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Synthesis of arginine-based surfactants in highly concentrated water-in-oil emulsions

Aurora Pinazo, Maria R. Infante, Paqui Izquierdo and Conxita Solans *

Departament de Tecnologia de Tensioactius, Institut d'Investigacions Químiques i Ambientals de Barcelona (IIQAB/CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain

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Synthesis of the cationic surfactant N^α-lauroylarginine methyl ester hydrochloride (LAM) has been carried out using highly concentrated water-in-oil emulsions as new reaction media. The influence of reaction time, ArgOMe–dodecanoic acid molar ratio, temperature and the volume fraction of the dispersed phase on reaction yield has been studied. A new HPLC analysis procedure and a MPLC purification for LAM is described.

Introduction

Owing to the trend toward greater environmental awareness and protection, in recent years surfactants with biodegradable and non-toxic properties have become increasingly important.[1] One novel strategy proposed to minimise the environmental effects involves designing new molecules that mimic natural amphiphilic structures.[2] In this context, a new class of amino acid-based surfactants, mimics of natural lipoaminoacids, has been developed by Infante *et al.*[3,4] These amphiphilic molecules, in particular the N^α-dodecanoylarginine methyl ester hydrochloride salt (N^α-lauroylarginine methyl ester hydrochloride salt, LAM throughout the paper) (Fig. 1), are an important new class of cationic surface active compounds with rich self-aggregation properties, bactericidal activity, biodegradability and low irritation to skin.[5,6] All these properties make them good candidates for potential applications in the food, pharmaceutical or cosmetic technology domains.

The chemical synthesis of LAM can be achieved by a coupling reaction between the carboxylic group of dodecanoic acid (oil soluble) and the α-amino group of the arginine methyl ester monohydrochloride derivative (water soluble), ArgOMe·HCl (ArgOMe throughout the paper), with dicyclohexylcarbodiimide (DCC) as the condensing reagent (Scheme 1). The reaction yields, in addition to LAM, the dicyclohexyl urea (DCU) as a by-product.

Classically, the reaction is carried out by mixing equimolecular amounts of ArgOMe·HCl, dodecanoic acid, and DCC in a dimethylformamide (DMF) solution at room temperature and in the presence of an organic base, such as triethylamine.[3] This synthesis is a typical example in which non-polar and polar organic compounds can be dissolved in an unique phase system to achieve good reaction rate and yield. DMF is an useful polar aprotic solvent for this type of reaction (at lab-scale) because of its capacity for dissolving both non-polar and polar compounds. Nevertheless, it is not recommended for large-scale work due to its toxicity and difficulties in its removal from the reaction product.

These problems lead us to investigate alternative, more environmentally friendly, reaction media for the synthesis of LAM. A good reaction medium should meet three requirements: 1) it should be capable of dissolving all reactants involved, 2) the system should be stable during the reaction, and 3) recovering the reaction product should be straightforward.

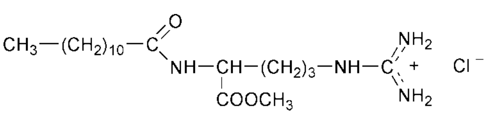
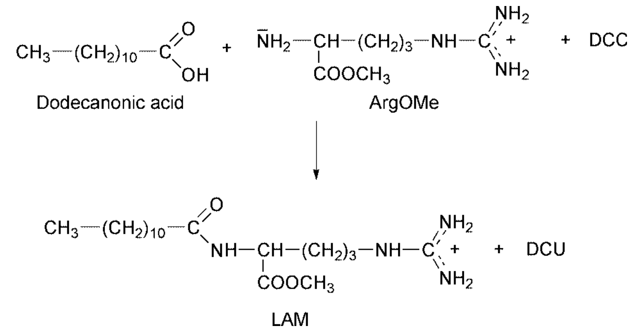


Fig. 1 Chemical structure of the N^α-lauroylarginine methyl ester derivative surfactant (LAM).



Scheme 1

Microemulsions have been described as alternative reaction media for preparing amphiphilic compounds or carrying enzymatic reactions.[7] Microemulsions are used for the synthesis of nonionic surfactants from sucrose and fatty acid methyl esters; in this case the microemulsion process avoids the use of DMF.[8] The interest in microemulsions stems from their characteristic properties, namely ultralow interfacial tensions, large interfacial areas, and high solubilization capacity for both water- and oil-soluble compounds.[9]

Another type of colloidal system, some of which have received a great deal of attention as novel reaction media for the preparation of polymeric materials,[10–13] is the so called high-internal-phase-ratio emulsion (HIPRE). These highly concentrated emulsions, or gel emulsions, can be formulated with a large amount of water (*i.e.* higher than 99% w/w) and very low surfactant concentration (*i.e.* lower than 0.5%).[14–27] The internal volume fraction in this type of emulsion exceeds the critical value, $\varphi_c = 0.74$, of the most compact arrangement of uniform, undeformed spherical droplets.[20] Consequently, the structure of highly concentrated water-in-oil emulsions consists of close-packed water droplets, with typical radii of a few microns, separated by a thin film of continuous phase.[20,21] It is known that the nature of the components, the volume fraction of the dispersed phase, the oil-to-surfactant weight ratio, the temperature and the presence of additives affect the stability of highly concentrated emulsions.[22–27]

In this paper we investigate highly concentrated water-in-oil emulsions as environmentally-friendly reaction media, an

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alternative to DMF, for the synthesis of LAM. Gel emulsions exhibit the desired properties for a reaction medium. Since the dispersed and the continuous phases are aqueous and oily phases respectively, both aqueous or oil soluble reactants can be incorporated in the system. The influence of several factors that have an effect on the yield of the LAM synthesis using highly concentrated water-in-oil emulsions as reaction media are reported. Specifically, we have studied the influence of ArgOMe : lauric acid molar ratio, temperature, volume fraction of the dispersed phase and, reaction scaling up. We also report a new high performance liquid chromatography (HPLC) analysis procedure and a medium pressure liquid chromatography (MPLC) preparative procedure to analyse and to recover respectively the LAM surfactant synthesised. For comparative purposes the results of the same reaction carried out in DMF are also reported.

Experimental procedures

Materials

Ethoxylated tetradecanol with an average ethylene oxide (EO) content of 4 mol per molecule, abbreviated as $C_{14}E_4$, was from Albright & Wilson (Spain). Pure decane (purity >99%) was from Fluka. Dodecanoic acid and DCC were synthetic grade from Merck. The ArgOMe derivative was prepared as described in ref. 2. Pure Millipore water from a Milli-Q four-bowl system was used. For HPLC analysis, acetonitrile (ACN) and spectroscopic grade trifluoroacetic acid (TFA) were from Merck.

Methods

Reaction in highly concentrated emulsions. The reaction media consisted of water-in-oil gel emulsions prepared with 90 wt% aqueous solution of ArgOMe and 10 wt% of a surfactant–oil–dodecanoic acid solution unless otherwise stated. A 1 : 1 molar ratio of ArgOMe and dodecanoic acid was used unless otherwise stated. The $C_{14}E_4$–oil weight ratio was kept constant and equal to 30 : 70. In order to avoid hydrolysis of DCC, this reagent was added to the system after the gel emulsion. Gel emulsions were prepared in the following way: dodecanoic acid was dissolved in solutions of appropriate amounts of decane and $C_{14}E_4$. ArgOMe was dissolved in the required amount of water. The concentrated emulsion was formed by slow addition of the internal phase (ArgOMe aqueous solution) to the external oil phase (decane, $C_{14}E_4$ and dodecanoic acid). After all the aqueous component had been added, gel emulsions were shaken with a vortex for a few minutes. The agitation time and vortex speed were the same in all cases. Once the emulsion was formed, the condensing reagent (DCC), diluted in decane, was added. The emulsion was manually shaken for 30 s. This point was taken as time zero for the reaction. The reaction was allowed to evolve at constant temperature without any further agitation of the reaction medium.

HPLC analysis. *Analysis of LAM.* Chromatographic determinations of LAM were performed on a Merck-Hitachi system which consisted of an injection valve fitted with a 20 µl loop, an Intelligent Pump L-6200, and a UV-VIS detector L-4250 at 215 nm wavelength. A Lichrocart 250 × 4 mm, Lichrospher 100CN (propylcyano) (particle size 5 µm) column was used at room temperature. The flow-rate through the HPLC column was 1.0 ml min^{-1}. Elution was performed in a gradient system of acetonitrile–water, 25/75 to 95/5, in the presence of 0.1% of trifluoroacetic acid, over 25 min. All samples were prepared by dilution (1 : 10) with methanol. Standard samples of emulsion components and reaction components were analysed separately in order to determine their retention times. The retention times obtained are shown in Table 1. Solutions of LAM in MeOH were prepared and analysed under the conditions described above. The results were used to fit a calibration curve that was used to perform LAM quantitative analysis during the reaction at several measuring times.

Table 1 HLPC retention times of emulsion components, reactants and products[a]

Compound	Retention time/min
ArgOMe	With the elution front
DCC	10.60
DCU	12.67
Dodecanoic acid	15.28
LAM	16.10

[a] HPLC column was a Lichrospher 100 CN, and the gradient system acetonitrile–water was 25 : 75–95 : 5 over 25 min.

Analysis of ArgOMe. Chromatographic determinations of ArgOMe were performed on a Waters LC Module I system with a UV-VIS detector at 215 nm wavelength. A Lichrospher 100 RP-18 (particle size 5 µm) in a Lichrocart 250 × 4 mm column was used at room temperature. The flow-rate through the HPLC column was 1.0 ml min^{-1}. Elution was performed in an isocratic system consisting of 98% of water and 2% of an acetonitrile–water mixture 60 : 40, in presence of 0.1% of trifluoroacetic acid. All samples were prepared by dilution (1 : 10) with water.

Highly concentrated emulsions containing different concentrations of ArgOMe were prepared in order to analyse ArgOMe by HPLC under the conditions described above. The results were used to fit a calibration curve that was used to perform ArgOMe quantitative analysis.

Recovery and purification of LAM by MPLC. After reaction completion the emulsion separated into two phases, one oily and one aqueous. To the aqueous phase a mixture of $CHCl_3$–MeOH was added, to break the emulsion completely. LAM was recovered and partially purified with several $CHCl_3$–MeOH (9 : 0.5) extractions. Final LAM purification was achieved by medium pressure liquid chromatography (MPLC). This was performed on a MPLC Büchi system model B-680 with a UV-VIS Knauer at 215 nm wavelength and a B-685 standard column of silica 60 Å (particle size 15–40 µm). The column was used at room temperature. The column pressure was controlled at 10 bars and the flow-rate through the MPLC column was 25.0 ml min^{-1}. Elution was performed by a methanol–chloromethane gradient (5–15%) over 45 min. The purification procedure allows isolation of LAM in the order of multigrams with a purity close to 90%, which was subsequently crystallised from water to obtain LAM compound with a purity of 99%.

Characterisation of LAM. Melting point was determined with a FB81 measuring cell of the FP90 Mettler System. Optical rotation was measured with a 141 Perkin-Elmer spectropolarimeter (Norwalk, CT). The structure of LAM was checked by ^{1}H-NMR analysis, which was recorded with a Varian 200 MHz spectrometer; the chemical shifts are reported in parts per million (δ, in ppm) downfield from tetramethylsilane (TMS). The IR spectra were recorded on an FTIR Nicolet 510 spectrometer, and fast-atom bombardment mass spectra (EM-FAB) were recorded with an AutoSpec-Q, VG-Organic. Satisfactory melting point, optical rotation and elemental analysis were obtained. FAB-MS, H^1-NMR and FTIR spectra were consistent with those reported earlier for LAM.[3]

Results and discussion

Stability of reactants in the reaction media

A factor that can affect the evolution and yield of the reaction is the stability of the reactants in the emulsion media. ArgOMe

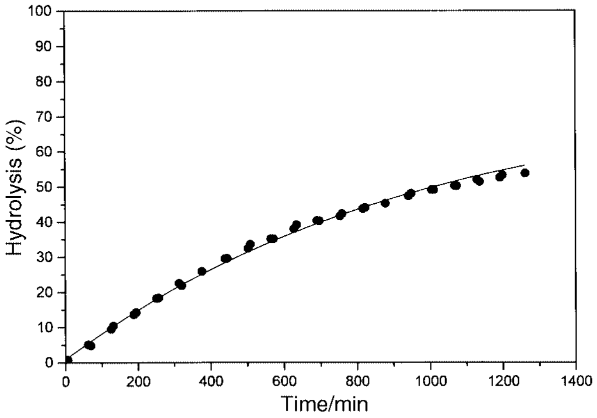

Fig. 2 HPLC analysis of ArgOMe hydrolysis as a function of time on highly concentrated emulsion media with 89 wt% water content of the dispersed phase at 25 °C.

is a very stable compound in the solid state but in aqueous media the compound shows a short lifetime because of the displacement of the OMe group by the hydroxide ions from water, yielding free arginine. To find out whether hydrolysis of ArgOMe affects the yield of LAM in this new media, the percentage of ArgOMe hydrolysis was measured. To this end, samples of the reaction medium with the reactants (dodecanoic acid and ArgOMe) but without DCC were prepared. They were analysed by HPLC over 24 h. The amount of hydrolysed ArgOMe (in %) is depicted in Fig. 2. It is worth noting that during the first 60 minutes the amount of hydrolysed ArgOMe is lower than 5%. As it is described below, after one hour of reaction, 80% of the total ArgOMe has been consumed. Therefore, it can be concluded that hydrolysis has a negligible effect on the LAM synthesis in gel emulsion media.

Synthesis of N^{α}-lauroylarginine methyl ester hydrochloride (LAM)

We selected a water–surfactant–oil model system, giving kinetically stable gel emulsion, in which to carry out the reaction. The model system chosen was the water–$C_{14}E_4$–decane system. We have already proved that in this system the long-term emulsion stability is not affected when additives, DCC, ArgOMe and dodecanoic acid are present.[28] The appearance of the emulsion in the presence of the reactants is the same as it is without them. Optical microscopy revealed a structure of close-packed water droplets separated by a thin film of continuous phase.

The reaction was monitored by HPLC analysis. Fig. 3 shows a typical chromatogram where the peak with label 5 corresponds to LAM formation. Comparing the retention times in the chromatograms with the standard retention times in Table 1, all peaks could be identified except that with label 6 in Fig. 3 showing a retention time of 21 min. To identify this peak, purification by a micropreparative HPLC technique was carried out. The isolated product, formed by an intramolecular acyl-transfer reaction, was identified as *N*-dodecylurea by H^1-NMR and mass spectroscopy.[29]

Quantitative analysis of LAM formation and ArgOMe conversion were carried out by HPLC after 1, 3 and 24 h of reaction. The results are shown in Table 2. For comparison purposes, Table 2 also includes the results obtained when the reaction is performed conventionally in DMF. The final yield is slightly higher in DMF. Comparing the yields at 1 h it can be seen that the reaction in highly concentrated emulsions gives relatively faster initial reaction rates. Note that a yield of 51% over a final yield of 53% shows a higher conversion rate than 46% over a final yield of 66%. The higher conversion rate in gel emulsion media can be attributed to the large interfacial contact area. Also, the presence of an oil–water interface may induce a proper orientation of reactants at the interface, which in turn may affect the conversion rate.[30]

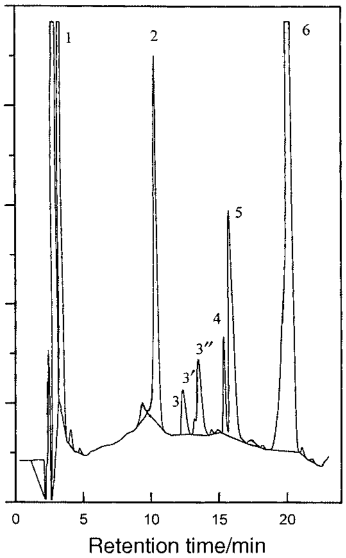

Fig. 3 Typical HPLC chromatogram of the LAM synthesis reaction mixture on highly concentrated emulsion media. 1 (ArgOMe), 2 (DCC), 3, 3′ and 3″ (DCU), 4 (dodecanoic acid), 5 (LAM), 6 (*N*-dodecylurea).

Table 2 Effect of reaction time on the LAM yield, and ArgOMe conversion in DMF and highly concentrated reaction media at 25 °C

	DMF		Highly concentrated emulsion[a]	
Reaction time/h	LAM yield (%)	ArgOMe conversion (%)	LAM yield (%)	ArgOMe conversion (%)
1	46.0	60.0	51.0	48.0
3	49.0	62.0	51.5	48.4
24	66.0	62.0	53.0	52.0

[a] Water content of the dispersed phase in highly concentrated emulsions was 89%.

Table 2 also shows that, in general, the conversion of ArgOMe agrees with the LAM formation ratio. As expected from results shown in Fig. 2, ArgOMe hydrolysis rate is slow compared with LAM formation rate and for this reason does not affect LAM formation.

The influence of temperature on the reaction yield was determined using ArgOMe–dodecanoic acid molar ratios of 1 : 1 and temperatures between 15 and 40 °C. The yields after 24 h at different temperatures are shown in Fig. 4. The reaction yield increases with temperature, reaching a maximum at 25 °C, then it decreases. This behaviour can be explained taking into account two different factors, which depend on the temperature: reactant diffusion and emulsion stability. Reactant diffusion increases with temperature, favouring increasing yields. In the type of emulsions we are dealing with, stability also increases with temperature, reaching its optimum for values of 25–30 °C over the hydrophile-lipophile balance (HLB) temperature. However, at higher temperatures stability decreases considerably.[26,27] Since the HLB temperature for the water–$C_{14}E_4$–decane system, using pure nonionic surfactant, is 8.8 °C,[31] the increasing yields for temperatures in the range 15–25 °C can be attributed to the combined effect of both factors, diffusion and stability. At higher temperatures these factors are opposing, and the unfavourable destabilisation would predominate because when emulsions break, interfacial area decreases and the contact between reactants is disfavoured.

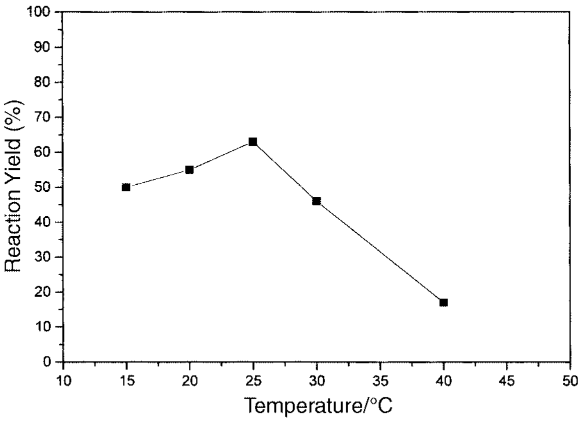

Fig. 4 Influence of temperature on the LAM reaction yield after 24 h reaction time in highly concentrated emulsion media.

Table 3 Effect of water content of the dispersed phase on the reaction yield as a function of reaction time at 25 °C

Water content (%)	Reaction time/h	LAM yield (%)	ArgOMe conversion (%)
80	1	43.4	52.5
	3	44.4	53.2
	24	56.4	55.0
82	1	45.0	37.0
	3	46.0	38.0
	24	53.0	40.0
85	1	56.0	59.5
	3	59.0	69.0
	24	60.7	74.0
90	1	47.4	48.0
	3	51.9	49.0
	24	54.4	52.0
95	1	43.0	50.0
	3	45.0	58.0
	24	49.0	60.0

Table 3 shows yield values of LAM formation and ArgOMe conversion after 1, 3 and 24 h of reaction for gel emulsions with water content of the dispersed phase of 80, 82, 85, 90 and 95%. It can be seen that in all cases LAM yield as a function of time has the same pattern and after 24 h the yield slightly decreases when the amount of water in the media increases. Assuming that the reaction takes place at the oil–water interface, this behaviour can be explained as follows. Increasing the water content in the highly concentrated emulsion produces an increase in droplet size[22,25] and, consequently, a decrease in interfacial area. As a result, the ability of ArgOMe molecules to react in the interfacial region, where reaction with the dodecanoic acid should take place, would be reduced.

The molar ratio of reactants can also influence the reaction yield. ArgOMe–dodecanoic acid molar ratios of 1 : 1, 2 : 1 and 1 : 2 were considered to determine this influence. The results are shown in Table 4. As the ArgOMe–dodecanoic acid molar ratio increases the experimental reaction yield increases slightly. As discussed in ref. 32, the cessation of reaction before all the reactants have been consumed may be seen as an interfacial phenomenon. Emulsions can be destabilised by changing of some of the system parameters. Since the reaction product, LAM, is a cationic hydrophilic surfactant, it accumulates at the interfaces, conferring instability and breaking the water–oil emulsion.

An initial assessment of the reaction scaling up at a laboratory scale was carried out by repeating the reaction using a total amount of 3, 5 and 10 g of reaction media. In all cases the water content of the dispersed phase was 89% and the temperature was 25 °C. The resulting yields are shown in Table 4. It can be seen that the yield slightly increases with the amount of reaction media. We do not have a clear explanation for this behaviour and plan to carry out more research into it.

Table 4 Effect of molar ratio and total amount of gel emulsion medium[a] on the reaction yield after 24 h reaction time at 25 °C

Molar ratio ArgOMe–lauric acid	Amount of reaction media/g	LAM yield (%)
1 : 1	10	63
2 : 1	10	68
1 : 2	10	53
1 : 1	3	39
1 : 1	5	55

[a] Water content of the dispersed phase was 89%.

Conclusions

In this paper we have investigated for the first time gel emulsions as alternative reaction media for the synthesis of LAM. The influence of a number of factors that have an effect on the yield of LAM have been studied.

It has been found that reaction yield increases with temperature reaching a maximum at 25 °C, which has been related mainly to emulsion stability. Increasing the water content results in larger droplet size; this means a smaller interfacial area and, therefore, a smaller reaction yield. The molar ratio of reactants has a slight influence on the yield reaction. It has been proved that in gel emulsion media, the ArgOMe hydrolysis rate is slow compared with that of LAM formation. This explains that the yields of LAM synthesised in highly concentrated emulsions are comparable to those obtained in conventional media.

The results reported in this paper show that highly concentrated water-in-oil emulsions could be used as a useful new reaction medium for the synthesis of LAM.

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