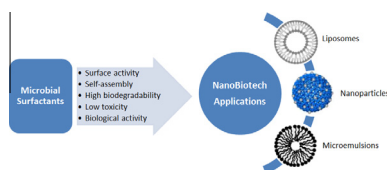# Microbial surfactants: Fundamentals and applicability in the formulation of nano-sized drug delivery vectors

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GRAPHICAL ABSTRACT

ARTICLE INFO

ABSTRACT

Microbial surfactants, so-called biosurfactants, comprise a wide variety of structurally distinct amphipathic molecules produced by several microorganisms. Besides exhibiting surface activity at the interfaces, these molecules present powerful characteristics including high biodegradability, low toxicity and special biological activities (e.g. antimicrobial, antiviral, anticancer, among others), that make them an alternative to their chemical counterparts. Several medical-related applications have been suggested for these molecules, including some reports on their potential use in the formulation of nano-sized drug delivery vectors. However, despite their promises, due to the generalized lack of knowledge on microbial surfactants phase behavior and stability under diverse physicochemical conditions, these applications remain largely unexplored, thus representing an exciting field of research. These nano-sized vectors are a powerful approach towards the current medical challenges regarding the development of efficient and targeted treatments for several diseases. In this review, a special emphasis will be given to nanoparticles and microemulsions. Nanoparticles are very auspicious as their size, shape and stability can be manipulated by changing the environmental conditions. On the other hand, the easiness of formulation, as well as the broad possibilities of administration justifies the recent popularity of the microemulsions. Notwithstanding, both vector types still require further developments to overcome some critical limitations related with toxicity and costs, among others. Such developments may include the search for other system components, as the microbial surfactants, that can display improved features.

1. Introduction

A wide variety of structurally diverse compounds from microbial origin exhibiting surface activity at the interfaces, the so-called microbial surfactants or biosurfactants, has been reported [1]. These amphipathic compounds resemble the chemical surfactants, containing both hydrophilic and hydrophobic moieties in the same

molecule. Microbial surfactants can be categorized according to their mode of action, molecular weight and general physicochemical properties [2,3]. In heterogeneous systems, microbial surfactants form a molecular interfacial film that affects the wettability and surface energy of the original surface. Besides lowering the liquids surface tension, these films can also greatly impact the interfacial behavior and mass transfer, as they also lower the interfacial tension between different liquid phases on the interfacial boundary existing between immiscible phases. Once at interfaces, the hydrophobic moiety aggregates at the surface facing the

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hydrophobic phase (oil phase), while the hydrophilic moiety is oriented towards the hydrophilic phase (water phase). This property makes microbial surfactants good foam stabilizers and emulsifiers, which represent the motifs behind their common use as detergents or food additives. Due to their varied functional properties including emulsification, wetting, foaming, cleansing and surface activity, among others, microbial surfactants find applications in many industrial sectors.

Several research efforts have been undertaken to establish the microbial surfactants as viable alternatives to their chemical counterparts [1,4–7]. Indeed, microbial surfactants present numerous advantages over the chemical ones, namely regarding their biodegradability, mild production conditions, environmental compatibility, low toxicity, high selectivity and specific activity at extreme temperatures, pH and salinities [1]. Moreover, several potential biomedical therapeutic and prophylactic applications have been emphasized for these compounds [4–8]. Consequently, besides their usefulness for chemical and environmental applications, their unique features have been capturing the attention of cosmetic, biomedical and pharmaceutical industries [2,9]. Actually, promising uses of microbial surfactants in the formulation of nano-sized drug delivery vectors have been reported [5]. It is well known that the synthesis of stable and effective nanoparticles with well-defined sizes is still not fully established. Besides, the synthesis processes are expensive and generally lead to the production of harmful wastes, thus it is of utmost importance finding viable alternative processes [10]. Microbial surfactant-mediated processes can be considered as an alternative for the rapid synthesis of nano-sized materials (particulate dispersions or solid particles within a size range of 10–1000 nm).

Despite all the advances in the drug delivery science field, the oral bioavailability of a great number of drugs exhibiting poor gastrointestinal adsorption remains a challenge [11]. Some strategies have been pursued towards the development of delivery systems able to overcome such limitation [12]. The self-emulsifying drug delivery systems (DDS) constitute a promising example of those strategies as, besides facilitating adsorption of drugs via intestinal lymphatic pathways, they have small size, globular shape, solubilize hydrophobic drugs, present formulation advantages and are easily scalable to industrial setups [13]. Formulations of liquid self-emulsifying DDS contain oils, surfactants, co-surfactants, and/or co-solvents. Surfactants are essential for these formulations, namely they will assist the drug solubilization if used in relatively large amounts, not only due to the reduced interfacial tension, but also to the increased permeability of the drugs [14]. An adequate selection of the relative proportions of the formulation components is critical to accomplish the desired physicochemical and pharmaceutical features, such as maximum drug solubilization capacity, high emulsification efficiency, acceptable intestinal permeability and high drug stability for prolonged periods of time [12].

The use of microbial surfactants in liquid self-emulsifying formulations alternatively to synthetic surfactants represents a very promising approach that can minimize the toxicity and gastric irritation usually caused by the synthetic surfactants [13]. Indeed, conventional self-emulsifying DDS commonly contain great amounts of synthetic surfactants that are recognized to damage the normal gastric mucosa lining [15]. On the other hand, as mentioned, microbial surfactants present high biocompatibility and low toxicity. Rhamnolipid, surfactin, iturin and pumilacidin are some of the microbial surfactants generally used for preparing oral lipid-based formulations of therapeutic agents [5]. For instance, surfactin has been used to prepare a self-microemulsifying DDS of vitamin E in order to enhance its pharmaceutical performance. The referred system exhibited a remarkable increase in the emulsification efficiency, dissociation rate, and consequently oral bioavailability of the therapeutic agent [13].

This review discusses the fundamentals of microbial surfactants as compared to their chemical counterparts, highlighting its potential applicability as novel nano-sized drug delivery vectors.
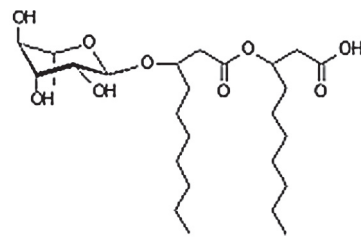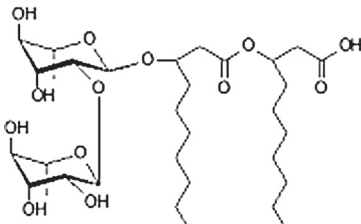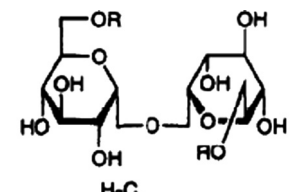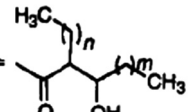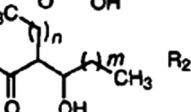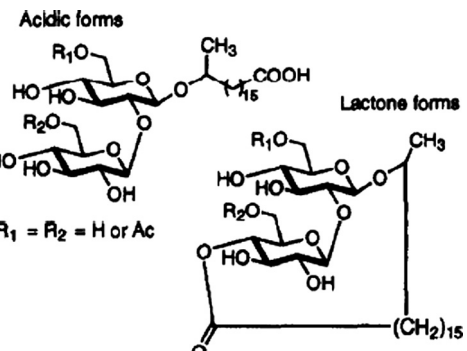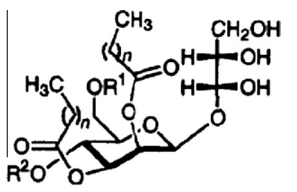
2. Microbial *versus* chemical surfactants

Surfactants are a class of chemical compounds that display affinity for both aqueous and oily phases, thus possessing both hydrophilic and hydrophobic properties. A typical surfactant consists of a lipophilic group, generally a long-chain hydrocarbon moiety, and a hydrophilic group, which is either a charged or an uncharged polar group. These molecules tend to migrate to interfaces, so that the hydrophilic moiety can stay in the phase with stronger polarity, and at the same time the lipophilic moiety is placed in the phase with comparatively weaker polarity, consequently minimizing the Gibbs energy of the system. This amphiphilic character is behind the hydrophobic effect, which constitutes the key thermodynamic driving force for surfactant self-assembly [16]. Distinct classes of surfactants can be defined according to the charge of their head groups, namely cationic (positive charge); anionic (negative charge); non-ionic (non-charged and highly hydrophilic); and zwitterionic (presence of both negative and positive charge centers, and a net charge equal to zero).

Similarly, microbial surfactants embrace a set of varied amphipathic molecules with distinct chemical structures that are naturally produced by a number of microorganisms. However, unlike chemically syn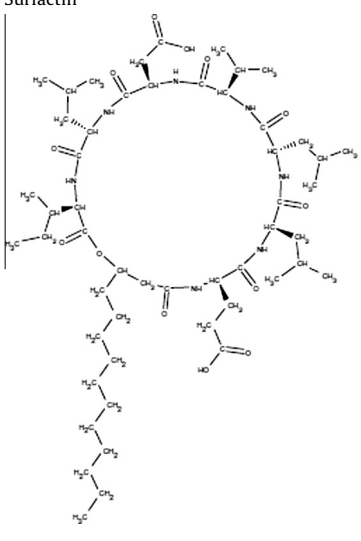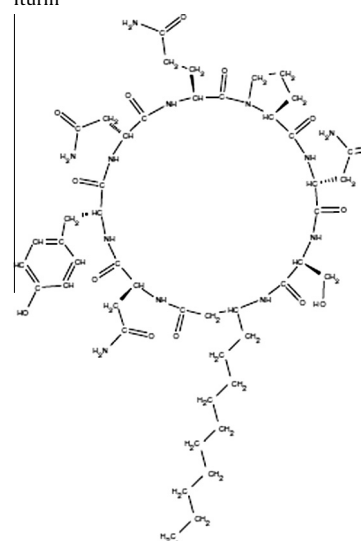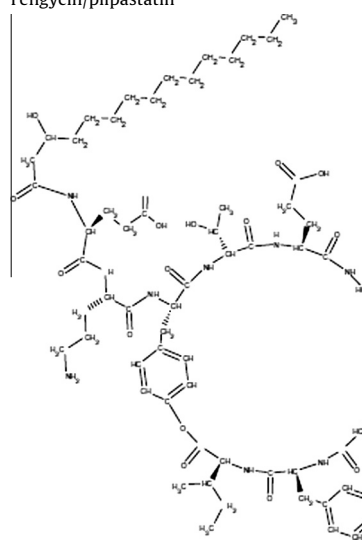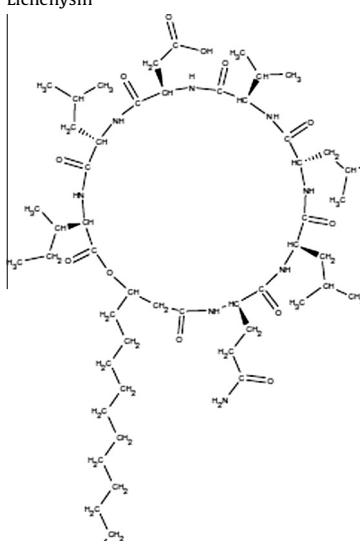thesized surfactants which are classified by their head group, microbial surfactants are generally categorized by their chemical composition and molecular weight, as low (e.g. glycolipids and lipopeptides) and high molecular weight (e.g. polysaccharides, proteins and lipoproteins) surfactants. In general, the amphiphilic and polyphilic polymers are usually more effective in stabilizing emulsions, while the low molecular weight microbial surfactants have simpler structures that lead to good surface active properties [1,6]. Their hydrophilic moiety is mainly comprised of an acid, peptide cations, or anions, mono-, di- or polysaccharides, while their hydrophobic moiety can be an unsaturated or saturated hydrocarbon chains or fatty acids [2]. The structural orientation of the molecule on the surfaces and at the interfaces is responsible for its properties, such as the ability to lower surface and interfacial tension of liquids and the formation of micelles and microemulsions between these different phases. The main types of microbial surfactants are presented in Table 1. Glycolipids and lipopeptides are the best-studied microbial surfactants [1]. Examples of glycolipids include rhamnolipids produced by *Pseudomonas aeruginosa*, trehalolipids produced by *Rhodococcus erythopolis*, sophorolipids produced by *Candida bombicola* and mannosylerythritol lipids (MEL) produced by *Pseudozyma* yeasts, which contain mono- or disaccharides, combined with long-chain aliphatic acids or hydroxyaliphatic acids. Among the lipopeptides, examples comprise surfactin, iturin and fengicyn cyclic lipopeptides produced by *Bacillus* species as antibiotic molecules.

Microbial surfactants, which are mainly formed as secondary metabolites, have been implicated in the survival of their producing microorganisms since they facilitate nutrient transport and uptake, interfere in microbe-host interactions and quorum sensing mechanisms, and even act as biocide agents [6]. Due to their diverse chemical structures and surface properties, different groups of microbial surfactants may display different natural roles in the producing microorganisms, thus being difficult to generalize those roles in microbial physiology. The physiological roles of microbial surface-active compounds have been reviewed by [22,23]. Some microbial surfactants are essential for the motility of microorganisms, for example surfactin is crucial for the swarming motility in *Bacillus subtilis*. Other microbial surfactants affect

Table 1
Main types of microbial surfactants and their producer microorganisms (adapted from [17–21]).

Class	Glycolipids			
Type	Rhamnolipids (RL)	Trehalose lipids (TL)	Sophorolipids (SL)	Mannosylerythritol lipids (MEL)
Structure	Mono-rhamnolipid Di-rhamnolipid	TL-1: $R_1 = R_2 =$ ... TL-2: $R_1 =$ ... $R_2 = H$ $(m + n = 27$ to $31)$	Acidic forms $R_1 = R_2 = H$ or Ac Lactone forms SL-1: $R_1 = R_2 = Ac$ SL-2: $R_1 = Ac$, $R_2 = H$ SL-3: $R_1 = H$, $R_2 = Ac$ SL-4: $R_1 = R_2 = H$	MEL-A: $R^1 = R^2 = Ac$ MEL-B: $R^1 = Ac$, $R^2 = H$ MEL-C: $R^1 = H$, $R^2 = Ac$ $(n = 6$ to $10)$
Producer	*Pseudomonas aeruginosa*	*Rhodococcus erithropolis* *Arthobacter* sp.	*Candida bombicola* *Candida apicola*	*Candida antartica* *Pseudozyma*
Class	Lipopeptides			
Type	Surfactin	Iturin	Fengycin/plipastatin	Lichenysin
Structure				
Producer	*Bacillus subtilis*	*Bacillus subtilis*	*Bacillus subtilis*	*Bacillus licheniformis*

the attachment–detachment of microorganisms to and from surfaces. Also, these molecules are involved in cell-to-cell interactions such as bacterial pathogenesis, quorum sensing and biofilm formation, maintenance and maturation. For instance, rhamnolipids are essential to maintain the architecture of the biofilms and are considered as one of the virulence factors in *Pseudomonas* sp. Rhamnolipids, mannosylerythritol lipid and surfactin exhibit antimicrobial and antibiotic properties thus conferring a competitive advantage to the microorganism during colonization and cell–cell competition. Additional roles attributed to microbial surfactants include cellular differentiation, access to insoluble substrates and resistance to toxic compounds.

Currently, an intense research activity is being directed towards the identification of new microbial surfactants and characterization of their chemical and biological properties. Actually, a great amount of work on their applications in medical-related fields has been encouraged by their accepted potential and biological nature (reviewed by [5,7]). As previously mentioned, given their microbial origin, diversity, biodegradability, and low toxicity, microbial surfactants are considered superior to their chemical counterparts [6]. Accordingly, their application in the food and cosmetics industries, enhanced oil recovery and bioremediation has been broadly investigated [10,24–26].

The microbial surfactants ability to effectively lower the surface tension of water has been generally recognized. An efficient biosurfactant can reduce the surface tension of water and air from 72 mN/m to less than 30 mN/m. Surfactin is one of the most powerful microbial surfactants reported, as it can reduce the surface tension of water to 27 mN/m at a concentration as low as 10 µM [27]. Likewise, rhamnolipids have been reported to achieve the same levels of reduction [19]. The sophorolipids from *C. bombicola* have been reported to reduce the surface tension to 33 mN/m [28], while MEL and trehalose lipids to less than 30 mN/m [18,29].

As surfactant monomers are added into solution, the surface and interfacial tension will decrease until the microbial surfactant reaches the critical micelle concentration (CMC), i.e. the minimum concentration necessary to initiate the formation of micelles. At this concentration, surfactant monomers begin to spontaneously associate into structured aggregates such as micelles, vesicles or continuous bilayers. These aggregates are produced as a result of weak chemical interactions such as hydrophobic, van der Waals and hydrogen bonding [30,31]. These structures are fluid-like and can easily move from one state to another as conditions such as electrolyte concentration and temperature are changed [32]. The aggregate structure will depend on the polarity of the solvent in which the surfactant is dissolved. In aqueous solution, the polar head groups of a micelle will be oriented outward towards the aqueous phase, while the hydrophobic tails will associate in the core of the micelle [3]. Efficient surfactants have low CMCs, i.e. less surfactant is necessary to decrease the surface tension to a given level. Microbial surfactants are most effective and efficient at their CMC which can be 10–40 times lower than that of chemical surfactants, thus less surfactant is necessary to get a maximum decrease in surface tension [2]. Another important feature of microbial surfactants for industrial applications is their stability and unaltered activity when exposed to extreme environmental conditions. Surfactin was found to be stable after autoclaving (121 °C/20 min), after 6 months at −18 °C, at a pH range from 5 to 11 and NaCl concentrations up to 20% [28].

Additionally, microbial surfactants are easily degraded and are generally considered as low or non-toxic compounds. Ivshina and co-workers [33] reported concentrations of microbial surfactants from *Rhodococcus* spp. and *P. aeruginosa* strains at which 50% of their maximal effect (EC50 values) is observed between 50 and 650 mg/l. Moreover, Lima et al. [34] reported EC20 values for different microbial surfactants (including lipopeptides and glycolipids)

between 261 and 736 mg/l, higher than the obtained for SDS, 25 mg/l. Franzetti and collaborators [35] also reported a low toxicity for the bioemulsifier produced by *Variovorax paradoxus* 7bCT5 against *Vibrio fisheri*, with an inhibition of 34 ± 2% after 15 min of exposure to the highest concentration tested (500 mg/l). Although the available data on the toxicity of microbial surfactants is limited, some reports suggest that they are less toxic than their chemical counterparts. The synthetic anionic surfactant (Corexit) showed a much higher toxicity than microbial surfactants, as it exhibited an LC50 (lethal concentration to 50% of test species) against *Photobacterium phosphoreum* at approximately ten times lower concentrations than that for rhamnolipids [28]. Moreover, rhamnolipids were found to be less toxic than the widely used synthetic surfactant Marlon A-350 [2].

Understanding the functional mechanisms of microbial surfactants is of utmost relevance to develop innovative applications. Microbial surfactants are known to partition at interfaces of fluid phases with distinct polarities and hydrogen bonding, thus affecting the adhesion of microorganisms [7]. Also, these molecules can disrupt cell membranes leading to their lysis [22,36–39]. For instance, surfactin is known to destabilize membranes disturbing their integrity and permeability. This is due to changes in the physical membrane structure or through the disruption of protein conformations which alter important membrane functions such as transport and energy generation [22]. The molecular mechanisms of surfactin interactions with membrane structures were described by [40,27]. An important step for membrane destabilization and leakage is the dimerization of surfactin into the bilayer [41]. The hypothetical mechanisms of surfactin interactions with membranes involve the insertion into the lipid bilayers, chelating mono- and divalent cations, modification of membrane permeability by channel formation or membrane solubilization by a detergent-like mechanism. The incorporation of surfactin into the membrane leads to dehydration of the phospholipid polar head groups and the perturbation of lipid packaging which strongly compromises the bilayer stability, leading to the disturbance of the membrane barrier properties. These structural instabilities may explain the primary mode of the antibiotic action, as well as other important biological effects of this lipopeptide. The extent of perturbation of the phospholipid bilayer depends on the surfactin concentration. At low concentrations, surfactin penetrates readily into the cell membrane, where it is completely miscible with the phospholipids and forms mixed micelles. At moderate concentrations, the lipopeptide forms domains segregated within the phospholipid bilayer that may contribute to the formation of ion-conducting pores in the membrane leading to membrane disruption and permeabilization at high concentrations. Surfactin has demonstrated a stronger activity than Triton [42]. Indeed, microbial surfactants have been recognized by their potent properties that include antibacterial, antifungal and antiviral activities, hence making them relevant molecules for uses in medical-related fields [4]. Some of these molecules have been suggested as harmless and effective substitutes of drugs and antimicrobials. For instance, these molecules can be used in gene transfection or as adjuvants for antigens, as well as anti-adhesive coatings for biomaterials or incorporated in probiotic preparations for the treatment and prevention of urogenital infections [4]. In addition, the microbial surfactants have been evaluated for their potential effects against cancer cells (as reviewed in [5]).

Moreover, several other therapeutic applications have been advocated for microbial surfactants including novel and striking uses in nanotechnology mainly based on their flexible self-assembling [43,44]. Microbial surfactants tend to self-assemble into hierarchically ordered structures using hydrogen bonding, hydrophobic and van der Waals interactions [43]. Glycolipids, particularly MEL, are well known for their self-assembling properties

that are influenced by the stereochemistry of the saccharide head groups [45]. Some glycolipids, possessing relatively large hydrophilic head groups as compared to the hydrophobic part, generally form micelles in dilute aqueous solutions. Besides spherical micelles, these surfactants also form disk-like and rod-like structures [46]. As the surfactant concentration further increases, glycolipid/water systems start to form a range of liquid crystalline phases. In particular, glycolipid microbial surfactants spontaneously self-assemble into a variety of molecular assemblies with well-defined and/or unique structures, such as sponge (L3), cubic (V2), hexagonal (H2), or lamellar (Lα) configurations [47]. The Fontell scheme (Fig. 1) illustrates the natural sequence of self-assembled structures and phases as a function of the surfactant concentration [48]. Among these molecular assemblies, vesicles are the most studied ones. MEL, due to their efficient molecular orientation and effective balance between hydrophilic and hydrophobic groups, are able to form giant vesicles with diameters larger than 10 μm [8]. It is important to mention that the formation of giant vesicles is not straightforward, since the vesicle structure requires strictly balanced hydrophobic and hydrophilic groups. Some synthetic and natural glycolipids bearing a disaccharide or larger hydrophilic head group have been reported to form vesicular systems by themselves. However, with the only exception of rhamnolipids, microbial glycolipids have not been reported to do the same [8]. Comparatively, rhamnolipids show a pH-sensitive conversion of molecular assemblies due to the presence of a carboxyl group on the side chain. Hence, rhamnolipids form micelles at pH values higher than 6.8, lipid particles at pH values between 6.2 and 6.6, lamella structures at pH 6.0–6.5, and finally vesicles sized 50–100 nm at pH 4.3–5.8. A di-rhamnolipid has been reported as a bilayer stabilizer in phosphatidylethanolamide (PE) systems, thus making this molecule a good candidate to form pH-sensitive vesicles in combination with PE [49]. Glycolipid biosurfactant-based vesicles or bilayer membranes appear to be very promising for exploiting useful nanostructured materials and/or systems. Examples include the work reported by Maitani and co-workers [50] that developed a liposome vector containing beta-sitosterol beta-D-glucoside microbial surfactant-complexed DNA which was successfully validated for herpes simplex virus thymidine kinase gene therapy. Moreover, nanovectors containing a microbial surfactant have been shown to greatly increase the efficacy of gene transfection [51]. Additionally, rhamnolipid and surfactin have been used to develop biodegradable core–shell polystyrene/biosurfactant bionanocomposites for protein drug release using an emulsion polymerization approach [52]. On the other hand, the usefulness of microbial surfactants for the biological synthesis of nanoparticles has been described [53,54]. For example, silver nanoparticles were synthesized using a rapid microbial surfactant-mediated synthesis [55]. However, although microbial surfactants are apparently valuable, versatile, multiuse and handy molecules for therapeutic applications, some of them may represent a risk for humans and should be cautiously examined. A simple illustration of this issue is the case of the glycolipids being produced by *P. aeruginosa* that present a great potential for several therapeutic uses, although it is well-known that this strain is responsible for serious nosocomial infections [56,57].

3. Microbial surfactants: properties and phase behavior

Prior knowledge about the features of a system and its components through the proper assessment of various parameters such as the surface properties (i.e. surface and interfacial tensions, CMC), hydrophilic–lypophilic balance (*HLB*), Israelachvili–Ninham packing parameter, *P* (also called critical packing parameter, *CPP*) and Winsor-*R* ratio would significantly reduce the complexity of a rational choice of the components that can lead to a successful formulation.

3.1. Surface properties

The addition of a surfactant to a given solution will lower its surface tension due to the surfactant adsorption at the interface. For a diluted solution, the more molecules adsorb at the interface, the lower the surface tension of the solution will be. Once the surface adsorption reaches its limit, the unimers in the bulk solution start to form aggregates as the Gibbs energy required for establishing non-polar chains in contact with water is higher than that of the repulsive head group interactions, chain packing restrictions in the aggregate core and creation of the interfacial region, which are associated with the formation of aggregates. Therefore, aggregation will be thermodynamically favored, and it will be a spontaneous and collaborative process. Several forms of surfactant adsorption and self-aggregation in aqueous solutions have been reported, however the simplest and most common type of aggregate formed is the micelle. As previously mentioned, the concentration needed to form a micelle is called the CMC. At this point, the surface tension remains nearly unchanged with increasing surfactant concentrations.
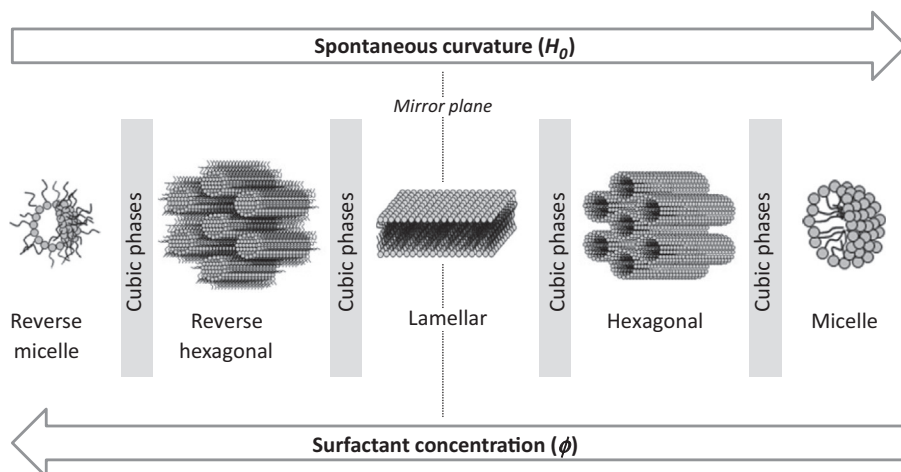

Fig. 1. Surfactants self-assembly as a function of surfactant concentration.

3.2. Hydrophilic–lypophilic balance (HLB)

HLB is a parameter known to affect the stability of an emulsion. It represents the relative contribution of the hydrophilic and lipophilic groups of the surfactant to the emulsion. As a general rule, low HLB values (3–6) favor the formation of W/O microemulsions (W – water; O – oil), whereas high HLB values (8–18) favor O/W microemulsions. For surfactants with very high HLB values (HLBs > 20), often a co-surfactant is required to reduce their effective HLB value. The HLB value is only applicable for non-ionic molecules. For ionic surfactants it has to be calculated experimentally in a relative basis.

3.3. Critical packing parameter (CPP)

In order to reduce the contact angle of water with hydrocarbon chains, surfactant molecules tend to form aggregates in solution with the chains oriented to the interior and enclosed in the aggregate. As mentioned, besides micelles, there are several other types of aggregates that can be formed. The volume of the hydrophobic carbon chains relative to the surfactant head group area at the interface will determine the type of aggregate being formed, as well as its aqueous behavior [58,59]. The concept of surfactant critical packing parameter (CPP) is used to illustrate such property. The packing parameter is significantly influenced by the different moieties of surfactant and the environmental conditions. Therefore, it is expected that surfactants exhibiting different CPP will tend to pack in different ways.

3.4. Spontaneous curvature (H_o)

Alternatively, one can rationalize aggregation on the basis of the so-called spontaneous curvature, H_o, of the surfactant film, i.e. the preferred mean curvature adopted by the film in the absence of mechanical constraints. Qualitatively, the concept of H_o is similar to the CPP as the preferred curvature will also depend on the relative proportion of polar and non-polar volumes. However, the theory behind it is based on the mechanical properties of the film as a whole. Generally, CPP is related with individual molecules, while H_o is related with a continuum with global physical properties.

3.5. Winsor-R ratio

The ratio of the total interaction energies (per unit area of interface) of the surfactant for the O and W phases is known as the Winsor-R ratio. Three possible situations can occur, namely $R < 1$, indicating that the water-surfactant interaction is stronger than the oil-surfactant interaction forming Winsor Type I microemulsions; $R > 1$, suggesting that the strength of oil-surfactant interaction is stronger than the water–surfactant interaction, forming Winsor Type II microemulsions; and $R = 1$, representing the situation in which the interactions are balanced, resulting in the formation of Winsor Type III microemulsions.

4. Microbial surfactants: self-assembly

Self-assembly consists in the ability of surfactant molecules to self-associate in a given solvent (water and a few other polar solvents), thus forming different types of aggregates and structures of colloidal dimensions. These structures can be of limited size (micelles, liposomes and microemulsion droplets) or large with connectivity in one, two or three dimensions (liquid crystals). The basic building blocks of all these discrete and infinite structures can be broadly divided into monolayer-based films or bilayer-based films.

4.1. Parameters influencing self-assembly

Similarly to adsorption, the surfactant self-assembly is mainly a consequence of the hydrophobic effect, although being also influenced by many other factors, including the repulsive interactions between polar head groups. Using the CPP and H_o parameters it is possible to qualitatively explain the preferential aggregates formed (Table 2.). However, the aggregate prediction is only valid when the interaction between aggregates is rather weak and can be neglected, which is usually the case for dilute systems. The structure and dynamics of self-assembled surfactant aggregates can differ broadly depending not only on the surfactants chemical structure, but also on the system variables, such as composition and temperature. The external parameters that strongly influence the CPP and H_o, and therefore the type of self-assembled structures formed, include temperature, salt, co-surfactants, type of oil and surfactant concentration. Further details on the effect of these parameters on the formation of the surfactant aggregates can be found in [16].

4.2. Self-assembled structures and phases

4.2.1. Bulk self-assembly

Depending on the molecular structures of the surfactants, as well as the abovementioned parameters that affect the CPP and H_o, a variety of self-organized structures can be formed in solution; and these are classified according to their phases as homogeneous (or single-phase) or heterogeneous (or multiphasic) systems [58,60].

Homogeneous systems can be further divided as: (a) solutions (e.g. micellar phase); (b) liquid-crystalline phases (e.g. lamellar phase); and (c) crystalline phases. Solutions are naturally disordered at both short- and long-range scales, although the existence of micellar aggregates presumes some degree of molecular organization in the bulk. Additionally, there is a residual liquid structure due to spatial correlations between the aggregates. Liquid-crystalline phases are disordered at short-range scales but present some type of orientational order and distinct translational order at long-range scales. Crystalline phases have both short- and long-range orders. Heterogeneous systems comprise emulsions, suspensions, foams, gels and adsorbed films.
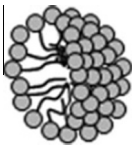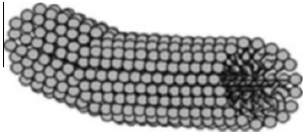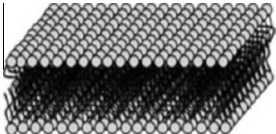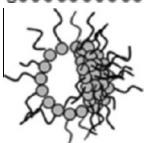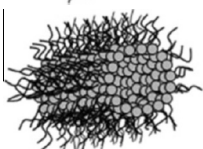
4.2.2. Self-assembly at interfaces

As expected, the surfactant self-assembly does not occur exclusively in the bulk but also at interfaces, such as gas–liquid and solid–liquid interfaces. Long-chain fatty acids and surfactants, water-insoluble compounds, can be spread from an organic solvent on an aqueous solution to form monomolecular films adsorbed at the gas–liquid interface, so-called monolayers. The molecules in monolayers can be self-organized in different ways, in particular when they are tightly packed, depending on the lateral forces to which they are subjected (Table 2.). It is also possible to build multilayers through successive deposition of monolayer films onto a solid substrate. The deposited films are named Langmuir–Blodgett films and are of great relevance for a number of applications (e.g. biosensors).

5. Microbial surfactants applications in nanobiotechnology

The unique properties of microbial surfactants, such as versatile self-assembling and biochemical properties, which are not usually observed in conventional chemical surfactants, have been attracting an increased attention in the field of bionanotechnology, namely for the design new functional structures and/or systems. MEL exhibit the most interesting self-assembling properties and

Table 2
Surfactants self-aggregation structures.

Aggregate	Structure	CPP	H_o
Spherical micelle		1/3	$1/R$
Cylindrical micelle		1/2	$1/2R$
Bilayer		1	≈ 0
Reverse spherical micelle		>1	$-1/R$
Reverse cylindrical micelle		>1	$-1/R$

R corresponds to the radii of curvature.

several related applications [43]. Several researchers [61–64] studied the kinetics of interactions in carbohydrate ligand systems composed of self-assembled monolayers of MEL-A serving as a high-affinity, easy to handle and low-cost ligand system from immunoglobulin G and M and lectins. Igarashi and collaborators [65] reported that MEL-A significantly increased the efficiency of gene transfection mediated by cationic liposomes. Additionally, Ueno et al. [66] found that MEL-A-containing liposomes exhibited high activity in DNA encapsulation and membrane fusion with anionic liposomes, which are important properties for gene transfection. On the other hand, MEL-B- and MEL-C-containing liposomes only increased either the encapsulation or the membrane fusion. Moreover, the same authors [67] suggested that MEL-A was capable of increasing and rapidly promoting the transfection efficiency of target cells by inducing membrane fusion between liposomes and the plasma membrane of these cells. Nanovectors containing a biosurfactant have been successfully used to increase the efficacy for gene transfection *in vitro* and *in vivo* [51]. State-of-the-art developments in the nanobiotechnology field using microbial surfactants are summarized in Table 3.

Another interesting application of microbial surfactants is the possibility to synthesize metal-bound nanoparticles using an environmentally friendly technology [76]. Moreover, Reddy and collaborators [95] synthesized, for the first time, surfactin-mediated gold nanoparticles.

5.1. Microbial surfactants-based liposome vectors and their applicability for gene transfection

Gene transfection into the cells is of utmost importance for clinical gene therapy [67]. Although several approaches have been studied for gene transfection, more efficient and safe systems are still required. Lipofection using cationic liposomes is considered to be a promising method for introducing foreign genes into the targeted cells due to their high transfection efficiency, low toxicity and immunogenicity, ease of preparation and targeted application [70]. The physicochemical properties of cationic liposomes, such as lipid packing density, shape and zeta potential have a significant effect on gene transfection efficiency. As previously discussed, microbial surfactants hold a number of interesting features that can be explored in the preparation of those cationic liposome vectors.

Inoh and co-workers [68] reported that MEL-A promoted DNA transfection efficiency mediated by cationic liposomes. Lipids and oligonucleotide DNA in MEL-A-containing liposome-DNA complex were found to distribute in the plasma membrane and nucleus of the target cells [69], thus suggesting that the biosurfactant induces the membrane fusion between the target cells and the cationic liposomes, accelerating the efficiency of gene transfection significantly. Likewise, Igarashi et al. [65] found that MEL-A significantly increased the efficacy of gene transfection mediated by cationic liposomes in human cervix carcinoma Hela cells. MEL-liposome complex was found to be distributed widely in the cytoplasm and the DNA presence was strongly detected in cytoplasm around the nucleus. These studies clearly demonstrate that MEL-A is able to increase gene expression by enhancing the association of the liposome complexes with the cells, thus suggesting that MEL-A-containing liposomes could be potentially used as a relevant non-viral vector for gene transfection and gene therapy.

Ueno et al. [66] found that MEL-A-containing liposomes exhibit a high DNA encapsulating activity, as well as membrane fusion with anionic liposomes. Additionally, MEL-B and MEL-C-containing liposomes only increased either the DNA encapsulation or the membrane fusion. On another study [67], the same authors further evaluated the transfection mechanisms mediated by cationic liposomes with NBD-conjugated MEL-A and reported that the biosur-

Table 3
Microbial surfactants applications in nanobiotechnology (adapted from [2]).

Microbial surfactant type	Activity/potential application	Reference
Mannosylerythritol lipids A	Ligand system for immunoglobulin G and M and lectins	[47,61–64]
	DNA encapsulation and membrane fusion with anionic liposomes	[66]
	In vitro and *in vivo* promotion of gene transfection mediated by cationic liposomes	[65,67–71]
	Delivery of siRNA into the cell cytosol through cationic liposomes	[72]
	Herpes simplex virus thymidine kinase gene therapy	[50]
	Stable water-in-oil microemulsions without the addition of a co-surfactant or salt	[73]
	Increased membrane fluidity of monolayers composed of L-α-dipalmitoylphosphatidylcholine (DPPC)	[43]
	Self-assembling and of formation of giant vesicles	[8]
Mannosylerythritol lipids-B	Self-assembling and vesicle-forming activity	[73]
Rhamnolipids and sophorolipids	Cadmium sulfide nanoparticles	[74]
	Biocompatible microemulsions of lecithin/rhamnolipid/sophorolipid biosurfactants	[75]
Rhamnolipids	Silver nanoparticles with antibioticmicrobial activity	[55]
	Nickel oxide nanoparticles by microemulsion technique	[76,77]
	Silver nanoparticles	[78]
	ZnS nanoparticles	[79]
	Stable microemulsions	[80–82]
	Alcohol-free microemulsions	[83]
	pH-sensitive vesicles in combination with phosphatidylethanolamide and dioleoylphosphatidylethanolamide	[49,84]
	Safe adsorption enhancers for oral dugs	[85]
	Dispersing nanoparticles	[86]
	Nanozirconia particles	[87]
	Enhancer of drug release from a lipid-polymer coated hybrid nanoparticle	[88]
Sophorolipids	Cobalt and silver nanoparticles	[89]
	Sophorolipid-coated silver and gold nanoparticles with antibacterial activity	[90,91]
Rhamnolipids and surfactin	Biodegradable core-shell polystyrene/biosurfactant bionanocomposites for protein drug release	[52]
Fengycin and surfactin	Enhancers for the skin accumulation of aciclovir	[92]
Surfactin	Cadmium sulfide nanoparticles	[74]
	Surfactin-mediated synthesis of gold nanoparticles	[93–95]
	Microemulsions of vitamin E	[13]
	Nanospheres and nanorods brushite particles synthesized in reverse microemulsions of surfactin	[96]
	Cationic surfactin liposomes enhanced the delivery of siRNA to HeLa cells	[97]

factant distributed on the intracellular membranes through the plasma membranes of the target cells, while the cationic liposomes with MEL-A fused to the plasma membranes. Afterwards, the authors noted that the DNA released from the vesicles was immediately transferred to the nucleus. Hence, those results suggested that MEL-A was capable of promoting the transfection efficiency of target cells by inducing membrane fusion between liposomes and the cells plasma membrane.

In addition, Kitamoto and collaborators [43] demonstrated that monolayers composed of L-α-dipalmitoylphosphatidylcholine (DPPC) containing MEL-A had greater membrane fluidity than those containing only DPPC. Moreover, it was found that the unsaturated fatty acids in MEL-A greatly influenced the surface pressure and packing density in the monolayer and consequently, the physicochemical properties of MEL-A and MEL-A/lipids [63]. Nanovectors containing MEL-A were further evaluated *in vivo* regarding their transfection efficiency [71]. When a complex of the nanovectors with MEL-A and plasmid DNA was injected into C57BL/6J mice bearing B16/BL6 tumors, the biosurfactant significantly increased liposome-mediated gene transfection to the mouse tumor cells. The transfection efficiency of the plasmids into the solid tumors by the cationic liposomes of cholesteryl-3-beta-carboxyamidoethylene-N-hydroxyethylamine (OH-Chol) with MEL-A increased by approximately 100-fold compared to that by the commercially available DC-Chol cationic liposomes without MEL-A. These results strongly suggest that non-viral vectors with MEL-A can be successfully used for gene transfection *in vivo*. Nakanishi and collaborators [51] reviewed the mechanisms of gene delivery by MEL-A based nanovectors.

The effects of unsaturated fatty acid ratio within the MEL-A biosurfactant on the physicochemical properties and gene delivery into cells of MEL-A based cationic liposomes were further studied by [70]. The MEL-A (21.5% unsaturated fatty acids)-containing cationic liposomes induced highly efficient membrane fusion after addition of anionic liposomes and led to subsequent DNA release.

These liposomes were found to fuse with the plasma membrane and delivered DNA into the nucleus of NIH-3T3 cells. It is important to notice that a higher unsaturated fatty acid ratio liposomes was able to fuse the plasma membrane but did not delivered DNA into the nucleus, while a lower unsaturated fatty acid ratio liposomes neither fused with the plasma membrane nor delivered DNA into the nucleus. Therefore, it may be suggested that MEL-A unsaturated fatty acid ratio significantly affects the transfection efficiency due to changes in membrane fusion activity and the efficiency of DNA release from the liposomes. Furthermore, the same authors [72] suggested that the ability to rapidly and directly deliver siRNA into the cytosol using MEL-A-containing cationic liposomes reduces immune responses, cytotoxicity and other side effects caused by viral vectors in clinical applications.

On the other hand, MEL B containing a different configuration of the erythritol moiety has been reported to self-assemble into a lamellar phase over a wide concentration and temperature range, while MEL-A self-assembles into various kinds of lyotropic liquid crystalline phases. Additionally, MEL-B showed great potential as a vesicle-forming lipid, thus suggesting its potential application in drug and gene delivery, as well as in transdermal delivery systems [73].

5.2. Microbial surfactants as auspicious agents for drug delivery

Microbial surfactants, given some of their properties that include emulsification, foaming, detergency and dispersion, encompass an interesting group of molecules with potential application in the field of drug delivery [44]. For instance, MEL have shown a much higher emulsifying activity with soybean oil and tetradecane than polysorbate 80 [43], as well as an interesting ability to form stable W/O microemulsions without the addition of a co-surfactant or salt [73]. On the other hand, it has been reported that rhamnolipids and sophorolipids can be mixed with lecithins to prepare biocompatible microemulsions in which the phase

behavior is almost insensitive to changes in temperature and salt concentration, thus making them desirable for cosmetic and drug delivery applications [75]. Rhamnolipid liposomes were patented as drug delivery systems, useful as microcapsules for drugs, proteins, nucleic acids, dyes and other compounds. These novel liposomes were described as safe and biologically decomposable, with suitable affinity for biological organisms, stable and with long service and shelf-life [2]. Furthermore, rhamnolipids have been reported as safe adsorption enhancers for oral drugs. These microbial surfactants at low concentrations not only enhanced paracellular and transcellular transport pathways in Caco-2 cells (*in vitro* model of the human small intestinal epithelium), but also inhibited P-gp activity. Besides, rhamnolipids showed low toxicity to Caco-2 cells and erythrocytes [85].

Additionally, the potential of fengycin and surfactin to act as enhancers for the transdermal penetration and skin accumulation of aciclovir was evaluated [92]. It was found that these lipopeptides did not enhance aciclovir transport across the skin although they increased aciclovir concentration in the epidermis by a factor of 2, thus demonstrating the potential of these microbial surfactants for drug delivery applications. Moreover, Onaizi and collaborators [98] studied the micellization and interfacial behavior of a mixture of surfactin and sodium dodecylbenzylsulphonate and demonstrated that the formation of mixed micelles was thermodynamically feasible.

Microemulsions obtained using microbial surfactants are thermodynamically stable and their isotropic systems that form spontaneously, in addition to their long-term stability, easy preparation and high solubilization capacity are considered to be very promising liquid vehicles for future drug delivery systems [99]. Moreover, microbial surfactants could also be considered as interesting components of an emulgel, which consists in an emerging topical drug delivery system. Emulgels are either emulsion of oil-in-water or water-in-oil type, which is gelled by mixing it with a gelling agent. The incorporation of an emulsion into a gel increases its stability and efficiency as compared to other topical drug delivery systems [100].

5.3. Microbial surfactants role in the development and production of nanoparticles

Nanoparticles are characterized by their unique size and stable shape [101]. The production of nanoparticles over a wide variety of physical structures and with high monodispersity still remains a challenge [10]. Actually, the available techniques are capital intensive, produce hazardous wastes and unstable nanoparticles with reduced targeted activity. Therefore, clean, non-toxic, size-controlled and environmentally acceptable synthesis procedures are crucial to encourage the large scale production of nanoparticles for several applications, including targeted drug delivery and biotechnology [78,102,103]. Given the need for greener bio-processes and new synthesis schemes microbial-based, microbial surfactants are evolving as interesting alternatives for the rapid synthesis of nanoparticles [89,95,104].

Gold nanoparticles are being increasingly used in the field of drug and gene delivery, targeted therapy and imaging technologies [105,106]. Potential therapeutic applications of such particles also include anti-HIV activity, anti-angiogenesis, antimalarial activity, anti-arthritic activity and biohydrogen production. Similarly, silver nanoparticles also possess anti-fungal activity, anti-inflammatory effect, anti-viral, anti-angiogenesis and anti-platelet activity [107]. Microbial surfactants can be used for high-performance nanomaterial production, since they easily form a variety of liquid crystals in aqueous solutions. Reddy and collaborators [95] stabilized the synthesis of silver nanoparticles with surfactin. Besides, these authors [93] successfully synthesized surfactin-mediated gold nanoparticles

and studied the effect of proton concentrations and temperature on the morphology of the obtained nanoparticles. Nanoparticles synthesized at pH 7 and 9 remained stable for 2 months, while aggregates were observed at pH 5 within 24 h. Moreover, the nanoparticles formed at pH 7 were uniform in shape and size and were polydispersed and anisotropic at pH 5 and 9. Nanoparticles synthesized at room temperature were found to be monodispersed and were more uniform as compared to those synthesized at 4 °C. In another study, the authors carried out a biological synthesis of gold and silver nanoparticles using the bacteria *B. subtilis* [94]. Gold nanoparticles were synthesized both intra- and extracellularly, while silver nanoparticles were exclusively formed extracellularly. The results suggest that the nanoparticles were stabilized by surface-active molecules, namely surfactin or other biomolecules released by *B. subtilis*. Surfactin produced by *Bacillus amyloliquefaciens* KSU-109 was also used for the synthesis of cadmium sulfide nanoparticles which remained stable up to 6 months without compromising their functionality [74]. Additionally, Maity and collaborators [96] reported the synthesis of brushite particles (nanospheres and nanorods) in reverse microemulsions of surfactin.

Rhamnolipids and sophorolipids have also been successfully used for the synthesis and stabilization of metal-bound nanoparticles. Purified rhamnolipids from *P. aeruginosa* strain BS-161R were used to synthesize silver nanoparticles which exhibited good antibiotic activity against both Gram-positive and Gram-negative pathogens and *Candida albicans*, thus suggesting their broad spectrum antimicrobial activity [55]. In another work, a glycolipid microbial surfactant produced by *Brevibacterium casei* MSA19 was used as a stabilizer for the synthesis of stable and uniform silver nanoparticles [102]. The microbial surfactant acted as a stabilization agent and prevented the formation of aggregates.

Palanisamy and Raichur [76] reported a simple method for synthesizing spherical nickel oxide nanoparticles by microemulsion technique using rhamnolipids. The synthesized nanoparticles were found to be fully crystalline and spherical in shape with uniform distribution and increasing the pH of the solution decreased the size of the nanoparticles. Xie et al. [78] synthesized silver nanoparticles in rhamnolipid reverse micelles, while in another study rhamnolipids were used as capping agents for the synthesis of ZnS nanoparticles in aqueous medium [79].

Sophorolipids have also been evaluated for the synthesis of nanoparticles and were reported to be good reducing and capping agents for cobalt and silver particles [89]. Singh and collaborators [90] proved the antibacterial activity of sophorolipid-coated silver and gold nanoparticles against both Gram-positive and -negative bacteria. Furthermore, the authors found that sophorolipid-coated gold nanoparticles were more cyto and geno-compatible as compared to silver nanoparticles [91].

Methodologies for the biological synthesis of metal nanoparticles using microbes have been described [94,107,108]. Indeed, several researchers have recently suggested the usefulness of living cells and natural products [81,109–111] in the synthesis of nanoparticles. For example, *Aspergillus fumigatus* has been implicated in the extracellular production of silver nanoparticles [112], but many other fungi and bacteria have also been reported to produce gold and silver nanoparticles, either intra or extracellular [113–118]. Silver nanoparticle synthesis was accomplished using *Pseudomonas stutzeri* AG259 [119], *Fusarium oxysporum* [115], *Phanerochaete chrysosporium* [120], *Plectonema boryanum* UTEX 485 and *Klebsiella pneumonia* [121]. Some of these whole organisms are also able to produce microbial surfactants, thus contributing to the synthesis process.

5.4. Microbial surfactants as constituents of microemulsion systems

The essential elements to produce a microemulsion-based colloidal DDS comprise an aqueous phase, an oil phase, a surfactant

and usually a co-surfactant or co-solvent. The surfactant self-aggregates to form varying structures. These structures are able to encapsulate and/or solubilize hydrophobic or hydrophilic drugs in the presence of a dispersed phase (oil for O/W or water for W/O microemulsions) within its structural core, thus partitioning the dispersed phase from the continuous phase [122]. A global micro-emulsion system can exhibit a wide range of structures of diverse nano-sized geometries (e.g. worm-like, bi-continuous sponge-like, liquid crystalline, among others). These systems are thermodynamically stable and exhibit high solubilization capacity and ultra-low interfacial tensions of oil and water, thus making them desirable for drug delivery applications [75]. The results from Xie and collaborators [80] suggest that rhamnolipids could be successfully used to form microemulsions using medium chain alcohols as co-surfactant. Moreover, the phase behavior and microstructure of these microemulsions were rational to the conformational changes of rhamnolipid molecules at the interface of O/W [82].

Microemulsion techniques using oil–water-surfactant mixtures have been found to be very useful in the production of nanoparticles and can be used to synthesize different types of particles. Silver nanoparticles have been synthesised using a rapid microbial surfactant-mediated synthesis, by mixing the surfactant and $AgNO_3$ solutions, followed by NaBH4 and vigorous stirring. Also, the silver nanoparticle production was accomplished *in situ* in the W/O microemulsion phase as described by Xie and collaborators [78]. For that purpose, aqueous solutions of $AgNO_3$, purified biosurfactant, n-butanol and n-heptane were vigorously stirred until homogeneous reverse micelles were formed. Then, $NaBH_4$ was used to form other reverse micelles. The two reverse micelle solutions were mixed and afterwards ethanol was added to the reaction mixture to break the reverse micelles. Finally, the silver nanoparticles were precipitated from the solution and isolated by centrifugation.

Ramnolipids have been successfully used to synthesize spherical nickel oxide nanoparticles (NiO nanorods) by this technique [76,77]. A first microemulsion was prepared by vigorously mixing rhamnolipid with heptane, followed by the addition of $NiCl_2$. A second microemulsion was prepared in NH_4OH. Subsequently, the two microemulsions were mixed and stirred to precipitate nickel hydroxide. The synthesized nanoparticles were found to be stable at different pH and temperatures [78,95]. Contrarily, experiments conducted with a different microbial surfactant (brevifactin, a novel lipopeptide biosurfactant produced by the marine actinobacterium *Brevibacterium aureum* MSA13) showed that both pH and temperature significantly affected the stabilization process [123]. Indeed, the reduction of metallic ions was found to be sensitive to the pH as it could affect the shape and size of the nanoparticles. The synthesis occurred under alkaline conditions, which was also reported by Sanghi and Verma [124].

Rhamnolipids have additionally been reported as dispersants for nanoparticles [86]. Fatty acids have been suggested to play a double role in the synthesis of nanoparticles, by stabilizing O/W emulsions thereby aligning the reducing groups on the outer side; and/or by reducing metal salts into nanometals [125,126]. Nguyen and Sabatini [83] formulated alcohol-free microemulsions using rhamnolipids. Also, microemulsions of lecithin/rhamnolipid/sophorolipid microbial surfactants using a range of oils have been developed and evaluated [75]. The sophorolipid played an important role as the hydrophobic component in these formulations and the phase behavior of these biocompatible microemulsions did not change significantly with changing temperature and electrolyte concentration, making them desirable for drug delivery applications.

6. Challenges & future perspectives

One of the present challenges on the medical field involves the search for new drugs and development of DDS with enhanced efficacies capable to significantly affect the outcome of several types of diseases [127]. An ideal DDS must exhibit two key features, namely an optimal drug loading capacity, which will lead to an increased drug bioavailability and ability to reach the target; and the subsequent release of the drug in a controlled and timely manner [5]. In this sense, diverse types of drug delivery vectors have been developed, including polymeric, particulate, macromolecular and cellular carriers. Under the particulate type occurring in a dispersed colloidal form, several structures can be found comprising microspheres, nanoparticles, micelles and liposomes, among others [128]. Particularly, microemulsions, mainly because they are easy to formulate, have become popular as new DDS that can be used through diverse routes including oral, nasal, ocular, topical and intravenous [127]. Nonetheless, systematic and pre-clinical studies must be conducted before an optimal formulation can meet the safety and efficacy criteria required. Given the thermodynamic stability of microemulsion systems [129], more caution has been used in the formulation of self-microemulsifying DDS, specifically for oral or parental routes. Indeed, most DDS fail when these routes of drug administration are used [130], particularly because of the poor efficacy in the drug delivery and the drug precipitation before it can reach the target site, besides the biocompatibility and biodegradability of the materials used.

In the particular case of microemulsions, an increased effort to search acceptable excipients to be used in the design of safer microemulsions for drug delivery applications has been registered lately. Prior attempts have usually applied synthetic hydrocarbon oils (e.g. heptanes, dodecane and cyclic oils) and surfactants (e.g. sodium dodecyl sulphate and tetraethylene glycol monododecyl ether) that are not approved for pharmaceutical applications and can be toxic [131]. Biocompatibility has been assured by the alternative use of lecithins and non-ionic surfactants (e.g. Brijs, Tweens and AOT) [132]. Simultaneously, a recent trend in the formulation of microemulsions is to use natural oils and natural and/or microbial surfactants. Natural oils are receiving an increased interest (e.g. linseed oil, soybean oil, jojoba bean oil, among others), but it is relatively difficult to solubilize them in microemulsions [133,134]. Likewise, natural and/or microbial surfactants have emerged as alternatives to their synthetic counterparts. Particularly, the non-ionic surfactants such as sucrose esters have been widely used in the production of microemulsions [135,136], although many other examples can be highlighted. Although the use of microbial surfactants appears to be an exciting alternative, these biomolecules also present some limitations that must first be addressed before considering them for drug delivery applications, for example in what concerns their purity and safety.

Although it is very challenging to predict the nature and stability of a microemulsion-based DDS, the current knowledge available in the literature can support the selection of the most adequate oil/biosurfactant systems. Similarly to synthetic surfactants, also microbial surfactants can be affected by the environmental conditions, and thereby their self-assembly. As previously discussed rhamnolipids and surfactin are the most studied microbial surfactants, thus a great amount of data on their structural aspects at different interfaces and solutions has been reported [137–140]. Nevertheless, microbial surfactants have very complex head groups which further complicate the accurate evaluation of their structures, since they can assume diverse structures with only minor changes in the environment.

In general, it can be anticipated that these microbial surfactants are non-ionic at low pH values, and anionic at high pH values due to the presence of carboxylic groups [80]. Moreover, structure transition from micellar to lamellar upon electrolyte addition has been reported [141,142]. The possibility of manipulating structure transition opens a great opportunity for the development of tailored DDS, as well as for the design of smart DDS that can respond to

particular environmental stimuli (pH, temperature or salt concentration) in a controlled way [143].

The aggregation of surfactants in lamellar arrangements can occur if one of the next conditions is observed [144]. High surfactant concentrations lead to a lamellar liquid-crystalline phase, while double-tailed amphiphiles commonly form bilayer sheets. Upon closing, these sheets form vesicles. Besides, these aggregates can be obtained from mixtures of anionic and cationic surfactants in water, mixtures of ionic surfactants and long-chain alcohols in water, or electrolyte solution. Moreover, in the presence of high salt concentrations some surfactants in aqueous solution spontaneously change from micelles to lamellar aggregates.

The lack of information on microbial surfactant microemulsion systems, such as phase behavior and its stability under diverse physicochemical conditions, has limited so far the application of these molecules in DDS. Nonetheless, an adequate evaluation of some system parameters such as the *HLB*, *CPP*, H_o and Winsor-*R* ratio, would greatly assist a rational choice of the components that ultimately lead to a successful formulation. Additionally, these parameters constitute powerful tools for the design of drug formulations envisaging a specific administration route.

In addition to their potential for the formulation of microemulsions, microbial surfactants have been suggested as valuable molecules for the synthesis of nanoparticles and liposomes. Xie and collaborators [78] reported a promising approach for nanoparticle synthesis through microemulsion technique involving the use of an oil–water-surfactant mixture. Despite the high potential of chemical surfactants, these can present some toxicity to the environment and therefore, the possible use of microbial surfactants represents an interesting alternative. Microbial surfactants are composed of mostly sugar and fatty acid moieties; possess higher biodegradability, lower toxicity, present remarkable biological activities (e.g. antimicrobial, antiviral, anticancer, among others), and have been reported as "green" candidates for the synthesis and/or stabilization of nanoparticles [10]. Lately, an increased interest on biosurfactant-mediated processes has been reported, mainly due to their potential role on the synthesis of silver nanoparticles and NiO nanorods [10,77].

The biological synthesis of nanoparticles is apparently superior to the chemical one, however it is important to take into account that this process is much slower comparing to approaches in which reducing agents are used. Besides, recovering the nanoparticles from the bacterial mass or natural extracts is somewhat complex, hence this is also a shortcoming of the biological synthesis of nanoparticles [10]. In this sense, the microbial surfactants comprise an interesting and greener alternative to the bacteria- or fungi-mediated synthesis processes, as these molecules reduce the formation of aggregates due to the electrostatic force of attraction and facilitate the uniform morphology of nanoparticles.

Regardless of the well-known potentialities of nanoparticles, they also present some limitations, as for example their small size and large surface area that can lead to aggregation, thus making their handling challenging in liquid and dry forms. Also, nanoparticles may be limited regarding their drug loading and release capacity. Metal nanoparticle properties including size, morphology, stability and physicochemical properties are strongly dependent on the experimental conditions, kinetics of interaction between metal ions and reducing agents, and adsorption mechanism between the stabilizing agent and the metal nanoparticles [10]. Therefore, the development of synthesis procedures that enable controlling the abovementioned properties is highly desirable [145]. Taking also into account the need to produce stable nanoparticles for unique applications, e.g. drug delivery [146]; the microbial surfactant-mediated synthesis appears to be a promising approach.

As mentioned, several microbial surfactants have been tested in the synthesis of nanoparticles. For instance, the rhamnolipids effect on the synthesis/stabilization of nanozirconia particles has been reported [87]. However, since pH and temperature affect the nanoparticles stability, screening and development of new microbial surfactants that could be stable over a range of pH, temperature and salinity concentrations would be highly advantageous for the synthesis and stabilization of nanoparticles. Moreover, although the microbial surfactant-mediated processes are highly effective, these are still not cost-effective. Hence, it is of utmost importance to develop scalable and economic bioprocesses for the production of novel microbial surfactants.

The most efficient method for the fast synthesis of great amounts of nanoparticles is through chemical reduction [10]. This method allows manipulating the nanoparticles shapes by changing the reaction conditions. However, the nanoparticles are unstable and tend to aggregate into larger structures, thus leading to some loss of their original characteristics and activity. Moreover, the reactants used are toxic with potential environmental and health risks [147]. Therefore, an alternative method to overcome the chemical reduction limitations and prevent nanoparticles aggregation is through reverse microemulsion which enables obtaining uniform and size-controllable nanoparticles [148,149]. The synthesis of spherical nanoparticles in W/O microemulsions remains the most common surfactant-mediated process. In microemulsions, the water soluble molecules in the droplet are kept inside and the droplet functions as a "reactor". By increasing the surfactant concentration, the size of the droplet is decreased, thereby decreasing the particle size. The water content dissolved in the microemulsion will dictate the morphology and size of the resulting nanoparticles. Han and collaborators [150] demonstrated the influence of the molar ratio of water to surfactant on the particle size distribution and monodispersity. It has been hypothesized that when the particle size is similar to that of the water pool, the surfactant molecules will adsorb on the particle surface to inhibit the particles from aggregating, thus controlling their size and shape. For instance, in a microemulsion-mediated borohydride reduction method, the interactive forces among the reverse micelles lead to collisions among the micelles that result in the exchange of reactants. Consequently, the monomeric silver nuclei start to form in the micelles and grow to a size dependent on the water core of the microemulsion.

Despite all the recent progress in the development of DDS, the perfect vector remains to be designed. This vector must be able to overcome the current limitations of such systems, namely regarding safety, bioavailability and efficacy [13]. Besides, the release of the drug in a controlled and timely manner is highly desirable. Although some successful examples have been reported in the literature on the use of triggered systems [151]; future research should continue to focus on the design and engineering of such triggers and switchable systems. Microbial surfactants have been recognized as versatile and useful molecules for several applications including targeted drug delivery. Although these molecules have proven their added value in the development of microemulsion-based drug formulations [5], as well as in the synthesis of nanoparticles [10], this imminent potential remains unexplored. Given their low toxicity, high biodegradability and biological activities, besides their performance in the production of the abovementioned DDS, microbial surfactants are promising alternatives to their chemical counterparts [4]. Besides, being produced by a number of microorganisms, these molecules present a wide variety of chemical structures which can be seen as an opportunity for the design of novel vectors. Moreover, microbial surfactants can potentially be used in triggered and targeted drug delivery. Shim and collaborators [97] successfully demonstrated the enhanced delivery of siRNA in HeLa cells using cationic surfactin liposomes.

Also, it was found that the surfactin-containing liposomes improved the specific silencing of the gene of interest. Additionally, MEL have also been evaluated for gene delivery, as they could form thermodynamically stable vesicles [152]. Indeed, MEL-containing vesicles dramatically increased the transfection efficacy of cationic liposomes leading to considerably higher levels of gene expression as compared with lipid cationic-containing commercially available kits [64,65]. Recently, rhamnolipids from a *P. aeruginosa* biofilm were evaluated for their ability to trigger the release of a drug encapsulated in lipid-polymer coated hybrid nanoparticles [88]. This system enabled triggering the drug release in the vicinity of the *P. aeruginosa* colonies, thus improving the nanoparticles anti-bacterial effectiveness. Moreover, pH-sensitive liposomes containing rhamnolipids and dioleoylphosphatidylethanolamine (DOPE) have been described as efficient systems for cytoplasmic delivery of molecules into cells. DOPE in combination with di-rhamnolipids formed stable multilamellar and unilamellar liposomes. Acidification of the liposomes led to membrane destabilization, fusion and release of entrapped aqueous liposomes contents; thus these pH responsive liposomes represent a promising means to deliver foreign substances into living cells in a controlled way [88]. All these examples strongly support the concept that targeted and triggered drug release using microbial surfactants can be further explored to develop superior drug delivery systems.

Acknowledgments

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