Ecotoxicological Assessment of Mixtures of Anionic/Non-ionic Surfactants in the Aquatic Environment

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Abstract

The purpose of this study was to discuss the effect of the chemical structure of anionic and non-ionic surfactants and their mixtures, and surface activity over toxicity. Single and binary mixtures of three ether carboxylic derivative surfactants and three amine-oxide-based non-ionic surfactants have been used. Toxicity has been determined using three test organisms: freshwater crustaceans, luminescent bacteria, and microalgae. The toxicity of surfactants is related to the hydrophobic alkyl chain, the degree of ethoxylation, and the critical micelle concentration of surfactants. Relationships found agreed with the fact that shortening the alkyl chain length lowers toxicity. There is a strong relation between surface activity and toxicity, so that the greater the surface activity the stronger the toxicity: the toxicity increased as the CMC of the surfactant or mixtures of surfactants decreased. The most sensitive microorganism to variations of the CMC was the microalga *S. capricornutum* and the least sensitive was *V. fischeri*. The results have given rise to a classification of the different surfactants and their mixtures according to the organism test, as safe, harmful or toxic. Taking into account the microorganism assayed, the bacterium *Vibrio fischeri* was in general the most sensitive to toxic effect from the surfactants, followed by *Daphnia magna*, while microalgae were more tolerant. These results can be useful for selecting technically efficient surfactants and their mixtures with a lower ecotoxicity on the aquatic environment.

Keywords Anionic surfactants, Microalgae, Non-ionic surfactants, Synergism, Toxicity.

1. Introduction

Surfactants constitute an important family of industrial chemical products that are widely used in practically all facets of modern industry. During the last decade, the global demand for surfactants has grown some 300% and their current annual world production exceeds three million tonnes. From this production, around 54% are used in detergents for textile products and cleaning products for the home, with only some 32% dedicated to industrial uses [1].

The twelve principles of green chemistry stated by Anastas and Kirchhoff [2] include: 3) "Synthetic methodologies should be designed to use and generate substances that possess little or no toxicity to human health and the environment" 4) "Chemical products should be designed to preserve efficacy of function while reducing toxicity" and 10) "Chemical products should be designed so that at the end of their function they do not persist in the environment and break down into innocuous degradation products". The potential environmental impact of chemicals is often determined by their ecotoxicity: toxicity and environmental exposure. The first is relatively high in the case of surfactants as a result of surface activity and the action against biological membranes [3]. Most surfactants are not acutely toxic to organisms at environmental concentrations, chronic aquatic toxicity of surfactants occurring at concentrations usually greater than 0.1 mg/L [4]. Of the many studies concerning biodegradability and toxicity of surfactants, most concern toxicity to small crustaceans such as *Daphnia magna* [5]. There has been an emphasis in recent years to develop non-polluting surfactants and builders with improved biodegradability [6]. This growing concern has promoted the development and use of more environmentally friendly surfactants such as the ether carboxylic derivative surfactants and the amine-oxide-based surfactants.

The ether carboxylic derivative surfactants tested in the present work are anionic surfactants with the general formula $R\text{-}O(CH_2\text{-}CH_2O)_E\text{-}CH_2\text{-}COO^-X$, where R is the alkyl chain and $X= H^+$ or Na. These surfactants improve the foaming quality of the detergent, reducing the irritation level, and therefore

they are used as co-surfactants in detergents which have to be in contact with the skin. Amine-oxide-based surfactants constitute a particular class of nitrogen non-ionic surfactants that exhibit cationic behaviour in acid solution. They show good foaming properties and are skin compatible [7]. These compounds, the consumption of which is estimated at 14 ktons year-^{1} [8] only in Westeren Europe, are widely used in detergents, toiletry, and antistatic preparations, usually together with other surfactants. They are compatible with anionic surfactants and offer synergistic advantages to formulations [9, 10].

For continued advancement in the search for relationships between toxicity and structural parameters in the field of surfactants, in the present work the ecotoxicity assay with luminescent bacteria, *Daphnia magna*, and microalgae is applied to different surfactants: ether carboxylic derivative surfactants and amine-oxide-based surfactants. Because a combination surfactant system usually exhibits better detergency performance than the composition containing single-surfactant [6], the objective of this study is to evaluate the individual and combined toxicity of different anionic/non-ionic surfactants to assess the toxicological interactions between the surfactants, which take place in natural environments, and how they can affect the toxicity of the mixture, especially when acting in synergism. Although a substantial body of data is available on the aquatic toxicity of various surfactants, few reported data are available on the synergism in binary mixtures 1:1 weight of surfactants related to aquatic toxicity. The results can be useful for the selection of technically efficient surfactants with a lower impact on the aquatic environment.

2. Materials and Experimentals

2.1. Surfactants

The surfactants used in this study are the commercial ether carboxylic derivative surfactants EC-R_8E_8, EC-$R_{12\text{-}14}E_3$ and EC-$R_{12\text{-}14}E_{10}$, together with the amine-oxide-based surfactants AO-R_{14}, AO-R_{12} and AOP-Cocoamido (supplied by Kao Corporation S.A., Tokyo, Japan). Table 1 shows the degree

of ethoxylation (E), the alkyl chain length (R), the % of active matter, and the critical micelle concentration (CMC) of the surfactants. The rest of the reagents used were supplied by Panreac. The chemical structure of the amine-oxide-based surfactants is shown in Figure S1 in the supplementary information.

2.2. Surface-Tension Measurements

CMC values were established by measuring the surface tension of surfactant solutions with different concentrations at 25ºC and pH =7, using a tensiometer model Tensiometer K11 (KRÜSS GmbH) equipped with a 2 cm platinum plate. The platinum plate in all cases was cleaned and heated to a reddish-orange colour with a Bunsen burner before use. At least three assays were made for each experimental datum. CMC data for the ether carboxylic derivative surfactants and amine-oxide-based surfactants are shown in Table 1.

The surface tension was measured also for the different mixtures of surfactants. The surface-tension data plotted on a semi-log plot for a surfactant has an approximately linear drop in surface tension followed by a plateau. The concentration at which this discontinuous change in slope occurs is the CMC.

2.3. Conductivity Measurements

In order to corroborate the surface tension measurements used to determine the CMC values, the CMC were also estimated by measuring conductivity. A minimum of 20 surfactant solutions in deionised water, 10 above and 10 below the expected CMC of each surfactant, were prepared in order to make the correct CMC determination. These solutions were placed in a constant-temperature bath of 25ºC for at least 20 min before the measurements were taken. The electrical conductivity was measured with a conductometer, model CDM210 from Radiometer Analytical, operated at 1 kHz. A two-pole conductivity cell, model CDC641T, was used. The cell constant, 0.847 cm^{-1}, was determined by calibration with potassium chloride standards (0.01 and 0.005 M). Accuracy was

±0.2% of the reading. Good agreement was found between the two technical procedures. Figure 1 presents the determination of CMC using the two methods for the mixture EC-R_8E_8 + EC-$R_{12-14}E_{10}$.

2.3. Toxicity Tests

Three toxicity tests were undertaken: the LumiStox® 300 test which employs the luminescent bacterium *Vibrio fischeri*, the 24-h immobilization test with *Daphnia magna* (freshwater crustacean), and the 72-h algal growth-inhibition test with *Selenastrum capricornutum*. In the first test, measurements were taken with LumiStox® 300, an instrument for measuring bioluminescence, and an incubation unit according to the UNE-EN ISO 11348-2 guideline [11]. The toxicity measurement was based on the luminous intensity of the marine bacteria of the strain *V. fisheri* NRRL-B-11177 after a certain exposure time to a toxic substance. The luminescent bacteria, dehydrated and frozen at -18ºC, were reactivated with the suspension supplied by Dr. Lange (Dr. Bruno Lange GmbH & Co., Düsseldorf, Germany). The assay conditions were pH 7.0 and NaCl concentration of 2%, with all the measurements duplicated for an incubation time of 15 min. When necessary, the sample was filtered prior to the assay. The toxicity values were measured as EC_{50}, which is the surfactant concentration that inhibits 50% after 15 min of exposure.

For each selected surfactant, the concentrations required for a mobility inhibition of the 50% of *Daphnia* population, were determined. Acute toxicity tests with *D. magna* were performed in Standard Reference Water (SRW) according to the UNE-EN ISO 6341 guideline [12]. The tests were performed in 100 mL polystyrene vessels, with 50 mL of SRW in each one. 20 neonates (<24 h) were transferred to vessels containing different concentrations of the test chemical, and the vessels were closed with a polyethylene cap. The neonates were separated from adults daily. There was no feeding and no aeration during the tests and the tests were run at 20±1ºC. Immobility was determined visually after 24 h. For each surfactant, controls and at least five concentrations were used for the determination of the mobility inhibition of 50% of *Daphnia* population (IC_{50}). The 72-h algal

growth-inhibition test with the microalgae *S. capricornutum* was administered according to the OECD 201 guideline [13]. The procedure consists of filling culture vials with appropriate volumes of nutrient medium and solutions of the surfactant being tested. At the beginning of the test, inocula of algae were added to the vials to be tested as well as to the control vials, and were kept under stable and predetermined incubation conditions.

Inocula were cultivated at 23±1ºC and constant uniform illumination (8000 lux). After 24, 48, and 72 h the algal density was determined to establish whether growth had been inhibited or stimulated with respect to control. Cell density was estimated by the optical density of the culture at 670 nm. The medium pH was kept constant = 7.0 for all measurements.

3. Results and Discussion

3.1. Individual Toxicity for the Surfactants

The toxicity of the ether carboxylic derivative surfactants and the amine-oxide-based surfactants was measured. Toxicity values of the surfactants were determined by applying the 24-h immobilization test with *D. magna*, the LumiStox® 300 test, which employs the luminescent bacteria *V. fischeri* and the 72-h algal growth-inhibition test.

For LumiStox® system, the initial values of luminous intensity measured were corrected by a factor that takes into account the natural decrease in luminous intensity, even in the absence of the toxic sample [14]:

$$fk = I_t(0) / I_0(0) \qquad \text{Eq.1}$$

with $I_0(0)$ and $I_t(0)$ being the readings of luminous intensity in the well containing concentration 0 at time 0 and t.

The percentage of inhibition (inhibitory effect) was calculated by the expression:

$$H_t = \frac{(I_{0t}(c) - I_t(c))}{I_{0t}(c)} 100 \qquad \text{Eq.2}$$

where

$$I_{0t}(c) = \overline{fk}\, I_0(c) \qquad \text{Eq.3}$$

with $\overline{fk}$ being the average correction factor of the control samples, $I_0(c)$ and $I_t(c)$ being readings of light intensity in the well containing concentration c at time 0 and t.

The Gamma function, the ratio between the light intensity lost by the bacterial solution and that remaining after exposure to the toxic sample, can be determined by the equation:

$$\Gamma_t = \frac{\overline{H}_t}{100 - \overline{H}_t} = \frac{f_k \cdot I_0(c) - I_t(c)}{I_t(c)} \qquad \text{Eq.4}$$

From the results, a linear relationship can be deduced between the function Γ and the concentration of the surfactant used, in the following form:

$$\log(c) = b \cdot \log(\Gamma) + \log(a) \qquad \text{Eq.5}$$

The values of EC_{50}, expressed as mg/L, are the concentrations of surfactant that inhibit 50%, and are calculated by giving Γ a value of 1.

IC_{50} values for the tests with *D. magna* were calculated using linear-regression analysis after transformation of dose-response curves by logarithmic transformation of the concentrations.

EC_{50} values for the tests with the microalgae were calculated using linear-regression analysis based on the dosage-response curves. Figure 2 provides an example of the linearization for the surfactant AO-R_{12} using *D. magna* and the mixture AO-R_{12}+AO-R_{14} using microalgae.

Table 2 shows the toxicity values for the tests with *V. fischeri*, *D. magna*, and microalgae, for the different surfactants assayed.

For all the tests, the surfactant concentration and one control were performed in triplicate for each organism tested. The surfactant concentration in the aquatic bioassays, at the beginning and at the

end of the tests, was measured using a TOC analyser. The aim of these measurements was to ensure that the test organisms do not use the surfactants as sources of carbon and that the adsorption to glassware, adsorption to the test organism, and biodegradation of test materials could be disregarded during the test period. All the concentrations were within 20% of nominal, so that it was acceptable to use the nominal value in order to calculate EC_{50} and IC_{50}.

The data shown in Table 2 indicate that for ether carboxylic derivative surfactants the toxic effect on the bacterium and on *D. magna* were similar, these organisms being more sensitive than the microalgae (almost double the toxicity). The least toxic surfactant of the ether carboxylic derivatives assayed was EC-R_8E_8, with the shortest alkyl chain length and a toxicity range from 76.26 mg/L to 134.59 mg/L (Table 2 and Figure S2 in the supplementary information).

For the amine-oxide-based surfactants, the toxicity values ranged from 0.35 to 155.02 mg/L. The toxicity and classification depended on the microorganism used (Table 2 and Figure S2 in the supplementary information). The most sensitive organism was the bioluminescent bacterium *V. fischeri*, followed by *D. magna*, while the least sensitive was the microalgae. A special case was the surfactant AOP-Cocoamido, where the toxicity data was reversed: this surfactant proved more sensitive for microalgae, the toxicity ranging from 11.43 mg/L to 85.86 mg/L.

The acute toxicity values of the ether carboxylic derivative surfactants ranged from 3.58 mg/L to 7.08 mg/L for the surfactant EC-$R_{12\text{-}14}E_3$, from 14.18 mg/L to 26.01 mg/L for theEC-$R_{12\text{-}14}E_{10}$, according to the European Union Directive No. 67/548/EEC [15] with the respective amendment No. 7, the results of toxicity for the different surfactants assayed allow the classification of the surfactants and the different mixtures formulated (Figure S2 in the supplementary information), taking into account the intervals shown in the Table 2. EC-$R_{12\text{-}14}E_3$ was the second toxicity class (R51), which is regarded as toxic against aquatic organisms. Meanwhile, the surfactants EC-$R_{12\text{-}14}E_{10}$ and EC-R_8E_8 were classified as harmful [third toxicity class (R52)] and safe, respectively. For the amine-oxide-

based surfactants the toxicity values ranged from 3.39 mg/L to 57.77 mg/L for the surfactant AO-R_{14}, from 0.35 mg/L to 155.02 mg/L for the AO-R_{12} and from 11.43 mg/L to 85.86 mg/L for the AOP-Cocoamido. According to the European Union Directive the above results classify the surfactant AOP-Cocoamido as harmful whereas for the surfactants AO-R_{14} and AO-R_{12} this classification depended on the organism tested.

According to the literature, anionic and non-ionic surfactants are toxic to various aquatic organisms at the concentrations from 0.0025 to 300 mg/L and from 0.3 to 200 mg/L, respectively [16]. For ecological safety, it is further assumed that the theoretically calculated concentration of a surfactant in the natural environment should be 100-fold lower than the values of IC_{50} and EC_{50} determined experimentally. In this case, no negative environmental impact of the surfactant would be expected. The results of the toxicity tests are typically much higher compared to values that might be found in the environment [17].

For the ether carboxylic derivative surfactants, the toxicity values appear to depend on the critical micelle concentration (CMC) and the length of the alkyl chain; toxicity falling as the CMC of the surfactant rose. These results agree with the data in the literature for the non-ionic surfactants alkylpolyglucosides [18]. Also, relationships were consistent with the fact that lower alkyl chain lengths result in lower toxicity (Figure 3). Similar studies have also shown that the homologues of alkylpolyglucosides of the longest alkyl chain presented the highest ecotoxicity values [3]. The degree of ethoxylation (E) does not present a clear effect on the toxicity, although it has been stated that toxicity increases when E increases [19].

For the amine-oxide-based surfactants the toxicity values ranged from 0.35 to 155.02 mg/L. The toxicity to *D. magna* and microalgae increased with the alkyl chain length of the amine oxide. However, toxicity for bacteria did not intensify when the hydrophobicity of the surfactant increased, probably because of the reduced solubility of AO-R_{14} in the salt medium of the tests. Also, the

presence of an amide group in the fatty alkyl chain significantly lowered toxicity, presumably as a result of the accentuated hydrophilic character of the surfactant. These results are consistent with the data in the literature for amine-oxide-based surfactants [7].

3.2. Toxicity for the mixtures of surfactants

The toxicity for surfactant mixtures were studied in order to evaluate the interactions between different types of surfactants, to determine the reason for such interactions and to establish the best way to determine which surfactants will work better together [20]. Surfactants are often used as co-surfactants in detergent formulas, and therefore the toxicological interactions in the binary mixtures 1:1 weight of ether carboxylic derivative surfactants and amide oxide surfactants were investigated. The results presented in Table 3 and in Figure 3 (see also Figure S2 in the supplementary information) reflect that *D. magna* was more sensitive to toxic effects from binary mixtures of ether carboxylic derivative surfactants than was *V. fischeri* and microalgae. Microalgae were less sensitive to toxic effects from binary mixtures of ether carboxylic derivative surfactants than to the individual surfactants. Figure S2 in the supporting information and Tables 2 and 3 show that in the making surfactant mixtures, it is possible to reduce the toxicity. The surfactant EC-$R_{12\text{-}14}E_3$, initially classified as "toxic" against aquatic organisms, may be transformed into "harmful" when mixed with EC- $R_{12\text{-}14}E_{10}$ or EC-R_8E_8, respectively, both surfactants registering a lower level of toxicity.

The least toxic mixtures were formed by the surfactants having lower individual toxicity (Table 3). This result highlights the synergism in the co-occurrence of these types of surfactants. Also, a Toxicity Unit (TU) was calculated as the ratio between the actual EC_{50} value and the average of the individual values, because the mixtures used at the proportion 1:1. TU values > 1 show that the actual EC_{50} was greater than the average, so that this mixture presents synergism to decrease in toxicity.

Figure S2 in the supplementary information presents the behaviour of mixtures of amine-oxide-based surfactants as well as the mixtures of ether carboxylic derivative surfactants and AOP-Cocoamido surfactant. The addition, the latter surfactant reduces the toxicity presented by the bacterium *V. fischeri* and *D. magna*, and in general intensifies toxicity for the microalgae.

CMC for mixtures of surfactants were determined by Surface-Tension Measurements (CMC data are collected in the Table S3 in the supplementary information). These results show a synergy in CMC for the binary mixtures of each class of surfactants tested. The explanation for this synergism is found in the screening of the electrostatic repulsion between the negatively charged head groups of the anionic surfactants by insertion of nonionic head groups.

There are many examples of surfactant mixtures in the household market. Mixtures are generally between anionic/anionic, cationic/cationic, non-ionic/non-ionic, amphoteric/amphoteric surfactants. However, synergism increases with the degree of charge difference [21], signifying that synergism between anionic/anionic or non-ionic/non-ionic is less than between anionic/non-ionic or cationic/non-ionic surfactants [22]. The binary mixture of the anionic surfactant with the shortest alkyl chain (EC-R_8E_8) and the non-ionic surfactant with the amide group in the fatty alkyl chain (AOP-Cocoamido) exhibited synergism which was stronger for the test using *D. magna*. Ionic surfactants show synergism with non-ionic surfactants, because non-ionic headgroups electrostatically shield the ionic headgroups on the surfactant molecules at interfaces so that they can stay closer together with less effect from repulsion [23].

Hisamo and Oya [19] tried to relate the surface activity and its change in mixtures with the toxicity, but they did not arrive at clear conclusions. We measured the CMC for mixtures and the synergism in this property. The CMC Unit (CMCU) was calculated as the ratio between the actual CMC value and the average of the individual values, because the mixtures used at the proportion 1:1. CMCU

values < 1 showed that the actual CMC was lower than the average, and therefore this mixture presents synergism in the sense that the surface activity increased.

In Figure 4 all the toxicity data measured for individual surfactants, together with other surfactants assayed in previous works (LAS, alkylpolyglucosides and glycerine polyoxyethylene esters), and their mixtures are represented against their CMC. It can be seen that this property is clearly related to toxicity: the lower the CMC (the greater the surface activity) the more toxic, depending on the organism assayed. In general, the most sensitive microorganism to CMC variation was the microalga *S. capricornutum*, and the least sensitive *V. fischeri*.

Binary mixture measurements indicate that the least toxic mixture was formed by the surfactant having lower individual toxicity. This finding allows us to formulate surfactant mixtures with reduced toxicity, especially if surfactants EC-R_8E_8 and AOP-Cocoamido are incorporated. Moreover, the test results for *V. fischeri* and *D. magna* indicate synergism in the co-occurrence of these surfactants. These results imply that the surfactants assayed, at low concentrations may be considered safe for the environment.

References

1. CNAE (2009) Observatorio Industrial del Sector Químico. Tendencias en el uso de la Biotecnología en el Sector Químico (2009) http://www.fedit.com/Spanish /DocumentosInformes/Portal/Publico/DocumentosEInformes/ObservatoriosIndustriales/Jornadas%20Difusi%C3%B3n%202009/OI-Qu%C3%ADmico.pdf

2. Anastas PT, Kirchhoff MM (2002) Origins, current status, and future challenges of green chemistry. Acc Chem Res 35: 686-694

3. Steber J, Guhl W, Steker N, Schröder FR (1995) Alkyl polyglycosides-ecological evaluation

of a new generation of nonionic surfactants. Tenside Surfact Det 32: 515-521

4. Ying GG (2005) Fate, behavior and effects of surfactants and their degradation products in the environment. Environ Int 32: 417-431

5. Liwarska-Bizukocj E, Miksch K, Malachowska-Jutsz A, Kalka J (2005) Acute toxicity and genotoxcity of five selected anionic and non-ionic surfactants. Chemosphere 58: 1249-1253

6. Yu Y, Zhao J, Bayly A (2008) Development of Surfactants and Builders in Detergent Formulations. Chinese J Chem Eng 16: 517-527

7. García MT, Campos E, Ribosa I (2007) Biodegradability and ecotoxicity of amine oxide based surfactants. Chemosphere 69: 1574-1578

8. Merrettig-Bruns U, Jelen E (2003) Anaerobic biodegradation of detergent surfactants. Final Report. Fraunhofer Institute for Environmental, Safety and Energy Technology UMSICHT, Oberhausen

9. Cross J (1994) Cationic Surfactants. In: Cross J and Singer J, eds, Analytical and Biological Evaluation, Surfactant Science Series vol. 53, Marcel Dekker, New York, USA, pp 140-175

10. Domingo A (1995) A guide to the surfactants World. Editorial Proa, Barcelona, Spain

11. UNE EN ISO 11348-2 (NORMA UNE EN ISO 11348-2) (2009) Determinación del efecto inhibidor de muestras de agua sobre la luminiscencia de *Vibrio fischeri* (Ensayo de bacterias luminiscentes). Spanish Association for Standardisation and Certification, Madrid

12. UNE-EN ISO 6341 (NORMA UNE EN ISO 6341) (1996) Determinación de la inhibición de la movilidad de *Daphnia magna* Straus (Cladocera, Crustacea) (Ensayo de toxicidad aguda). Spanish Association for Standardisation and Certification, Madrid

13. OECD Organization for Economic Cooperation and Development (1984) OECD Guidelines for the Testing of Chemicals. Alga growth inhibition test, OECD. Paris. France

14. Jurado E, Fernández-Serrano M, Núñez-Olea J, Luzón G, Lechuga M (2009) Acute toxicity and relationship between metabolites and ecotoxicity during the biodegration process of non-ionic surfactants: fatty-alcohol ethoxylates, nonylphenol polyethoxylate abd alkylpolyglucosides. Water Sci Technol 59 (12): 2351-2358

15. European Economic Community (EEC) (1967) Council Directive of 27th June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (67/548/EEC)

16. Pettersson A, Adamsson M, Dave G (2000) Toxicity and detoxification of Swedish detergents and softener products. Chemosphere 41: 1611-1620

17. Szwach I, Hreczvch W Fochtman P (2002) Comparative evaluation of environmental impact of fatty alcohol ethoxylates and fatty acid methyl ester ethoxylates as nonionic surfactants. Proceedings, 5th World Conference on Detergents: reinventing the industry: opportunities and challenges, October, 13th-17th, 2002, Montreux, Switzerland, 163-165

18. Uppgård L, Sjöström M, Wold S (2000) Multivariate quantitative structure-activity relationships for the aquatic toxicity of alkyl polyglucosides. Tenside Surfact Det 37: 131-138

19. Hisano N, Oya M, (2010) Effects of surface activity on aquatic toxicity of binary surfactant mixtures. J Oleo Sci 59 (11): 589-599

20. Hua XY, Rosen MJ (1982) Synergism in binary-mixtures of surfactants 1. Theoretical analysis. J Colloid Interf Sci 90: 212-219

21. Werts KM, Grady BP (2011) Mixtures of nonionic surfactants made from renewable

resources with alkyl sulfates: Comparison of headgroups. J Surfactants Deterg 14: 77-84

22. Kume G, Gallotti M, Nunes G (2008) Review on anionic/cationic surfactant mixtures. J Surfactants Deterg 11: 1-11

23. Zhou Q, Rosen MJ (2003) Molecular interactions of surfactants in mixed monolayers at the air/aqueous solution interface and in mixed micelles in aqueous media: the regular solution approach. Langmuir 19: 4555-4562

Figure and table legends

Figure S1. Chemical structure of the amine-oxide-based surfactants used in the tests: Myristyl dimethyl amine oxide and Lauryl dimethyl amine oxide (left); Cocoamidopropyl dimethyl amine oxide (right).

Figure 1. Linear regression for calculating CMC for the binary mixture EC-R8E8 + EC-R12-14E10 T= 25ºC. a) Surface tension vs. concentration. b) Conductivity vs. concentration. Arrows indicate the CMC of the single surfactants.

Figure 2. Linear-regression analysis for calculating toxicity: (a) Surfactant AO-R12 using D. magna. (b) Mixture of surfactants AO-R12 + AO-R14 using microalgae.

Figure S2. Toxicity of ether carboxylic derivative surfactants and their mixtures (up), and amine oxide and their mixtures (bottom) with the classification of different microorganisms according to European Union Directive Nº 67/548/EEC.

Figure 3. Variation in toxicity for the ether carboxylic derivative surfactants with R

Figure 4. CMC vs. toxicity for all the individual surfactants and binary mixtures assayed using V. fischeri, D. magna, and microalgae

Table 1. Description of the surfactants used in the tests

Table 2. Toxicity values (95% CI) in mg/L for the tests with *Vibrio fischeri*, *Daphnia magna* and Microalgae

Table 3. Toxicity values (95% CI) in mg/L for the mixtures of surfactants

Table S3. CMC values (95% CI) in mg/L for the binary mixtures surfactants

Authors Biographies

Dra. M Fernández-Serrano was born in 1967 and studied chemistry at the University of Granada. She was awarded a 4-year fellowship at the same university and received her PhD in 1995. She became an associate professor at the University of Granada in 1999. Her research activities include searching for new groups of biodegradable surfactants and the study of ecotoxicological properties of surfactants as biodegradability and toxicity. At present, she is head of the Chemical Engineering Department at the University of Granada.

Dra. E. Jurado was born in 1951 and graduated from the University of Granada in 1975. She obtained her PhD in 1980 and became full professor of Chemical Engineering in the Science Faculty at the same University in 1996. She was head of the Chemical Engineering Department (1997-2012). At present, she also directs the "Surfactants, Enzymes and Emulsions" research group. Her main research areas are: enzymes, kinetic enzymatic, biodegradation of surfactants, emulsions, and the physical chemistry and applications of surfactants. At present, she is head of the Chemical Engineering Department at the University of Granada. She has published over 120 papers in different fields.

Dr. A Fernández-Arteaga was born in 1978 and graduated in Chemical Engineering of the University of Granada in 2002. He earned his Ph.D. in 2006 from the same University. He is currently working there as an assistant lecturer in the Chemical Engineering Department, after a post-doc stay at the Colloidal & Interfacial Chemistry Research Center (Prof. Solans) in Barcelona. He is conducting research on properties of surfactants and synthesis of bioemulsifiers.

F. Ríos, born in 1985, graduated in Chemical Engineering at the University of Granada in 2009. Currently, he works on a project entitled "Environmental Impact of commercial surfactants" for his Ph.D.

Dra. M. Lechuga was born in 1976. She graduated from the University of Granada in Chemical Engineering and then worked on an investigation project entitled “Formulation of Liquid Detergents Specifically for the Industrial Agrofood and Hotel Sector” directed by E. Jurado. She received her PhD in 2005 and is currently an associate professor at the University of Granada. Her research interests include surfactants, their applications and the study of ecotoxicological properties.

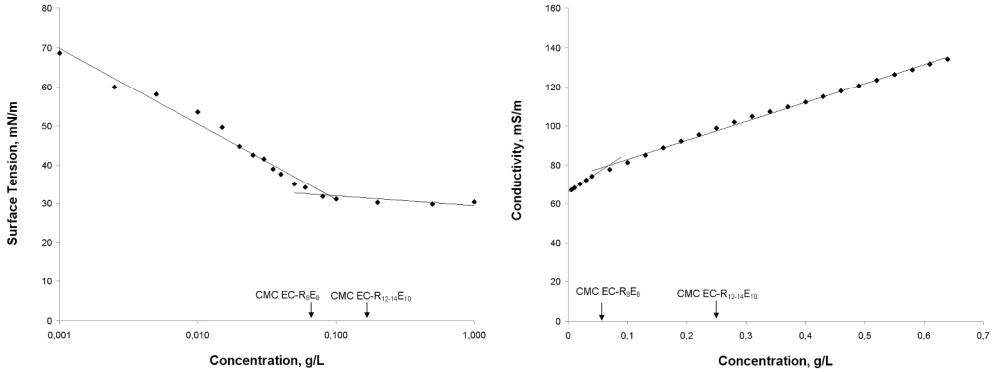

Figure 1
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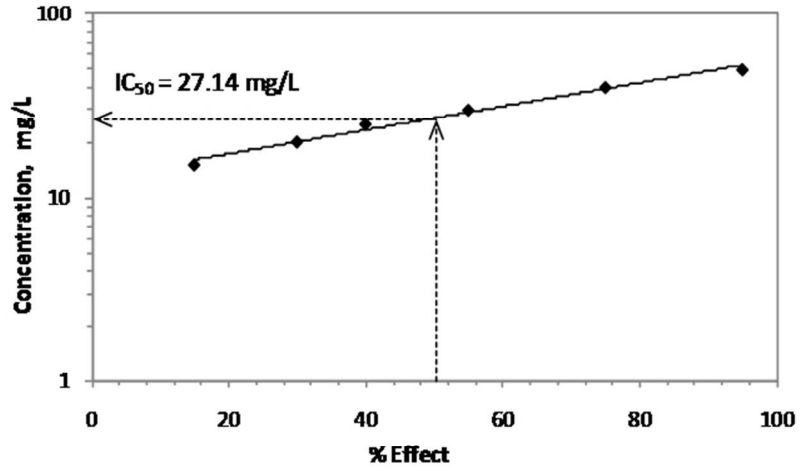

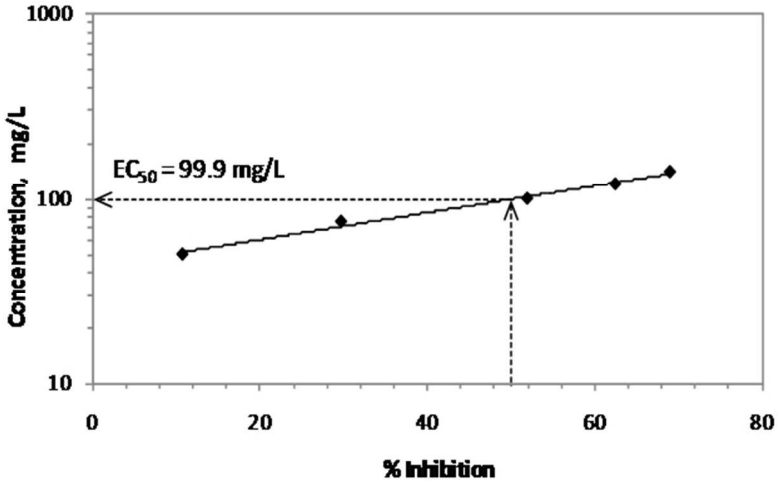

Figure 2
190x254mm (96 x 96 DPI)

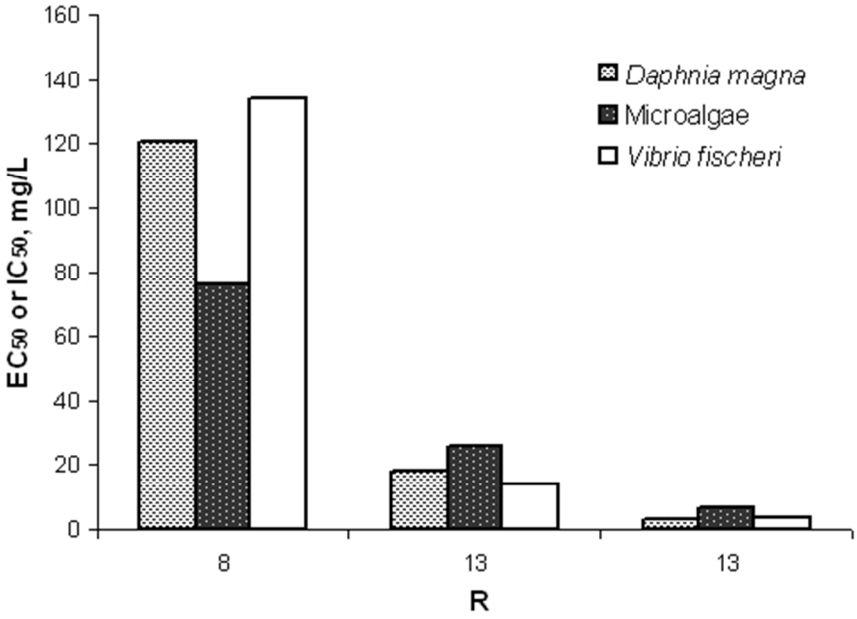

Figure 3
158x113mm (96 x 96 DPI)

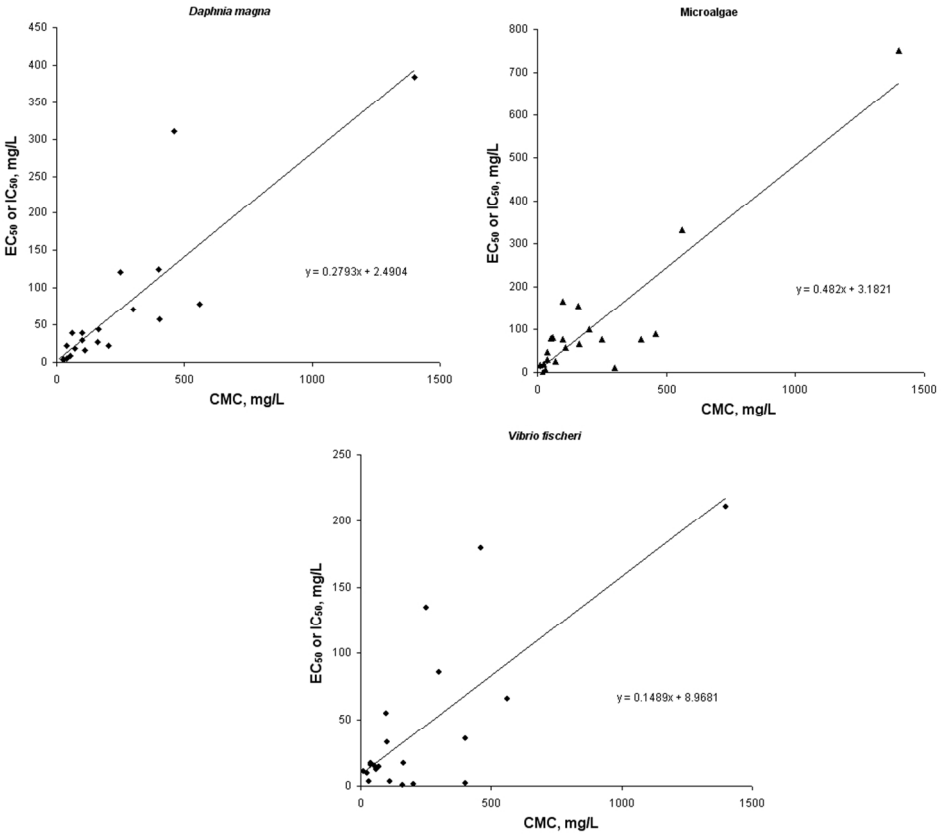

Figure 4
291x243mm (96 x 96 DPI)

Table 1. Description of the surfactants used in the tests

Surfactant	Family	Abbreviation	Structure	Active Matter [1], %	CMC [2], mg/L
Capryleth-9 carboxylic acid	Anionic	EC-R_8E_8	R:8. E:8	89	243.4
Laureth-4 carboxylic acid	Anionic	EC-$R_{12\text{-}14}E_3$	R:12-14. E:3	94	33.2
Laureth-11 carboxylic acid	Anionic	EC-$R_{12\text{-}14}E_{10}$	R:12-14. E:10	94	70.80
Myristyl dimethyl amine oxide	Non-ionic	AO-R_{14}	R:14	30	107.7
Lauryl dimethyl amine oxide	Non-ionic	AO-R_{12}	R:12	30	160.0
Cocoamidopropyl dimethyl amine oxide	Non-ionic	AOP-Cocoamido	R'CONH $(CH_2)_3$ R`=12	30	309.9

E: degree of ethoxylation.

R: alkyl chain length.

[1] The % of active matter is supplied by the manufacturer

[2] CMC measured at 25ºC using the commercial surfactant undried

Table 2. Toxicity values (95% CI) in mg/L for the tests with *Vibrio fischeri*, *Daphnia magna* and Microalgae

	Vibrio fischeri	***Daphnia magna***	**Microalgae**
Surfactant	**EC_{50} (15 min), mg/L**	**IC_{50}, mg/L**	**EC_{50}, mg/L**
EC-$R_{12-14}E_3$	3.58 (3.19-3.97)	3.47 (2.81-4.14)	7.08 (5.08-9.08)
EC-$R_{12-14}E_{10}$	14.18 (11.35-17.02)	18.74 (17.27-20.21)	26.01 (19.38-32.64)
EC-R_8E_8	134.59 (125.26-143.93)	120.95 (93.35-148.55)	76.26 (64.85-87.67)
AO-R_{14}	3.39 (3.24-3.54)	15.46 (11.26-19.66)	57.77 (47.01-68.53)
AO-R_{12}	0.35 (0.34-0.37)	27.14 (25.45-28.83)	155.02 (141.58-168.46)
AOP-Cocoamido	85.86 (83.53-88.20)	71.24 (66.43-76.05)	11.43 (11.15-11.71)

Table 3. Toxicity values (95% CI) in mg/L for the mixtures of surfactants

	Vibrio fischeri	*Daphnia magna*	Microalgae	*V. fischeri*	*D. magna*	Microalgae
Surfactants	**EC_{50} (15 min), mg/L**	**IC_{50}, mg/L**	**EC_{50}, mg/L**	**TU**	**TU**	**TU**
EC-$R_{12\text{-}14}E_3$+ EC-R_8E_8	14.96 (9.69-20.23)	8.04 (5.81-10.27)	78.14 (67.62-88.66)	0.22	0.13	1.88
EC-$R_{12\text{-}14}E_3$+ EC-$R_{12\text{-}14}E_{10}$	17.04 (13.50-20.57)	5.04 (4.57-5.51)	29.02 (24.38-33.66)	1.92	0.45	1.75
EC-R_8E_8+ EC-$R_{12\text{-}14}E_{10}$	54.70 (46.90-62.49)	39.31 (33.34-45.28)	166.57 (149.93-183.21)	0.74	0.56	3.26
AO-R_{14}+AO-R_{12}	1.30 (1.21-1.39)	22.06 (16.56-27.56)	99.88 (92.00-107.76)	0.70	1.04	0.94
AO-R_{14}+ AOP-Cocoamido	17.06 (16.85-17.27)	44.59 (37.23-51.95)	65.90 (61.42-70.38)	0.38	1.03	1.90
AO-R_{12}+ AOP-Cocoamido	2.20 (2.09-2.31)	57.82 (54.10-61.54)	77.34 (65.66-89.02)	0.05	1.18	0.93
EC-R_8E_8+ AOP-Cocoamido	179.55 (165.68-193.42)	310.96 (302.74-319.18)	19.82 (14.42-25.22)	1.63	3.24	0.45
EC-$R_{12\text{-}14}E_3$+ AO-R_{14}	9.54 (8.28-10.80)	3.14 (2.22-4.06)	89.76 (75.78-103.74)	2.74	0.33	2.77

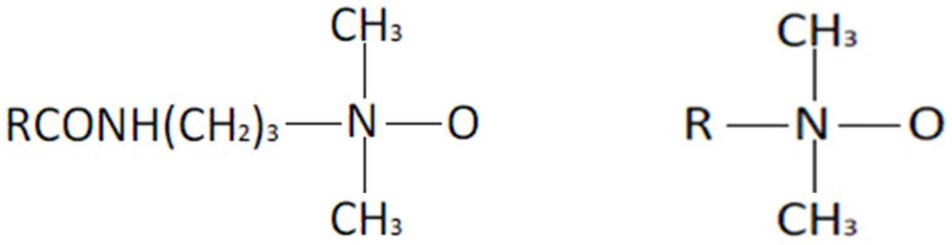

Figure S1
132x42mm (96 x 96 DPI)

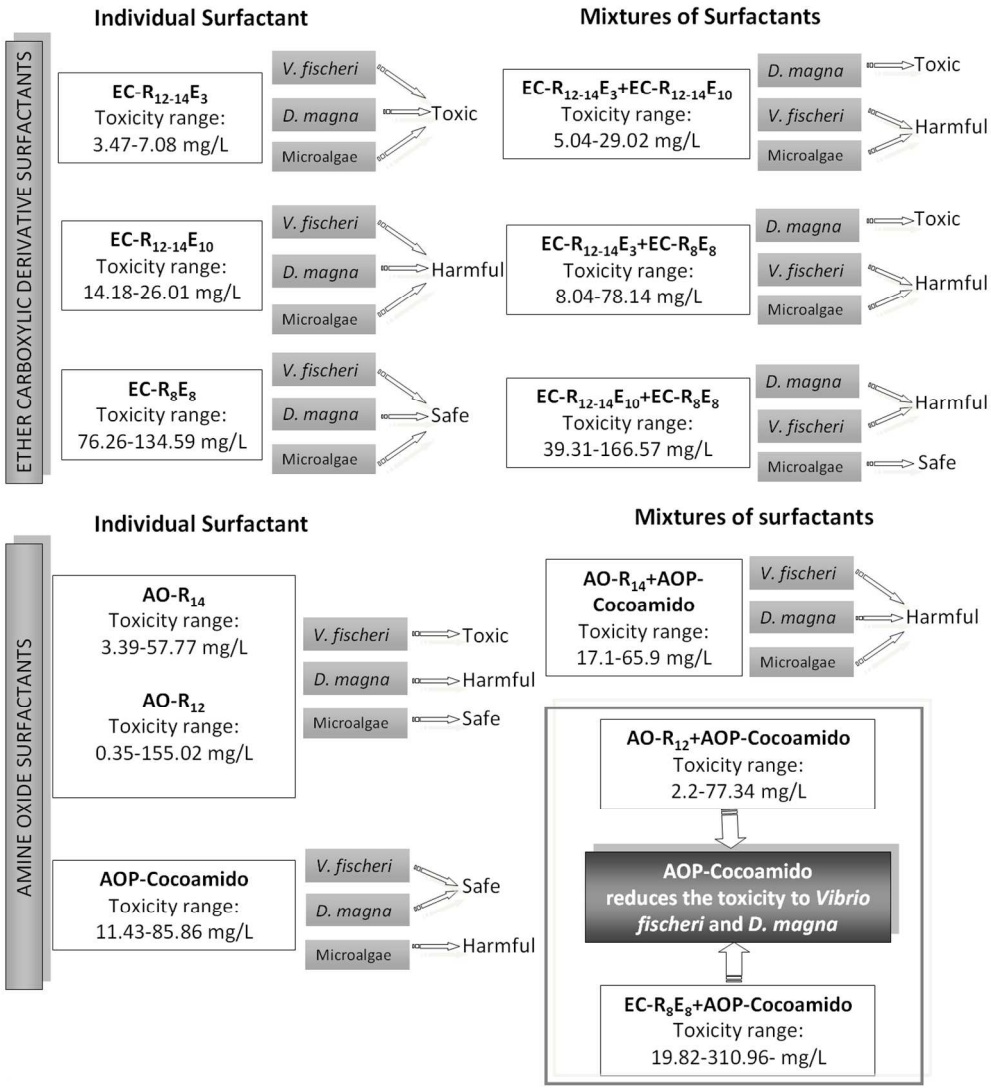

Figure S2
383x431mm (96 x 96 DPI)

Table S3. CMC values (95% CI) in mg/L for the binary mixtures surfactants

Surfactants	CMC, mg/L	CMCU
EC-$R_{12-14}E_3$+EC-R_8E_8	52.66 (52.03-53.29)	0.34
EC-$R_{12-14}E_3$+EC-$R_{12-14}E_{10}$	39.64 (39.30-39.98)	0.29
EC-R_8E_8+EC-$R_{12-14}E_{10}$	98.63 (98.12-99.14)	1.90
AO-R_{14}+AO-R_{12}	201.55 (200.48-202.62)	1.51
AO-R_{14}+AOP-Cocoamido	163.11 (162.21-164.01)	0.78
AO-R_{12}+AOP-Cocoamido	401.87 (400.86-402.88)	1.71
EC-R_8E_8+AOP-Cocoamido	460.06 (459.58-460.54)	2.42
EC-$R_{12-14}E_3$+AO-R_{14}	26.05 (25.59-26.51)	0.15