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(71) Applicant (for all designated States except US):  
**LAMIRSA S.A.** [ES/ES]; Pol. Industrial Can Parellada, c/  
Geminis, n° 4, E-08228 Les Fonts de Terrassa/Barcelona  
(ES).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **URGELL BEL-  
TRAN, Joan, Baptista** [ES/ES]; c/ Cardenal Vives i  
Tuto 37-39 3° 3a, E-08034 Barcelona (ES). **SEGUER  
BONAVENTURA, Joan** [ES/ES]; c/ Riera Blanca 182  
5°2a, E-08902 L'Hospitalet de Llobregat/Barcelona (ES).

(74) Agent: **GILLE HRABAL STRUCK NEIDLEIN PROP  
ROOS**; Brucknerstrasse 20, E-40593 Duesseldorf (DE).

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ance Notes on Codes and Abbreviations" appearing at the begin-  
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(54) Title: USE OF CATIONIC SURFACTANTS IN COSMETIC PREPARATIONS

(57) Abstract: The invention relates to a new use of cationic surfactants derived from the condensation of fatty acids and esterified dibasic amino acids, according to the following formula: where: X<sup>-</sup> is Br, Cl, or HSO<sub>4</sub><sup>-</sup>; R<sub>1</sub>: is linear alkyl chain from an saturated fatty acid, or hydroxyacid from 8 to 14 atoms of carbon bonded to the α- amino acid group through amidic bond. R<sub>2</sub>: is a lineal or branched alkyl chain from 1 to 18 carbon atoms or aromatic R<sub>3</sub>: is: and n can be from 0 to 4. This class of compounds turned out to be highly suitable for use as preservatives in cosmetic or dermatological preparations. A particularly suitable compound is the ethyl ester of the lauramide of arginine hydrochloride (LAE).



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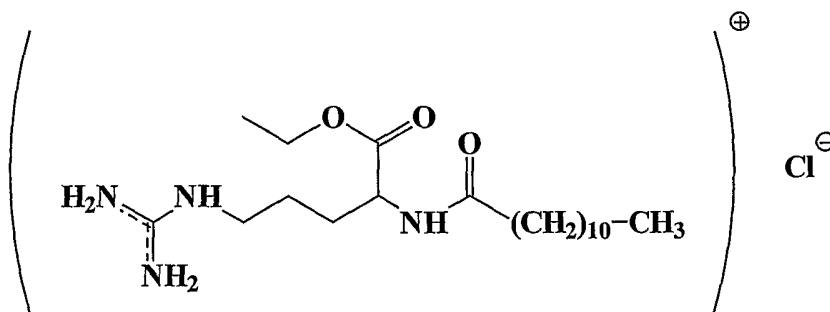
## USE OF CATIONIC SURFACTANTS IN COSMETIC PREPARATIONS

### DESCRIPTION

This invention relates to a novel use of cationic surfactants and preparations according to this novel use.

Due to their composition, many cosmetic products are susceptible to act as a culture medium for microorganisms, and this can cause possible alterations to the cosmetic preparation and constitute a possible risk to human health as well. Thus, a cosmetic composition requires good protection against microbiological contamination.

A well-known substance used for the protection against microorganisms is a cationic surfactant derived from lauric acid and arginine, in particular, the ethyl ester of the lauramide of the arginine monohydrochloride, hereafter named LAE. The chemical structure is described in the following formula:



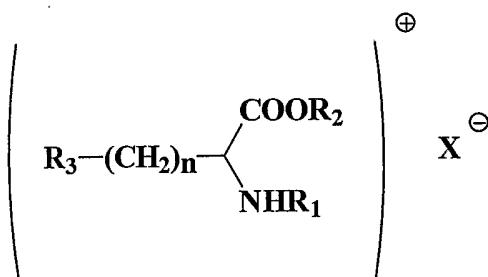
This compound is remarkable for its activity against different microorganisms, such as bacteria, fungi and yeasts and its use is known in food and feed preparations. The compound is well-known to be harmless to animals and humans. The minimum inhibitory concentrations of LAE are shown in the following table 1.

Table 1

Kind	Microorganism	M.I.C. (ppm)
Gram + Bacteria	<i>Arthrobacter oxydans</i> ATCC 8010	64
	<i>Bacillus cereus</i> var <i>mycoide</i> ATCC 11778	32
	<i>Bacillus subtilis</i> ATCC 6633	16
	<i>Clostridium perfringens</i> ATCC 77454	16
	<i>Listeria monocytogenes</i> ATCC 7644	10
	<i>Staphylococcus aureus</i> ATCC 6538	32
	<i>Micrococcus luteus</i> ATCC 9631	128
	<i>Lactobacillus delbrueckii</i> ssp <i>lactis</i> CECT 372	16
	<i>Leuconostoc mesenteroides</i> CECT 912	32
Gram – Bacteria	<i>Alcaligenes faecalis</i> ATCC 8750	64
	<i>Bordetella bronchiseptica</i> ATCC 4617	128
	<i>Citrobacter freundii</i> ATCC 22636	64
	<i>Enterobacter aerogenes</i> CECT 689	32
	<i>Escherichia coli</i> ATCC 8739	32
	<i>Escherichia coli</i> 0157H7	20
	<i>Klebsiella pneumoniae</i> var <i>pneumoniae</i> CECT 178	32
	<i>Proteus mirabilis</i> CECT 170	32
	<i>Pseudomonas aeruginosa</i> ATCC 9027	64
	<i>Salmonella typhimurium</i> ATCC16028	32
	<i>Serratia marcescens</i> CECT 274	32
	<i>Mycobacterium phlei</i> ATCC 41423	2
Fungi	<i>Aspergillus niger</i> ATCC14604	32
	<i>Aureobasidium pullulans</i> ATCC 9348	16
	<i>Gliocadium virens</i> ATCC 4645	32
	<i>Chaetomium globosum</i> ATCC 6205	16
	<i>Penicillium chrysogenum</i> CECT 2802	128
	<i>Penicillium funiculosum</i> CECT 2914	16
Yeast	<i>Candida albicans</i> ATCC 10231	16
	<i>Rhodotorula rubra</i> CECT 1158	16
	<i>Saccharomyces cerevisiae</i> ATCC 9763	32

It has now been detected that the product LAE and related compounds are particularly suitable to be used in cosmetic preparations.

The use of the invention relates to cationic surfactants derived from the condensation of fatty acids and esterified dibasic amino acids, according to the following formula:



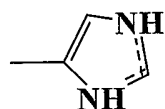
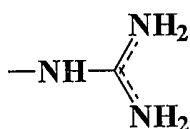
where:

$\text{X}^-$  is  $\text{Br}^-$ ,  $\text{Cl}^-$ , or  $\text{HSO}_4^-$

$\text{R}_1$ : is a linear alkyl chain from a saturated fatty acid or hydroxyacid from 8 to 14 atoms of carbon bonded to the  $\alpha$ - amino acid group through an amidic bond.

$\text{R}_2$ : is a linear or branched alkyl chain from 1 to 18 carbon atoms or an aromatic group.

$\text{R}_3$ : is:



and n can be from 0 to 4.

The most preferred compound of the above class of compounds is LAE.

LAE can be used in cosmetic formulations and preparations that are applied in the epidermis, the capillary system, lips, nails, external genital organs, or in the teeth and mouth cavity mucous, in order to clean, perfume, or modify its aspect and/or change corporal smells and/or protect a good

physical fitness. At the same time LAE inhibits the growth of microorganisms in the cosmetic formulations and preparations in which they are susceptible to develop, and also from the microorganisms that can be introduced by the practical use of the customer.

The compositions of the invention have a medium which is compatible with the skin, the mucous membranes, and hair. These compositions can have the classical components such as: fatty compounds such as mineral oil, animals oil, vegetal oil, synthesis and silicon oils, and also alcohols, fatty acids and waxes; organic solvents, surface active agents, solubilizers and ionic and non ionic emulsifiers, thickening agents and jellying hydrophilic agents such as carboxyvinilic polymers (e.g.. carbomer), acrylic copolymers (e.g.. acrylates and alkylacrylates), polyacrylamides, polysaccharides, natural gums (e.g.. xanthan gum); thickening agents and jellying lipophilic agents such as modified clays (e.g.. bentonite), fatty acid metallic salts, hydrophobic silica and polyethylene; perfumes and essential oils; softening agents; excipients; antioxidants; sequestering agents; opacifiers; filters; colouring compounds which may be either hydrosoluble or liposoluble, and pigments; and hydrophilic or lipophilic active ingredients. These compositions can also contain further preservatives besides the ones used according to the invention.

The proportions of the components mentioned in the previous paragraph are the ones normally used in the mentioned applications. These components have to be applied without changing the preservative system of the invention.

According to the invention the compositions can be in different cosmetic forms suitable for a topic application, such as:

a) Monophasic systems:

- Aqueous or hydroglycolic solution that contain one or more surfactants to be used for the cleaning of the skin, hair and mucous membranes;
- Aqueous, hydroalcoholic, hydroglycolic or oily solution that can contain other additives to be used in the general care and/or protection for skin and/or mucous membranes;
- Aqueous, hydroalcoholic, hydroglycolic or oily gel that can contain other additives to be used in general care and/or protection for skin and/or mucous membranes;
- Solid anhydride products that can contain other additives to be used in the general care and/or protection for skin and/or mucous membranes;

b) Biphasic systems:

- Aqueous, hydroalcoholic, hydroglycolic or oily gel that can contain other additives to be used in general care and/or protection for skin and/or mucous membranes;

- Solid anhydride products that can contain other additives to be used in the general care and/or protection for skin and/or mucous membranes;
  - Emulsions formed by dispersion of a oil phase in a water phase (O/W) or inverse phase (W/O), to be used in general care and/or protection of the face skin, body, hands and/or mucous; cleaning and/or removal of make-up from the skin, mucous membranes, hair and/or mouth cavity; protection and/or skin care against solar radiation effects; colouring support and pigment to be applied to the skin.
- c) And combinations of the other systems that form multiphasic systems, suspensions and microemulsions.

The compositions previously mentioned can be used in different forms such as foam, spray, or aerosol compositions and can contain a propulsion agent under pressure.

Thus, the compositions of the invention can have the aspect of a cream, a lotion, a milk, an emulsion, a gel or an oil for the skin, a beauty mask, a salt, a gel, a foam/spray or an oil for bath and shower, or for making up and making-up cleaner for the face and eyes and any other aspect known in the art.

The compositions according to the invention have been prepared according to the techniques well known for a person skilled in the art.

#### Procedure to evaluate the preservative efficacy for LAE

The method is based on the *Antimicrobial Effectiveness Testing* USP 24<sup>th</sup> Edition, 1999 (pp. 1809-1811), in order to demonstrate that the antimicrobial activity of the compound aim of the patent is enough to avoid the microbial growth that could have in the storage and use of the preparation, preventing the adverse effects of the contamination (Real Farmacopea Española, 1<sup>st</sup> Edición, 1997).

This assay consists of the contamination of the protecting formulations with an inoculum mixture of  $10^8$  cfu/mL concentration, for each of the microorganisms, and the determination of the number of viable cells in the time. This inoculum mixture is composed of the following microorganisms:

<i>Pseudomonas aeruginosa</i>	ATCC 9027
<i>Staphylococcus aureus</i>	ATCC 6538
<i>Candida albicans</i>	ATCC 10231
<i>Aspergillus niger</i>	ATCC 16404
<i>Escherichia coli</i>	ATCC 8739

The cosmetic composition to be analysed is divided into sterile containers with 50 g of product for each flask. Each container is inoculated with 0.5 mL of inoculum ( $10^8$  cfu/mL). The target concentration is  $10^6$  cfu/mL, approximately. All the containers are kept at a temperature between 20-25° C and are protected from light.

The level of the microbial contamination is checked at 0 hours, 7 days, 14 days and 28 days. The number of colonies is evaluated by dilution in buffer peptone with the appropriate neutraliser agent of the preservative. The culture media used for counting the microorganisms are: Soya triptone (35-37 °C, 48 hours) for the determination of bacteria; Sabouraud agar with chloramphenicol for fungi and yeast (25°C, 3-5 days).

According to *Antimicrobial Effectiveness Testing* USP 24<sup>th</sup> Edition, 1999 (pp. 1809-1811), an antimicrobial preservative is considered to be effective in topically used products made with aqueous bases or vehicles, non-sterile nasal products and emulsions, including those applied to mucous membranes, if:

- Not less than 2,0 logarithm reduction from the initial calculated bacteria's count is reached at 14 days and no increase from the 14 days' count at 28 days is detected; and
- No increase from the initial calculated count of yeast and moulds is observed.

## EXAMPLES

Different examples of cosmetic preparations and formulations are provided where the product has been assayed. Theses examples are a part of the preparations and formulations assayed.

### ▪ Example 1:

The composition of the cosmetic formulation in oil-in-water emulsion with non-ionic surfactant, is (in g):

- Polysorbate 60 ..... 3,00
- Sorbitan stearate ..... 2,00
- Cetyl alcohol ..... 1,00
- Paraffinum ..... 3,00
- Isopropyl mirystate ..... 3,00
- Caprylic-caproic triglycerides ..... 3,00
- Dimethicone ..... 0,50
- Propylene glycol ..... 3,00
- Cellulose gum ..... 0,25
- Carbomer 940 ..... 0,10
- Triethanolamine ..... 0,10
- Aqua ..... 100 c.s.p.

This formulation is completed with 0,20 g of LAE and its capacity of preservation is evaluated against the formulation without LAE. The results are shown in the table 2.

Table 2

	Microorganism	Without LAE (cfu/mL)	With LAE (cfu/mL)
Initial	Aerobes	$2,1 \cdot 10^6$	$1,1 \cdot 10^6$
	Fungi	$1,6 \cdot 10^4$	$1,7 \cdot 10^4$
	Yeast	$3,7 \cdot 10^5$	$5,6 \cdot 10^5$
7 days	Aerobes	$2,1 \cdot 10^6$	$3,1 \cdot 10^3$
	Fungi	$7,0 \cdot 10^2$	$< 9,9 \cdot 10^1$
	Yeast	$8,2 \cdot 10^3$	$9,5 \cdot 10^2$
14 days	Aerobes	$6,2 \cdot 10^6$	$3,3 \cdot 10^2$
	Fungi	$5,9 \cdot 10^2$	$< 9,9 \cdot 10^1$
	Yeast	$4,8 \cdot 10^3$	$4,0 \cdot 10^2$

At 28 days no increase has been detected from the 14 days' count.



▪ **Example 2:**

The composition of an oil-in-water emulsion with an ionic emulsifier, used as cosmetic formulation, is (in g):

- Stearic acid..... 1,70
- Glyceryl stearate S.E..... 2,50
- Cetyl alcohol..... 1,50
- Paraffinum ..... 3,00
- Isopropyl myristate ..... 3,00
- Caprylic-caproic triglycerides ..... 3,00
- Dimethicone..... 0,50
- Propylene glycol..... 3,00
- Cellulose gum..... 0,50
- Triethanolamine..... 1,03
- Aqua ..... 100 c.s.p.

This formulation is completed with 0,20 g of LAE and its capacity of preservation is evaluated against the formulation without LAE. The results are shown in the table 3.

**Table 3**

	Microorganism	Without LAE (cfu/mL)	With LAE (cfu/mL)
Initial	Aerobes	$7,4 \cdot 10^6$	$2,7 \cdot 10^6$
	Fungi	$2,0 \cdot 10^4$	$1,4 \cdot 10^4$
	Yeast	$3,6 \cdot 10^4$	$3,6 \cdot 10^4$
7 days	Aerobes	$5,2 \cdot 10^6$	$1,6 \cdot 10^4$
	Fungi	$8,8 \cdot 10^2$	$< 9,9 \cdot 10^1$
	Yeast	$4,7 \cdot 10^4$	$1,0 \cdot 10^2$
14 days	Aerobes	$1,7 \cdot 10^7$	$6,5 \cdot 10^2$
	Fungi	$7,0 \cdot 10^2$	$< 9,9 \cdot 10^1$
	Yeast	$1,0 \cdot 10^4$	$1,0 \cdot 10^2$

At 28 days no increase has been detected from the 14 days' count.

▪ **Example 3:**

The general composition for a cosmetic formulation, in water in oil emulsion with non-ionic emulsifiers, is (in g)

- Cetyl Dimethicone copolyol ..... 3,00
- Isohexadecane ..... 6,00
- Paraffinum ..... 8,00
- Isopropyl myristate ..... 6,00
- Caprylic-caproic triglycerides ..... 4,00
- Glycerin ..... 5,00
- Sodium chloride ..... 0,50
- Aqua ..... 100 c.s.p.

This formulation is completed with 0,20 g of LAE and its capacity of preservation is evaluated against the formulation without LAE. The results are shown in the Table 4.

**Table 4**

	Microorganism	Without LAE (cfu/mL)	With LAE (cfu/mL)
Initial	Aerobes	$1,6 \cdot 10^6$	$6,2 \cdot 10^6$
	Fungi	$2,0 \cdot 10^4$	$1,0 \cdot 10^4$
	Yeast	$3,8 \cdot 10^4$	$7,0 \cdot 10^4$
7 days	Aerobes	$1,1 \cdot 10^6$	$1,8 \cdot 10^3$
	Fungi	$5,0 \cdot 10^2$	$< 9,9 \cdot 10^1$
	Yeast	$9,0 \cdot 10^2$	$< 9,9 \cdot 10^1$
14 days	Aerobes	$8,7 \cdot 10^6$	$< 9,9 \cdot 10^1$
	Fungi	$3,0 \cdot 10^2$	$< 9,9 \cdot 10^1$
	Yeast	$3,0 \cdot 10^2$	$< 9,9 \cdot 10^1$

At 28 days no increase has been detected from the 14 days' count.

▪ **Example 4:**

The composition of a formulation to obtain an aqueous solution with a surfactants' mixture, is (in g):

- Sodium lauryl sulfate (sol. 27%)..... 14,00
- Cocamidopropyl betaine..... 6,00
- Disodium cocoamfoacetate ..... 6,00
- Lactic acid..... 0,25
- Sodium chloride..... 0,50
- Aqua ..... 100 c.s.p.

This formulation is applied in bath gels.

This formulation is completed with 0,20 g of LAE and its capacity of preservation is evaluated against the formulation without LAE. The results are shown in the Table 5.

**Table 5**

	Microorganism	Without LAE (cfu/mL)	With LAE (cfu/mL)
Initial	Aerobes	$3,6 \cdot 10^6$	$3,3 \cdot 10^6$
	Fungi	$1,6 \cdot 10^4$	$1,9 \cdot 10^4$
	Yeast	$3,9 \cdot 10^4$	$4,6 \cdot 10^4$
7 days	Aerobes	$4,2 \cdot 10^6$	$5,8 \cdot 10^3$
	Fungi	$2,7 \cdot 10^3$	$2,7 \cdot 10^2$
	Yeast	$4,5 \cdot 10^3$	$< 9,9 \cdot 10^1$
14 days	Aerobes	$5,5 \cdot 10^6$	$< 9,9 \cdot 10^1$
	Fungi	$3,4 \cdot 10^3$	$< 9,9 \cdot 10^1$
	Yeast	$5,9 \cdot 10^3$	$< 9,9 \cdot 10^1$

At 28 days no increase has been detected from the 14 days' count.

▪ **Example 5:**

The composition of a formulation to obtain an aqueous solution with a surfactants' mixture, is (in g):

- Sodium lauryl sulfate (sol. 27%)..... 14,00
- Cocamidopropyl betaine..... 6,00
- Disodium lauryl sulfosuccinate..... 6,00
- Lactic acid..... 0,25
- Sodium chloride..... 0,50
- Aqua ..... 100 c.s.p.

This formulation is applied in bath gels.

This formulation is completed with 0,20 g of LAE and its capacity of preservation is evaluated against the formulation without LAE. The results are shown in the Table 6.

**Table 6**

	Microorganism	Without LAE (cfu/mL)	With LAE (cfu/mL)
Initial	Aerobes	$8,6 \cdot 10^6$	$6,7 \cdot 10^6$
	Fungi	$1,1 \cdot 10^4$	$1,4 \cdot 10^4$
	Yeast	$3,2 \cdot 10^4$	$3,6 \cdot 10^4$
7 days	Aerobes	$3,9 \cdot 10^7$	$4,8 \cdot 10^2$
	Fungi	$1,6 \cdot 10^3$	$< 9,9 \cdot 10^1$
	Yeast	$3,1 \cdot 10^3$	$< 9,9 \cdot 10^1$
14 days	Aerobes	$3,3 \cdot 10^6$	$1,3 \cdot 10^2$
	Fungi	$8,3 \cdot 10^3$	$< 9,9 \cdot 10^1$
	Yeast	$3,9 \cdot 10^4$	$< 9,9 \cdot 10^1$

At 28 days no increase has been detected from the 14 days' count.

▪ **Example 6:**

The composition of a formulation to obtain a hydroalcoholic gel, is (in g):

- Hydroxyethyl cellulose..... 0,40
- Carbomer 940. .... 0,40
- Glycerin ..... 8,00
- Alcohol denat..... 30,00
- PEG 40 hydrogenated castor oil ..... 1,50
- Parfum ..... 0,75
- Triethanolamine..... 0,25
- Aqua ..... 100 c.s.p.

This formulation is applied in lotions for after-shaving skin care.

This formulation is completed with 0,20 g of LAE and its capacity of preservation is evaluated against the formulation without LAE. The results are shown in the Table 7.

**Table 7**

	Microorganism	Without LAE (cfu/mL)	With LAE (cfu/mL)
Initial	Aerobes	$1,1 \cdot 10^6$	$3,7 \cdot 10^6$
	Fungi	$8,7 \cdot 10^4$	$9,1 \cdot 10^4$
	Yeast	$3,9 \cdot 10^4$	$4,2 \cdot 10^4$
7 days	Aerobes	$4,6 \cdot 10^6$	$6,9 \cdot 10^3$
	Fungi	$9,1 \cdot 10^3$	$4,1 \cdot 10^2$
	Yeast	$8,6 \cdot 10^2$	$1,6 \cdot 10^2$
14 days	Aerobes	$7,3 \cdot 10^6$	$< 9,9 \cdot 10^1$
	Fungi	$1,7 \cdot 10^3$	$< 9,9 \cdot 10^1$
	Yeast	$1,2 \cdot 10^3$	$< 9,9 \cdot 10^1$

At 28 days no increase has been detected from the 14 days' count.

▪ **Example 7:**

The composition of a formulation to obtain a facial tonic, is (in g):

- Hydroxyethyl cellulose..... 0,20

- Caprylic-caproic triglycerides ..... 1,00
- PEG 40 hydrogenated castor oil.. ..... 6,00
- Lactic acid..... 1,00
- Sodium chloride..... 0,35
- Glycerin ..... 3,00
- *Chamomilla Recutita* extract ..... 3,00
- Aqua ..... 100 c.s.p.

This formulation is completed with 0,20 g of LAE and its capacity of preservation is evaluated against the formulation without LAE. The results are shown in the Table 8.

Table 8

	Microorganism	Without LAE (cfu/mL)	With LAE (cfu/mL)
Initial	Aerobes	$6,7 \cdot 10^6$	$2,7 \cdot 10^6$
	Fungi	$4,1 \cdot 10^4$	$2,1 \cdot 10^4$
	Yeast	$3,2 \cdot 10^4$	$1,2 \cdot 10^4$
7 days	Aerobes	$3,7 \cdot 10^7$	$3,6 \cdot 10^3$
	Fungi	$9,1 \cdot 10^3$	$1,3 \cdot 10^2$
	Yeast	$4,2 \cdot 10^3$	$1,1 \cdot 10^2$
14 days	Aerobes	$8,7 \cdot 10^7$	$5,9 \cdot 10^2$
	Fungi	$2,1 \cdot 10^4$	$< 9,9 \cdot 10^1$
	Yeast	$1,2 \cdot 10^4$	$< 9,9 \cdot 10^1$

At 28 days no increase has been detected from the 14 days' count.

#### ▪ Example 8:

The composition of a formulation to obtain a mask-up cleaner, is (in g):

- Stearic acid..... 2,00
- Glyceryl stearate S.E..... 2,50
- Cetyl alcohol..... 1,50
- Paraffinum ..... 6,00

- Isopropyl myristate ..... 1,50
- Caprylic-caproic triglycerides ..... 1,50
- Dimethicone..... 0,50
- Propylene glycol ..... 3,00
- Triethanolamine..... 1,20
- Aqua ..... 100 c.s.p.

This formulation is completed with 0,20 g of LAE, and the preservative capacity is evaluated and it is compared with the formulation without LAE. The results are shown in the Table 9.

Table 9

	Microorganism	Without LAE (cfu/mL)	With LAE (cfu/mL)
Initial	Aerobes	$5,5 \cdot 10^6$	$4,5 \cdot 10^6$
	Fungi	$7,9 \cdot 10^4$	$7,6 \cdot 10^4$
	Yeast	$8,4 \cdot 10^4$	$7,2 \cdot 10^4$
7 days	Aerobes	$6,5 \cdot 10^6$	$3,8 \cdot 10^3$
	Fungi	$8,2 \cdot 10^2$	$3,5 \cdot 10^2$
	Yeast	$8,8 \cdot 10^2$	$1,8 \cdot 10^2$
14 days	Aerobes	$9,5 \cdot 10^6$	$6,7 \cdot 10^2$
	Fungi	$2,9 \cdot 10^3$	$< 9,9 \cdot 10^1$
	Yeast	$1,8 \cdot 10^3$	$< 9,9 \cdot 10^1$

At 28 days no increase has been detected from the 14 days' count.

#### ▪ Example 9:

The composition of a formulation to obtain a fluid oil-in-water emulsion with non-ionic surfactants, is (in g):

- Polysorbate 60 ..... 3,00
- Sorbitan stearate ..... 2,00
- Cetyl alcohol..... 0,75
- Paraffinum ..... 3,00

- Isopropyl myristate ..... 2,50
- Caprylic-caproic triglycerides ..... 2,00
- Dimethicone..... 0,50
- Propylene glycol ..... 3,00
- Aqua ..... 100 c.s.p.

This formulation is applied in body oil.

This formulation is completed with 0,20 g of LAE, and the preservative capacity is evaluated and it is compared with the formulation without LAE. The results are shown in the Table 10.

**Table 10**

	Microorganism	Without LAE (cfu/mL)	With LAE (cfu/mL)
Initial	Aerobes	$1,5 \cdot 10^6$	$4,5 \cdot 10^6$
	Fungi	$2,6 \cdot 10^4$	$7,6 \cdot 10^4$
	Yeast	$3,2 \cdot 10^4$	$7,2 \cdot 10^4$
7 days	Aerobes	$4,5 \cdot 10^6$	$7,6 \cdot 10^3$
	Fungi	$7,7 \cdot 10^3$	$1,4 \cdot 10^2$
	Yeast	$8,4 \cdot 10^3$	$2,3 \cdot 10^2$
14 days	Aerobes	$6,3 \cdot 10^6$	$< 9,9 \cdot 10^1$
	Fungi	$1,6 \cdot 10^4$	$< 9,9 \cdot 10^1$
	Yeast	$7,9 \cdot 10^3$	$< 9,9 \cdot 10^1$

At 28 days no increase has been detected from the 14 days' count.

▪ **Example 10:**

The composition of a toothpaste formulation is (in g):

- Calcium carbonate ..... 16,00
- Dicalcium phosphate ..... 24,00
- Silica ..... 2,00
- Petrolatum ..... 10,00
- Glycerine ..... 20,00
- Sodium fluoride ..... 0,20



- Hydroxyethyl cellulose..... 1,00
- Lauroyl sarcosine ..... 2,00
- Aqua ..... 100 c.s.p.

This formulation is completed with 0,20 g of LAE, and the preservative capacity is evaluated and it is compared with the formulation without LAE. The results are shown in the Table 11.

Table 11

	Microorganism	Without LAE (cfu/mL)	With LAE (cfu/mL)
Initial	Aerobes	$6,5 \cdot 10^6$	$4,5 \cdot 10^6$
	Fungi	$9,6 \cdot 10^4$	$5,6 \cdot 10^4$
	Yeast	$4,8 \cdot 10^4$	$4,2 \cdot 10^4$
7 days	Aerobes	$3,5 \cdot 10^7$	$3,3 \cdot 10^3$
	Fungi	$1,6 \cdot 10^4$	$1,6 \cdot 10^2$
	Yeast	$5,2 \cdot 10^3$	$1,2 \cdot 10^2$
14 days	Aerobes	$6,5 \cdot 10^7$	$8,0 \cdot 10^2$
	Fungi	$1,7 \cdot 10^4$	$< 9,9 \cdot 10^1$
	Yeast	$4,8 \cdot 10^3$	$< 9,9 \cdot 10^1$

At 28 days no increase has been detected from the 14 days' count.

▪ **Example 11:**

The composition of a formulation to obtain an aqueous solution with surfactants, is (in g):

- Sodium lauryl sulfate (sol. 27%)..... 12,00
- Cocamidopropyl betaine..... 5,00
- Disodium cocoamfoacetate ..... 5,00
- Polyquaternium11 ..... 1,00
- Lactic acid..... 0,25
- Sodium chloride..... 0,50
- Aqua ..... 100 c.s.p.

This formulation is applied in shampoos cosmetic formulations.

This formulation is completed with 0,20 g of LAE, and the preservative capacity is evaluated and it is compared with the formulation without LAE. The results are shown in the Table 12.

**Table 12**

	Microorganism	Without LAE (cfu/mL)	With LAE (cfu/mL)
Initial	Aerobes	$3,7 \cdot 10^6$	$3,3 \cdot 10^6$
	Fungi	$7,6 \cdot 10^4$	$9,6 \cdot 10^4$
	Yeast	$3,2 \cdot 10^4$	$4,2 \cdot 10^4$
7 days	Aerobes	$5,9 \cdot 10^6$	$4,8 \cdot 10^3$
	Fungi	$9,6 \cdot 10^2$	$1,1 \cdot 10^2$
	Yeast	$4,9 \cdot 10^3$	$1,2 \cdot 10^2$
14 days	Aerobes	$6,3 \cdot 10^6$	$7,0 \cdot 10^2$
	Fungi	$1,1 \cdot 10^3$	$< 9,9 \cdot 10^1$
	Yeast	$5,2 \cdot 10^3$	$< 9,9 \cdot 10^1$

At 28 days no increase has been detected from the 14 days' count.

▪ **Example 12:**

The composition of a formulation to obtain an oil-in-water emulsion with non-ionic surfactants, is (in g):

- Glyceryl stearate + PEG 100 stearate..... 4,00
- Cetyl alcohol + sodium cetyl sulfate ..... 2,00
- Caprylic-caproic triglycerides ..... 4,00
- Isopropyl mirystate ..... 2,50
- Paraffinum ..... 2,00
- Dimethicone..... 0,50
- Glycerin ..... 3,00

- Wheat (*triticum vulgare*) germ protein ..... 2,00
- Aqua ..... 100 c.s.p.

This formulation is applied in a face cream for skin care.

This formulation is completed with 0,20 g of LAE and its capacity of preservation is evaluated against the formulation without LAE. The results are shown in the Table 13.

**Table 13**

	Microorganism	Without LAE (cfu/mL)	With LAE (cfu/mL)
Initial	Aerobes	$3,3 \cdot 10^6$	$1,1 \cdot 10^6$
	Fungi	$1,6 \cdot 10^4$	$1,7 \cdot 10^4$
	Yeast	$2,2 \cdot 10^4$	$3,2 \cdot 10^4$
7 days	Aerobes	$4,3 \cdot 10^6$	$3,7 \cdot 10^4$
	Fungi	$1,9 \cdot 10^2$	$8,7 \cdot 10^2$
	Yeast	$2,5 \cdot 10^2$	$9,2 \cdot 10^2$
14 days	Aerobes	$4,3 \cdot 10^6$	$1,9 \cdot 10^3$
	Fungi	$1,8 \cdot 10^2$	$< 9,9 \cdot 10^1$
	Yeast	$2,9 \cdot 10^2$	$< 9,9 \cdot 10^1$

At 28 days no increase has been detected from the 14 days' count.

▪ **Example 13:**

The composition of a formulation to obtain an oil-in-water emulsion with non-ionic surfactants, is (in g):

- Polysorbate 60 ..... 3,00
- Sorbitan stearate ..... 2,00
- Cetyl alcohol ..... 2,50
- Paraffinum ..... 2,00
- Caprylic-caproic triglycerides ..... 2,00
- Ethyl hexyl methoxycinnamate ..... 5,00
- Benzophenone 3 ..... 1,00
- Dimethicone ..... 0,50
- Propylene glycol ..... 3,00

- Aqua ..... 100 c.s.p.

This formulation is applied in a sun protector cosmetic formulator.

This formulation is completed with 0,20 g of LAE and its capacity of preservation is evaluated against the formulation without LAE. The results are shown in the Table 14.

**Table 14**

	Microorganism	Without LAE (cfu/mL)	With LAE (cfu/mL)
Initial	Aerobes	$4,4 \cdot 10^6$	$3,1 \cdot 10^6$
	Fungi	$5,7 \cdot 10^4$	$4,9 \cdot 10^4$
	Yeast	$2,7 \cdot 10^4$	$3,8 \cdot 10^4$
7 days	Aerobes	$6,3 \cdot 10^6$	$8,4 \cdot 10^3$
	Fungi	$5,1 \cdot 10^2$	$2,7 \cdot 10^2$
	Yeast	$2,3 \cdot 10^2$	$4,2 \cdot 10^2$
14 days	Aerobes	$7,2 \cdot 10^6$	$7,5 \cdot 10^2$
	Fungi	$5,9 \cdot 10^2$	$< 9,9 \cdot 10^1$
	Yeast	$2,8 \cdot 10^2$	$< 9,9 \cdot 10^1$

At 28 days no increase has been detected from the 14 days' count.

▪ **Example 14:**

The composition of a formulation to obtain an oil-in-water emulsion with non-ionic surfactants is (in g):

- Cetyl Dimethicone copolyol ..... 3,00
- Isohexadecane. .... 4,00
- Paraffinum ..... 5,00
- Isopropyl myristate ..... 3,00
- Caprylic-caproic triglycerides ..... 3,00
- Ethyl hexyl methoxycinnamate..... 5,00
- Benzophenone 3 ..... 1,00
- Glycerin ..... 3,00
- Sodium chloride..... 0,50
- Aqua ..... 100 c.s.p.

This formulation is applied in a sun protector cosmetic product.

This formulation is completed with 0,20 g of LAE, and the preservative capacity is evaluated and it is compared with the formulation without LAE. The results are shown in the Table 15.

**Table 15**

	Microorganism	Without LAE (cfu/mL)	With LAE (cfu/mL)
Initial	Aerobes	$2,8 \cdot 10^6$	$1,4 \cdot 10^6$
	Fungi	$5,5 \cdot 10^4$	$5,3 \cdot 10^4$
	Yeast	$7,7 \cdot 10^4$	$7,9 \cdot 10^4$
7 days	Aerobes	$4,4 \cdot 10^6$	$9,4 \cdot 10^2$
	Fungi	$8,6 \cdot 10^2$	$6,7 \cdot 10^2$
	Yeast	$8,3 \cdot 10^2$	$4,5 \cdot 10^2$
14 days	Aerobes	$7,2 \cdot 10^6$	$8,7 \cdot 10^2$
	Fungi	$5,8 \cdot 10^2$	$< 9,9 \cdot 10^1$
	Yeast	$7,9 \cdot 10^2$	$< 9,9 \cdot 10^1$

At 28 days no increase has been detected from the 14 days' count.

▪ **Example 15:**

The composition of a formulation to obtain an emulsion for hands care, is (in g):

- Cetyl alcohol + ceteareth 20..... 6,00
- Isopropyl myristate ..... 2,00
- Caprylic-caproic triglycerides ..... 1,00
- Dimethicone..... 1,00
- Benzophenone 3 ..... 1,00
- Glycerin ..... 6,00
- Carbomer 940 ..... 0,10
- Triethanolamine..... 0,10
- Aqua ..... 100 c.s.p.

This formulation is completed with 0,20 g of LAE, and the preservative capacity is evaluated and it is compared with the formulation without LAE. The results are shown in the Table 16.

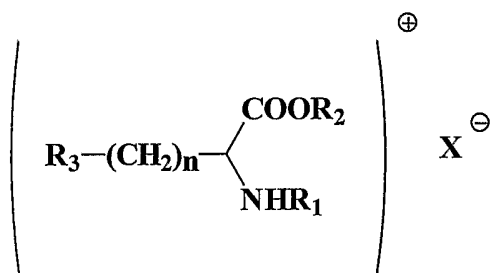
Table 16

	Microorganism	Without LAE (cfu/mL)	With LAE (cfu/mL)
Initial	Aerobes	$4,5 \cdot 10^6$	$4,4 \cdot 10^6$
	Fungi	$6,1 \cdot 10^4$	$5,8 \cdot 10^4$
	Yeast	$8,8 \cdot 10^4$	$8,6 \cdot 10^4$
7 days	Aerobes	$7,4 \cdot 10^6$	$3,3 \cdot 10^3$
	Fungi	$7,8 \cdot 10^2$	$8,7 \cdot 10^2$
	Yeast	$8,9 \cdot 10^2$	$7,2 \cdot 10^2$
14 days	Aerobes	$4,4 \cdot 10^6$	$< 9,9 \cdot 10^1$
	Fungi	$9,8 \cdot 10^2$	$< 9,9 \cdot 10^1$
	Yeast	$1,2 \cdot 10^3$	$< 9,9 \cdot 10^1$

At 28 days no increase has been detected from the 14 days' count.

## CLAIMS

1. Use of cationic surfactants derived from the condensation of fatty acids and esterified dibasic amino acids, according to the following formula:



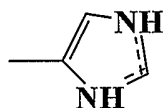
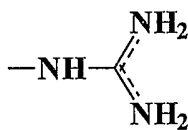
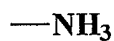
where:

$\text{X}^-$  is  $\text{Br}^-$ ,  $\text{Cl}^-$ , or  $\text{HSO}_4^-$

$\text{R}_1$ : is linear alkyl chain from an saturated fatty acid, or hydroxyacid from 8 to 14 atoms of carbon bonded to the  $\alpha$ - amino acid group through amidic bond.

$\text{R}_2$ : is a lineal or branched alkyl chain from 1 to 18 carbon atoms or aromatic.

$\text{R}_3$ : is:



and n can be from 0 to 4,

as preservatives in cosmetic or dermatological preparations.

2. The use according to Claim 1, wherein the cationic surfactant is the ethyl ester of the lauramide of arginine hydrochloride (LAE).

3. A cosmetic or dermatological composition comprising the cationic surfactant defined in claim 1 or 2 as a preservative.
4. The cosmetic or dermatological composition of claim 3, wherein the cationic surfactant is LAE at a concentration of from 0,001 to 2%.
5. Composition according to the claim 3 or 4 further comprising fatty compounds such as mineral oil, animals, vegetal, from synthesis and silicon, and also alcohol, fatty acids and waxes; organic solvents, surface active agents, solubilizers and ionic and non ionic emulsifiers, thickening agents and jellying hydrophilic agents such as carboxyvinilic polymers (e.g.. carbomer), acrylic copolymers (e.g.. acrylates and alkylacrylates), polyacrylamides, polysaccharides, natural gums (e.g.. xanthan gum); thickening agents and jellying lipophilic agents such as modified clays (e.g.. bentonite), fatty acid metallic salts, hydrophobic silica and polyethylene; perfumes and essential oils; softening agents; excipients; antioxidants; sequestering agents; opacifiers; filters; colouring compounds hydrosolubles and liposolubles, and pigments; and hydrophilic or lipophilic active ingredients.
6. Composition according to the claims 3 to 5 in the form of an aqueous, hydroalcoholic, or hydroglycolic solution, of an emulsion, microemulsion, aqueous or anhydrous gel or of a vesicle dispersion.
7. Composition according to any of claims 3 to 6 for skin care.



# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 01/09198

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K7/40 A61K7/48 A61K7/50 A61K7/16 A61K7/46

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, PAJ, CHEM ABS Data, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 749 960 A (MIRET LAB) 27 December 1996 (1996-12-27) claims 8,9	1,3
X	GB 1 352 420 A (AJINOMOTO KK) 8 May 1974 (1974-05-08) page 2, left-hand column, line 7 - line 14; claims 1,13; examples 2,8,9; tables 3,4	1,3

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Voyiazoglou, D

## INTERNATIONAL SEARCH REPORT

International Application No

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	<p>---  PATENT ABSTRACTS OF JAPAN  vol. 1998, no. 01,  30 January 1998 (1998-01-30)  &amp; JP 09 255518 A (NOEVIR CO LTD),  30 September 1997 (1997-09-30)  abstract</p>	1,3
A	<p>---  DATABASE CA 'Online!  CHEMICAL ABSTRACTS SERVICE, COLUMBUS,  OHIO, US;  GARCIA DOMINGUEZ, J. ET AL: "Cationic  surfactants with antimicrobial activity"  retrieved from STN  Database accession no. 107:79974  XP002196810  abstract  &amp; ES 530 051 A (CONSEJO SUPERIOR DE  INVESTIGACIONES CIENTIFICAS, SPAIN)  1 May 1985 (1985-05-01)</p>	1,3
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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 01/09198

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>DATABASE CA 'Online!            CHEMICAL ABSTRACTS SERVICE, COLUMBUS,            OHIO, US;            GARCIA DOMINGUEZ, J. J. ET AL:            "N.alpha.-Acyl-L-alkylaminoguanidinic            acids and their salts as surfactants with            antimicrobial action"            retrieved from STN            Database accession no. 99:122920            XP002196912            abstract            &amp; ES 512 643 A (ASOCIACION DE            INVESTIGACION DE DETERGENTES, SPAIN)            16 February 1983 (1983-02-16)</p>	1,3
A	<p>PATENT ABSTRACTS OF JAPAN            vol. 007, no. 118 (C-167),            21 May 1983 (1983-05-21)            &amp; JP 58 039651 A (AJINOMOTO KK),            8 March 1983 (1983-03-08)            abstract</p>	1

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Information on patent family members

International Application No

PCT/EP 01/09198

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