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# Acute toxicity of anionic and non-ionic surfactants to aquatic organisms

M. Lechuga, M. Fernández-Serrano *, E. Jurado, J. Núñez-Olea, F. Ríos

*Department of Chemical Engineering, Faculty of Sciences, University of Granada, Campus Fuentenueva s/n, 18071 Granada, Spain*

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ABSTRACT

The environmental risk of surfactants requires toxicity measurements. As different test organisms have different sensitivity to the toxics, it is necessary to establish the most appropriate organism to classify the surfactant as very toxic, toxic, harmful or safe, in order to establish the maximum permissible concentrations in aquatic ecosystems. We have determined the toxicity values of various anionic surfactants ether carboxylic derivatives using four test organisms: the freshwater crustacean *Daphnia magna*, the luminescent bacterium *Vibrio fischeri*, the microalgae *Selenastrum capricornutum* (freshwater algae) and *Phaeodactylum tricornutum* (seawater algae). In addition, in order to compare and classify the different families of surfactants, we have included a compilation of toxicity data of surfactants collected from literature. The results indicated that *V. fischeri* was more sensitive to the toxic effects of the surfactants than was *D. magna* or the microalgae, which was the least sensitive. This result shows that the most suitable toxicity assay for surfactants may be the one using *V. fischeri*. The toxicity data revealed considerable variation in toxicity responses with the structure of the surfactants regardless of the species tested. The toxicity data have been related to the structure of the surfactants, giving a mathematical relationship that helps to predict the toxic potential of a surfactant from its structure. Model-predicted toxicity agreed well with toxicity values reported in the literature for several surfactants previously studied. Predictive models of toxicity is a handy tool for providing a risk assessment that can be useful to establish the toxicity range for each surfactant and the different test organisms in order to select efficient surfactants with a lower impact on the aquatic environment.



## 1. Introduction

Surfactants have the characteristic of one or several hydrocarbon chains that form the lipophilic part of the molecule and one or several polar groups that form the hydrophilic part (Ying, 2006). Surfactants, also called surface-active agents, can possess different lengths and different degrees of unsaturation in the hydrocarbon chains as well as different polar groups, a fact that gives rise to a great variety of surfactant compounds with different properties and varied applications (Holmberg, 2001). Surfactants are widely used in household and industrial applications. More than 4.2 million tons of detergent products and 1.2 million tons of softener products were used annually in Western Europe 10 years ago (Petersson et al., 2000), and surfactants are one of the most important components of this products, comprising from 15% to 40% of the total detergent formulation (Scheibel, 2004).

After use, surfactants as well as their products are mainly discharged into sewage-treatment plants and then dispersed into the environment by releasing effluents into surface waters and by sludge disposal on land. Different surfactants vary in behaviour and fate in the environment.

The environmental risk of surfactants depends on the final concentration reached in the aquatic medium. The surfactant concentration and therefore its possible toxic effect are reduced by degradation of surfactants through microbial activity, the primary transformation occurring in the environment. Nevertheless, the toxic products released in the biodegradation process can bioaccumulate and their long-term effects are not sufficiently well known. Also, soil absorption is important, as this may give rise to ground-water contamination by high concentrations of surfactants.

In addition, the surfactant degradation depends on the conditions under which biodegradation occurs. Under aerobic conditions, most surfactants i.e. LAS, fatty alcohol ethoxylates (FAEs), alcohol ethoxylate (AE), alkylphenol ethoxylate (APE), and the cationic surfactant dimethyl ammonium chloride (DTDMAC), are degradable or readily degradable. However, under anaerobic

* Corresponding author.
*E-mail address:* mferse@ugr.es (M. Fernández-Serrano).

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conditions, LAS, FAEs, and DTDMAC are persistent and alkylphenol ethoxylate is partially degradable (Ying, 2006).

The massive use of surfactants in detergents and cosmetic formulations and their subsequent disposal in aquatic systems require surfactants to be as environmentally friendly as possible. This implies the need for low-toxicity and biodegradable surfactants. The environmental impact of chemicals is often determined by the ecotoxicity, which is relatively high in the case of surfactants as a result of surface activity and the action against biological membranes (Steber et al., 1995).

Ecotoxicity studies examine the toxic effects caused by physicochemical agents on living beings. In a laboratory assay it is very difficult to represent the diversity of circumstances that can coincide in a natural setting and therefore, today, a series of normalized assays are used in order to assess the impact of a spill in the environment. Because of its ease of application to study aquatic toxicity of surfactants, a number of *in vitro* assays have become widespread, using as test organisms bacteria, algae, macrophytes, micro- as well as macro-crustaceans, and fish. Basically, all the toxicity assays are based on exposing a population of aquatic organisms to the substance to be evaluated. These tests provide an estimation of the dosage that affects 50% of the population exposed (mortality, mobility inhibition, reproduction interference, reduction of respiration, etc.). Aquatic toxicity is expressed by $EC_{50}$ (effective concentration) or $LC_{50}$ (lethal dose).

The aims of the present work are to study the aquatic toxicity of some commercially important anionic and non-ionic surfactants, the selection of the most appropriate organism to classify the surfactant as very toxic, toxic, harmful or safe, in order to establish the maximum permissible concentrations in aquatic ecosystems, and to analyze the mathematical relationship that helps to predict the toxic potential of a surfactant from its structure. For this purpose, this paper includes the toxicity evaluation of the anionic surfactants ether carboxylic derivatives. Toxicity tests have been employed using different test organisms: the luminescent bacterium *Vibrio fischeri*, the freshwater crustacean *Daphnia magna*, the freshwater algae *Selenastrum capricornutum* and the seawater algae *Phaeodactylum tricornutum*. Toxicity data have been related to the structure of the surfactants giving a mathematical relationship. Moreover, in order to compare the different families of surfactants, a compilation of toxicity data of surfactants found in literature and their structure-toxicity relationship have been also included. The knowledge of toxicity properties is useful for the selection of technically efficient surfactants with a lower impact on the aquatic environment.

## 2. Materials and methods

### 2.1. Surfactants

Six ether carboxylic derivative surfactants were tested: EC-$R_{4\text{-}8}E_{1\text{-}8}$, EC-$R_{6\text{-}8}E_{3\text{-}8}$, EC-$R_8E_5$, EC-$R_8E_8$, EC-$R_{12\text{-}14}E_3$ and EC-$R_{12\text{-}14}E_{10}$. These are commercial anionic surfactants provided by Kao Corporation (Tokyo, Japan) under the commercial name AYKYPO®. Their chemical structure is R-O($CH_2$-$CH_2O$)$_E$-$CH_2$-$COO^-$. Each of them has different chain length (R) and degree of ethoxylation (E) that make them suitable for many different applications. These anionic surfactants improve the foaming quality of detergent, reducing the irritation level, and therefore they are used as co-surfactants for general detergents and cleaners, which have to be in contact with eyes and/or skin. Table S1 in supplementary material shows the structural parameters, the % of active matter, the critical micelle concentration (CMC) and the CAS number of the surfactants used.

In previous works, we have tested the toxicity of three of them, EC-$R_8E_8$, EC-$R_{12\text{-}14}E_3$ and EC-$R_{12\text{-}14}E_{10}$ and the toxicity of binary mixtures of them, using the toxicity tests with bacteria *V. fischeri*, crustaceans *D. magna* and microalgae *S. capricornutum* (Jurado et al., 2011; Fernández-Serrano et al., 2014). In this study, we incorporate three new ether carboxylic derivative surfactants EC-$R_{4\text{-}8}E_{1\text{-}8}$, EC-$R_{6\text{-}8}E_{3\text{-}8}$ and EC-$R_8E_5$, to assessment their toxicity with the same organisms aforementioned, and with marine algal *Phaeodactylum tricornutum* for the ether carboxylic derivate surfactants EC-$R_8E_8$, EC-$R_{12\text{-}14}E_3$ and EC-$R_{12\text{-}14}E_{10}$.

### 2.2. Toxicity tests

Four toxicity tests were undertaken: the LumiStox® 300 test, which employs the luminescent bacterium *Vibrio fischeri*; the 24-hr immobilization test with *Daphnia magna* (freshwater crustacean); the 72-hr algal growth-inhibition test with *Selenastrum capricornutum* (freshwater algae, renamed as *Pseudokirchneriella subcapitata*) and *Phaeodactylum tricornutum* (seawater algae). Table S2 in supplementary material shows the characteristics and conditions of the toxicity tests employed.

#### 2.2.1. Toxicity to bacteria

Measurements were taken with the LumiStox® 300 system, which consists of an instrument for measuring bioluminescence and an incubation unit according to the UNE-EN ISO 11348-2 (2009) guideline (UNE-EN ISO 11348-2, 2009). The toxicity measurement is based on the luminous intensity of the marine bacteria of the strain *V. fischeri* NRRL-B-11177 after a certain exposure time to a toxic substance. The luminescent bacteria, dehydrated and frozen at $-18$ °C, were reactivated with the suspension supplied by Dr. Lange (Dr. Bruno Lange GmbH & Co., Düsseldorf, Germany). The assay conditions were pH 7.0, NaCl concentration of 2%, all the measurements duplicated for an incubation time of 15 min. The toxicity values were measured as $EC_{50}$, which is the surfactant concentration that inhibits 50% of luminescence after 15 min of exposure.

#### 2.2.2. Toxicity to crustaceans

Acute-toxicity tests with *Daphnia magna* were performed in Standard Reference Water (SRW) according to the UNE-EN ISO 6341 (2013) guideline (UNE-EN ISO 6341, 2013).

The tests were performed in 100 mL polystyrene vessels, each with 50 mL of SRW. 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 every day. There was no feeding and no aeration during the tests and the tests were run at $20\pm1$ °C. 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 ($EC_{50}$).

#### 2.2.3. Toxicity to algae

The 72-hr algal growth-inhibition test with the microalgae *Selenastrum capricornutum* was administered according to the UNE-EN ISO 8692 (2012) guideline (UNE-EN ISO 8692, 2012). The procedure consisted of filling culture vials with appropriate volumes of nutrient medium and solutions of the surfactant being tested. At the beginning of the test, the inoculums 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. The inoculum was cultivated at $23\pm1$ °C and constant uniform illumination (8000 lx). After 24, 48, and 72 h the algal density was determined in order 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.