Synthesis and properties of lipoamino acid-fatty acid mixtures: influence of the amphiphilic structure

C. Rondel, Isabelle Alric, Zephirin Z. Mouloungui, Jean-François Blanco, Françoise Silvestre

Synthesis and properties of lipoamino acid/fatty acid mixtures.

Influence of the amphiphilic structure.

Caroline Rondel: caroline.rondel@ensiacet.fr

Isabelle Alric: isabelle.alric@ensiacet.fr

Zéphirin Mouloungui*: zephirin.mouloungui@ensiacet.fr

Jean-François Blanco[1]: jeanfrancois.blanco@ensiacet.fr

Françoise Silvestre: francoise.silvestre@ensiacet.fr

Université de Toulouse - UMR1010 Chimie Agro-Industrielle, ENSIACET, INPT, INRA,

118 Route de Narbonne, F- 31077 Toulouse Cedex 4

* Corresponding author, tel.: +33 5 62 88 57 27, fax: +33 5 62 88 57 30

[1] Université de Toulouse - UMR 5503 Laboratoire de Génie Chimique, ENSIACET, INPT, CNRS, UPS, BP 1301, 5 rue Paulin Talabot, F-31106 Toulouse Cedex 1

Abstract The acylation of amino acids by acid chlorides with from 8 to 12 carbon atoms, in alkaline aqueous medium following Shotten-Baumann reaction, results in sodium salts of N^{α}-acylamino acids and fatty acids mixture. These lastest are present in proportion from 40 to 60%. These compositions represent mixtures of amphiphilic anionic surfactants. They contribute together to the properties of the formulation. Measurements of the surface-active properties of these formulations, such as critical micelle concentration (CMC), surface tension at the CMC (TS), foaming capacity (FC) and foaming stability (FS), show that surfactant mixtures with the longest chain have the most desirable properties. They are comparable to commercial petroleum-based surfactants. Thus, the CMC, TS and CM values of the formulation obtained starting from leucine and dodecanoyl chloride (310 mg/L, 30.1 mN/m and 200%, respectively) are similar, even better than, sodium dodecylsulfate (290 mg/L, 39.1 mN/m and 230%, respectively).

Keywords: Surfactants, amino acids, acylation, critical micelle concentration (CMC), foaming properties

Introduction

Surfactants, or surface-active agents, are among the most widespread and widely used chemical products in the world today. In 2002, total surfactant production volume worldwide was 11 million tonnes. Derivatives of plant origin accounted for approximately 20% of this production. Within a context of sustainable development, there is an urgent need to produce environmentally-friendly surfactants.

These surfactants can be obtained from molecules that mimic natural amphiphilic structures. The association of a polar amino acid (hydrophilic moiety) and a non polar long chain compound (hydrophobic moiety) to built amphiphilic structures allows to obtain molecules with a high surface activity (*1*). Thus, lipoamino acids obtained from natural raw materials are the choice surface-active molecules for applications in the food, pharmaceutical and cosmetic industries. As a result of their structure, they have a low degree of toxicity, improved resistance to hard water, antimicrobial activity (*2*), are gentle and not irritating to the skin and are easily biodegradable (*1, 3*).

If we consider the chemical structure of an amino acid, the fatty chain can be introduced via the amine or carboxylic function. However, the reactivity of the amine function in aqueous medium is widely higher than the one of carboxylic acid. We were interested in N^{α}-acylamino acids obtained by fat chain grafting on the amine function of amino acid. Many synthesis pathways use organic solvents (*3-6*). Another pathway consists of synthesis by acylation using an acid chloride in water, following the Schotten-Baumann reaction (*2, 7, 8*). In parallel, in operating conditions (alkaline aqueous medium), the acid chloride hydrolyses in carboxylate ion (Figure 1). It is therefore necessary to determine the quantity of fatty acid formed during the secondary reaction in order to determine the composition of the final mixture. The aim is to acylate the greatest proportion of amino acid possible. To do this, an excess of acid chloride in relation to amino acids, is added. The presence of sodium (do)decanoate and

octanoate contributes also to the mixture properties, because they have an amphiphilic capacity.

We were particularly interested in the acylation of a neutral amino acid with a non-ionic side chain (leucine), of an amino acid with a negatively-charged side chain (glutamic acid) and a basic amino acid with a positively-charged side chain (arginine), and acid chlorides with from 8 to 12 carbon atoms. The N^{α}-acylamino acids/fatty acid sodium salts ratio of the mixture obtained was determined by NMR and UV spectroscopy methods. Then, we studied the surfactant properties of these different formulations. Indeed, only few of these surfactants were studied: Takehara *et al.* (*9-11*) and George *et al*. (*2*) were interested in the surface-active properties of few N^{α}-acylamino acid salts.

Experimental Procedures

Materials

L-leucine (99%), L-glutamic acid (99%), L-arginine (98%), octanoyl chloride (>97%), decanoyl chloride (98%), dodecanoyl chloride (98%), o-phtaldialdehyde (OPA), sodium phosphate, sodium tetraborate, sodium dodecyl sulphate (SDS), β-mercaptoethanol and hexadecyl-trimethylammoniumbromid (CTAB) were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). D_2O (99.90%) was supplied by Eurisotop (Gif-sur-Yvette, France).

Preparation of N^{α}-acylamino acid sodium salts

Sodium hydroxide (2.72 g/68 mmol) – except for synthesis with glutamic acid (4.08 g/102 mmol) – and amino acid (34 mmol) were dissolved in 30 mL of water in a 250-

mL two-necked flask equipped with a cooling agent and mechanical stirring. The mixture was stirred at room temperature. Acid chloride (51 mmol) was added after the amino acid and the sodium hydroxide were totally dissolved. The reaction temperature was thus maintained at 50°C for 3 h. At the end of the reaction, the pH was adjusted to 2-3 with a 4N hydrochloric acid solution. Water was eliminated using a rotary evaporator. A purification step of reaction mixture in order to eliminate the sodium chloride was then performed. For that purpose, 50 mL of ethanol was added to the mixture. Then, the ethyl-alcoholic solution was heated under reflux for 30 min. The mixture is vacuum-filtered. The dry matter and mineral content of the solid (obtained after the former filtration) was determined using the AFNOR standard, NF VO3-706, and the insoluble fraction was analysed by infrared spectrometry. The ethanol was then eliminated from the filtrate using the rotary evaporator. The residue obtained was dissolved in a saturated sodium hydroxide aqueous solution (4 to 6 g) and in 10 to 20 mL of ethanol. The N^{α}-acylamino acid and fatty acid of sodium salts mixture were precipitated after adding 30 mL of acetone (*2*). Finally, a filtration is performed in order to recover and vacuum dry the product mixture.

Determination of the dry matter and mineral content by AFNOR standard, NF VO3-706

2.5 g of product was put down in porcelain crucible. It was placed in an incubator at 105°C until to a constant weight. Then, it cooled in a desiccator. The percentage in dry matter is determined by the relation:

$$\%MS = m_{dry} / mi \times 100$$

mi: masse of the initial product (g)

m_{dry}: masse of the dry product after a stay in an incubator at 105°C (g)

After the determination of the dry matter rate, the crucibles are placed in an oven at 550°C for 3 hours. The percentage of mineral is figured by the following relation:

$$\%MM = \frac{m_{ashes}}{m_{dry}} \times 100$$

m_{dry}: masse of the dry product after a stay in an incubator at 105°C (g)

m_{ashes}: masse of ashes from dry product after calcination at 550°C (g)

Analysis of amino groups and determination of the acylation rate

Free amino acid groups were quantified using the reliable OPA method (*12*), described by Frister *et al*. (*13*). The sample to be analysed was dissolved in a buffer solution of 12.5 mM sodium tetraborate and 2% SDS (w:w) at pH 8.5. 2 mL of a reagent solution prepared the same day were added to 1 mL of unknown solution. The reagent solution was prepared by introducing the following into a 50 mL volumetric flask: 25 mL 0.1 M sodium tetraborate solution (pH 9.2), 2.5 mL 20% SDS (w:w), 40 mg OPA dissolved in 1 mL methanol, 100 µg β-mercaptoethanol. The final volume was completed up to 50 mL with demineralised water. The absorbance of the mixture was measured at 340 nm after 2 min incubation. The number of amine groups was calculated using a calibration curve obtained with L-leucine. The acylation rate (AR) was determined using the following formula:

$$AR = (Ni-Nf)/Ni*100$$

where Ni is the number of free amine groups in the amino acid before acylation, and Nf is the number of free amine groups in the final product determined by OPA.

Nuclear magnetic resonance (^{1}H and ^{13}C NMR) analysis

All the NMR measurements were performed with in 7.05 Tesla Bruker AVANCE 300 spectrometer. All the proton and carbon-13 spectra were acquired at 300.1312 MHz and 75.4764 MHz, respectively, in a 5 mm direct probe (PH QNP-300SB F/P/C-H-D -05 – Zgrad

-BTO 2000). The experiments were carried out at 293.1 ± 0.1 K. The sample (20-50 mg of salt) was dissolved in 0.6 mL of D_2O. The chemical shifts were referenced to tetramethylsilane (TMS) for ^{1}H and ^{13}C nuclei. The proton spectra were collected with a solvent suppression experiment (Pulse programme ^{1}H: zgpr) with a 90° (P1=2.70 µs ; PL1=0 dB and PL9=45 dB) pulse as 32K points over 6.172 KHz spectral width.

All the carbon-13 spectra were acquired using standard proton–decoupled carbon-13 acquisition with pulse-gradient (Pulse programme ^{13}C: zgpg30). For this purpose, a carbon-13 length pulse of typically 9.70 µs (PL=-1 dB) and 18.115 KHz proton decoupling were applied during acquisition. The number of scan is 1,536 for carbon-13 experiments and 64 for proton analysis.

Measurement of the Critical Micelle Concentration (CMC)

Surface tension was measured using a GBX-TEN 089 tensiometer equipped with a Wilhelmy plate. Mixtures consisting of N^{α}-acylamino acid and fatty acid sodium salts (2.5 g/L) were dissolved in a 0.1 M sodium phosphate buffer solution at pH 7.0 to obtain 200 mL of solution. Drops of 400 µL of this solution were added to 50 mL of the same phosphate buffer solution. Surface tension was continuously measured and recorded at 20°C until a constant surface tension value was observed. The CMC was obtained at the breaking point of the surface tension curve in relation to the logarithm of the mixture concentration.

Measurement of foaming properties

Foaming properties were measured using the method described by Padmashree *et al.* (*14*). 3 g of surfactants were mixed with 300 mL of water in a graduated 1-L cylinder. The solution was

stirred at 1600 r/min. The volume was measured 30 s after stirring. The foaming capacity (FC) was expressed as the percentage of volume according to the following formula:

$$FC = \frac{Volume\ after\ stirring\ -\ Volume\ before\ stirring}{Volume\ before\ stirring} \times 100$$

The foam volume was recorded at 5, 30, 60, 120 and 300 min after stirring. Foaming stability (FS) was calculated using the following formula:

$$FS = \frac{Foam\ volume\ after\ a\ time\ "t"}{Initial\ \ foam\ volume} \times 100$$

Results and discussion

Purification of acylation reaction products

The aim of this step is to eliminate all compounds that have no surface-active properties (sodium chloride, amino acids). After the elimination of water, the reaction products are dissolved in ethanol under reflux. By simple filtration of the medium, the totality of sodium chloride, not soluble in ethanol is eliminated. The mineral matter determination of solid obtained gives values of about 100% for synthesis performed with leucine and glutamic acid and above 60% for the synthesis carried out with arginine. In the last case, the infrared showed that the precipitate also contained an arginine fraction, slightly soluble in ethanol.

The second step of purification consists in obtaining the surface-active molecules under salt form and it permits to eliminate the amino acids that have not reacted. These latest do not precipitate during acetone addition. Therefore, the final mixture contains only the N^{α}-acylamino acid sodium salts and the fatty acid sodium salts. In the case of mixture obtained starting from glutamic acid, the precipitation of this amino acid is observed in acetone. The surfactant mixtures are thus contaminated by glutamic acid (Table1).

Determination of the conversion rate of the acylation reaction and the final formulation composition

In the operating conditions involving 1.5 equivalent of acid chloride for 1 equivalent of amino acid in presence of 2 equivalents of sodium hydroxide (3 for the synthesis with glutamic acid in reason of the presence of two carboxylic functions), the conversion in relation to amino acid (η_{AA}) is determined by OPA method, for the different reactions. η_{AA} are raised for arginine (77-90%) and for leucine (87-88%) and lowered for glutamic acid (53-75%) (Table1). This lower conversion rate is due to higher basicity of reaction medium during the synthesis with glutamic acid, because of the presence of its two carboxylate functions, favouring the chloride hydrolysis. The influence of hydrophilic element is perceptible in the case of arginine acylation because the acylation rate is proportional to the fatty chain length. The N^{α}-acylation by acid chloride is an efficient way to constitute amphiphilic compounds.

The ratio of N^{α}-acylamino acids to the total quantity of N^{α}-acylamino acids and fatty acid salts formed in the final composition (R_{AA}) and the fatty acid salts to the total quantity of N^{α}-acylamino acids and fatty acid salts formed in the final composition (R_{FA}) is determined by NMR. A preliminary study of ^{1}H (zgpr) and ^{13}C (zgig) signals, completed with 2D studies (COSY, HSQC, HMBC) led to the attribution of the HNMR signals. To allow quantitative HNMR determination, all NMR measurements have been carried out with a delay time D1 = 60 sec. This delay time permits the full relaxation of all the nuclei concerned by the assay. The values of integration on the ^{1}H spectra are determined in order to allow the calculation of the assay of our mixtures. I_1 and I_2 the value of the integral corresponding to one proton of N^{α}-acylamino acid sodium salts and fatty acid sodium salts, respectively. For the three N^{α}-acylleucine acids, the N^{α}-octanoylarginine acid and the N^{α}-decanoylarginine acid-based

mixtures, the integrals chosen correspond to the CH_2 (protons H3) in alpha of the amide function (integral I_1) or in alpha of carboxylic function (integral I_2) for the N^{α}-acylamino acid sodium salts or the fatty acid sodium salts, respectively (Figure 2). Concerning the N^{α}-dodecanoylarginine acid sodium salt and the three N^{α}-acylglutamic acid sodium salt-based mixtures, the integrals chosen correspond to the terminal methyl group (protons H1) of the hydrophobic part of our components (integral I_1+I_2) and to the proton (H4) signals grafted on the asymmetric carbon of the N^{α}-acylamino acid sodium salts (integral I_1). The integrals I_1 and I_2 of the spectrum of the mixture containing the N^{α}-octanoylglutamate sodium salt and the octanoate sodium salt are illustrated on Figure 3. The assay of the mixture is expressed by the yield (R_{AA} and R_{FA}) calculated with the following formula:

$$R_{AA} = \frac{I_1}{I_1 + I_2} \times 100$$

$$R_{FA} = \frac{I_2}{I_1 + I_2} \times 100$$

R_{AA} and R_{FA} are gathered in Table 1. The yield (R_{AA}) obtained after purification are different from conversion rate (η_{AA}) obtained by OPA before purification. According to our experimental conditions, R_{AA} can not exceed 66%. The lower yields observed for the derivates of glutamic acid may be explained by a content of sodium hydroxide greater during the synthesis with this amino acid that would lead to a premature hydrolysis of the chloride acid. The chain length does not seem to have any influence on the yield of the synthesis, regarding the uncertainty of about 10% on these values.

Measurement of the critical micelle concentration

A linear decrease in surface tension is observed when the concentration of the mixture is increased for all surfactants, up to the CMC (Figure 4), beyond which there is no observable change in surface tension. This behaviour is common to surfactants in solution. The high CMC values observed for the N^{α}-octanoylamino acid-based formulations are due to the fact that the N^{α}-octanoylamino acids have a less hydrophobic chain than that of N^{α}-decanoylamino and N^{α}-lauroylamino acids (Table 2). In fact, a longer, more hydrophobic chain results in lower surface tension. This is attributed to the increase in the affinity of lipophilic molecules for interfaces (*15*). Regardless of the nature of the hydrophilic head of the amino acid, N^{α}-lauroylamino acids have the lowest CMC. The charge of the polar head also seems to have an influence on the CMC. For the same chain length, the CMC of the leucine derivates mixture is inferior to the one of arginine derivates, which is inferior to the one of glutamic acid derivates. The N^{α}-acylleucine sodium salts have an anionic hydrophilic head, whereas N^{α}-acylarginine sodium salts have an amphoteric one and N^{α}-glutamic acid sodium salts have one with two negative charges. Likewise, the higher values of CMC for the glutamic acid derivates are explained by the presence of residual glutamic acid. This amino acid has no surface-active properties, so its presence increases the CMC values

N^{α}-lauroylamino acid- and N^{α}-decanoylamino acid-based formulations have a surface tension at the CMC comparable and sometimes even lower than that of SDS and CTAB. They therefore have interesting surface-active properties.

Measurements of foaming properties

The results of foaming properties are given on Table 3. Regardless of the amino acid used, the shorter the chain is, the lower the FC will be, implying that less foam will be produced. By extending the length of the chain, the lipophilic character of the molecule is increased and the

tension at the water/air interface is lowered at the same time. The N^{α}-lauroylarginine sodium salt-based mixtures and the N^{α}-decanoylarginine sodium salt-based mixtures have a good foaming capacity. Indeed, the anionic and amphoteric surfactants are known for their good foaming capacity (*16*). The foam volume value obtained for these mixtures is comparable to the value obtained with commercial SDS. This is proof that these are good foaming agents. Moreover, some N^{α}-acylamino acids are sold as cosmetic ingredients for shower gels and cleansers (*17*). The stirring with N^{α}-acylglutamate sodium salts have less good foaming capacity for the same reasons that previously (presence of sodium glutamate in the mixture).

A second major parameter when studying foaming properties concerns their stability (Figure 5). Foams are thermodynamically unstable systems. Their stability and their fracture depend on a series of complex phenomena that begin with the hydrodynamic drainage of the liquid, the dilution of the aqueous film and the coalescence of bubbles (*18*). An arginine hydrophilic head combined with a C10 or C12 hydrophobic chain confer good stability on N^{α}-acylarginine due to the amphoteric nature of their polar head. Indeed, the amphoteric surfactants improve the foaming capacity, as well as the foaming stability (*19*).

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Illustrations and Tables

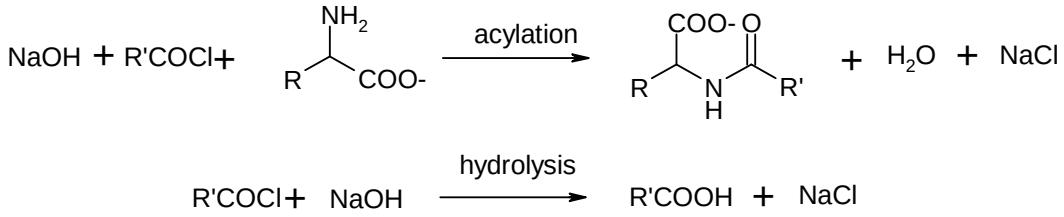

Figure 1: Two competitive reactions: acylation and hydrolysis of acid chloride

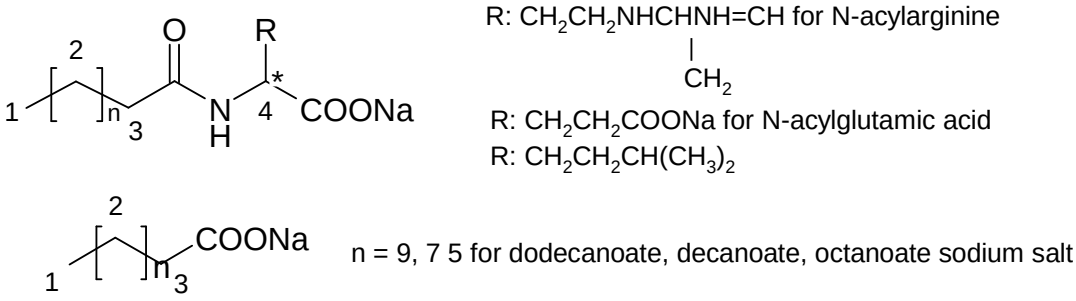

Figure 2 : N^{α}-acylamino acid and alcanoate sodium salts

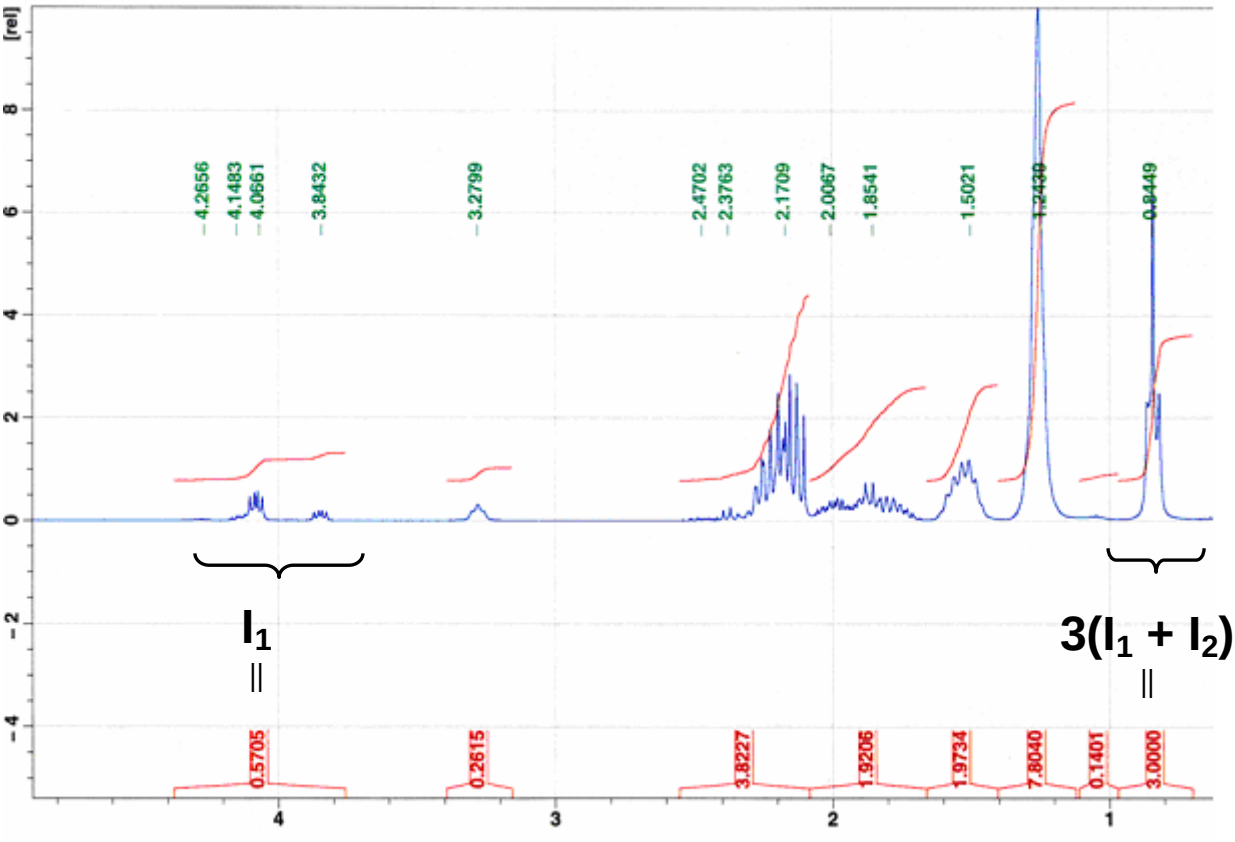

Figure 3: ^{1}H spectrum of C8-glutamic acid

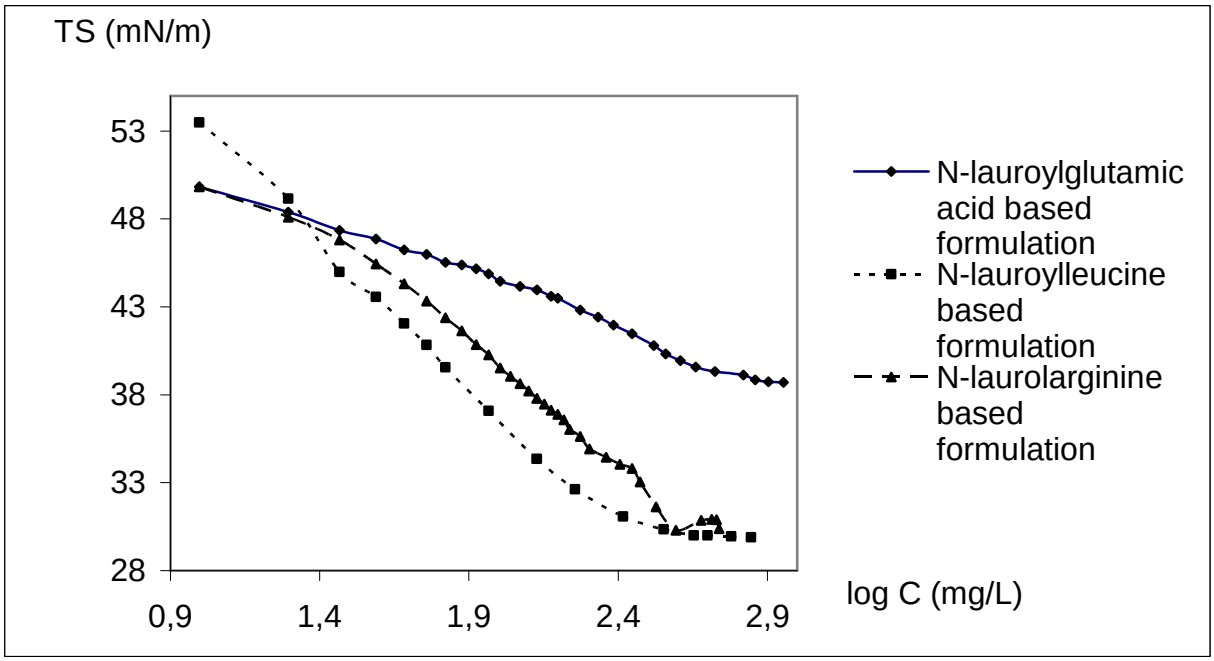

Figure 4: Surface tension of N^{α}-lauroylamino acid based formulations

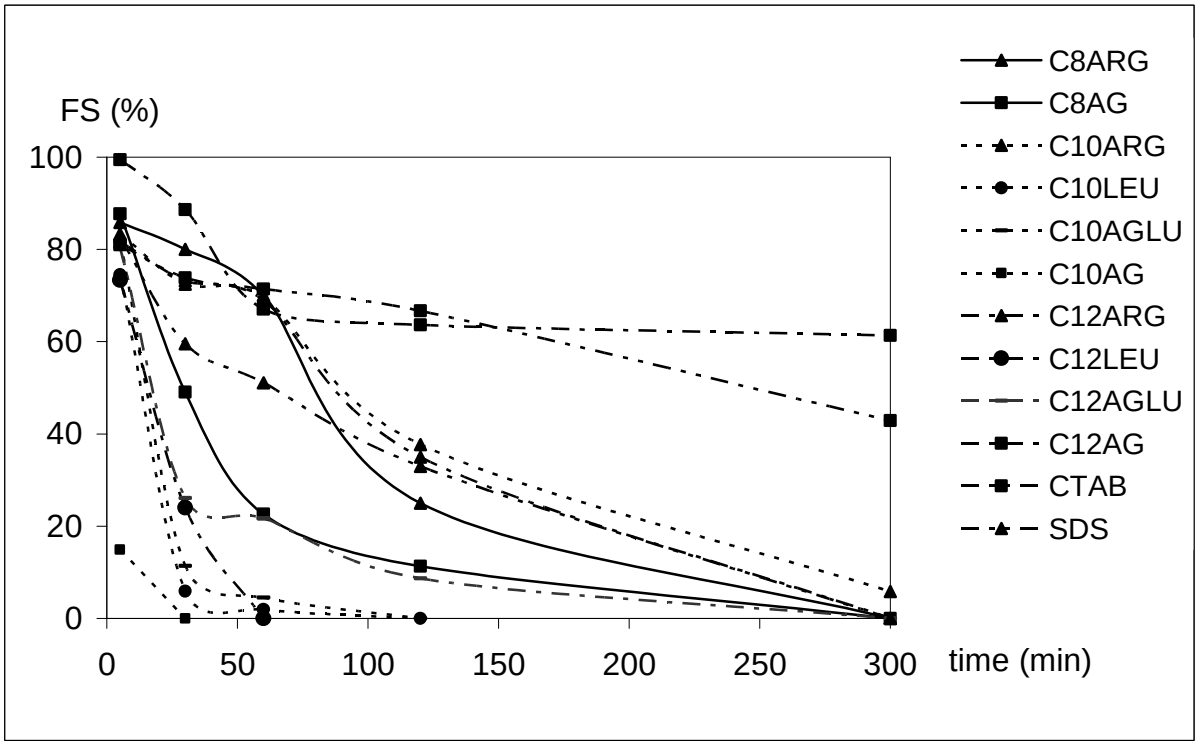

Figure 5: Measurement of foaming stability of amino acid based formulations and references

	arginine			leucine			glutamic acid			
	η_{AA}	R_{AA}	R_{FA}	η_{AA}	R_{AA}	R_{FA}	η_{AA}	R_{AA}	R_{FA}	residual acid glutamic (%)
C8	77	45	55	88	45	55	75	55	45	40
C10	84	42	58	87	51	49	53	24	76	46
C12	90	39	61	87	55	45	64	38	62	43

η_{AA}: conversion rate of acylation reaction in relation to the amino acid determined by OPA

R_{AA}: molar composition of the final mixture (ratio of N^{α}-acylated amino acids to the total quantity of N^{α}-acylated amino acids and fatty acid salt) determined by NMR

R_{FA}: molar composition of the final mixture (ratio of fatty acid salt to the total quantity of N^{α}-acylated amino acids and fatty acid salt) determined by NMR

residual acid glutamic (%): percentage of residual glutamic acid in relation to N^{α}-acylglutamic acid determined by NMR

Table1: Acylation reaction yield and the final composition of the mixture in relation to the amino acid and acid chloride used

	arginine		leucine		glutamic acid		carboxylic acid	
	CMC (mg/L)	TS (mN/m)	CMC (mg/L)	TS (mN/m)	CMC (mg/L)	TS (mN/m)	CMC (mg/L)	TS (mN/m)
C8	>2500	/	1100±100	34.1±0.5	>2500	/	>2500	/
C10	1500±150	26.0±0.5	560±40	37.8±0.5	1930±90	27.5	2700±150	27.7±0.5
C12	410±40	30.2±0.5	310±80	30.1±0.5	800±60	38.7	140±50	31.0±0.5

SDS: CMC = 290±30 mg/L; TS = 39.1 mN/m

CTAB: CMC = 280±30 mg/L; TS = 37.2 mN/m

Table 2: Critical micelle concentration of N^{α}-acylamino acid-based formulations and references

	arginine	leucine	glutamic acid	carboxylic acid
C8	160±5	0	0	150±5
C10	180±5	140±5	70±5	190±5
C12	180±5	200±5	120±5	200±5

SDS: 230±5%

CTAB: 210±5%

Table 3: Foaming capacity (%) of N^{α}-acylamino acid-based formulations and references

Author Biographies

Caroline Rondel worked at the Laboratory of Agroindustrial Chemistry, under Pr. Françoise Silvestre's management. She is working on the synthesis of new surfactants from vegetable protein and the study of their properties. She passed a chemistry engineer degree and a Search Master of Chemistry at the Toulouse University in 2005 and a Ph D of Chemistry in February 2009.

Dr. Isabelle Alric is a assistant professor at the ENSIACET, INP Toulouse. She is teaching organic chemistry and is working in the- UMR1010 Chimie Agro-Industrielle, ENSIACET, INPT, INRA, in the field of chemical modification of proteins.

Dr Jean-François BLANCO, research engineer in physicochemical analyses at the Laboratoire de Génie Chimique (ENSIACET / UPS-CNRS-INP de Toulouse) is specialised in NMR spectroscopy since September 2001. J-F Blanco owns a PhD in polymer science and membrane process delivered by the University of Rouen. He obtained, thanks to several collaborations with various scientific entities, 9 publications and 14 oral communications.

Françoise Silvestre is Professor at the University of INP/ENSIACET Toulouse. She is teaching organic chemistry and she is working in the Laboratory of Agro-Industrial Chemistry - UMR 1010 INRA/INPT-ENSIACET Toulouse-France - in the field of chemical reactivity of proteins and biodegradability of bioproducts.

Zephirin Mouloungui is a research director at the Laboratory of Agro-Industrial Chemistry - UMR 1010 INRA/INPT-ENSIACET Toulouse-France. He is working in the field of LipoChemistry, in particular glycerol chemistry. He is the author of hundred of publications.