www.rsc.org/greenchem

"Green" amino acid-based surfactants

Ma Carmen Morán,[a] Aurora Pinazo,[a] Lourdes Pérez,[a] Pere Clapés,[a] Marta Angelet,[a] Ma Teresa García,[a] Ma Pilar Vinardell[b] and Ma Rosa Infante*[a]

[a] *Instituto de Investigaciones Químicas y Ambientales de Barcelona-C.S.I.C., Jordi Girona 18-26, 08034 Barcelona, Spain. E-mail: rimste@cid.csic.es; Fax: +34 932045904; Tel: +34 934006 100*

[b] *Facultad de Farmacia, Universidad de Barcelona, Unidad Asociada de Investigacion del CSIC, Avd. Juan XXIII s/n. Edificio B 3ª planta, 08028 Barcelona, Spain; Fax: +34 934035901; Tel: +34 934024505*

Received 9th January 2004, Accepted 8th March 2004
First published as an Advance Article on the web 26th March 2004

The value of amino acids and vegetable oil derivatives as raw materials for the preparation of surfactants was recognized as soon as they were discovered early in the last century. Amino acid-based surfactants, which have an amino acid residue as a hydrophilic moiety, are reviewed with respect to their synthesis, properties and some applications. The review covers three main categories of amino acid-based surfactants: N^{α}-acyl, *N*-alkyl amide and *O*-alkyl ester derivatives among the linear or single chain amino acid-based surfactants; N^{α}, N^{ω}-bis(N^{α}-acylarginine)α,ω-alkylendiamides, which are *gemini* amino acid-based surfactants; and 1-monoacyl-*rac*-glycero-3-*O*-(N^{α}-acetyl-L-amino acid) and 1,2-diacyl-*rac*-glycero-3-*O*-(N^{α}-acetyl-L-amino acid), both amino acid-based surfactants with glycerolipid-like structures.

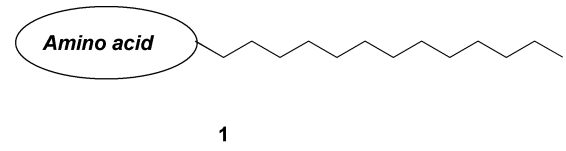
1 Introduction

Surfactants are one of the most representative chemical products, which are consumed in large quantities every day on a worldwide scale. Since it is known that surface-active compounds can adversely affect the aquatic environment, the biodegradability and biocompatibility of surfactants have become almost as important as their functional performance to the consumer. Because of this, there is a pressing need for development of efficient novel surfactants that are biodegradable and biocompatible.[1]

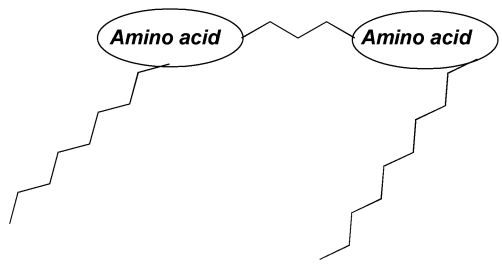
Surfactants of this kind can be obtained by designing molecules that mimic natural amphiphilic structures, *e.g.* phospholipids,[2] N^{α}-acyl amino acids[3] and alkyl-glucosides.[4] Surfactant molecules from renewable raw materials that mimic natural lipoamino acids are one of the preferred choices for food, pharmaceutical and cosmetic applications. Given their natural and simple structure they show low toxicity and quick biodegradation.[5] They can be produced by biotechnological and chemical methods using renewable raw materials such as amino acids and vegetable oils.[6–12] The value of amino acids as raw materials for the preparation of surfactants was recognized as soon as they were discovered early in the last century.[13] Initially they were used as preservatives for medical and cosmetic applications. Moreover, they were found to be active against various disease-causing bacteria, tumors, and viruses.[14–16] The combination of polar amino acids/peptides (hydrophilic moiety) and non-polar long chain compounds (hydrophobic moiety) for building up the amphiphilic structure has produced molecules with high surface activity. There is a large variety of amino acid/peptide structures. Moreover, the fatty acid chains can vary in their structure, length and number. These facts explain their wide structural diversity and different physicochemical and biological properties.[5,10,17,18]

The amino acid or peptide moiety determines the main differences of adsorption, aggregation and biological activity between the amino acid/peptide-based surfactants. Hence, cationic, anionic, non-ionic and amphoteric surfactants can be obtained depending on the free functional groups. Further modification of these groups allows a fine-tuning of their properties to meet almost every particular application.

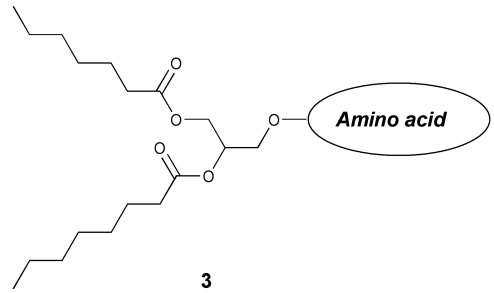
The amino acids and long aliphatic chains can be combined with each other to generate three main structures (Scheme 1) of amino acid-based surfactants namely linear or single chain **1**, dimeric or *gemini* **2** and glycerolipid-like structures **3**. Linear structures **1**



1



2



3

Scheme 1 Structures of amino acid-based surfactants: (**1**) Linear or single chain, (**2**) Dimeric or *Gemini* and (3) Glycerolipid-like structures. The amino acid constitutes the polar head of the surfactant. The hydrocarbon alkyl chain constitutes the hydrophobic moiety.

DOI: 10.1039/b400293h

consist of an amino acid bearing at least one hydrophobic tail. *Gemini* or dimeric are amphipathic structures **2** with two polar heads (*i.e.* two amino acids) and two hydrophobic tails per molecule separated by a covalently bonded spacer. Glycerolipid-like structures **3** can be considered analogues of mono-, diglycerides and phospholipids, and consist of one polar head and one or two hydrophobic moieties linked together through a glycerol skeleton.

This review is concerned with the preparation and the structure–activity relationship studies of amino acid-based surfactants of single chain, double chain *gemini* and glycerolipid-like structures for adsorption, self-assembling, and biological properties.

2 Single chain amino acid/peptide surfactants

2.1 Synthesis

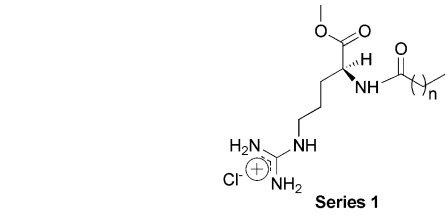
Acidic, basic, or neutral amino acids such as aspartic acid, glutamic acid, arginina, alanine, glycine, leucine, proline, serine, and protein hydrolysates have been used as starting materials to synthesize single chain amino acid surfactants commercially and experimentally.[10]

Amino acids are linked to long aliphatic chains through the α-amino, α-COOH or side chain groups (Scheme 2).

Thus, fatty acids or alkyl halides can react with amino groups yielding the corresponding *N*-acyl and *N*-alkyl derivatives (Scheme 2, compounds **1** and **2**), respectively. Alternatively, the carboxyl group of the amino acid can be condensed with alkyl amines or aliphatic alcohols to give *N*-alkyl amides and esters (Scheme 2, compounds **3** and **4**, respectively).

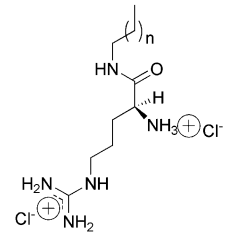
Among the different types of linkages between the long aliphatic chain and the amino acid, the *N*-acyl (Scheme 3, series 1), *N*-alkyl amides (Scheme 3, series 2) and *O*- alkyl esters (Scheme 3, series 3) of arginine have attracted much interest in our group due to their low toxicity and high biodegradability in combination with their antimicrobial activity.[19–25]

The *N*-acylation of the amino terminal arginine (series 1) was prepared by condensation of fatty acids to arginine methyl ester hydrochloride using classical chemical methods.[5] The application of biotechnological procedures was not efficient for these compounds.[25] Series 2 was at first prepared by chemical procedures,[23] however papain from *Carica papaya* latex was found to be a suitable catalyst for the formation of amide (series 2) and ester bonds (series 3) between Cbz–Arg–OMe and various long chain alkyl amines and fatty alcohols.[24] In all cases, papain deposited onto polyamide was found to be the best biocatalyst configuration. The preparation of arginine alkyl esters was carried out in solvent free systems using the same alcohol reagent. Both series were enzymatically synthesized at multigram scale with a purity higher than 99%.



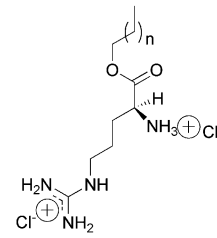
N^{α}-acyl-Arginine-methyl ester hydrochloride

CAM: n= 8
LAM: n= 10
MAM: n= 12
PAM: n= 14



Arginine-N-alkyl amide dihydrochloride

ACA: n= 8
ALA: n= 10
AMA: n= 12



Arginine-O-alkyl ester dihydrochloride

AOE: n= 6
ACE: n= 8
ALE: n= 10

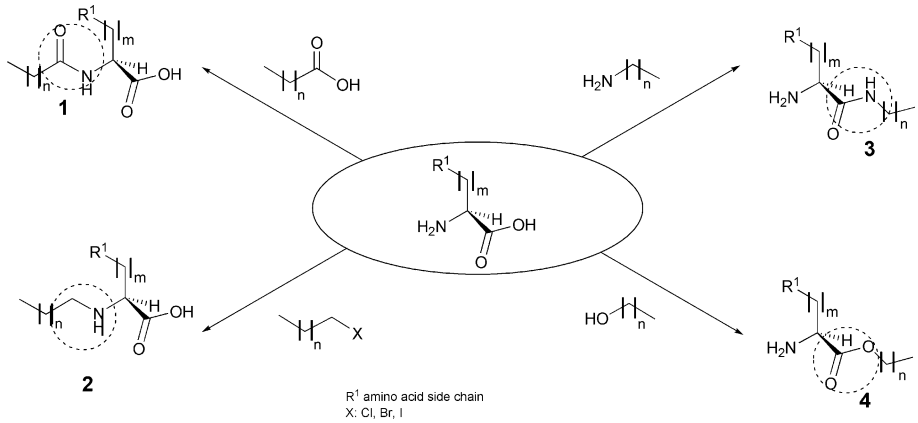
Scheme 3 Chemical structure of single chain arginine-based cationic surfactants.

N-Alkyl amide and ester derivatives of N^{α}-protected amino acids have also been prepared by lipases. In a study carried out with *Candida antarctica* and *Rhizomucor miehei* lipases, it was found that these enzymes could readily catalyse the condensation of a number of N^{α}-Cbz-amino acids with α,ω-alkyldiamines or fatty alcohols.[26]

N-Alkyl amide and side chain-substituted lipoamino acids have also been prepared by proteases and lipases. It was found that these enzymes could readily catalyse the condensation of a number of amino acids with fatty acids and triglycerides.[27]

2.2 Properties

Our group has reported that long chain N^{α}-acyl arginine methyl ester compounds (series 1 Scheme 3) are cationic surfactants with a satisfactory toxicity profile, high biodegradability and a surface activity comparable to that of conventional long chain quaternary ammonium salts. We have demonstrated that the morphology of



Scheme 2 Different types of linkages between an amino acid and a hydrophobic alkyl chain: acyl bond derivatives (**1**), alkyl bond derivatives (**2**), amide bond derivatives (**3**) and ester bond derivatives (**4**).

their micelle aggregates and lyotropic phases depends on the hydrophobic moiety, temperature, composition and electrolyte content in the system. As a result, we have found that compounds of series 1 show a rich and unusual phase behavior.[28–30] For instance, reversed vesicles (dispersion of lamellar liquid crystals in non-polar media) with biocompatible properties occurred in the lecithin-LAM–squalane system.[31] The PAM homologue was the only compound that showed lamellar lyotropic liquid crystals in the binary water–surfactant system.[30] These properties make them good alternatives for a wide range of industrial applications in the personal care, pharmaceutical and food sectors as well as in the design and synthesis of biomaterials. Furthermore, the arginine residue gives antimicrobial activity to the amphipathic molecule, a valuable property for a biocompatible surfactant.[32]

Compared to series 1, the series 2 and 3 compounds (Scheme 3) have two positive charged groups in the hydrophilic moiety, one in the primary amine and a second in the guanidine function. From the surface tension/concentration curves at 25 °C the critical micelle concentration (CMC) which is associated with the hydrophobicity of the molecule, the maximum surface excess concentration at the air–aqueous solution interface (Γ_m) and the area per molecule whose value indicates the minimum area per surfactant molecule at the air–aqueous solution interface (A_{min}) were calculated. Table 1

Table 1 Surface active properties of single chain surfactants from arginine at 25 °C[27–29,39]

Compound	γ_{cmc}[a]/ 10^{-3} N m^{-1}	CMC[b]/ 10^{-3} mol dm^{-3}	Γ_m[c]/ 10^{14} mol m^{-2}	A_{min}[d]/ 10^2 nm^2
CAM[e]	40	16		
LAM[e]	32	5.8		67
MAM[e]	32	2		62
ACA[f]	35	26	1.79	62
ALA[f]	37	1.8	1.24	90
AMA[f]	33	0.7	0.97	114
AOE[g]	35	38	1.15	96
ACE[g]	34	13	1.54	72
ALE[g]	30	5	0.91	122

[a] Surface tension at the critical micelle concentration. [b] Critical micelle concentration. [c] Maximum surface excess concentration at the air–aqueous solution interface. [d] Minimum area per surfactant molecule at the air–aqueous solution interface. [e] Compounds of series 1 (Scheme 3). [f] Compounds of series 2 (Scheme 3). [g] Compounds of series 3 (Scheme 3).

summarizes the surface parameters of the three series of compounds using the Gibbs adsorption equation.[33] In all cases they have the ability to decrease the surface tension of water until a constant value is reached, γ_{CMC}, and show a clear CMC in the surface tension–log C curves in the millimolar range. This indicates that they can be classified as surfactants with a surface activity similar to that of conventional cationic ones. Table 1 shows that the CMC depends on the straight alkyl chain and the nature of the hydrophilic moiety. For the three series of surfactants, the larger the number of methylene groups in the alkyl chain the lower the CMC, as would be expected from the increase in the hydrophobic character of the molecule. The smaller the A_{min}, the more effective its adsorption at the interfaces. We found that the A_{min} values for series 2 and 3 (62–114 $\times$ 10^{-2} nm^2 and 96–122 $\times$ 10^{-2} nm^2, respectively) were higher than that for series 1 with the same alkyl chain length (67 and 62 $\times$ 10 nm^2). This result indicates that the new molecules are less packed at the interface than those of series 1. The two charged groups in series 2 and 3 tend to spread themselves out on the interface due to an increase in the inter–intramolecular electrostatic repulsion forces.

One important milestone in our research is the design and development of new amino acid-based surfactants with antimicrobial properties, which mimic natural amphiphilic cationic peptides.[21,34] To this end, Lys and Arg derivatives of long chain N^{α}-acyl, COO-ester and *N*-alkyl amide have been prepared. In particular, the N^{α}-acyl arginine methyl ester derivatives series 1 (Scheme 3) have turned out to be an important class of cationic surface active compounds with a wide bactericidal activity, high biodegradability and low toxicity profile. The antimicrobial activities were determined *in vitro* on the basis of the minimum inhibitory concentration (MIC) values, defined as the lowest concentration of antimicrobial agent which inhibits the development of visible growth after 24 h of incubation at 37 °C. We have shown that essential structural factors for their antimicrobial activity include both the length of the fatty residue (akin with their solubility and surface activity) and the presence of the protonated guanidine function.[34,35] Amphoteric lipopeptide surfactants with antimicrobial activity comparable to those of LAM were found when neutral Gly or Phe amino acids were condensed to the N^{α}-lauroyl arginine.[10] More interestingly, condensation of a N^{α}-acyl-arginine residue to an acid-hydrolyzed collagen gave rise to a family of amphoteric protein-based surfactants with antimicrobial activity, the homologue of C_{14} carbon atoms being the most active.[10] The activity of all these amphoteric N^{α}-acyl arginine surfactants could be due to the presence of the long chain N^{α}-acyl-arginine residue and to the absence of intramolecular ionic interactions in the molecule. The free guanidine group together with the surface activity of these compounds could interact with the polyanionic components of the cell surface triggering the biocide mechanisms of these surfactants. In accordance with Ferguson's principle,[36] the antimicrobial activity might be related to the combination of several physicochemical properties such as surface activity, adsorption and solubility.

The ultimate biodegradability[37] measurements for the three series of arginine-based cationic surfactants showed that all homologues (except AMA) can be considered biodegradable.[38] Interestingly, using ester type bonds (series 3, Scheme 3) to link the hydrophobic and hydrophilic moieties accelerates their biodegradation considerably. This fact has also been described for sugar-based surfactants.[39]

The haemolytic activity, HC_{50}, which is the concentration of surfactant that causes 50% of haemolysis of red blood cells from healthy human donors[40] and the HC_{50}/D ratio, where D is the haemoglobin denaturising index, are the parameters commonly measured for evaluating the potential toxicity of the surfactants. The HC_{50}/D or L/D is used for predicting the potential ocular irritation relative to sodium dodecyl sulfate (SDS) (L/D_{SDS}: 0.44; irritant). The values of HC_{50} for series 1, 2 and 3 (Scheme 3) demonstrated that these compounds can be considered non-haemolysing agents (HC_{50} < 1000 μg mL^{-1}). For comparison's sake, commercial cationic surfactants have HC_{50} ranging from 4 to 15 μg mL^{-1}. Furthermore, according to the results of the L/D ratio first and by the *in vivo* eye irritation Draize test later, these linear arginine-based surfactants have no irritant effect on the eyes (non eye-irritants, L/D > 100).[40]

2.3 Applications

The application of synthetic acyl amino acid/peptide vesicles as drug carriers as well as for the preparation of functional liposomes with lipopeptide ligands have been examined by several authors in recent years.[41,42] Vesicles of long aliphatic chain N^{α} acyl amino acids showed encapsulation efficiencies for solutes comparable to that of conventional liposomes of lecithin. Recently, a new technology has emerged for the transfer of foreign DNA into cells forming non-toxic hydrophobic ion-paired complexes between long chain arginine alkyl esters (Scheme 3 series 3) with DNA.[43]

Lipoamino acids are also particularly attractive as antiviral agents. Certain acyl amino acid derivatives have been found to produce inhibition on influenza neuraminidase.[44] A number of N^{α}-palmitoylated amino acids/peptides have been incorporated into model membranes affecting the transition temperature between the

bilayer to hexagonal aggregation, a property associated with antiviral activity against the Cantell strain of the Sendai virus (parainfluenza type 1).[45]

Polymeric amino acid-based surfactants for enantiomeric separations using capillary electrokinetic chromatography (EKC) has been reported in the bibliography.[46–48] The effect of the position and number of chiral centers, amino acid order and steric effects have been evaluated.[48]

3 Amino acid-based *gemini* surfactants

Gemini surfactants are a novel class of amphipathic compounds consisting of two hydrophilic and two hydrophobic groups per molecule linked through a spacer chain. These molecules can be considered dimers of the single chain conventional surfactants of one hydrophilic and one hydrophobic group. Their interest lies in the number of unexpected surface activity properties, which make them superior to the conventional surfactants. These molecules show extremely low CMC (CMC, a fundamental parameter of surfactants close related to their hydrophobicity), solubilizing, wetting, foaming, antimicrobial and lime soap dispersion properties.[49–56]

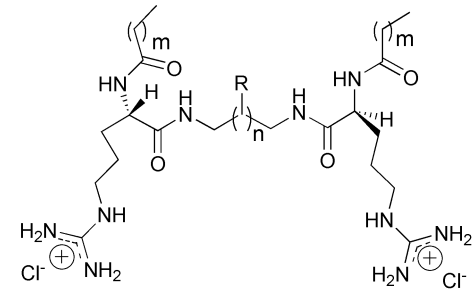
3.1 Synthesis

In the past 15 years, many different types of *gemini* surfactants have been synthesized.[57] Reviews concerning the synthesis and structures of *gemini* surfactants have been published.[58–61]

A number of surfactant structures with a variety of hydrophilic head groups and hydrophobic tails of different chain lengths have been reported in an effort to increase their surface-active properties.[62–66] Several papers have been published addressing the synthesis and properties of a new type of bifunctional amphiphilic cationic compounds with an effective surfactant and antimicrobial behaviour of the *gemini* type called bis quaternary ammonium halide surfactants, or bis(Quats).[67] These are double-headed surfactants in which two alkyldimethyl quaternary ammonium groups per molecule are linked by a hydrocarbon spacer chain. Owing to their extraordinary activity, they are regarded as an outstanding new generation of cationic surfactants with excellent performance with regard to solubilization, soil cleaning, and oil recovery. However, they are very stable molecules with a questionable chemical and biological degradability.[68]

A strategy to reduce the environmental impact and potential toxicity is to build up *gemini* structures from environmentally friendly single chain arginine-based surfactants. To this end, our group has chemically synthesized and studied a new class of *gemini* cationic surfactants derived from the arginine: the N^{α},N^{ω}-bis(N^{α}-acylarginine)α,ω-alkylendiamides or bis(Args).[25,69–73] These compounds consist of two symmetrical long chain N^{α}-acyl-L-arginine residues of twelve N^{α},N^{ω}-bis(N$^{\alpha}$-lauroylarginine)α,ω-alkylendiamides [$C_n(LA)_2$ series], and ten carbons atoms, N^{α},N^{ω}-bis(N$^{\alpha}$-caproylarginine)α,ω-alkylendiamides [$C_n(CA)_2$ series], linked by amide bonds to an α,ω-alkylenediamine spacer chain of varying length (n = 2–10) (Scheme 4). This particular alkylenediamine spacer chain was chosen to control the distance between the charged sites of the molecule which modify the inter- and intra-hydrophilic–hydrophobic interactions. The bis(Args) were investigated in an attempt to develop a new class of environmentally friendly amino acid-based surfactants with surface activity exceeding that of CAM and LAM, and with at least the same antimicrobial, toxicity and biodegradability properties.

Recently a series of bis(Args) have been prepared at multigram scale by a chemoenzymatic approach using papain deposited onto Celite for the best results[70,74] (Scheme 4 right).



$C_3(OA)_2$: m=6; n=1; R= H
$C_2(CA)_2$: m=8; n=0; R=H
$C_3(CA)_2$: m=8; n=1; R=H
$C_4(CA)_2$: m=8; n=2; R=H
$C_6(CA)_2$: m=8; n=4; R=H
$C_9(CA)_2$: m=8; n=7; R=H
$C_{10}(CA)_2$: m=8; n=8; R=H
$C_2LA)_2$: m=10; n=0; R=H
$C_3(LA)_2$: m=10; n=1; R=H
$C_4(LA)_2$: m=10; n=2; R=H
$C_6(LA)_2$: m=10; n=4; R=H
$C_9(LA)_2$: m=10; n=7; R=H
$C_{10}(LA)_2$: m=10; n=8; R=H

$C_3(OA)_2$: m=6, n=1, R: H
$C_3(CA)_2$: m=8, n=1, R: H
$C_3(LA)_2$: m=10, n=1, R: H
$C_3OH(LA)_2$: m=10, n=1, R: OH
$C_6(CA)_2$: m=8, n=4, R: H
$C_9(CA)_2$: m=8, n=7, R: H

Scheme 4 Structures of bis(Args) *gemini* cationic surfactants prepared by chemical procedures (left) and chemo-enzymatic procedures (right).

3.2 Properties

In the light of our studies, most *gemini* surfactant properties are superior to the corresponding conventional monomeric surfactants. They were found to be more efficient surface active molecules than the single chain structures: Their CMC are at least one order of magnitude lower than that of the corresponding monomeric surfactants[71] and they are 10–100 times more efficient at reducing the surface tension of water and in foam stability.[75]

To establish the effect of the dimerization on the antimicrobial activity as well as the influence of the alkyl and the spacer chain length of bis(Args), the evaluation of *in vivo* membrane-disrupting properties was made using cell bacteria as biological membranes. The dilution antimicrobial susceptibility test was carried out and the minimum inhibitory concentration (MIC) values were determined. Bis(Args) exhibited a broad spectrum of preservation capacity at MIC values in the range from 4 to 125 μg mL^{-1}.[69] The dimerization enhanced the antimicrobial activity for the *gemini* $C_n(CA)_2$ compared with CAM. Given the peculiar structure of bis(Args), the compounds of the $C_n(CA)_2$ series have a hydrophobicity which is more than twice the hydrophobicity of CAM. However, the presence of two ionic arginine groups in one molecule of $C_n(CA)_2$ can make a positive contribution to the degree of the hydration of this series, showing a water-solubility similar to that of CAM. These two characteristics in the molecule can result in a more effective adsorption and diffusion of $C_n(CA)_2$ on the cell interface, resulting in an antimicrobial action at lower concentrations.

Bearing in mind that biological membranes are essentially nonpolar interfaces, evidence exists that the toxicity of surfactants against the aquatic species tested is caused by the ability of the monomers to disrupt the integral membrane by a hydrophobic/ionic adsorption phenomenon at the cell membrane–water interface in a similar way to that of the antimicrobial mode action. Acute toxicity tests on freshwater crustacea (*Daphnia magna*), a very sensitive invertebrate,[76] as well as on saltwater bacteria (*Photobacterium phosphoreum*)[77] were carried out to assess the aquatic toxicity. These standard tests represent two of the trophic levels which can be exposed to the cationic surfactants. Concentration values that cause inmobilization in 50% of the Daphnia after 24 hours exposure (IC_{50}) and 50% reduction in the light emitted by the bacteria after 30 minutes exposure (EC_{50}) were determined. Values of IC_{50} and EC_{50} for the bis(Args) together with those of LAM and CAM are summarised in Table 2.[78] Values reported for two series of

Table 2 Aquatic toxicity values of $C_n(LA)_2$, $C_n(CA)_2$ series and CAM, LAM, DTAB and HTAB.[77]

	Daphnia magna IC_{50}[a]/mg L^{-1}		*Photobacterium phosphoreum* EC_{50}[b]/mg L^{-1}	
Compounds	mean	95% confidence range	mean	95% confidence range
$C_2(LA)_2$	4.4	(3.5–5.3)	28	(18–43)
$C_3(LA)_2$	2.1	(1.8–2.4)	2.4	(2.2–2.7)
$C_4(LA)_2$	4.6	(3.9–5.2)	5.8	(4.3–8.0)
$C_6(LA)_2$	2.4	(1.9–2.5)	3.0	(2.0–4.5)
$C_9(LA)_2$	2.2	(1.9–2.5)	13	(10–17)
$C_{10}(LA)_2$	16	(11–20)	20	(15–28)
LAM	15	(12–18)	12	(10–14)
DTAB	0.38	(0.36–0.40)	0.24	(0.20–0.30)
HTAB	0.13	(0.11–0.15)	0.63	(0.40–0.98)
$C_2(CA)_2$	16	(11–20)	1.5	(1.2–1.9)
$C_3(CA)_2$	16	(11–20)	1.1	(0.5–2.1)
$C_4(CA)_2$	12	(7–17)	0.9	(0.4–2.2)
$C_6(CA)_2$	15	(11–19)	1.3	(0.3–5.6)
$C_9(CA)_2$	5.5	(2.7–8.2)	1.1	(0.7–1.7)
$C_{10}(CA)_2$	7.5	(5.0–10)	2.7	(1.2–5.8)
CAM	77	(56–98)	4.0	(3.1–5.3)

[a] Concentration values that cause 50% inhibition in the crustacean mobility after 24 h of exposure. [b] Concentration values that cause 50% reduction in the light emitted by the bacteria after 30 min of exposure.

conventional mono(Quats) dodecyltriammonium bromide (DTAB) and hexadecyltriammonium bromide (HTAB) are also indicated. When increasing the hydrophobicity of the molecule, the acute toxicity raised for each series of surfactants is in agreement with their CMC values, Table 3. Thus, $C_n(CA)_2$ were less toxic than the

Table 3 CMC values (g L^{-1}) and HC_{50} (mg L^{-1}) of CAM, LAM and bis(Args) homologues on human red blood cells.[77]

Compound	CMC[a]/g L^{-1}	HC_{50}[b]/mg L^{-1}
CAM	6.05	38.5
$C_3(CA)_2$	1.53	110.5
$C_6(CA)_2$	1.29	9.0
$C_9(CA)_2$	9.35	8.7
LAM	2.44	20.8
$C_3(LA)_2$	4.60	80.7
$C_3(OH)(LA)_2$	5.89	> 200
$C_3(OA)_2$	49.77	> 200

[a] Critical micelle concentration. [b] Hemolysis value.

$C_n(LA)_2$ compounds due to their lower hydrophobic character. Interestingly, IC_{50} values for $C_n(CA)_2$ compounds were similar to that of LAM. Furthermore, all of them were one order of magnitude less toxic than the conventional mono(Quats).

Due to the complexity and hydrophobicity of the *gemini* compared with the single chain structures, the biodegradation rate of single chain structures such as LAM (90% in 14 days), was higher than that of the bis(Args) (50–90% in 14 days). The biodegradation rate of bis(Args) decreased when both the spacer chain and the alkyl chain length increased. Hence, the higher the hydrophobicity of the surfactants the lower their biodegradation rate[78].

The haemolysis test showed again that the highest HC_{50} values were obtained for the compounds with the highest hydrophobic character, namely, those with the longest alkyl and spacer chain lengths. There is considerable difference between the HC_{50} of these new *gemini* surfactants and those bearing a quaternary ammonium group at the polar head. MonoQuats have HC_{50} values between 0.05 and 0.1 μg m^{-1}. The introduction of a hydroxyl function to the spacer chain make the compound more hydrophilic, in consequence the CMC increases and the HC_{50} increases.

Dimerization of LAM and CAM yields environmentally friendly antimicrobial *gemini* surfactants with lower hemolytic activity, aquatic toxicity and efficient surface activity than other cationic surfactants, (*i.e.* monoQuats). The increase of hydrophobicity of these molecules is a negative structural parameter for their environmental behaviour.

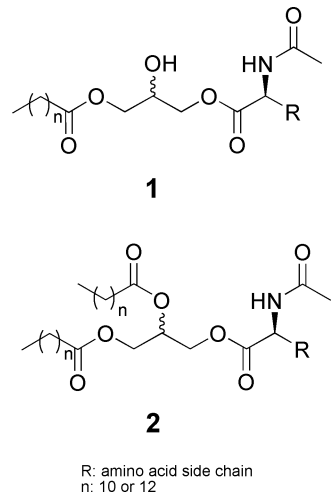
3.3 Applications

Gene therapy is an important topic in life science. It depends on effective techniques for safe introduction of the selected gene into living cells. Recently, *Gemini* surfactants have been found to be particularly promising as potential vehicles for the transport of bioactive molecules. Lysine and 2,4-diaminobutyric acid form polycationic *gemini* surfactants, by relatively simple synthesis using standard peptide chemistry.[79,80] These polycationic *gemini* surfactants and spermine-based *gemini* surfactants are attracting much attention as efficient agents in gene delivery.[81–86]

4 Amino acid glyceride conjugates

4.1 Synthesis

Amino acid glyceride conjugates (*i.e.* glycero amino acids) constitute a novel class of lipoamino acids, which can be considered analogues of mono and diacylglycerides and phospholipids (1 and 2 Scheme 5). They consist of one or two aliphatic chains and one amino acid, as the polar head, linked together through ester bonds in the glycerol backbone.

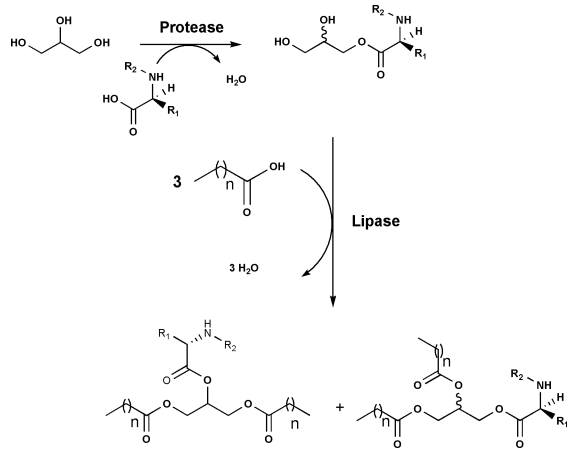


Scheme 5 Structures of amino acid glyceride conjugates: mono acyl derivatives (**1**) and diacyl derivatives (**2**).

Our group has synthesized these products using chemical and enzymatic methodologies.[25,87–89] The first enzymochemical synthetic strategy of amino acid glyceride conjugates was described by Valivety *et al.*[90] The synthesis started with the preparation of amino acid glyceryl esters namely 1-*O*-(N^{α}-protected-aminoacyl)glycerols. The preparation of these compounds was performed using an acid catalyst such as BF_3 at elevated temperatures in glycerol, which served as solvent and reactant. Alternatively, they can be obtained by enzymatic methodology using hydrolases (Scheme 6). Proteases and lipases were found to be a versatile catalyst for this reaction.[91–92] A variety of protected amino acid glyceryl ester derivatives were obtained in 46–98% yield under mild and selective conditions.

In a second step, the free hydroxyl groups of the glyceryl moiety were acylated with fatty acid using lipases as catalyst. According to Valivety *et al.*,[60,90] both Novozyme and Lipozyme can be successfully used as biocatalysts showing a high regioselectivity towards the primary hydroxyl group of the amino acid glyceryl ester derivative. Hence, by this methodology only the 1(3)-aminoacyl monoglycerides are likely to be prepared.

We have developed a novel methodology to obtain both 1-monoacyl- and 1,2-diacyl-3-aminoacyl glycerol[88] (see Scheme 5,



Scheme 6 Reaction pathway for the synthesis of mono and diacylglycerol amino acid conjugates.

1 and 2, respectively). Mono and diacylation of amino acid glyceryl ester may be carried out using selective lipases by taking advantage of the spontaneous intramolecular acyl-migration reaction that occurs in partial glycerides.[93–95] Thus, the 1(3)-acylated product may undergo intramolecular 1(3)→2 acyl migration and the resulting 1,2(2,3)-isomer subsequently be acylated at the free primary hydroxyl group by the lipase. Accordingly, the yield of diacylated product will depend on both the rate of intramolecular acyl-migration and the enzymatic esterification of the newly free primary hydroxyl of the monoacylated derivative. Both processes are influenced by the reaction conditions, such as solvent, support for enzyme immobilization, buffer salts and by the amino acid glyceryl ester derivative. All the enzymatic acylations were carried out in solvent free media, at a temperature around the melting point of the corresponding fatty acid.

We have found that the 1,2-diacyl-3-aminoacyl glycerol derivatives were in fact a mixture of two regioisomers: 1,2-diacyl-*rac*-glycero-3-(amino acid) derivative as the major one, and 1,3-dilauroyl-glycero-2-(amino acid) derivative. With this methodology a series of mono and dilauroylated glycerol derivatives of arginine, aspartic acid, glutamic acid, asparagine, glutamine and tyrosine were prepared.[88] All of them were mixtures of diastereoisomers and regioisomers except the dilauroylated derivative of glutamine. Here, both 1,3-dilauroyl-glycero-2-*O*-(N^{α}-acetyl-L-glutamine) and 1,2-dilauroyl-*rac*-glycero-3-*O*-(N^{α}-acetyl-L-glutamine) were separarated by flash chromatography on silica.

Chemically, two biocompatible cationic surfactants from the amino acid argininine were prepared (Scheme 5, **2**, *n*: 10, 12).[96] The first compound is 1,2-dilauroyl-*rac*-glycero-3-*O*-(N^{α}-acetyl-L-arginine), with 12 carbon atoms in the alkyl chains (Scheme 5, **2**, *n*: 10) . The second one is the 1,2-dimiristoyl-*rac*-glycero-3-*O*-(N^{α}-acetyl-L-arginine), with 14 carbon atoms in the alkyl chains (Scheme 5, **2**, **n**: 12). Henceforth, we shall refer to these compounds as 1212RAc and 1414RAc, respectively.

The synthesis of 1212RAc and 1414RAc compounds was achieved following a two step procedure. The first step involves the enzymatic preparation of the arginine glyceryl ester derivative as described in Morán *et al.*[92] This reaction consisted of the selective protease-catalysed esterification of one of the primary hydroxyl groups of the glycerol with the carboxylate group of the N^{α}-protected amino acid. The reaction yield was 80%. The second step consists of the preparation of 1,2-diacyl-*rac*-glycero-3-*O*-(N^{α}-acetyl-L-arginine) by acylation of the two remaining free hydroxyls groups of the arginine glyceryl ester derivative with the corresponding long chain acid chloride.[96] The progress control of the reaction showed that the arginine glyceryl ester derivative was first esterified with 1 mol of acyl chloride to give the corresponding monoacyl derivatives. With a molar ratio acid chloride : arginine glyceryl ester derivative (1 : 3), the main reaction products were 1212RAc and 1414RAc with conversions higher than 98%. The overall yield of these reactions after purification was in the range of about 85–70%.

New surfactants that are analogues of lecithin have been synthesized[97–101] The structure of these non-ionic amphiphilic compounds is based on natural dibasic (lysine) or diacidic (glutamic or aspartic acids) amino acids. As with the lecithins, these amino acid-based compounds have two hydrophobic tails and one hydrophilic head. However, in the latter compounds the polar head is of the non-ionic type (one or two chains of monomethyletherpolyoxyethylene glycol) whereas in the lecithins it is of the zwitterionic type. The central pivot in the structure of lecithins, *i.e.* the glycerol, is imitated by the natural trifunctional amino acids lysine,[91–101] aspartic acid[99,101] and glutamic acid.[99,101] The hydrophobic (fatty acids or fatty amines) and hydrophilic (monomethyletherpolyoxyethylene amine or monomethyletherpolyethylene carboxylic acid) moieties were introduced into their amino or carboxylic functions through amine bonds, in place of the ester bonds in lecithins. The latest variation on this theme contains two different hydrophobes; thus creating an asymmetric structure that displays lower water solubility and higher surface activity as well as a slightly increased toxicity.

4.2 Properties

The physicochemical and biological properties of amino acid glyceride conjugates have not been extensively explored yet. From preliminary observations carried out in our lab, these novel compounds combine the advantages of both partial glycerides and lipoamino acids.

The influence of the temperature as a function of time on the stability properties was studied in order to ascertain whether chemical degradation of 1212RAc and 1414RAc compounds occurred.[102] As expected, the hydrolysis rate was higher when the temperature increases, reaching 10% in the case of the 1414RAc and 8.9% in the case of the 1212RAc at 40 °C after 144 hours. The hydrolysis of 1212RAc and 1414RAc compounds is related to their structure in which three ester bonds are present. The ester bonds hydrolyzed under acid pH conditions. Owing to the presence of a weak acid group, the guanidine group, aqueous solutions of these surfactants have a slightly acid pH (pH = 4). Slow hydrolysis of the ester bonds was promoted at this pH.

The aqueous aggregation behavior, studied by conductivity, showed a linear increase with concentration up to a break point of 0.12 mM for 1212RAc and 0.09 mM for 14141RAc. Results for similar nonacetylated compounds suggest that the break points from conductivity for this new family of compounds do not correspond to a true CMC (monomer to micelle) but some other transition (*i.e.* vesicle to ribbon).[102]

Qualitative phase behavior studies applying the flooding method revealed the formation of anisotropic phases in all the binary surfactant systems studied. The compound 1212RAc forms lamellar liquid crystals at room temperature (25 °C) and this structure is stable and remains stable until high temperatures are reached. The 1414RAc also forms anisotropic phases that developed in lamellar liquid crystals at 45 °C. Dispersions of 1212RAc and 1414RAc 0.1% in water at 35 °C revealed that their lamellar structures (Fig. 1) spontaneously form stable multilamellar vesicles (Fig. 2) spontaneously and easily, of diverse size and number of bilayers.

The 1212RAc and 1414RAc surfactants can simultaneously stabilize water-in-oil (W/O) droplets and oil-in-water droplets (O/W), forming multiple emulsions. On account of this behavior, these surfactants constitute an interesting alternative to the diglycerides and lecithins in formulations that need antimicrobial properties.

The phase behaviour in the dry state of pure and regioisomeric mixtures has been studied by differential scanning calorimetry and small-angle X-ray diffraction complemented by polarized light microscopy.[103] The study showed the influence of the bilayer packing as a function of the temperature.

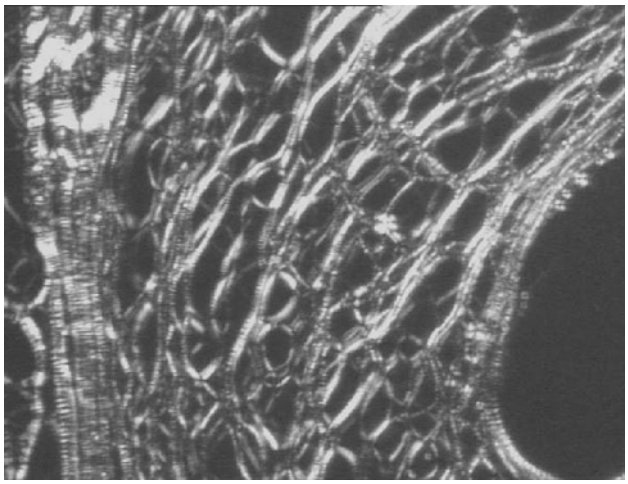


Fig. 1 Polarized microscopy of lamellar liquid crystals for the 1414RAc/H_2O system (**1**).

Fig. 2 Polarized microscopy of vesicles for the 1212RAc/H_2O system (**2**).

We have shown that a novel family of 1,2-diacylglycerol amino acid-based surfactants exhibits a monolayer behaviour similar to that found in natural phospholipids, suggesting that they may be viable substitutes for these compounds in industrial applications, which need multifunctional compounds.[104] Use of Brewster Angle Microscopy (BAM) image analysis of the inner textures has revealed that condensed phases of the diacyl glycerol compounds with 14 carbon atoms in the hydrocarbon chain exhibit hexatic order. Variations in the chain length introduce similar changes to those commonly found in lipid monolayers. Generally, compounds with a 1,2-substitution pattern in the glycerol backbone exhibit a tendency to form continuous monolayers (expanded phases) at significantly lower molecular densities than their natural phospholipid counterparts.

Our investigations with a compound bearing a 1,3-substitution pattern [1,3-dilauroyl-glycero-2-(N^{α}-acetyl-L-glutamine)] show that it forms rough and compact LC domains, very different from those observed in phospholipid monolayers.[104]

In summary, amino acid-based surfactants constitute a class of bio-based surfactants with excellent surface properties, wide biological activity, low potential toxicity and low environmental impact. Moreover, they can be prepared efficiently by chemical and enzymatic catalysis. All these features make them an outstanding clean and safe alternative to conventional specialty surfactants. Hence, this new generation of surfactants will contribute to meet the increasing demand for environmentally friendly surfactants for pharmaceutical and food industries.

Acknowledgement

This work was supported by the MEC, Projects QUI97-0570 and PPQ2000-1687-CO2-01 and UA-CSIC *Interaccion de Tensioactivos con membranas.*

References

1 K. Holmberg, Novel Surfactants. Preparation, Applications and Biodegradability, Second Edition, Revised and Expanded, *Surfactant Science Series*, ed. K. Holberg, Marcel Dekker, New York, 2004.
2 Y. Okahata, S. Tammamachi, M. Magai, T. Kunitake and J. Colloid, *Interface Sci.*, 1981, **82**, 401.
3 Ma R. Infante, J. Molinero, P. Erra, R. Juliá, J. J. García Domínguez and M. Robert, *Fette Seifen Anstrichm.*, 1986, **88**, 108.
4 T. Kida, N. Morishima, A. Masuyama and Y. Nakatsuji, *J. Am. Oil Chem. Soc.*, 1994, **71**, 705.
5 Ma R. Infante, A. Pinazo and J. Seguer, *Colloids Surf., A*, 1997, **123–124**, 49.
6 T. Furutani, H. Ooshima and J. Kato, *Enzyme Microb. Technol.*, 1997, **20**, 214.
7 B. Gallot and H. H. Hassan, *Mol. Cryst. Liq. Cryst.*, 1989, **170**, 195.
8 S. E. Godtfredsen and F. Bjoerkling, World Patent No. 90/14429, 1990.
9 A. Nagao and M. Kito, *J. Am. Oil Chem. Soc.*, 1989, **66**, 710.
10 I. A. Nnanna and J. Xia. Protein Based Surfactants: Synthesis, *Physicochemical Properties and Applications*, Marcel Dekker, New York, 2001.
11 A. Vonderhagen, H.-C. Raths and E. Eilers, Ger. Offen. DE *19749555 A1 12 May* Henkel K.-G.a.A., Germany, 1999, 4.
12 R. Valivety, P. Jauregui and E. N. Vulfson, *J. Am. Oil Chem. Soc.*, 1997, **74**, 879.
13 W. Heutrich, H. Keppler and K. Hintzmann, Ger Patent 635522, 1936.
14 H. Yokota, K. Sagawa, Ch. Eguchi and M. Takehara, *J. Am. Oil Chem. Soc.*, 1985, **62**, 1716.
15 G. Baschang, A. Hartmann and O. Wacker, US patent 4666886 A, 1987.
16 D. B. Braun, *Cosmet. Toiletries*, 1989, **104**, 92.
17 P. Presenz, *Pharmazie*, 1996, **51**, 755.
18 M. Takehara, *Colloids Surf.*, 1989, **38**, 149.
19 Ma R. Infante, J. J. García Domínguez, P. Erra, Ma R. Juliá and M. Prats, *Int. J. Cosmet. Sci.*, 1984, **6**, 27.
20 Ma R. Infante, J. Molinero, P. Bosch, Ma R. Juliá and P. Erra, *J. Am. Oil Chem. Soc.*, 1992, **69**, 647.
21 Ma R. Infante and V. Moses, *Int. J. Pept. Protein Res.*, 1994, **43**, 173.
22 J. Seguer, J. Molinero, A. Manresa, J. Caelles and Ma R. Infante, *J. Soc. Cosmet. Chem.*, 1994, **45**, 53.
23 E. Piera, F. Comelles, P. Erra and Ma R. Infante, *J. Chem. Soc., Perkin Trans. 2*, 1998, 335.
24 P. Clapés, C. Morán and Ma R. Infante, *Biotechnol. Bioeng.*, 1999, **63**, 333.
25 P. Clapés and Ma R. Infante, *Biocatal. Biotransform.*, 2002, **20**, 215.
26 R. Valivety, I. S. Gill and E. N. Vulfson, *J. Surf. Deterg.*, 1998, **1**, 177.
27 E. L. Soo, A. B. Salleh, M. Basri, R. N. Z. R. A. Rahman and K. Kamaruddin, *J. Biosci. Bioeng.*, 2003, **95**(4), 361.
28 C. Solans, N. Azemar, Ma R. Infante and T. Warnheim, *Prog. Colloid Polym. Sci.*, 1989, **79**, 70.
29 H. Fördedal, J. Sjöblom and Ma R. Infante, *Colloids Surf., A*, 1993, **79**, 81.
30 M. A. Pés. Doctoral Thesis, University of Barcelona, 1992.
31 H. Kunieda, K. Nakamura, Ma R. Infante and C. Solans, *Adv. Mater.*, 1992, **4**, 291.
32 T. J. Franklin and G. A. Snow, *Biochemistry of Antimicrobial Action*, 3rd edn., Chapman & Hall, New York, 1981.
33 M. J. Rosen, *Surfactants and Interfacial Phenomena*, Wiley & Sons, New York, 1987.
34 M. R. Infante, J. Molinero, P. Erra, M. R. Juliá and J. J. García Dominguez, *Fett. Wiss. Technol.*, 1998, **87**, 309.
35 H. Gibson and J. T. Holah, in *Preservation of Surfactant Formulations*, ed. F. F. Morpeth, Blackie Academic and Professional, Glasgow, 1995, p. 30.
36 J. Ferguson, *Proc. R. Soc. London, Ser. B.*, 1939, **127**, 387.
37 Organisation for Economic Cooperation and Development (OECD) Chemicals Group, Revised Guidelines for tests for Ready Biodegradability, 301E. Paris, 1993 and OECD guidelines for testing of Chemicals. Vol. 1, section 2.

38 C. Morán, P. Clapés, F. Comelles, M. T. García, L. Pérez, Ma Vinardell, M. Mitjans and Ma R. Infante, *Langmuir*, 2001, **17**, 5071.
39 O. Kirk, F. D. Pedersen and C. C. Fuglsang, *J. Surfactants Deterg.*, 1998, **1**, 37.
40 W. J. W. Pape and U. Hopper, *Drug Res.*, 1990, **4**, 498.
41 C. Boeckler, B. Frisch and F. Schuber, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 2055.
42 N. Yagi, Y. Ogawa, M. Kodaka, T. Okada, T. Tomohiro, T. Konakahara and H. Okuno, *Lipids*, 2000, **35**, 673.
43 D. J. Claffey, J. D. Meyer, R. Beauvais, T. Brandt, E. Shefter, D. J. Kroll, J. A. Ruth and M. C. Manning, *Biochem. Cell Biol.*, 2000, **78**, 59.
44 M. Kondoh, T. Furutani, M. Azuma, H. Oshima and J. Kato, *Biosci., Biotechnol., Biochem.*, 1997, **61**, 870.
45 R. F. Epand, Ma R. Infante, T. D. Flanagan and R. M. Epand, *BBA-Biomembranes*, 1998, **1373**, 67.
46 J. Wang and I. M. Warner, *Anal. Chem.*, 1994, **66**, 3773.
47 S. Hara and A. Dobashi, Jpn. Pat. 04 149 205 1993.
48 E. Billiot and I. Warner, *Anal. Chem.*, 2000, **72**, 1740.
49 M. J. Rosen, *CHEMTECH*, 1993, 30.
50 R. Zana, in *Specialist Surfactants*, ed. D. Robb, Blackie, London, 1997, p. 81.
51 F. M. Menger and C. A. Littau, *J. Am. Chem. Soc.*, 1993, **115**, 10083.
52 F. Devinsky, I. Lacko, F. Bittererova and D. Mlynarcik, *Chem. Pap.*, 1987, **41**, 803.
53 M. El Achouri, Ma R. Infante, F. Izquierdo, F. Kertit, H. M. Gouttaya and B. Nciri, *Corros. Sci.*, 2001, **43**, 19.
54 F. M. Menger and J. S. Keiper, *Angew. Chem., Int. Ed.*, 2000, **39**, 1906.
55 A. Pinazo, M. Diz, A. Pés, P. Erra and M. R. Infante, *J. Am. Oil Chem. Soc.*, 1993, **70**, 37.
56 M. Diz, A. Manresa, A. Pinazo, P. Erra and M. R. Infante, *J. Chem. Soc., Perkin. Trans. 2*, 1994, 1871.
57 I. Ikeda, in Novel Surfactants. Synthesis of Gemini (Dimeric) and Related Surfactants, *Surfactant Science Series*, ed. K. Holberg, Marcel Dekker, New York, 2004, pp. 9–35.
58 M. J. Rosen, *CHEMTECH*, 1993, **23**, 30; Y. Nakatsuji and I. Ikeda, *Chim. Oggi.*, 1997, 40.
59 R. Zana, in Novel Surfactants. Preparation, Applications and Biodegradability, *Surfactant Science Series*, ed. K. Holberg, Marcel Dekker, New York, 1998, pp. 241–277.
60 E. Vulfson, in Novel Surfactants. Enzymatic Synthesis of Surfactants, *Surfactant Science Series*, K. Holberg, Marcel Dekker, New York, 2004, pp. 279–300 (and references therein).
61 T. W. Davey, in Gemini Surfactants. Special Gemini Surfactants: Nonionic, Zwitterionic, Fluorinated, and Amino Acid Based, *Surfactant Science Series*, K. Holberg, Marcel Dekker, New York, 2004, pp. 253–280 (and references therein).
62 M. Brock, *Tenside Surfactants Deterg.*, 1993, **30**, 394.
63 R. Puchta, P. Krigs and F. Schambil, Comite Español De La Detergencia Tensioactivos Y Afines (C.E.D.), Barcelona, 1993.
64 Kationische Zuckertenside, *Seifen Oele Fette Waschse*, 1994, **120**, 423.
65 R. Pi Subirana, N. Bonastre, E. Prat Queralt and J. Bigorra Lloses, Ger. Patent 195 39 876 to Henkel K._G.a.A. 1996.
66 R. Valivety, I. S. Gill and E. N. Vulfson, *J. Surfactants Deterg.*, 1998, **1**(2), 177.
67 M. Biermann, F. Lange, R. Piorr, U. Ploog, H. Rutzen, J. Schindler and R. Schmidt, in *Surfactants in Consumer Products*, ed. J. Falbe, Springer-Verlag, Heidelberg, 1987, pp. 110–114.
68 L. Edebo, M. Lindstedt, S. Allenmark and R. A. Thompson, *Antimicrob. Agents Chemother.*, 1990, **34**, 1949.
69 L. Pérez, J. L. Torres, A. Manresa, C. Solans and Ma R. Infante, *Langmuir*, 1996, **12**, 5296.
70 E. Piera, Ma R. Infante and P. Clapés, *Biotechnol. Bioeng.*, 2000, **70**, 323.
71 L. Pérez, A. Pinazo, M. J. Rosen and M. R. Infante, *Langmuir*, 1998, **14**, 2307.
72 A. Pinazo, X. Wen, L. Pérez, M. R. Infante and E. I. Franses, *Langmuir*, 1999, **15**, 3134.
73 L. Pérez, Doctoral Thesis, University of Barcelona, 1997.
74 E. Piera, Doctoral Thesis, University of Barcelona, 2000.
75 A. Pinazo, L. Pérez, M. R. Infante and E. I. Franses, *Colloids Surf., A*, 2001, **189**, 225.
76 OECD guidelines for testing of Chemicals. Vol. 1, section 2: *Effects on Biotic system*, 202, Paris, France, 1993.
77 J. M. Ribó and K. L. M. Kaiser, *Toxic. Assess.*, 1987, **2**, 305.
78 L. Perez, T. García;, I. Ribosa, P. Vinardell, A. Manresa and Ma R. Infante, *Environ. Toxicol. Chem.*, 2002, **21**, 1279.
79 C. McGregor, C. Perrin, M. Monck, P. Camilleri and A. Kirby, *J. Am. Chem. Soc.*, 2001, **123**, 6215.
80 P. Camilleri, A. Kremer, A. J. Edwards, K. H. Jennings, O. Jenkins and I. Marshall, *Chem. Commun.*, 2000, 1253.
81 C. McGregor, C. Perrin, M. Monck, P. Camilleri and A. Kirby, *J. Am. Chem. Soc.*, 2001, **123**, 6215.
82 G. Ronsin, C. Perrin, P. Guedat, A. Kremer, P. Camilleri and A. Kirby, *Chem. Commun.*, 2001, 2234.
83 G. Ronsin, A. J. Kirby, S. Rittenhouse, G. Woodnutt and P. Camilleri, *J. Chem. Soc., Perkin. Trans. 2*, 2002, 1302.
84 P. Camilleri, A. Kremer, A. J. Edwards, K. H. Jennings, O. Jenkins and I. Marshall, *Chem. Commun.*, 2000, 1253.
85 P. Camilleri, C. McGregor, A. J. Kirby and C. Perrin, WO 0230957, September 2002.
86 K. H. Jennings, I. C. B. Marshall, M. Wilkinson, A. Kremer, A. J. Kirby and P. Camilleri, *Langmuir*, 2002, **18**, 2426.
87 S. Pegiadou, L. Pérez and Ma R. Infante, *J. Surfactants Deterg.*, 2000, **3**, 517.
88 C. Morán, Ma R. Infante and P. Clapés, *J. Chem. Soc., Perkin. Trans. 2*, 2001, 2063.
89 M. C. Morán, Doctoral Thesis, University of Barcelona, 2002.
90 R. Valivety, P. Jauregui and E. N. Vulfson, *J. Am. Oil Chem. Soc.*, 1997, **74**, 879.
91 Y. V. Mitin, K. Braun and P. Kuhl, *Biotechnol. Bioeng.*, 1997, **54**, 287.
92 C. Morán, M. R. Infante and P. Clapés, *J. Chem. Soc., Perkin Trans. 1*, 2001, 2063.
93 M. Berger, K. Laumen and M. P. Schneider, *J. Am. Oil Chem. Soc.*, 1992, **69**, 955.
94 A. Millqvist-Fureby, C. Virto, P. Adlercreutz and B. Mattiason, *Biocatal. Biotransform.*, 1996, **14**, 89.
95 C. Virto, I. Svensson and P. Adlercreutz, *Enzyme Microb. Technol.*, 1999, **24**, 651.
96 L. Pérez, M. R. Infante, R. Pons, C. Moran, P. Vinardell, M. Mitjans and A. Pinazo, *Colloids Surf., B*, 2003, in press.
97 M. R. Infante, J. Seguer, A. Pinazo and M. P. Vinardell, *J. Dispersion Sci. Technol.*, 1999, **20**, 621.
98 J. Seguer, C. Selve, M. Allouch and M. R. Infante, *J. Am. Oil Chem. Soc.*, 1996, **73**(1), 79.
99 J. Seguer, M. R. Infante, M. Allouch, L. Mansuy, C. Selve and P. Vinardell, *New J. Chem.*, 1994, **18**, 765.
100 M. Macian, J. Seguer, Ma R. Infante, C. Selve and Ma P. Vinardell, *Toxicology*, 1996, **106**, 1.
101 J. Seguer, Doctoral Thesis, UB, 1993.
102 A. Pinazo, L. Perez, M. R. Infante and R. Pons, *Phys. Chem. Chem. Phys.*, 2004, **6**, in press10.1039/b313313n.
103 M. C. Morán, A. Pinazo, P. Clapés, L. Pérez, M. R. Infante and R. Pons, *J. Phys. Chem. B*, 2004, (submitted).
104 R. Albalat, J. Claret, J. Ignés-Mullol, F. Sagués, C. Morán, L. Pérez, P. Clapés and A. Pinazo, *Langmuir*, 2003, **19**, 10878.