A. Pinazo

Departament de Tecnologia de Tensioactius

Institut d'Investigacions Químiques i Ambientals de Barcelona, CSIC

Jordi Girona 18-26 Barcelona, Spain

e-mail: apgste@cid.csic.es

Tel: 34 93 400 61 64

fax: 34 93 204 59 04

Eliminado: ¶

Con formato: Normal, Izquierda, Interlineado: 1,5 líneas

Con formato: Fuente: Sin Cursiva, Inglés (Reino Unido)

Con formato: Izquierda, Interlineado: 1,5 líneas

Con formato: Interlineado: 1,5 líneas

Eliminado: ¶

Con formato: Interlineado: sencillo

Eliminado: ¶
¶
¶

A synthetic alternative to natural lecithins with antimicrobial properties

Eliminado: MONOCATIONIC ARGI

Eliminado: NI

Eliminado: NINE-DIGLYCERIDE SURFACTANTS FOR FOOD AND COSMETIC APPLICATIONS

Eliminado: ¶

Eliminado: ¶
¶
¶

L.Pérez [a], M.R.Infante [a], R.Pons [a], C.Moran [a], P.Vinardell [b,c], M. Mitjans[b],

A.Pinazo [a] *

[a] *Departament de Tecnologia de Tensioactius*

Institut d'Investigacions Químiques i Ambientals de Barcelona, CSIC

Jordi Girona 18-26 Barcelona, Spain

[b] *Departament de Fisiologia - Divisió IV*

Facultat de Farmàcia, Universitat de Barcelona

Avinguda Joan XXIII, s/n Barcelona, Spain

[c] *Unidad Asociada CSIC-8004, Spain*

* To whom correspondence should be addressed.

Eliminado: ¶
¶
¶
¶
¶
¶
¶

Comentario [.1]:

Abstract

Two soft biocompatible cationic surfactants from the amino acid arginine, 1, 2 dilauroil-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

Eliminado: R…has bee ... [1]
Eliminado: .
Eliminado: . A…in one ... [2]
Eliminado: (
Eliminado: 1)
Con formato ... [3]
Eliminado: […] ... [4]
Con formato ... [5]
Con formato ... [6]
Eliminado:
Eliminado: Alain Guyot ... [7]
Con formato ... [8]
Con formato ... [9]
Eliminado: (7) [3] Nie ... [10]
Eliminado: With this a ... [11]
Eliminado: They are r ... [12]
Eliminado: (CAL UNA ... [13]
Eliminado: . Unfortuna ... [14]
Eliminado:
Eliminado: is small ar ... [15]
Eliminado: ¶
Eliminado: Designi…r ... [16]
Eliminado:
Eliminado: ive
Eliminado:
Eliminado: great ... [17]
Eliminado: With this a ... [18]
Eliminado: (Referencia
Eliminado: []
Eliminado:)
Eliminado: , Gemini []
Eliminado: (referencia)
Eliminado: and diglyc ... [19]
Eliminado: (Referencia)
Eliminado: . In all
Eliminado:
Eliminado: cases they ... [20]
Eliminado: be
Eliminado: high
Eliminado: ly
Eliminado: biodegradability,
Eliminado: les
Eliminado:
Eliminado: with
Eliminado: low toxicit ... [21]
Eliminado: on the…ar ... [22]

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.

Eliminado: y

Eliminado:

Eliminado:

Eliminado:

Eliminado: ,

Eliminado: bonded

Eliminado: l

Eliminado: i

Eliminado: an

Eliminado: 1

Eliminado: i

Eliminado: 3

Eliminado: In

Eliminado:

Eliminado: what follows

Eliminado: will

Eliminado: by

Eliminado: The structures are shown in Figure 1.

Eliminado: It is expected that the new synthetic compounds will be an interesting alternative to lecithin with better solubility properties and antimicrobial activity ¶

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

2.1. Materials

All the solvents were reagent grade and were used without further purification. 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 fosfatidyl choline (DLPC) and the hexadecyl trimethyl ammonium bromide (HTAB) were from Sigma. Lauroyl arginine methyl ester (LAM) was synthesized as described in [12].

Span 20 was provided by ICI. Lecithin was from Lipoid GmbH (Ludwigshafen, 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 prepared by enzymatic methods and analysed as described in Morán et al. [13].

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

Eliminado: PROCEDURES
Eliminado: ¶
Eliminado: r
Eliminado: from
Eliminado: ethyl
Con formato: Color de fuente: Automático
Eliminado: supplied for LAMIRSA. SA.
Eliminado: from now on designed by
Eliminado: (
Eliminado:)
Eliminado: ribed in Ref C
Eliminado:
Eliminado: .
Eliminado: .[
Con formato: Sin Superíndice / Subíndice
Eliminado: 9]
Con formato: Color de fuente: Automático
Eliminado:
Con formato: Color de fuente: Automático, Sin Superíndice / Subíndice

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 H_2O-CH_3CN. Eluent

Eliminado: ¶
(9) Morán, C., Infante, M.R., Clapés, P., "Synthesis of glycero amino acid-based surfactants. Part 1. Enzimatic preparation of rac-1-O-(N$^{\alpha}$-acetil-L-aminoacyl)glycerol derivatives" J.Chem.Soc., Perkin Trans. 1:2063-2070, 2001.¶

Eliminado: ¶

Eliminado: The reaction

Eliminado: The reaction evolution

Eliminado: progress

Eliminado: was monitored by HPLC with a

Eliminado: using

Eliminado: an L-4250 UV-VIS detector at 215 nm. ¶
¶
Analysis of 00RAc¶

Eliminado: .

Eliminado: to control the

Eliminado: disappearance of the 00RAc.

Eliminado:

Eliminado: The mobile phase was

Eliminado: ¶

Eliminado: ¶

Eliminado: ¶
Analysis of 1212RAc and 1414RAc¶

Eliminado:

Eliminado: ¶

Eliminado: as

Eliminado: checked

Eliminado: sistem

Eliminado: . A gradient elution profile with

Eliminado: of

Eliminado: two eluents was used.

A was 0.1% (vol/vol) TFA in H_2O, and eluent B was 0.085 % TFA in H_2O/CH_3CN 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 ^{1}H and ^{13}C 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

Eliminado:
Eliminado:
Eliminado:
Eliminado:
Eliminado:
Eliminado: were
Eliminado: for the 1414RAc min
Eliminado: ¶
Eliminado: ¶
Eliminado: ¶
Eliminado: Afegir: DLPC, lecitina, HTAB, Teo-bet, LAE, 12 i 14 acid chlorides¶ ¶
Eliminado: ¶ ... [23]
Eliminado: checked
Eliminado: i
Eliminado: (MS)
Eliminado: analysis of ... [24]
Eliminado: Synthesis ... [25]
Eliminado: e
Eliminado: s (5g) of 0 ... [26]
Eliminado: –
Eliminado: laury
Eliminado: i
Eliminado: l choride f ... [27]
Eliminado: i
Eliminado: l chloride f ... [28]
Eliminado: --
Eliminado: --
Eliminado: was then ... [29]
Eliminado: .
Eliminado:
Eliminado: Stirring was kept
Eliminado: through 4 ... [30]
Eliminado: i
Eliminado: c/myristic acid
Eliminado: excess
Eliminado: . The solve ... [31]
Eliminado: redi
Eliminado: solved in ... [32]
Eliminado: ¶ ... [33]
Eliminado: washing th ... [34]
Eliminado: ¶ ... [35]
Eliminado: The fractio ... [36]
Eliminado: ¶
Eliminado: ¶

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 25ºC 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.

Eliminado: ¶
¶

Eliminado: ¶
Eliminado:
Con formato: Inglés (Reino Unido)
Eliminado:
Eliminado:
Con formato: Inglés (Reino Unido), Sin Superíndice / Subíndice
Eliminado: [8]
Con formato: Inglés (Reino Unido)
Eliminado: (10) Laughlin, R.G. (1992) Adv. Coll. Interface Sci. 41, 57-79.¶
Eliminado: ,
Con formato: Inglés (Reino Unido)
Con formato: Color de fuente: Automático, Inglés (Reino Unido)
Con formato: Color de fuente: Automático
Con formato: Fuente: Cursiva, Color de fuente: Automático
Eliminado:
Eliminado: used
Eliminado: For the image capture a
Eliminado: the softhware
Eliminado: as
Eliminado: developed around

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-refringent phases.

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 Na_2HPO_4, 5.6 mmol/L KH_2PO_4, 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 10^9 cell / mL.

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

Eliminado:
Eliminado:
Eliminado: pyrex
Eliminado: appearance
Eliminado: ¶
Stability¶
¶
The stability of 1212RAc and 1414RAc as a function of the pH and the temperature was evaluated
Con formato
Eliminado: by HPLC analysis as described in the HPLC section. The surfactants were dissolved in water, ethanol or DMF at the appropriate pH and temperature. Analysis of these solutions were carried out every 24 hours, monitoring the decrease of the peak corresponding to the surfactant under study.¶
¶
Eliminado: ¶
Eliminado: : h
Eliminado:
Eliminado: The
Eliminado: e
Eliminado:
Eliminado: cells
Eliminado:
Eliminado: ¶
Eliminado: He*molysis*
Eliminado: :
Eliminado: D
Eliminado: from 10 to 80 µL
Con formato: Color de fuente: Automático
Eliminado:
Eliminado: ipetted
Eliminado: to
Eliminado: . A
Eliminado: s
Eliminado: ere
Eliminado: the
Eliminado: s and
Eliminado:
Eliminado: , whilst shaking, at room temperature.

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 μL of test sample were added to tubes containing 875 μL of PBS. Next, 25 μL 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 37ºC. 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 aereus* ATCC 25178, *Staphylococcus epidermidis* ATCC 155-1, *Micrococcus luteus* ATCC 9341. Yeast: *Candida albicans* ATCC 10231.

Eliminado: and t
Eliminado: percentage of
Eliminado:
Eliminado: :
Eliminado:
Eliminado: Then
Eliminado: and
Eliminado: erytrhocyte
Eliminado: the
Eliminado: After incubating the tubes for s and incubated for 10 minutes, t
Eliminado: y
Eliminado: . The tubes were
Eliminado: and t
Eliminado: s'
Eliminado: absorbances
Eliminado: ere
Eliminado: was
Eliminado:
Eliminado: the supernatant at
Eliminado: The ratio of absorbance at 575 nm and 540 nm allowed the calculation of the hemoglobin denaturation index (DI) which was compared with the SDS as the internal standard. ¶
Eliminado: ¶
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 to10 moderately irritant, L/** ... [37]
Eliminado: ¶
Eliminado:
Eliminado: The a
Eliminado:
Con formato ... [38]
Eliminado: [10]
Con formato ... [39]
Eliminado: *Jones RN,* ... [40]
Eliminado: (11) Jones ... [41]
Con formato ... [42]
Eliminado:

3. RESULTS AND DISCUSSION

3.1. Synthesis

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 (00RAc) as described in Morán et al. [13]. This reaction consisted in 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 in the preparation of 1, 2-diacyl-3-O-(N acetylarginyl - rac-glicero) by acylation of the two remaining free hydroxyls of 00RAc groups with the corresponding long chain acid chloride. One factor that can affect the evolution and yield of the reaction is the stability of the reactants in the reaction media. A number of methods of preparation described in the literature for the acylation of the hydroxyl groups of glycerol have been carried out in the presence of basic or acid catalysts [18] and at temperatures around 100ºC.

Under these conditions, hydrolysis of the ester bond between the arginine and the glycerol may occur. In order to avoid the hydrolysis of the ester bond, the esterification of the two glycerol hydroxyl groups was carried out with fatty acyl chloride in pyridine as solvent at room temperature. Under these mild conditions, the pH of the reaction mixture was kept in the range 5 to 7. At these values the 00RAc starting compound was stable.

Eliminado: Stability¶ ... [43]
Eliminado: the
Eliminado: 1212RA
Eliminado: a
Eliminado: c and 1414RAc
Eliminado: compound
Eliminado: as a funct ... [44]
Eliminado: 1212Rac ... [45]
Eliminado: were diss ... [46]
Eliminado: e
Eliminado: s of these ... [47]
Eliminado: studied.
Eliminado: ¶
Eliminado: ¶ ... [48]
Eliminado: ¶ ... [49]
Eliminado: Physicoch ... [50]
Eliminado: ¶
Eliminado: Samples f ... [51]
Eliminado: ¶
Eliminado: Conductiv ... [52]
Eliminado: ized
Eliminado: parallel pl ... [53]
Eliminado: M
Eliminado: easureme ... [54]
Eliminado:
Eliminado: ¶
Eliminado: to minimiz ... [55]
Eliminado: ¶ ... [56]
Eliminado: *Method for q*
Eliminado: *ualitative p* ... [57]
Eliminado: ¶
Eliminado: Qualitative ... [58]
Eliminado: a
Eliminado: c and ... [59]
Eliminado: ,
Eliminado: systems a ... [60]
Eliminado: as
Eliminado: studied by ... [61]
Eliminado: examinations
Eliminado: were perf ... [62]
Eliminado: or "
Eliminado: penetration)
Eliminado: "
Eliminado: method of ... [63]
Eliminado: ,
Eliminado: and
Eliminado: the
Eliminado: different ... [64]
Eliminado: as separate
Eliminado: around cr ... [65]
Eliminado: ¶ ... [66]
Eliminado: *Emulsions.*
Eliminado: ¶
Eliminado: Emulsions ... [67]
Eliminado: drop wise
Eliminado: to a water ... [68]
... [69]
... [70]

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 ^{13}CNMR.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

Eliminado: by the addition of a molar ratio acid clhoride/00RAC (1:1.5) we obtained mainly the monoacilated compound 120RAc or 140RAc, due to the different reactivity of the primary and secondary hydroxyl group of the 00RAc and probably also to esteric impediments. By addition of acid clhoride untilluntil a molar ratio acid clhoride/00RAc (1/3) the main product formed was the diacylated compounds in good yields (85-90%) with a starting compound conversion higher than 98%. The product obtained by this method consisted in a mixture of two positional isomers characterised by ^{13}CNMR

Eliminado: : 7% of

Eliminado: and 93% of

Eliminado: ¶
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 superiorshigher than to 98%. was obtained.¶
¶
¶
... [73]

Eliminado: ¶

Eliminado: about

Eliminado:

Eliminado: 5

Eliminado: these two surfactants

Eliminado: presented

Eliminado: I

Eliminado: Satisfactory

Eliminado:

Eliminado: i

Eliminado: e

Eliminado: ¶

Eliminado: were

Eliminado:

Eliminado: also

Eliminado: S

Eliminado: spectrum have

Eliminado: been

Eliminado: C

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

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 25ºC 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 40ºC 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 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 25ºC 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

Eliminado: obtained from these materials given FABMS and NMR spectra which were consistent with the
Eliminado: ,
Eliminado: desired
Con formato: Inglés (Reino Unido)
Con formato: Inglés (Reino Unido)
Con formato: Inglés (Reino Unido)
Con formato: Inglés (Reino Unido)
Con formato: Color de fuente: Automático, Inglés (Reino Unido)
Eliminado: .....
Con formato: Inglés (Reino Unido)
Con formato: Inglés (Reino Unido)
Con formato ... [74]
Con formato ... [75]
Con formato ... [76]
Con formato ... [77]
Con formato ... [78]
Con formato ... [79]
Eliminado: lower
Con formato ... [80]
Eliminado: higher
Eliminado: ¶
Eliminado: at
Eliminado: ¶
Eliminado: P
Eliminado: compoun ... [81]
Eliminado: [13]¶ ... [82]
Con formato ... [83]
Eliminado:)¶ ... [84]
Eliminado: ¶
Eliminado: ¶
Eliminado: [14],¶ ... [85]
Con formato ... [86]
Eliminado: For
Eliminado: ing
Eliminado: ance
Eliminado: ere
Con formato ... [87]
Eliminado: 2
Eliminado:

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 -CH_2 - 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 nonacetilated 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].

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 zwiterionic surfactants [19].

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-

Eliminado: 0

Con formato: Color de fuente: Automático, Inglés (Reino Unido)

Con formato: Color de fuente: Automático, Inglés (Reino Unido)

Con formato: Color de fuente: Automático, Inglés (Reino Unido), Sin Superíndice / Subíndice

Con formato: Color de fuente: Automático

Con formato: Inglés (Reino Unido)

Con formato: Inglés (Reino Unido), Sin Superíndice / Subíndice

Con formato: Inglés (Reino Unido)

Eliminado: ¶

Eliminado: ¶
The CMC of

Eliminado: se

Eliminado: is similar to those of the short-chain

Eliminado: lecithine

Eliminado: lecithin with alkyl chain of 8 carbons atoms (0.24 mM)

Eliminado: [15

Eliminado: 7

Eliminado:].

Con formato: Inglés (Reino Unido)

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).

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.

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 (25ºC) and this structure is stable, until reaching high temperatures. The 1414RAc forms anisotropic phases that developed in lamellar liquid crystals at 45ºC (Figure 4a). The different temperature at which 1212RAc and 1414RAc

Eliminado: fact
Con formato: Inglés (Reino Unido)
Eliminado: instead of a cmc, ….This needs ... [88]
Eliminado: F is warranted These surfactants have higher alkyl chain that theseis phospholiipids but posses cationic character in the polar group, so that, the hydrophobic/hydrophiylic balance is probably similar.
Eliminado: Optical microscopy
Eliminado: due to… to form micelles ... [89]
Eliminado: i
Eliminado: surrounding the dispersed particles …the particles with ... [90]
Con formato ... [91]
Eliminado: This presents the coalescence
Eliminado: that resists the coalescence
Eliminado: ,
Eliminado: preventing
Eliminado: impeding sufficient
Eliminado: enough
Con formato: Inglés (Reino Unido)
Eliminado: the [14].
Con formato ... [92]
Eliminado:
Con formato ... [93]
Eliminado: A
Con formato: Color de fuente: Automático, Inglés (Reino Unido)
Eliminado: that form... ... [94]
Eliminado: that… formation those surfactants will tent to form compact films at ... [95]
Eliminado: (Rosen, Ilibre)
Eliminado: ¶ ... [96]
Eliminado: remains
Con formato ... [97]
Eliminado: also …at r ... [98]
Con formato ... [99]
Eliminado:
Eliminado: warming ... [100]
Con formato ... [101]

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 ºC 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

Eliminado: 1

Con formato: Inglés (Reino Unido), Sin Superíndice / Subíndice

Eliminado: [6]

Con formato: Inglés (Reino Unido)

Con formato: Color de fuente: Automático, Inglés (Reino Unido), Sin Superíndice / Subíndice

Con formato: Color de fuente: Automático, Inglés (Reino Unido)

Eliminado: [Ref]

Eliminado: (Physics of Amphiphiles: Micelles,Vesicles and Microemulsions, pg 34, 1985, Editors V. Degiorgio and M. Corti, Editorial Elsevier Science Publishers, Amsterdam)¶

Eliminado: [16].

Eliminado:

Eliminado: ¶

Eliminado: spontaneously

Eliminado: easily

Eliminado: C

Eliminado: vesicles as in the case of the phospholipids [18]. Figure Y is a picture example taken with an optical microscope that shows the vesicles corresponding to the 1212RAc compound.

Eliminado: o

Eliminado: , this

Eliminado: involve

Eliminado: that they

Eliminado: spontaneously are

Eliminado: o

Eliminado: ,

Eliminado: n

Eliminado: have

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.

Con formato: Inglés (Reino Unido)
Con formato: Inglés (Reino Unido)
Eliminado: 7
Eliminado: 9
Con formato: Inglés (Reino Unido), Sin Superíndice / Subíndice
Con formato: Color de fuente: Automático, Inglés (Reino Unido)
Con formato: Inglés (Reino Unido)
Eliminado: at
Eliminado: It is not common t
Eliminado: that is
Eliminado: 's both
Con formato: Inglés (Reino Unido)
Con formato: Inglés (Reino Unido), Sin Superíndice / Subíndice
Con formato: Inglés (Reino Unido)
Eliminado:
Con formato: Color de fuente: Automático, Inglés (Reino Unido)
Con formato: Inglés (Reino Unido)
Eliminado: did
Eliminado: efficiency
Eliminado: r
Eliminado: quite
Eliminado: quite
Eliminado: elated
Eliminado: assessed
Eliminado: also
Eliminado: make

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

Eliminado: 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 lecythins.¶ ... [102]
Eliminado: (MIC)
Eliminado:

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* , wile the 1414RAc has a stronger activity agains *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.

Eliminado:
Eliminado: (
Eliminado: 3
Eliminado:)
Eliminado: ice
Eliminado: I…XX…c … [103]
Eliminado: all…e … [104]
Eliminado: (Table X).
Eliminado: ¶ … [105]
Eliminado: e
Eliminado: ,
Eliminado: due to
Eliminado: v…s… … [106]
Eliminado: almost like
Eliminado: T…,…to … [107]
Eliminado:
Eliminado: suitable f … [108]
Eliminado: Due to… … [109]
Eliminado: lower
Eliminado: Figure
Eliminado: .
Eliminado: X depict … [110]
Eliminado: shows th … [111]
Eliminado: Clearly,
Eliminado: As we can see,
Eliminado: t
Eliminado:
Eliminado: the … [112]
Eliminado: ow
Eliminado: possess … [113]
Eliminado: M
Eliminado: i…to ach … [114]
Eliminado: are gene … [115]
Eliminado: sufficient … [116]
Eliminado: , because … [117]
Eliminado: growth
Eliminado: Taking in … [118]
Eliminado: …….
Eliminado: nd
Eliminado:
Eliminado: consequently
Eliminado: s…is … [119]
Eliminado: for the 12 … [120]
Eliminado: ,
Eliminado:
Eliminado: … … [121]
Eliminado: for the 1414RAc
Eliminado: , i
Eliminado: s

This behaviour has also been described for other surfactants from arginine [26]. When comparing the antimicrobial activity of these two new surfactants with those reported recently for diglyceride surfactants from arginine (which have the free α-NH_2 group of the arginine as hydrochloride and consequently two positive charges), a similar behaviour is observed [27]. The number of cationic charges in the polar head does not affect the antimicrobial activity in this type of surfactant.

3.5. Hemolysis

Traditionally, the evaluation of the potential ocular irritation of compounds has been determined in vivo by the Draize [28] test in which the product is instilled into the conjunctival sac of the eye of a rabbit, and the damage induced to iris, conjunctiva and cornea is recorded. A number of in vitro procedures have been developed to replace this aggressive methodology. One of these procedures is the Red Blood Cell Test (RCB), which has been described specifically for surfactants [15, 16].This test consists of two steps. First, the HC50 hemolytic concentration, i.e. the concentration inducing the hemolysis of 50% of the erythrocytes is determined. This value is used as a measure of the damage caused by the compound to the membranes. In a second step, the denaturation of the hemoglobin after cell hemolysis is determined. This value is known as the denaturation index (DI). Whenever an eye is damaged there is a denaturation of the protein of the cornea. The denaturation index is a measure of eye damage when comparison is made with the effects caused by a well known irritant surfactant, e.g. sodium dodecyl sulphate (SDS), used as the internal standard.

Eliminado: same
Eliminado: .
Con formato ... [122]
Eliminado: (
Con formato ... [123]
Eliminado: 19)
Eliminado: ref).
Eliminado:
Con formato ... [124]
Con formato ... [125]
Eliminado: For the s ... [126]
Eliminado: C…T ... [127]
Eliminado: are also s ... [128]
Eliminado: , so that
Eliminado: ,
Eliminado: type of surfactant
Eliminado: s
Con formato ... [129]
Eliminado:
Eliminado: so that th ... [130]
Con formato ... [131]
Eliminado: […0 ... [132]
Con formato ... [133]
Eliminado: 8
Eliminado:]
Con formato ... [134]
Eliminado:
Eliminado:
Con formato ... [135]
Eliminado: may be observed
Eliminado: ese
Eliminado: From the ... [136]
Con formato ... [137]
Eliminado: in this
Eliminado:
Eliminado: n't… of th ... [138]
Eliminado: It is well k ... [139]
Eliminado: The antir ... [140]
Eliminado: Neverthe ... [141]
Eliminado: ¶ ... [142]
Eliminado: The opti ... [143]
Eliminado: ¶ ... [144]
Eliminado: ve
Eliminado: e known ... [145]
Eliminado: , classifyi ... [146]
Eliminado: mounts
Eliminado: ¶ ... [147]
Eliminado: T
Eliminado: ,
Eliminado: several i
Eliminado: lternatives i
Eliminado: n vitro
Eliminado:
Eliminado: procedu ... [148]
Eliminado: to replac ... [149]
Eliminado: the … [1 ... [150]
Con formato ... [151]
... [152]
... [153]

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 to10 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

Eliminado: □□□ÕÀ□ ... [155]
Eliminado: □□!
Eliminado: □□□-X□ ... [156]
Eliminado: □□□□□ ... [157]
Eliminado: □□□□□f
Eliminado: Š
Eliminado: □□□□□ ... [158]
Eliminado: measures
Eliminado:
Eliminado: [11,12]… ... [159]
Eliminado: From the ... [160]
Eliminado: t
Eliminado: 4
Eliminado: 4
Eliminado:
Eliminado: ing…in ... [161]
Eliminado: the …s… ... [162]
Eliminado: Due that
Eliminado: , some of ... [163]
Eliminado: presents in
Eliminado: the mark ... [164]
Eliminado: The
Eliminado: say
Eliminado: [
Con formato ... [165]
Eliminado: 3
Con formato ... [166]
Eliminado: 16
Eliminado:]
Con formato ... [167]
Eliminado: that…t… ... [168]
Eliminado: aine that
Eliminado: are
Eliminado: in some cases
Con formato ... [169]
Eliminado: s
Eliminado: 24
Con formato ... [170]
Eliminado: 15…hose ... [171]
Eliminado: (
Con formato ... [172]
Eliminado: 19)
Con formato ... [173]
Eliminado: also deriv ... [174]
Eliminado: already
Eliminado: ¶
Eliminado: Taking in ... [175]
Eliminado: the same
Eliminado: similar … ... [176]

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.

Eliminado: think
Eliminado: …cutt ... [177]
Eliminado: Because ... [178]
Eliminado: Although
Eliminado: T
Eliminado: the irrita ... [179]
Eliminado: those
Eliminado: that shown
Eliminado: ed
Eliminado: by non i ... [180]
Eliminado: 5
Eliminado:),
Eliminado: but
Eliminado: it is simil ... [181]
Eliminado:
Eliminado: Ref
Eliminado:).
Eliminado: From the ... [182]
Eliminado: Thus, the ... [183]
Eliminado: with
Eliminado: the mole ... [184]
Eliminado: hemolytic ... [185]
Eliminado: igure 2). ... [186]
Eliminado: XXRAc
Eliminado: solutions ... [187]
Eliminado: increasing
Eliminado: concentr ... [188]
Eliminado: ----
Eliminado: mM for 1 ... [189]
Eliminado: high solubility
Eliminado: of the po ... [190]
Eliminado: the new ... [191]
Eliminado: increase ... [192]
Eliminado: due to th ... [193]
Eliminado: . The sol ... [194]
Eliminado: ¶
Eliminado: The phas ... [195]
Con formato ... [196]
Eliminado: Critical r ... [197]
Eliminado: the new ... [198]
Eliminado: at 25ºC w
Eliminado: a
Eliminado: ere
Eliminado: s
Eliminado: measure ... [199]
Eliminado: f
Eliminado: but
Eliminado: in this
Eliminado: ese
Eliminado: case it is difficult
Eliminado: not easy t
Eliminado: to identify
Eliminado: ier
Eliminado: the
Eliminado: type of
Eliminado: structure ... [200]
... [201]

REFERENCES

[1] A. Guyot, in K. Holmberg (Ed), Polymerizable Surfactants in Novel Surfactants, Surfactant Science Series, vol 74, p. 279. Marcel Dekker, Inc., 1998, New York.

[2] K. Holmberg, in K. Holmberg (Ed) Cleavable Surfactants in *Novel Surfactants*, Surfactant Science Series, vol 74, p. 333 , Marcel Dekker, Inc., 1998, New York.

[3] J. Seguer, M.R. Infante, M. Allouch, L. Mansuy, C. Selve, P. Vinardell, New J. Chem., 18 (1994) 765.

[4] L. Pérez, J.L. Torres, A. Manresa, C. Solan, M.R. Infante, Langmuir 12 (1996) 5296.

[5] L.Pérez, M.T. Garcia, I.Ribosa, M.P. Vinardell, A. Manresa, M.R Infante, Environ. Tox. Chem., 21 (2002) 1279.

[6] L.Pérez, A.Pinazo, P.Vinardell, P.Clapés, M.Angelet, M.R.Infante, New Jour. Chem.,26 (2002) 1221.

[7] A. Pinazo, M.R. Infante, P. Izquierdo, C. Solans, J. Chem. Soc. Perkin Trans. 2 (2000) 1535.

[8] E. Piera, F. Comelles, P. Erra and M.R. Infante, J. Chem. Soc. Perkin Trans. 2 (1998) 335.

[9] Niels Krog, in F. D. Gunstone and F.B. Padley (Eds) Food Emulsifiers in Lipid Technologies and Applications, p. 521, Maecel Dekker Inc., 1997, New York.

Con formato: Inglés (Reino Unido)

Con formato: Inglés (Reino Unido)

Con formato: Inglés (Reino Unido)

Con formato: Fuente: Sin Cursiva

Con formato: Fuente: Sin Cursiva

Con formato: Fuente: Sin Cursiva

Con formato: Fuente: Sin Cursiva

Con formato: Inglés (Reino Unido)

Con formato: Inglés (Reino Unido)

Con formato: Fuente: Sin Cursiva, Inglés (Reino Unido)

Con formato: Inglés (Reino Unido)

Con formato: Inglés (Reino Unido)

Con formato: Inglés (Reino Unido)

Con formato: Inglés (Reino Unido)

Con formato: Inglés (Reino Unido)

Con formato: Fuente: Sin Cursiva, Inglés (Reino Unido)

Con formato: Fuente: Sin Cursiva, Inglés (Reino Unido)

Con formato: Inglés (Reino Unido)

Con formato: Inglés (Reino Unido)

Con formato: Inglés (Reino Unido)

Con formato: Inglés (Reino Unido)

[10] Niels J. Krog, in S.E. Friberg, K. Larsson (Eds), Food Emulsifiers and Their Chemical and Physical Properties in Food Emulsions, p. 141, Marcel Dekker Inc., 1997, New York.

[11] M. Schneider, in F.D. Gunstone, F.B. Padley (Eds), Phospholipids in Lipid Technologies and Applications, p. 51, Marcel Dekker, Inc. 1997, New York.

[12] M.R. Infante, J. Garcia Dominguez, P. Erra, R. Julia, M. Prats, Int. J. Cosm. Sci., 6 (1984) 275.

[13] C. Morán, M.R. Infante, P. Clapés, J.Chem.Soc., Perkin Trans. 1 (2001) 2063.

[14] R. G. Laughlin, Adv. Coll. Interface Sci., 41 (1992) 57.

[15] R. N. Jones, A. L. Barry, T. L. Gavan, J. A. Washington in E.H. Lennette, A. Ballows , W. J. Hauser, H. J. Shadomy,in (Eds.), Manual of Clinical Microbiology p. 972, American Society for Microbiology, Washington, USA, 1980

[16] E. Jungermann, Cos. Sci. Technol. Ser., 11 (glycerine) (1991) 97.

[17] M. J. Rosen, Surfactants and Interfacial Phenomena, John Wiley & Sons, New York, 1978.

[18] L. Pérez, M.R. Infante, M. Angelet, P. Clapés, A. Pinazo Prog. Colloid Surf., 2003, accepted.

[19] A. Pinazo, L. Perez, M.R. Infante, R. Pons. Physical Chemistry Chemical Physics, 2003 submitted.

Con formato: Fuente: Sin Cursiva

Con formato: Color de fuente: Automático

Con formato: Color de fuente: Automático

Con formato: Color de fuente: Automático, Inglés (Reino Unido)

Con formato: Color de fuente: Automático

Con formato: Inglés (Reino Unido)

Con formato: Inglés (Reino Unido)

Con formato: Fuente: Sin Cursiva, Inglés (Reino Unido)

Con formato: Fuente: Sin Cursiva

Con formato: Inglés (Reino Unido)

Con formato: Inglés (Reino Unido)

Con formato: Fuente: Sin Cursiva

Con formato: Fuente: Sin Cursiva

Con formato: Fuente: Sin Cursiva

Con formato: Color de fuente: Automático

Con formato: Fuente: Sin Cursiva

Con formato: Color de fuente: Automático

Con formato: Color de fuente: Automático

Con formato: Color de fuente: Automático, Inglés (Reino Unido)

Con formato: Fuente: Sin Cursiva, Color de fuente: Automático, Inglés (Reino Unido)

[20] R.J.M. Tausk, J. Karmiggelt, C. Oudshoorn, J.Th.G.Overbeek, Biophys. Chem. 1 (1974) 175.

[21] V. Degiorgio and M. Corti (Eds.) ,Physics of Amphiphiles: Micelles,Vesicles and Microemulsions, p.38 Elsevier Science Publishers, Amsterdam, 1985.

[22] B.P. Binks in B.P. Binks (Ed.), Emulsions, Recent Advances in Understanding in Modern Aspects of Emulsion Science, p.1, The Royal Society of Chemistry, Cambridge, 1998.

[23] A. Pinazo, X. Wen, Y-Ch. Liao, A. J. Prosser, E. I. Franses, Langmuir 18 (2002) 8888.

[24] C.Morán, P.Clapés, F.Comelles, T.García, L.Pérez, P. Vinardell, M.Mitjans, M.R.Infante, Langmuir, 17 (2001) 5071.

[25] L.Pérez, A.Pinazo, P.Vinardell, P.Clapés, M.Angelet, M.R. Infante, New J. Chem., 26 (2002) 1221.

[26] J. H. Draize in *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetic*, Assoc. Food & Drug Officials of USA, Austin, TX, 1959.

[27] W. J. W. Pape, U. Pfannenbecker, U. Hopper, Mol Toxicol. 1 (1987) 525.

[28] W.J. W. Pape, U. Hopper, *Drug. Res.* 4 (1990) 498.

[29] Y. Okamoto, K. Ohkoshi, H. Itagaki, T. Tsuda, H. Kakishima, T. Ogawa, Y. Kasai, J. Ohuchi, H. Kojima, A. Kurishita, T. Kaneko, Y. Matsushima, Y. Iwabuchi, Y. Oho, Toxicol. in Vitro 13 (1999) 115.

Con formato: Fuente: Sin Cursiva

Con formato ... [203]

Con formato ... [204]

Con formato: Inglés (Reino Unido)

Con formato ... [205]

Con formato ... [206]

Con formato ... [207]

Con formato: Inglés (Reino Unido)

Eliminado: 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.¶
¶
¶
(3) Seguer, J., Infante, MR., Allouch, M., Mansuy, L., Selve, C., Vinardell, *P., "Synthesis and evaluation of non-ionic amphiphilic compounds from aminoacids: molecular mimics of lecithins" New J. Chem.*, **18**, 765-774,1994,¶
¶
(4) Pérez L, Torres JL, Manresa A, Solan C, Infante MR. *"Synthesis, aggregation and biological properties of a new class of gemini cationic amphiphilic compounds from arginine, bis(Args)". Langmuir* **12**:5296-5301, 1996.¶
¶
(5) L.Pérez, M.T. Garcia, I.Ribosa, M.P. Vinardell, A. Manresa, M.R Infante "Biological properties of arginine-based gemini cationic surfactants". *Environmental Toxicology and Chem*istry, **21(6):** 1279-1285, 2002.¶
¶
(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 Chem*istry, ... [208]

Con formato: Color de fuente: Automático

Con formato: Interlineado: 1,5 líneas

Con formato ... [209]

Eliminado: ¶

Eliminado: ¶

[30] M.Macián, J.Seguer, M.R.Infante, M.P.Vinardell, Rev.Toxicol. 11 (1994) 127.

Eliminado: [
Con formato: Sangría: Izquierda: 0 pto, Sangría francesa: 21,3 pto, Interlineado: 1,5 líneas
Eliminado: 4]
Eliminado: :
Eliminado: (10) Laughlin, R.G. (1992) *Adv. Coll. Interface Sci.* **41**, 57-7 ... [210]
Con formato ... [211]
Con formato ... [212]
Con formato ... [213]
Con formato ... [214]
Con formato ... [215]
Con formato ... [216]
Eliminado: 12 (E. ... [217]
Con formato ... [218]
Con formato ... [219]
Con formato ... [220]
Con formato: Fuente: Cursiva
Con formato ... [221]
Eliminado: 24. Betainas¶
Con formato ... [222]
Con formato ... [223]
Con formato ... [224]
Con formato ... [225]
Con formato ... [226]
Con formato ... [227]
Con formato ... [228]
Con formato ... [229]
Con formato ... [230]
Con formato ... [231]
Con formato ... [232]
Con formato ... [233]
Con formato ... [234]
Con formato ... [235]
Con formato: Fuente: Cursiva
Con formato: Fuente: Negrita
Con formato ... [236]
Con formato ... [237]
Con formato ... [238]
Con formato ... [239]
Con formato: Fuente: Cursiva
Con formato: Fuente: Negrita
Con formato: Fuente: Cursiva
Con formato: Fuente: Negrita
Eliminado: –Salto de página–

Table1: Analytical data for 1212RAc and 1414RAc compounds

Compound	Molecular Formula / Molecular Weight (g/mol)	Melting point (°C)	Yield (%)	HPLC retention time (min)	Elemental analysis Calculated (with 1 mol H_2O) / found			
					%C	%H	%N	%Cl
1212RAc	$C_{35}H_{67}N_4O_7Cl$ 690.5	100 -104	68	20.6	59.24/ 59.38	9.73/ 9.70	7.90/ 7.83	5.01/5.28
1414RAc	$C_{39}H_{75}N_4O_7Cl$ 746.5	85 - 88	70	21.8	61.21/61.44	10.07/10.46	7.32/ 7.31	4.64/ 4.61

Con formato ... [240]
Eliminado: ¶ ... [241]
Eliminado:
Eliminado: REFERE ... [242]
Eliminado: (
Eliminado: Balzer, D ... [243]
Eliminado: 31
Eliminado: 169-206,
Eliminado: Wolfgang ... [244]
Eliminado: Ed. I.D. F ... [245]
Eliminado: ¶
Eliminado: [2] Jiding ... [246]
Eliminado: Novel Ca ... [247]
Eliminado: Surfactan ... [248]
Eliminado: 74
Eliminado: ,
Eliminado: edited by ... [249]
Eliminado: Marcel D ... [250]
Eliminado: 1998
Eliminado:).¶ ... [251]
Eliminado: [3] Niels ... [252]
Eliminado: Data of 1 ... [253]
Con formato ... [254]
Eliminado: ¶
Eliminado: [9] Morár ... [255]
Eliminado: ¶
Eliminado: [10] Jone ... [256]
Eliminado: ¶
Eliminado: ¶ ... [257]
Eliminado: [12] Mora ... [258]
Con formato ... [259]
Eliminado: [3] Y. Oka ... [260]
Eliminado: ¶ ... [261]
Eliminado: [11] Clin ... [262]
Eliminado: ¶ ... [263]
Eliminado: ¶ ... [264]
Eliminado: T
Eliminado: ABLE
Eliminado: 1

Con formato ... [265]
Tabla con formato ... [266]
... [267]

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		

Eliminado: ---Salto de página---
Eliminado: ¶
¶
Eliminado: ¶
¶
Eliminado: ABLE 2
Eliminado: 2
Eliminado: ¶
Eliminado: Compounds
Tabla con formato
Eliminado: ¶
Eliminado: a
Eliminado: ¶
Eliminado: a
Eliminado: ¶
Eliminado: ¶
Con formato: Centrado
Eliminado: ¶
Con formato: Izquierda
Eliminado: ¶
Con formato: Izquierda
Eliminado: ¶
Eliminado: ¶
¶
Eliminado: ,
Eliminado: ,
Eliminado: (
Eliminado:)
Con formato: Centrado
Eliminado: ,
Eliminado: ,
Eliminado: (
Eliminado:)

Table 3. Composition of the tested emulsions and morphological characteristics

Sample code	Water content[1] (weight fraction)	Oil content[1] (weight fraction)	Surfactant content[1] (weight fraction)	Dominant droplet size (μm)	Emulsion type
E1	0.5	0.5 decane	0.00223, 1212RAc	5 / 50 [2] aggregates	W/O/W
E2	0.5	0.5 decane	0.00223, 1414RAc	5 / 50 [2]	W/O/W
E3	0.09	0.91 decane	0.00022, 1212RAc	5 / 50 [3]	O/W/O
E4	0.5	0.5 decane	0.0023, DLPC	20	O/W
E5	0.77	0.23 squalane	0.02 Span20+ 0.00045 RAc 1212	10 / 10	O/W and W/O
E6	0.77	0.23 squalane	0.01, Span 20	10	O/W
E7	0.5	0.5 decane	0.025, Lecithin	10 aggregates	W/O

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

Eliminado: [a] Chemicals ships and integration are refereed to the 12,12 RAc compound. Similar spectras have been obtained for the homologue 14,14,RAc. Assignments corresponding to scheme 1.¶ ¶

Eliminado: Salto de página 3: ... [269]

Eliminado: ABLE 3¶ ¶

Eliminado: Table 3:

Eliminado: ¶

Eliminado:) of the XXR compounds¶ ¶

Eliminado: ¶ ¶ ... [270]

Con formato: Inglés (Reino Unido)

Tabla con formato

Eliminado: ¶ ... [271]

Eliminado: ¶ ... [272]

Eliminado: ¶ ... [273]

Eliminado: ¶ ... [274]

Eliminado: ¶

Eliminado: ¶

Eliminado: ¶ ... [275]

Eliminado:

Eliminado: ¶ ¶ ... [276]

Eliminado: ¶ ... [277]

Eliminado: ¶ ... [278]

Eliminado:

Eliminado: ¶ ... [279]

Eliminado: ¶ ... [280]

Eliminado: ¶ ... [281]

Eliminado: ¶ ... [282]

Eliminado: ¶ ... [283]

Eliminado: ¶ ... [284]

Eliminado: ¶ …¶ ... [285]

Table 5. Hemolytic activity

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

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

Eliminado: ¶
¶
Eliminado: 4:
Eliminado: ABLE 4¶
¶
Eliminado: ¶
¶
Table 4:
Eliminado:
Eliminado: of the XXR compounds
Con formato: Centrado, Interlineado: sencillo
Tabla con formato
Con formato: Interlineado: sencillo
Eliminado: ,
Eliminado: E
Con formato: Centrado, Interlineado: sencillo
Con formato: Interlineado: sencillo
Eliminado: ,… (1,9) … [286]
Con formato: Sin Elevado / Disminuido
Con formato: Normal, Centrado, Interlineado: sencillo
Eliminado: I
Con formato … [287]
Eliminado:
Con formato … [288]
Eliminado: ,… … [289]
Eliminado:
Con formato … [290]
Eliminado: ,… … [291]
Con formato … [292]
Con formato … [293]
Eliminado: Moderad … [294]
Eliminado: Clasificación
Con formato … [295]

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 25ºC, (▲)1212RAc 40ºC, (▼)1414RAc 25ºC, (♦)1414RAc 40ºC.

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

Figure 4. a) polarized microscopy of lamellar liquid crystals for the 1414RAc/H_2O system. b) Polarized microscopy of vesicles for the 1212RAc/H_2O system

Eliminado: ¶
¶

Eliminado: Table

Eliminado: ABLE

Eliminado: 5:

Eliminado: ¶

Eliminado: Composition of the tested emulsions

Eliminado: ¶
Sample code ... [296]

Tabla con formato

Con formato: Inglés (Reino Unido)

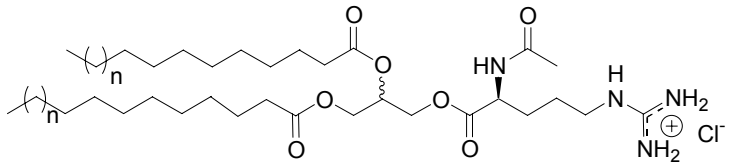
Con formato: Inglés (Reino Unido)

Eliminado: Salto de página ¶
¶

Eliminado: ¶

Eliminado: ¶

Figure 1



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

Eliminado: ¶

Con formato: Normal, Interlineado: sencillo

Con formato: Fuente: Negrita

Figure 2

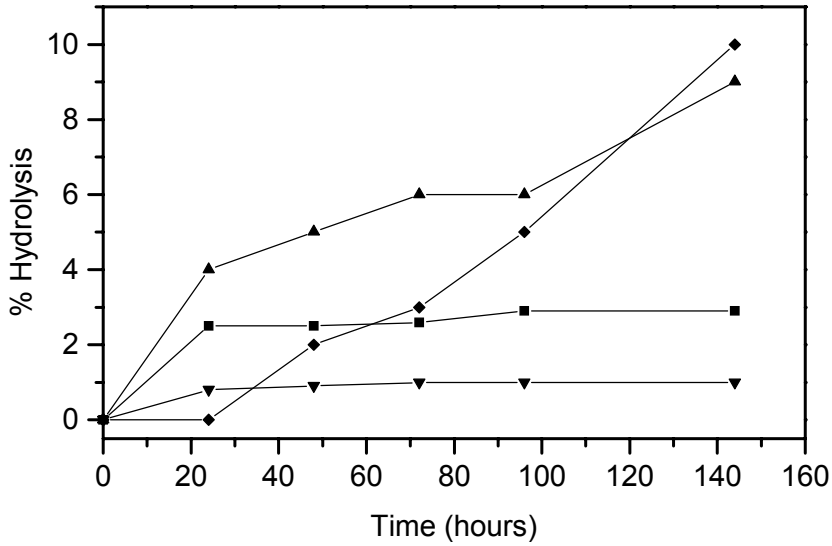
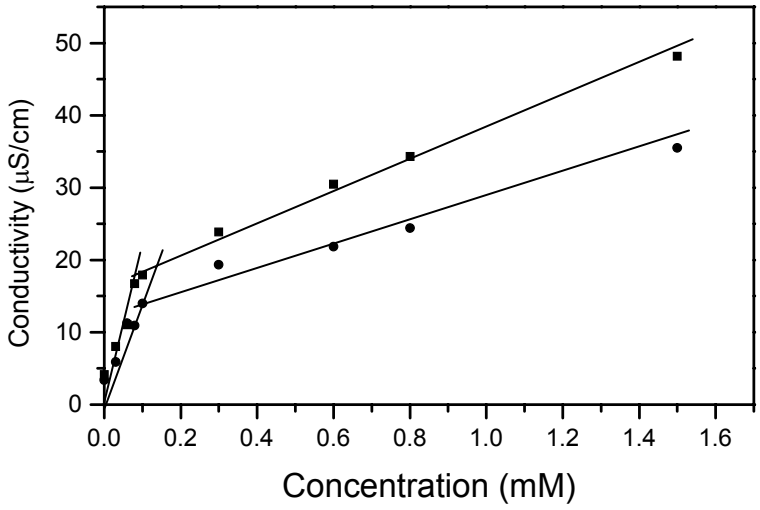


Figure 3



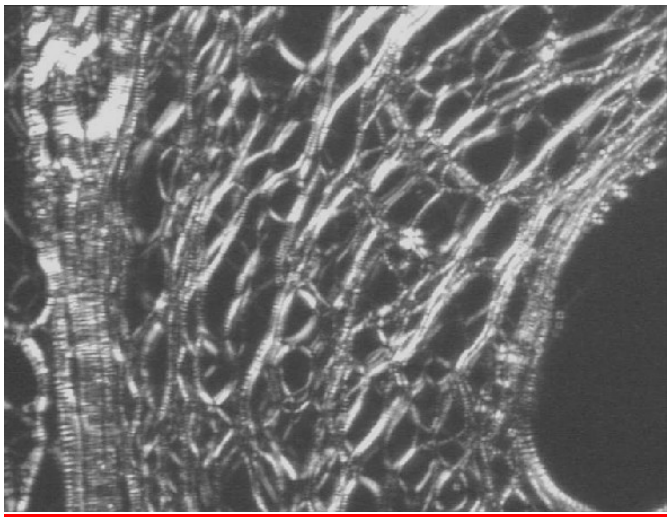
Con formato: Fuente: Negrita

Con formato: Centrado

Con formato: Izquierda

Figure 4

a



b

Con formato: Fuente: Negrita

Con formato: Fuente: 16 pt

Con formato: Centrado

Con formato: Fuente: 16 pt

Con formato: Izquierda

Con formato: Fuente: 16 pt

Con formato: Centrado

Con formato: Centrado

Eliminado: REFERENCES¶
¶
<#>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.¶
¶
(2) Hopewell, P.C. and Murray, J. (1977) In Respiratory Emergencies, Shibel, E.M. and Moser, K.M. (eds.), pp 101-128, C.V. Mosby, St. Louis.¶
¶
(3) Rinaldo, J.E. and Rogers, R.M. (1982) N. Engl. J. Med. 306, 900-909.¶
¶
(4) Holm, B.A. and Notter, R.H. (1989) in Surfactant Replacement Therapy. Shapiro, D.L. and Notter, R.H., eds., pp. 273-304, A.R. Liss, New York).¶
(5) (Notter, R.H. and Shapiro, D.L. (1987) Clinics in Perinatology, 14, 433-479.¶
¶
(6) Notter, R. H. (1989) The physical chemistry and physiological activity of pulmonary surfactants. in Surfactant Replacement Therapy (Shapiro, D.L. and Notter, R.H., eds.) pp. 19 -70, A.R. Liss, New York.¶
¶
(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.).¶
¶
(8) (Perez et al. in preparation)¶
¶
¶
(12) A. Pinazo, M.R. Infante, S.Y. Park, E.I. Franses. Colloids and Surfaces B: Biointerfaces 8 (1996) 1-11. ¶
¶
¶
... [297]

Página 3: [1] Eliminado **Man209** **28/02/2003 14:08:00**

R

Página 3: [1] Eliminado **Man209** **03/03/2003 11:27:00**

has been intense

Página 3: [1] Eliminado **Man209** **28/02/2003 15:27:00**

benign to the environment

Página 3: [2] Eliminado **Man209** **28/02/2003 15:27:00**

. A

Página 3: [2] Eliminado **Man209** **28/02/2003 15:28:00**

in one molecule

Página 3: [2] Eliminado **Man209** **28/02/2003 15:28:00**

such as

Página 3: [3] Con formato **Man209** **18/03/2003 11:49:00**

Color de fuente: Automático, Sin Superíndice / Subíndice

Página 3: [4] Eliminado **Man209** **12/03/2003 15:52:00**

[

Página 3: [4] Eliminado **Man209** **12/03/2003 15:52:00**

]

Página 3: [5] Con formato **Man209** **18/03/2003 11:49:00**

Color de fuente: Automático

Página 3: [6] Con formato **Man209** **18/03/2003 11:49:00**

Color de fuente: Automático, Sin Superíndice / Subíndice

Página 3: [6] Con formato **Man209** **18/03/2003 11:49:00**

Color de fuente: Automático, Sin Superíndice / Subíndice

Página 3: [7] Eliminado **RMN** **04/03/2003 12:22:00**

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.

(3) Seguer, J., Infante, MR., Allouch, M., Mansuy, L., Selve, C., Vinardell, P., *"Synthesis and evaluation of non-ionic amphiphilic compounds from aminoacids: molecular mimics of lecithins"* New J. Chem., 18, 765-774,1994,

(4) Pérez L, Torres JL, Manresa A, Solan C, Infante MR. *"Synthesis, aggregation and biological properties of a new class of gemini cationic amphiphilic compounds from arginine, bis(Args)"*. Langmuir 12:5296-5301, 1996.

(5) L.Pérez, M.T. Garcia, I.Ribosa, M.P. Vinardell, A. Manresa, M.R Infante "Biological properties of arginine-based gemini cationic surfactants". Environmental Toxicology and Chemistry, 21(6): 1279-1285, 2002.

(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.

Página 3: [8] Con formato **Lourdes1** **06/03/2003 13:54:00**

Color de fuente: Automático, Inglés (Reino Unido)

Página 3: [9] Con formato **Man209** **18/03/2003 11:50:00**

Color de fuente: Automático

Página 3: [10] Eliminado **RMN** **04/03/2003 12:25:00**

(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.

(8) Michael Schneider “Phospholipids” in *Lipid Technologies and Applications* pp: 51-78, Ed. F.D. Gunstone, F.B. Padley, Marcel Dekker, Inc. New York, 1997.

With this aim, our group has synthesied 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 probed to have high biodegradability, low toxicity and antimicrobial activity.

Environmentally friendly Ssurfactants that mimic natural amphiphatic structures such as lipoaminoacids, glicolipids and glyicerolipids 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 aditives. 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.

Página 3: [12] Eliminado . 17/12/2002 15:39:00

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

Página 3: [13] Eliminado aurora 09/12/2002 12:11:00

(CAL UNA REFERENCIAReferencia)

Página 3: [14] Eliminado . 17/12/2002 15:39:00

. Unfortunately their water solubility

Página 3: [15] Eliminado . 17/12/2002 15:40:00

is small and they are expensive.

Página 3: [16] Eliminado Man209 28/02/2003 15:32:00

Designi

Página 3: [16] Eliminado Man209 28/02/2003 15:32:00

ng

Página 3: [16] Eliminado Man209 28/02/2003 15:32:00

zing

Página 3: [16] Eliminado Man209 28/02/2003 15:32:00

which

Página 3: [16] Eliminado Man209 30/10/2003 16:18:00

both glycerides and

Página 3: [16] Eliminado Man209 28/02/2003 15:33:00

e

Página 3: [17] Eliminado Man209 28/02/2003 15:34:00

great accomplishment

Página 3: [18] Eliminado . 17/12/2002 15:30:00

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

Página 3: [19] Eliminado . 17/12/2002 15:30:00

and diglyceride bicationic structures []

Página 3: [20] Eliminado . 17/12/2002 15:30:00

cases they have probed to have

Página 3: [21] Eliminado . 17/12/2002 15:30:00

low toxicity and antimicrobial activity.

Página 3: [22] Eliminado Man209 28/02/2003 15:35:00

on the

Página 3: [22] Eliminado Man209 19/02/2003 15:34:00

and the properties

Página 7: [23] Eliminado . 14/01/2003 13:47:00

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

analysis of the final compounds were

Página 7: [25] Eliminado Man209 06/02/2003 13:55:00

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 00RAc 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

l choride for 1212RAc and myristoy

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

l 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

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 to10 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

Sin Superíndice / Subíndice

Página 10: [39] Con formato Man209 18/03/2003 11:54:00

Color de fuente: Automático

Página 10: [40] Eliminado . 10/12/2002 12:02:00

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

(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

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 H_2O) 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

easurements 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

***ualitative 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 H_2O) 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 superiorshigher 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 Tthe main peak (retention time 20.61 min). Isolation of thhe main peak was isolated in two steps. fFirst we applied chromatography possible by chromatography with aon 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 smallminor peak correspondsed to the positional isomer 1,3-diacyl-2-acetylarginyl-rac-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**

13)

Página 13: [83] Con formato	**Man209**	**18/03/2003 11:55:00**

Fuente: Cursiva, Color de fuente: Automático

Página 13: [84] Eliminado	**RMN**	**04/03/2003 12:44:00**

)

(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

[14],

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

Color de fuente: Automático

Página 13: [87] Con formato Man209 04/11/2003 16:38:00

Inglés (Reino Unido)

Página 15: [88] Eliminado RMN 04/03/2003 10:01:00

instead of a cmc,

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

.

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

This needs

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

due to

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

to form micelles

Página 15: [90] Eliminado RMN 04/03/2003 10:06:00

surrounding the dispersed particles

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

the particles with

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

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

Página 15: [91] Con formato **Man209** **18/03/2003 11:56:00**
Color de fuente: Automático, Inglés (Reino Unido)

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

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

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

Página 15: [93] Con formato **Man209** **18/03/2003 11:56:00**
Color de fuente: Automático, Inglés (Reino Unido)

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

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

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

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

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

Página 15: [96] Eliminado **RMN** **04/03/2003 16:27:00**

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

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

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

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

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

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

Página 15: [99] Con formato Man209 18/03/2003 11:56:00
Color de fuente: Automático, Inglés (Reino Unido)

Página 15: [100] Eliminado Man209 06/02/2003 16:36:00
warming the sample up to ---ºC lamellar liquid crystals become apparent

Página 15: [100] Eliminado Man209 17/03/2003 12:18:00

Página 15: [100] Eliminado Man209 12/03/2003 16:16:00

Página 15: [101] Con formato Man209 18/03/2003 11:56:00
Color de fuente: Automático, Inglés (Reino Unido)

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

Página 17: [102] Eliminado Man209 10/02/2003 12:47:00
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 lecythins.

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 25ºC 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 50ºC. On the other hand, when we add to the solution a low volume of organic solvent (ethanol o DMF), the hydrolysis is also higher du 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 week 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 hydrolisehydrolyzed.

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

l

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

XX

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

compounds

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

exhibited

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

all

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

e

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

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

n

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

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**

,

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**

a

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

compound

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

possess antimicrobial activity

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

the

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

i

Página 18: [114] Eliminado **Man209** **14/03/2003 10:49:00**

to achieve a

Página 18: [115] Eliminado **RMN** **04/03/2003 10:45:00**

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**

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**

, because of that, the solubility play a important role in the antimicrobial properties.

Página 18: [117] Eliminado **RMN** **04/03/2003 10:47:00**

is also noticeable

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

Taking into account

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

that

Página 18: [118] Eliminado **RMN** **04/03/2003 10:48:00**

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

s

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

is

Página 18: [120] Eliminado **RMN** **04/03/2003 10:48:00**

for the 1212RAc and

Página 18: [121] Eliminado **Man209** **10/03/2003 14:00:00**

Página 18: [121] Eliminado **Man209** **10/03/2003 14:00:00**

Página 19: [122] Con formato **Man209** **18/03/2003 11:58:00**

Sin Expandido / Comprimido

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

Sin Expandido / Comprimido

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

Color de fuente: Automático, Sin Expandido / Comprimido

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

Sin Expandido / Comprimido

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

Sin Superíndice / Subíndice , Sin Expandido / Comprimido

Página 19: [124] Con formato **Man209** **18/03/2003 11:58:00**

Color de fuente: Automático, Sin Expandido / Comprimido

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

Sin Expandido / Comprimido

Página 19: [126] Eliminado **RMN** **04/03/2003 10:52:00**

For the same alkyl chain lengths,

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

C

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

T

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

are also similar to

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

that

Página 19: [128] Eliminado **RMN** **04/03/2003 10:54:00**

Página 19: [129] Con formato **Man209** **05/11/2003 14:05:00**

Sin Expandido / Comprimido

Página 19: [130] Eliminado **RMN** **04/03/2003 10:55:00**

so that they possess two positive charges in the polar head

Página 19: [131] Con formato **Man209** **05/11/2003 14:05:00**

Color de fuente: Automático, Sin Superíndice / Subíndice , Sin Expandido / Comprimido

Página 19: [132] Eliminado **Man209** **12/03/2003 16:20:00**

[

Página 19: [132] Eliminado **Man209** **12/03/2003 16:37:00**

0

Página 19: [133] Con formato **Man209** **18/03/2003 11:58:00**

Color de fuente: Automático, Sin Expandido / Comprimido

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

Sin Expandido / Comprimido

Página 19: [135] Con formato **Man209** **18/03/2003 11:58:00**

Color de fuente: Automático

Página 19: [136] Eliminado **RMN** **04/03/2003 10:58:00**

From these results we can conclude

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

Sin Expandido / Comprimido

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

n’t

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

of them

Página 19: [139] Eliminado **Lourdes1** **19/12/2002 17:00:00**

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, tIt is well Know that the antimicrobial activity of

homologues surfactants with different alkyl chain length reaches a maximum for a determinated 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 that 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

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

T

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

e known

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

(Ref)

Página 19: [145] Eliminado Man209 14/03/2003 10:53:00

Página 19: [146] Eliminado RMN 04/03/2003 11:01:00

, classifying compounds depending on the damage of the eye

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

A

Página 19: [148] Eliminado RMN 04/03/2003 11:02:00

procedures have been developed

Página 19: [149] Eliminado aurora 23/08/2002 14:36:00

to replace this aggressive methodology.

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

alternatives

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

the

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

[12

Página 19: [151] Con formato **Man209** **05/11/2003 14:09:00**

Color de fuente: Automático

Página 19: [152] Eliminado **.** **10/12/2002 12:32:00**

(CAL UNA REFERENCIA)

Página 19: [153] Eliminado **aurora** **23/08/2002 14:11:00**

present

Página 19: [153] Eliminado **aurora** **23/08/2002 18:21:00**

wo

Página 19: [154] Eliminado **aurora** **23/08/2002 14:11:00**

parts, the

Página 19: [154] Eliminado **aurora** **23/08/2002 14:11:00**

f

Página 19: [154] Eliminado **aurora** **23/08/2002 14:12:00**

is to determine

Página 20: [155] Eliminado **Man209** **17/02/2003 16:33:00**

□□□ÕÀ□□

Salto de columna

□bjbjÏ2Ï2□□□□□□□□□□□□□□□□□□□□

Salto de página

Página 20: [156] Eliminado **Man209** **17/02/2003 16:16:00**

□□□-X□□-X□□¸¸□□□□□□□□□□□□□□□□□□□

Página 20: [157] Eliminado **aurora** **23/08/2002 14:41:00**

□□□□□□□□□□□□ÿÿ□□□□□□□□□□□ÿÿ□□□□□□□□□□□ÿÿ□□□□□□□□□□□

□□□□□□□ˆ□□□□□p□□□□□□□p□□□p□□□□□□□p□□□□□□□p□□□□□□□

p□□□□□□□p□□□□□□□□□□□□□□□z□□□□□□□fŠ□

Página 20: [158] Eliminado **Man209** **17/02/2003 16:17:00**

□□□□□□fŠ□□8□□□□Š□□ì□50 hemolysis and the DI is called

Página 20: [159] Eliminado **.** **10/12/2002 12:32:00**

Página 20: [159] Eliminado . 10/12/2002 12:32:00

Página 20: [160] Eliminado aurora 23/08/2002 18:33:00

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

ing

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

in

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

the

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

s

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

Hence

Página 20: [163] Eliminado Man209 10/03/2003 14:12:00

, some of the surfactants currently on

Página 20: [164] Eliminado Man209 10/03/2003 14:11:00

the market, mainly the cationic surfactants are classified as irritant compounds.

Página 20: [165] Con formato Man209 05/11/2003 14:12:00

Color de fuente: Automático

Página 20: [166] Con formato Man209 18/03/2003 11:59:00

Color de fuente: Automático, Superíndice

Página 20: [167] Con formato Man209 18/03/2003 11:59:00

Color de fuente: Automático

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

that

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

t

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

similar

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

Sin Superíndice / Subíndice

Página 20: [170] Con formato **Man209** **18/03/2003 11:59:00**

Color de fuente: Automático

Página 20: [171] Eliminado **RMN** **04/03/2003 13:39:00**

15

Página 20: [171] Eliminado **RMN** **04/03/2003 11:08:00**

hose

Página 20: [172] Con formato **Man209** **18/03/2003 11:59:00**

Color de fuente: Automático

Página 20: [173] Con formato **Man209** **18/03/2003 11:59:00**

Color de fuente: Automático

Página 20: [174] Eliminado **Man209** **10/03/2003 14:15:00**

also derived also from

Página 20: [175] Eliminado **RMN** **04/03/2003 11:10:00**

Taking into account

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

due

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

ve

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

similar

Página 20: [176] Eliminado **RMN** **04/03/2003 11:12:00**

the

Página 21: [177] Eliminado **Man209** **18/03/2003 11:59:00**

Página 21: [177] Eliminado **Man209** **19/02/2003 11:57:00**

cutt

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

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 lab[1] [8,12] a. This hemolytic activity nd 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 thein 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 hemolityc 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 μmg/mLL)[21] [16] whereas 1212RAc and 1414RAc only these surfactants show hemolytic effects at concentrations higher than ranges between 6.019-60 μmg/mL and . 1.9 μg/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
igure 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 …

Página 21: [189] Eliminado . 17/12/2002 16:30:00
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

Página 21: [190] Eliminado . 10/12/2002 13:48:00
of the polar group 1212RAc and 1414RAc that

Página 21: [191] Eliminado aurora 23/08/2002 19:12:00
the new compounds

Página 21: [192] Eliminado . 10/12/2002 13:50:00
increases the solubility

Página 21: [193] Eliminado aurora 23/08/2002 19:14:00
due to the cationic character of the polar group

Página 21: [194] Eliminado . 10/12/2002 13:50:00
. The solubility in monomers in water for XXRAc is ------ while for dilauroyl phosphatidylcoline is----- .

Optical microscopy

Página 21: [195] Eliminado . 17/12/2002 16:30:00
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

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

Página 21: [196] Con formato Lourdes1 06/03/2003 13:54:00
Inglés (Reino Unido)

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

Critical micellar concentration

The conductance of aqueous solutions of 1212RAc and 1414RAc

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

Página 21: [199] Eliminado . 17/12/2002 16:30:00
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
structure type. However,

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

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

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

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

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

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

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

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

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

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

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

Fuente: Sin Cursiva

Página 24: [205] Con formato	**Man209**	**05/11/2003 13:50:00**

Inglés (Reino Unido)

Página 24: [205] Con formato	**Man209**	**05/11/2003 13:50:00**

Inglés (Reino Unido)

Página 24: [205] Con formato	**Man209**	**05/11/2003 13:50:00**

Fuente: Sin Cursiva, Inglés (Reino Unido)

Página 24: [205] Con formato	**Man209**	**05/11/2003 13:50:00**

Inglés (Reino Unido)

Página 24: [205] Con formato	**Man209**	**05/11/2003 13:50:00**

Inglés (Reino Unido)

Página 24: [205] Con formato	**Man209**	**05/11/2003 13:50:00**

Inglés (Reino Unido)

Página 24: [206] Con formato	**Man209**	**05/11/2003 14:05:00**

Inglés (Reino Unido)

Página 24: [206] Con formato	**Man209**	**05/11/2003 14:05:00**

Fuente: Sin Cursiva, Inglés (Reino Unido)

Página 24: [206] Con formato	**Man209**	**05/11/2003 14:05:00**

Inglés (Reino Unido)

Página 24: [206] Con formato	**Man209**	**05/11/2003 14:05:00**

Inglés (Reino Unido)

Página 24: [206] Con formato	**Man209**	**05/11/2003 14:05:00**

Inglés (Reino Unido)

Página 24: [206] Con formato	**Man209**	**05/11/2003 14:05:00**

Inglés (Reino Unido)

Página 24: [207] Con formato	**Man209**	**05/11/2003 14:06:00**

Inglés (Reino Unido)

Página 24: [207] Con formato	**Man209**	**05/11/2003 14:06:00**

Fuente: Sin Cursiva

Página 24: [208] Eliminado	**Man209**	**30/10/2003 15:55:00**

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.

(3) Seguer, J., Infante, MR., Allouch, M., Mansuy, L., Selve, C., Vinardell, *P., "Synthesis and evaluation of non-ionic amphiphilic compounds from aminoacids: molecular mimics of lecithins" New J. Chem.*, **18**, 765-774,1994,

(4) Pérez L, Torres JL, Manresa A, Solan C, Infante MR. *"Synthesis, aggregation and biological properties of a new class of gemini cationic amphiphilic compounds from arginine, bis(Args)". Langmuir* **12**:5296-5301, 1996.

(5) L.Pérez, M.T. Garcia, I.Ribosa, M.P. Vinardell, A. Manresa, M.R Infante "Biological properties of arginine-based gemini cationic surfactants". *Environmental Toxicology and Chem*istry, **21(6):** 1279-1285, 2002.

(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 Chem*istry, **26**: 1221-1227, 2002.

(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.

(8) Michael Schneider "Phospholipids" in *Lipid Technologies and Applications* pp: 51-78, Ed. F.D. Gunstone, F.B. Padley, Marcel Dekker, Inc. New York, 1997.

(9) Morán, C., Infante, M.R., Clapés, P., "Synthesis of glycero amino acid-based surfactants. Part 1. Enzimatic preparation of rac-1-O-(N^{α}-acetil-L-aminoacyl)glycerol derivatives" *J.Chem.Soc., Perkin Trans. 1*:2063-2070, 2001.

Página 24: [209] Con formato Man209 17/03/2003 13:50:00

Fuente: Cursiva, Color de fuente: Automático

Página 24: [209] Con formato Man209 17/03/2003 13:50:00

Color de fuente: Automático

Página 24: [209] Con formato Man209 17/03/2003 13:50:00

Fuente: Cursiva, Color de fuente: Automático

Página 24: [209] Con formato Man209 17/03/2003 13:50:00

Color de fuente: Automático

Página 24: [209] Con formato Man209 17/03/2003 13:50:00

Fuente: Cursiva, Color de fuente: Automático

Página 24: [209] Con formato Man209 17/03/2003 13:50:00

Color de fuente: Automático

Página 24: [209] Con formato	**Man209**	**17/03/2003 13:50:00**

Fuente: Cursiva, Color de fuente: Automático

Página 24: [209] Con formato	**Man209**	**17/03/2003 13:50:00**

Color de fuente: Automático

Página 24: [209] Con formato	**Man209**	**17/03/2003 13:54:00**

Fuente: Negrita, Color de fuente: Automático

Página 24: [209] Con formato	**Man209**	**17/03/2003 13:50:00**

Color de fuente: Automático

Página 24: [209] Con formato	**Man209**	**18/03/2003 12:05:00**

Inglés (Reino Unido)

Página 24: [209] Con formato	**Man209**	**18/03/2003 12:05:00**

Color de fuente: Automático, Inglés (Reino Unido)

Página 24: [209] Con formato	**Man209**	**18/03/2003 12:05:00**

Color de fuente: Automático

Página 24: [209] Con formato	**Man209**	**18/03/2003 12:05:00**

Fuente: Cursiva, Color de fuente: Automático

Página 24: [209] Con formato	**Man209**	**18/03/2003 12:05:00**

Fuente: Negrita, Color de fuente: Automático

Página 24: [209] Con formato	**Man209**	**18/03/2003 12:05:00**

Color de fuente: Automático

Página 24: [209] Con formato	**Man209**	**18/03/2003 12:05:00**

Inglés (Reino Unido)

Página 24: [209] Con formato	**Man209**	**18/03/2003 12:05:00**

Color de fuente: Automático

Página 24: [209] Con formato	**Man209**	**18/03/2003 12:05:00**

Fuente: Cursiva, Color de fuente: Automático

Página 24: [209] Con formato	**Man209**	**17/03/2003 13:50:00**

Color de fuente: Automático

Página 24: [209] Con formato	**Man209**	**17/03/2003 13:54:00**

Fuente: Negrita, Color de fuente: Automático

Página 24: [209] Con formato	**Man209**	**17/03/2003 13:50:00**

Color de fuente: Automático

Página 24: [209] Con formato	**Man209**	**17/03/2003 13:50:00**

Fuente: Cursiva, Color de fuente: Automático

Página 24: [209] Con formato	**Man209**	**17/03/2003 13:50:00**

Color de fuente: Automático

Página 24: [209] Con formato	**Man209**	**17/03/2003 14:01:00**

Fuente: Negrita, Color de fuente: Automático

Página 24: [209] Con formato	**Man209**	**17/03/2003 13:50:00**

Color de fuente: Automático

Página 24: [209] Con formato	**Man209**	**17/03/2003 13:50:00**

Color de fuente: Automático

Página 24: [209] Con formato	**Man209**	**17/03/2003 13:50:00**

Color de fuente: Automático

Página 24: [209] Con formato	**Man209**	**31/10/2003 13:35:00**

Fuente: Cursiva, Color de fuente: Automático

Página 24: [209] Con formato Man209 17/03/2003 13:50:00
Color de fuente: Automático
Página 25: [210] Eliminado Man209 17/03/2003 13:01:00
(10) Laughlin, R.G. (1992) *Adv. Coll. Interface Sci.* **41**, 57-79.

(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, 1980

Página 25: [211] Con formato Man209 17/03/2003 13:50:00
Color de fuente: Automático, Inglés (Reino Unido)
Página 25: [212] Con formato Man209 17/03/2003 13:50:00
Color de fuente: Automático
Página 25: [213] Con formato Man209 17/03/2003 13:50:00
Fuente: Cursiva, Color de fuente: Automático
Página 25: [214] Con formato Man209 17/03/2003 13:50:00
Color de fuente: Automático
Página 25: [215] Con formato Man209 17/03/2003 15:53:00
Fuente: Negrita, Color de fuente: Automático
Página 25: [216] Con formato Man209 17/03/2003 13:50:00
Color de fuente: Automático
Página 25: [217] Eliminado Man209 31/10/2003 14:23:00
12 (E. Jungermann, *Cosmetic Sci. Technol. Ser.*, 1991, **11** (Glycerine), 97-112)

13 (Ref: Laughlin, R.G., *The Aqueous Phase Behaviour of Surfactants*, Academic Press, New York (1972).

14 (Rosen, *M. J. (1978). Surfactants and Interfacial Phenomena*. John Wiley & Sons, New York.)

15 R.J.M. Tausk, J.Karmiggelt, C. Oudshoorn, J.Th.G.Overbeek, *Biophysical Chemistry* **1** (1974), 175-183.

16 *(Physics of Amphiphiles: Micelles,Vesicles and Microemulsions*, pg 34, 1985, Editors V. Degiorgio and M. Corti, Editorial Elsevier Science Publishers, Amsterdam)

17 Bancroft's rule [17][19] B.P. Binks " Emulsions, Recent Advatages in Understanding" in *Modern Aspects of Emulsion Scince* Ed. B.P. Binks, The Royal Society of Chemistry, Canbridge, 1998.

19. C.Morán, P.Clapés, F.Comelles, T.García, L.Pérez, P. Vinardell, M.Mitjans, M.R.Infante, *Langmuir*, 2001, 5071-5075

20. L.Pérez, A.Pinazo, P.Vinardell, P.Clapés, M.Angelet, M.R. Infante*, New J. Chem.,* 2002, **26**, 1221-1227.

[21] Pape WJW, Pfannenbecker U, Hopper U. "Validation of the red blood cell test system as in vitro assay for the rapid screening of irritation potential of surfactants" . Mol Toxicol **1**: 525-536, 1987.

[22] Pape WJW, Hopper U. "Standardisation of an in vitro red blood cell test for evaluating the acute cytoxic potential of tensides" *Drug. Res.* **4**:498-502, 1990.

[23] Okamoto, Y. Ohkoshi, K., Itagaki, H., Tsuda, T., Kakishima, H., Ogawa, T., Kasai, Y., Ohuchi, J., Kojima, H., Kurishita, A., Kaneko, T., Matsushima, Y., Iwabuchi, Y., Oho, Y*. "Interlaborator validation of the in vitro eye irritation tests for cosmetic ingredints. (3) evaluation of the haemolysis test"*. *Toxicol. in vitro*, **13**:115-124, 19

Página 25: [218] Con formato **Man209** **17/03/2003 13:50:00**
Color de fuente: Automático

Página 25: [219] Con formato **Man209** **17/03/2003 13:50:00**
Fuente: Cursiva, Color de fuente: Automático

Página 25: [220] Con formato **Man209** **17/03/2003 13:50:00**
Color de fuente: Automático

Página 25: [221] Con formato **Man209** **17/03/2003 13:50:00**
Color de fuente: Automático

Página 25: [222] Con formato **Man209** **17/03/2003 13:50:00**
Fuente: Cursiva, Color de fuente: Automático

Página 25: [223] Con formato **Man209** **17/03/2003 13:50:00**
Color de fuente: Automático

Página 25: [224] Con formato **Man209** **17/03/2003 15:53:00**
Fuente: Negrita, Color de fuente: Automático

Página 25: [225] Con formato **Man209** **17/03/2003 13:50:00**
Fuente: Cursiva, Color de fuente: Automático

Página 25: [226] Con formato **Man209** **17/03/2003 13:50:00**
Color de fuente: Automático

Página 25: [227] Con formato **Man209** **17/03/2003 15:54:00**

Fuente: Negrita, Color de fuente: Automático

Página 25: [228] Con formato **Man209** **17/03/2003 13:50:00**

Color de fuente: Automático

Página 25: [229] Con formato **Man209** **17/03/2003 13:50:00**

Fuente: Cursiva, Color de fuente: Automático, Inglés (Reino Unido)

Página 25: [230] Con formato **Man209** **17/03/2003 13:50:00**

Color de fuente: Automático, Inglés (Reino Unido)

Página 25: [231] Con formato **Man209** **17/03/2003 13:49:00**

Inglés (Reino Unido)

Página 25: [232] Con formato **Man209** **17/03/2003 13:49:00**

Color de fuente: Automático, Inglés (Reino Unido)

Página 25: [233] Con formato **Man209** **17/03/2003 13:49:00**

Color de fuente: Automático

Página 25: [234] Con formato **Man209** **17/03/2003 14:06:00**

Fuente: Cursiva, Color de fuente: Automático

Página 25: [235] Con formato **Man209** **17/03/2003 14:06:00**

Color de fuente: Automático

Página 25: [236] Con formato **Man209** **05/11/2003 14:12:00**

Fuente: Sin Cursiva

Página 25: [237] Con formato **Man209** **05/11/2003 14:12:00**

Fuente: Negrita, Sin Cursiva

Página 25: [238] Con formato **Man209** **05/11/2003 14:12:00**

Fuente: Sin Cursiva

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**

REFERENCES

[1]

Página 26: [243] Eliminado **RMN** **04/03/2003 13:46:00**

Balzer, D., "Alkyl Polyglucosides" in *Specialist Surfactants* pg

Página 26: [244] Eliminado aurora 09/12/2002 11:15:00

Wolfgang von Rybinski and Karlheinz in Novel Surfactants, Surfactant Science Series vol 74, edited by Krister Holmberg, Marcel Deker , 1998.)

Página 26: [245] Eliminado RMN 04/03/2003 13:46:00

Ed. I.D. Robb, Blackie Academic & Profesional, 1997, London.

Página 26: [246] Eliminado RMN 04/03/2003 13:46:00

[2] Jiding Xia, Ifendu A. Nnanna, Kazutami Sakamoto "Amino Acid Surfactants" in Protein-Based Surfactants: Synthesis, Physicochemical Properties and Applications" pp: 75-122, Ed. Ifendu A.Nnanna, Jiding Xia,

Página 26: [247] Eliminado aurora 09/12/2002 11:59:00

Novel Cationic Surfactants from Arginine pg 8, in Novel Surfactants,

Página 26: [248] Eliminado RMN 04/03/2003 13:46:00

Surfactant Science Series vol 101

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

Página 26: [251] Eliminado RMN 04/03/2003 13:46:00

).

Página 26: [252] Eliminado Man209 19/02/2003 16:42:00

[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.

[4] Michael Schneider “Phospholipids” in *Lipid Technologies and Applications* pp: 51-78, Ed. F.D. Gunstone, F.B. Padley, Marcel Dekker, Inc. New York, 1997.

[5] Seguer, J., Infante, MR., Allouch, M., Mansuy, L., Selve, C., Vinardell, P., *“Synthesis and evaluation of non-ionic amphiphilic compounds from aminoacids: molecular mimics of lecithins”* New J. Chem., 18, 765-774,1994,

[6] Pérez L, Torres JL, Manresa A, Solan C, Infante MR. *“Synthesis, aggregation and biological properties of a new class of gemini cationic amphiphilic compounds from arginine, bis(Args)”*. Langmuir 12:5296-5301, 1996.

[7] L.Pérez, M.T. Garcia, I.Ribosa, M.P. Vinardell, A. Manresa, M.R Infante “Biological properties of arginine-based gemini cationic surfactants”. Environmental Toxicology and Chemistry, 21(6): 1279-1285, 2002.

[8] 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.

Página 26: [253] Eliminado Man209 17/03/2003 16:48:00

Data of 1,2-Diacyl 3-acetylarginyl-glycerols

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)

Página 26: [255] Eliminado Man209 19/02/2003 16:46:00

[9] Morán, C., Infante, M.R., Clapés, P., “Synthesis of glycero amino acid-based surfactants. Part 1. Enzimatic preparation of rac-1-O-(N$^{\alpha}$-acetil-L-aminoacyl)glycerol derivatives” J.Chem.Soc., Perkin Trans. 1:2063-2070, 2001.

Página 26: [256] Eliminado Man209 19/02/2003 17:00:00

[10] 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, 1980.

Página 26: [257] Eliminado Man209 19/02/2003 16:49:00

[11] Lawrence, A.C.S. Chem.Ind., 1961. 1764

Página 26: [258] Eliminado RMN 04/03/2003 13:46:00

[12] Morán, C., Clapés, P., Comelles, F., García, T., Pérez, L., Vinardell, P., Mitjans, M., Infante, M.R. *“Chemical structure/properties relationship in single-chain arginine surfactants”* Langmuir,17, 5071-5075, 2001.

[13] *Pape WJW, Pfannenbecker U, Hopper U. “Validation of the red blood cell test system as in vitro assay for the rapid screening of irritation potential of surfactants” . Mol Toxicol 1: 525-536, 1987.*

[14] *Pape WJW, Hopper U. “Standardisation of an in vitro red blood cell test for evaluating the acute cytoxic potential of tensides”* Drug. Res. 4:498-502, 1990.

[15] Betainas

[16] Okamoto et al., 1999, Okamoto, Y. Ohkoshi, K., Itagaki, H., Tsuda, T., Kakishima, H., Ogawa, T., Kasai, Y., Ohuchi, J., Kojima, H., Kurishita, A.,

Kaneko, T., Matsushima, Y., Iwabuchi, Y., Oho, Y. *"Interlaborator validation of the in vitro eye irritation tests for cosmetic ingredints. (3) evaluation of the haemolysis test"*. Toxicol. in vitro, 13:115-124, 1999.

[17] Tausk R.J.M., Karmiggelt, J., Oudshoorn C., Overbeek J. Th. G., Biophysical Chemistry 1: 175-183 (1974).

[18] A. Pinazo, X. Wen, Y-C. Liao, A.J. Prosser, E.Franses, " A comparison of DLPC and DPPC in controling the dynamic adsorption and surface tension of their aqueous dispersions", Langmuir, 2003. (in press)

[19] B.P. Binks " Emulsions, Recent Advatages in Understanding" in *Modern Aspects of Emulsion Scince* Ed. B.P. Binks, The Royal Society of Chemistry, Canbridge, 1998.

Salto de página

Página 26: [259] Con formato **Lourdes1** **06/03/2003 14:14:00**
Inglés (Reino Unido)

Página 26: [259] Con formato **Lourdes1** **06/03/2003 14:14:00**
Inglés (Reino Unido)

Página 26: [260] Eliminado **.** **11/12/2002 15:25:00**
[3] Y. Okata, S. Tanamachi, M. Nagai and Kunitake, J. Colloid Int. Sci. 82: 401 (1981).

[4] M.R. Infante, J.J. Garcia Dominguez, P. Erra, M.R. Julia and M. Prats, Int. J. Cosm. Sci. 6: 275 (1984).

[5] L. Perez, J.L. Torres, A. Manresa, C. Solans and M.R. Infante, Langmuir 12: 5296 (1996).

[6] M.R. Infante, J.Seguer, A. Pinazo Colloids and Surfaces 49:123(1997).

[7] Kalyanasundaram, K.; Thomas, J. K., J. Am. Chem. Soc. 99: 2039 (1977).

[8] Rendall, K., G.J.T. Tidyy and M.A. Trevetham, J. Chem. Soc. Faraday Trans. I 79:673 (1983).

[9] Jones, R.N., Barry, A. L., Gavan, T. L., Washintong, J. A., II Manual of Clinical Microbiology; Lennette, E.H., Ballows, A., Hauser, W.J., Eds.; American Society for Microbiology: Washington, DC, 1985; p 972.

[10] Tausk R.J.M., Karmiggelt, J., Oudshoorn C., Overbeek J. Th. G., Biophysical Chemistry 1: 175-183 (1974).

[11] Clint, J. H. and T. Walker J. Chem. Soc. Faraday Trans. 1, 71: 946 (1975).

[12] Surfactants and Interfacial Phenomena, Milton J. Rosen, 1988, John Wiley and Sons.

[13] Helmut Hauser, BBA 1508: 164-181 (2000).

[14] M. Diz, A. Manresa, A. Pinazo, P. Erra and M.R. Infante, J.Chem. Perkin Trans 2, 8: 1871 (1994).

Página 26: [263] Eliminado	**aurora**	**23/08/2002 18:38:00**

Salto de página

Página 26: [264] Eliminado	**.**	**11/12/2002 15:26:00**

Página 26: [265] Con formato **Man209** **17/03/2003 16:40:00**

Centrado

Página 26: [266] Cambio **Man209** **17/03/2003 16:42:00**

Tabla con formato

Página 26: [267] Eliminado **aurora** **23/08/2002 21:17:00**

Página 26: [268] Eliminado **aurora** **23/08/2002 21:06:00**

m

Página 26: [268] Eliminado **aurora** **23/08/2002 21:06:00**

w

Página 29: [269] Eliminado **Man209** **05/11/2003 13:57:00**

Salto de página

Página 29: [269] Eliminado **Man209** **05/11/2003 13:57:00**

3:

Página 29: [270] Eliminado **RMN** **04/03/2003 13:47:00**

Página 29: [270] Eliminado **RMN** **04/03/2003 13:47:00**

Página 29: [270] Eliminado **RMN** **04/03/2003 13:47:00**

Página 29: [271] Eliminado **RMN** **04/03/2003 13:47:00**

Página 29: [271] Eliminado **RMN** **04/03/2003 13:47:00**

Página 29: [271] Eliminado	RMN	04/03/2003 13:47:00
Página 29: [272] Eliminado	RMN	04/03/2003 13:47:00
Página 29: [272] Eliminado	RMN	04/03/2003 13:47:00
Página 29: [272] Eliminado	RMN	04/03/2003 13:47:00
Página 29: [273] Eliminado	RMN	04/03/2003 13:47:00
Página 29: [273] Eliminado	RMN	04/03/2003 13:48:00
Página 29: [273] Eliminado	RMN	04/03/2003 13:48:00
Página 29: [274] Eliminado	RMN	04/03/2003 13:48:00
Página 29: [274] Eliminado	RMN	04/03/2003 13:48:00
Página 29: [274] Eliminado	RMN	04/03/2003 13:48:00
Página 29: [275] Eliminado	RMN	04/03/2003 13:48:00
Página 29: [275] Eliminado	RMN	04/03/2003 13:48:00
Página 29: [276] Eliminado	RMN	04/03/2003 13:48:00
Página 29: [276] Eliminado	RMN	04/03/2003 13:48:00
Página 29: [276] Eliminado	RMN	04/03/2003 13:48:00

Página 29: [277] Eliminado	RMN	04/03/2003 13:48:00
Página 29: [277] Eliminado	RMN	04/03/2003 13:48:00
Página 29: [277] Eliminado	RMN	04/03/2003 13:48:00
Página 29: [278] Eliminado	RMN	04/03/2003 13:48:00
Página 29: [278] Eliminado	RMN	04/03/2003 13:48:00
Página 29: [278] Eliminado	RMN	04/03/2003 13:48:00
Página 29: [279] Eliminado	RMN	04/03/2003 13:48:00
Página 29: [279] Eliminado	RMN	04/03/2003 13:48:00
Página 29: [279] Eliminado	RMN	04/03/2003 13:48:00
Página 29: [280] Eliminado	RMN	04/03/2003 13:48:00
Página 29: [280] Eliminado	RMN	04/03/2003 13:48:00
Página 29: [280] Eliminado	RMN	04/03/2003 13:48:00
Página 29: [281] Eliminado	RMN	04/03/2003 13:48:00
Página 29: [281] Eliminado	RMN	04/03/2003 13:48:00
Página 29: [281] Eliminado	RMN	04/03/2003 13:48:00
Página 29: [282] Eliminado	RMN	04/03/2003 13:48:00

Página 29: [282] Eliminado	**RMN**	**04/03/2003 13:49:00**
Página 29: [282] Eliminado	**RMN**	**04/03/2003 13:49:00**
Página 29: [283] Eliminado	**RMN**	**04/03/2003 13:49:00**
Página 29: [283] Eliminado	**RMN**	**04/03/2003 13:49:00**
Página 29: [283] Eliminado	**RMN**	**04/03/2003 13:49:00**
Página 29: [284] Eliminado	**RMN**	**04/03/2003 13:49:00**
Página 29: [284] Eliminado	**RMN**	**04/03/2003 13:49:00**
Página 29: [284] Eliminado	**RMN**	**04/03/2003 13:49:00**
Página 29: [285] Eliminado	**RMN**	**04/03/2003 16:20:00**
Página 29: [285] Eliminado	**RMN**	**04/03/2003 13:56:00**
Página 29: [285] Eliminado	**RMN**	**04/03/2003 16:20:00**
Página 29: [285] Eliminado	**RMN**	**04/03/2003 16:19:00**
Página 30: [286] Eliminado	**Man209**	**18/03/2003 16:23:00**

,

Página 30: [286] Eliminado	**Man209**	**18/03/2003 16:21:00**

(1,9)

Página 30: [286] Eliminado	**Man209**	**18/03/2003 16:24:00**

,

Página 30: [286] Eliminado **Man209** **18/03/2003 16:21:00**

(0,2)

Página 30: [286] Eliminado **Man209** **18/03/2003 16:26:00**

,

Página 30: [286] Eliminado **Man209** **18/03/2003 16:26:00**

,

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**

,

Página 30: [289] Eliminado **Man209** **18/03/2003 16:24:00**

,

Página 30: [289] Eliminado **Man209** **18/03/2003 16:26:00**

,

Página 30: [289] Eliminado **Man209** **18/03/2003 16:26:00**

,

Página 30: [290] Con formato **Man209** **17/03/2003 16:56:00**

Centrado, Interlineado: sencillo

Página 30: [290] Con formato **Man209** **17/03/2003 16:56:00**

Interlineado: sencillo

Página 30: [291] Eliminado **Man209** **18/03/2003 16:23:00**

,

Página 30: [291] Eliminado **Man209** **18/03/2003 16:24:00**

,

Página 30: [291] Eliminado **Man209** **18/03/2003 16:25:00**

,

Página 30: [291] Eliminado **Man209** **18/03/2003 16:27:00**

,

Página 30: [292] Con formato **Man209** **17/03/2003 16:56:00**

Centrado, Interlineado: sencillo

Página 30: [293] Con formato **Man209** **17/03/2003 16:56:00**

Centrado, Interlineado: sencillo

Página 30: [294] Eliminado aurora 23/08/2002 14:18:00

Moderado

Página 30: [294] Eliminado aurora 23/08/2002 14:17:00

Irritante

Página 30: [294] Eliminado aurora 23/08/2002 14:19:00

Moderado

Página 30: [294] Eliminado aurora 23/08/2002 14:19:00

Moderado

Página 30: [295] Con formato Man209 17/03/2003 16:55:00

Interlineado: sencillo

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

decane aggregates

REFERENCES

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.

(2) Hopewell, P.C. and Murray, J. (1977) In Respiratory Emergencies, Shibel, E.M. and Moser, K.M. (eds.), pp 101-128, C.V. Mosby, St. Louis.

(3) Rinaldo, J.E. and Rogers, R.M. (1982) N. Engl. J. Med. 306, 900-909.

(4) Holm, B.A. and Notter, R.H. (1989) in Surfactant Replacement Therapy. Shapiro, D.L. and Notter, R.H., eds., pp. 273-304, A.R. Liss, New York).

(5) (Notter, R.H. and Shapiro, D.L. (1987) Clinics in Perinatology, 14, 433-479.

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

(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.).

(8) (Perez et al. in preparation)

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