being ideal for intestinal delivery preventing stomach release [194].

Anti-cancer drugs were also encapsulated in LLCs for oral administration. For example, Waghule et al. used LCNPs to encapsulate temozolomide (TMZ) for the treatment of glioblastoma. In this case, LCNPs protected the drug from the degradation by the plasma pH, resulting in a prolongation of the brain bioavailability, reducing the dosages and toxicities not associated with the therapeutic target. Related to liposomes, the LCNPs showed longer release as well as smaller size and better entrapment [189].

4.1.5. Other routes of administration

4.1.5.1. Periodontal injection.
This route of administration is essential for the treatment of periodontitis, inflammatory diseases of the tissue that supports the teeth. Over time it can lead to tooth loss. It is caused by a multifactorial infection and therefore one of the possible treatments is the use of antimicrobials. Due to the appearance of periodontal pockets as a pathological manifestation, periodontal injection and its various formulations have emerged as a possible treatment [195]. For example, the phytantriol-based platform for periodontal administration, developed by Jiang et al. was an injectable system against chronic periodontitis, as it contained minocycline hydrochloride. The system, based on reversed hexagonal *in situ* LLCs, demonstrated higher cumulative releases. Studies on specific pathogen free rats showed it to be an effective system in comparison with the commercially available treatment, Periocline ® [196].

4.1.5.2. Inhalation.
The inhalation administration is a non-invasive technique unlike systemic administration and can avoid the drawbacks of systemic administration. In the case of anticancer drugs, oral and intravenous administration are accompanied by high accumulation in the kidneys, liver and spleen and low accumulation in lungs. Attempts to administer higher doses result in serious adverse effects and even the development of multidrug-resistant tumours. Inhalation administration localises the action and limits adverse effects [197]. Abdelaziz et al. developed an inhalable monoolein formulation to treat noninvasive lung cancer. LLCs encapsulating resveratrol and anti-cancer drug pemetrexed were coated by chondroitin sulfate and lactoferrin through layer-by-

layer technique. This coating enables the tumour-targeting to cells overexpressing CD44 (chondroitin sulfate) as well as possessing anti-cancer properties (lactoferrin). Nanoparticles were then encapsulated inside microparticles to obtain inhalable particles and showed a lung deposition in *in vivo* experiments with mice. These carriers provoked a reduction in the tumour size, reduction in the vascular endothelial growth factor (VEGF) expression and activation of caspase-3, suggesting to be suitable for non-invasive treatment of lung cancer in addition to not causing functional damage to the liver and kidneys [198]. More *in vivo* studies will determine whether LLCs can really be an improvement in this route of administration over other existing formulations.

4.2. Biomacromolecules delivery

LLCs have also being used for the encapsulation of biomacromolecules such as peptides [145,146,199] or proteins [146,200] (Table 4).

4.2.1. Nucleic acids
One type of biomacromolecule where lipid LLCs have been successfully applied is nucleic acids [201]. An interesting approach consists of the encapsulation of synthetic interference RNA (siRNA). There is a therapy that consists of the gene suppression or silencing of specific sequences using interference RNA therapy using double-stranded RNA or siRNA [202]. This therapeutic strategy has been already shown its effectiveness against cancer or viral infections [203]. However, the main limitation of this technology is its high molecular weight and negative charges that hinders its entry through the cell membrane [204], requiring the use of viral and non-viral vectors with high costs and immunogenic problems. LLCs can enhance the efficacy of siRNA by release free siRNA directly into the cytoplasm upon dissociation of free siRNA from LLCs due to fusion of the latter with the membrane [205]. For instance, GMO nanodispersions have been used for the topical treatment of vitiligo [206]. Vitiligo, depigmentation due to melanocyte loss, is thought to be caused by autoimmunity against Tyrosinase-related protein-1 (TyRP-1), a melanocyte surface protein that is thought to behave as an antigen in those suffering from this disease. In this case, siRNA against TyRP-1 was encapsulated in the LLCs. To complex the siRNA, a cationic polymer, branched poly(ethyleneimine) (PEI) was used which, due to its positive charge, retained the negatively charged siRNA. This formulation showed that siRNA could reach the cytoplasm of melan-A cells and inhibit the expression of TyRP-1 unlike naked siRNA [207]. Another example are the LCNPs that have been complexed with short-interfering RNAs (siRNAs) and functionalized with cell

Table 4
Recent examples of the use of liquid crystals for the delivery of biomacromolecules.

Structure	Administration route	Lipid	Biomacromolecule	Use	Ref
Reverse hexagonal structure	Topical	GMO	siRNA	Skin diseases	[215]
Cubosomes	Unexplored	GMO	siRNA	Gene therapy	[216]
Cubosomes	Unexplored	GMO	Neurotrophin brain-derived neurotrophic factor	Neuroprotective	[217]
LCNPs	Unexplored	GMO, Phytantriol	Glycoside hydrolase	Infection-directed therapy	[218]
Lamellar	Vaginal administration	Oleic acid	Ergosterol (D2 vitamin)	Vulvovaginal candidiasis by *Candida albicans*	[219]
Bicontinuous- cubic	Unexplored	Lipidic zinc (II)-bis(dipicolylamine) (Zn2BDPA)) complexes admixed with GMO	siRNA	RNA therapeutic delivery	[220]
Cubic	Topical skin	GMO	Recombinant human epidermal growth factor (rhEGF)	Chronic wound	[221]
Lipid sponge (l3) phase	Unexplored	GMO, diglycerolmonooleate (DGMO)	Aspartic Protease	Unexplored	[222]
Cubosomes	Oral	GMO	Coenzyme Q10	Hepatoprotective	[223]
Lamellar	Topical skin	L-α-phosphatidylcholine, glycerol trioleate	Lysozyme	Unexplored	[224]
Hexagonal phases, cubic phases	Intratumoral	GMO	siRNA	Promising anticancer platform	[225]
Cubic IA3D	Transcutaneous	Monolinolein, GMO	Peptid antigen	Transcutaneous vaccination	[226]

penetration peptides (TAT or penetratin) by Petrilli et al. The hexagonal phase LCNPs encapsulating siRNA for inhibiting tumour necrosis factor α (TNF-α) and decorated with TAT have shown a reduction in the expression of the pro-inflammatory cytokine TNF-α in rabbit and mouse models of inflammatory disease. The results obtained indicate that TAT decorated LCNPs can improve the internalization of these NPs, improve the cellular uptake of the siRNA, without irritating the skin [201]. These findings suggest that LLCs could be an interesting approach for RNA delivery in vaccines or the treatment of diseases such as chronic inflammatory diseases or cancer. Another alternative that is beginning to be explored is the use of double-stranded DNA, with results that seem to indicate that these structures do not undergo structural modifications inside liquid-crystalline structures [208], although there is still much research to be done on the possibilities of LLCs in gene therapy.

4.2.2. Proteins

LLCs have also proved their efficacy for the delivery of proteins. Ki et al. developed an injectable LC-forming system based on phosphatidylcholine and loaded with leuprolide acetate, an hormone analogue which is used subcutaneously against prostate cancer. *In vitro* assay showed higher IC50 for the LLC formulation than the drug commercial formulation, indicating its safety. *In vivo* studies in rabbits and rats also confirmed LLCs safety of LLCs, as no lesions were found (Fig. 7A). The formulation was transforming into hexagonal LCs when subcutaneous administered due to the presence of local water. This transformation allowed that there was no initial burst release of the payloads in

comparison with commercial poly (lactic-coglycolic acid)-based formulation, as demonstrated in pharmacokinetic studies on rats (Fig. 7B) and beagles (Fig. 7B), without changing its therapeutic effect. Although there were no major differences in release over time, except for the burst release of the commercial formulation and therefore a higher maximum concentration, the ease of preparation is a great advantage over the commercial formulation, as it is only a mixture and dissolution, thus proving to be very useful [209].

There are many other examples of attempts to introduce proteins into LLCs. VEGF was incorporated into a GMO precursor solution which, in contact with liquid, forms a self-assembled gel of bicontinuous cubic inverse LLCs to regenerate the vasculature of lesions. VEGF when free in the blood, has a short half-life, however, when encapsulated in this platform, it prolonged its half-life due to the sustained release capacity of the LLCs for 7 days. In addition, the gel form allows it to completely occupy the lesion, making it a promising system. LLCs showed a higher tube formation and cell migration than free VEGF on human umbilical vein endothelial cells (HUVECs), suggesting a better angiogenic potential of LLCs. In addition, VEGF-LLC when injected subcutaneously into rats caused a mild inflammatory response that spontaneously reversed and did not cause damage to vital organs after 14 days of treatment but did show a higher angiogenic capacity of the system when compared to the same but empty LLCs, as more and larger diameter blood vessels were observed in the VEGF-LLC [210]. LLCs also allows to encapsulate proteins under mild conditions, avoiding the harsh conditions that might occur in other delivery platforms. For instance, Chung and

Fig. 7. Injectable LLC precursor forming LLCs made of phosphatidylcholine and the hormone analogue leuprolide acetate. (A) Safety of the formulation in rats and rabbits. From left to right, LLCs formed after subcutaneous administration and the tissues around them in rats and rabbits, respectively, over 7, 14 and 28 days. (B) Plasma concentrations over time of leuprolide in rats (left) and beagles (right) after subcutaneous injection of the commercial formulation of PLGA *vs* LLCs (LCFS). Reprinted from Ref. [209] with the permission from Elsevier.

colleagues fabricated cubic LLCs encapsulating insulin, avoiding the use of high temperature that could damage the protein integrity. This LLCs formulation was able to control the glucose levels in diabetic rats, maintaining the insulin levels in serum above the baseline up to 6 h [211].

Antibodies have also been introduced into LLCs. Zhai et al. developed LCNPs against the epidermal growth factor receptor (EGFR). This NPs had an internal LLC structure, thus possessing cell penetration and drug loading capacity inside. On their surface they had conjugated anti-EGFR Fab fragments retaining their activity and a poly(ethylene glycol) corona to avoid the recognition by the immune system. The efficacy of the conjugation was assessed by a competitive binding assay to sEGFR501.Fc, a protein with high affinity to anti-EGFR ligands, showing high affinity [212]. Lipid LLCs have even proved to be an effective system for producing vaccines, by encapsulating antigens into them. Sánchez Vallecillo et al. showed that LLCs system modulated the antigen release kinetics as well as the interaction between the system and the immune system. The formulation of LLCs with a lamellar structure was formed by 6-O-ascorbyl palmitate (ASC16) which self-assembled due to cooling in water, called Coa-ASC16 (or coagel). The coagel is detected by innate immunity through the MyD88 protein [213], without the need of any immunoadjuvant. Thus, altering inflammatory activity itself and being a valid system for the development of vaccines [214].

4.3. Imaging carriers

LCs have been successfully used in cellular and molecular imaging, as imaging agents can be easily encapsulated in LLCs. Some examples of interest are listed in Table 5. Within medical imaging, one field that LLCs have a great potential is magnetic resonance imaging (MRI). This non-invasive technique generally requires the use of contrast agents to improve its sensitivity. Imaging agents can undergo changes in their performance in lipid environments, so they need to be assessed to stablish the optimal charge [227], measuring its relaxivity which is their relaxation of water normalized to the concentration of the contrast agent [228,229] and indicates the effectiveness of these agents [11,228]. Among contrast agents, positive charged agents that change T1 (paramagnetic gadolinium (Gd), manganese II (Mn)…) and negative charged that change T2 (nanomaterials based on superparamagnetic iron oxide) can be introduced into LLCs. Cubosomes are of great interest for MRI application because their bicontinuous cubic structure facilitate the coordination of water molecules around the metal ion. They can also facilitate the transferring of water molecules that provokes an increase in the rate of relaxation, as well as prevent some rotation movements of the metallic ions. This will change the water conditions to which the contrast agent is subjected [228].

Paramagnetic Gd and Mn ions are the most used for clinical MRI [35], and they have introduced in LLCs such as lamellar [230,231], hexagonal [232], reverse hexagonal [231], micellar cubic [231]. Today, chelated Gd is usually one of the components of contrast agents, but as a free trivalent ion it is highly toxic [11,35,228,233]. Gd was subjected to different chelation processes with phytantyl-ethylenediaminetetraacetic acid (EDTA) derivatives and diethylenetriaminepentaacetic acid (DTPA) can be later incorporated into systems formed by GMO and phytantriol [228]. An example of this was reported by Gupta et al. Researchers developed cubosomes based on Gd (III) and GMO chelated DTPA. LLCs formulations had higher relaxivities than Magnevist, a commercial contrast agent [234].

As discussed above, the use of paramagnetic NPs, such as iron oxide NPs, is also a common practice when using them as contrast agents, and they have also been locked into cubosomes [228]. Sun et al. developed a phytantriol-based lipid LLCs loaded with iron oxide NPs and sodium fluorescein as a model drug. In *in vitro* studies, an alternating magnetic field was applied after 1 h to start releasing the drug and its action was maintained for 2 h. A decay of the release due to the alternating magnetic field was observed, which is believed to be due to a transition from V2 to V2/H2 (mixed), with respect to the control, without iron oxide nanoparticles. Thus, it was possible to obtain a system able to react and decrease its release rate to external magnetic forces and to return to a fast release state when these forces disappear. It appears to be a promising platform for programmable release/imaging [118].

Using metal-free nitroxide lipid contrast agent systems, also paramagnetic [11,228,229], is unconventional [228]. Muir et al. used this system, introducing these contrast agents into lyotropic LCNPs, based either on GMO (Myverol®) or on phytantriol. The relaxivity study showed an improvement in T1, compared to substances in clinical use, such as omniscan, which contains Gd. *In vitro* toxicity tests on Chinese hamster ovaries, as well as subsequent pharmacokinetic tests on rats (Fig. 8A), showed a longer half-life and lower toxicity of the GMO-formed systems. In addition, the GMO-formulated system showed effectiveness at the hepatic level in rats without the use of Gd (Fig. 8B), which as previously discussed is toxic. It was observed that the more the concentration of nitroxide was increased, the conformation changed from cubic to hexagonal (Fig. 8C), resulting in a detrimental effect on relaxivity [229].

All these finding suggest that LLCs are promising platforms for high field contrast agent's encapsulation as well as showing possibilities as a delivery agent, thus could be powerful theragnostic agents.

Lipid LLCs have also been used for fluorescence imaging. This technique is unexpensive, safe, accurate and allow detection of early stages cancer [245]. It is based on the ability of some compounds, known as fluorophores, to emit light at longer wavelengths, which they had previously absorbed [246]. Within this field, Aggregation-Induced Emission (AIE), which is based on the formation of aggregates to

Table 5

Lipid platforms LLCs as carriers for imaging agents and theragnostic agents.

| Structure	Administration route	Lipid	Detection substance	Use	Ref
inverse bicontinuous cubic mesophase	Unexplored	GMO	Carbon nanodots	Fluorescence imaging	[235]
HII	Intravenous	Phytantriol	MnO nanoparticles	Breast cancer Fluorescence imaging and tratment	[236]
Hexosomes (H_{II})	Subcutaneously	Phytantriol, Oleic acid	Technetium-99 m [99 m Tc]-labeled	Regional lymph node theragnostic agent	[237]
cubosome	No studied	GMO	DPP-ZnP-NH2	Theragnostic for cancer (fluorescent)	[238]
cubosomes	Unexplored	GMO	NaYF4, Er3+, Yb3+	Theragnostic for cancer (fluorescent	[239]
Not disclosed	Unexplored	Cholesterol	3,3′-dioctadecyloxacarbocyanine perchlorate (DiO)	Fluorescence imaging	[240]
Not disclosed	Unexplored	Cholesterol	Samarium (III)	Fluorescent imaging	[241]
Reverse hexagonal	Unexplored	GMO	Rhodamine (+ docetaxel)	Theragnostic for cancer (fluorescent)	[242]
Cubosomes	Unexplored	GMO	Rhodamine (+ Docetaxel)	Theragnostic for cancer (fluorescent)	[243]
Cubosomes, hexosomes	Intravenous	GMO, capric acid	Gadolinium, lipophilic near infrared fluorescent	MRI and fluorescent imaging	[244]
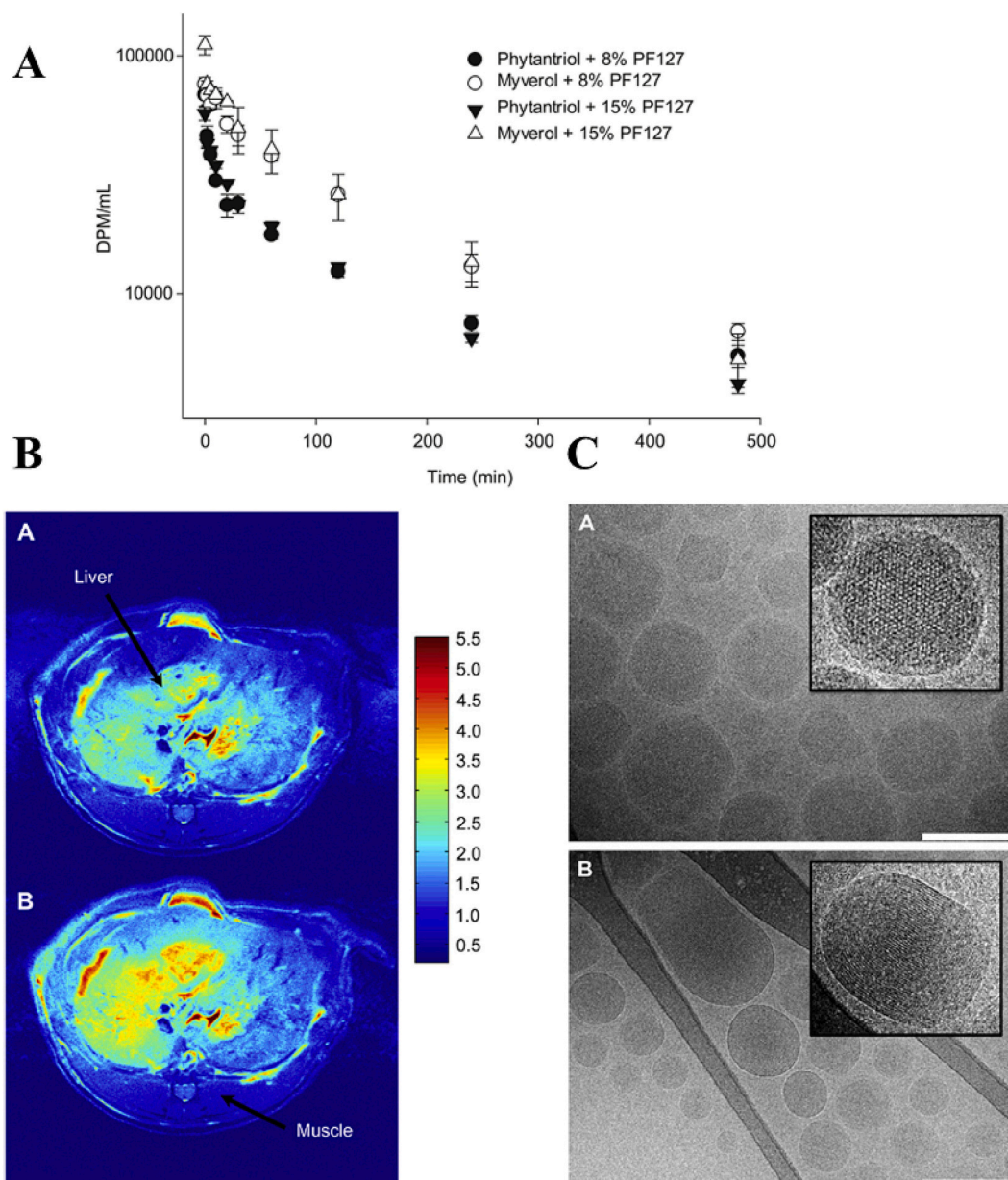
Fig. 8. GMO (Myverol®) or phytantriol LLCs containing nitroxide lipids contrast agent. (A) Time decays of phytantriol and Myverol LLCs containing 8% and 15% pluronic F127 after intravenous injection into rats (0,5 mCi 3H-dioleilfosfatidilcholine was the radioactive tracer. (B) Rat liver before (top) and after (down) intravenous injection of GMO LLCs hexosomes (14.5% nitroxide lipids). (C) Cryo-TEM images of cubosomes (2% nitroxidised lipids) and hexosomes (14.5% nitroxidised lipids). Reprinted from Ref. [229] with the permission from Elsevier.

increase light emission, has emerged relatively recently [247]. Agents with AIE properties do not have problems such as cytotoxicity and low photostability that inorganic contrast agents have. This type of agents have been incorporated into lipid LLCs systems, such as GMO-based hexagonal LCNPs. Urandur et al. used this platform as a theragnostic agent for breast cancer. LLCs were loaded with tetraphenylethane (TPE), an optical AIE beacon and an anti-cancer phytoestrogen, formononetin (FMN). The LCCs were also coated with anisamide (AA), to target the sigma receptor of cancer cells.

The formulation was hemocompatibility and safe *in vitro* and *in vivo*, allowing its intravenous administration. LLCs decorated with AA enhanced the anticancer activity and cell uptake in cancer cells and did not affect normal cells in comparison with the free drug and non-decorated LLCs encapsulating FMN. *In vivo* experiments in mice also confirmed the higher efficacy of AA-decorated LLCs, thus showing its potential use as a theragnostic agent in breast cancer [245].

4.4. Tissue engineering

The reversible transformations and flexibility of the lipidic LLCs as well as their biocompatibility and the possibility to encapsulate bio-macromolecules inside them, make them suitable materials to fabricate scaffolds (Table 6). In addition, they can react to different external stimulus, and they allow anisotropic growth, informing about the cell growth thanks to birefringence or liquid-crystalline molecular alignment [248,249]. LLCs scaffolds can be fabricated by electrospinning and 3D-printing among others [250], and have been mainly used in skin regeneration due to the similarities of LLCs with the lipids of the stratum corneum. For instance, Soon et al. developed a cholesterol ester-based LLCs for the fabrication of skin grafts using human keratinocytes (HaCaTs). The extracellular space of the stratum corneum presents cholesteryl ester varying from 0 to 20% [251], which can be transformed into cholesterol by hydrolysis, and it is used for the formation of

Table 6
Multi-purpose scaffolds made from lipid LLCs. LCEs refers to liquid crystal elastomers.

Scaffold type	LC type	Lipid	Aplication	Reference
LC films	Not disclosed	Cholesteryl-oligo (lactic acid)	Tissue regeneration	[254]
LCE foams	Smectic-A	Cholesterol	Neural regeneration (SH-SY5Y neuroblastoma cells)	[255]
LCE foams	Smectic-A phase	Cholesterol	Neuronal growth (SH-SY5Y)	[256]
LCE scaffolds and foams	Not disclosed	Cholesterol	Vascularization of tissues (resembles vascular networks observed in tissue)	[257]
LCE	Smectic-A	Cholesterol	Scaffolds for human myoblast (C2C12) and human neuroblastoma (SH-Sy5Y)	[258]
LCE	Smectic-A	Cholesterol	Scaffold for human dermal fibroblast and mouse skeletal myoblasts (C2C12) culture	[259]
Biomimetic scaffold	Hexagonal to cubic phases	GMO	Cartilage defect regeneration	[260]
LLCs	Lamellar and cubic phases	GMO	Cardiac tissue regeneration	[261]

lipoproteins and for the cell membrane [252]. The scaffold allowed the organization of keratinocytes into 3D microtissues with a good cell adhesion, cell viability and proliferation after 20 days and even showed cell migration within the scaffold. The main advantage of these 3D platform is that it preserved the native function of the cells, forming stratified keratino-spheroids and it is suitable for studying cell migrations [252].

LLCs have also been used in the field of bone tissue engineering. LLCs based on cholesterol chloroformate were introduced into a polyurethane based porous scaffold by means of a soaking swell technique. Polyurethane provides elasticity, which is important for bone regeneration, while LLCs improve its osteogenic capacity. The scaffold high porosity and high pore size favored the bone growth. Human mesenchymal stem cells (hMSCs) showed good cellular adhesion without cytotoxicity in these scaffolds. The presence of LLCs in the scaffolds increased the cell growth and adhesion of hMSCs was observed when LLCs were present in the scaffolds. In addition, an increase in the alkaline phosphatase activity, calcium deposits formation and gene expression related with hMSCs differentiation was observed, which confirmed the cell differentiation onto osteoblast [253].

4.5. Other biomedical uses

Lipidic LLCs have also been used for the creation of platforms against post-operative adhesion. For example, Murakami et al. developed a platform made of squalene and C17 glycerin ester and tested it against lateral lesions in the peritoneum of rats and its effectiveness was comparable with Seprafilm®, a system already marketed for the same purpose. The incorporation of squalene into the LLCs promotes the transition from cubosomes to hexosomes and provides greater stability to the dispersion. The tissue postoperative adhesion was reduced by 77.6% with the higher lipid percentage formulations (21 and 25%), and 35.0% with Seprafilm® against untreated lesions. It is believed that the adhesion of the hexosome is due to the hydrophobic chains around it. Although further research of the biocompatibility and metabolism of C17 glycerin ester is needed, these results are promising for preventing

postoperative adhesion [262].

4.6. Limitations in biomedicine of lipid LLCs

The research in nanocarriers as drug delivery systems is continuously growing, being even some examples authorized by regulatory agencies. However, there is still no LLCs-based nanocarrier close to be used in patients. One of the reasons is that most of the NPs based on LLCs tend to lead to rapid release, especially when dealing with small molecules. Therefore, dispersed phases of LCs (cubosomes and hexosomes) have a much higher release rate of small water-soluble drugs than non-dispersed ones, due to the shorter diffusion distances of the molecules [263]. This phenomenon can be overcome in the case of small fat-soluble drugs and proteins, where normally a slow and sustained release can be achieved due to interaction with hydrophobic domains and the absence of the partition coefficient effect, respectively [263]. Nevertheless, proteins are in general not suitable payloads for LLCs due to high temperatures needed for the fabrication of the formulation. Interestingly, some metals can form tight bounds with LLCs, such as gadolinium in cubosomes. This property makes LCs optimal platforms for imaging applications, due to the stability of the contrast agents [264].

Another limitation of LLCs is their biocompatibility. Some studies have suggested that cubosomes need a stabilizing polymeric barrier, such as Pluronic F127, to ensure low cytotoxicity. However, it is not yet known why their cytotoxicity is reduced [265]. Despite being Pluronic F127 the gold standard in cubosomes stabilization, it has been evidenced that it does not have the capacity to preserve the integrity of the LCs in long-term. Indeed, molecules such as 1,2-distearoyl-sn-glycero-3-phosphoethanolamine conjugated with poly(ethylene glycol) (DSPE-PEGMW) have shown lower toxicity than Pluronic F127 [266]. Resultantly, efforts are underway to develop new stabilizers for lipid LLC systems [267]. Perhaps for all these reasons, it would be of interest to continue with the study of bulk phases in those cases where nanoparticles do not seem to provide advantages in drug release, whenever possible.

Regarding the use of LCs in tissue engineering, lipid LLCs scaffolds offer multiple advantages in skin regeneration, such as its safety, biocompatibility and biodegradability, or shapes and pattern versatility. Indeed, they can be adaptable to cell growth and act as adaptive scaffolds [268]. However, this is a new area with only few examples published, and only time and future studies will show their limitations in this area.

5. Conclusions and future perspectives

Throughout this review, the main biomedical applications of lipid-based LLCs have been discussed. Lipidic LLCs have evidenced to be optimal platforms for drug and biomacromolecules delivery, scaffolds, imaging carriers and even for cell therapy. The main biomaterials used for the fabrication of LLCs in this field are GMO and phytantriol, maybe because these materials have already been the subject of many drug delivery studies and toxicological tests. However, studies suggest that GMO has a short-term storage stability due to hydrolysis and/or oxidation processes in its structure whereas phytantriol is prone to cause haemolysis [45], which is why recently new biomaterials have been formulated in LLCs, especially those lipids naturally present in biological systems, to find out a greater biocompatibility and stability.

Top-down and bottom-up methods can be followed to prepare lipid LLCs. Top-down are generally faster, but they can only be used with payloads that are not sensitive with temperature and in small scale [45]. On the other side, in the bottom-up approach is more suitable to encapsulate thermos-sensitive payloads and at larger scales, but it generates solvent residues, and the LCs size is less controllable [45]. Therefore, the method followed for the preparation of the LCs should be selected according to the properties of the encapsulant.

Lipid LLCs exhibit a wide structural diversity, and it needs to be considered when designing delivery platforms. Therefore, differences in structure are responsible for the physicochemical properties and behavior of the LLCs. For example, hexagonal and lamellar phase, can encapsulate biomacromolecules, possess high loading capacity, and high *in vivo* stability. On the other hand, cubic phases show higher stiffness than the other phases, they are easy to preparate, but when they are dispersed in water (cubosomes) they cannot sustain the release of water-soluble active ingredients [269]. In some cases, super-swollen structures, which are those with wider water channels, must be used for the delivery of biomacromolecules [44]. For this reason, the structure of the LLCs must also take into consideration when designing platforms for biomedical applications.

The authors of this review consider that the future of lipid LLCs will be dominated by platforms that respond to changes in the environment, such as those sensitive to pH or magnetic fields. This stimuli-sensitivity ensures the design of platforms with a programmed release, as well as Pulsatile Drug Delivery Systems, which enables an intelligent release dependent on internal stimuli. Perhaps more novel lines of research are also possible, such as chronotherapeutics [270], *i.e.* treatments for diseases dependent on circadian rhythms, encompassed within pulsatile drug delivery systems. Indeed, the authors of this review encourage researchers to develop platforms that are sensitive to stimuli, as the results obtained so far by multiple research groups seem to confirm their effectiveness. Theragnostic LLCs will also play an important role. The possibility of incorporating an imaging agent and one or several active substances in the same platform could be very relevant for diseases such as cancer, neurodegenerative diseases and others that require a very localized action and supervision by a medical team. Within biomacromolecules delivery, the most promising results are related to the release of proteins and peptides and siRNA. To the authors' knowledge, there do not seem to be many studies on carbohydrate delivery that could be used as pharmacological chaperones. The biocompatibility, morphological diversity, and biodegradability that lipid LLCs bring to tissue engineering do not seem to be taken into account, according to the authors' opinion, as neither the most recent reviews on the subject nor the authors of this review seem to find many examples in this respect. As is well known, 3D-printing is one of the current trends in biomedicine, and according to the authors, the characteristics of these systems could be exploited to formulate novel scaffolds through this technology.

Another application that may represent the near future of lipid LLCs is biosensing, something that has been reported in LCs [271] and in chromonic LLCs [53] for detecting of bacteria or viruses, but not well studied in lipid LLCs. For example, by incorporating mobile redox mediators in a GMO-water system, it ensures a rapid communication with the electrode [272]. For instance, a platform based on phytantriol cubosomes was used to detect cholera toxin B subunit and neutravidin, demonstrating its capacity as a biosensing platform [273]. Another approach consists in the transformation of the LC in the presence of CO_2, such as monoolein LLCs and looks like a promising biosensing platform [274]. Therefore, with the fabrication of novel LLCs with new lipid biomaterials, new applications could arise. In conclusion, lipid LLCs are a relevant platform in drug/biomacromolecules delivery and seem to provide very interesting features in the field of bioimaging and tissue engineering. Therefore, we hope that this review will serve as a guide in this fascinating field of research.

CRediT authorship contribution statement

Guillermo Blanco-Fernandez: Conceptualization, Writing – original draft. **Barbara Blanco-Fernandez:** Conceptualization, Writing – review & editing. **Anxo Fernández-Ferreiro:** Conceptualization, Writing – review & editing, Supervision. **Francisco J. Otero-Espinar:** Conceptualization, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Data availability

No data was used for the research described in the article.

Acknowledgement

References

[1] Mounir M. Construcción y aplicaciones de macromoléculas de Au, Pd y Pt con ligandos fluoroarílicos (PhD Thesis),. Barcelona: University of Barcelona; 2005.

[2] García Velarde M, Salan Santos J. Mesofases de la materia los cristales líquidos. El Basilisco 1979;7:46–52.

[3] Reinitzer F. Beitr ge zur Kenntniss des Gholesterins. Monatsh Chem n.d.;9(1): 421–41.

[4] Lehmann O. Über fliessende Krystalle. Z Phys Chem n.d.;4U(1. Doi: https://doi. org/10.1515/zpch-1889-0434.

[5] Chavez R., Santiago J. Cristales líquidos parte I: Cristales líquidos termotrópicos. Rev Química n.d.;13(2):53–70.

[6] Lyon LA. Handbook of Applied Surface and Colloid Chemistry. 1st ed. West Sussex: John Wiley & Sons; Chalmers University of Technology. West Sussex: John Wiley & Sons; 2002.

[7] Pasquali RC., Bregni C., Serrao R. Características e identificación de los cristales líquidos liotrópicos. Rev Mex Cienc Farm n.d.;2:38–53.

[8] Barón M., Noël C., Shibaev VP., Hess M., Jenkins AD., Jin JI., et al. Definitions of basic terms relating to low-molar-mass and polymer liquid crystals (IUPAC recommendations 2001). Pure Appl Chem n.d.;73(5):845–95. Doi: https://doi. org/10.1351/pac200173050845.

[9] Goodby JW., Gray GW. Guide to the nomenclature and classification of liquid crystals. In: Demus D, Goodby J, Gray GW, Spiess HW, Vill V, editors. Handbook of liquid crystals. Weinheim: Wiley-VCH; n.d.;vol. 1:17–23.

[10] Pasquali RC., Bregni C., Serrao R. Estructura de las principales fases líquido-cristalinas liotrópicas. Acta Farm Bonaer 2005:453–7.

[11] Mulet X, Boyd BJ, Drummond CJ. Advances in drug delivery and medical imaging using colloidal lyotropic liquid crystalline dispersions. J Colloid Interface Sci 2013;393(1):1–20. https://doi.org/10.1016/j.jcis.2012.10.014.

[12] Roland JC, Reis D, Vian B. Liquid crystal order and turbulence in the planar twist of the growing plant cell walls. Tissue Cell 1992;24(3).

[13] Knight D., Vollrath F. Hexagonal columnar liquid crystal in the cells secreting spider silk. Tissue Cell n.d.;31(6):617–20. Doi: https://doi.org/10.1054/tice. 1999.0076.

[14] Fontell K. Liquid Behavior. Prog Chem Fats Other Lipids 1978:145–62.

[15] Reis D, Vian B, Chanzy H, Roland JC. Liquid crystal-type assembly of native cellulose-glucuronoxylans extracted from plant cell wall. Biol Cell 1991;73(2–3): 173–8.

[16] Giraud-Guille MM. Liquid crystalline phases of sonicated type I collagen. Biol Cell n.d.;67(1):97–101. Doi: https://doi.org/10.1111/j.1768-322x.1989.tb03014.x.

[17] Lagerwall JPF., Scalia G. A new era for liquid crystal research: applications of liquid crystals in soft matter nano-, bio- and microtechnology. Curr Appl Phys n. d.;12(6):1387–412. Doi: https://doi.org/10.1016/j.cap.2012.03.019.

[18] Franklin RE, Gosling RG. Molecular configuration in sodium thymonucleate. Nature 1953:740–1.

[19] Zanchetta G., Bellini T., Nakata M., Clark NA. Physical polymerization and liquid crystallization of RNA oligomers. J Am Chem Soc n.d.;130(39):12864–5. Doi: http s://doi.org/10.1021/ja804718c.

[20] Leforestier A, Livolant F. The bacteriophage genome undergoes a succession of intracapsid phase transitions upon DNA ejection. J Mol Biol 2010;396(2):384–95. https://doi.org/10.1016/j.jmb.2009.11.047.

[21] Larsson M, Larsson K. Periodic minimal surface organizations of the lipid bilayer at the lung surface and in cubic cytomembrane assemblies. Adv Colloid Interf Sci 2014;205:68–73. https://doi.org/10.1016/j.cis.2013.07.003.

[22] Quirino-barreda CT, Gazga-Urioste C, Juárez JJ, Faustino-vega A, Noguez-méndez NA, Macín-cabrera SA. Cristales líquidos liotrópicos. Nanoestructuras biomiméticas para uso tópico medicinal. *Mundo*. Nano 2017;10(19):7–25.

[23] Haasen C, Linden M, Tiberg F. Pharmacokinetics and pharmacodynamics of a buprenorphine subcutaneous depot formulation (CAM2038) for once-weekly dosing in patients with opioid use disorder. J Subst Abus Treat 2017;78:22–9. https://doi.org/10.1016/j.jsat.2017.04.008.

[24] Mezzenga R., Seddon JM., Drummond CJ., Boyd BJ., Schröder-Turk GE., Sagalowicz L. Nature-inspired design and application of Lipidic Lyotropic liquid crystals. Adv Mater n.d.;31(35):1–19. Doi: https://doi.org/10.1002/adma. 201900818.

[25] Feng S, Nie L, Zou P, Suo J. Effects of drug and polymer molecular weight on drug release from PLGA-mPEG microspheres. J Appl Polym Sci 2015;132(6):1–8. https://doi.org/10.1002/app.41431.

[26] Zhai J, Sarkar S, Tran N, Pandiancherri S, Greaves TL, Drummond CJ. Tuning nanostructured Lyotropic liquid crystalline Mesophases in lipid nanoparticles with Protic ionic liquids. J Phys Chem 2020;12:399–404.

[27] Sadhu VR, Beram NS, Kantamneni P. A review on cubosome: the novel drug delivery system. GSC Biol Pharm Sci 2018;5(1):076–81. https://doi.org/10.30574/gscbps.2018.5.1.0089.

[28] Singhvi G, Banerjee S, Khosa A. In: Grumezescu AM, editor. Lyotropic liquid crystal nanoparticles: A novel improved lipidic drug delivery system. Amsterdam: Elsevier Inc.; 2018.

[29] Ramos Riesco M, Martínez Casado MJ, García Pérez MV, Redondo-Yélamos MI, Cristales Cheda JAR. Líquidos: Otro Estado de la Materia. In: Cañón Gabriel Pinto, editor. Jornadas sobre Didáctica de la Química y Vida Cotidiana; 2003. p. 267–74.

[30] Vidal Bonifácio B, Bento da Silva P, dos Santos Ramos MA, Silveira Negri KM, Bauab TM, Chorilli M. Nanotechnology-based drug delivery systems and herbal medicines : a review. Int J Nanomedicine 2014;9:1–15.

[31] Martínez Rodríguez M. Formación y caracterización de emulsiones altamente concentradas de betún en agua (PhD Thesis),. Barcelona: University of Barcelona; 2014.

[32] Cano M. Organizaciones supramoleculares de compuestos con estructura "tipo banana": preparación y caracterización. 8(PhD Thesis). Universidad de Zaragoza; 2013.

[33] Muñoz J., Alfaro MC. Rheological and phase behaviour of amphiphilic lipids. Grasas Aceites n.d.;51(1–2):6–25. Doi: https://doi.org/10.3989/gya.2000.v51.i1-2.403.

[34] Amar-Yuli I, Libster D, Aserin A, Garti N. Solubilization of food bioactives within lyotropic liquid crystalline mesophases. Curr Opin Colloid Interface Sci 2009;14 (1):21–32. https://doi.org/10.1016/j.cocis.2008.02.001.

[35] Mulet X, Conn CE, Fong C, Kennedy DF, Moghaddam MJ, Drummond CJ. High-throughput development of amphiphile self-assembly materials: fast-tracking synthesis, characterization, formulation, application, and understanding. Acc Chem Res 2013;46(7):1497–505. https://doi.org/10.1021/ar300285u.

[36] Urandur S, Marwaha D, Gautam S, Banala VT, Sharma M, Mishra PR. Nonlamellar liquid crystals: A new paradigm for the delivery of small molecules and bio-macromolecules. Ther Deliv 2018;9(9):667–89. https://doi.org/10.4155/tde-2018-0038.

[37] Kaasgaard T, Drummond CJ. Ordered 2-D and 3-D nanostructured amphiphile self-assembly materials stable in excess solvent. Phys Chem Chem Phys 2006;8 (43):4957–75. https://doi.org/10.1039/b609510k.

[38] Rodrigues L, Schneider F, Zhang X, Larsson E, Moodie LWK, Dietz H. Cellular uptake of self-assembled phytantriol-based hexosomes is independent of major endocytic machineries. J Colloid Interface Sci 2019;553:820–33. https://doi.org/10.1016/j.jcis.2019.06.045.

[39] Popov N, Honaker LW, Popova M, Usol'tseva N, Mann EK, Jákli A, et al. Thermotropic liquid crystal-assisted chemical and biological sensors. Materials 2017. https://doi.org/10.3390/ma11010020.

[40] Zhang L, Qu Y, Gu J, Liu Y, Tang Z, Zhang C, et al. Photoswitchable solvent-free DNA thermotropic liquid crystals toward self-erasable shape information recording biomaterials. Mater Today Bio 2021;12. https://doi.org/10.1016/j.mtbio.2021.100140.

[41] Hui L, Wang D, Liu Z, Zhao Y, Ji Z, Zhang M, et al. The cell-isolation capsules with rod-like channels ensure the survival and response of cancer cells to their microenvironment. Adv Healthc Mater 2022;11(2). https://doi.org/10.1002/adhm.202101723.

[42] Ju HK, Kim JW, Han SH, Chang IS, Kim HK, Kang HH, et al. Thermotropic liquid-crystal/polymer microcapsules prepared by in situ suspension polymerization. Colloid Polym Sci 2002;280(10):879–85. https://doi.org/10.1007/s00396-002-0696-x.

[43] Waghule T, Gorantla S, Rapalli VK, Shah P, Dubey SK, Saha RN, et al. Emerging trends in topical delivery of curcumin through lipid nanocarriers: effectiveness in skin disorders. AAPS PharmSciTech 2020. https://doi.org/10.1208/s12249-020-01831-9.

[44] Zhai J, Sarkar S, Conn CE, Drummond CJ. Molecular engineering of super-swollen inverse bicontinuous cubic and sponge lipid phases for biomedical applications. Mol Syst Des Eng 2020:1354–75. https://doi.org/10.1039/d0me00076k.

[45] Chountoulesi M, Pispas S, Tseti IK, Demetzos C. Lyotropic liquid crystalline nanostructures as drug delivery systems and vaccine platforms. Pharmaceuticals 2022. https://doi.org/10.3390/ph15040429.

[46] Li F, Lin L, Chi J, Wang H, Du M, Feng D, et al. Guanidinium-rich lipopeptide functionalized bacteria-absorbing sponge as an effective trap-and-kill system for the elimination of focal bacterial infection. Acta Biomater 2022;148:106–18. https://doi.org/10.1016/j.actbio.2022.05.052.

[47] Kumari S, Goyal A, Garg M, Antonescu A, Sindhu RK. Lyotropic liquid crystal system for drug delivery of Astaxanthin: physical characterization and enhanced antioxidant potential. Crystals (Basel) 2023;13(1):142. https://doi.org/10.3390/cryst13010142.

[48] Waheed A, Zameer S, Sultana N, Ali A, Aqil M, Sultana Y, et al. Engineering of QbD driven and ultrasonically shaped lyotropic liquid crystalline nanoparticles for Apigenin in the management of skin cancer. Eur J Pharm Biopharm 2022;180: 269–80. https://doi.org/10.1016/j.ejpb.2022.10.015.

[49] Bunjes H, Rades T. Thermotropic liquid crystalline drugs. J Pharm Pharmacol 2010;57(7):807–16. https://doi.org/10.1211/0022357056208.

[50] Devadiga D, Ahipa TN. Liquid crystal-based water treatment membranes. Adv Mater Interfaces 2022. https://doi.org/10.1002/admi.202101276.

[51] Oladepo SA. Development and application of liquid crystals as stimuli-responsive sensors. Molecules 2022. https://doi.org/10.3390/molecules27041453.

[52] Wang H, Xu T, Fu Y, Wang Z, Leeson MS, Jiang J, et al. Liquid crystal biosensors: principles, structure and applications. Biosensors (Basel) 2022. https://doi.org/10.3390/bios12080639.

[53] Popov P, Mann EK, Jákli A. Thermotropic liquid crystal films for biosensors and beyond. J Mater Chem B 2017:5061–78. https://doi.org/10.1039/c7tb00809k.

[54] Tam-Chang SW, Huang L. Chromonic liquid crystals: properties and applications as functional materials. Chem Commun 2008:1957–67. https://doi.org/10.1039/b714319b.

[55] Lin YC, Li GS, Yu PJ, Ercan E, Chen WC. Organic liquid crystals in optoelectronic device applications: field-effect transistors, nonvolatile memory, and photovoltaics. J Chin Chem Soc 2022:1289–304. https://doi.org/10.1002/jccs.202200061.

[56] Zhang C, Zhang L, Cao Z, Lian C, Gao S, Zhang D, et al. Fluorescent DNA thermotropic liquid crystal showing thermostability and water-resistance. Dyes Pigments 2022:204. https://doi.org/10.1016/j.dyepig.2022.110449.

[57] Sahu S, Schwindt NS, Coscia BJ, Shirts MR. Obtaining and characterizing stable Bicontinuous cubic morphologies and their nanochannels in lyotropic liquid crystal membranes. J Phys Chem B 2022;126(48):10098–110. https://doi.org/10.1021/acs.jpcb.2c06119.

[58] Cao Y, Wang PX, D'Acierno F, Hamad WY, Michal CA, MJ MacLachlan. Tunable diffraction gratings from biosourced Lyotropic liquid crystals. Adv Mater 2020;32 (19). https://doi.org/10.1002/adma.201907376.

[59] Neelamma MK, Holla SR, Selvakumar M, Chandran PA, De S. Bentonite clay liquid crystals for high-performance Supercapacitors. J Electron Mater 2022;51 (5):2192–202. https://doi.org/10.1007/s11664-022-09469-y.

[60] Amirzhanova A, Akmanşen N, Karakaya I, Dag Ö. Mesoporous MnCo2O4, NiCo2O4, and ZnCo2O4Thin-film electrodes as Electrocatalysts for the oxygen evolution reaction in alkaline solutions. ACS Appl Energy Mater 2021;4(3): 2769–85. https://doi.org/10.1021/acsaem.1c00064.

[61] Topuzlu EY, Ulgut B, Dag Ö. Role of water in the lyotropic liquid crystalline lithium iodide-iodine-water-C12E10Mesophase as a gel electrolyte in a dye-sensitized solar cell. Langmuir 2021;37(27):8305–13. https://doi.org/10.1021/acs.langmuir.1c01094.

[62] Xu Y, Rather AM, Song S, Fang JC, Dupont RL, Kara UI, et al. Ultrasensitive and selective detection of SARS-CoV-2 using Thermotropic liquid crystals and image-based machine learning. Cell Rep Phys Sci 2020;1(12). https://doi.org/10.1016/j.xcrp.2020.100276.

[63] Ortiz BJ, Boursier ME, Barrett KL, Manson DE, Amador-Noguez D, Abbott NL, et al. Liquid crystal emulsions that intercept and report on bacterial quorum sensing. ACS Appl Mater Interfaces 2020;12(26):29056–65. https://doi.org/10.1021/acsami.0c05792.

[64] Li L, Dong X, Li M, Jiang Y, Xu J, Li Q, et al. Development of low-temperature driven high-strength interpenetrating polyurethane-liquid crystal elastomer actuators with self-sensing property. Sens Actuators A Phys 2023:349. https://doi.org/10.1016/j.sna.2022.114069.

[65] Chen T, Han JY, Okonski DA, Kazerooni D, Ju L, Baird DG. Thermotropic liquid crystalline polymer reinforced polyamide composite for fused filament fabrication. Addit Manuf 2021:40. https://doi.org/10.1016/j.addma.2021.101931.

[66] Liu H, Wang L, Hu Y, Huang Z, Sun Y, Dong S, et al. DNA thermotropic liquid crystals controlled by positively charged catanionic bilayer vesicles. Chem Commun 2020;56(24):3484–7. https://doi.org/10.1039/d0cc00980f.

[67] el Nokaly M, Friberg SE, Larsen DW. Lyotropic liquid crystals from lecithin, water, and polyethylene glycol. J Colloid Interface Sci 1984;98(1):274–6.

[68] Lee J, Kellaway IW. Buccal permeation of [D-Ala2, D-Leu5]enkephalin from liquid crystalline phases of glyceryl monooleate. Int J Pharm 2000;195(1–2): 35–8.

[69] Libster D, serin A., Garti N.. Interactions of biomacromolecules with reverse hexagonal liquid crystals: drug delivery and crystallization applications. J Colloid Interface Sci 2011;356(2):375–86. https://doi.org/10.1016/j.jcis.2011.01.047.

[70] Chountoulesi M, Perinelli DR, Pippa N, Chrysostomou V, Forys A, Otulakowski L. Physicochemical, morphological and thermal evaluation of lyotropic lipidic liquid crystalline nanoparticles: the effect of stimuli-responsive polymeric stabilizer. Colloids Surf A Physicochem Eng Asp 2020:595. https://doi.org/10.1016/j.colsurfa.2020.124678.

[71] Sawada T, Yanagimachi M, Serizawa T. Controlled release of antibody proteins from liquid crystalline hydrogels composed of genetically engineered filamentous viruses. Mater Chem Front 2017;1(1):146–51. https://doi.org/10.1039/c6qm00140h.

[72] Xie Y, Liu X, Hu Z, Hou Z, Guo Z, Chen Z, et al. Synthesis, self-assembly, and drug-release properties of new amphipathic liquid crystal polycarbonates. Nanomaterials 2018;8(4). https://doi.org/10.3390/nano8040195.

[73] Andrade FF, Santos ODH, Oliveira WP, Rocha-Filho PA. Influence of PEG-12 Dimethicone addition on stability and formation of emulsions containing liquid crystal. Int J Cosmet Sci 2007;29(3):211–8. https://doi.org/10.1111/j.1467-2494.2007.00374.x.

[74] Akbar S, Anwar A, Ayish A, Elliott JM, Squires AM. Phytantriol based smart nano-carriers for drug delivery applications. Eur J Pharm Sci 2017;101:31–42. https://doi.org/10.1016/j.ejps.2017.01.035.

[75] Wibroe PP, Mat Azmi ID, Nilsson C, Yaghmur A, Moghimi SM. Citrem modulates internal nanostructure of glyceryl monooleate dispersions and bypasses complement activation: towards development of safe tunable intravenous lipid

nanocarriers. Nanomedicine 2015;11(8):1909–14. https://doi.org/10.1016/j.nano.2015.08.003.

[76] Engelskirchen S, Maurer R, Glatter O. Effect of glycerol addition on the internal structure and thermal stability of hexosomes prepared from phytantriol. Colloids Surf A Physicochem Eng Asp 2011;391(1–3):95–100. https://doi.org/10.1016/j.colsurfa.2011.10.001.

[77] Yaghmur A, Rappolt M. The micellar cubic Fd3m phase. Recent advances in the structural characterization and potential applications. Adv Planar Lipid Bilayers Liposomes 2013:111–45.

[78] Fouad EA, El-badry M, Mahrous GM, Alsarra IA, Alashban Z, Alanazi FK. In vitro investigation for embedding dextromethorphan in lipids using spray drying. Dig J Nanomater Biostructures 2011:1129–39.

[79] Müller RH, Mäder K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery - a review of the state of the art. Eur J Pharm Biopharm 2000;50:161–77.

[80] Masson DS, Morais GG, Morais JM, Andrade FF, dos Santos ODH, Oliveira WP. Polyhydroxy alcohols and peach oil addition influence on liquid crystal formation and rheological behavior of O/W emulsions. J Dispers Sci Technol 2006;26(4):463–8.

[81] Santos ODH, Morais JM, Andrade FF, Aguiar TA, Rocha Filho PA. Development of vegetable oil emulsions with lamellar liquid-crystalline structures. J Dispers Sci Technol 2011;32(3):433–8. https://doi.org/10.1080/01932691003662464.

[82] Cuerva De Alaíz C. Metalomesógenos : cristales líquidos en las nuevas tecnologías. MoleQla 2013;12:3–5.

[83] Giménez R, Lydon DP, Serrano JL. Metallomesogens: A promise or a fact? Curr Opin Solid State Mater Sci 2002;6(6):527–35.

[84] Wang X, Zhang Y, Gui S, Huang J, Cao J, Li Z. Characterization of lipid-based Lyotropic liquid crystal and effects of guest molecules on its microstructure: a systematic review. AAPS PharmSciTech 2018;19(5):2023–40. https://doi.org/10.1208/s12249-018-1069-1.

[85] Wang D, Cao Y, Cao M, Sun Y, Wang J, Hao J. Dual-responsive viscoelastic lyotropic liquid crystal fluids to control the diffusion of hydrophilic and hydrophobic molecules. ChemPhysChem 2016;17(13):2079–87. https://doi.org/10.1002/cphc.201600066.

[86] Guo C, Wang J, Cao F, Lee RJ, Zhai G. Lyotropic liquid crystal systems in drug delivery. Drug Discov Today 2016;15(23–24):1032–40. https://doi.org/10.1016/j.drudis.2010.09.006.

[87] Chountoulesi M, Pippa N, Pispas S, Chrysina ED, Forys A, Trzebicka B. Cubic lyotropic liquid crystals as drug delivery carriers: physicochemical and morphological studies. Int J Pharm 2018;550(1–2):57–70. https://doi.org/10.1016/j.ijpharm.2018.08.003.

[88] Wörle G, Drechsler M, Koch MHJ, Siekmann B, Westesen K, Bunjes H. Influence of composition and preparation parameters on the properties of aqueous monoolein dispersions. Int J Pharm 2007;329(1–2):150–7. https://doi.org/10.1016/j.ijpharm.2006.08.023.

[89] Bhosale RR, Osmani RA, Harkare B, Ghodake PP. Cubosomes: the inimitable Nanoparticulate drug carriers. Sch Acad J Pharm 2013;2(6):481–6.

[90] Balata GF, Amin M, Alhalabi F, Alansari S. Cubosomes: A novel approach for delivery of anticancer drugs. Am J PharmTech Res 2016:1–14.

[91] Bodratti AM, Alexandridis P. Amphiphilic block copolymers in drug delivery: advances in formulation structure and performance. Expert Opin Drug Deliv 2018;15(11):1085–104. https://doi.org/10.1080/17425247.2018.1529756.

[92] Molly BA, Prasanthi NL. Cubic liquid crystalline nanoparticles(cubosomes): A novel carrier for drug delivery. Int J Pharm Sci Res 2018;10(3):973–84.

[93] Hirlekar R, Jain S, Patel M, Garse H, Kadam V. Hexosomes: a novel drug delivery system. Curr Drug Deliv 2010;7(1):28–35.

[94] Wu H, Cui Z, Huo Y, Sun Y, Zhang X, Guan J, et al. Influence of drug-carrier compatibility and preparation method on the properties of paclitaxel-loaded lipid liquid crystalline nanoparticles. J Pharm Sci 2021;110(7):2800–7. https://doi.org/10.1016/j.xphs.2021.03.016.

[95] Caffrey M. Kinetics and mechanism of transitions involving the lamellar, cubic, inverted hexagonal, and fluid isotropic phases of hydrated Monoacylglycerides monitored by time-resolved X-ray diffraction. Biochemistry 1987;26(20):6349–63. https://doi.org/10.1021/bi00394a008.

[96] Rosevear FB. Liquid crystals: The mesomorphic phases of surfactant compositions. J Soc Cosmet Chem 1968;19:581–94.

[97] Larsson K. Lyotropic liquid crystals and their dispersions relevant in foods. Curr Opin Colloid Interface Sci 2009;14(1):16–20. https://doi.org/10.1016/j.cocis.2008.01.006.

[98] Larsson K. Cubic lipid-water phases: structures and biomembrane aspects. J Phys Chem 1989;93(21):7304–14. https://doi.org/10.1021/j100358a010.

[99] Manaia EB, Abuçafy MP, Chiari-Andréo BG, Silva BL, Oshiro Junior JA, Chiavacci LA. Physicochemical characterization of drug nanocarriers. Int J Nanomedicine 2017;12:4991–5011. https://doi.org/10.2147/IJN.S133832.

[100] Azmi IDM, Moghimi SM, Yaghmur A. Cubosomes and hexosomes as versatile platforms for drug delivery. Ther Deliv 2015;6(12):1347–64. https://doi.org/10.4155/tde.15.81.

[101] Lindblom G, Orädd G. NMR studies of translational diffusion in lyotropic liquid crystals and lipid membranes. Prog Nucl Magn Reson Spectrosc 1994;26(PART 5). 10.1016 0079–6565 94 80014–6.

[102] Israelachvili JN., Mitchell DJ., Ninham BW. Theory of self-assembly of hydrocarbon amphiphiles into micelles and bilayers. J Chem Soc 1976;2:1525–68. Doi: https://doi.org/10.1039/F29767201525.

[103] Srivastava S, Padmaja GV. Study of nano-sized micelles formation with surfactant critical packing parameter and its applications. Int J Res Eng Appl Manag 2018:1–6. https://doi.org/10.18231/2454-9150.2018.0742.

[104] Sadeghpour A, Rappolt M, Misra S, Kulkarni CV. Bile salts caught in the act: from emulsification to nanostructural reorganization of lipid self-assemblies. Langmuir 2018;34(45):13626–37. https://doi.org/10.1021/acs.langmuir.8b02343.

[105] Salim M, Minamikawa H, Sugimura A, Hashim R. Amphiphilic designer nano-carriers for controlled release: from drug delivery to diagnostics. Medchemcomm 2014;5(11):1602–18. https://doi.org/10.1039/c4md00085d.

[106] Qiu H, Caffrey M. The phase diagram of the monoolein/water system: Metastability and equilibrium aspects. Biomaterials 2000;21(3):223–34.

[107] Burrell J, Dymond MK, Gillams RJ, Parker DJ, Langley GJ, Labrador A. Using curvature power to map the domain of inverse micellar cubic phases: the case of aliphatic aldehydes in 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine. Langmuir 2017;33(44):12804–13. https://doi.org/10.1021/acs.langmuir.7b02998.

[108] Jennings J, Carter MCD, Son CY, Cui Q, Lynn DM, Mahanthappa MK. Protonation-driven aqueous lyotropic self-assembly of synthetic six-tail lipidoids. Langmuir 2020;36(28):8240–52. https://doi.org/10.1021/acs.langmuir.0c01369.

[109] Mendozza M, Montis C, Caselli L, Wolf M, Baglioni P, Berti D. On the thermotropic and magnetotropic phase behavior of lipid liquid crystals containing magnetic nanoparticles. Nanoscale 2018;10(7):3480–8. https://doi.org/10.1039/c7nr08478a.

[110] Yesylevskyy SO, Ramseyer C. Determination of mean and Gaussian curvatures of highly curved asymmetric lipid bilayers: the case study of the influence of cholesterol on the membrane shape. Phys Chem Chem Phys 2014;16(32):17052–61. https://doi.org/10.1039/c4cp01544d.

[111] Siegel DP, Kozlov MM. The Gaussian curvature elastic modulus of N-monomethylated dioleoylphosphatidylethanolamine: relevance to membrane fusion and lipid phase behavior. Biophys J 2004;87(1):366–74. https://doi.org/10.1529/biophysj.104.040782.

[112] Separovic F, Pax R, Cornell B. Nmr order parameter analysis of a peptide plane aligned in a lyotropic liquid crystal. Mol Phys 1992;78(2):357–69. https://doi.org/10.1080/00268979300100281.

[113] Sivasri J, Pardhasaradhi P, Madhav BTP, Tejaswi M, Manepalli RKNR. Birefringence studies on alkoxy benzoic acids with dispersed Fe3O4 nanoparticles. Liq Cryst 2018;47(3):330–44. https://doi.org/10.1080/02678292.2019.1647571.

[114] Müller-Goymann CC. Physicochemical characterization of colloidal drug delivery systems such as reverse micelles, vesicles, liquid crystals and nanoparticles for topical administration. Eur J Pharm Biopharm 2004;58(2):343–56. https://doi.org/10.1016/j.ejpb.2004.03.028.

[115] Negrini R, Fong WK, Boyd BJ, Mezzenga R. PH-responsive lyotropic liquid crystals and their potential therapeutic role in cancer treatment. Chem Commun 2015;51(30):6671–4. https://doi.org/10.1039/c4cc10274f.

[116] Silvestrini AVP, Caron AL, Viegas J, Praça FG, Bentley MVLB. Advances in lyotropic liquid crystal systems for skin drug delivery. Expert Opin Drug Deliv 2020;17(12):1781–805. https://doi.org/10.1080/17425247.2020.1819979.

[117] Salentinig S, Tangso KJ, Hawley A, Boyd BJ. pH-driven colloidal transformations based on the vasoactive drug nicergoline. Langmuir 2014;30(49):14776–81. https://doi.org/10.1021/la503824z.

[118] Sun X, Alcaraz N, Qiao R, Hawley A, Tan A, Boyd BJ. Magnetically-stimulated transformations in nanostructure of lipid mesophases: effect of structure of iron oxide nanoparticles. Colloids Surf B: Biointerfaces 2020;191(March). https://doi.org/10.1016/j.colsurfb.2020.110965.

[119] Chen Y, Ma P, Gui S. Cubic and hexagonal liquid crystals as drug delivery systems. Biomed Res Int 2014;nd.

[120] Blanco-Fernandez G, Blanco-Fernández B, Fernández-Ferreiro A, Otero-Espinar F. Bringing lipidic lyotropic liquid crystal technology into biomedicine. Trends Pharmacol Sci 2022:1–4. https://doi.org/10.1016/j.tips.2022.03.004.

[121] Fan Y, Chen H, Huang Z, Zhu J, Wan F, Peng T, et al. Taste-masking and colloidal-stable cubosomes loaded with Cefpodoxime proxetil for pediatric oral delivery. Int J Pharm 2020:575. https://doi.org/10.1016/j.ijpharm.2019.118875.

[122] Chen J, Wang H, Mei L, Wang B, Huang Y, Quan G, et al. A pirfenidone loaded spray dressing based on lyotropic liquid crystals for deep partial thickness burn treatment: healing promotion and scar prophylaxis. J Mater Chem B 2020;8(13):2573–88. https://doi.org/10.1039/c9tb02929j.

[123] Loo YS, Madheswaran T, Rajendran R, Bose RJ. Encapsulation of berberine into liquid crystalline nanoparticles to enhance its solubility and anticancer activity in MCF7 human breast cancer cells. J Drug Deliv Sci Technol 2020:57. https://doi.org/10.1016/j.jddst.2020.101756.

[124] Pramanik A, Xu Z, Shamsuddin SH, Khaled YS, Ingram N, Maisey T, et al. Affimer tagged Cubosomes: targeting of carcinoembryonic antigen expressing colorectal cancer cells using in vitro and in vivo models. ACS Appl Mater Interfaces 2022;14(9):11078–91. https://doi.org/10.1021/acsami.1c21655.

[125] Hosny KM, Rizg WY, Alkhalidi HM, Abualsunun WA, Bakhaidar RB, Almehmady AM, et al. Nanocubosomal based in situ gel loaded with natamycin for ocular fungal diseases: development, optimization, in-vitro, and in-vivo assessment. Drug Deliv 2021;28(1):1836–48. https://doi.org/10.1080/10717544.2021.1965675.

[126] de Souza IFF, dos Santos TQ, Placido RV, Mangerona BA, Carvalho FC, Boralli VB, et al. The liquid crystalline phase behaviour of a nasal formulation modifies the brain disposition of donepezil in rats in the treatment of Alzheimer's disease. Colloids Surf B: Biointerfaces 2021:203. https://doi.org/10.1016/j.colsurfb.2021.111721.

[127] Tian C, Liu L, Xia M, Qin Chu X. The evaluations of menthol and propylene glycol on the transdermal delivery system of dual drug–loaded lyotropic liquid crystalline gels. AAPS PharmSciTech 2020;21(6). https://doi.org/10.1208/s12249-020-01762-5.

[128] De Bastiani FWM de S, Spadari C de C, de Matos JKR, Salata GC, Lopes LB, Ishida K. Nanocarriers provide sustained antifungal activity for amphotericin B and Miltefosine in the topical treatment of murine vaginal candidiasis. Front Microbiol 2020:10. https://doi.org/10.3389/fmicb.2019.02976.

[129] Chountoulesi M, Perinelli DR, Forys A, Chrysostomou V, Kaminari A, Bonacucina G, et al. Development of stimuli-responsive lyotropic liquid crystalline nanoparticles targeting lysosomes: physicochemical, morphological and drug release studies. Int J Pharm 2023:630. https://doi.org/10.1016/j.ijpharm.2022.122440.

[130] Rajesh S, Zhai J, Drummond CJ, Tran N. Novel pH-responsive cubosome and hexosome lipid nanocarriers of SN-38 are prospective for cancer therapy. Pharmaceutics 2022;14(10). https://doi.org/10.3390/pharmaceutics14102175.

[131] Tarsitano M, Mancuso A, Cristiano MC, Paolino D, Fresta M. In situ swelling formulation of glycerol-monooleate-derived lyotropic liquid crystals proposed for local vaginal application. Molecules 2022;27(19). https://doi.org/10.3390/molecules27196295.

[132] Nasr M, Almawash S, al Saqr A, Bazeed AY, Saber S, Elagamy HI. Bioavailability and antidiabetic activity of gliclazide-loaded cubosomal nanoparticles. Pharmaceuticals 2021;14(8). https://doi.org/10.3390/ph14080786.

[133] Saklani R, Yadav PK, Nengroo MA, Gawali SL, Hassan PA, Datta D, et al. An injectable in situ depot-forming lipidic lyotropic liquid crystal system for localized intratumoral drug delivery. Mol Pharm 2022;19(3):831–42. https://doi.org/10.1021/acs.molpharmaceut.1c00782.

[134] Mohammad Y, Prentice RN, Boyd BJ, Rizwan SB. Comparison of cubosomes and hexosomes for the delivery of phenytoin to the brain. J Colloid Interface Sci 2022; 605:146–54. https://doi.org/10.1016/j.jcis.2021.07.070.

[135] Rajesh S, Gangadoo S, Nguyen H, Zhai J, Dekiwadia C, Drummond CJ, et al. Application of fluconazole-loaded pH-sensitive lipid nanoparticles for enhanced antifungal therapy. ACS Appl Mater Interfaces 2022. https://doi.org/10.1021/acsami.2c05165.

[136] Valente F, Simoni E, Gentilin E, Martini A, Zanoletti E, Marioni G, et al. Liquid crystalline nanoparticles conjugated with dexamethasone prevent cisplatin ototoxicity in vitro. Int J Mol Sci 2022;23(23). https://doi.org/10.3390/ijms232314881.

[137] Bor G, Lin JH, Lin KY, Chen HC, Prajnamitra RP, Salentinig S, et al. PEGylation of phosphatidylglycerol/docosahexaenoic acid hexosomes with d -α-tocopheryl succinate poly(ethylene glycol)2000Induces morphological transformation into vesicles with prolonged circulation times. ACS Appl Mater Interfaces 2022;14 (43):48449–63. https://doi.org/10.1021/acsami.2c14375.

[138] Thorn CR, Carvalho-Wodarz C de S, Horstmann JC, Lehr CM, Prestige CA, Thomas N. Tobramycin liquid crystal nanoparticles eradicate cystic fibrosis-related *Pseudomonas aeruginosa* biofilms. Small 2021;17(24). https://doi.org/10.1002/smll.202100531.

[139] Bessone CDV, Akhlaghi SP, Tártara LI, Quinteros DA, Loh W, Allemandi DA. Latanoprost-loaded phytantriol cubosomes for the treatment of glaucoma. Eur J Pharm Sci 2021;160. https://doi.org/10.1016/j.ejps.2021.105748.

[140] Hiwale P, Lampis S, Murgia S, Monduzzi M. Liquid-crystal based formulations for topical drug delivery. J Dispers Sci Technol 2013:1286–93. https://doi.org/10.1080/01932691.2012.738118.

[141] Monteiro e Silva SA, Lacerda R, Bernegossi J, Chorilli M, Ricci Leonardi G. Development of nanotechnology-based drug delivery systems with olive vegetable oil for cutaneous application. Braz J Pharm Sci 2016;52(1):211–9. https://doi.org/10.1590/S1984-82502016000100023.

[142] Oyafuso MH, Carvalho FC, Chiavacci LA, Daflon Gremião MP, Chorilli M. Design and characterization of silicone and surfactant based systems for topical drug delivery. J Nanosci Nanotechnol 2015;15(1):817–26. https://doi.org/10.1166/jnn.2015.9181.

[143] Carvalho FC, Rocha e Silva H, Marielli da Luz G, da Silva Barbi M, Silveira Landgraf D, Chiavacci LA, et al. Rheological, mechanical and adhesive properties of surfactant-containing systems designed as a potential platform for topical drug delivery. J Biomed Nanotechnol 2012;8(2):280–9. https://doi.org/10.1166/jbn.2012.1373.

[144] Fonseca-Santos B, Satake CY, Fioramonti Calixto GM, dos Santos AM, Chorilli M. Trans-resveratrol-loaded nonionic lamellar liquid-crystalline systems: structural, rheological, mechanical, textural, and bioadhesive characterization and evaluation of in vivo anti-inflammatory activity. Int J Nanomedicine 2017;12: 6883–93. https://doi.org/10.2147/IJN.S138629.

[145] Mo J, Milleret G, Nagaraj M. Liquid crystal nanoparticles for commercial drug delivery. Liq Cryst Rev 2017;5(2):69–85. https://doi.org/10.1080/21680396.2017.1361874.

[146] Keservani RK, Sharma AK, Kesharwani RK. Liquid crystal systems in drug delivery. In: Chaudhary KK, Kannojia P, Mishra N, editors. Novel Approaches for Drug Delivery; 2017.

[147] Yamada K, Yamashita J, Todo H, Miyamoto K, Hashimoto S, Tokudome Y. Preparation and evaluation of liquid-crystal formulations with skin-permeation-enhancing abilities for entrapped drugs. J Oleo Sci 2011;60(1):31–40. https://doi.org/10.5650/jos.60.31.

[148] Aytekin M, Gursoy RN, Ide S, Soylu EH, Hekimoglu S. Formulation and characterization of liquid crystal systems containing azelaic acid for topical delivery. Drug Dev Ind Pharm 2013;39(2):228–39. https://doi.org/10.3109/03639045.2012.671829.

[149] Norlén L, Al-Amoudi A. Stratum corneum keratin structure, function, and formation: the cubic rod-packing and membrane templating model. J Invest Dermatol 2004;123(4):715–32. https://doi.org/10.1111/j.0022-202X.2004.23213.x.

[150] Lafleur M. Phase behaviour of model stratum corneum lipid mixtures: an infrared spectroscopy investigation. Can J Chem 1998;76(11):1501–11. https://doi.org/10.1139/v98-114.

[151] Gazga-Urioste C, Rivera-Becerril E, Pérez-Hernández G, Angélica Noguez-Méndez N, Faustino-Vega A, Tomás Quirino-Barreda C. Physicochemical characterization and thermal behavior of hexosomes containing ketoconazole as potential topical antifungal delivery system. Drug Dev Ind Pharm 2019;45(1):168–76. https://doi.org/10.1080/03639045.2018.1526188.

[152] Rajabalaya R, Nuh Musa M, Kifli N, David SR. Oral and transdermal drug delivery systems: role of lipid-based lyotropic liquid crystals. Drug Des Devel Ther 2017; 11:393–406. https://doi.org/10.2147/DDDT.S103505.

[153] Rocha e Silva H, Kolenyak dos Santos F, Marielli da Luz G, Chorilli M, Daflon Gremião MP. Validation of high-performance liquid chromatographic method for analysis of fluconazole in microemulsions and liquid crystals. Braz. J Pharm Sci 2014;50(2):381–9.

[154] Ribeiro de Souza AL, Kiill CP, Kolenyak dos Santos F, da Luz GM, Rocha e Silva H, Chorilli M, et al. Nanotechnology-based drug delivery systems for dermatomycosis treatment. Curr Nanosci 2012;8(4):512–9. https://doi.org/10.2174/157341312801784311.

[155] Carboni M, Falchi AM, Lampis S, Sinico C, Manca ML, Schmidt J. Physicochemical, cytotoxic, and dermal release features of a novel cationic liposome nanocarrier. Adv Healthc Mater 2012;2(5):692–701. https://doi.org/10.1002/adhm.201200302.

[156] el Maghraby GM. Self-microemulsifying and microemulsion systems for transdermal delivery of indomethacin: effect of phase transition. Colloids Surf B: Biointerfaces 2010;75(2):595–600. https://doi.org/10.1016/j.colsurfb.2009.10.003.

[157] Vučinić-Milanković N, Savić S, Vuleta G, Vučinić S. Natural surfactant-based emulsion vehicles: A correlation between colloidal structure and in vitro release of diclofenac diethylamine. J Dispers Sci Technol 2010;31(8):1077–84. https://doi.org/10.1080/01932690903224904.

[158] Savić S, Tamburić S, Savić MM. From conventional towards new natural surfactants in drug delivery systems design: current status and perspectives. Expert Opin Drug Deliv 2010;7(3):353–69. https://doi.org/10.1517/17425240903535833.

[159] Pande V, Nayak S, Kendre P, Patil P, Sagar K. Fabrication and characterization of zaltoprofen loaded lyotropic liquid crystalline gel. Indian Drugs 2015;53(3): 17–23.

[160] Thorn CR, Clulow AJ, Boyd BJ, Prestidge CA, Thomas N. Bacterial lipase triggers the release of antibiotics from digestible liquid crystal nanoparticles. J Control Release 2020;319:168–82. https://doi.org/10.1016/j.jconrel.2019.12.037.

[161] de Souza ALR, Fonseca M, Barboza Ferreira LM, Kurokawa SSS, Kiill CP, Ferreira NN, et al. Meglumine-based supra-amphiphile self-assembled in water as a skin drug delivery system: influence of unfrozen bound water in the system bioadhesiveness. Colloids Surf B: Biointerfaces 2019;184(June). https://doi.org/10.1016/j.colsurfb.2019.110523.

[162] Lopes LB, García MTJ, Bentley MVLB. Chemical penetration enhancers. Ther Deliv 2015;7(2):1053–61.

[163] Musa MN, David SR, Zulkipli IN, Mahadi AH, Chakravarthi S, Rajabalaya R. Development and evaluation of exemestane-loaded lyotropic liquid crystalline gel formulations. BioImpacts 2017;7(4):227–39. https://doi.org/10.15171/bi.2017.27.

[164] Cohen-Avrahami M, Libster D, Aserin A, Garti N. Penetratin-induced transdermal delivery from H II mesophases of sodium diclofenac. J Control Release 2012;159 (3):419–28. https://doi.org/10.1016/j.jconrel.2012.01.025.

[165] Lee J, Young SA, Kellaway IW. Water quantitatively induces the mucoadhesion of liquid crystalline phases of glyceryl monooleate. J Pharm Pharmacol 2001;53(5): 629–36. https://doi.org/10.1211/0022357011775956.

[166] Nguyen TH, Hanley T, Porter CJH, Boyd BJ. Nanostructured reverse hexagonal liquid crystals sustain plasma concentrations for a poorly water-soluble drug after oral administration. Drug Deliv Transl Res 2011;1(6):429–38. https://doi.org/10.1007/s13346-011-0045-z.

[167] Souza C, Watanabe E, Borgheti-Cardoso LN, Fantini MCA, Lara MG. Mucoadhesive system formed by liquid crystals for buccal administration of poly (hexamethylene biguanide) hydrochloride. J Pharm Sci 2014;103(12):3914–23. https://doi.org/10.1002/jps.24198.

[168] du JD, Liu Q, Salentinig S, Nguyen TH, Boyd BJ. A novel approach to enhance the mucoadhesion of lipid drug nanocarriers for improved drug delivery to the buccal mucosa. Int J Pharm 2014;471(1–2):358–65. https://doi.org/10.1016/j.ijpharm.2014.05.044.

[169] Swarnakar NK, Jain V, Dubey V, Mishra D, Jain NK. Enhanced oromucosal delivery of progesterone via hexosomes. Pharm Res 2007;24(12):2223–30. https://doi.org/10.1007/s11095-007-9409-y.

[170] Nielsen LS, Schubert L, Hansen J. Bioadhesive drug delivery systems I. Characterisation of mucoadhesive properties of systems based on glyceryl mono-oleate and glyceryl monolinoleate. Eur J Pharm Sci 1998;6:231–9.

[171] Jie H, Liu L, Shuangying G, Xingqi W, Rongfeng H, Yong Z. A novel phytantriol-based in situ liquid crystal gel for vaginal delivery. AAPS PharmSciTech 2019;20 (5). https://doi.org/10.1208/s12249-019-1393-0.

[172] Cervin C, Tinzl M, Johnsson M, Abrahamsson PA, Tiberg F, Dizeyi N. Properties and effects of a novel liquid crystal nanoparticle formulation of docetaxel in a prostate cancer mouse model. Eur J Pharm Sci 2010;41(2):369–75. https://doi.org/10.1016/j.ejps.2010.07.003.

[173] Li J, Wu L, Wu W, Wang B, Wang Z, Xin H. A potential carrier based on liquid crystal nanoparticles for ophthalmic delivery of pilocarpine nitrate. Int J Pharm 2013;455(1–2):75–84. https://doi.org/10.1016/j.ijpharm.2013.07.057.

[174] Chen Y, Lu Y, Zhong Y, Wang Q, Wu W, Gao S. Ocular delivery of cyclosporine A based on glyceryl monooleate/poloxamer 407 liquid crystalline nanoparticles: preparation, characterization, in vitro corneal penetration and ocular irritation. J Drug Target 2012;20(10):856–63. https://doi.org/10.3109/1061186X.2012.723214.

[175] Carvalho FC, Campos ML, Gonçalves Peccinini RG, Daflon Gremião MP. Nasal administration of liquid crystal precursor mucoadhesive vehicle as an alternative antiretroviral therapy. Eur J Pharm Biopharm 2013;84(1):219–27. https://doi.org/10.1016/j.ejpb.2012.11.021.

[176] Li Y, Angelova A, Liu J, Garamus VM, Li N, Drechsler M. In situ phase transition of microemulsions for parenteral injection yielding lyotropic liquid crystalline carriers of the antitumor drug bufalin. Colloids Surf B: Biointerfaces 2019;173: 217–25. https://doi.org/10.1016/j.colsurfb.2018.09.023.

[177] Streck L, Sarmento VHV, Machado PRL, Farias KJS, Fernandes-Pedrosa MF, da Silva-Júnior AA. Phase transitions of isotropic to anisotropic biocompatible lipid-based drug delivery systems overcoming insoluble benznidazole loading. Int J Mol Sci 2016;17(7):1–18. https://doi.org/10.3390/ijms17070981.

[178] Barauskas J, Cervin C, Jankunec M, Špandyreva M, Ribokaite K, Tiberg F. Interactions of lipid-based liquid crystalline nanoparticles with model and cell membranes. Int J Pharm 2010;391(1–2):284–91. https://doi.org/10.1016/j.ijpharm.2010.03.016.

[179] Abdelaziz HM, Elzoghby AO, Helmy MW, Samaha MW, Fang JY, Freag MS. Liquid crystalline assembly for potential combinatorial chemo–herbal drug delivery to lung cancer cells. Int J Nanomedicine 2019;14:499–517. https://doi.org/10.2147/IJN.S188335.

[180] Xia MQ, Liu L, Tian CL, Li Q, Hu RF, Gui SY, et al. Pharmacokinetics of sinomenine hydrochloride cubic liquid crystal injection based on microdialysis technology. J Drug Deliv Sci Technol 2019;52(1):553–8. https://doi.org/10.1016/j.jddst.2019.05.021.

[181] Fong WK, Hanley TL, Thierry B, Hawley A, Boyd BJ, Landersdorfer CB. External manipulation of nanostructure in photoresponsive lipid depot matrix to control and predict drug release in vivo. J Control Release 2016;228:67–73. https://doi.org/10.1016/j.jconrel.2016.02.042.

[182] Fong WK, Hanley T, Boyd BJ. Stimuli responsive liquid crystals provide "on-demand" drug delivery in vitro and in vivo. J Control Release 2009;135(3): 218–26. https://doi.org/10.1016/j.jconrel.2009.01.009.

[183] Fang X, Shen A. Preparation and evaluation of phytantriol liquid crystal as a liquid embolic agent. Pharm Dev Technol 2020;25(5):610–6. https://doi.org/10.1080/10837450.2020.1725046.

[184] Rajak P, Nath LK, Bhuyan B. Liquid crystals: an approach in drug delivery. Indian J Pharm Sci 2019;81(1):11–21.

[185] Nguyen TH, Hanley T, Porter CJH, Larson I, Boyd BJ. Phytantriol and glyceryl monooleate cubic liquid crystalline phases as sustained-release oral drug delivery systems for poorly water-soluble drugs II. In-vivo evaluation. J Pharm Pharmacol 2010;62(7):856–65. https://doi.org/10.1211/jpp.62.07.0006.

[186] Nguyen TH, Hanley T, Porter CJH, Larson I, Boyd BJ. Phytantriol and glyceryl monooleate cubic liquid crystalline phases as sustained-release oral drug delivery systems for poorly water soluble drugs I. Phase behaviour in physiologically-relevant media. J Pharm Pharmacol 2010;62(7):844–55. https://doi.org/10.1211/jpp.62.07.0005.

[187] Liu X, Wang Z, Wei L, Wei X. Curcumin-encapsulated hexagonal liquid crystalline formed by Brij 97/NaDC mixtures. J Dispers Sci Technol 2013;35(12):1699–708. https://doi.org/10.1080/01932691.2013.870042.

[188] Wen J, du Y., Li D., Alany R. Development of water-in-oil microemulsions with the potential of prolonged release for oral delivery of L-glutathione. Pharm Dev Technol 2013;18(6):1424–9. https://doi.org/10.3109/10837450.2012.734518.

[189] Waghule T, Rapalli VK, Singhvi G, Gorantla S, Khosa A, Dubey SK. Design of temozolomide-loaded proliposomes and lipid crystal nanoparticles with industrial feasible approaches: comparative assessment of drug loading, entrapment efficiency, and stability at plasma pH. J Liposome Res 2021;31(2):158–68. https://doi.org/10.1080/08982104.2020.1748648.

[190] Singh A, Kumar A, Verma RK, Shukla R. Silymarin encapsulated nanoliquid crystals for improved activity against beta amyloid induced cytotoxicity. Int J Biol Macromol 2020;149:1198–206. https://doi.org/10.1016/j.ijbiomac.2020.02.041.

[191] Yang Z, Tan Y, Chen M, Dian L, Shan Z, Peng X. Development of amphotericin B-loaded cubosomes through the SolEmuls technology for enhancing the oral bioavailability. AAPS PharmSciTech 2012;13(4):1483–91. https://doi.org/10.1208/s12249-012-9876-2.

[192] Patil S, Kadam C, Pokharkar V. QbD based approach for optimization of Tenofovir disoproxil fumarate loaded liquid crystal precursor with improved permeability. J Adv Res 2017;8(6):607–16. https://doi.org/10.1016/j.jare.2017.07.005.

[193] Pham AC, Hong L, Montagnat O, Nowell CJ, Nguyen TH, Boyd BJ. In vivo formation of cubic phase in situ after oral administration of cubic phase precursor formulation provides long duration gastric retention and absorption for poorly water-soluble drugs. Mol Pharm 2016;13(1):280–6. https://doi.org/10.1021/acs.molpharmaceut.5b00784.

[194] Negrini R, Mezzenga R. PH-responsive lyotropic liquid crystals for controlled drug delivery. Langmuir 2011;27(9):5296–303. https://doi.org/10.1021/la200591u.

[195] Nair SC, Anoop KR. Intraperiodontal pocket: an ideal route for local antimicrobial drug delivery. J Adv Pharm Technol Res 2012;3(1):9–15.

[196] Jiang X, Liang X, Wang S, Guo J, Tao Y, Gui S. An injectable in situ hexagonal mesophase system for local delivery of minocycline hydrochloride: preparation and pharmacodynamics in rats. Pharmazie 2017;72(5):249–56. https://doi.org/10.1691/ph.2017.6190.

[197] Lee WH, Loo CY, Ghadiri M, Leong CR, Young PM, Traini D. The potential to treat lung cancer via inhalation of repurposed drugs. Adv Drug Deliv Rev 2018;133: 107–30. https://doi.org/10.1016/j.addr.2018.08.012.

[198] Abdelaziz HM, Elzoghby AO, Helmy MW, Abdelfattah EZA, Fang JY, Samaha MW. Inhalable Lactoferrin/chondroitin-functionalized monoolein nanocomposites for localized lung cancer targeting. ACS Biomater Sci Eng 2020;6 (2):1030–42. https://doi.org/10.1021/acsbiomaterials.9b01639.

[199] Libster D, Aserin A, Yariv D, Shoham G, Garti N. Concentration- and temperature-induced effects of incorporated desmopressin on the properties of reverse hexagonal mesophase. J Phys Chem B 2009;113(18):6336–46. https://doi.org/10.1021/jp810309d.

[200] Chemelli A, Maurer M, Geier R, Glatter O. Optimized loading and sustained release of hydrophilic proteins from internally nanostructured particles. Langmuir 2012;28(49):16788–97. https://doi.org/10.1021/la303373q.

[201] Petrilli R, Eloy JO, Praça FSG, del Ciampo JO, Fantini MAC, Fonseca MJV. Liquid crystalline nanodispersions functionalized with cell-penetrating peptides for topical delivery of short-interfering RNAs: A proposal for silencing a pro-inflammatory cytokine in cutaneous diseases. J Biomed Nanotechnol 2016;12(5): 1063–75. https://doi.org/10.1166/jbn.2016.2211.

[202] Swamy MN, Wu H, Shankar P. Recent advances in RNAi-based strategies for therapy and prevention of HIV-1/AIDS. Adv Drug Deliv Rev 2016;103:174–86. https://doi.org/10.1016/j.addr.2016.03.005.

[203] Whitehead KA, Langer R, Anderson DG. Knocking down barriers: advances in siRNA delivery. Nat Rev Drug Discov 2009;8(2):129–38. https://doi.org/10.1038/nrd2742.

[204] Wang J, Lu Z, Wientjes MG, Au JLS. Delivery of siRNA therapeutics: barriers and carriers. AAPS J 2010;12(4):492–503. https://doi.org/10.1208/s12248-010-9210-4.

[205] He S, Fan W, Wu N, Zhu J, Miao Y, Miao X. Lipid-based liquid crystalline nanoparticles facilitate cytosolic delivery of siRNA via structural transformation. Nano Lett 2018;18(4):2411–9. https://doi.org/10.1021/acs.nanolett.7b05430.

[206] Ross K. Towards topical microRNA-directed therapy for epidermal disorders. J Control Release November 2017;2018(269):136–47. https://doi.org/10.1016/j.jconrel.2017.11.013.

[207] Tofani LB, Depieri LV, Campos PM, Riul TB, Antonietto KS, Abreu Fantini MC. In vitro TyRP-1 knockdown based on siRNA carried by liquid crystalline Nanodispersions: an alternative approach for topical treatment of vitiligo. Pharm Res 2018;35(5). https://doi.org/10.1007/s11095-017-2330-0.

[208] Sarkar S, Tran N, Soni SK, Conn CE, Drummond CJ. Size-dependent encapsulation and release of dsDNA from cationic lyotropic liquid crystalline cubic phases. ACS Biomater Sci Eng 2020;6(8):4401–13. https://doi.org/10.1021/acsbiomaterials.0c00085.

[209] Ki MH, Lim JL, Ko JY, Park SH, Kim JE, Cho HJ. A new injectable liquid crystal system for one month delivery of leuprolide. J Control Release 2014;185(1): 62–70. https://doi.org/10.1016/j.jconrel.2014.04.034.

[210] Wang B, Huang Y, Huang Z, Wang H, Chen J, Pan X. Self-assembling in situ gel based on lyotropic liquid crystals containing VEGF for tissue regeneration. Acta Biomater 2019;99:84–99. https://doi.org/10.1016/j.actbio.2019.09.011.

[211] Chung H, Kim J, Um JY, Kwon IC, Jeong SY. Self-assembled "nanocubicle" as a carrier for peroral insulin delivery. Diabetologia 2002;45(3):448–51. https://doi.org/10.1007/s00125-001-0751-z.

[212] Zhai J, Scoble JA, Li N, Lovrecz G, Waddington LJ, Tran N. Epidermal growth factor receptor-targeted lipid nanoparticles retain self-assembled nanostructures and provide high specificity. Nanoscale 2015;7(7):2905–13. https://doi.org/10.1039/c4nr05200e.

[213] Rakoff-Nahoum S, Medzhitov R. Regulation of spontaneous intestinal tumorigenesis through the adaptor protein MyD88. Science (1979) 2007;317 (5834):124–7. https://doi.org/10.1126/science.1140488.

[214] Sánchez Vallecillo MF, Minguito de la Escalera MM, Aguirre MV, Ullio Gamboa GV, Palma SD, González-Cintado L, et al. A liquid crystal of ascorbyl palmitate, used as vaccine platform, provides sustained release of antigen and has intrinsic pro-inflammatory and adjuvant activities which are dependent on MyD88 adaptor protein. J Control Release 2015;214:12–22. https://doi.org/10.1016/j.jconrel.2015.07.008.

[215] Kawai M, Ibaraki H, Takashima Y, Kanazawa T, Okada H. Development of a liquid crystal formulation that can penetrate the stratum Corneum for intradermal delivery of small interfering RNA. Mol Pharm 2021;18(3):1038–47. https://doi.org/10.1021/acs.molpharmaceut.0c00997.

[216] Sarkar S, Tran N, Soni SK, Nasa Z, Drummond CJ, Conn CE. Cuboplex-mediated nonviral delivery of functional siRNA to Chinese Hamster ovary (CHO) cells. ACS Appl Mater Interfaces 2021;13(2):2336–45. https://doi.org/10.1021/acsami.0c20956.

[217] Rakotoarisoa M, Angelov B, Drechsler M, Nicolas V, Bizien T, Gorshkova YE, et al. Liquid crystalline lipid nanoparticles for combined delivery of curcumin, fish oil and BDNF: in vitro neuroprotective potential in a cellular model of tunicamycin-induced endoplasmic reticulum stress. Smart Mater Med 2022;3:274–88. https://doi.org/10.1016/j.smaim.2022.03.001.

[218] Thorn CR, Raju D, Lacdao I, Gilbert S, Sivarajah P, Howell PL, et al. Protective liquid crystal nanoparticles for targeted delivery of PslG: A biofilm dispersing enzyme. ACS Infect Dis 2021;7(8):2102–15. https://doi.org/10.1021/acsinfecdis.1c00014.

[219] Rodero CF, Fioramonti Calixto GM, Cristina Dos Santos K, Sato MR, Dos Santos Aparecido, Ramos M, et al. Curcumin-loaded liquid crystalline systems for controlled drug release and improved treatment of vulvovaginal candidiasis. Mol Pharm 2018;15(10):4491–504. https://doi.org/10.1021/acs.molpharmaceut.8b00507.

[220] Tajik-Ahmadabad B, Chollet L, White J, Separovic F, Polyzos A. Metallo-Cubosomes: zinc-functionalized cubic nanoparticles for therapeutic nucleotide delivery. Mol Pharm 2019;16(3):978–86. https://doi.org/10.1021/acs.molpharmaceut.8b00890.

[221] Zhou C, Huang Z, Huang Y, Wang B, Yang P, Fan Y. In situ gelation of rhEGF-containing liquid crystalline precursor with good cargo stability and system mechanical properties: A novel delivery system for chronic wounds treatment. Biomater Sci 2019;7(3):995–1010. https://doi.org/10.1039/c8bm01196f.

[222] Valldeperas M, Talaikis M, Dhayal SK, Velička M, Barauskas J, Niaura G. Encapsulation of aspartic protease in nonlamellar lipid liquid crystalline phases. Biophys J 2019;117(5):829–43. https://doi.org/10.1016/j.bpj.2019.07.031.

[223] Mohsen AM, Younis MM, Salama A, Darwish AB. Cubosomes as a potential oral drug delivery system for enhancing the hepatoprotective effect of coenzyme Q10. J Pharm Sci 2021;110(7):2677–86. https://doi.org/10.1016/j.xphs.2021.02.007.

[224] Lampis S, Carboni M, Steri D, Murgia S, Monduzzi M. Lipid based liquid-crystalline stabilized formulations for the sustained release of bioactive hydrophilic molecules. Colloids Surf B: Biointerfaces 2018;168:35–42. https://doi.org/10.1016/j.colsurfb.2018.03.002.

[225] Borgheti-Cardoso LN, Kooijmans SAA, Fens MHAM, Meel R, Vicentini FTMC, Fantini MCA. In situ gelling liquid crystalline system as local siRNA delivery system. Mol Pharm 2017;14(5):1681–90. https://doi.org/10.1021/acs.molpharmaceut.6b01141.

[226] Kozaka S, Wakabayashi R, Kamiya N, Goto M. Lyotropic liquid crystal-based transcutaneous peptide delivery system: evaluation of skin permeability and potential for transcutaneous vaccination. Acta Biomater 2022;138:273–84. https://doi.org/10.1016/j.actbio.2021.11.008.

[227] Kraft JC, Ho RJY. Interactions of indocyanine green and lipid in enhancing near-infrared fluorescence properties: the basis for near-infrared imaging in vivo. Biochemistry 2014;53(8):1275–83. https://doi.org/10.1021/bi500021j.

[228] Alcaraz N, Boyd BJ. Cubosomes as carriers for MRI contrast agents. Curr Med Chem 2017;24(5):470–82. https://doi.org/10.2174/0929867323666160817141556.

[229] Muir BW, Acharya DP, Kennedy DF, Mulet X, Evans RA, Pereira SM. Metal-free and MRI visible theranostic lyotropic liquid crystal nitroxide-based nanoparticles. Biomaterials 2012;33(9):2723–33. https://doi.org/10.1016/j.biomaterials.2011.12.018.

[230] Moghaddam MJ, Campo L, Waddington LJ, Weerawardena A, Kirby N, Drummond CJ. Chelating oleyl-EDTA amphiphiles: self-assembly, colloidal particles, complexation with paramagnetic metal ions and promise as magnetic resonance imaging contrast agents. Soft Matter 2011;7(22):10994–1005. https://doi.org/10.1039/c1sm05957b.

[231] Moghaddam MJ, Campo L, Waddington LJ, Drummond CJ. Chelating phytanyl-EDTA amphiphiles: self-assembly and promise as contrast agents for medical imaging. Soft Matter 2010;6(23):5915–29. https://doi.org/10.1039/c0sm00586j.

[232] Conn CE, Panchagnula V, Weerawardena A, Waddington LJ, Kennedy DF, Drummond CJ. Lanthanide phytanates: liquid-crystalline phase behavior, colloidal particle dispersions, and potential as medical imaging agents. Langmuir 2010;26(9):6240–9. https://doi.org/10.1021/la904006q.

[233] Natalin RA, Prince MR, Grossman ME, Silvers D, Landman J. Contemporary applications and limitations of magnetic resonance imaging contrast materials. J Urol 2010;183(1):27–33. https://doi.org/10.1016/j.juro.2009.09.029.

[234] Gupta A, Stait-Gardner T, Campo L, Waddington LJ, Kirby N, Price WS. Nanoassemblies of Gd-DTPA-monooleyl and glycerol monooleate amphiphiles as potential MRI contrast agents. J Mater Chem B 2014;2(9):1225–33. https://doi.org/10.1039/c3tb21069c.

[235] Zhai J, Bao L, Walduck AK, Dyett BP, Cai X, Li M, et al. Enhancing the photoluminescence and cellular uptake of fluorescent carbon nanodots via cubosome lipid nanocarriers. Nanoscale 2022;14(48):17940–54.

[236] Urandur S, Banala VT, Shukla RP, Gautam S, Marwaha D, Rai N, et al. Theranostic lyotropic liquid crystalline nanostructures for selective breast cancer imaging and therapy. Acta Biomater 2020;113:522–40. https://doi.org/10.1016/j.actbio.2020.06.023.

[237] Helvig SY, Andersen H, Antopolsky M, Airaksinen AJ, Urtti A, Yaghmur A. Hexosome engineering for targeting of regional lymph nodes. Materialia (Oxf) 2020:11. https://doi.org/10.1016/j.mtla.2020.100705.

[238] Jenni S, Picci G, Fornasier M, Mamusa M, Schmidt J, Talmon Y, et al. Multifunctional cubic liquid crystalline nanoparticles for chemo- A nd photodynamic synergistic cancer therapy. Photochem Photobiol Sci 2020;19(5):674–80. https://doi.org/10.1039/c9pp00449a.

[239] Bazylińska U, Wawrzyńczyk D, Kulbacka J, Picci G, Manni LS, Handschin S, et al. Hybrid theranostic cubosomes for efficient NIR-induced photodynamic therapy. ACS Nano 2022;16(4):5427–38. https://doi.org/10.1021/acsnano.1c09367.

[240] Nag OK, Naciri J, Oh E, Spillmann CM, Delehanty JB. Targeted plasma membrane delivery of a hydrophobic cargo encapsulated in a liquid crystal nanoparticle carrier. J Vis Exp 2017;120:1–8. https://doi.org/10.3791/55181.

[241] Yao B, Cong Y, Zhang B, Wang Z, Chen F, Sun C. Novel luminescent chiral network liquid-crystalline polymers containing Sm(iii) ions. RSC Adv 2016;6(48):42745–50. https://doi.org/10.1039/c6ra07388c.

[242] Meli V, Caltagirone C, Sinico C, Lai F, Falchi AM, Monduzzi M. Theranostic hexosomes for cancer treatments: an in vitro study. New J Chem 2017;41(4):1558–65. https://doi.org/10.1039/c6nj03232j.

[243] Meli V, Caltagirone C, Falchi AM, Hyde ST, Lippolis V, Monduzzi M. Docetaxel-loaded fluorescent liquid-crystalline nanoparticles for cancer theranostics. Langmuir 2015;31(35):9566–75. https://doi.org/10.1021/acs.langmuir.5b02101.

[244] Tran N, Bye N, Moffat BA, Wright DK, Cuddihy A, Hinton TM. Dual-modality NIRF-MRI cubosomes and hexosomes: high throughput formulation and in vivo biodistribution. Mater Sci Eng C 2017;71(1):584–93. https://doi.org/10.1016/j.msec.2016.10.028.

[245] Urandur S, Banala VT, Shukla, Mittapelly RP, Pandey G, Kalleti N. Anisamide-anchored lyotropic nano-liquid crystalline particles with AIE effect: A smart optical Beacon for tumor imaging and therapy. ACS Appl Mater Interfaces 2018;10(15):12960–74. https://doi.org/10.1021/acsami.7b19109.

[246] Shin D, Vigneswaran N, Gillenwater A, Richards-Kortum R. Advances in fluorescence imaging techniques to detect oral cancer and its precursors. Future Oncol 2010;6(7):1143–54.

[247] He Z, Ke C, Tang BZ. Journey of aggregation-induced emission research. ACS Omega 2018;3(3):3267–77. https://doi.org/10.1021/acsomega.8b00062.

[248] Prévôt M, Hegmann E. Biomimetic, and Polymer to Biodegradable and Biocompatible Liquid Crystal Elastomer Cell Scaffolds. In: Ito Y, Chen X, Kang IK, editors. Advances in Bioinspired and Biomedical Materials. ACS Symp Ser; 2017. p. 3–45. https://doi.org/10.1021/bk-2017-1253.ch001.

[249] Martella D, Parmeggiani C. Advances in cell scaffolds for tissue engineering: the value of liquid crystalline elastomers. Chem Eur J 2018;24(47):12206–20. https://doi.org/10.1002/chem.201800477.

[250] Prévôt ME, Ustunel S, Hegmann E. Liquid crystal elastomers-A path to biocompatible and biodegradable 3D-LCE scaffolds for tissue regeneration. Materials 2018;11(3). https://doi.org/10.3390/ma11030377.

[251] Norlén L, Gil IP, Simonsen A, Descouts P. Human stratum corneum lipid organization as observed by atomic force microscopy on Langmuir-Blodgett films. J Struct Biol 2007;158(3):386–400. https://doi.org/10.1016/j.jsb.2006.12.006.

[252] Soon CF, Thong KT, Tee KS, Ismail AB, Denyer M, Ahmad MK. A scaffoldless technique for self-generation of three-dimensional keratinospheroids on liquid crystal surfaces. Biotech Histochem 2016;91(4):283–95. https://doi.org/10.3109/10520295.2016.1158865.

[253] Xie W, Ouyang R, Wang H, Zhou C. Construction and biocompatibility of three-dimensional composite polyurethane scaffolds in liquid crystal state. ACS Biomater Sci Eng 2020;6(4):2312–22. https://doi.org/10.1021/acsbiomaterials.9b01838.

[254] Chen J, Yang Y, Wu J, Rui X, Wang W, Ren R. Spatiotemporal variations of contact stress between liquid-crystal films and fibroblasts guide cell fate and skin regeneration. Colloids Surf B: Biointerfaces 2020:188. https://doi.org/10.1016/j.colsurfb.2019.110745.

[255] Prévôt ME, Ustunel S, Bergquist LE, Cukelj R, Gao Y, Mori T. Synthesis of biocompatible liquid crystal elastomer foams as cell scaffolds for 3D spatial cell cultures. J Vis Exp 2017;122:1–11. https://doi.org/10.3791/55452.

[256] Prévôt ME, Andro H, Alexander SLM, Ustunel S, Zhu C, Nikolov Z. Liquid crystal elastomer foams with elastic properties specifically engineered as biodegradable brain tissue scaffolds. Soft Matter 2018;14(3):354–60. https://doi.org/10.1039/c7sm01949a.

[257] Gao Y, Mori T, Manning S, Zhao Y, Nielsen AD, Neshat A. Biocompatible 3D liquid crystal elastomer cell scaffolds and foams with primary and secondary porous architecture. ACS Macro Lett 2016;5(1):4–9. https://doi.org/10.1021/acsmacrolett.5b00729.

[258] Sharma A, Neshat A, Mahnen CJ, Nielsen AD, Snyder J, Stankovich TL. Biocompatible, biodegradable and porous liquid crystal elastomer scaffolds for spatial cell cultures. Macromol Biosci 2015;15(2):200–14. https://doi.org/10.1002/mabi.201400325.

[259] Sharma A, Mori T, Mahnen CJ, Everson HR, Leslie MT, Nielsen AD, et al. Effects of structural variations on the cellular response and mechanical properties of biocompatible, biodegradable, and porous Smectic liquid crystal elastomers. Macromol Biosci 2017;17(2):1–14. https://doi.org/10.1002/mabi.201600278.

[260] Wang H, Peng T, Wu H, Chen J, Chen M, Mei L, et al. In situ biomimetic lyotropic liquid crystal gel for full-thickness cartilage defect regeneration. J Control Release 2021;338:623–32. https://doi.org/10.1016/j.jconrel.2021.08.062.

[261] Mancuso A, Cianflone E, Cristiano MC, Salerno N, Tarsitano M, Marino F, et al. Lyotropic liquid crystals: A biocompatible and safe material for local cardiac application. Pharmaceutics 2022;14(2). https://doi.org/10.3390/pharmaceutics14020452.

[262] Murakami T, Hijikuro I, Yamashita K, Tsunoda S, Hirai K, Suzuki T. Antiadhesion effect of the C17 glycerin ester of isoprenoid-type lipid forming a nonlamellar liquid crystal. Acta Biomater 2019;84:257–67. https://doi.org/10.1016/j.actbio.2018.12.009.

[263] Lancelot A, Sierra T, Serrano JL. Nanostructured liquid-crystalline particles for drug delivery. Expert Opin Drug Deliv 2014:547–64. https://doi.org/10.1517/17425247.2014.884556.

[264] Fong WK, Negrini R, Vallooran JJ, Mezzenga R, Boyd BJ. Responsive self-assembled nanostructured lipid systems for drug delivery and diagnostics. J Colloid Interface Sci 2016:320–39. https://doi.org/10.1016/j.jcis.2016.08.077.

[265] Zhai J, Suryadinata R, Luan B, Tran N, Hinton TM, Ratcliffe J, et al. Amphiphilic brush polymers produced using the RAFT polymerisation method stabilise and reduce the cell cytotoxicity of lipid lyotropic liquid crystalline nanoparticles. Faraday Discuss 2016;191:545–63. https://doi.org/10.1039/c6fd00039h.

[266] Zhai J, Hinton TM, Waddington LJ, Fong C, Tran N, Mulet X, et al. Lipid-PEG conjugates sterically stabilize and reduce the toxicity of phytantriol-based lyotropic liquid crystalline nanoparticles. Langmuir 2015;31(39):10871–80. https://doi.org/10.1021/acs.langmuir.5b02797.

[267] Chong JYT, Mulet X, Keddie DJ, Waddington L, Mudie ST, Boyd BJ, et al. Novel steric stabilizers for lyotropic liquid crystalline nanoparticles: PEGylated-phytanyl copolymers. Langmuir 2015;31(9):2615–29. https://doi.org/10.1021/la501471z.

[268] Shah S, Joga R, Kolipaka T, Sabnis Dushyantrao C, Khairnar P, Simran, et al. Paradigm of lyotropic liquid crystals in tissue regeneration. Int J Pharm 2023; 634:122633. https://doi.org/10.1016/j.ijpharm.2023.122633.

[269] Rapalli VK, Waghule T, Hans N, Mahmood A, Gorantla S, Dubey SK, et al. Insights of lyotropic liquid crystals in topical drug delivery for targeting various skin disorders. J Mol Liq 2020. https://doi.org/10.1016/j.molliq.2020.113771.

[270] Lin SY. Thermoresponsive gating membranes embedded with liquid crystal(s) for pulsatile transdermal drug delivery: an overview and perspectives. J Control Release 2020;319:450–74. https://doi.org/10.1016/j.jconrel.2019.12.046.

[271] Sivakumar S, Wark KL, Gupta JK, Abbott NL, Caruso F. Liquid crystal emulsions as the basis of biological sensors for the optical detection of bacteria and viruses.

Adv Funct Mater 2009;19(14):2260–5. https://doi.org/10.1002/adfm.200900399.

[272] Rowiński P, Korytkowska A, Bilewicz R. Diffusion of hydrophilic probes in bicontinuous lipidic cubic phase. Chem Phys Lipids 2003;124(2):147–56. https://doi.org/10.1016/S0009-3084(03)00051-3.

[273] Fraser SJ, Mulet X, Martin L, Praporski S, Mechler A, Hartley PG, et al. Surface immobilization of bio-functionalized cubosomes: sensing of proteins by quartz crystal microbalance. Langmuir 2012;28(1):620–7. https://doi.org/10.1021/la2032994.

[274] Debas MVM, Freire R, Salentinig S. Supramolecular design of CO2-responsive lipid nanomaterials. J Colloid Interface Sci 2023. https://doi.org/10.1016/j.jcis.2023.01.060.