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A synthetic alternative to natural lecithins with antimicrobial properties

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Abstract

Two soft biocompatible cationic surfactants from the amino acid arginine, 1, 2 dilauoil-3-acetylarginyl-rac-glycero (1212RAc) and 1,2 dimirystoil-3-acetylarginyl-rac-glycero (1414RAc), were prepared. Their physicochemical properties show that they can be classified as multifunctional surfactants with self-aggregation behavior comparable to that of short-chain lecithins. The two surfactants can simultaneously stabilize water-in-oil droplets and oil-in-water droplets, forming multiple emulsions. They have antimicrobial activity similar to that of conventional cationic surfactants and are as harmless as amphoteric betaines. These surfactants constitute an interesting alternative to the diglycerides and lecithins in formulations that require antimicrobial properties.

1. INTRODUCTION

Considerable research has been focused on new surfactants and emulsifiers in recent years. The main driving force behind the development of novel surfactants is the search for environmentally friendly products. Another incentive for the development of new surfactants is to combine surface activity and another property in one molecule, i.e. polymerizability [1], susceptibility to cleavage by some specific mechanism [2] or antimicrobial properties [3-8].

Food emulsifiers are polar lipids needed to increase colloidal stability and provide interfacial interactions between food components such as lipids, proteins and carbohydrates. Such interactions are important factors in obtaining emulsion stability, foam formation of whipped products, and increased shelf life in many foods [9]. Lecithin (1,2-diacyl-3-phosphorylcholine) and Lysolecithin (1(2)-acyl-3-phosphorylcholine) are well known food and cosmetic additives [10]. They are natural environmentally friendly products, which exhibit a number of desirable properties such as low toxicity, high biodegradability and compatibility with a number of pharmaceutical products [11]. However, they have low water solubility and are very costly (i.e. in rape and soybean oils only about 0.3 - 2.5% phospholipids are extracted from the oil fraction on a dry basis).

The design and synthesis of surfactants that exhibit the properties of lecithins, along with a significant water solubility and antimicrobial activity would be a considerable step forward. In this work we report a new chemical synthesis of

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two cationic diglyceride surfactants derived from arginine. The new products have an acetyl arginine head group, linked to a glycerol by an ester bond, and two saturated alkyl chains. The first compound is the 1,2 dilauroil-3-acetylarginyl-rac-glycerol with 12 carbon atoms alkyl chains. The second compound is the 1,2 dimirystoil-3-acetylarginyl-rac-glycerol with 14 carbon atoms in the alkyl chains. Henceforth, we shall refer to these compounds as 1212RAc and 1414RAc, respectively (Figure 1). Results on chemical stability, aggregation behaviour, liquid crystals, emulsion formation, toxicity and antimicrobial activity are included in this work.

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- Eliminado: First we describe the chemical procedure developed to synthesize the surfactants. Then we characterize the surfactant properties including stability, antimicrobial activity, toxicity, as well as the qualitative phase and emulsion behaviour. ¶¶

2. EXPERIMENTAL SECTION

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2.1. Materials

All the solvents were reagent grade and were used without further purification.

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Anhydrous Glycerol and the fatty acyl chlorides with 12 and 14 carbon atoms

were from Fluka. Tego®-bet betaine was provided by Goldsmith. The dilauroyl

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fosfatidyl choline (DLPC) and the hexadecyl trimethyl ammonium bromide

(HTAB) were from Sigma. Lauroyl arginine methyl ester (LAM) was synthesized

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Span 20 was provided by ICI. Lecithin was from Lipoid GmbH (Ludwigshafen,

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Germany). Decane was from Merck with a purity > 99% and Squalane puriss p.a.

for G. C. was from Fluka.

The product 1-acetylarginyl-glycerol, henceforth referred to as 00RAc, was

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prepared by enzymatic methods and analysed as described in Morán et al. [13].

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2.2. Synthesis Procedure

A solution of pyridine (80 mL) containing 0.015 mols (5g) of 00RAc was placed

in a round-bottom flask. To this solution, 0.05 mols of lauroyl chloride for

1212RAc or myristoyl chloride for 1414RAc were then added dropwise at room

temperature while stirring continuously for 4 hours. The pH of the reaction

mixture was kept in the range 5 to 7 measuring it with a pH indicator paper from

Merck. Once the reaction was completed, pyridine was removed by a vacuum

pump. The resulting solid was dissolved in methanol and extracted three times

with petroleum ether to remove the extra lauric / myristic acid. The solvent was evaporated and the remaining solid dissolved in chloroform. Water soluble impurities were eliminated by successive extractions of the chloroform solution with water. Next, the product was purified by silica acid flash chromatography using graded chloroform/methanol as eluent. The fractions containing the desired product were pooled and crystallized twice from methanol/acetone. The precipitate was dissolved in water and dried in the freeze dryer yielding a white solid.

2.3. HPLC analysis

High performance liquid chromatography (HPLC) analysis was performed on a Merck-Hitachi D-2500 system which consisted of an injection valve fitted with a 20 µl loop, an intelligent pump L-6200 and a UV-VIS detector at 215 nm wavelengths. A Lichrocart 125-4, Lichrospher 100 RP 18 column was used at room temperature to analyze 00RAC. The flow-rate through the HPLC column was 1.0 ml min⁻¹. Elution was performed in an isocratic system of 0.1 % trifluoroacetic acid (TFA) in water. All samples were prepared by dilution (1:10) with methanol. Quantitative analysis of 00RAC was performed from peak areas by means of the external standard method. The elution retention time was 12 min.

The formation of the diacyl compounds, 1212RAC and 1414RAC, in the reaction mixture as well as the purity of the final products were verified using a Lichrospher 100 CN (propylcyano) 5-µm, 250 x 4 mm column at room temperature. Elution was performed in a gradient system of H₂O-CH₃CN. Eluent

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(9) Morán, C., Infante, M.R., Clapés, P., "Synthesis of glycerol amino acid-based surfactants. Part 1. Enzymatic preparation of rac-1-O-(N^ε-acetyl-L-aminoacyl)glycerol derivatives" J.Chem.Soc., Perkin Trans. 1:2063-2070, 2001.¶

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A was 0.1% (vol/vol) TFA in H₂O, and eluent B was 0.085 % TFA in H₂O/CH₃CN 1:4. The initial composition A/B of the gradient was 75/25 (by volume), changing over 24 min to a final composition of 5/95. The flow-rate through the column was 1.0 ml min⁻¹. The elution retention times for 1212RAC and 1414RAC compounds were 20.6 min and 21.8 min, respectively.

2.4. Characterization of 1212RAC and 1414RAC

Melting points were determined on a hot stage Koffler apparatus and were not corrected. The structures of the pure compounds were verified by ¹H and ¹³C RMN analyses, which were recorded with a Gemini 300 MHz spectrometer. Chemical shifts are reported in parts per million (δ , in ppm) downfield from tetramethylsilane (TMS). Mass spectroscopy spectra with fast atom bombardment (FAB-MS) or electrospray techniques were also conducted with a VG-QUATTRO from Fisons Instruments. Elemental analysis of the final compounds was also performed.

The purity of the products was analyzed with HPLC, RMN, FAB-MA and elemental analysis. All results indicate that the purity is higher than 99.6%.

2.5. Stability

The stability of 1212RAC and 1414RAC surfactants as a function of temperature was evaluated. A 10 mM solutions of the new surfactants were prepared in water. Solutions were allowed to evolve at different temperatures in a

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thermostatic bath. Samples were analysed for 160 hours by HPLC under the conditions described above.

2.6. Physicochemical Characterisation

2.6.1. Conductivity

Samples for conductivity measurements were prepared by weight in Millipore ultrapure water. Conductivity was measured at 25°C using a Crisson 525 with platinized parallel plates with a constant of 0.998 cm⁻¹ and working at 1 kHz. The cell constant was calibrated periodically with standard sodium chloride solutions. To minimize errors from possible electrode contamination, measurements were made at increasing concentrations.

2.6.2. Qualitative phase behavior

Qualitative phase behavior of binary water/1212Rac and water/1414Rac systems as a function of temperature were studied by optical microscopy. Optical observations were performed according to the "flooding" (penetration) method of Lawrence [14]. A polarizing microscope Reichert Polyvar[®] 2 Leica equipped with a hot stage was employed. A videocamera and a PC with Leica IM 500 software were used for the image capture. In a flooding experiment, water was allowed to diffuse into an anhydrous surfactant placed between a slide and a cover slip. After a short time, gradients in composition were produced and different separated mesophases developed around the crystalline surfactant.

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2.6.3. Emulsions

Emulsions were prepared by adding dropwise a volume of oil to a water-surfactant mixture. During the addition, the samples were stirred with a Heidolph Reax Top Vibromixer at full speed (2400 rpm). The emulsions were prepared in 15 mm diameter Pyrex tubes and were examined by optical microscopy. The type of emulsion was determined by measuring the differences in the refractive index between dispersed and continuous media. The general aspect (droplet size) and flocculation state were also noted. Examination of these emulsions under crossed polarized illumination did not show the presence of bi-refrinct phases.

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The stability of 1212RAC and 1414RAC as a function of the pH and the temperature was evaluated

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2.7. Hemolytic Activity

2.7.1. Erythrocytes preparation

Human blood was obtained from the Blood Bank of the Hospital Clínic (Barcelona). Blood was drained into heparinized tubes. Erythrocytes were washed three times in phosphate buffer isotonic saline (PBS), containing 22.2 mmol/L Na₂HPO₄, 5.6 mmol/L KH₂PO₄, 123.3 mmol/L NaCl, Glucose 10.0 mmol/L in distilled water (pH = 7.4). The erythrocytes were then suspended in PBS at a cell density of 8 x 10⁹ cell/mL.

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2.7.2. Hemolysis assay (HC50)

A series of different volumes of surfactant solution in PBS (1 mg/ml), ranging from 10 to 80 µL, were placed in polystyrene tubes and an aliquot of 25 µL of erythrocyte suspension was added to each tube. The tubes were incubated at room temperature while shaking for 10 minutes. Following incubation, the tubes

were centrifuged (5 min at 5000 rpm). The hemolysis degree was determined by comparing the absorbance (540 nm) of the supernatant with that of the control samples totally hemolysed with distilled water.

2.7.3. Protein denaturation.

Aliquots of 100 μ L of test sample were added to tubes containing 875 μ L of PBS. Next, 25 μ L of erythrocyte suspension were added to each tube. The tubes were centrifuged after incubation for 10 minutes. The supernatant absorbance was determined at 575 nm and 540 nm in a dual-beam UV/VIS spectrophotometer [15, 16].

2.8. Antimicrobial activity

Antimicrobial activities were determined “in vitro” on the basis of the minimum inhibitory concentration (MIC) values [17] defined as the lowest concentration of antimicrobial agent which inhibits the development of visible growth after 24 h of incubation at 37°C. The micro-organisms used (15 bacteria and one yeast) were the following: Gram-negative bacteria: *Alcaligenes faecalis* ATCC 8750, *Bordetella bronchiseptica* ATCC 4617, *Citrobacter freundii* ATCC11606, *Enterobacter aerogenes* ATCC 10938, *Salmonella typhimurium* ATCC 14028, *Streptococcus faecalis* ATCC 1054, *Escherichia coli* ATCC 27325, *Klebsiella pneumoniae* ATCC 9721, *Pseudomonas aeruginosa* ATCC 9721, *Arthrobacter oxydans* ATCC 8010. Gram-positive bacteria: *Bacillus cereus* var. *mycoides* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25178, *Staphylococcus epidermidis* ATCC 155-1, *Micrococcus luteus* ATCC 9341. Yeast: *Candida albicans* ATCC 10231.

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The progress control of the reaction showed that 00RAC was first esterified with 1 mol of acyl chloride to give either 120RAC or 140RAC. With a molar ratio acid chloride/00RAC (1:3), the main reaction products were 1212RAC and 1414RAC with conversions higher than 98%.

For the two compounds synthesized, two peaks - a main peak and a minor peak- were observed on the HPLC chromatogram at the end of the reaction. In the case of the 1212RAC compound, the retention times were 20.61 and 21.22 min, respectively, and 21.8 and 22.42 min in the case of the 1414RAC compound. After purification the compounds were quantified and structurally characterised by ¹³CNMR. The minor peak corresponds to the products with the fatty chains in the positions 1 and 3 of the glycerol, 1,3-diacyl-2-acetylarginyl-rac-glycerol (12RAC12 or 14RAC14), and is a 7% of the overall yield. The main peak is the desired product, 1,2-diacyl-3-acetylarginyl-rac-glycerol (1212RAC or 1414RAC), and accounts for 93% of the overall yield.

The overall yield of these reactions after purification was in the range 68 - 70%. The analytical data and elemental analysis for 1212RAC and 1414RAC are summarized in Table 1. Results yielded by FABMS and NMR spectral analyses are given in Table 2. Chemical shifts and integration are referred to 1212RAC.

For chemical assignments see Figure 1. A similar spectrum was obtained for 1414RAC. The mass spectrum of the compounds showed a molecular ion peak corresponding to the [M]⁺ without the counterion chloride. Analytical and

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Isolation and purification of the desired isomer was carried out by two steps: The control of the reaction by HPLC showed that the 3-acetylarginyl-glycerol was first esterified with 1 mol of acyl chloride to give either 1212RAC or 1414RAC-acyl-3-acetylarginyl-rac-glycerols. With a convenient acyl chloride excess, conversions of the starting compound conversions were superior higher than to 98%. was obtained. ¶

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spectral results confirm that 1212RAC and 1414RAC have the desired structure depicted in Figure 1.

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3.2. Stability

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 (Figure 2). At 25°C in aqueous solutions, the rate of decomposition is low (1-3%) after 168 hours. As expected, the hydrolysis rate is 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.

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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 is promoted at this pH.

3.3. Physicochemical characterization

3.3.1. Critical micellar concentration

To investigate the aggregation behaviour, the conductivity of aqueous solutions of 1212RAC and 1414RAC at 25°C was measured for solutions in concentrations ranging from 0.01 mM to 1.5 mM (Figure 3). The conductivities of the aqueous solutions increased linearly with the concentration up to break

points of 0.12 mM for 1212Rac, and 0.09 mM for 1414Rac. The ratio of these cmc values is 1.33. For ammonium bromide surfactants after an increase of four -CH₂ - groups the cmc ratio would be about 17 for single chain surfactants and 10 for double chain surfactants [19]. This anomalous behaviour observed for the 1212Rac and 1414Rac is similar to that encountered for nonacetylated compounds [20]. The cmc ratio for nonacetylated compounds with similar fatty chain (1212R and 1414R) was 1.2 and the cmc ratio was 4 for nonacetylated compounds with shorter fatty acid chain (1010R and 88R). This results suggests that the break points from conductivity for this new family of compounds does not corresponds to a true cmc (monomer to micelle) but to some other transition (i.e. vesicle to ribbon) as found for 1010R from Static Light Scattering results [21].

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The structure of the surfactant molecule affects micellization. The balance between the character of the hydrophilic head group and the hydrophobic tail determines the cmc values. In general, the cmc value in aqueous media decreases as the hydrophobic character of the surfactant increases, and ionic surfactants have much higher cmc than non-ionic or zwitterionic surfactants [19].

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In the case of 1212Rac and 1414Rac they have a cationic polar group, yielding cmc values that are higher than those corresponding to the lecithins with equivalent hydrophobic groups. Comparison of values obtained for the new surfactants with those determined for synthetic short-chain lecithins by surface tension measurements shows that the new surfactants have values that are similar to those of the synthetic short-chain lecithin [22]. Unlike synthetic short-

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chain lecithins, no dependence of the cmc value on the chain length was observed for the 1212Rac and 1414Rac compounds. This reinforces that the break point observed on the conductivity curve is a critical aggregation concentration (instead of a cmc).

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3.3.2. *Liquid crystals and Emulsions*

Conventional surfactants aggregate in solution to form micelles because of the hydrophobic effect. At higher concentrations, the micelles become ordered, forming lyotropic liquid crystals. Liquid crystal formation can stabilize the emulsions. By accumulating at the interface, liquid crystals form a high-viscosity region. This protects the coalescence of individual droplets, acting as a steric barrier and impeding the dispersed particles from coming too close to one another with the result that the van der Waals forces of attraction do not operate [19]. Surfactants will tend to form compact films at interfaces at concentrations that are lower than those forming liquid crystals.

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In this section, the results of liquid crystal and emulsion formation are presented. The phases of the binary 1212Rac / water and 1414Rac / water systems were determined by visual observation of the samples through crossed polarized microscopy. Qualitative phase behaviour studies applying the flooding method revealed the formation of anisotropic phases in all the binary surfactant systems studied. 1212Rac forms lamellar liquid crystals at room temperature (25°C) and this structure is stable until reaching high temperatures. The 1414Rac forms anisotropic phases that developed in lamellar liquid crystals at 45°C (Figure 4a). The different temperature at which 1212Rac and 1414Rac

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form lamellar liquid crystals depends on the relationship between the cross sectional area of the head group and the chains yielding different packing adaptations [23].

Dispersions of 1212RAC and 1414RAC 0.1% in water at 35 °C revealed that their lamellar structures spontaneously form stable multilamellar vesicles of diverse size and number of bilayers. Figure 4b shows vesicles of 60 μm diameter corresponding to the 1212RAC compound. The curvature of the surfactant bilayer, which is *inter alia* determined by packing geometries, limits the smallest possible size of the bilayer. As in the case of diacylphosphatidyl choline, the 1212RAC and 1414RAC are double chained surfactants with a large head group area. These structural characteristics imply that the vesicle size formed is in the order of μm.

One of the widest applications of surfactants is to solubilise or disperse water-insoluble substances (generally organic compounds) in water in the form of emulsions. In a two-phase emulsion, one liquid is dispersed into another in the form of droplets. They are called oil-in-water (O/W) emulsions if the continuous phase is water; the opposite arrangement is called a water-in-oil emulsion (W/O). In order to check for the possibilities of these surfactants as emulsifiers, several emulsions of 1212RAC and 1414RAC were prepared with decane as the oil phase and the type and stability of the emulsion were studied. The results are shown in Table 3.

Both 1212RAC and 1414RAC can simultaneously stabilize water-in-oil (W/O) droplets and oil-in-water droplets (O/W), forming multiple emulsions. We first

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dissolved the surfactants in water and then added the oil, thus favouring the formation of oil-in-water emulsions. Bancroft's rule [24], states that the preferred continuous phase of an emulsion will be the one in which the surfactant is more soluble. A refinement of Bancroft's rule states that the preferred type of emulsion will be that in which surfactant self aggregation takes place. The situation of a single surfactant stabilizing both types of droplets is uncommon. This capability may be related to their ability to form vesicles, i.e., to stabilize planar or quasi-planar interfaces, together with very low solubility in water and in oil. DLPC and lecithin are also vesicle forming surfactants [25]. However, in our experiments, they only stabilized the O/W emulsion in the case of the shorter hydrophobic chain DLPC (sample E4), and the W/O emulsion in the case of the longer hydrophobic chain lecithin (sample E7). The ability of 1212RAC and 1414RAC surfactants to form emulsions is fairly good. The concentrations used in this work were very small, and the emulsions formed were fairly stable with respect to coalescence, which was corroborated by optical microscopy. The addition of one of these surfactants to the anionic (span20, sample E5) O/W forming surfactant also results in some W/O droplets. The ability to stabilize both W/O and O/W droplets could render these surfactants suitable for use as additives for W/O, O/W and for both types of multiple emulsions.

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3. 4. Antimicrobial activity

The dilution antimicrobial susceptibility test was carried out and the minimum inhibitory concentration values were determined. The resulting values are given in

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Table 4. Note that, in contrast to short-chain lecithins, 1212RAC and 1414RAC exhibited antimicrobial activity against some of the microorganisms tested.

As expected, these surfactants show low antimicrobial activity against the Gram negative microorganisms because of the different cell wall composition between these two types of bacteria. The Gram-positive bacteria have relatively simple walls while the structure of a typical Gram-negative cell wall is more complex, with multiple layers resembling geological strata containing phospholipids, protein and lipopolysaccharides forming a barrier to the permeability of charged substances in the medium. On account of this behaviour, and given the presence of ester linkages in the molecules, these compounds would readily be biodegradable.

Owing to the raised aqueous solubility, the 1212RAC has a stronger activity against *B. cereus*, *B. subtilis*, *M. luteus*, *S. typhimudium*, *A. oxidans* and *S. faecalis*, while the 1414RAC has a stronger activity against *S. epidermidis*, *C. albicans*, *Alc. faecalis* and *Kl. pneumoniae*. Given that the natural habitat of microorganisms is the aqueous phase, there must be an effective concentration in this phase for there to be antimicrobial activity. Thus, solubility plays an important role in antimicrobial properties. It should also be noted that these surfactants are only active against the microorganisms that need concentrations lower than 64 µg/mL to produce the inhibitory effect of the growth. Given that the cmc values of these two surfactants are 72.2 µg / mL and 62,3 µg / mL, the highest monomer concentrations of surfactant in the solution are 72.2 µg / mL and 62,3 µg / mL, respectively. It may thus be concluded that the surfactant monomers are the species that interact with the cells and not the aggregates.

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The ratio between the HC50 and the DI is called the Lysis / Denaturation ratio, L/D, whose value indicates the potential irritation power of a surfactant. The surfactants were classified according to the following criteria: L/D < 0.1 very irritant, L/D from 0.1 to 1 irritant, L/D from 1 to 10 moderately irritant, L/D from 10 to 100 slightly irritant and L/D > 100 non irritant.

Hemolytic activity tests were performed on human red blood cell suspensions.

The results are given in Table 5. The amphiphilic character of the surfactants allows them to interact with the cellular membranes that consist of lamellar structures formed by lipid compounds. In contrast to the very irritant character of other commercially available surfactants, for example, SDS, benzalkonium chloride, or cetyltrimethylammonium chloride [29], which can produce lysis at concentrations as low as 0.5 µg/mL, the 1212Rac can be classified as moderately irritant, with an L/D value higher than that of Tego®-bet, which is considered to be a soft amphoteric surfactant [29]. The irritant character of this surfactant is also similar to that obtained for the lauroyl arginine ethyl ester [24], a cationic surfactant based on arginine, which has already been commercialized as a food additive. However the potential irritation values are higher than those shown by lecithin mimics derived from lysine with non-ionic character [30]. These results indicate that the irritant character is related to the cationic charge in these compounds and not to the molecular structure.

Considering the L/D value obtained for the 1414Rac, this compound can be classified as a non irritant. Nevertheless, given that this surfactant has a chemical structure and physicochemical properties that are similar to those of

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1212Rac, we believe that this value is due to a solubility cut off around this chain length in the test medium. Thus, it is possible that the effective concentration of this surfactant in the test medium was lower than the nominal concentration, resulting in this anomalous L/D value.

4. CONCLUSIONS

Novel multifunctional soft biocompatible cationic surfactants from diacyl glycerides and the amino acid arginine were synthesized. The physicochemical study shows that these surfactants have a self-aggregation behavior similar to that of lecithins. Moreover, these compounds show antimicrobial activity. 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.

ACKNOWLEDGEMENTS

We are grateful to N. Pages for her assistance in the emulsion preparation. This work was partially supported by the CICYT under Grant PPQ2000-1687-CO2-01.

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(6) L.Pérez, A.Pinazo, P.Vinardell, P.Clapés, M.Angelet, M.R.Infante "Synthesis and biological properties of dicationic arginine-diglycerides". *New Journal of Chemistry*, ... [208]

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[30] M.Macián, J.Seguir, M.R.Infante, M.P.Vinardell, Rev.Toxicol. 11 (1994)

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Table 1: Analytical data for 1212RAC and 1414RAC compounds

Compound	Molecular Formula	Melting point	Yield (%)	HPLC retention time	Elemental analysis			
	Molecular Weight (g/mol)	(°C)		(min)	Calculated (with 1 mol H ₂ O) / found			
					%C	%H	%N	%Cl
1212RAC	C ₃₅ H ₆₇ N ₄ O ₇ Cl	100 - 104	68	20.6	59.24 / 59.38	9.73 / 9.70	7.90 / 7.83	5.01 / 5.28
	690.5							
1414RAC	C ₃₉ H ₇₅ N ₄ O ₇ Cl	85 - 88	70	21.8	61.21 / 61.44	10.07 / 10.46	7.32 / 7.31	4.64 / 4.61
	746.5							

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Table 2: Spectral Assignments for 1212RAc and 1414RAc compounds

Compound	m/e (M ⁺)	¹ H NMR (DMSO), δ ppm	¹³ C NMR (DMSO), δ ppm
1212RAc	655.60	0.83 [tr, 6H (21,22)]; 1.1-1.7 [m, (9,19,20)]; 1.84 [s, 3H (8)]; 2.26 [tr, 4H (17,18)]; 3.09 [m, 2H (10)]; 4.01-4.3 [m, 5H (1,3,5)]; 5.19 [m, 1H (2)]; 7.17 [m, 4H, 13, 14); 7.86 [tr, 1H (11)]; 8.31 [d, 1H (6)].	13.71 (12,22); 21.97 (8); 51.59 (5); 61.69 (1); 62.22 (3); 68.52 (2); 157.02 (12); 169.42 (7); 171.48 (4); 172.02 (15); 172.31 (16).
1414RAc	712.15		

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Table 3. Composition of the tested emulsions and morphological characteristics

<u>Sample code</u>	<u>Water content¹ (weight fraction)</u>	<u>Oil content¹ (weight fraction)</u>	<u>Surfactant content¹ (weight fraction)</u>	<u>Dominant droplet size (μm)</u>	<u>Emulsion type</u>
<u>E1</u>	<u>0.5</u>	<u>0.5 decane</u>	<u>0.00223, 1212RAc</u>	<u>5 / 50² aggregates</u>	<u>W/O/W</u>
<u>E2</u>	<u>0.5</u>	<u>0.5 decane</u>	<u>0.00223, 1414RAc</u>	<u>5 / 50²</u>	<u>W/O/W</u>
<u>E3</u>	<u>0.09</u>	<u>0.91 decane</u>	<u>0.00022, 1212RAc</u>	<u>5 / 50³</u>	<u>O/W/O</u>
<u>E4</u>	<u>0.5</u>	<u>0.5 decane</u>	<u>0.0023, DLPC</u>	<u>20</u>	<u>O/W</u>
<u>E5</u>	<u>0.77</u>	<u>0.23 squalane</u>	<u>0.02 Span20+ 0.00045 RAc 1212</u>	<u>10 / 10</u>	<u>O/W and W/O</u>
<u>E6</u>	<u>0.77</u>	<u>0.23 squalane</u>	<u>0.01, Span 20</u>	<u>10</u>	<u>O/W</u>
<u>E7</u>	<u>0.5</u>	<u>0.5 decane</u>	<u>0.025, Lecithin</u>	<u>10 aggregates</u>	<u>W/O</u>

¹ The rounded weight fraction sum more than unity

² The first figure corresponds to water droplets within oil which droplet size corresponds to the second figure.

³ The same as note 2 with oil and water roles reversed.

Table 4. Minimum Inhibitory Concentration (µg/mL)

	Microorganism	1212Rac	1414Rac
Gram-positives	<i>Bacillus cereus var. mycoide</i> ATCC 11778	4	>256
	<i>Bacillus subtilis</i> ATCC 6633	32	-
	<i>Staphylococcus aureus</i> ATCC 6538	32	32
	<i>Staphylococcus epidermidis</i> ATCC 12228	>256	8
	<i>Micrococcus luteus</i> ATCC 9341	2	4
	<i>Candida albicans</i> ATCC 10231	>256	8
Gram-negatives	<i>Salmonella typhimurium</i> ATCC 14028	32	>256
	<i>Pseudomonas aeruginosa</i> ATCC 9027	>256	>256
	<i>Escherichia coli</i> ATCC 8793	>256	>256
	<i>Arthrobacter oxidans</i> ATCC 8010	16	>256
	<i>Streptococcus faecalis</i> ATCC 19434	1	8
	<i>Bodetella bronchiseptica</i> ATCC 4617	>256	>256
	<i>Citrobacter freundii</i> ATCC 22636	>256	>256
	<i>Alcaligenes faecalis</i> ATCC 8750	16	2
	<i>Enterobacter aerogenes</i> CECT 689	32	32
	<i>Klebsiella pneumoniae v. pneumoniae</i> CIP 104216	8	1

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Table 5. Hemolytic activity

	<u>1212 RAc</u>	<u>1414 RAc</u>	<u>HTAB</u>	<u>Tego®-Bet</u>	<u>LAM</u>
HC50* (µg/mL)	<u>75.1</u>	<u>44.7</u>	<u>11.6</u>	<u>14.4</u>	<u>14.7</u>
DI** (%)	<u>10.7</u>	<u>4.5</u>	<u>46.5</u>	<u>34.4</u>	<u>38.4</u>
L/D***	<u>6.4</u>	<u>9.9</u>	<u>0.2</u>	<u>2.4</u>	<u>2.6</u>
<u>Irritability</u>	<u>Moderate</u>	<u>Moderate</u>	<u>High</u>	<u>Moderate</u>	<u>Moderate</u>

* Hemolytic concentration, ** Denaturation index, *** Lysis Denaturation ratio

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Figure captions

Figure 1. Molecular structure of 1, 2 dilauroil-3-acetylarginyl-rac-glycerol (1212RAc) and 1, 2 dimirystoil-3-acetylarginyl-rac-glycerol (1414RAc).

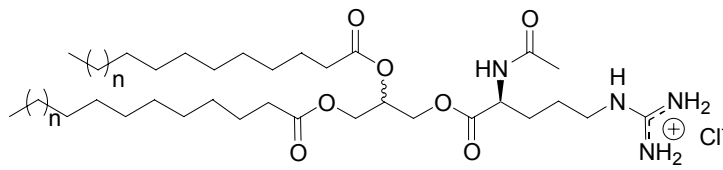
Figure 2. Influence of temperature on % of hydrolysis as a function of time for aqueous solutions of 1212RAc and 1414RAc compounds. (■) 1212RAc 25°C, (▲)1212RAc 40°C, (▼)1414RAc 25°C, (◆)1414RAc 40°C.

Figure 3. Conductivities of aqueous solutions of 1212RAc (●) and 1414RAc (■) solutions at 25°C

Figure 4. a) polarized microscopy of lamellar liquid crystals for the 1414RAc/H₂O system. b) Polarized microscopy of vesicles for the 1212RAc/H₂O system

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Figure 1



(n=2) **1212RAc**
(n=4) **1414RAc**

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Figure 2

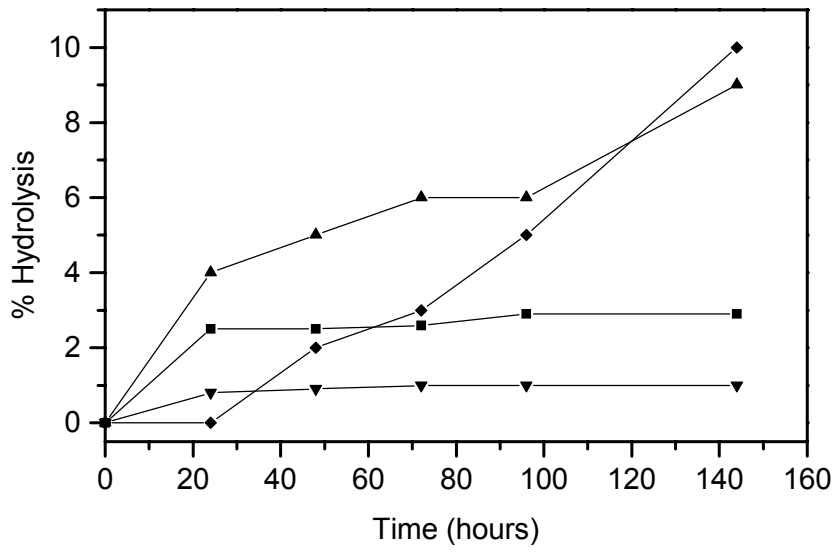
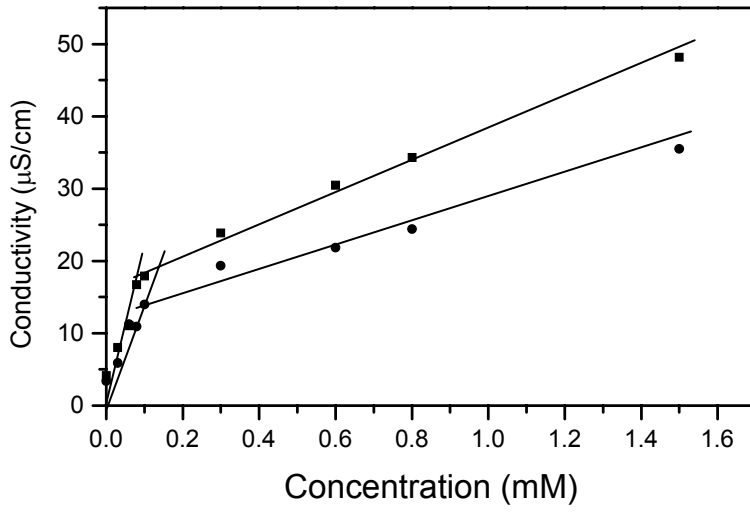


Figure 3



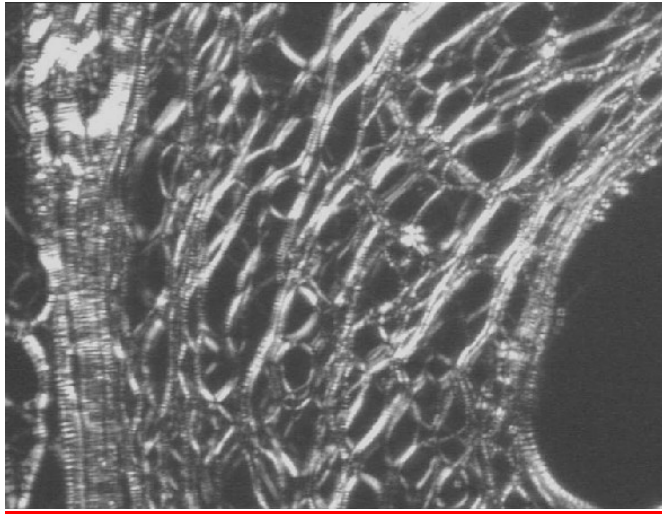
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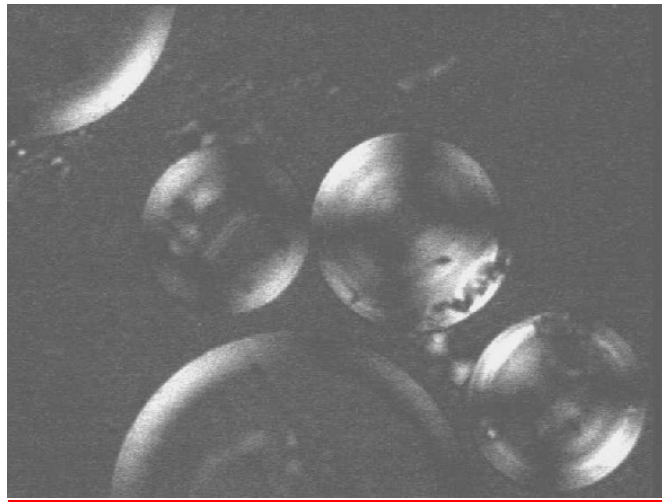
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<#>Farrell, P.M. 1982 Lung Development: Biological and Clinical perspectives (ed.). Vol. II, Academic Press, New York.; Robertson, B., Van Golde, L.M.G. and Batenburg, J.J. (1984) Pulmonary Surfactant (eds.) Elsevier. Amsterdam.; Shapiro, D.L. and Notter, R.H. (1989) Surfactant replacement therapy (eds.) A.R. Liss. Inc., New York.¶

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(7) Notter, R.H. (1989) The physical chemistry and physiological activity of pulmonary surfactant Replacement Therapy (Shapiro, D.L. and Notter, R.H., eds.), pp 19-70, A.R. Liss, New York.).¶

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(12) A. Pinazo, M.R. Infante, S.Y. Park, E.I. Franses. Colloids and Surfaces B: Biointerfaces 8 (1996) 1-11. ¶

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Alain Guyot, Polymerizable Surfactants pg 279 in Novel Surfactants, Surfactant Science Series, vol 74, edited by Krister Holmberg, Marcel Dekker, Inc., New York.

Krister Holmberg, Cleavable Surfactants, pg 333 in Novel Surfactants, Surfactant Science Series, vol 74, edited by Krister Holmberg, Marcel Dekker, Inc., New York.

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(6) L.Pérez, A.Pinazo, P.Vinardell, P.Clapés, M.Angelet, M.R.Infante “*Synthesis and biological properties of dicationic arginine-diglycerides*”. *New Journal of Chemistry*, 26: 1221-1227, 2002.

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(7) [3] Niels J. Krog “*Food Emulsifiers and Their Chemical and Physical Properties*” in *Food Emulsions* pp:141-188, Ed. Stig E. Friberg, Kare Larsson, Marcel Dekker, Inc. New York, 1997.

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With this aim, our group has synthesised and studied a number of different arginine based surfactants including monomeric [5], Gemini [6,7] and diglyceride bicationic structures [8]. In all cases they have proved to have high biodegradability, low toxicity and antimicrobial activity.

Environmentally friendly surfactants that mimic natural amphiphatic structures such as lipoaminoacids, glycolipids and glycerolipids have become increasingly important specially in fields like food and cosmetic applications[1,2]. (Ref. Novel Surfactants: Preparation, Applications and Biodegradability, K. Holmberg, Marcel Dekker, 1998). Specifically, mixtures of mono- and diglycerides are widely used as emulsifiers in fat-based products such as margarine, spread and bakery fats [3] (CAL UNA REFERENCIAreferencia). One of the main drawbacks is their low water solubility. To overcome this problem, in some applications, they are used in combination with other more hydrophilic emulsifiers.

Lecithin (1,2-diacyl-3-phosphorylcholine) and Lysolecithin ((1(2)-acyl-3-phosphorylcholine) are also well recognised food additives. They are natural amphiphatic compounds which exhibit a number of desirable properties like low toxicity, high biodegradability and compatibility with many pharmacy products

[4]. However their water solubility is small and they are very costly products (i.e. in rape and soybean oils about 0.3 - 2.5% phospholipids are only extracted from the oil fraction, on dry basis). Cosmetics and medicine industries use lecithines as surfactants.

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They are natural amphiphatic compounds which exhibit a number of desirable properties like low toxicity, high biodegradability and compatibility with many pharmacy products []

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. Unfortunately their water solubility

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is small and they are expensive.

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great accomplishment

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With this aim, our group has synthesised and studied a number of different arginine based surfactants including monomeric

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Página 7: [24] Eliminado Man209 23/01/2003 12:22:00

analysis of the final compounds were

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Synthesis Procedure

A solution of pyridine (80 mL) containing 0.015 mol

Página 7: [26] Eliminado Man209 31/10/2003 13:37:00

s (5g) of OORAc was placed in a round-bottom flask. To this solution, 0.05 mol of acyl chloride,

Página 7: [27] Eliminado Man209 31/10/2003 13:37:00

I chloride for 1212RAc and myristoy

Página 7: [28] Eliminado Man209 31/10/2003 13:37:00

I chloride for 1414RAc,

Página 7: [29] Eliminado Man209 31/10/2003 13:37:00

was then added drop wise at room temperature while stirring continuously

Página 7: [30] Eliminado Man209 28/02/2003 16:04:00

through 4 hours. Once the reaction was completed, pyridine was removed by a vacuum pump. The resulting solid was dissolved in methanol and extracted three times with petroleum ether to remove the extra lauri

Página 7: [31] Eliminado Man209 31/10/2003 13:37:00

. The solvent was evaporated and the remaining solid dis

Página 7: [32] Eliminado Man209 31/10/2003 13:37:00

solved in chloroform. Water soluble impurities were eliminated by

Página 7: [33] Eliminado Man209 31/10/2003 13:37:00

W

Página 7: [34] Eliminado Man209 23/01/2003 12:36:00

washing the solution with water. After that, the product was purified by silica acid flash chromatography using graded chloroform/methanol as eluent.

Página 7: [35] Eliminado Man209 31/10/2003 13:37:00

Página 7: [36] Eliminado Man209 31/10/2003 13:37:00

The fractions containing the desired product were pooled and crystallized twice from methanol/acetone. The precipitated was dissolved in water and dried in the freeze dryer yielding a white solid with a purity of 99.6 or higher.

Página 10: [37] Eliminado	aurora	23/08/2002 18:18:00
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L/D ratio: The relationship between hemolysis (HC50) and the denaturation index (DI) is defined as the lysis/denaturation quotient or L/D ratio used to predict the potential ocular irritation of the surfactant^{11,12}. The surfactants were classified in accordance with the following criteria: L/D < 0.1 very irritant, L/D from 0.1 to 1 irritant, L/D from 1 to 10 moderately irritant, L/D from 10 to 100 slightly irritant and L/D > 100 non irritant.

Página 10: [38] Con formato	Man209	31/10/2003 14:18:00
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Sin Superíndice / Subíndice

Página 10: [39] Con formato	Man209	18/03/2003 11:54:00
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Página 10: [40] Eliminado	.	10/12/2002 12:02:00
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Jones RN, Barry AL, Gavan, TL, Washington JA. 1980. *II Manual of Clinical Microbiology*. Lennette EH, Ballows A, Hauser WJ, Shadomy HJ, eds. American Society for Microbiology, Washington, USA.],

Página 10: [41] Eliminado	RMN	04/03/2003 12:32:00
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(11) Jones RN, Barry AL, Gavan, TL, Washington JA. 1980. *II Manual of Clinical Microbiology*. Lennette EH, Ballows A, Hauser WJ, Shadomy HJ, eds. American Society for Microbiology, Washington, USA, 198

Página 10: [42] Con formato	Man209	31/10/2003 14:20:00
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Color de fuente: Automático

Página 11: [43] Eliminado . 17/12/2002 15:50:00

Stability

The stability of

Página 11: [44] Eliminado . 17/12/2002 15:50:00

as a function of the pH and the temperature was evaluated by HPLC

analysis. The surfactants

Página 11: [45] Eliminado aurora 22/08/2002 21:16:00

1212Rac or 1414Rac

Página 11: [46] Eliminado . 17/12/2002 15:50:00

were dissolved in water, ethanol or DMF at the appropriate pH and

temperature. HPLC analysi

Página 11: [47] Eliminado . 17/12/2002 15:50:00

s of these solutions were carried out every 24 hours, monitoring the

decrease of the peak corresponding to the surfactant under study.

Página 11: [48] Eliminado aurora 23/08/2002 13:49:00

Página 11: [49] Eliminado Man209 19/02/2003 11:09:00

Página 11: [50] Eliminado . 17/12/2002 15:49:00

Physicochemical Characterisation

Conductivity.

Página 11: [51] Eliminado . 17/12/2002 15:49:00

Samples for conductivity measurements were prepared by weight in Millipore ultrapure water. The dispersions (0.1% w/v in H₂O) were prepared with the flowing protocol. First the dispersion was hand shaken for about 1min. Then it was sonicated for 15 min in a sonicator bath (Branson Cleaning Equipment Co., Shelton, Connecticut). Dispersed particles were removed from the samples by filtering them two times with 0.2 μm Nucleopore membranes.

Página 11: [52] Eliminado . 17/12/2002 15:49:00

Conductivity was measured using a Crisson 525 with platinized

Página 11: [53] Eliminado . 17/12/2002 15:49:00

parallel plates with a constant of 0.998 cm⁻¹ and working at 1 KHz. The cell constant was calibrated periodically with sodium chloride solutions.

To minimize errors from possible electrode contamination, m

Página 11: [54] Eliminado . 17/12/2002 15:49:00

measurements were made at increasing concentrations.

Página 11: [55] Eliminado aurora 22/08/2002 21:19:00

to minimize errors from possible contamination from electrode.

Página 11: [56] Eliminado . 17/12/2002 15:49:00

Q

Página 11: [57] Eliminado . 17/12/2002 15:49:00

qualitative phase behavior.

Página 11: [58] Eliminado . 17/12/2002 15:49:00

Qualitative phase behaviors of binary water/1212RA

Página 11: [59] Eliminado . 17/12/2002 15:49:00

c and water/1414RAc

Página 11: [60] Eliminado . 17/12/2002 15:49:00

systems as a function of temperature were

Página 11: [61] Eliminado . 17/12/2002 15:49:00

studied by optical microscopy. Optical observations

Página 11: [62] Eliminado . 17/12/2002 15:49:00

were performed according to the "flooding" (

Página 11: [63] Eliminado . 17/12/2002 15:49:00

method of Lawrence [8]. A polarizing microscope equipped with a hot

stage was used. In a flooding experiment, water was allowed to diffuse an

anhydrous surfactant placed between a slide and a cover slip. After a

short time, gradients in composition were produced

Página 11: [64] Eliminado . 17/12/2002 15:49:00

different separated mesophases developed

Página 11: [65] Eliminado . 17/12/2002 15:49:00

around crystalline surfactant.

Página 11: [66] Eliminado aurora 23/08/2002 13:52:00

Sample preparation

Samples for conductivity measurements were prepared by weight in Millipore ultrapure water.

The dispersions (0.1% w/v in H₂O) were prepared with the flowing protocol. First the dispersion was shaken by hand for aprox. 1min then sonicated for 15 min in a sonicator bath (Branson Cleaning Equipment Co., Shelton, Connecticut). Dispersed particles from samples were removed filtering the samples two times using 0.2 μm Nucleopore membranes.

Página 11: [67] Eliminado . 17/12/2002 15:49:00

Emulsions were prepared by adding drop wise a volume of oil

Página 11: [68] Eliminado . 17/12/2002 15:49:00

to a water-surfactant mixture. During the

Página 11: [69] Eliminado . 17/12/2002 15:49:00

addition, the samples were stirred with

Página 11: [70] Eliminado . 17/12/2002 15:49:00

a Heidolph Reax Top Vibromixer at full speed (2400 r

Página 11: [71] Eliminado . 17/12/2002 15:49:00

). The emulsions were prepared in 15mm diameter pyrex tubes and

Página 11: [72] Eliminado . 17/12/2002 15:49:00

were examined by optical microscopy. The type of emulsion was determined measuring

Página 12: [73] Eliminado Man209 27/01/2003 12:02:00

Isolation and purification of the desired isomer was carried out by two steps: The control of the reaction by HPLC showed that the 3-acetylarginyl-glycerol was first esterified with 1 mol of acyl chloride to give either 1212RAc or 1414RAc1-acyl-3-acetylarginyl-rac-glycerols. With a convenient acyl chloride excess, conversions of the starting compound conversions were superior higher than to 98%. was obtained.

Injections of each compound in the HPLC showed two peaks: The main peak with a retention time of 20.61 min, and a small peakh (pick area equal to 7% of the main pick area) with a at there was a little peak (retention time of 21.22

min., 7%) together to the main peak (retention time 20.61 min). Isolation of the main peak was isolated in two steps. First we applied chromatography possible by chromatography with a silica gel column chromatography (Chromagel 60 A CC, 70-230 mesh) using as eluent a gradient from chloroform to chloroform/methanol. Then and finally, a crystallization of the compounds were crystallized in methanol/acetone. RMN studies showed that the small minor peak corresponded to the positional isomer 1,3-diacyl-2-acetylglyceryl-glycerol.

Página 13: [74] Con formato	Man209	18/03/2003 11:55:00
Inglés (Reino Unido)		
Página 13: [75] Con formato	Man209	18/03/2003 11:55:00
Inglés (Reino Unido)		
Página 13: [76] Con formato	Man209	18/03/2003 11:55:00
Inglés (Reino Unido)		
Página 13: [77] Con formato	Man209	18/03/2003 11:55:00
Inglés (Reino Unido)		
Página 13: [78] Con formato	Man209	18/03/2003 11:55:00
Color de fuente: Automático, Inglés (Reino Unido)		
Página 13: [79] Con formato	Man209	18/03/2003 11:55:00
Inglés (Reino Unido)		
Página 13: [80] Con formato	Man209	18/03/2003 11:55:00
Inglés (Reino Unido)		
Página 13: [81] Eliminado	aurora	23/08/2002 19:59:00
compounds (Table II).		

Página 13: [82] Eliminado	Man209	04/11/2003 15:49:00
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Página 13: [83] Con formato	Man209	18/03/2003 11:55:00
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Página 13: [84] Eliminado	RMN	04/03/2003 12:44:00
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(Ref: Laughlin, R.G., *The Aqueous Phase Behaviour of Surfactants*, Academic Press, New York (1972)).

Página 13: [85] Eliminado	Man209	04/11/2003 15:50:00
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Surfactants molecules are amphiphilic, normally having a polar hydrophilic head group attached to a nonpolar hydrophobic portion. This leads to a diverse solution and phase behavior, including the formation of aggregates and other microstructures with water (Ref: Laughlin, R.G., The Aqueous Phase Behaviour of Surfactants, Academic Press, New York (1972)). **The mass spectrum of the compounds showed a molecular ion peak corresponding to the [M]⁺ without the counterion Chloride.**

Página 13: [86] Con formato	Man209	18/03/2003 11:55:00
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Página 13: [87] Con formato	Man209	04/11/2003 16:38:00
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Página 15: [88] Eliminado	RMN	04/03/2003 10:01:00
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instead of a cmc,

Página 15: [88] Eliminado	RMN	04/03/2003 10:02:00
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Página 15: [88] Eliminado	RMN	04/03/2003 10:02:00
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This needs

Página 15: [89] Eliminado	RMN	04/03/2003 10:03:00
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due to

Página 15: [89] Eliminado	RMN	04/03/2003 10:03:00
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to form micelles

Página 15: [90] Eliminado	RMN	04/03/2003 10:06:00
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surrounding the dispersed particles

Página 15: [90] Eliminado	RMN	04/03/2003 10:07:00
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the particles with

Página 15: [91] Con formato	Man209	18/03/2003 11:56:00
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Inglés (Reino Unido)

Página 15: [91] Con formato	Man209	05/11/2003 12:50:00
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Inglés (Reino Unido)

Página 15: [91] Con formato	Man209	18/03/2003 11:56:00
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Página 15: [91] Con formato	Man209	18/03/2003 11:56:00
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Inglés (Reino Unido)

Página 15: [92] Con formato	Man209	18/03/2003 11:56:00
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Color de fuente: Automático, Inglés (Reino Unido)

Página 15: [92] Con formato	Man209	18/03/2003 11:56:00
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Inglés (Reino Unido)

Página 15: [93] Con formato	Man209	18/03/2003 11:56:00
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Página 15: [93] Con formato	Man209	18/03/2003 11:56:00
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Inglés (Reino Unido)

Página 15: [94] Eliminado	Man209	10/03/2003 12:50:00
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that form

Página 15: [94] Eliminado	Man209	10/03/2003 12:50:00
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Página 15: [95] Eliminado	RMN	04/03/2003 10:12:00
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that

Página 15: [95] Eliminado	RMN	04/03/2003 10:12:00
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formation those surfactants will tent to form compact films at interfaces

Página 15: [96] Eliminado	RMN	04/03/2003 16:27:00
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Página 15: [96] Eliminado	RMN	04/03/2003 10:13:00
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have

Página 15: [96] Eliminado	RMN	04/03/2003 10:13:00
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been

Página 15: [97] Con formato	Man209	18/03/2003 11:56:00
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Inglés (Reino Unido)

Página 15: [98] Eliminado	Man209	06/02/2003 16:18:00
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also

Página 15: [98] Eliminado	Man209	06/02/2003 16:20:00
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at room temperature but

Página 15: [98] Eliminado	Man209	06/02/2003 16:36:00
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in this case it is difficult to identify the structure type. However,

Página 15: [99] Con formato	Man209	18/03/2003 11:56:00
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Página 15: [100] Eliminado	Man209	06/02/2003 16:36:00
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warming the sample up to ---°C lamellar liquid crystals become apparent

Página 15: [100] Eliminado	Man209	17/03/2003 12:18:00
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Página 15: [100] Eliminado	Man209	12/03/2003 16:16:00
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Página 15: [101] Con formato	Man209	18/03/2003 11:56:00
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Página 15: [101] Con formato	Man209	18/03/2003 11:56:00
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Inglés (Reino Unido)

Página 17: [102] Eliminado	Man209	10/02/2003 12:47:00
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The formation of lamellar phases has been also described for other surfactants from arginine, but they need higher temperatures to form this type of structures. The main difference of these compounds with the other arginine surfactants is that they can form easily vesicles due that their chemical structure is similar to those of lecithins.

Stability

In order to Know the application conditions of these compounds we have study the stability of them as a function of the temperature and solvents. At 25°C in aqueous solutions, the compounds decompose in very low percentage (2-4%). As expected the hydrolysis percentage is higher when

the temperature increases reaching a 50% in the case of the 1414RAC at 50°C. On the other hand, when we add to the solution a low volume of organic solvent (ethanol o DMF), the hydrolysis is also higher due to the better solubility of the surfactants in these mediums. The decompositions of these compounds is due to the presence in the molecules of a weak acid group, the protonated guanidine group. Due that, the pH of the aqueous solutions of these surfactants is slowly acid (pH=4) , at these pH values some of the ester linkages of the molecules can be hydrolyzed.

Página 18: [103] Eliminado	aurora	23/08/2002 20:23:00
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I

Página 18: [103] Eliminado	aurora	23/08/2002 11:50:00
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XX

Página 18: [103] Eliminado	aurora	23/08/2002 11:54:00
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compounds

Página 18: [103] Eliminado	aurora	23/08/2002 11:54:00
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exhibited

Página 18: [104] Eliminado	.	10/12/2002 12:17:00
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all

Página 18: [104] Eliminado	.	10/12/2002 12:20:00
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e

Página 18: [105] Eliminado	Man209	19/02/2003 11:53:00
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Página 18: [105] Eliminado	Man209	19/02/2003 11:53:00
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n

Página 18: [106] Eliminado	Man209	17/02/2003 16:14:00
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V

Página 18: [106] Eliminado Man209 14/03/2003 10:37:00

S

Página 18: [106] Eliminado Man209 17/02/2003 16:13:00

Página 18: [107] Eliminado Man209 10/03/2003 12:48:00

T

Página 18: [107] Eliminado Man209 14/03/2003 10:45:00

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Página 18: [107] Eliminado Man209 14/03/2003 10:39:00

together with

Página 18: [107] Eliminado Man209 14/03/2003 10:40:00

can make

Página 18: [107] Eliminado Man209 17/02/2003 16:13:00

i

Página 18: [108] Eliminado Man209 14/03/2003 10:44:00

suitable for the subsequent biodegradability process of these surfactants

Página 18: [108] Eliminado Man209 18/03/2003 11:57:00

Página 18: [109] Eliminado RMN 04/03/2003 10:41:00

Due to

Página 18: [109] Eliminado RMN 04/03/2003 10:41:00

higher

Página 18: [110] Eliminado . 11/12/2002 9:59:00

X depicts the ratio of microorganisms inhibited by each surfactant.

Página 18: [111] Eliminado aurora 23/08/2002 11:55:00

shows the percentage of microorganismes inhibited fro these two surfactants.

Página 18: [112] Eliminado aurora 23/08/2002 11:51:00

the

Página 18: [112] Eliminado	aurora	23/08/2002 11:51:00
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a

Página 18: [112] Eliminado	aurora	23/08/2002 11:51:00
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compound

Página 18: [113] Eliminado	aurora	23/08/2002 11:51:00
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possess antimicrobial activity

Página 18: [113] Eliminado	aurora	23/08/2002 11:51:00
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the

Página 18: [114] Eliminado	Man209	10/03/2003 13:58:00
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i

Página 18: [114] Eliminado	Man209	14/03/2003 10:49:00
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to achieve a

Página 18: [115] Eliminado	RMN	04/03/2003 10:45:00
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are generally present in the aqueous phase, therefore there must be an effective concentration in the aqueous phase to achieve rapid kill

Página 18: [116] Eliminado	Lourdes1	20/12/2002 9:11:00
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sufficiently soluble in water to achieve adequate concentrations in the aqueous phase of the system

Página 18: [117] Eliminado	RMN	04/03/2003 10:45:00
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, because of that, the solubility play a important role in the antimicrobial properties.

Página 18: [117] Eliminado	RMN	04/03/2003 10:47:00
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is also noticeable

Página 18: [118] Eliminado	RMN	04/03/2003 10:47:00
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Taking into account

Página 18: [118] Eliminado	RMN	04/03/2003 10:47:00
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that

Página 18: [118] Eliminado	RMN	04/03/2003 10:48:00
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Página 18: [119] Eliminado	Man209	14/03/2003 10:50:00
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S

Página 18: [119] Eliminado	Man209	14/03/2003 10:50:00
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is

Página 18: [120] Eliminado	RMN	04/03/2003 10:48:00
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for the 1212RAc and

Página 18: [121] Eliminado	Man209	10/03/2003 14:00:00
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Página 18: [121] Eliminado	Man209	10/03/2003 14:00:00
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Página 19: [122] Con formato	Man209	18/03/2003 11:58:00
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Sin Expandido / Comprimido

Página 19: [123] Con formato	Man209	05/11/2003 14:02:00
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Sin Expandido / Comprimido

Página 19: [123] Con formato	Man209	05/11/2003 14:02:00
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Página 19: [123] Con formato	Man209	05/11/2003 14:02:00
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Sin Expandido / Comprimido

Página 19: [123] Con formato	Man209	05/11/2003 14:02:00
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Sin Superíndice / Subíndice , Sin Expandido / Comprimido

Página 19: [124] Con formato	Man209	18/03/2003 11:58:00
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Color de fuente: Automático, Sin Expandido / Comprimido

Página 19: [125] Con formato	Man209	18/03/2003 11:58:00
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Sin Expandido / Comprimido

Página 19: [126] Eliminado	RMN	04/03/2003 10:52:00
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For the same alkyl chain lengths,

Página 19: [127] Eliminado	Man209	14/03/2003 10:51:00
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C

Página 19: [127] Eliminado	Man209	19/02/2003 11:54:00
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T

Página 19: [128] Eliminado	RMN	04/03/2003 10:53:00
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are also similar to

Página 19: [128] Eliminado	RMN	04/03/2003 10:53:00
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that

Página 19: [128] Eliminado	RMN	04/03/2003 10:54:00
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Página 19: [129] Con formato	Man209	05/11/2003 14:05:00
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Sin Expandido / Comprimido

Página 19: [130] Eliminado	RMN	04/03/2003 10:55:00
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so that they possess two positive charges in the polar head

Página 19: [131] Con formato	Man209	05/11/2003 14:05:00
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Página 19: [132] Eliminado	Man209	12/03/2003 16:20:00
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Página 19: [132] Eliminado	Man209	12/03/2003 16:37:00
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Página 19: [133] Con formato	Man209	18/03/2003 11:58:00
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Color de fuente: Automático, Sin Expandido / Comprimido

Página 19: [134] Con formato	Man209	18/03/2003 11:58:00
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Sin Expandido / Comprimido

Página 19: [135] Con formato	Man209	18/03/2003 11:58:00
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Color de fuente: Automático

Página 19: [136] Eliminado	RMN	04/03/2003 10:58:00
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From these results we can conclude

Página 19: [137] Con formato	Man209	18/03/2003 11:58:00
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Sin Expandido / Comprimido

Página 19: [138] Eliminado	RMN	04/03/2003 10:57:00
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n't

Página 19: [138] Eliminado	RMN	04/03/2003 10:57:00
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of them

Página 19: [139] Eliminado	Lourdes1	19/12/2002 17:00:00
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It is well know that the antimicrobial activity of homologues surfactants with different alkyl chain length reaches a maximum for a given chain length (CAL UNA REFERENCIA) [12]. This behaviour can be attributed to the combination of several physicochemical properties like hydrophobicity, CMC, water solubility, etc. In our case, as expected, tlt is well Know that the antimicrobial activity of

homologues surfactants with different alkyl chain length reaches a maximum for a determined chain length. This can be attributed to the combination of several physicochemical parameters: hydrophobicity, CMC, aqueous solubility, etc

hee antimicrobial activities of 1212RAC and 1414RAC are smaller than that of their dicationic homologues with 8 and 10 carbon atoms in the alkyl chain, but similar to those reported for diglyceride surfactants from arginine with two ionic charges in the polar group and the same alkyl chain [8]. (CAL UNA REFERENCIA).

Página 19: [140] Eliminado aurora 23/08/2002 20:33:00

The antimicrobial activity of these compounds are similar to those reported for diglyceride surfactants from arginine with two ionic charges in the polar group (XXR).

Página 19: [141] Eliminado aurora 23/08/2002 20:37:00

Nevertheless, monoalkyl chain surfactants from arginine with two positive ionic groups in the polar head possess higher antimicrobial activity than their homologues with only one ionic group.

Página 19: [142] Eliminado . 09/12/2002 8:57:00

Página 19: [143] Eliminado aurora 23/08/2002 20:20:00

The optimum biological activity for the XXR compounds was found for the surfactants with 8 carbon atoms on the alkyl chains. This surfactant presented activity against all microorganismes tested. From these results we could predict that the optimum activity for these new surfactants will be the same This maximum is lower than those of monoalkyl surfactants from arginine(12 carbons atoms in the alkyl chain) and also than those reported

on antimicrobial activity of gemini surfactants bisQuats type (10-12 carbon atoms) and gemini surfactants from arginine (10 carbons atoms).

Página 19: [144] Eliminado	aurora	23/08/2002 14:09:00
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Página 19: [144] Eliminado	aurora	23/08/2002 14:09:00
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T

Página 19: [145] Eliminado	Man209	10/03/2003 14:04:00
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e known

Página 19: [145] Eliminado	Man209	14/03/2003 12:27:00
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(Ref)

Página 19: [145] Eliminado	Man209	14/03/2003 10:53:00
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Página 19: [146] Eliminado	RMN	04/03/2003 11:01:00
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, classifying compounds depending on the damage of the eye

Página 19: [147] Eliminado	aurora	23/08/2002 14:09:00
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A

Página 19: [148] Eliminado	RMN	04/03/2003 11:02:00
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procedures have been developed

Página 19: [149] Eliminado	aurora	23/08/2002 14:36:00
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to replace this aggressive methodology.

Página 19: [149] Eliminado	aurora	23/08/2002 14:10:00
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alternatives

Página 19: [150] Eliminado	Man209	10/03/2003 14:08:00
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the

Página 19: [150] Eliminado	Man209	12/03/2003 16:38:00
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[12

Página 20: [159] Eliminado	.	10/12/2002 12:32:00
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Página 20: [160] Eliminado	aurora	23/08/2002 18:33:00
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From these two parts we can determine the relationship between hemolysis (HC50) and the denaturation index (DI) that is defined as the lysis/denaturation quotient or L/D ratio used to predict the potential ocular irritation of surfactants.

Página 20: [161] Eliminado	RMN	04/03/2003 11:06:00
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ing

Página 20: [161] Eliminado	RMN	04/03/2003 11:06:00
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in

Página 20: [162] Eliminado	Man209	14/03/2003 10:57:00
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the

Página 20: [162] Eliminado	Man209	10/03/2003 14:11:00
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S

Página 20: [162] Eliminado	Man209	10/03/2003 14:12:00
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Hence

Página 20: [163] Eliminado	Man209	10/03/2003 14:12:00
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, some of the surfactants currently on

Página 20: [164] Eliminado	Man209	10/03/2003 14:11:00
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the market, mainly the cationic surfactants are classified as irritant compounds.

Página 20: [165] Con formato	Man209	05/11/2003 14:12:00
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Página 20: [166] Con formato	Man209	18/03/2003 11:59:00
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Página 20: [167] Con formato	Man209	18/03/2003 11:59:00
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Página 20: [168] Eliminado	RMN	04/03/2003 11:07:00
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that

Página 20: [168] Eliminado	RMN	04/03/2003 11:07:00
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t

Página 20: [168] Eliminado	RMN	04/03/2003 11:08:00
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similar

Página 20: [169] Con formato	Man209	18/03/2003 11:59:00
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Sin Superíndice / Subíndice

Página 20: [170] Con formato	Man209	18/03/2003 11:59:00
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Página 20: [171] Eliminado	RMN	04/03/2003 13:39:00
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Página 20: [174] Eliminado	Man209	10/03/2003 14:15:00
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also derived also from

Página 20: [175] Eliminado	RMN	04/03/2003 11:10:00
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Taking into account

Página 20: [175] Eliminado	RMN	04/03/2003 11:11:00
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due

Página 20: [175] Eliminado	RMN	04/03/2003 11:11:00
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Página 20: [176] Eliminado	RMN	04/03/2003 11:11:00
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similar

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the

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cutt

Página 21: [178] Eliminado	RMN	04/03/2003 11:13:00
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Because of that

Página 21: [178] Eliminado RMN 04/03/2003 11:13:00
given

Página 21: [178] Eliminado RMN 04/03/2003 11:13:00
this

Página 21: [179] Eliminado Man209 10/03/2003 14:20:00
the irritation character of these surfactants is higher than

Página 21: [180] Eliminado Man209 10/03/2003 14:20:00
by non ionic surfactants from lysine with an analogue chemical structure (24

Página 21: [181] Eliminado Man209 10/03/2003 14:20:00
it is similar to the other cationic surfactants from arginine with different
molecular structures. (19, 5

Página 21: [182] Eliminado Man209 10/03/2003 14:20:00
From these results we can conclude that the

Página 21: [183] Eliminado Man209 10/03/2003 14:20:00
Thus, the irritant character is related to the cationic charge in these compounds
and not to

Página 21: [184] Eliminado Man209 10/03/2003 14:20:00
the molecular structure.

Página 21: [185] Eliminado Lourdes1 07/01/2003 13:42:00
hemolytic activity is of the same order for the two compounds studied, 1212Rac
and 1414Rac, and. This is similar to the hemolytic activities of other arginine
based cationic surfactants synthesised in our lab¹ [8,12] a. This hemolytic
activity and is also similar to some betaines which are considered in some cases
to be soft amphoteric surfactants[15] (CAL UNA REFERENCIA). The potential
ocular irritation given predicted by the red blood cell method classifies indicates

that 1212RActhese and 1414Rac as moderate irritants surfactants like them the same category of betaines, that is, they surfactants are moderate irritants, such as betaine in contrast to the very irritant action of other commercially available surfactants, say (SDS, benzalkonium chloride, or cetyltrimethylammonium chloride[16])²¹.

There is a considerable difference between the hemolytic potency of 1212Rac and 1414Rac are cationic surfactants but their hemolytic power is much smaller than that of MonoQuats, cationic surfactants these new surfactants and that of those with a quaternary group in the polar head (Okamoto 1999), . MonoQuats are capable of inducing lysis of erythrocytes at low concentrations as low as (0.05 -0.1 $\mu\text{mg/mL}$)²¹ [16] whereas 1212Rac and 1414Rac only these surfactants show hemolytic effects at concentrations higher than ranges between 6.019-60 $\mu\text{mg/mL}$ and . 1.9 $\mu\text{g/mL}$, respectively. See Table 4.

In summary we can conclude that the biological activity of diglyceride surfactants from arginine is not affected for the number of ionic charges in the polar head.

Página 21: [186] Eliminado . 17/12/2002 16:30:00
figure 2). The conductivities of the aqueous

Página 21: [187] Eliminado . 17/12/2002 16:30:00
solutions increased linearly with the

Página 21: [188] Eliminado . 17/12/2002 16:30:00
concentration up to break points of nM for 1212Rac and ...

mM for 1414Rac (Figure X).

The new surfactants differ from short-chain lecithines in terms of aggregation behavior. Short chain phospholipids aggregate in water forming micelles at concentrations of about one order of magnitude higher. This increment can be attributed to the cationic character

of the polar group 1212Rac and 1414Rac that

the new compounds

increases the solubility

due to the cationic character of the polar group

. The solubility in monomers in water for XXRac is ----- while for dilauroyl phosphatidylcoline is----- .

Optical microscopy

The phases of the binary 1212Rac/water and 1414Rac/water systems have been determined by visual observation of the samples through crossed polarized microscopy. Qualitative phase behavior studies applying the flooding method revealed the formation of anisotropic phases in all the binary surfactant systems studied. 1212Rac forms lamellar liquid crystals at room temperature

(25°C) and this structure remains until reaching high temperatures. The 1414RAc also forms anisotropic phases at room temperature but

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Página 21: [197] Eliminado	.	17/12/2002 16:30:00
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Critical micellar concentration

The conductance of aqueous solutions of 1212RAc and 1414RAc

Página 21: [198] Eliminado	aurora	23/08/2002 19:09:00
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the new compounds

Página 21: [199] Eliminado	.	17/12/2002 16:30:00
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measured for solutions in concentrations ranging from 0.01 mM to 1.5 mM (F

Página 21: [200] Eliminado	.	17/12/2002 16:30:00
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structure type. However,

Página 21: [201] Eliminado	aurora	23/08/2002 19:18:00
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it can be also observed the

Página 21: [202] Eliminado	.	17/12/2002 16:30:00
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quid crystals become apparent (Figure

Página 24: [203] Con formato	Man209	05/11/2003 13:46:00
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Página 24: [203] Con formato	Man209	05/11/2003 13:46:00
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Inglés (Reino Unido)

Página 24: [203] Con formato	Man209	05/11/2003 13:46:00
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Fuente: Sin Cursiva, Inglés (Reino Unido)

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Página 24: [203] Con formato	Man209	05/11/2003 13:46:00
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Inglés (Reino Unido)

Página 24: [204] Con formato	Man209	05/11/2003 13:48:00
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Página 24: [204] Con formato	Man209	05/11/2003 13:48:00
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Inglés (Reino Unido)

Página 24: [204] Con formato	Man209	05/11/2003 13:48:00
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Fuente: Sin Cursiva

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Fuente: Sin Cursiva

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Página 24: [205] Con formato	Man209	05/11/2003 13:50:00
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Fuente: Sin Cursiva, Inglés (Reino Unido)

Página 24: [205] Con formato	Man209	05/11/2003 13:50:00
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Página 24: [206] Con formato	Man209	05/11/2003 14:05:00
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Fuente: Sin Cursiva, Inglés (Reino Unido)

Página 24: [206] Con formato	Man209	05/11/2003 14:05:00
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Página 24: [206] Con formato	Man209	05/11/2003 14:05:00
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Fuente: Sin Cursiva

Página 24: [208] Eliminado	Man209	30/10/2003 15:55:00
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Página 24: [209] Con formato	Man209	17/03/2003 13:50:00
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Página 24: [209] Con formato	Man209	17/03/2003 13:50:00
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Página 24: [209] Con formato	Man209	17/03/2003 13:50:00
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Página 24: [209] Con formato	Man209	17/03/2003 13:54:00
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Página 24: [209] Con formato	Man209	18/03/2003 12:05:00
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Página 24: [209] Con formato	Man209	17/03/2003 13:50:00
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Página 24: [209] Con formato	Man209	17/03/2003 13:54:00
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Página 24: [209] Con formato	Man209	17/03/2003 13:50:00
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Página 25: [210] Eliminado	Man209	17/03/2003 13:01:00
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Página 25: [211] Con formato	Man209	17/03/2003 13:50:00
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Página 25: [212] Con formato	Man209	17/03/2003 13:50:00
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Página 25: [217] Eliminado	Man209	31/10/2003 14:23:00
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Página 25: [218] Con formato	Man209	17/03/2003 13:50:00
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Página 25: [219] Con formato	Man209	17/03/2003 13:50:00
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Página 25: [222] Con formato	Man209	17/03/2003 13:50:00
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Página 25: [223] Con formato	Man209	17/03/2003 13:50:00
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Página 25: [224] Con formato	Man209	17/03/2003 15:53:00
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Página 25: [225] Con formato	Man209	17/03/2003 13:50:00
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Página 25: [226] Con formato	Man209	17/03/2003 13:50:00
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Página 25: [238] Con formato	Man209	05/11/2003 14:12:00
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Página 25: [239] Con formato	Man209	05/11/2003 14:12:00
Fuente: Sin Cursiva		
Página 26: [240] Con formato	Man209	20/03/2003 15:53:00
Izquierda: 70,9 pto, Derecha: 70,9 pto, Arriba: 85,05 pto, Abajo: 85,05 pto, Inicio de sección: Nueva columna, Ancho: 841,9 pto, Alto: 595,3 pto		
Página 26: [241] Eliminado	.	11/12/2002 9:42:00

Página 26: [242] Eliminado	RMN	04/03/2003 13:46:00
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Página 26: [244] Eliminado aurora 09/12/2002 11:15:00

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Página 26: [249] Eliminado aurora 09/12/2002 12:00:00

edited by Krister Holmberg,

Página 26: [250] Eliminado RMN 04/03/2003 13:46:00

Marcel Deker (2001

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Página 26: [254] Con formato	Lourdes1	06/03/2003 14:14:00
Inglés (Reino Unido)		
Página 26: [254] Con formato	Lourdes1	06/03/2003 14:14:00
Inglés (Reino Unido)		
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Página 26: [259] Con formato	Lourdes1	06/03/2003 14:14:00
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Página 26: [259] Con formato	Lourdes1	06/03/2003 14:14:00
Inglés (Reino Unido)		
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Página 26: [265] Con formato	Man209	17/03/2003 16:40:00
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Centrado

Página 26: [266] Cambio	Man209	17/03/2003 16:42:00
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Tabla con formato

Página 26: [267] Eliminado	aurora	23/08/2002 21:17:00
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Página 26: [268] Eliminado	aurora	23/08/2002 21:06:00
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Página 26: [268] Eliminado	aurora	23/08/2002 21:06:00
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Página 29: [269] Eliminado	Man209	05/11/2003 13:57:00
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Página 29: [269] Eliminado	Man209	05/11/2003 13:57:00
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Página 29: [270] Eliminado	RMN	04/03/2003 13:47:00
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Página 29: [272] Eliminado	RMN	04/03/2003 13:47:00
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Página 29: [285] Eliminado	RMN	04/03/2003 16:20:00
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Página 29: [285] Eliminado	RMN	04/03/2003 13:56:00
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Página 29: [285] Eliminado	RMN	04/03/2003 16:20:00
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Página 29: [285] Eliminado	RMN	04/03/2003 16:19:00
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Página 30: [286] Eliminado	Man209	18/03/2003 16:23:00
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Página 30: [286] Eliminado	Man209	18/03/2003 16:21:00
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Página 30: [286] Eliminado	Man209	18/03/2003 16:24:00
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Página 30: [286] Eliminado	Man209	18/03/2003 16:21:00
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Página 30: [286] Eliminado	Man209	18/03/2003 16:26:00
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Página 30: [287] Con formato	Man209	17/03/2003 16:56:00
Centrado, Interlineado: sencillo		
Página 30: [288] Con formato	Man209	17/03/2003 16:56:00
Interlineado: sencillo		
Página 30: [289] Eliminado	Man209	18/03/2003 16:23:00
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Centrado, Interlineado: sencillo		

Página 30: [294] Eliminado	aurora	23/08/2002 14:18:00
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Página 30: [294] Eliminado	aurora	23/08/2002 14:17:00
Irritante		
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Moderado		
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Moderado		
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Página 31: [296] Eliminado	Man209	05/11/2003 13:53:00

Sample code	Water content	Oil content and name	Surfactant content and name	Dominant droplet size	Emulsion type
E1	0.5	0.5 decane	0.00223, Rac 1212Rac	50µm/5µm agregates	W/O/W
E2	0.5	0.5 decane	0.00223, Rac 1414Rac	50µm/5µm	W/O/W
E3	0.09	0.91 decane	0.00022, Rac 1212Rac	50µm/5µm	O/W/O
E4	0.5	0.5 decane	0.0023, DLPC	20µm	O/W
E5	0.77	0.23 squalane	0.02 Span20+ 0.00045 Rac 1212	10µm/10µm	O/W and W/O
E6	0.77	0.23 squalane	0.01, Span 20	10µm/10µm	O/W
E7	0.5	0.5	0.025, Lecithin	10µm	W/O

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