Chapter 7

# Amino Acid-Based Surfactants for Biomedical Applications

Lídia Pinheiro and Célia Faustino

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67977

**Abstract**

The growing demand for surfactants worldwide has a profound impact on the environment and public health. The quest for environmentally friendly "green" surfactants has driven research toward bio-based surfactants from renewable sources with improved performances and low toxicity. Amino acid-based surfactants (AAS) are a promising class of biocompatible and biodegradable surfactants for biomedical applications due to their improved safety profiles that meet the requirements of both physiological and ecological compatibility. Natural amino acids are chiral compounds and important raw materials for production of AAS. The amino acid pool allows the synthesis of multifunctional surfactants with chiral properties that can be tailored for specific technological and/or biomedical applications. The nature of the amino acid residue, the chirality, and the ability for hydrogen bond formation strongly influences the surface active properties and self-assembly behavior of AAS. This review summarizes recent developments in AAS structure-property relationships providing valuable information for modulation of the surface active and biological properties of AAS to meet specific biomedical applications. The interaction of AAS with biointerfaces and biological molecules is also addressed concerning cellular toxicity and potential therapeutic applications of AAS as antimicrobial agents, drug delivery vehicles, and a promising alternative to viral vectors in gene therapy.

**Keywords:** amino acid, surfactant, micelles, drug delivery, gene delivery

## 1. Introduction

Surfactants are surface active molecules characterized by a polar headgroup linked to a long hydrocarbon chain. According to the nature of their headgroup, surfactants are classified as nonionic, anionic, cationic, or zwitterionic [1]. The amphiphilic nature of surfactants is the

basis of their characteristic properties, such as the ability to adsorb at interfaces, lower the surface tension of water or spontaneously self-assembly in aqueous systems forming micelles once the critical micelle concentration (CMC) is reached.

Surfactants are widely used as wetting agents, detergents, emulsifiers, and softeners in a wide range of industries [1]. The type of application is determined by the balance between the hydrophilic and hydrophobic regions in the surfactant molecule. Petrochemical-based surfactants represent the large majority of surfactants in the market today [2, 3]. The huge consumption of surfactants worldwide calls for sustainable surfactant production from natural renewable sources in order to reduce the impact on the environment, answers consumers' demand, and keeps up with increasing regulatory pressure. Thus, there is an urgent need for the development of novel environmentally friendly surfactants with low toxicity and improved performances based on natural building blocks that can be produced by clean and sustainable technologies.

Natural amino acids are potential building blocks for surfactant synthesis due to their availability, biocompatibility, and multifunctionality [4–6]. Protein hydrolysates from waste proteins are ideal raw materials for the production of amino acid-based surfactants thus contributing to the valorization of secondary products, to the reduction of pollution load, and to the sustainable development of the bioindustry [7, 8].

Amino acid surfactants (AAS) are biocompatible and biodegradable surfactants obtained by condensation of natural amino acids with fatty acids (or their derivatives) of oleochemical source [5, 9, 10]. Hydrolysis of triglycerides from animal fat or vegetable oils furnishes a wide variety of saturated and unsaturated fatty acids with straight hydrocarbon chains and an even number of carbon atoms due to their biosynthetic route [3, 11, 12]. The use of non-edible waste cooking oils is also a viable alternative, and further contributes to reduce the environmental burden [13].

Moreover, AAS can be produced in large scale by green chemistry approaches, including enzyme-catalyzed synthesis using immobilized lipases and proteases, although chemical processes still prevail due to high yields and low production costs [14–19]. Being specialty surfactants, AAS still represent a low market share, but the trend toward green surfactants entirely produced from natural renewable sources by environmentally friendly technologies can change this scenario [3].

## 2. Chemical structure and classification

The presence of an amino acid as the polar headgroup characterizes AAS. The 20 standard amino acids used as the building blocks of proteins are the natural choice as raw materials for the production of AAS. The proteinogenic amino acids are L-$\alpha$-amino acids (except gycline, which is achiral) classified according to the nature of their side-chain. Due to the presence of both amino and carboxylic acid groups, amino acids are amphoteric compounds and exist as zwitterions at physiological pH.

http://dx.doi.org/10.5772/67977

The wide diversity of AAS, with different structures and properties, is only possible due to the nature and multifunctionality of the amino acid residue. According to the site of introduction of the hydrophobic chain and to the charge of the amino acid side-chain, anionic, cationic, or zwitterionic AAS can be obtained.

The hydrophobic chain can be introduced through acyl, ester, amide, or alkyl linkage [5, 9]. Due to the stability of the alkyl bond compared to biodegradable amide and ester bonds, it will not be considered further. Thus, introduction of the hydrophobic chain at the amino group by acylation with a fatty acid leads to anionic *N*-acyl AAS, while condensation of the carboxyl group of the amino acid with fatty alcohols or fatty amines produces cationic alkyl ester and alkyl amide AAS, respectively (**Figure 1**).

Moreover, according to the number of hydrophobic chains introduced, single-chain, double-chain, or dimeric (gemini) AAS can be obtained. Gemini AAS are made of two amino acid headgroups and two hydrophobic chains per molecule joined by a spacer chain at or near the

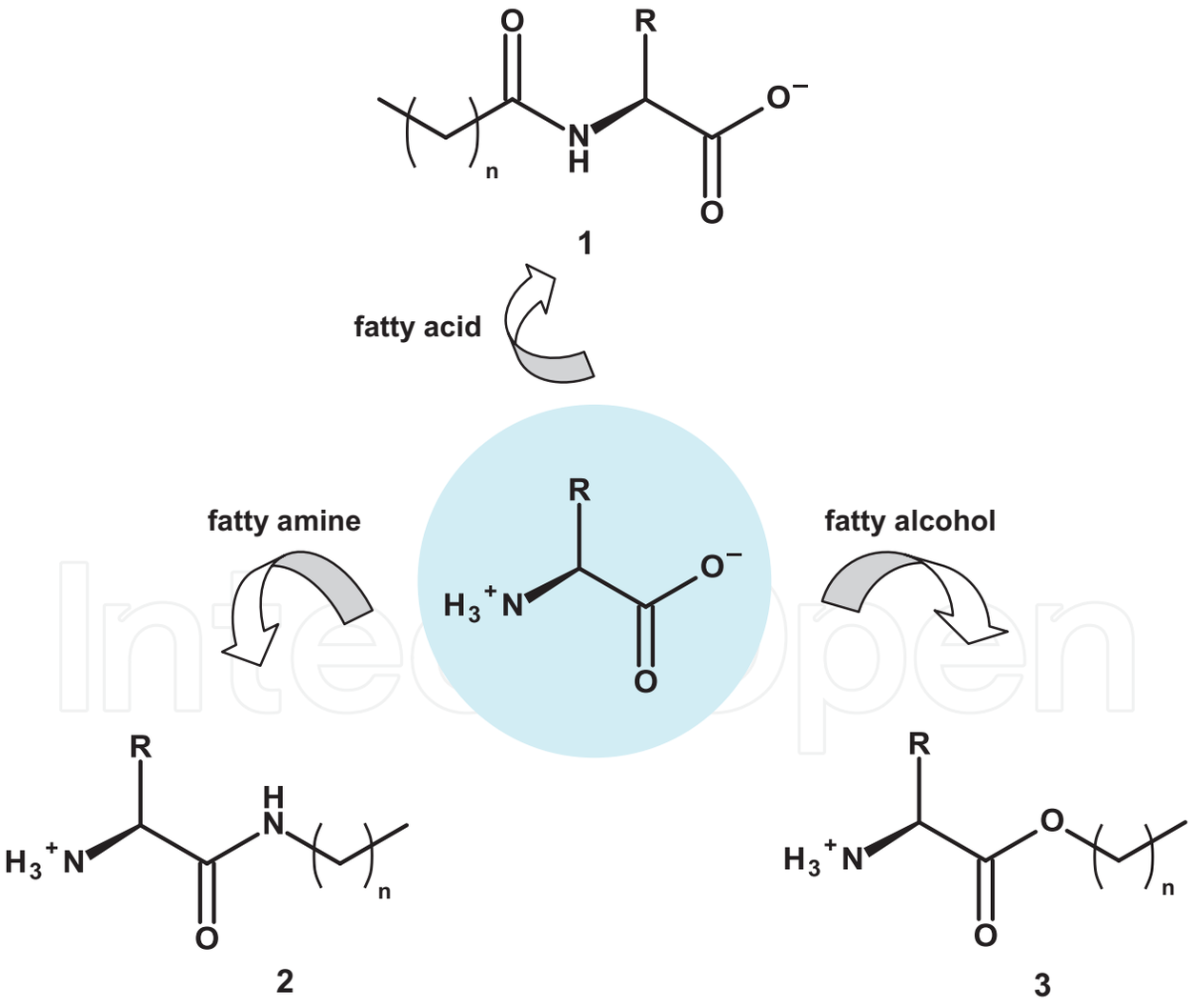


**Figure 1.** Types of amino acid-based surfactants: acyl (**1**), ester (**2**), and amide (**3**) bond derivatives.

headgroups. Gemini AAS show better performance compared to their monomeric counterparts, such as lower CMC, higher efficiency in surface and interfacial tension reduction, and higher solubilization capacity [10, 20]. Cystine, the dimer of cysteine where the thiol groups have been oxidized to form a disulfide bond, is a potential raw material for the synthesis of gemini AAS.

## 3. Properties and self-assembly behavior

### 3.1. Single-chain surfactants

The nature of the amino acid residue determines the main differences on the adsorption, aggregation, and biological properties among the AAS. The self-assembly of surfactants in aqueous media has been extensively studied and some correlations between AAS structure and surfactant properties have been established. Thus, increasing the length of the hydrophobic chain is usually accompanied by a decrease in the CMC, similarly to conventional surfactants, where the hydrophobic interaction is the driving force for the self-assembly process. For the AAS, non-covalent interactions arising from the side-chains of the amino acid residues provide additional contributions, and effective solvation of the headgroups is also a relevant factor influencing self-assembly [6, 10, 21].

The CMC is an important parameter for the biomedical application of AAS, since interactions with biointerfaces and cellular components largely differ in the presence of micelles or monomeric surfactant molecules. Although the CMC often decreases with increasing hydrophobicity of the amino acid, specific intra- and/or intermolecular interactions between the amino acid residues may alter this trend [22, 23].

The presence of aromatic or bulky substituents and the presence of hydrogen bond donor/acceptor groups can strongly influence molecular packing at interfaces and micelle stabilization. Intermolecular hydrogen bonding interactions between headgroups can occur for AAS with amino acid residues with hydroxyl, amido, amino, and carboxylic groups in their side-chains, contributing to their adsorption and micellization properties in solution [6, 10, 21].

The *N*-acyl phenylalanine AAS usually show lower CMC than the other *N*-acyl amino acids with the same acyl-chain length due to intramicellar $\pi$-$\pi$ interactions between the aromatic rings of the amino acid side-chains that contribute for micelle stabilization. These interactions also occur in the solid state, contributing to the higher Kraft temperature of phenylalanine AAS. On the other hand, the complex self-assembly behavior of proline AAS is associated with van der Waals repulsions between the bulky side-chains while the incapability to form hydrogen bonds is reflected in high surface area as a result of less compact molecular packing at the air/water interface [10, 21, 23, 24].

The CMC values found for *N*-decanoyl leucine, methionine, proline, and serine followed a trend related to the hydrophilicity of the amino acid headgroup, with the less hydrophilic surfactant showing the lower CMC [24]. DLS measurements showed that the leucine and methionine derivatives formed spherical micelles with sizes around 3–5 nm, while the serine and

proline surfactants formed larger supramolecular aggregates (11–14 nm), probably elongated rod-like micelles, due to the presence of the hydroxyl group on the serine AAS and conformational rigidity in the proline AAS. The steric hindrance of the pyrrolidine moiety in the proline AAS as well as the hydroxyl-containing side-chain in the serine derivative that prevent simple insertion of the hydrophobic chain inside red cell membrane were also responsible for their lower hemolytic activity [24].

The hydroxyl in the phenol ring of tyrosine dodecyl ester hydrochloride has a strong influence on the conformation of the molecule, providing more compact structures at the interface and contributing to lower interfacial area relatively to the more hydrophobic phenylalanine derivative, as demonstrated from molecular modeling studies [25].

For the surfactants with amide bonds, such as the *N*-acyl AAS, the capacity of the amide bond to participate in intra- and intermolecular hydrogen bonding can strongly influence the surfactant properties both at interfaces and in solution. The role of the amide bond for the self-assembly of AAS in solution, and their adsorption at the air/water interface and at solid surfaces, was studied by different techniques using sodium *N*-lauroyl glycinate and sodium *N*-lauroylsarcosinate as model surfactants [26]. The former can form intermolecular hydrogen bonds via the amide group but not the latter, due to the methylation of the amide nitrogen. The amide bond was found to contribute to the hydrophilicity of both surfactants, with slightly lower CMC being observed for the sarcosinate derivative due to the hydrophobicity of the additional methyl group. The ability of the glycinate surfactant to form intermolecular hydrogen bonds led to tighter packing at the air/water interface and at hydrophobic surfaces. The higher ionization degree found for the sarcosinate surfactant was also in accordance with a less tight packing of the surfactant in micelles in aqueous solution.

Besides micelles, AAS can form other supramolecular structures in aqueous medium due to the chiral nature of the amino acid residue, which may induce molecular packing into self-assembled tubules, rods, helical and twisted ribbons, or fibers. Moreover, the chiral nature of AAS allows them to interact selectively with enantiomers of chiral solutes, which has important practical implications. Chiral AAS are routinely employed as column-packing material in chiral chromatography for the separation of racemic mixtures and as enantiodiscriminating NMR solvents. The chiral discrimination of AAS has been reported in the solid state, in liquid crystalline phases, in Langmuir monolayers, and even in micelles [6, 10].

Racemic mixtures of the sodium salts of *N*-lauroyl phenylalanine and *N*-lauroyl valine, as well as *N*-acyl glutamic acid disodium salts, exhibited higher CMC than that of the pure enantiomers. The effect was attributed to the differences in the conformation of the amino acid moiety of surfactants at the micelle surface. Racemic *N*-stearoylserine also showed slightly higher CMC compared to the pure L- or D- enantiomers and strong variations in the circular dichroism spectra of the enantiomerically pure micelles suggested formation of a repetitive arrangement of the polar headgroups at the micellar surface stabilized by intermolecular hydrogen bonds between the amide groups [6, 10]. The preference for homochiral (L-L or D-D) over heterochiral (D-L) interaction depends on the stability of the hydrogen bond formed between the amino acid residues in combination with stereochemical effects.

The influence of chirality on the micellar properties of AAS seems to be dependent on structural effects related to the amino acid residue, such as size and stereochemical hindrance, since no differences have been found between the CMC of the racemic mixtures and that of enantiomerically pure enantiomers for the sodium salts of *N*-palmitoyl-phenylalanine, leucine, threonine, methionine, and proline, and potassium salts of *N*-lauroylalanine or cationic surfactants derived from *N*-lauroylarginine [23]. Supramolecular chirality has been successfully employed in asymmetric organic synthesis [27].

The AAS developed at the industrial scale are mainly anionic *N*-acyl AAS due to their mild properties, low toxicity, biodegradability, and facile synthesis by the Schotten-Baumman process involving condensation of the amino acid with a fatty acyl chloride (from a fatty acid) under aqueous alkaline conditions. Moreover, many *N*-acyl AAS have emulsifying and antimicrobial properties with potential value as additives for formulations in the food, pharmaceutical, and cosmetic industries.

Although most AAS reported in the literature are based on compounds made from condensation of an amino acid with pure fatty acids, many commercial AAS are produced from amino acid and/or fatty acid mixtures obtained from protein hydrolysates or triglyceride hydrolysis, respectively, due to cost advantage [28]. Often AAS with mixed fatty acid chains have superior performance when compared to pure compounds because of synergistic interactions. Cocoyl glycinate exhibited considerably lower CMC than the sodium salts of lauroyl glycinate, lauroyl sarcosinate, and *N*-stearoyl amino acids, which may be related to the presence of acyl glycinates of different acyl chain lenghts [19].

The sodium salts of *N*-acyl phenylalanines and *N*-acyl isoleucines prepared from fatty acid mixtures obtained from coconut, palm, palm kernel, jathropa, karanja, *Sterculia foetida*, castor, and high oleic sunflower oils exhibited superior surface active properties like surface tension, CMC, calcium tolerance, wetting power, foaming, and emulsion stability compared to reference surfactant sodium lauryl sulphate (SLS) [12, 29]. Except for the *N*-acylphenylalaninate from coconut oil, all the other *N*-acyl phenylalanines showed promising cytotoxicity against human cancer cell lines [12].

The *N*-acyl AAS from aspartic and glutamic acid are mild surfactants widely used in cosmetics and personal care formulations due to their low toxicity and mildness to the skin and eyes. The disodium salts of *N*-lauroyl aspartate and *N*-lauroyl glutamate show CMC values of 73 and 74 mmol/L, respectively, much higher than that of sodium *N*-lauroyl glycinate (14 mmol/L) due to the presence of the additional carboxylate group [30]. The CMC and the micellar ionization degree were also influenced by the choice of monovalent counterion, increasing in the order $Li^+ < Na^+ < K^+$ which is the opposite trend usually found for anionic surfactants in solution. These results were interpreted according to the hard and soft acid-base concept, i.e., the hard carboxylate headgroup binds stronger to the harder lithium cation, in agreement with the Hofmeister series [31].

Significant differences were found in the ability of the dicarboxylate surfactants to chelate calcium [30]. Lauroyl aspartate formed an intramolecular complex with calcium ions while lauroyl glutamate formed an intermolecular complex which resulted in higher calcium tolerance,

tight packing at the air/water interface, and very low surface tension values (below 30 mN/m). On the other hand, the ability to form intramolecular chelates favored adsorption at calcium-containing surfaces with lauroyl aspartate adsorbing strongly on hydroxyapatite while lauroyl glutamate showed weak adsorption [30].

The degree of neutralization also influences the properties of the dicarboxylate AAS. According to TEM studies, a gel network structure was formed both in the bulk phase and in the foam films of sodium lauroyl glutamate at a certain temperature and pH range [32]. Variation of gel strength in foam film with changes in pH and temperature influenced foam stability. Formation of a weak gel in foam film favored foam stability while formation of hard gel had the opposite effect. The highest foam stability, as well as minimum surface tension, were found at pH 7 when no gel was formed in solution suggesting a more dense arrangement of the surfactant molecules at the air/water interface of the foam film as described by molecular dynamics simulation.

$N^{\alpha}$,$N^{\varepsilon}$-Dioctyl lysinate salts with different counterions ($Li^+$, $Na^+$, $K^+$, $Lys^+$, and $Tris^+$) have been studied both in the dry state and in aqueous solution. The CMC was found to be nearly independent of the counterion, which had a strong influence in hemolytic activity. Surfactants interact with erythrocyte membranes in a biphasic way by protecting against hypotonic hemolysis at low concentrations but inducing hemolysis at higher concentrations [33]. All the compounds protected erythrocytes against hypotonic hemolysis, with $HC_{50}$ values (concentration of surfactant that induces 50% hemolysis in isotonic medium) in the range 260.7–560.0 μg/mL that increased in the order $Na^+ < K^+ < Lys^+ < Li^+ < Tris^+$. The maximum protective concentration of each surfactant was close to its $HC_{50}$ value and below the CMC value while the antihemolytic potency was around 35% except for the potassium salt which showed a value of 76% [33].

The structure of the dioctyl lysinate salts in the dry state depended on the size of the counterion. Large organic counterions favored lamellar arrangements while small inorganic counterions favored bicontinuous cubic structures [34]. The influence of acyl chain length on the dry state structure of diacyl lysine surfactants showed that long alkyl chains favored a lamellar structure while medium length chains produced cubic bicontinuous structures. On the other hand, short chains promoted formation of reverse hexagonal structure similar to that of Aerosol-OT in the dry state, a behavior that was attributed to lack of flexibility of the chain to adopt a packed conformation.

AAS can spontaneously form vesicles with other amphiphiles in aqueous media. Catanionic mixtures of sodium $N^{\alpha}$,$N^{\varepsilon}$-dilauroyl lysinate and dodecyltrimethylammonium bromide (DTAB) exhibited several single and multiphase regions [35]. Addition of increasing concentrations of the lysinate surfactant to pure DTAB solution leads to mixed micelle formation and micellar growth until a given mixing ration at which vesicles assemble and coexist with small micelles. In the DTAB-rich system, stable unilamellar vesicles were observed with an average size in the order of 30–40 nm according to self-diffusion measurements and cryo-TEM imaging.

On the other hand, the pure lysinate surfactant crystallized into micrometer-sized tubules upon cooling from an isotropic micellar solution that induce gelation of the system [36].

Hydrophobic interactions and hydrogen-bonding between the polar headgroups contributed to the stability and overall rigidity of the tubules. The chiral center in the amino acid headgroup was held responsible for tubular self-assembly; however, electrostatic interactions also played a role in the process since tubules were not formed at low pH when the surfactant exists mainly in the neutral form. The phase behavior of the lysine AAS showed the phase sequence micellar → hexagonal → lamellar → hydrated crystals which is expected for single-chain surfactants, suggesting that the double-chain lysinate AAS adopts an overall cone-shaped configuration instead of a cylindrical one [36].

Long chain cationic AAS from arginine are usually biodegradable surfactants showing antimicrobial activity and low toxicity. Several cationic single-chain arginine AAS have been studied, including $N^{\alpha}$-acyl arginine methyl ester hydrochloride, arginine *N*-alkyl amide dihydrochloride, and arginine *O*-alkyl ester dihydrochloride, obtained by the synthetic pathways shown in **Figure 1**. For all the surfactants studied, increasing the hydrophobic chain length was accompanied by a decrease in the CMC, as expected. The CMC values and the surface tension at the CMC were lower than the ones found for commercial quaternary ammonium surfactants with the same alkyl chain length, where the cationic charge is closer to the $\alpha$-carbon of the hydrophobic chain than in the arginine surfactants. For the same alkyl chain length, CMC was lower and molecular surface area at the air/water interface was higher for the dicationic surfactants compared to the monovalent compound which indicates less tight packing at the interface due to an increase in the inter- and intramolecular electrostatic repulsions among the headgroups [5].

Considering the type of linkage between the arginine headgroups and the hydrophobic chain, the ester bond resulted in surfactants with higher biodegradation rates when compared to the ones with amide bonds. Based on the hemolytic activity measured by $HC_{50}$, both the monocationic esters and the dicationic amides were classified as non-hemolyzing agents ($HC_{50}$ < 1000 μg/mL compared to 4–15 μg/mL for commercial cationic surfactants) and nonirritating to the eyes according to the *in vivo* eye irritation Draize test [14].

The phase behavior of $N^{\alpha}$-acyl arginine methyl ester hydrochloride showed the classical phase progression hexagonal → cubic → lamellar liquid crystal typical of single-chain surfactants, like DTAB, with increasing concentration in the micellar solution phase. Reversed vesicles in the lecithin- $N^{\alpha}$-lauroyl arginine methyl ester-squalene-water system have also been reported [5].

Amino acid-glyceride conjugates with a glycerolipid structure have also been studied. The 1-monoacyl glyceroarginine AAS formed micelles with CMC values in the range 0.2–6 mmol/L that decreased with increasing acyl chain length [5]. The 1,2-diacyl glyceroarginine derivatives formed lamellar liquid crystals and their dispersions at 0.1% in water led to spontaneously self-assembly into stable multilamellar vesicles. The diacyl glyceroarginine AAS were also found to stabilize both water-in-oil (w/o) and oil-in-water (o/w) droplets, forming multiple emulsions that constitute potential alternatives to diglycerides and lecithins with additional antimicrobial properties [5, 14].

### 3.2. Gemini surfactants

Gemini AAS surfactants usually show better performance, such as lower CMC, lower surface tension, lower Kraft temperature, and higher solubilization power, when compared to their

monomeric counterparts. The solubilization power of micelles has pharmaceutical relevance since micellar solubilization of hydrophobic drugs improves their water-solubility and stability against chemical and/or enzymatic degradation, thus enhancing drug bioavailability. Gemini AAS also show a rich polymorphic phase behavior and a variety of self-assembled aggregates has been observed, such as micelles, bilayers, and vesicles, depending on the nature of the amino acid polar headgroup and on the lengths of the hydrophobic tail and the spacer chain [5, 10].

The properties of anionic gemini AAS were first reported by Tsubone and coworkers who studied the sodium salts of *N*-acyl AAS derived from aspartic acid [37]. Very low CMC values, in the micromolar range, were observed for these geminis as well as an inversion of the tendency of the CMC to decrease with increasing acyl chain length for surfactants with acyl chains longer than 14 carbons accompanied by an increase in surface tension values, due to the formation of small size soluble aggregates (dimmers) below the CMC. The premicellar aggregates were devoided of surface activity but decreased the concentration of free monomers, thus reducing the surface activity. Other unusual findings were the absence of a break in the conductivity-surfactant concentration plots for the aspartate geminis and an increase in the pH of the solution with surfactant concentration in the CMC neighborhood. These findings suggested protonation of the carboxylate anion with simultaneous release of $Na^+$ during micellization. Hydrogen bond formation between the carboxyl and the amide groups, leading to an increase in the size of the headgroup, was responsible for the inhibition of micellization. The phenomenon had already been observed for monomeric AAS of the kind and results from the characteristic surfactant structure containing both *N*-acyl amide and carboxylate groups [38]. Moreover, the skin irritation potential of the gemini surfactant was lower than that of the monomeric counterpart or the nontoxic sodium sarcosinate surfactant as determined from human response to *in vivo* closed patch tests.

Several gemini surfactants formerly derived from cysteine have been synthesized since the nucleophilic thiol group of cysteine can be readily oxidized to cystine by the formation of a disulfide bond. Cystine is a potential building block for gemini surfactants for biomedical applications, including controlled-release drug delivery systems. Depending on the hydrophobic chain length, either micelles or vesicles can be formed, and the disulfide bond can be easily cleaved by endogenous reduction agents, such as glutathione, thus regenerating the free thiol group and liberating the encapsulated bioactive agents. Sodium *N*,*N*′-didecanoyl- and *N*,*N*′-dilauroyl cystine has been prepared and the ease of reduction of the disulfide bonds of the gemini surfactants was used to control the surface properties and aggregation behavior of these switchable surfactants [39]. Reduction of the gemini surfactants with dithiothreitol led to vesicle disruption while oxidation of the corresponding monomers to gemini surfactants regenerated the vesicles.

Faustino and coworkers synthesized anionic *N*,*N*′-dicarbamoyl gemini AAS from cystine by condensing the disodium salt of the dimeric amino acid with octylisocyanate. Their behavior in aqueous media at physiological pH and interaction with biomolecules of pharmaceutical relevance was characterized by conductivity, surface tension, and fluorescence quenching methods with pyrene as probe. The gemini AAS were found to interact with bile acids, membrane phospholipids, oligosaccharides, and serum albumin protein [10, 40–42].

The gemini surfactant was less efficient in surface tension reduction than its monomeric counterpart, which was attributed to the film formation by the hydrocarbon chains of the former at the air/water interface so that they cannot adsorb effectively at the interface, an unusual phenomenon also found for other gemini surfactants from cystine with the same alkyl chain lenght [43]. Chirality was found to influence the surface active properties of the gemini AAS and their interaction with chiral biomacromolecules but not their micellar properties in solution since similar CMC values and Gibbs energy of micellization were obtained for gemini AAS derived from L- and D-cystine, and their racemate [40, 41]. On the other hand, a less favorable packing at the air/water interface for the racemic mixture compared to the pure enantiomers was suggested by the higher surface tension and the higher Gibbs energy of adsorption observed for the former [40].

The surfactant cystine dioctyl ester dihydrochloride showed remarkable surface activity with a CMC value of 14.2 μmol/L, which was about one order of magnitude lower than that reported for other cystine surfactants with the same alkyl chain length [44]. Results from Langmuir film balance experiments showed that the cationic gemini forms stable viscoelastic films at the interface with molecular modeling studies pointing to a tilted orientation of the surfactant at the interface. SEM studies suggest that the gemini surfactant forms elongated micelles in aqueous solution.

Cationic gemini surfactants derived from arginine by linking two long chain $N^{\alpha}$-acyl-L-arginine residues through amide bonds to a diamine spacer of variable chain length, bis(Args), have been studied. Unlike their monomeric counterparts, aqueous solutions of the geminis exhibited unconventional aggregation behavior and two distinct CMC values were obtained from surface tension ($CMC_1$) and conductivity measurements ($CMC_2$). The higher $CMC_2$ values were consistent with the formation of regular micelles while the very low $CMC_1$ values could be attributed to non-globular small-size aggregates or to big lamellar-type aggregates according to the length of the spacer chain [5].

Recently, cationic gemini surfactants derived from lysine intended for biomedical applications were synthesized from $N^{\alpha}$-lauroyl lysine or $N^{\varepsilon}$-lauroyl lysine by linking the monomers through amide bonds to 1,6-hexanediamine or spermidine as spacers. Their CMC values were similar to the ones obtained by conductivity measurements for cationic arginine geminis of the same alkyl chain and spacer lengths, and about one order of magnitude lower than the ones for their similar cationic monomeric counterparts, the $N^{\alpha}$- and $N^{\varepsilon}$-lauroyl lysine methyl esters, respectively. For both type of lysine geminis, the position of the cationic charge, located either at the $\alpha$-amino or at the side-chain ε-amino group of the lysine residue, did not significantly affect the CMC which was dependent only on the hydrophobic character of the surfactants [45].

## 4. Biomedical applications

### 4.1. Antimicrobial agents

Development of new antimicrobial agents is mandatory, on the face of the fast growth of drug-resistant bacteria and fungi [46]. Cationic amino acid-based surfactants, which mimic natural antimicrobial peptides, can be seen as promising alternative antibacterial and antifungal agents

when compared to the currently used antibiotic compounds [14, 46]. Antibacterial monocatenary, dicatenary and gemini surfactants, with a creative molecular design that goes through new modes of action and diverse targets makes the difference to the existing conventional antibiotics.

These cationic antimicrobial AAS show an optimal association between the cationic charge and the hydrophobic moiety, which is the key to its activity against bacteria, fungi, and yeast [46]. Conventional antibiotics usually target specific enzymes or DNA. However, cationic AAS interacts with cellular membranes, leading to depolarization, lysis, and cell death, probably resulting from an advantageous incorporation into the hydrophobic lipid bilayer, which hinder bacterial resistance [46–48].

Cationic AAS represent promising alternatives to the typical cationic surfactants derived from quaternary ammonium salts that had been long-time utilized as biocidal agents (being part of antiseptics, dressing, catheters, and sutures). The hemolytic activity and cytotoxicity of the latter does not make them convenient for biomedical applications. Unlike cationic AAS, they are not easily biodegradable, hence toxic to aquatic organisms. The antimicrobial activity of cationic AAS depends on their structures and size (namely, the amino acid residue and the chain length as key parameters), the molecule hydrophilic/lipophilic balance, and the cationic charge density [46].

The arginine amino acid is an optimum raw material to prepare cationic surfactants with significant antiseptic and biocidal properties, due to the presence of a guanidine side chain [14, 46, 49]. Pinazo and coworkers [14, 46, 50] developed different synthetic routes (chemical, enzymatic, or a combination of both) to prepare a broad range of single chain and gemini arginine-based surfactants. The obtained minimum inhibitory concentration (MIC) were lower than the corresponding CMC values, which suggests that the species interacting with the bacterial membrane are monomers instead of aggregates. The synthesized compounds, with a broad range of action, exhibited good inhibitory activity against Gram-positive bacteria, Gram-negative bacteria, and yeast.

In the single-chain arginine-based surfactants, the alkyl chain length influenced the antimicrobial efficiency. For long chain $N^{\alpha}$-acyl arginine methyl or ethyl ester, the maximum activity of surfactants was obtained for an alkyl chain length of 12 carbons [14, 46]. For the arginine-*N*-alkyl amide surfactants the variation of MIC with the alkyl chain was less noticeable than in the former compounds (whose structural difference from the amide surfactants lies in the number of positive charges per molecule). In contradiction with the preceding compounds, arginine-*O*-alkyl ester dihydrochlorides showed a very pronounced reduction in the antimicrobial activity, as the ester bond (instead of an amide bond) linking the hydrophobic group with the polar head can be easily hydrolyzed by bacteria.

On the other hand, conjugates of arginine with 1-monoacyl- and 1,2-diacyl-glycerolipids which can be considered analogs of partial glycerides and phospholipids, combine the physicochemical properties of glycerol derivatives and polar arginine-based surfactants. These attributes confers them some advantages over common phospholipids, namely, endowing the amphiphilic structure with antimicrobial activity due to the cationic features related to arginine [46].

Cationic lysine-based surfactants, in which the hydrophobic part is connected to the carboxylic lysine group through an ester or amide bond, have also been studied [46, 50, 51]. Several lysine-derived surfactants such as a lauroyl amide, a guanidinylated lauroyl amide, and a polyol-modified carbohydrate-templated lauroyl amide of lysine were tested using a group of clinically relevant isolates of Gram-positive (including MRSA and MRSE) and Gram-negative bacteria [51]. The results revealed that the substitution of lysine by carbohydrate-templated lysine analogs improved the hydrophobicity of the polar group and reduced the antibacterial potency of the corresponding cationic lipid. However, enhancement of the antibacterial activity was observed by guanidinylation of the two lysine amino groups.

Other studies concerning the effects of the position of the cationic charge on the biological properties of these compounds have been performed with cationic $N^{\varepsilon}$-acyl lysine methyl ester, $N^{\alpha}$-lauroyl lisine methyl and ethyl ester hydrochloride analogs, $N^{\alpha}$-lauroyl-$N^{\varepsilon}$-trimethyl lysine derivatives, and $N^{\varepsilon}$-miristoyl-$N^{\alpha}$-trimethyl lysine methyl ester surfactants [46, 52]. The $N^{\alpha}$-lauroyl lysine methyl and ethyl ester hydrochlorides presented MIC values in the same range as those of arginine analogs, explaining the wide spectrum of antimicrobial activity against Gram-positive and Gram-negative bacteria of cationic AAS. The results obtained showed that the stereochemistry of these surfactants did not influence their antimicrobial behavior. The maintenance of bioactivity regardless of optical purity is an advantageous issue, considering the difficult task in isolation of pure diastereoisomers [46].

$N^{\alpha}$-Lauroyl-$N^{\varepsilon}$-trimethyl lysine derivatives displayed similar antimicrobial activity, in spite of the introduction of three methyl groups into the amino group of these molecules, probably to their identical cationic charge density. However, considering the p$K_a$ values of the $N^{\varepsilon}$-acyl lysine methyl ester derivatives, their cationic charge density was expected to be lower than the $N^{\alpha}$-lauroyl-$N^{\varepsilon}$-trimethyl lysine surfactants. Transferring the alkyl chain from the $\alpha$-amino to the ε-amino group of lysine, considerably weakened the antimicrobial activity of $N^{\varepsilon}$-acyl lysine methyl ester derivatives, displaying no inhibitory effects upon Gram-negative bacteria. Furthermore, fixing the cationic charge in the $N^{\varepsilon}$-miristoyl-$N^{\alpha}$-trimethyl lysine methyl ester surfactants, led to an enhanced antimicrobial activity.

Thereby for amino acid-based cationic surfactants in which the cationic charge is found on a protonated amine group, the antimicrobial activity is influenced by the p$K_a$ of the latter [46]. Good inhibitory effects can be observed for surfactants with p$K_a$ values higher than 9, whereas for those with p$K_a$ values lower than 7, the antimicrobial efficacy is less pronounced, especially against Gram-negative microorganisms [46]. Generally, antimicrobial surfactants have frequently less impact on Gram-negative bacteria, because the lipopolysaccharide-packed outer envelopes hamper the access of amphiphilic compounds.

Cationic surfactants based on L-tryptophan and on L-tyrosine have been proved to be excellent gelators, revealing notable bactericidal properties [46]. Considering the cationic surfactants based on L-tryptophan with chloride as the counterion, optimum inhibitory features (against Gram-positive and particularly against Gram-negative with MICs of 0.5–5.0 μg/mL) were found for alkyl chain lengths of 10–14 carbons. However, molecules with alkyl chain length of 15 and 17 carbons still showed activity against Gram-positive bacteria, but not inhibiting the growth of Gram-negative bacteria. Exchanging the chloride counter-ion for the more

hydrophobic organic carboxylates increased the activity against Gram-positive bacteria and fungi, improving also the biocompatibility towards eukaryotic cells [46].

Hydrogelator surfactants based on L-tryptophan and on L-tyrosine were used as templates for *in situ* synthesis of silver nanoparticles in order to increase the antimicrobial power. Since pure compounds only disturbed Gram-positive bacteria, the supramolecular assemblies of silver nanoparticles allowed the development of soft nanocomposites showing a wider bioactivity range for both Gram-positive and Gram-negative bacteria [53].

Antimicrobial activity for cationic surfactants from phenylalanine and tyrosine, in which the alkyl chain is linked to the carboxylic group of the amino through an ester bond, has been reported [54]. The antimicrobial activity was high for Gram-positive bacteria and low for Gram-negative bacteria. Antibacterial properties were affected by the alkyl chain length (increasing with this descriptor) and by both electrostatic and hydrophobic interactions between surfactants and the bacterial membranes.

The enhanced antimicrobial activity of cationic gemini AAS when compared with their monomeric counterparts can possibly be explained by their low CMC values, good solubility, the presence of two positively charged headgroups, and two hydrophobic chains per molecule [14, 20, 46, 47].

The antimicrobial behavior of gemini surfactants is influenced by a number of factors such as the length of the spacer chain, the length of the alkyl chain, the site where the cationic charge is positioned, and the net cationic charge of the molecules. According to the majority of the studies reported [46], the antimicrobial activity of the gemini surfactants is generally higher than of the corresponding monocatenary compounds, on account of their structural and functional characteristics. Growth inhibition of a comprehensive array of microorganisms (including Gram-positive and Gram-negative bacteria) was observed for a cationic gemini surfactant prepared by condensation of *N*-lauroyl glycine betaine with cystine dimethyl ester hydrochloride, with MICs ranging from 0.125 to 16 μg/mL [46].

Cationic gemini surfactant from arginine (consisting of $N^{\alpha}$-acyl arginine linked by amide bonds to a polymethylene spacer chain) and from lysine (consisting of $N^{\alpha}$-acyl- $N^{\varepsilon}$-acyl lysine with a hexamethylene or a spermidine spacer linked by amide bonds to the carboxyl groups) also displayed antimicrobial activity against a wide range of Gram-positive and Gram-negative bacteria [45, 46]. When the acyl chain was kept constant, the antimicrobial activity was shown to decrease with long spacer chains: the longer the spacers, the greater the ability to form viscous solutions enclosing large aggregates. Since big aggregates hardly interact with erythrocyte membranes, gemini surfactants with long spacers are much less hemolytic than their single-chain counterparts [55].

Concerning the alkyl chain length (whose influence is similar in monocatenary homologs) gemini surfactants with 10–12 carbon tails showed the best performance in terms of antimicrobial behavior.

Regarding the location of the cationic charge, arginine-based gemini surfactants usually display a higher antimicrobial efficiency when compared with the monomeric compounds.

The net cationic charge appreciably affected the antimicrobial activity of several gemini compounds, increasing with the p$K_a$ value of the molecules, and modulating their capacity to disrupt the bacterial membrane, a similar pattern to that shown by their monomeric counterparts [20, 45, 46].

Antimicrobial gemini surfactants from arginine and lysine have shown lower hemolytic activity than their single chain homologs [45, 46], which seem to depend on the alkyl chain length, and also on the spacer length and cationic charge density (for the same alkyl chain length). Aggregate size in solution (which depends on the molecular architecture of the surfactants) is another feature influencing the hemolytic activity; big micellar aggregates in aqueous medium hinder the interaction with biological membranes [46, 55]. Gemini surfactants with short spacer chains are more hemolytic than their single chain homolog, whereas gemini surfactants with long spacers are much less hemolytic than their single chain counterpart [55].

Catanionic mixtures of oppositely charged surfactants have shown to improve physicochemical and biological properties, when compared to the individual components. Within this framework, mixtures of lichenysin (an anionic biosurfactant) and two amino acid-based gemini cationic surfactants ($N^{\alpha}$,$N^{\omega}$-bis($N^{\alpha}$-lauroyl lysine) $\alpha$,$\omega$-hexylendiamide and $N^{\alpha}$,$N^{\omega}$-bis($N^{\alpha}$-lauroyl $\alpha$,$\omega$-propylendiamide) were explored [56]. Lichenysin is a cyclic lipopeptide produced by *Bacillus licheniformis* similarly to surfactin, with a high surface tension activity in water (29 mN/m) and a very low CMC (15 mg/L), but without its antimicrobial activity.

The antimicrobial activity of the surfactant binary systems were evaluated *in vitro* against a wide range of Gram-negative and Gram-positive bacteria (including MRSA strains) and *Candida albicans*. A significant bacterial growth inhibition was observed for the 8:2 lichenysin-$N^{\alpha}$,$N^{\omega}$-bis($N^{\alpha}$-lauroyl $\alpha$,$\omega$-propylendiamide) mixture, showing a clearly synergistic effect. The differences between the two cationic AAS mixtures with lichenysin, in terms of the synergistic effect, can be ascribed to the different p$K_a$ values of the surfactants. These results suggested a "hybrid surfactant" formation which could produce a more powerful hydrophobic interaction with the lipid bilayer, with additional stronger electrostatic interaction due to the presence of the guanidine group, present in the mixture and acting similar to a cationic surfactant [56].

Moreover, the antimicrobial properties of cationic gemini AAS may be enhanced by the use of cosurfactants, reinforcing the potential for biomedical applications of amino acid-derived surfactants.

On the other hand, as the negative charge density at the cell membrane in fungi is lower than in bacteria, the majority of cationic AAS do not exhibit antifungal properties. However, alanine-based Gemini surfactants were effective in preventing the formation of mycoses on mucous membranes of patients with suppressed immunity caused by different strains of *Candida albicans* with deletions of gene-encoded multidrug resistance transporters [57]. The obtained good results suggested their use as surface-coating agents against fungal colonization.

Cationic amino acid-based surfactants have also been proved to have antiviral activity [14]. Acyl amino acid derivatives produced inhibition on influenza neuraminidase. On the other hand, several $N^{\alpha}$-palmitoyl amino acids, which have been incorporated into model membranes, seemed to influence the transition temperature between the bilayer to hexagonal

phase, a property linked to antiviral activity against the Cantell strain of the Sendai virus (parainfluenza type 1) [14].

### 4.2. Drug delivery

To enable controlled or responsive self-assembly systems with special characteristics, new functional surfactants or mixtures of different types of surfactants are constantly being developed and formulated.

Nanocarriers have gained recent widespread interest due to their targeted drug delivery, hence positively impacting on the systemic side effects often seen by avoiding other organs, having also the advantage of protecting the drugs from degradation and increasing drug solubility. AAS can be promising novel biomaterials in drug delivery systems, given their biocompatible properties and low cytotoxicity.

Cells usually take up drug carriers through endocytosis that limits the internalized active compounds to vesicles (endosomes). Surface properties, such as hydrophobicity and surface charge, have a major impact on cellular uptake of particulate drug delivery systems, therefore the incorporation of charged surfactants into these carriers might improve targeting to specific cells. In addition, surfactants with pH-responsive membrane-disruptive activity may further destabilize endosomal compartments [58].

The membrane-disruptive activity of $N^{\varepsilon}$-myristoyl lysine methyl ester and $N^{\varepsilon}$-palmitoyl lysine methyl ester surfactants was evaluated using erythrocytes as a model of an endosomal membrane [58]. Due to the positive charge on the $\alpha$-amino group of lysine, both surfactants showed pH-responsive hemolytic activity. The overall hemolysis results suggested that both surfactants might achieve maximum membrane lytic activity in the late endosomes, and the hemolytic kinetics demonstrated their ability to disrupt endosomal membranes before vesicular evolution from endosomes to lysosomes. These outcomes identify these lysine surfactants as potential bioactive excipients in drug delivery systems [58].

In the same context, a series of chitosan–tripolyphosphate nanoparticles for intracellular drug delivery were designed using two pH-sensitive cationic AAS from the family of $N^{\alpha}$,$N^{\varepsilon}$-dioctanoyl lysine as bioactive compounds [59]. The results showed that by inserting the lysine-based amphiphiles into chitosan nanoparticles, pH-sensitive membranolytic and potentially endosomolytic nanocarriers were developed, which, therefore, demonstrated ideal viability for intracellular drug delivery. The enhanced kinetics of the hemolytic activity supported the ability of these functional nanodevices to disrupt endosomal membranes before vesicular evolution from endosomes to lysosomes, where many drugs may suffer degradation.

On the whole, the results suggested the possible potential of these pH responsive nanocarriers to promote an improved delivery of bioactive compounds to the intracellular compartments, although further *in vitro* and *in vivo* studies are needed to substantiate this hypothesis [59].

Cationic vesicular systems prepared from biocompatible diacylglyceroarginine surfactants can be eventually used as carriers in controlled drug release formulations [46, 60]. These vesicles were able to encapsulate drugs such as ciprofloxacin, with percentage of encapsulated drug

depending on both the physicochemical properties of the carrier and the nature of the drug. Antimicrobial activity of empty and ciprofloxacin-loaded vesicles (against some Gram-positive and Gram-negative bacteria) was noticeable, with drug-loaded vesicles showing similar or higher bioactivity than the free drug solution. Additionally, the encapsulated ciprofloxacin preserved its antimicrobial activity. Adding dipalmitoyl phosphatidylcholine as a membrane additive diminished the antimicrobial power of the cationic vesicles without drug, but improved the antimicrobial activity of vesicles loaded with ciprofloxacin. The dual pharmacological functions (related to the nature of the encapsulated drug and related to the intrinsic antibacterial properties of the surfactant-based carriers) turned these formulations into innovative potential candidates for drug delivery.

A synergistic formulation, combining a natural antimicrobial cationic surfactant from lysine ($N^{\varepsilon}$-myristoyl lysine methyl ester) with the sodium salt of hyaluronic acid was developed in order to be used as a coating for viscose fabric in wound healing and textile medicine [61]. The amount of amine groups deposited on the viscose fabric surface is a key factor when aiming for an antimicrobial functionalization of textiles. The interaction studies proved that the lysine-based surfactant and the biopolymer formed a complex bearing a slightly positive charge at neutral pH, and the viscose samples thus treated showed very pronounced antimicrobial properties when tested against several Gram-positive and Gram-negative bacteria, and some pathogenic fungi.

Novel AAS derived from bis(carboxymethyl) lysine with saturated and polyunsaturated fatty acyl chains of variable chain length and unsaturation degree attached at the ε-amino group were developed to improve solubilization of a water insoluble anticancer drug [62]. Their cytoxicity was evaluated *in vitro* by the MTT and LDH assays on endothelial cells. The arachidonoyl and pentacosanoyl derivatives were less cytotoxic than polysorbate 80 used as the model solubilizer. The alkyl chain length and the unsaturation degree strongly influenced toxicity. The saturated surfactants showed similar hemolytic activity, due to their low CMC values and the linear configuration of their hydrophobic chain. The arachidonoyl and 10,12-pentacosadinoyl derivatives were less hemolytic than polysorbate 80.

The nonadecanoyl, pentacosanoyl, and 10,12-pentacosadinoyl derivatives were found to increase drug solubility from <0.15 μg/mL up to 7 mg/mL, with 46% (w/w) drug loading, which was attributed to their linear and flexible hydrophobic chain configuration, in accordance with the molecular modeling studies. The potential use of these surfactants as solubilizers is dependent on the selection of the hydrophobic moiety based on the compromise between the strength of the hydrophobic interaction with the drug, leading to improved solubility, and the affinity for the cell membrane leading to toxicity [62].

As invasive fungal infections are a major cause of concern in immunocompromised patients, AAS can be considered interesting substitutes for the solubilization of amphotericin B (AmB), a hydrophobic polyene antibiotic used in the therapy of systemic fungal infections. Due to its poor water solubility, AmB is commercialized as a colloidal suspension using sodium deoxycholate as the solubilizing agent. However, severe toxic effects of this formulation are associated with AmB aggregation and thus more suitable delivery vehicles are required. The anionic *N,N′*-bis(octylcarbamoyl) gemini AAS derived from cystine was found to form micelles at a

lower CMC than the bile salt under physiological mimetic conditions, being also a better solubilizing agent for AmB [63]. The increased solubility of the drug in the gemini micellar solutions is due to the dimeric structure of the surfactant which contributes to a higher hydrophobicity and thus to a higher molar fraction of gemini surfactant in the micellar form as a result of a lower CMC. The gemini micelles solubilized AmB in a monomeric form, contributing to a less toxic formulation, although AmB efficacy was slightly reduced as indicated by MIC values.

Equimolar mixtures of the same anionic gemini AAS with bile salts sodium cholate and sodium deoxycholoate were evaluated as potential delivery agents for AmB [48]. Results showed that mixed micellar systems improved the solubilization of AmB (in its monomeric and less toxic form), and exhibited *in vitro* antifungal activity against *Candida albicans* comparable to that of commercial formulation. The potential safety profile of the gemini AAS and the possibility of reductive cleavage of the disulfide bond to control drug release from gemini AAS micelles, turn these formulations (either pure or in combination with other amphiphiles promoting synergistic interactions) into novel and promising drug delivery systems for the solubilization of amphiphilic drugs sparingly water-soluble other than AmB, contributing to increase the therapeutic window of the drug [48, 63].

### 4.3. Transfection vectors

DNA compaction in livings cells is a critical process, being important to regulate cellular activities through gene transcription, cellular proliferation, and differentiation. The ability to compact DNA protects it against enzymatic degradation and releases it after reaching the desired compartment in the target cell are crucial requirements for the design of efficient vectors for gene delivery.

Gene transfer vectors commonly used are mostly based on viruses which arises considerable related biosafety concerns associated with carcinogenicity, immunogenicity, and broad tropism. Hence, non-viral vectors such as cationic liposomes offer a nonimmunogenic and safe method for systemic gene delivery but are, in general, less efficient than viral vectors [14, 20, 46, 64].

DNA molecules are known to interact with single or double chain cationic surfactants, as well as with cationic gemini surfactants. Hydrophobic and electrostatic interactions occur between DNA and surfactants: the cationic group promotes the displacement of sodium cations nearby the nucleic acid, whereas hydrophobic interactions take place between the alkyl tails of surfactants; these two cooperative mechanisms promote the formation of complexes between DNA and surfactants with potential application in gene therapy [64].

AAS conjugated with biogenic (poly)amines, such as spermidine and spermine, with the amino acid residue acting as a linker between the hydrophobic chain(s) and the hydrophilic headgroup, have been developed as synthetic alternatives to viral vectors. The best known example of this class is the dioctadecylamidoglycylspermine (DOGS), an efficient transfection agent with glycine as spacer [20].

Cationic gemini AAS are able to bind DNA with several advantages compared to classic monovalent surfactants: lower cellular toxicity, lower CMC, and higher tendency to self-assemble [65]. Addition of a helper lipid (dioleyl phosphatidylethanolamine (DOPE)), induces polymorphic

phase behavior, with the appearance of inverted micellar and cubic structures, leading to an increased transfection efficiency.

Gemini surfactants, *N,N*-bis(dimethylalkyl)-$\alpha,\omega$-alkanediammonium halide derivatives, which are known to be flexible vectors for non-viral gene delivery, have been modified at the spacer chain by introduction of an amino acid (glycine or lysine) to improve transfection efficiency. Gene delivery efficiency was evaluated in epithelial cells for topical cutaneous and mucosal applications, in the presence of DOPE. The superior performance of these spacer-substituted gemini surfactants in transfecting epithelial cells might be attributed to their better flexibility and biocompatibility conferred by the amino acid residue, when compared to the surfactants possessing unsubstituted spacers. These results demonstrate the feasibility of using amino acid-substituted gemini surfactants as gene carriers for the treatment of diseases affecting epithelial tissue [65].

Transfection efficiency has also been attempted *in vitro* in several cell lines, including human hepatocarcinoma and human breast adenocarcinoma, using cationic AAS bearing serine, alanine, and β-alanine headgroups [66]. The transfection efficacy was more significant for cationic AAS with alanine and β-alanine headgroups than with their serine headgroup counterparts, presumably due to the enhanced sensitivity of DNA associated with the hydroxyl-containing serine headgroup [66]. Gene transfer efficacies of cationic amphiphiles can be significantly modulated by minor structural variations in both the polar headgroup and hydrophobic tail regions of the surfactant.

Cationic liposomal formulations composed of a mixture of dioleoyl trimethylammoniumpropane (DOTAP) and cholesterol (Chol), and a pH-sensitive formulation comprising DOTAP, Chol, DOPE, and cholesteryl hemisuccinate (CHEMS) were developed as a new gene delivery system to plasmid DNA precondensed with arginine *N*-lauroyl amide dihydrochloride, along with the incorporation of blood protein transferrin (Tf) [67].

The transfection efficiency of these systems was directly related with the presence of the nontoxic arginine surfactant and the lipidic composition. Better transfection profiles were found for the complexes based on the pH-sensitive liposomal formulation. The pair DOPE:CHEMS is believed to act synergistically with the arginine surfactant and Tf, contributing to the escape of DNA complexes from the endosome, therefore improving the transfection, when compared to complexes composed of DOTAP:Chol liposomes.

### 4.4. Interactions with biomolecules and biointerfaces

Interactions of AAS with biological systems are relevant for potential biotechnological and biomedical applications. In the biological domain, the interaction between proteins and surfactants is of great importance since it can clarify the action of surfactants as denaturants and solubilizing agents for proteins [20]. Proteins are known to cooperatively bind many surfactants forming a protein-surfactant complex where the hydrophobic moieties of the surfactant cause protein unfolding by interacting with the non-polar amino acid residues [40].

The different interactions between glutamic acid-based gemini surfactants and hemoglobin, when compared with their corresponding single-chain homolog, were reported in the literature [20]. The weaker denaturing ability of gemini surfactants concerning hemoglobin can be assigned to their large size, and the extension of denaturation decreased when spacer length increased.

http://dx.doi.org/10.5772/67977

Interactions between anionic cystine-based gemini surfactants with the globular protein BSA were investigated, proving to be influenced by temperature and pH [40]. The gemini surfactant stereochemistry also affected the interactions, since the association of enatiomerically pure compounds with BSA is favored when compared to the racemic mixture, and pure L-stereochemistry is preferred over D-stereochemistry. On the other hand, the cationic gemini surfactant cystine dilauroyl amide dihydrochloride, with longer dodecyl tails but a cationic headgroup, showed a weaker interaction with BSA, with an association constant of $1.68 \times 10^4$ L/mol according to fluorescence quenching data [63].

The interest in enhanced properties of amphiphilic compounds in the biomedical areas has led to the development of mixed surfactants systems formulations [10]. Mixed micelles comprising bile salts solubilize significant biological compounds, such as cholesterol and fatty acids, representing promising drug delivery systems. The mixed micelle formation in basic solutions between an anionic gemini AAS derived from cystine and bile salts sodium cholate and sodium deoxycholate has been studied [10]. Micellization in these mixtures was found to depend not only on the hydrophobic effect, which aims at minimizing the hydrophobic surface, but also on hydrogen bonding ability, which is determined by some structural factors, including the number, position, and orientation of the hydroxyl groups of bile salts and packing geometry restrictions.

Studies have also been performed with mixed systems comprising the anionic gemini AAS and phospholipids. Synergism was found for surfactant mixtures between the gemini and diheptanoyl phosphatidylcholine (DHPC, a micelle-forming lipid) as well as for surfactant mixtures with dimyristoyl phosphatidylcholine (DMPC, a vesicle-forming lipid). These findings were due to the reduction of electrostatic repulsions between the anionic headgroups of the surfactant as a result of intercalation of the zwitterionic phospholipids in the mixed micelles [42]. Structural effects can also be involved since the short disulfide spacer draws the two hydrophobic chains of the gemini molecule close together thus increasing alkyl chain density and also the charge density of the headgroups, leading to strong intermolecular interactions with other amphiphiles in solution.

Biocompatible thermoresponsive gels, produced by mixtures of the ethyl(hydroxyethyl)cellulose polysaccharide and both monomeric and gemini arginine surfactants bis(Args) have been described [68]. When compared with the monomeric surfactant, the gemini compound showed superior gel formation capacity (needed to induce a sol-gel transition), generating thermoresponsive gels at concentrations 1000-fold lower. The cytotoxicity of the polymer-surfactant systems, evaluated through *in vitro* experiments on a human epithelial cervical carcinoma cell line, was significantly compensated by their superior efficiency at low concentrations. This fact seems particularly interesting for applications requiring temperature-induced thickening useful in pharmaceutical and biomedical areas.

Aqueous dispersions of pure gemini surfactants from arginine or mixtures of bis(Args) with phospholipids lead to stable cationic colloidal systems with a promising use as drug delivery systems [20]. While single chain surfactants and gemini with short spacer chains promoted solutions with micellar aggregates, gemini with long spacers gave rise to large aggregates promoting viscous solutions or gels [55]. As big aggregates do not interact so easily with biological membranes, gemini surfactants with long spacers show lower cytotoxicity, thus antimicrobial and hemolytic activities are strongly affected by aggregates size.

## 5. Conclusions

AAS possess good surface active and emulsifying properties, low toxicity, and high biodegradability, which are attractive properties for applications in food, personal care products, and pharmaceutics. AAS are based on naturally occurring renewable sources having a strong influence in their environmental impact, and their preparation is economically feasible. Moreover, their wide structural diversity and different physicochemical and biological properties can be tailored to meet a specific application by appropriate choice of the amino acid residue and linkage of the hydrophobic chain.

The outcome of numerous studies about the cationic AAS, particularly gemini compounds, placed them as ideal candidates for biomedical applications that require positively charged amphiphiles, since they show promising biological properties, namely, antimicrobial and DNA transfecting. Given their distinctive physicochemical and biological properties, new possible pharmaceutical devices based on cationic AAS may be considered a viable alternative to the classical formulations, showing good stability, low hemolytic effects, and also a natural antimicrobial activity, which is not provided by conventional ones.

However, since many *in vitro* tests used to measure the toxicity of AAS are inconclusive, and that these formulations are intended for human use, more *in vivo* tests should continue to be conducted. The correct choice and combination of cell lines and bioassays in toxicity studies for a safe and reliable screen of AAS with potential interest in pharmaceutical industry is thus critical.

Notwithstanding, future perspectives point to the preparation of a larger library of compounds for better robustness in biomedical applications aiming at rationally designing and developing more effective therapeutic agents and delivery systems based on AAS.

## Author details

Lídia Pinheiro* and Célia Faustino

*Address all correspondence to: lpinheiro@ff.ulisboa.pt

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

## References

[1] Rosen MJ. Surfactants and Interfacial Phenomena. 3rd ed. New Jersey: Wiley Interscience; 2004. 444 p. DOI: 10.1002/0471670561.

[2] Morán C, Pérez L, Pons R, Pinazo A, Infante MR. Aminoacids, lactic acid and ascorbic acid as raw materials for biocompatible surfactants. In: Kjellin M, Johansson I, editors. Surfactants from Renewable Resources. Chichester: Wiley; 2010. p. 85-107. DOI: 10.1002/9780470686607.ch5.

[3] Foley P, Kermanshahi A, Beach ES, Zimmerman JB. Derivation and synthesisof renewable surfactants. Chem. Soc. Rev. 2012;**41**:1499-1518. DOI: 10.1039/C1CS15217C.

[4] Scott E, Peter F, Sanders J. Biomass in the manufacture of industrial products—the use of proteins and amino acids. Appl. Microbiol. Biotechnol. 2007;**75**:751-762. DOI: 10.1007/s00253-007-0932-x.

[5] Pinazo A, Pons R, Pérez L, Infante MR. Amino acids as raw material for biocompatible surfactants. Ind. Eng. Chem. Res. 2011;**50**:4805-4817. DOI: 10.1021/ie1014348.

[6] Bordes R, Holmberg K. Amino acid-based surfactants—do they deserve more attention?. Adv. Colloid Interface Sci. 2015;**222**:79-91. DOI: 10.1016/j.cis.2014.10.013.

[7] Wu M-H, Wan L-Z, Zhang Y-Q. A novel sodium N-fatty acyl amino acid surfactant using silkworm pupae as stock material. Sci. Rep. 2014;**4**:4428. DOI: 10.1038/srep04428.

[8] Bautista ME, Pérez L, García MT, Cuadros S, Marsal A. Valorization of tannery wastes: lipoamino acid surfactant mixtures from the protein fraction of process wastewater. Chem. Eng. J. 2015;**262**:399-408. DOI: 10.1016/j.cej.2014.10.004.

[9] Infante MR, Pérez L, Pinazo A, Clapés P, Morán MC, Angelet M, Garcia MT, Vinardell MP. Amino acid-based surfactants. C. R. Chim. 2004;**7**:583-592. DOI: 10.1016/j.crci.2004.02.009.

[10] Faustino CMC, Serafim CS, Ferreira IN, Branco MA, Calado ART, Garcia-Rio L. Mixed micelle formation between an amino acid-based anionic gemini surfactant and bile salts. Ind. Eng. Chem. Res. 2014;**53**:10112-10118. DOI: 10.1021/ie5003735.

[11] Hassanzadeh M, Kambarani M, Tayebi L, Yazdian F. Kinetic study of sodium cocoyl sarcosinate synthesis and factors affecting the reaction on bench and pilot scales. J. Surf. Deter. 2012;**15**:551-556. DOI: 10.1007/s11743-012-1333-4.

[12] Sreenu M, Prasad RBN, Sujitha P, Kumar CG. Synthesis and surface-active properties of sodium *N*-acylphenylalanines and their cytotoxicity. Ind. Chem. Eng. Res. 2015;**54**:2090-2098. DOI: 10.1021/ie503764v.

[13] Zhang Q-Q, Cai B-X, Xu W-J, Gang H-Z, Liu J-F, Yang S-Z, Mu B-Z. The rebirth of waste cooking oil to novel bio-based surfactants. Sci. Rep. 2015;**5**:9971. DOI: 10.1038/srep09971.

[14] Morán MC, Pinazo A, Pérez L, Pinazo A, Clapés P, Angelet M, Garcia MT, Vinardell MP, Infante MR. "Green" amino acid-based surfactants. Green Chem. 2004;**6**:233-240. DOI: 10.1039/b400293h.

[15] Villeneuve P. Lipases in lipophilization reactions. Biotechnol. Adv. 2007;**25**:515-536. DOI: 10.1016/j.biotechadv.2007.06.001.

[16] Morcelle SR, Liggieri CS, Bruno MA, Priolo N, Clapés P. Screening of plant peptidases for the synthesis of arginine-based surfactants. J. Mol. Catal. B: Enzymatic. 2009;**57**:177-182. DOI: 10.1016/j.molcatb.2008.08.013.

[17] Reznik GO, Vishwanath P, Pynn MA, Sitnik JM, Todd JJ, Wu J, Jiang Y, Keenan BG, Castle AB, Haskell RF, Smith TF, Somasundaran P, Jarrell KA. Use of sustainable chemistry to

produce an acyl amino acid surfactant. Appl. Microbiol. Biotechnol. 2010;**86**:1387-1397. DOI: 10.1007/s00253-009-2431-8.

[18] Franssen MCR, Steunenberg P, Scott EL, Zuilhofac H, Sanders JPM. Immobilised enzymes in biorenewables production. Chem. Soc. Rev. 2013;**42**:6491-6533. DOI: 10.1039/c3cs00004d.

[19] Zhang G, Xu B, Han F, Zhou Y, Liu H, Li Y, Cui L, Tan T, Wang N. Green synthesis, composition analysis and surface active properties of sodium cocoyl glycinate. Am. J. Anal. Chem. 2013;**4**:445-450. DOI: 10.4236/ajac.2013.49056.

[20] Pérez L, Pinazo A, Pons R, Infante MR. Gemini surfactants from natural amino acids. Adv. Colloid Interface Sci. 2014;**205**:134-155. DOI: 10.1016/j.cis.2013.10.020.

[21] Haldar S, Maji SK. Role of non-covalent interactions in the molecular organization of *N*-n-hexadecanoyl amino acid amphiphiles with hydrophobic $C_{\alpha}$-side chains in Tris buffer (pH 9.3). Colloids Surf A Physicochem. Eng. Aspects. 2013;**420**:10-21. DOI: 10.1016/j.colsurfa.2012.12.012.

[22] Ohta A, Toda K, Morimoto Y, Asakawa T, Miyagishi S. Effect of the side chain of *N*-acyl amino acid surfactants on micelle formation: an isothermal titration calorimetry study. Colloids Surf A Physicochem. Eng. Aspects. 2008;**317**:316-322. DOI: 10.1016/j.colsurfa.2007.10.028.

[23] Gerova M, Rodrigues F, Lamère J-F, Dobrev A, Fery-Forgues S. Self-assembly properties of some chiral *N*-palmitoyl amino acid surfactants in aqueous solution. J. Colloid Interface Sci. 2008:**319**:526-533. DOI: 10.1016/j.jcis.2007.12.004.

[24] Perinelli DR, Casettari L, Cespi M, Fini F, Man DKW, Giorgioni G, Canala S, Lam JKW, Bonacucina G, Palmieri GF. Chemical–physical properties and cytotoxicity of N-decanoyl amino acid-based surfactants: effect of polar heads. Colloids Surf A Physicochem. Eng. Aspects. 2016;**492**:38-46. DOI: 10.1016/j.colsurfa.2015.12.009.

[25] Vijay R, Angayarkanny S, Bhaska G. Amphiphilic dodecyl ester derivatives from aromatic amino acids: significance of chemical architecture in interfacial adsorption characteristics. Colloids Surf A Physicochem. Eng. Aspects. 2008;**317**:643-649. DOI: 10.1016/j.colsurfa.2007.11.059.

[26] Bordes R, Tropsch J, Holmberg K. Role of an amide bond for self-assembly of surfactants. Langmuir. 2010; **26**:3077-3083. DOI: 10.1021/la902979m.

[27] Roy S, Das D, Dasgupta A, Mitra RN, Das PK. Amino acid based cationic surfactants in aqueous solution: physicochemical study and application of supramolecular chirality in ketone reduction. Langmuir. 2005;**21**:10398-10404. DOI: 10.1021/la051548s.

[28] Xia J, Nnanna IA, Sakamoto K. Amino acid surfactants: chemistry, synthesis and properties. In: Nnanna IA, Xia J, editors. Protein-Based Surfactants. Synthesis, Physicochemical Properties and Application. Surfactant Science Series 101. New York: Marcel Dekker; 2001; 312 p. ISBN: 0-8247-0004-X.

[29] Sreenu M, Rao BVSK, Prasad BN, Sujitha P, Chityala GK. Synthesis, surface and biological properties of sodium N-acyl isoleucines. Eur. J. Lipid Sci. Technol. 2014;**116**:193-206. DOI: 10.1002/ejlt.201300189.

[30] Bordes R, Holmberg K. Physical chemical characteristics of dicarboxylic amino acid-based surfactants. Colloids Surf A Physicochem. Eng. Aspects. 2011;**391**:32-41. DOI: 10.1016/j.colsurfa.2011.03.023.

[31] Bordes R, Tropsch J, Holmberg K. Counterion specificity of surfactants based on dicarboxylic amino acids. J. Colloid Interface Sci. 2009;**338**:529-536. DOI: 10.1016/j.cis.2009.06.032.

[32] Zhang D, Sun Y, Deng Q, Qi X, Sun H, Li Y. Study of the environmental responsiveness of amino acid-based surfactant sodium lauroylglutamate and its foam characteristics. Colloids Surf A Physicochem. Eng. Aspects. 2016;**504**:384-392. DOI: 10.1016/j.colsurfa.2016.05.097.

[33] Sanchez L, Martinez V, Infante MR, Mitjans M, Vinardell MP. Haemolysis and antihaemolysis induced by amino acid-based surfactants. Toxicol. Lett. 2007;**169**:177-184. DOI: 10.1016/j.toxlet.2007.01.003.

[34] Pinazo A, Pérez L, Lozano M, Angelet M, Infante MR, Vinardell MP, Pons R. Aggregation properties of diacyl lysine surfactant compounds: Hydrophobic chain length and counterion effect. J. Phys. Chem. B. 2008;**112**:8578-8585. DOI: 10.1021/jcisp802193p.

[35] Brito RO, Marques EF, Gomes P, Falcão S, Söderman O. Self-assembly in a catanionic mixture with an amino acid-derived surfactant: from mixed micelles to spontaneous vesicles. J. Phys. Chem. B. 2006;**110**:18158-18165. DOI: 10.1021/jp063271q.

[36] Brito RO, Oliveira IS, Araújo MJ, Marques EF. Morphology, thermal behaviour, and stability of self-assembled supramolecular tubules from lysine-based surfactants. J. Phys. Chem. B. 2013;**117**:9400-9411. DOI: 10.1021/jp400127k.

[37] Tsubone K, Ogawa T, Mimura K. Surface and aqueous properties of anionic gemini surfactants having dialkyl amide, carboxyl, and carboxylate groups. J. Surfactants Deterg. 2003;**6**:39-46. DOI: 10.1007/s4743-003-0246-5.

[38] Tsubone K, Rosen MJ. Structural effect on surface activities of anionic surfactants having *N*-acyl-*N*-methylamide and carboxylate groups. J. Colloid Interface Sci. 2001;**244**:394-398. DOI: 10.1006/jcis.2001.7995.

[39] Fan H, Han F, Liu Z, Qin L, Li Z, Liang D, Ke F, Huang J, Fu H. Active control of surface properties and aggregation behaviour in amino acid-based gemini surfactant systems. J. Colloid Interface Sci. 2008;**321**:227-234. DOI: 10.1016/j.jcis.2008.01.039.

[40] Faustino CMC, Calado ART, Garcia-Rio L. Gemini surfactant-protein interactions: effect of pH, temperature, and surfactant stereochemistry. Biomacromolecules. 2009;**10**:2508-2514. DOI: 10.1021/bm9004723.

[41] Faustino CMC, Calado ART, Garcia-Rio L. Dimeric and monomeric surfactants derived from sulphur-containing amino acids. J. Colloid Interface Sci. 2010; **351**:472-477. DOI: 10.1016/j.jcis.2010.08.007.

[42] Faustino CMC, Calado ART, Garcia-Rio L. Mixed micelle formation between amino acid-based surfactants and phospholipids. J. Colloid Interface Sci. 2011;**359**:493-498. DOI: 10.1016/j.jcis.2011.04.016.

[43] Yoshimura T, Sakato A, Esumi K. Solution properties and emulsification properties of amino acid-based gemini surfactants derived from cysteine. J. Oleo Sci. 2013;**62**:579-586. DOI: 10.5650/jos.62.579.

[44] Angayarkanny S, Vijay R, Baskar G, Mandal AB. Self-organization at the interface and in aqueous solution of a cationic gemini surfactant from the dioctyl ester of cystine. J. Colloid Interface Sci. 2012;**367**:319-326. DOI: 10.1016/j.jcis.2011.10.043.

[45] Colomer A, Pinazo A, Manresa MA, Vinardell MP, Mitjans M, Infante MR, Pérez L. Cationic surfactants derived from lysine: effects of their structure and charge type on antimicrobial and hemolytic activities. J. Med. Chem. 2011;**54**:989-1002. DOI: 10.1021/jm101315k.

[46] Pinazo A, Manresa MA, Marques AM, Bustelo M, Espuny MJ, Pérez L. Amino acid–based surfactants: new antimicrobial agents. Adv. Colloid Interface Sci. 2016;**228**:17-39. DOI: 10.1016/j.cis.2015.11.007.

[47] Faustino C, Serafim C, Ferreira I, Pinheiro L, Calado A. Solubilization power of an amino acid-based gemini surfactant towards the hydrophobic drug amphotericin B. Colloids Surf A Physicochem. Eng. Aspects. 2015;**480**:426-432. DOI: 10.1016/j.colsurfa.2014.11.039.

[48] Serafim C, Ferreira I, Rijo P, Pinheiro L, Faustino C, Calado A, Garcia-Rio L. Lipoamino acid-based micelles as promising delivery vehicles for monomeric amphotericin B. Int. J. Pharm. 2016;**497**:23-35. DOI: 10.1016/j.ijpharm.2015.11 DOI:10.1021/ed4008364 .034.

[49] Castillo JÁ, Pinazo A, Carilla J, Infante MR, Alsina MA, Haro I, Clapés P. Interaction of antimicrobial arginine-based cationic surfactants with lipossomes and lipid monolayers. Langmuir. 2004;**20**:3379-3387. DOI: 10.1021/la036452h.

[50] Colomer A, Perez L, Pons R, Infante MR, Perez-Clos D, Manresa A, Espuny MJ, Pinazo A. Mixed monolayer of DPPC and Lysine-based cationic surfactants: an investigation into the antimicrobial activity. Langmuir. 2013;**29**:7912-7921. DOI: 10.1021/la401092j.

[51] Mondal D, Zhanel GG, Schweizer F. Synthesis and antibacterial properties of carbohydrate-templated lysine surfactants. Carbohydr. Res. 2011;**346**:588-594. DOI:10.1016/j.carres.2011.01.025.

[52] Pérez L, Pinazo A, Garcia T, Lozano M, Manresa A, Angelet M, Vinardell MP, Mitjans M, Pons R, Infante MR. Cationic surfactants from lysine: synthesis, micellization and biological evaluation. Eur. J. Med. Chem. 2009;**44**:1884-1892. DOI: 10.1016/j.ejmech.2008.11.003.

[53] Shome A, Dutta S, Maiti S, Das PK. In situ synthesized Ag nanoparticle in self-assemblies of amino acid based amphiphilic hydrogelators: development of antibacterial soft nanocomposites. Soft Matter 2011;**7**:3011-3022. DOI: 10.1039/c0sm01087a.

[54] Joondan N, Jhaumeer-Laulloo S, Camul P. A study of the antibacterial activity of l-phenylalanine and l-tyrosineesters in relation to their CMCs and their interactions with 1,2-dipalmitoyl-sn-glycero-3-phosphocholine,DPPC as model membrane. Microbiol. Res. 2014;**169**:675-685. DOI: 10.1016/j.micres.2014.02.010.

[55] Tavano L, Infante MR, Abo Riya M, Pinazo A, Vinardell MP, Mitjans M, Manresa MA, Pérez L. Role of aggregate size in the hemolytic and antimicrobial activity of colloidal solutions based on single and gemini surfactants from arginine. Soft Matter. 2013;**9**:306-319. DOI: 10.1039/c2sm26670a.

[56] Coronel-León J, Pinazo A, Pérez L, Espuny MJ, Marqués AM, Manresa A. Lichenysin-geminal amino acid-based surfactants: synergistic action of an unconventional antimicrobial mixture. Colloids Surf. B Biointerfaces. 2017;**149**:38-47. DOI: 10.1016/j.colsurfb.2016.10.008.

[57] Luczynski J, Frackowiak R, Szczepaniak J, Krasowska A. Chemodegradable gemini alanine-based cationic surfactants: synthesis and antifungal activity. Chem. Lett. 2012;**41**: 1176-1177. DOI: 10.1246/cl.2012.1176.

[58] Nogueira DR, Mitjans M, Morán MC, Pérez L, Vinardell MP. Membrane-destabilizing activity of pH-responsive cationic lysine-based surfactants: role of charge position and alkyl chain length. Amino Acids. 2012;**43**:1203-1215. DOI: 10.1007/s00726-011-1176-8.

[59] Nogueira DR, Scheeren LE, Vinardell MP, Mitjans M, Infante MR, Rolim CMB. Nanoparticles incorporating pH-responsive surfactants as a viable approach to improve the intracellular drug delivery. Mat. Sci. Eng. C. 2015;**57**:100-106. DOI: 10.1016/j.msec.2015.07.036.

[60] Tavano L, Pinazo A, Abo-Riya M, Infante MR, Manresa MA, Muzzalupo R, Pérez L. Cationic vesicles based on biocompatible diacyl glycerol-argininesurfactants: physicochemical properties, antimicrobial activity,encapsulation efficiency and drug release. Colloids Surf. B Biointerfaces. 2014;**120**:160-167. DOI: 10.1016/j.colsurfb.2014.04.009.

[61] Bračič M, Pérez L, Infante R, Kogej K, Hribernik S, Sauperl O, Pinazo A, Pons R, Infante MR. A novel synergistic formulation between a cationic surfactant from lysine and hyaluronic acid as an antimicrobial coating for advanced cellulose materials. Cellulose. 2014;**21**:2647-2663. DOI: 10.1007/s10570-014-0338-8.

[62] Ménard N, Tsapis N, Poirier C, Arnauld T, Moine L, Lefoulon F, Péan JM, Fattal E. Drug solubilization and in vitro toxicity evaluation of lipoamino acid surfactants. Int. J. Pharm. 2012;**423**:312-320. DOI: 10.1016/j.ijpharm.2011.11.030.

[63] Branco MA, Pinheiro L, Faustino C. Amino acid-based cationic gemini surfactant-protein interactions. Colloids Surf A Physicochem. Eng. Aspects. 2015;**480**:105-112. DOI: 10.1016/j.colsurfa.2014.12.022.

[64] Beliéres M, Dejugnat C, Chouini-Lalanne N. Histidine-based lipopeptides enhance cleavage of nucleic acids: interactions with DNA and hydrolytic properties. Bioconjugate Chem. 2015;**26**:2520-2529. DOI: 10.1021/acs.bioconjchem.5b00542.

[65] Yang P, Singh J, Wettig S, Foldvari M, Verral RE, Badea I. Enhanced gene expression in epithelial cells transfected with amino acid-substituted gemininanoparticles. Eur. J. Pharm. Biopharm. 2010;**75**:311-320. DOI: 10.1016/j.ejpb.2010.04.007.

[66] Sen J, Chaudhuri A. Gene transfer efficacies of novel cationic amphiphiles with alanine, β-alanine, and serine headgroups: a structure-activity investigation. Bioconjugate Chem. 2005;**16**:903-912. DOI: 10.1021/bc0500443.

[67] Rosa M, Penacho N, Simões S, Lima MCP, Lindman B, Miguel MG. DNA pre-condensation with an amino acidbased cationic amphiphile. A viable approach for liposome-based gene delivery. Mol. Membrane Biol. 2008;**25**:23-34. DOI: 10.1080/09687680701499451.

[68] Calejo MT, Kjøniksen AL, Pinazo A, Pérez L, Cardoso AMS, Pedroso de Lima MC, Jurado AS, Sande SA, Nyström B. Thermoresponsive hydrogels with low toxicity from mixtures of ethyl(hydroxyethyl) cellulose and arginine-based surfactants. Int. J. Pharm. 2012;**436**:454-462. DOI: 10.1016/j.ijpharm.2012.07.018.



IntechOpen