



Treball Final de Grau

Bibliographical study of water-in-water emulsions and their stabilization

Estudio bibliográfico de emulsiones agua-agua y su estabilización

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"El agua es un caos sensible."

Novalis

Quiero agradecer a José María Gutiérrez su tiempo y su tutela y en especial a mi compañera y a mi familia que han sido un apoyo incondicional y me han inspirado en todo momento.

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SUMMARY

The interactions of water-soluble biopolymers can cause the separation of hydrophilic phases and the dispersion of one phase in the other resulting in water-in-water emulsions. These water-in-water emulsions can have different applications in food because they allow traditional textures without the need to use unhealthy ingredients such as fats.

Water-in-water emulsions, unlike oil-water emulsions, cannot be stabilized by surfactants, which means that one of the biggest impediments in the use of water-in-water emulsions is their stabilization. Recently studies have determined that these emulsions can be stabilized from the addition of particles which are located at the interface of the two aqueous phases preventing a macroscopic separation of phases. The addition of particles in oil-water emulsions has been widely studied for years in contrast to water-in-water emulsions. The behaviour of water-in-water emulsions stabilized by the addition of particles is quite different from that of oil-water emulsions because the interfacial tension is much lower and the much larger length scale at which the interphase expresses itself.

This work deals with a bibliographic review of different water-in-water emulsion stabilization studies giving importance to emulsions with possible future applications in food and the factors on which this stabilization depends.

Keywords: Water-in-water emulsions, phase separation, stabilization, Pickering, addition of particles, functional foods.

RESUMEN

Las interacciones de biopolímeros solubles en agua pueden provocar la separación de fases hidrófilas y la dispersión de una fase en la otra resultando en emulsiones agua-en-agua. Estas emulsiones agua-en-agua pueden tener distintas aplicaciones en alimentación ya que permiten obtener texturas tradicionales sin la necesidad de utilizar ingredientes poco saludables como las grasas.

Las emulsiones agua-en-agua, al contrario que las emulsiones formadas por aceite y agua, no pueden ser estabilizadas por surfactantes ya que provoca que uno de los mayores impedimentos en el uso de emulsiones agua-en-agua sea su estabilización. Recientemente estudios han determinado que estas emulsiones pueden ser estabilizadas a partir de la adición de partículas las cuales se sitúan en la interfaz de las dos fases acuosas impidiendo que haya una separación macroscópica de fases. La adición de partículas en emulsiones de aceite y agua ha sido ampliamente estudiada durante años al contrario que las emulsiones agua-en-agua. El comportamiento de emulsiones agua-en-agua estabilizadas por adición de partículas es bastante diferente al de las emulsiones formadas por aceite y agua debido a que la tensión interfacial es mucho menor y mayor la escala de longitud a la que se expresa la interfaz.

Este trabajo trata de una revisión bibliográfica de distintos estudios sobre la estabilización de emulsiones agua-en-agua dando importancia a las posibles aplicaciones futuras en alimentación y a los factores de los que depende esta estabilización.

Palabras clave: Emulsiones agua-en-agua, separación de fases, estabilización, Pickering, adición de partículas, alimentos funcionales.

1. INTRODUCTION

In recent years, important changes are taking place in consumption habits driven by the continuous appearance of scientific evidences that show how through the diet and / or its components some specific physiological functions can be modulated in the organism and therefore promote wellness and health. There is great interest in search new systems that could serve as the base of next generation low-fat food. In this sense, continuous progress is being made in the development of healthier perceived foods. Food products are complex systems containing mainly different kinds of ingredients. Emulsions are part of our daily diets, think of mayonnaise, milk, creams or butter.

An emulsion is a thermodynamically unstable dispersion of two or more immiscible or partially miscible liquids. Emulsions are part of a more general class of two-phase systems of matter called colloids. Although the terms colloid and emulsion are sometimes used interchangeably, emulsion should be used when both phases, one dispersed and one continuous, are liquids. When we join two immiscible liquids they form two macroscopic phases, with mechanical agitation one phase can be dispersed in the other, that is what we call an emulsion, if the emulsion is not stabilized will end up in two phases again, losing the properties that had as emulsion. Generally they are stabilized with the presence of an emulsifying agent also called surfactant (figure 1). Concentrated emulsions are an important means for texturize foods.

Water-in-oil and oil-in-water emulsions are the most common emulsions used in food products, what means fat or oil droplets dispersed in a continuous water phase or vice versa. An inherent disadvantage of using oil-based emulsions in food is their high caloric content. Some of these studies consist in a way of find water-soluble components that are immiscible with each other. The objective of these studies is to replace oil-based emulsions with water-in-water emulsions just to make them healthier.

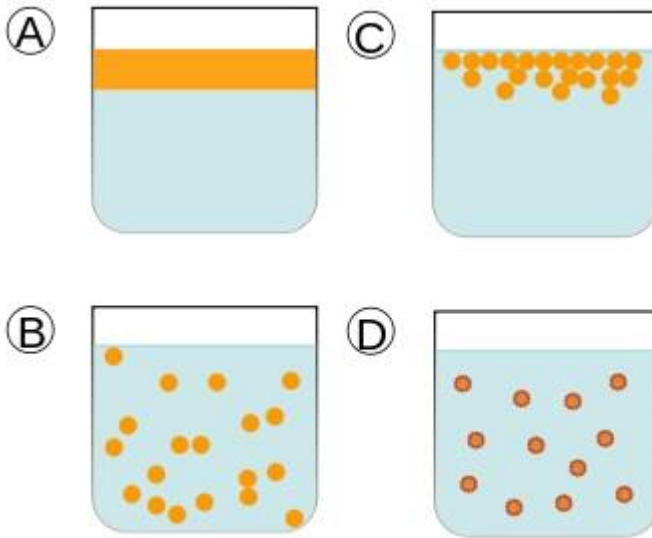


Figure 1. Blue region = Phase I, Orange region = Phase II, Purple surface = Surfactant.

A. Two immiscible liquids, not emulsified. B. Emulsion of Phase II dispersed in Phase I. C. The unstable emulsion progressively separates. D. Surfactant positions itself on interface between Phases I and II, stabilizing emulsion. (via Wikipedia)

Water-in-water emulsions are formed when aqueous solutions of at least two mutually incompatible water-soluble macromolecules are mixed. Leading to a dispersed phase enriched with one macromolecule and a continuous phase enriched with the other. The phase with smaller volume is the dispersed phase thus the phase with bigger volume becomes the continuous phase. This phenomenon has been known for a long time reported for first time by Beijerinck in 1896 ⁽⁵⁾, who discovered by serendipity, observing the appearance of droplets while he mixed starch solution with gelatine. He described that the emulsion was composed of two different aqueous solutions and that each of the phases were enriched with one of the two polymer (starch or gelatine) and could be inverted depending on the polymer ratio. He also realized that the droplet size could be controlled by agitation. Although he did not name this discovery as water-in-water emulsions.

Water-in-water have a wide range of applications due to their all-water nature, in absence of oils and surfactants. Water-in-water emulsions consequently can be used to encapsulate hydrophilic active components of drugs or at food formulations. Food products often contain mixtures of incompatible water soluble macromolecules such as proteins and polysaccharides. The purpose of replacing water-in-water emulsions with water oil is to achieve more nutritious and healthy properties for our organism. Certainly the field of water-in-water emulsions can improve organoleptic and health properties of food systems. This bibliographic review will be focused in water-in-water emulsions and their stabilization.

2. OBJECTIVES

The purpose of this bibliographic review is concentrated in the current progress in stabilization of water-in-water food grade emulsions area and the knowledge of the new methodologies to stabilize them.

Nowadays the published studies have been used from food, to pharmaceutical, cosmetic and personal care industries, due to their high potential for applications. For example, the goal in food area could be improve nutrients of aliments, reducing the fat component of common water-in-oil emulsions, or releasing the digestion maximizing the uptake of nutrients and delaying gastric emptying promoting satiety. On pharmaceutical area could be reducing the collateral damage of drugs.

This thesis pretends to get a conclusion about which are the best ways to stabilize water-in-water emulsions, and the factors that are in involved in the process. Making a recovery of important stabilization studies and recent studies that have been made. Giving importance to water-in-water emulsions that can be used in food formulation, to develop healthier aliments, etc.

3. PICKERING EMULSIONS

Stabilizing droplets against coalescence with colloidal particles was reported at the early 1900's for first time. This type of emulsions are commonly called Pickering emulsions, due to one of the discoverers of the phenomenon (1,2). Since this reports, particle-stabilized emulsions have been used in many fields. For oil-in-water emulsions the stabilizing effects of particles accumulation at the interface have been known for a long time. Accumulation in the interface of micro particles and the stabilization of water-in-water emulsion formed mixtures of incompatible polysaccharides was observed by Poortinga (3). In 2009 Firoozmand, Murray and Dickinson (6) reported that the addition of particles and their establishment on the interface was able to stop macroscopic phase separation and demonstrated that Pickering emulsions could be prepared in water-in-water emulsions.

The increase of the free energy (ΔG) formed by the increment of the interfacial area are between phase A and phase B produced by the departure of the particle, maintains the particle at the interface. The surface of the particle at the interphase is exposed to both phases, which means that the energy is only reduced when the interfacial tension of the particle with each phase is smaller than the interfacial tension between the two phases. The free energy (ΔG) needed to detach a spherical particle from the interface depends on the size of the particle (πR^2), the interfacial tension (γ_{AB}) and the contact angle of the particle with the interface (θ) (Eq.1).

$$\Delta G = -\pi R^2 \gamma_{AB} (1 - |\cos \theta|)^2 \quad (\text{Eq.1})$$

The adsorption contact angle of spherical particles was measured by Balakrishnan (18), adding particles to a Polyethylene oxide/dextran system. The contact angle depends on the difference between the interfacial tension of the particles with phase A (γ_{Ap}) or phase B (γ_{Bp}) (Eq.2).

$$\cos \theta = (\gamma_{Ap} - \gamma_{Bp})/\gamma_{AB} \quad (\text{Eq.2})$$

Generally oil-in-water emulsions free energy (ΔG) has greater values than the kinetic energy for nanoparticles. This effect is much smaller for water-in-water emulsions because the interfacial tensions between the two phases are lower than the oil-in-water values, and goes to zero in critical points. That is why Firoozmand ⁽⁶⁾ proposed that the accumulation of particles at the water-in-water emulsions interface was depletion of the particles from the polymer solution towards the solvent rich interface. The energy of adsorption strongly depends on the particle size (R^2) ^(eq.1) of a particle so taking realistic values even for water-in-water emulsions free energy (ΔG) can have greater values than kinetic energy when particles are not too small and mixtures too near to the critical point. Large particles are able to adsorb and stabilize while little ones are not. Balakrishnan ⁽¹⁸⁾ used latex particles with size around $1\mu\text{m}$ and concluded that this size is enough to anchor the particles at the interface and achieve a good stabilization ^(Figure.2).

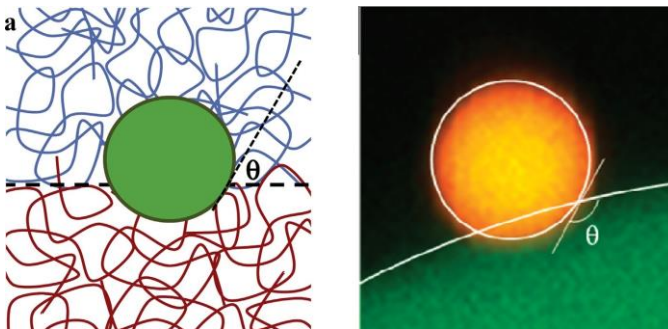


Figure. 2. a. Schematic drawing of a particle at the interface between two incompatible polymer solutions. θ is the contact angle of the particle with the interface. b. Latex particle ($R = 1\mu\text{m}$) at the surface of a large dextran drop in a continuous Polyethylene oxide phase (Balakrishnan, G., Nicolai, T., Benyahia, L., & Durand, D. (2012). ⁽¹⁸⁾).

In the last years the interest in water-in-water emulsions stabilization has increased, so far much of this researches have been done for non-food systems, although a number of food

grade emulsions have been investigated. As Poortinga and Firoozmand demonstrated, particles can accumulate at the interphase of water-in-water systems, this opens a new method for the stabilization of aqueous systems. In the following sections we will focus in water-in-water emulsions and we will see some researches made on water-in-water emulsions stabilization by using addition of particles (Pickering emulsions).

4. WATER-IN-WATER EMULSIONS

4.1. WATER-IN-WATER EMULSIONS FUNDAMENTS

Water-in-water separation can be found in a large variety of systems which include aqueous mixtures of polymers, polymers and surfactants, polymers and electrolytes and surfactant solutions.

As mentioned in the introduction, Beijerinck was the first to talk about water-in-water emulsions in 1986. He was able to invert the emulsion and modify the drops size by varying the polymer ratios and the agitation intensity.

Esquena suggested that the term water-in-water emulsions is preferable to aqueous two phase mixtures as it is self-defining, non-ambiguous and most common in recent works. Using this term also excludes other water-in-water systems just as colloidal dispersions of liquid crystals⁽⁷⁾.

Water-in-water emulsions are constituted by a mixture of two aqueous macromolecule solutions which are thermodynamically incompatible and therefore separate in two phases. Droplets will be formed by one aqueous phase dispersed into the other aqueous phase. In the end droplets will form coalesce until the two phases are macroscopically separated.

Nowadays is known that phase separation in aqueous mixtures of two hydrophilic polymers can occur in two different ways, segregative and associative. This depends on the repulsion or attraction between the two hydrophilic polymers and the hydration interaction between each polymer and water⁽¹²⁾.

Segregative phase separation is produced when hydrophilic polymers repel each other. When there are low polymer concentrations the mixture remains in a one phase solution containing both polymers. At some minimum concentrations, two immiscible phases appear in the solution with one phase enriched with one of the components and the other phase enriched with the other. In conditions where the two polymers have no opposite charges, the negative

entropy of mixing induce to the thermodynamic incompatibility. It's correct to say that phase separation occurs because of the thermodynamic incompatibility of the two components, above certain concentrations (figure 3).

Figure 3.a illustrates a schematic ternary phase diagram which illustrates segregative phase separation, where the immiscibility region is delimited by a bimodal line. Equilibrium compositions are indicated by tie-lines which converge in one point where the two immiscible phases become in one unique phase. Segregative phase commonly occur in mixtures of non-ionic polymers or in the case of one ionic polymer mixed with a non-ionic one. Also can be observed of mixtures of a surfactant with a polymer.

Associative phase separation is another phenomenon where mixtures of two components and water separate forming a solid precipitate and a supernatant solution. The precipitate contains high concentrations of the hydrophilic polymers, the supernatant solution has residual low concentration of the polymers. Associative phase separation is commonly produced by electrostatic complexation, due to the presence of opposite charged polymers. The precipitate is usually denominated as a coacervate, which can be solid-like particles or highly viscous droplets. Schematic ternary associative phase separation is shown in figure, Figure 3.b, we can observe that the tie-lines are practically vertical, as in segregative phase separation are horizontal.

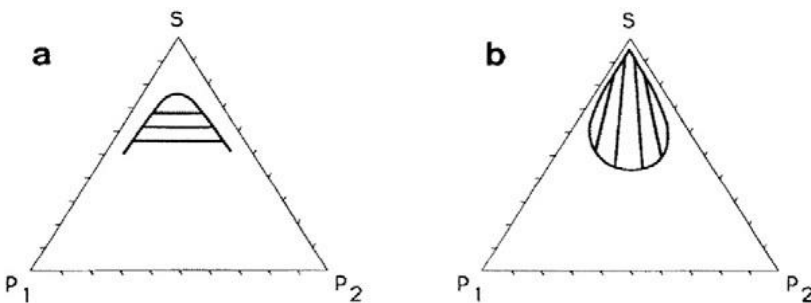


Figure 3. Schematic ternary phase diagrams, which illustrate segregative = (a) and associative = (b) phase separation in mixed hydrophilic polymer systems. S: solvent (water); P1: Polymer 1; P2: Polymer 2. (Piculell, L. & Lindman B. (1992) ⁽¹²⁾).

Phase separation can lead various phenomena depending in the interactions between the two components (figure 4).

At high macromolecule, a repulsive interaction between polymer molecules produce segregative phase separation, at low concentrations instead cosolubilization is formed. This is usually found in hydrophilic mixtures (7), Grimberg listed around a hundred different systems of polymers that form segregative phase separation.

Nature of the electrolyte, pH and ionic strength can greatly influence interactions between polymers thus can induce changes on phase behaviour. Segregation and associative phase separation can occur at the same time depending on pH and ionic strength

The mechanism behind associative and segregative phase separation are clearly different. Associative is induced by electrostatic attraction between charges of opposite sign, whereas segregation can arise from a negative entropy of mixing, which depends on molecular conformation. Repulsions between polymer chains are often restrictions in the free conformational movement of the polymers. Conformational freedom depends on molecular weights, short molecules tend to restrict free conformation of long linear chain molecules. Mechanism of segregative phase separation have been studied from various theoretical points of view, currently different theoretical approaches are used to predict the thermodynamic incompatibility between two hydrophilic polymer in aqueous solution (14).

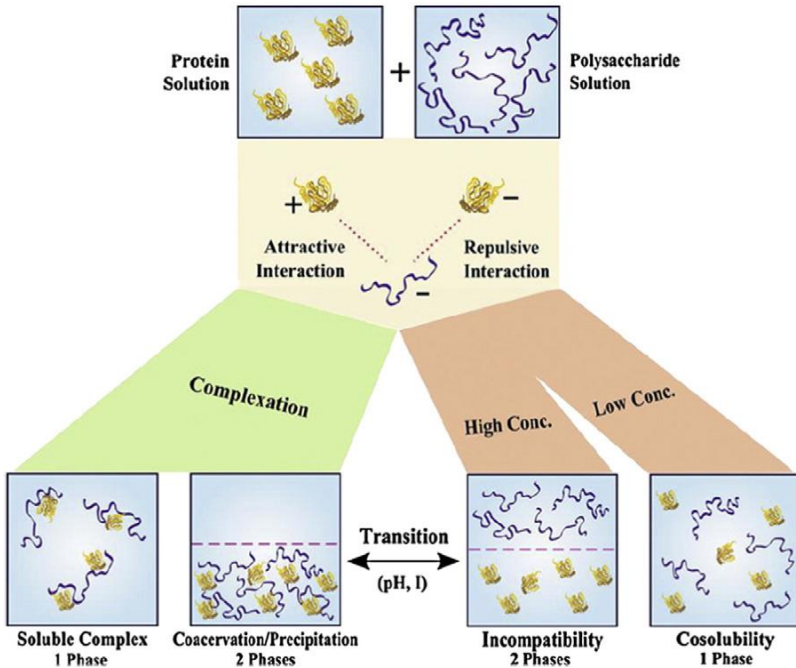


Figure 4. Scheme showing the four different phase situations, generated by either attraction or repulsion between macromolecules. The example refers to mixtures of proteins and polysaccharides, but the same behaviour can be extrapolated and is found in many other combinations of two different macromolecules (Matalanis, A., Jones, O.G. & McClements, D.J. (2011). ⁽¹³⁾).

Water-in-water emulsions can be formed by applying mechanical agitation in a two aqueous phase systems. The phase with small polymer volume becomes the phase dispersed in the continuous one which is the one that contains the highest concentration polymer. Phase inversion occurs at compositions which the two phases have approximately the same volume ratio. Figure 5 illustrates the different water-in-water emulsions that are formed in an aqueous gelatine and maltodextrin system depending on volume ratio of each component (Figure 5).

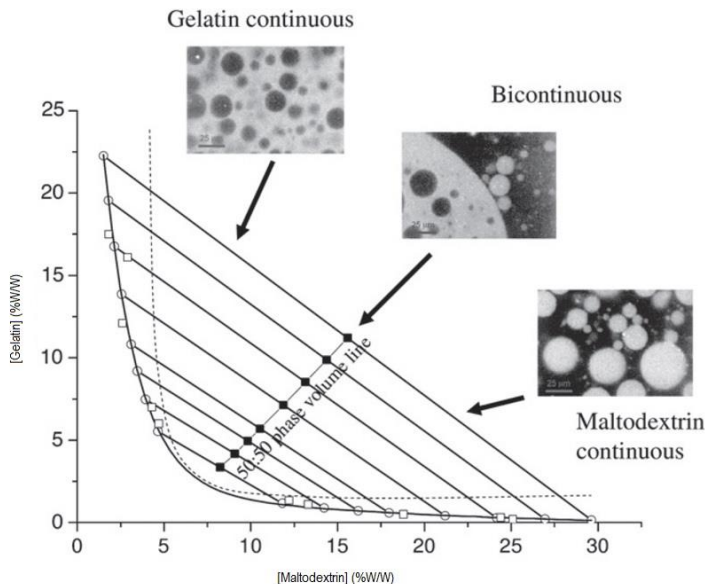


Figure 5. Scheme of water-in-water emulsion formation in the gelatine/maltodextrin system (Norton, I.T. & Frith, W.J. (2001))⁽¹⁵⁾.

Nguyen observed the influence of interactions between pH-sensitive microgels at the interface and its curvature of polyethylene oxide/dextran mixtures. He noticed that the particle where swelled when increased the pH from 6,5 to 7. But couldn't explain why the emulsions were stable at pH between 7 and 7,5 and destabilized at lower or higher pH. They attributed this to interactions between the microgel interactions and repulsion due to electrostatic interactions.

The interaction between two polymers can be modulated by temperature, pH and ionic strength, consequently causing changes in the phase behaviour. Also can be important the hydration capability of the electrolytes. Alves et al. (1999) declared that in gelatine/locust bean gum mixtures at high concentrations the conformational entropy constrains are the main factor determining phase separation⁽²⁷⁾.

Phase separation may be driven by conformational ordering resulting in a decreased entropy penalty for the formation of two phases. Temperature reduction will reduce the entropy of mixing leading to an increase in the incompatibility of the system⁽²⁸⁾.

Because of the low interfacial tensions in water-in-water emulsions it can be possible to modulate the drop size by varying the shear rate. If shear is applied while gelling the size and shape can be controlled creating non-spherical samples ^(29, 30). Demonstrated a clear influence of shear stress intensity while cooling on the microstructures of gelatine/guar and gellan/k-carrageen mixtures, forming spherical, long extended and irregular particles increasing the shear stress.

The non-spherical shapes can be stabilized by crosslinking and they morphology depends on temperature, molecular ordering and the relative phase volume of the equilibrium phases ^(31, 30).

This section helps us to understand the basics of formation of water-in-water emulsions to get a post understanding about the mechanism of water-in-water emulsion stabilization.

4.2. DIFFERENCE BETWEEN WATER-IN-WATER AND OIL-IN-WATER EMULSIONS

One of the greatest difference between water-in-water emulsions and oil-in-water or water-in-oil emulsions are the lower values of interfacial tension which are orders of magnitude lower for water-in-water emulsions values of dozens $\mu\text{m}/\text{N}$ for the former and values of dozens mN/N for the latter (16-23). The interfacial tensions tend to zero at the critical point. In water–sodium caseinate–sodium alginate systems the interfacial tension close to the critical point was $\sim 10\text{--}8$ N/m and it increased considerably to a value of up to 5.2×10^{-6} N/m farther from the critical point (24). To measure, different methods can be applied, analysis of the shape relaxation of individual droplets after cessation of shear, analysis of the shape of macroscopic interface near a vertical wall, using spinning drop tensiometers or by a rheo-optical methodology. (18, 20, 21, 24, 25).

Another peculiarity of water-in-water emulsions is that the interface length scale was comparable to the correlation length of the polymer solutions (4, 18) which is larger than molecular surfactant size. Contrary to water-in-oil or oil-in-water emulsions, water-in-water emulsions can be stabilized by amphiphilic molecules or surfactants (Figure 6).

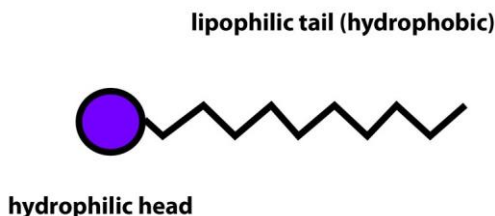


Figure 6. Surfactant molecule scheme (via Wikipedia).

Water-in-water emulsions are highly unstable and coalescence is fast as there are no repulsion forces between droplets. The common method to prevent macroscopic phase separation, i.e., maintaining the droplets in the continuous phase, for water-in-water emulsions is to gel one or both phases, preventing coalescence.

Stability of water-in-water emulsions has an important role in the characteristics and the stability of the foodstuffs that contain these systems. When a monolayer is formed by

coalescence of the dispersed phase the emulsion will be considered destabilized (Figure.7, figure 8). Creaming and sedimentation occur and the velocity depends on the viscosity of the continuous phase (η), difference of the density between the two phases ($\Delta\rho$) and the droplet radius:

$$\Delta G = g \Delta\rho 2R^2 / (9\eta) \quad (\text{Eq.3})$$

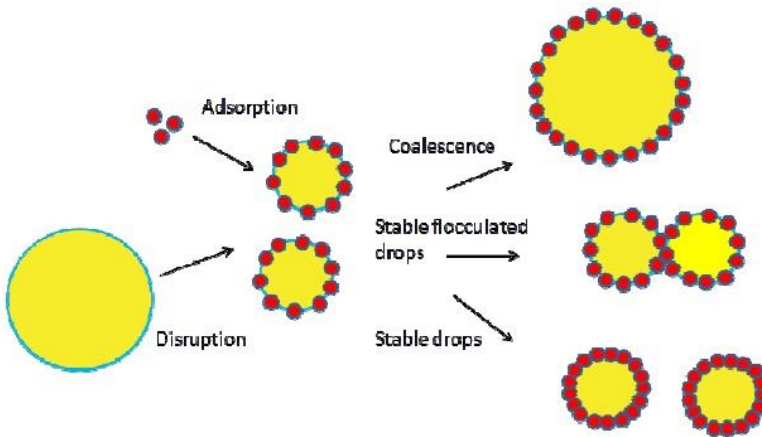


Figure. 7. Scheme of Pickering emulsions formation and evolution (González, A., & New, J. (2018) ⁽²⁶⁾).

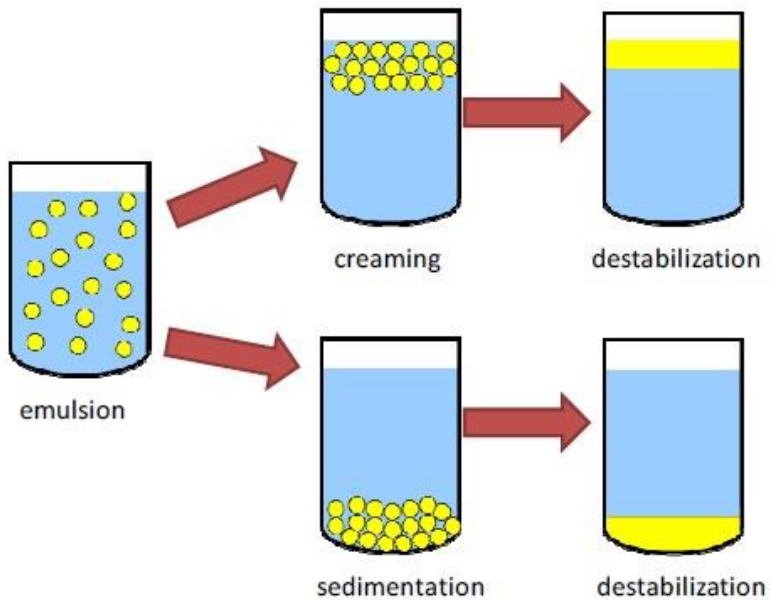


Figure. 8. Scheme of creaming, sedimentation and destabilization of emulsions (González, A., & New, J. (2018) ⁽²⁶⁾).

The most relevant differences between these two types of emulsions are the lower stability of water-in-water beside the oil and water emulsions and the lack of fats in the water-in-water system.

5. STABILIZATION OF WATER-IN-WATER EMULSIONS BY ADDITION OF PARTICLES

The main problem of water-in-water emulsions is their usual absence of stability. Fast coalescence or flocculation tend to occur leading to a two phase separation. Interfaces between the two phases are ill-defined and are usually thicker in comparison to oil-in-water interfaces. Surfactants are composed of a hydrophobic part and a hydrophilic, or water soluble, part. They are amphiphilic molecules. Therefore, small hydrophilic molecules do not encounter an interface when they move from one polymer phase to the other, as a consequence, small molecules do not adsorb on water-in-water interfaces, and thus, poor stability has been the main disadvantage for using water-in-water emulsions in practical applications. However, stable water-in-water emulsions are found in lots of food products because phase separation is inhibited by gelation of the continuous phase. The stabilization of water-in-water emulsions is of the utmost technological importance and finding methods for effective stabilization of water-in-water emulsions is a needed challenge. In the next section, a review of different food grade water-in-water emulsion stabilization by particle addition studies will be made.

5.1. PARTITION IN WATER-IN-WATER EMULSIONS

Particles in water-in-water emulsions may be located at the interphase, almost exclusively in one of the phases or have a partition between the two phases. Different partition behaviours have been observed by different authors. Murray and Phisarchanan ^(30, 31) observed that in a starch and locust bean gum/guar gum mixtures nanoparticles and whey protein isolated showed preference for the starch domains. Latex particles preference for gelatine in gelatine/oxide starch was perceived by Firoozmand ⁽⁶⁾.

pH can modify partition of different particles in water-in-water emulsions. Freitas et. al. ⁽³³⁾ noticed that β -lactoglobulin microgels switched their partition by reducing pH in amylopectin/xyloglucan phase.

In polyethylene oxide and dextran mixtures microgels partitioned in a non-monotonic manner way. As function of the pH and the ionic strength. Partition to the dextran phase was observed at pH between 7,2 and 7,8 and to the polyethylene oxide phase at lower and higher pH. They also found that 1mM NaCl was enough to invert the partition from polyethylene oxide phase to dextran phase (Nguyen et. al.) ⁽⁴⁾.

Partition depends on the protein type and its hydrophobicity, but also on surface charge, protein and polymer concentrations and molecular weights, pH and ionic strength ^(32, 34, 35).

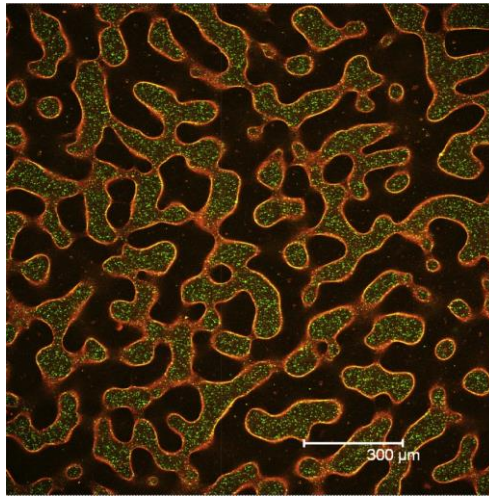


Fig. 9. CLSM image illustrating self-selective partitioning of colloidal particles in a 24 h old phase-separating mixture of 8% starch + 9% gelatin at 40°C. Dark regions = starch-rich phase, lighter regions = gelatine-rich phase. Red particles = polyethylene glycol coated polystyrene latex (0.32 μm diameter), green particles = COOH coated polystyrene latex particles (0.2 μm (diameter). polyethylene glycol coated particles at the water-in-water interface inhibit the phase separation (Firoozmand et al. (2009) ⁽⁶⁾).

5.2. STABILIZATION OF FOOD GRADE WATER-IN-WATER EMULSIONS BY PARTICLE ADDITION

As commented before, Grinberg and Tolstoguzov⁽⁸⁾ reviewed about a hundred protein-polysaccharide aqueous systems declaring that in some specific conditions any protein/polysaccharide system is spontaneously separated in two different phases with division of protein and polysaccharide. Doublier et. al. and Turgeon et. al. ^(14, 9) tried to clarify the kinetics, thermodynamic and structural aspects of these mixtures.

Water-in water emulsions in food have been studied for many years not necessary consciously thinking of the as emulsions and certainly not using particles to stabilize them. There have been many studies of water-in-oil and oil-in-water systems stabilized by particles. Many of these studies have involved food materials but a minority have used food grade particles. There are even less examples of water-in-water systems stabilized by particles and even less that use food grade particles which is the topic of these section. That is because it is difficult to find water-insoluble food grade particles for water-in-oil Pickering emulsions and even harder finding water-insoluble particles for water in water Pickering emulsions.

As mentioned in section 3, Poortinga was the first in describe water-in-water Pickering emulsions in food systems. He used several phase-separation solutions containing or two polysaccharides or one protein and one polysaccharide. Was apparently the first to identify particles affecting water-in-water particles of food systems ^(Figure 10). He took the following conclusions from his study. That encapsulates can be produced with a shell that can be responsive to the environment, thus allowing controlled release. The absence of organic solvents allows the use of delicate ingredients, most notably, biological material, both as active and as shell material. This further extends the possibility of producing capsules with an environmental responsiveness by making use of the many available biological ligand-receptor interactions to aggregate the particles forming the shell. And that, the low interfacial tension of water-in-water emulsions allows the formation of tubular structures ^{(3) (Figure 11)}.

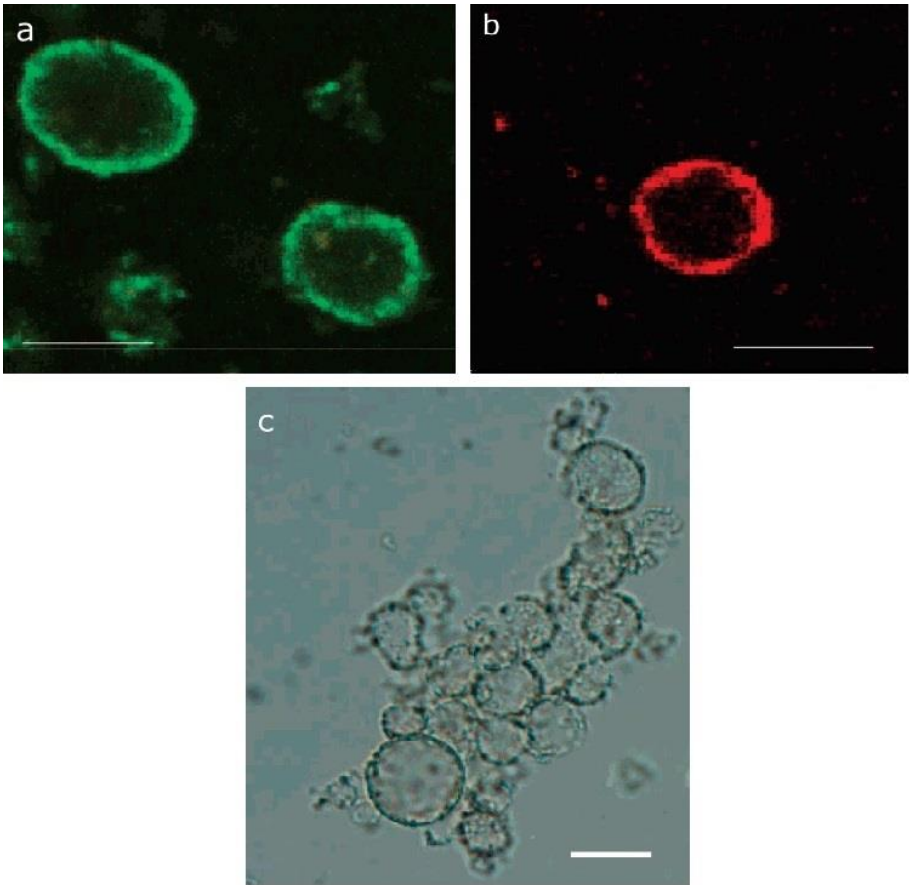


Figure 10. Microscopic images of several colloidosomes produced from dextran in methylcellulose emulsions containing fat particles after aggregation of the particles and removal of the phase separation. (a) Confocal scanning laser microscope (CSLM) picture of calcium aggregated particle shells (green = fat). (b) CSLM picture from a complex coacervated particle shell (red = fat). (c) Microscopic picture of complex coacervated particle shells. The scale bars denote 10 μm . (Poortinga (2008) ⁽³⁾)

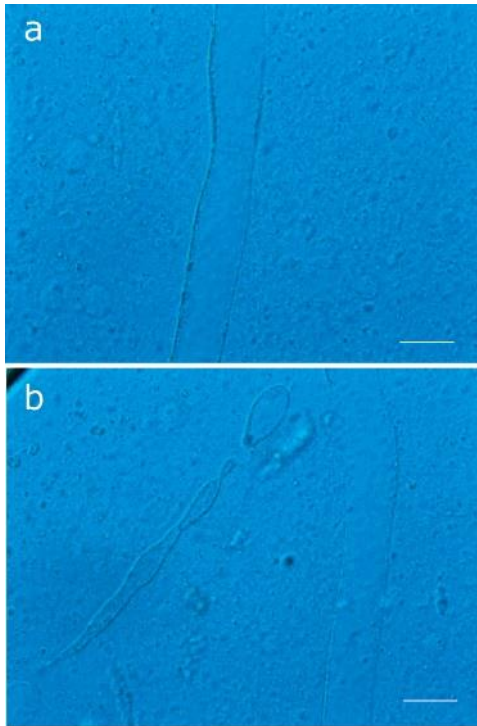


Figure 11. (a) Microscopic image of an elongated structure consisting of fat particles adsorbed at the dextran/methylcellulose solution interface. (b) Microscopic image of an elongated structure (at the left) consisting of fat particles adsorbed at the dextran/methylcellulose solution interface that is stable for hours without having aggregated the particles. The scale bars denote 10 μm . (*Poortinga (2008)* ⁽³⁾).

Firoozmand ⁽⁶⁾ studied systems composed of 7% gelatine in weight and 4% oxidized starch in weight with addition of monodisperse polystyrene latex particles added at a concentration of 0.35, 0.7, or 1.4 percent in weight. In this study it could be demonstrated that the addition of 1 percent in weight or less of latex particles could arrest spinodal type phase separation of the system. Also observed that highly stable emulsion droplets slowed phase separation. Although droplets slowed phase separation in a considerable way, could not slow it as much as latex particles could. It can be considered the first to talk about the idea of fluid oil liquid droplets as

water-insoluble particles, this might be applied to other food systems. Firoozmand study clearly demonstrated that Pickering emulsions could be prepared in water-in-water systems (Figure 12).

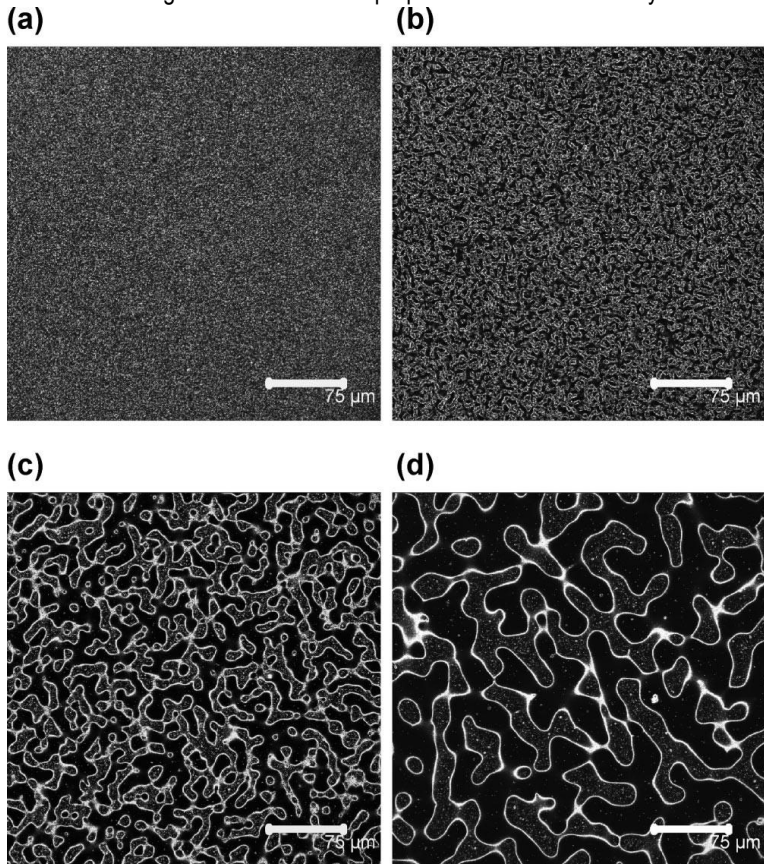


Figure 12. Confocal scanning laser microscope images showing the initial development of the microstructure of a phase-separated mixed biopolymer system (25.5 weight % sugar, 31.4% glucose syrup, 7 weight % gelatine, and 4 weight % oxidized starch) containing 0.7 weight % polystyrene latex particles. The sample was quenched from 90 to 1°C, held at 1°C for 10 min, heated to 40 at 6°C min⁻¹, and observed at 40°C for different times: (a) 2, (b) 4, (c) 8, (d) 16 min. (Firoozmand (2009) ⁽⁶⁾).

Firoozmand study was continued by Hanazzawa and Murray (2013, 2014) ^(37, 38) with a analysis of the phase separation, influenced by of oil droplets, of xanthan gum/sodium caseinate

system. In this system pH and concentration of calcium ions $[Ca^{+}]$ influence the phase separation. It was observed that the addition of droplets slowed down the phase separation, whenever pH and $[Ca^{+}]$ concentrations induce to precipitation of the casein and the flocculation of the droplets. In the second report mentioned Hanazzawa and Murray (2014) ⁽³⁸⁾ proved that with droplets addition, under conditions where microscopic phase separation was inhibited, increased the interfacial viscosity at the water-in-water interface. With an increase of a higher fat content of the droplets the effect improved.

Water-in-water systems that contain proteins have even more difficulty because proteins use to absorb most surface, so they will also absorb to any particle surface. This implies that the wetting and the tendency to aggregate of particles must change. Nguyen et. al. (2015) ⁽⁴⁾ studied Polyethylene and dextran solutions just because the none of the biopolymers is surface active, so it is easier to understand the particle control of water-in-water emulsions. This system also allows the addition of protein particles as stabilizers, just as Nguyen and co-workers made.

In a recent report whey protein isolated microgel particles added at a guar gum/ starch and locust bean gum/ starch systems were able to stop the phase separation of these systems. Even for a low concentration of protein particles (Murray and Phisancharnan (2016) ⁽³¹⁾). Same effect was seen by Nguyen and co-workers ⁽³⁹⁾ and Gornzalez Jordan et. al. (2016) ⁽³⁶⁾ in polyethylene oxide/dextran systems, where they could deduce that phase separation was slower at pH values near to isoelectric pH of the whey protein isolated particles. Due to reduced electrostatic repulsion between proteins different experiments concluded that aggregation of the microgel particles improved closer to the isoelectric pH.

Food grade silica and caseinate stabilized oil droplets were used in water-in-water emulsions but lower stabilization effects, although, with non-food grade silica nanoparticles showed really strong effects on water-in-water emulsions (Hannazawa an Murray (2013, 2014))^(37,38). Large particles size induce that particles cannot cover water-in-water emulsions fast enough to prevent the phase separation. As microgel particles are deformable will permit its adherence at the water-in-water interphase.

Segregative phase separation occurs in mixtures of xyloglucan and amylopectin. de Freitas et. al. (2016) ⁽³³⁾ used β -lactoglobulin microgels coated in polysaccharide to control stability of xyloglucan and starch water-in-water emulsions, just to use the idea of the bottom-up synthesis method, which can produce particles with smaller size than traditional top-down method.

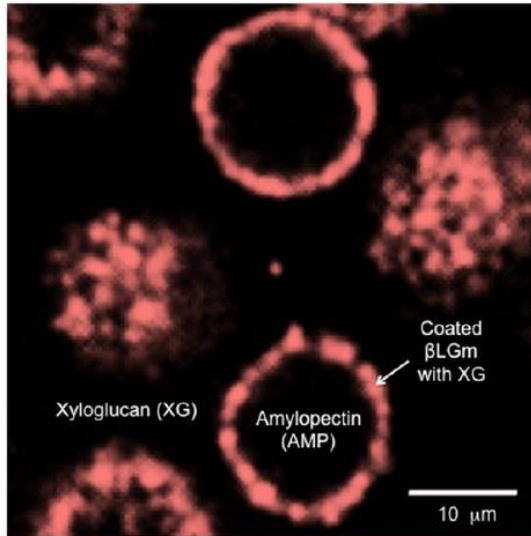


Figure 13. Close-up of a section of the confocal scanning laser microscope image at pH 5.0 after 24 h. For some droplets, the focus is at the interface layer, while for others it is closer to the centre of the droplets. Individual microgels can be distinguished at the interface in both views. The scale bar represents 10 μm (de Freitas, R.A. (2016) ⁽³³⁾).

Water-in-water emulsions of AMP droplets in a continuous xyloglucan phase can be stabilized by addition of protein particles for $\text{pH} \leq 5.0$. At higher pH, β -lactoglobulin microgels have a much stronger affinity for the AMP phase than the xyloglucan phase and therefore do not enter the interface between the two incompatible polysaccharide solutions. Below pH 5.5, xyloglucan binds to β -lactoglobulin, which inhibits large scale aggregation of the microgels close to the isoelectric point of the proteins. It also leads to an increase of the affinity of the microgels for the xyloglucan phase and as a consequence they enter the interface and stabilize the emulsions.

Firoozmand and Rosseau (2014) ⁽⁴⁰⁾ used edible single celled organisms particles to control stability of water-in-water emulsions. They used them in a gelatine/maltodextrin water-in-water system, where microstructure, rheology and system stability were controlled with a 2% weight

negative charged cells. This study showed that single-celled microorganisms can be used as micron-sized particles in thermodynamically-incompatible biopolymer solutions to create novel microstructures including for the first time, the formation of edible bi-continuous emulsions stabilized by solid particles. The presence of the microbes altered the rheological properties of gelatine/maltodextrin phase-separated gel by avoiding the reduction in gel elastic modulus normally seen with phase separation and salt. The results indicated that by using different proportions of these single-celled microorganisms, it should be possible to create unique gels with a variety of microstructures and rheological properties. These cell-containing gels should be considered as a new contribution to the collection of food gel types currently possible, providing diverse microstructures and uniquely-controllable rheological properties.

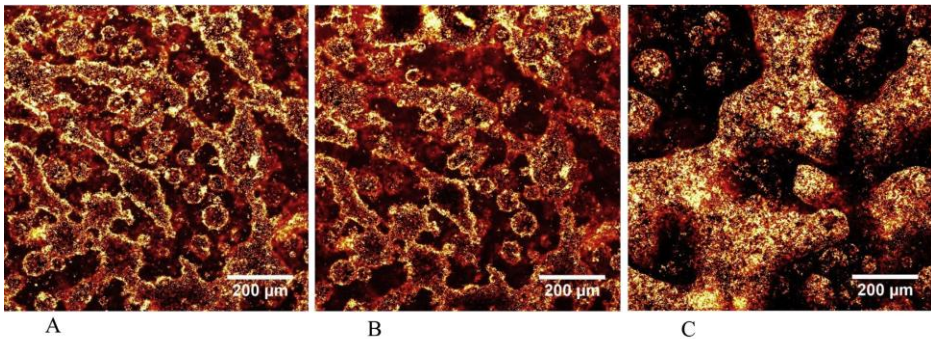


Fig. 14. CLSM of gels made of 6 wt % gelatin and 6 wt % maltodextrin containing 2 wt % yeast subjected to: (A) Th = 40 °C and Th = 30 s; (B) Th = 40 °C and th = 10 min; (C) Th = 40 °C and th = 30 s (Th =holding temperature, th = holding time with 1 wt % salt. The bright regions are gelatin-rich and yeast cells whereas the dark regions are maltodextrin-rich (Firoozmand, H., & Rousseau, D. (2014)⁽⁴⁰⁾).

Aqueous nature of emulsions and biocompatibility of the particles make this studies perfect for food industry applications and as we have seen in these studies particles can be really effective for water-in-water emulsion stabilization.

5.3. STABILIZATION OF WATER-IN-WATER EMULSIONS BY BLOCK COPOLYMERS

Buzza, D. M. A., Fletcher, P. D., Georgiou, T. K. & Ghasdian, N. (2013) ⁽²³⁾ studied addition of triblock copolymers to stabilize polyethylene glycol and dextran systems. Triblocks could stabilize polyethylene glycol-in-dextran as dextran-in-polyethylene glycol at neutral pH. Triblock polymer were formed of two different end blocks, each end prefers one of the two different phases. (Polyethylene glycol phase and dextran phase), connected by a central hydrophobic block. Large range of copolymers with different compositions were tested, emulsions were better stabilized when central block and the block favouring dextran phase were larger. Polyethylene glycol phase favouring blocks size did not seem to be relevant for stabilization and stabilized emulsions were formed even with the absence of this block. The author suggested that the block copolymer formed a layer at the interface with two of the end blocks in the phase it favours but this model cannot explain why diblocks were more effective than triblocks.

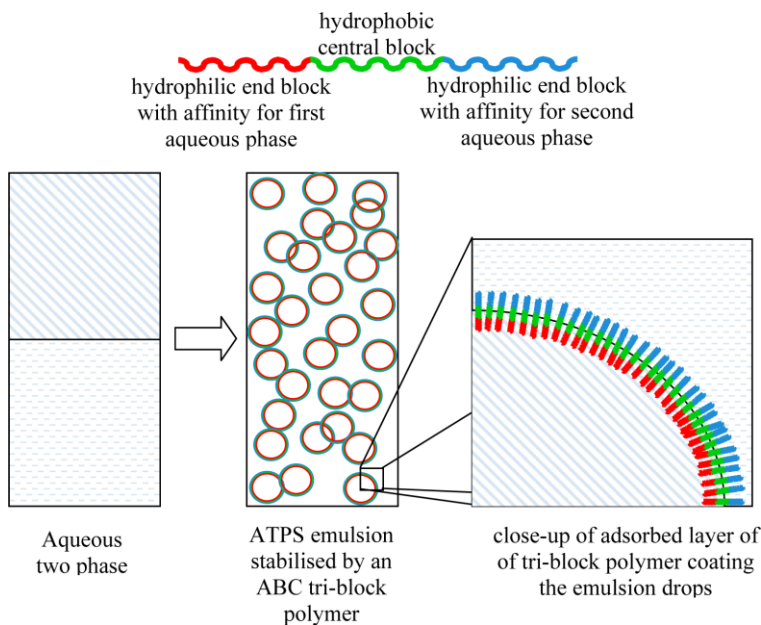


Figure 15. Design of the ABC triblock polymer suitable for stabilizing emulsions formed from aqueous two-phase systems containing a mixture of incompatible polymers (Buzza, D. M. A., Fletcher, P. D., Georgiou, T. K. & Ghasdian, N. (2013)) ⁽²³⁾.

Authors concluded that for the range of triblock copolymers used in the study, the emulsions containing equal volumes of dextran and Polyethylene glycol-rich phases are all of the dextran-in-polyethylene glycol type, have droplet sizes ranging from about 5–100 μm in diameter, and can be stable for up to several months in favourable cases. Drop size decreases and emulsion stability increases with increasing length of the hydrophobic block of the stabilizing polymer.

This novel process of forming polymersome-like structures by templating within water-in-water emulsions produces polymersome-like structures, which have some important differences to conventional polymersomes. Templated polymersomes are formed with only low energy input (i.e., only magnetic stirring of the mixtures is required and without the addition of organic solvents). Polysomersomes can be inverted from dextran-in-Polyethylene glycol to Polyethylene glycol to dextran type. Encapsulant solutes “self load” either into the droplets or the continuous phase with rapid mass transfer. Collapse of the water-in-water emulsion structure within a few minutes can be triggered by dilution of the emulsions with pure water. These differences are relevant to many possible applications.

Buzza, D. M. A., et. al. (2013) ⁽²³⁾ did not investigate the structure of the polymer in aqueous solution but most likely polymeric solutions were formed by diblocks with a hydrophobic core and a hydrophilic corona. Nicolai, T. & Murray, B. (2017) ⁽¹⁰⁾ suggested that the block copolymer formed polymeric micelles that adsorbed to the interphase and stabilize emulsions just as other type of particles. This suggestion would explain why the effective stabilization effect increased with the size of the copolymer and why it did not depend so much on its composition and why diblock copolymers were more effective. It is unclear if with two hydrophilic blocks (diblock) that prefer different water phases could stabilize the water-in-water emulsions. In this last case blocks should be larger than correlation length of the two phases.

5.4. WATER-IN-WATER EMULSIONS AS MICROREACTORS

Dextran-in-polyethylene glycol stabilized with the presence of spherical liposomes with an approximate radius of 65 nm were studied by Dewey, Strulson, Cacace, Bevilacqua and Keating (2014) ⁽⁴²⁾. Liposomes formed a layer around the dextran droplets that inhibited their coalescence. Liposomes maintained their structure and were relatively immobile due to electrostatic jamming. These structures provide favourable microenvironments that support accumulation and reactivity of bio-macromolecules as it was demonstrated with a ribozyme and can function as bioreactors. Although, the charge stabilization mechanism observed here poses some limits on reaction conditions (that is, ionic strength) for which the emulsion is stable, this has been overcome here by use of intermediate chelation and we anticipate that additional stabilization methods, for example, use of larger lipid assemblies that offer greater steric stabilization, can be employed to expand applicable conditions.

Mixing droplets covered with liposomes with different fluorescence demonstrated that if intact droplets were induced to coalesce liposomes of the droplets did not mix on the surface but if the emulsions were vigorously mixed the droplets were disrupting during the mixing indicated by the random distribution that liposomes made at their addition. The layer of liposomes allowed small DNA molecules to enter in the droplets and prevented other liposomes of entering.

These structures offer an easy encapsulation of macromolecular crowding agents and bio macromolecules by partitioning into the interior phase, excellent uniformity in droplet size and contents across a population and much greater access into/out of the interior volume. Authors exploited the strong partition of proteins and nucleotides to the dextran phase as micro-reactors for enzymatic reactions. Enzymes accumulate in the dextran droplet and catalyse the transformation of small reactants that could penetrate the droplet through liposome layer.

This demonstrate that water-in-water emulsions can be used for localized chemical reactions if one of the reactants strongly partitions to the dispersed phase and reacts cannot escape from it.

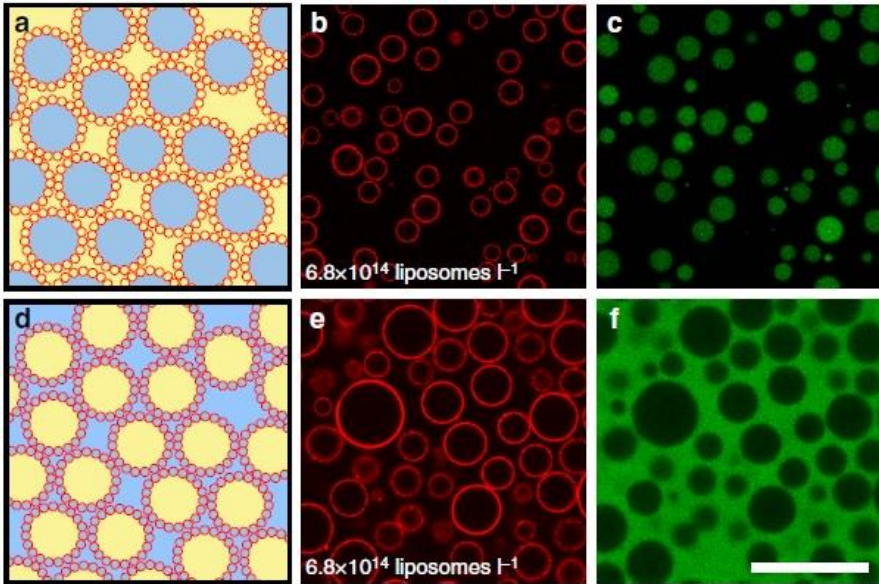


Figure 16. Liposome-stabilized ATPS emulsions. (a,b, and c) Dextran-rich droplets dispersed in polyethylene glycol-rich continuous phase. (a) Schematic illustration (red, liposomes; blue, dextran-rich phase; and yellow, polyethylene glycol-rich phase), (b) fluorescence image showing location of DOPE-Rhodamine-labelled liposomes at the aqueous/aqueous interface, (c) fluorescence image showing location of dextran-rich phase labelled with fluorescein isothiocyanate (FITC)-dextran 500 kDa. The inverted emulsion is depicted as (d) an illustration, (e) a fluorescence image showing location of DOPE-Rhodamine-labelled liposomes at the aqueous/aqueous interface, (f) a fluorescence image showing location of dextran-rich phase labeled with FITC-dextran 500 kDa. Liposome concentrations were 6.8×10^{14} liposomes l^{-1} . All image frames use the 25 mm scale bar (Dewey, Strulson, Cacace, Bevilacqua and Keating (2014) ⁽⁴²⁾).

5.5. INFLUENCE OF PH, PARTICLE MORPHOLOGY AND SIZE IN WATER-IN-WATER EMULSIONS STABILIZATION

In this section we will discuss the influence of particle morphology and size and the effect of pH in the stabilization of water-in-water emulsions.

Clearly particle size is an important aspect in water-in-water emulsions stabilization. If particles are too large they will not be able to cover the complete interfacial area of the water-in-water emulsions, as absorbing too slowly or sedimentation and creaming out too quick.

β -lactoglobulin microgels have already been shown to be able to stabilize Polyethylene oxide-in-dextran emulsions at neutral pH, but not dextran-in-Polyethylene oxide emulsions (Nguyen et al. 2013)⁽³⁹⁾. González, A. & New, J. (2018)⁽²⁶⁾ studied Polyethylene oxide and dextran systems with β -lactoglobulin stabilization. They wanted to find the effects of particle morphology and partition preference of the particles which directly depends on pH, as said in section 5.1. The study was made for pH 7 and pH 3 (Figure 17, Figure 18). Particles in the form of fibrils, microgels and fractal aggregates showed different efficacy to stabilize water-in-water emulsions. It depends not only on the concentration of protein particles, but also on their morphology, their affinity for each phase and their interaction with each other at the interface. However, the behavior at pH 7.0 and 3.0 was not totally opposite. The stability is also affected by interactions between the proteins at the interface which depends on particle morphology and pH.

At same concentration, the number aggregates is higher for fractals than for the dense microgels particles. This should make fractal aggregates more efficient to stabilize the emulsions. The spatial conformation of the fibrils could explain the inhibition of creaming of P/D emulsions at pH 7.0. It is possible that they increased the effective viscosity even though samples could flow when tilting. It is expected that fibrils form a very thin layer at the interface being parallel oriented to it.

Some questions could not be answered just like the optimal concentration of fractals to stabilize Polyethylene oxide-in-dextran emulsions at pH 7.0 and the fact that they were the most efficient aggregates at pH 3.0 whereas fibrils were most effective for Polyethylene oxide-in-dextran at pH 7.0.

The three different morphologies of protein particles, spheres, fractal aggregates and rod-like fibrils were able to stabilize Polyethylene oxide and dextran mixtures too different extents depending on the conditions. At neutral pH fibrils stabilized Polyethylene oxide-in-dextran

emulsions against coalescence better than microgels, whereas fractals were the less efficient. However, at pH 3.0 the same emulsions could not be stabilized by fibrils, but it was very stable when fractals were added. Contrary to neutral pH, where phase separation occurred very quickly for the three types of particles, stable dextran-in-Polyethylene oxide emulsions could be formed at pH 3.0. Significantly smaller drops were formed with fractals than with microgels and fibrils.

We can conclude that the stability of water-in-water emulsions formed by dextran and Polyethylene oxide depends on many factors protein particles concentration, morphology, the partition preference of the protein of each protein and the interaction between them.

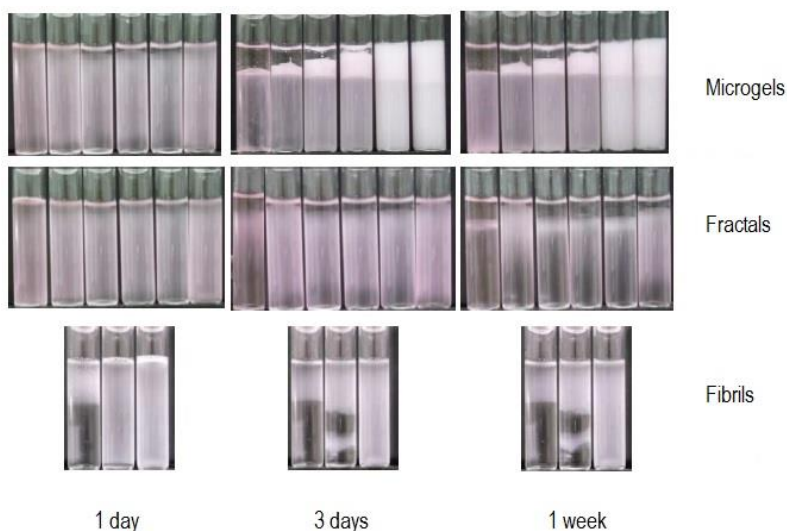


Figure 17. Photographs at different times after mixing polyethylene oxide into dextran emulsions at pH 7.0 containing different amounts of microgels or fractals (0.05, 0.1, 0.2, 0.3, 0.5, 0.75% (left to right)) or fibrils (0.05, 0.1, 0.3% (left to right)) ⁽²⁶⁾.

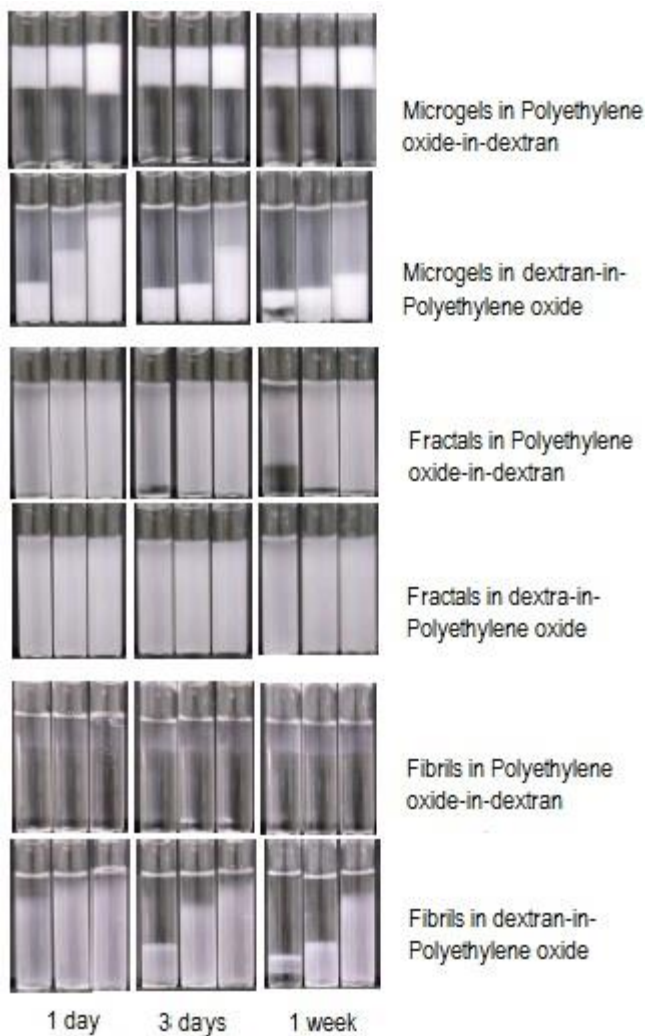


Figure 18. Photographs at different times after mixing polyethylene oxide in dextran (P/D) emulsions and dextran in polyethylene oxide (D/P) emulsions at pH 3.0 with different amounts of microgels, fractals, and fibrils: 0.05, 0.1, and 0.3% (left to right) ⁽²⁶⁾.

6. APPLICATIONS OF WATER-IN-WATER EMULSIONS

Mixtures of proteins and polysaccharides are common in a wide variety of food products. The kinetics of digestion of proteins can be altered when combined with certain polysaccharides, for example, by self-assembly causing gelification in the stomach at low pH conditions. An example is the combination of whey protein and alginate which slowed digestion by maximizing the absorption of nutrients and delaying gastric emptying promoting satiety (Norton et al., 2015) ⁽⁴¹⁾. Also, water-in-water emulsions can be used for the extraction and purification of biomolecules such as proteins in certain mixtures of proteins and polysaccharides, or to separate cells, because no organic solvents are used.

Water-in-water emulsions can be applied for many other things, for example, the protection of specific micronutrients and the modulation of their liberation. The use of encapsulation to protect, deliver and control the liberation of bio-actives in specific places of the gastrointestinal tract is commonly used in the pharmaceutical industry and is trying to be promoted to the food industry (Norton et al., 2015) ⁽⁴¹⁾. Water-in-water emulsions can be used as a way for transporting water-soluble substances, such as pigments, flavours, minerals, probiotics and vitamins, and for the targeted release of different active compounds to specific parts of the digestive system.

In the water-in-water interfaces different shells can be formed with specific properties. Polymers and colloidosomes that vary their permeability or stability depending on the environment have a good potential for controlled release. The shells of biological materials, such as cells, could be formed by a wide variety of biological interactions, with colloidosomes sensitive to specific environmental triggers (Poortinga 2008) ⁽³⁾.

In addition, water-in-water emulsions can also be used to deliver lipophilic components by encapsulating oil droplets into hydrogel particles. Matalanis et al (2011) ⁽¹³⁾ used segregative followed by aggregative phase separation of the pectin and casinade mixture to encapsulate lipid drops coated with casein. The oil was divided into the dispersed phase rich in caseinate at pH 7. When acidified at pH 5, the interactions between the two phases promoted the stabilization of

the biopolymer particles. Another application of the emulsion in water-in-water is its use as microreactors as described in section 5.4. Dewey et al. (2014) ⁽⁴²⁾ used dextran drops in Polyethylene oxide stabilized with liposomes for enzymatic reactions. The strong partition of nucleotides and proteins to the dextran drops and the ability of the interface of the liposomes to access inside and outside substrates and products allowed the use of the system for the cleavage reaction of the ribosome. They also applied the same mixture for the enzymatic synthesis of CaCO₃ nanoparticles. The urea entered the drop of dextran reacting with urease, which strongly led to the dextran phase, forming CO₃²⁻. CO₃²⁻ reacted with Ca²⁺ producing solid CaCO₃. Small molecule chelators with intermediate binding affinity were used to control the availability of Ca²⁺ during CaCO₃ mineralization, since liposomes were unstable in the presence of free Ca²⁺ (Cacace et al., 2015) ⁽⁴³⁾.

In addition, microgels and anisotropic particles can be formed using water-in-water emulsions. When one of the polymers is able to form a gel, it stops the process of phase separation at a specific point and allows to study the structures obtained. The relationship between phase separation and gel formation will determine the morphologies, which in turn modifies the rheological and sensory properties (Turgeon et al., 2003) ⁽⁹⁾. A new range of texturizing agents can be formed, including new nanoparticles, which can be more digestible and bio-accessible compared to microstructures (Norton et al., 2015) ⁽⁴¹⁾. Gelation under shear flow can give rise to different forms, from spherical to anisotropic, which influences the textural properties (Wolf et al., 2000, Wolf et al., 2001) ^(28, 29). In addition to the possibility to vary the shape, other advantages of the use water-in-water emulsions to form microgels are the absence of surfactant and the oil phase (Shewan & Stokes 2013) ⁽⁴⁴⁾. The main systems used are gellan and carrageenan mixtures (Wolf et al 2000, Wolf et al 2001) ^(28, 29); gelatine and slurry systems (Wolf et al., 2000) ⁽²⁸⁾ and gelatine and maltodextrin mixtures (Alevisopoulos et al., 1996; Stokes et al., 2001; Butler & Heppenstall-Butler, 2003) ^(45, 46, 47). The properties of gelatin, with a viscosity that depends largely on the temperature of gelation, makes it easy to control the size and morphology (Stokes et al., 2001; Matalanis et al., 2011; Shewan & Stokes. 2013) ^(45,13,44).

The microstructure of the food has a clear impact on the sensory and textural perception of the product. Therefore, water-in-water emulsions can lead to the development of new products in categories such as dairy, sauces, beverages, prepared dishes, nutraceuticals, etc.

Water-in-water emulsions have a great variety of possible applications greatly expanding over the years.

7. CONCLUSIONS

The research reported in this thesis was aimed at improving our understanding of stabilization of water-in-water emulsions by particles, which may help in the development of industrial applications of water-in-water emulsions in different areas such as food, pharmaceutical, cosmetic and personal care. Consisting in a bibliographic review of water-in-water emulsions focusing in their stabilization for grade emulsions.

Interfacial tensions between the two aqueous immiscible phases are very low, and depend on the difference in composition between the two aqueous phases, along the binodal line. Interfacial tension is virtually zero at the critical point where tie-lines converge, and immiscibility vanishes, producing a merging of the two phases into a single aqueous phase. Surfactant molecules poorly adsorb on water-in-water interfaces, and thus, the stabilization of water-in-water emulsions is an important challenge for physical chemists. It has been seen that by Pickering emulsions, addition of particles at the interphase of the two phases, water-in-water emulsions can be stabilized opening a new window for a large range of applications.

As seen in this report, stabilization by addition of particles depends on many factors; pH, concentration, size and morphology of the particle, etc. There are still many issues about the stabilization of Pickering water-in-water emulsions that require further research. It is necessary to do more studies trying to understand the effects of these factors, just like usage of particles for which the shape can be modified may help to understand the effect of the morphology on the stability of water-in-water emulsions.

I think it is necessary to find new types of bio-chemical or synthetic particles that could control the stabilization of water-in-water emulsions, something that can open new options for interesting applications.

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