Ecotoxicity of anionic surfactants AKYPO®

E. Jurado, M. Fernández-Serrano, J. Núñez-Olea,
M. Lechuga & F. Ríos
Department of Chemical Engineering, Faculty of Sciences,
University of Granada, Spain

Abstract

In this paper, the toxicity values of ether carboxylic derivatives surfactants with commercial name AKYPO®, and the anionic surfactant linear alkylbenzene sulfonate (LAS), have been determined by applying the 24-h immobilization test with *Daphnia magna* (freshwater crustacea), the LumiStox® 300 test which employs the luminescent bacterium *Photobacterium phosphoreum* of the strain *Vibrio fischeri,* and the 72-h algal growth-inhibition test with the microalgae *Selenastrum capricornutum*, using culture-growth inhibition as the effect criterion. Three AKYPO®, with different alkyl chain and degree of ethoxylation, and LAS have been tested. For all tests, the results indicated that *Vibrio fischeri* is more sensitive to toxic effects from AKYPO® and LAS than *Daphnia magna* or the microalgae. The results demonstrate that the toxicity is lower for the AKYPO® with the shortest alkyl chain. The anionic surfactant LAS presents intermediate toxicity values.

Keywords: anionic surfactants, ecotoxicity, AKYPO®, Daphnia magna, microalgae, Vibrio fischeri.

1 Introduction

The enormous worldwide use of surfactants in detergent and cosmetic formulations, which are generally dumped into water systems, requires them to be as inocuous as possible for the environment: low toxicity and easily biodegraded ones. The aspect of environmental impact of chemicals is mainly governed by their ecotoxicity which is relatively high in the case of surfactants because of their surface activity and the resulting action against biological membranes [1]. This increasing worry impels the development of new

WIT Transactions on Ecology and the Environment, Vol 144, © 2011 WIT Press
www.witpress.com, ISSN 1743-3541 (on-line)
doi:10.2495/ECO110431

surfactants. The amphoteric character of anionic surfactants facilitate their accumulation in living organisms. Anionic surfactants mainly show eye and skin irritation potentials. Because of the high number of surfactants in contact with humans, many in vitro methods have been developed for the prediction of the eye irritation potential of surfactants [2].

The anionic surfactants tested in this research, AKYPO® series from Kao Corporation S.A., improve the foam quality of the detergent reducing irritation level, therefore they are used as co-surfactants in detergents which have to be in contact with the skin. AKYPO® are ether carboxylic derivatives and are commercialised in concentrated acid form. Figure 1 shows the chemical structure of AKYPO®.

$$\mathbf{R\text{-}O(CH_2\text{-}CH_2O)_n\text{-}CH_2\text{-}COO^-X}$$

Figure 1: Chemical structure of AKYPO®.

The objective of this study is to compare the ecotoxicity of the anionic tensioactive linear alkylbenzene sulfonate (LAS) with these novel surfactants.

Many types of bioassays are available to establish the toxicity levels of compounds for aquatic organisms, but many of these tests are also time-consuming and not routinely applicable. Moreover, the use of higher organisms as test species may also be ethically undesirable. There is a need to replace acute toxicity tests on fish with more effective assays. Although several bioassays using microorganisms have been described, most of the bacterial screening tests have been based on luminiscence measurements, because in this way they are rapid, reproducible, and simple to use, they cause no ethical problems, and they are cost-effective [3]. The characteristics of speed, reliability, and normalization of the toxicity results by bioassays with luminescent bacteria make them ideal for gathering data on toxicity, which can be compared and statistically studied for establishing correlations between toxicity as well as the chemical structure and/or different properties of the compounds assayed. Assays using luminescent bacteria are gaining wide acceptance for the quick and simple determination of the toxicity of chemical compounds in surface water and wastewater as well as in extracts of solid matrices. This explains the fact that, together with the *Daphnia* assay, these are listed as an approved bioassay to characterize toxic and dangerous wastes. *Daphnia magna* has proved to be a sensitive and simple laboratory model for predictive toxicity studies [4].

In a general way, the use of toxicity data has been extended to invertebrates and fish, considering them more important and representative than the toxicity assays with primary producers, as the former are more sensitive to toxins. However, some studies [5] have shown that in some cases, plants are far more sensitive, for example in assays with metals, industrial effluents, pesticides, and cationic surfactants.

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 ether carboxylic derivatives surfactants. Surfactant toxicity

has been measured by the LumiStox® assay according to the UNE-EN ISO 11348-2 guideline [6], using luminescent bacteria of the strain *Vibrio fischeri* as test microorganisms and by applying the 24-h inmobilization test with *Daphnia magna* according to the UNE-EN ISO 6341 guideline [7]. Also, the 72-h algal growth-inhibition test was performed with the microalga *Selenastrum capricornutum*.

The results indicate that *Vibrio fischeri* is more sensitive to toxic effects originating from AKYPO® and LAS than is *Daphnia magna* and microalgae.

2 Materials and methods

2.1 Surfactants

The surfactants used in this study are the commercial ether carboxylic derivatives AKYPO® LF2, AKYPO® RLM-25 and AKYPO® RLM-100 supplied by Kao Corporation S.A. (Tokyo, Japan). Table 1 shows the degree of ethoxylation (n) and the alkyl chain length (R) of the surfactants. The surfactant LAS is also supplied by Kao Corporation S.A. (Tokyo, Japan). The rest of the reagents used were PA quality and supplied by Panreac.

Table 1: Description of the surfactants employed in the tests.

Commercial Name	Structure
AKYPO® LF 2	R: 8 n: 8
AKYPO® RLM-25	R: 12-14 n: 3
AKYPO® RLM-100	R: 12-14 n: 4.5

2.2 Acute toxicity tests

Three toxicity tests were undertaken: the LumiStox® 300 test which employs the luminescent bacterium *Photobacterium phosphoreum*, the 24-h immobilization test with *Daphnia magna* (freshwater crustacea), and the 72-h algal growth inhibition test with *Selenastrum capricornutum*.

In the first one, measurements were taken with the measuring system LumiStox® 300, which consists of an instrument for measuring bioluminescence and an incubation unit according to the UNE-EN ISO 11348-2 guideline [6]. The toxicity measurement is based on the luminous intensity of the marine bacteria of the strain *Vibrio 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. The assay conditions were pH 7.0, ClNa concentration of 2%, all the measurements duplicated for

incubation times of 15 and 30 min. When necessary, the sample was filtered prior to the assay.

The toxicity value was measured as EC_{50} or EC_{20}, which are, respectively, the surfactant concentrations that inhibit 50 and 20% after 15 and 30 min of exposure.

Acute toxicity tests with *Daphnia magna* were performed in Standard Reference Water (SRW) according to the UNE-EN ISO 6341 guideline [7]. 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 every day. There was no feeding and no aeration during the tests and the tests were run at 20±1ºC. Immobilization 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 microalga *Selenastrum capricornutum* was administered according to the OECD 201 guideline [8]. 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, an inoculum of algae was added to the vials to be tested and to the vials of control, and were kept under stable and predetermined incubation conditions. Inocula were cultivated at 25±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 670nm.

For all the tests, the surfactant concentration and one control were performed in triplicate for each organism tested.

3 Results and discussion

The toxicity was determined for different AKYPO®. The initial concentrations of the surfactant were between 100 and 500 mg/L, depending on the surfactant assayed. 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:

$$fk = \frac{I_t(0)}{I_0(0)} \tag{1}$$

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:

$$H_t = \frac{(I_{0t}(c) - I_t(c))}{I_{0t}(c)} 100 \tag{2}$$

where

$$I_{0t}(c) = \overline{fk}\, I_0(c) \tag{3}$$

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:

$$\Gamma_t = \frac{H_t}{100 - H_t} = \frac{fk \cdot I_0(c) - I_t(c)}{I_t(c)} \tag{4}$$

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

$$\log(c) = b \cdot \log(\Gamma) + \log(a) \tag{5}$$

Figure 2 provides an example of the linearization for AKYPO® LF2. The values of EC_{20} and EC_{50}, expressed as mg/L, are the concentrations of surfactant

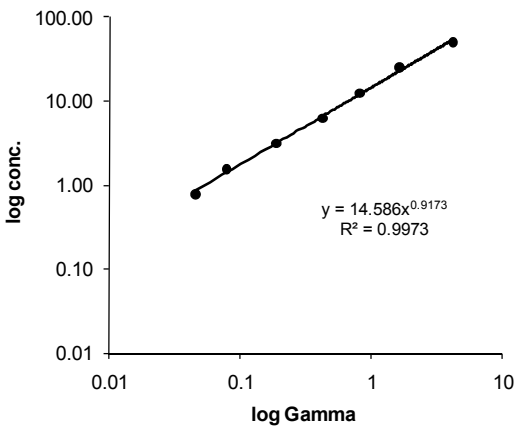

Figure 2: Linear relationship between the function Γ and concentration according to Eq. (5).

that inhibit 20 and 50%, and are calculated, giving Γ values of 0.25 and 1, respectively. Table 2 shows the results for the different surfactants, in decreasing order of toxicity, for incubation times of 15 and 30 min.

Table 2: Acute toxicity data for AKYPO® and LAS for the tests with *Vibrio fischeri* (values of EC_{50} and EC_{20} in mg/L).

Surfactant	EC_{20} (15 min)	EC_{50} (15 min)	EC_{20} (30 min)	EC_{50} (30 min)
AKYPO® RLM-25	0.42	3.58	1.90	4.74
AKYPO® RLM-100	4.51	14.18	4.39	15.19
LAS	9.41	27.58	8.29	26.50
AKYPO® LF2	22.93	134.59	36.44	181.65

IC_{50} values for the tests with *Daphnia magna* were calculated using linear-regression analysis after conversion of dose-response curves by logarithmic transformation of the concentrations (Figure 3). Table 3 shows (in decreasing order of toxicity) the IC_{50} values for the tests with *Daphnia magna,* for the different surfactants assayed.

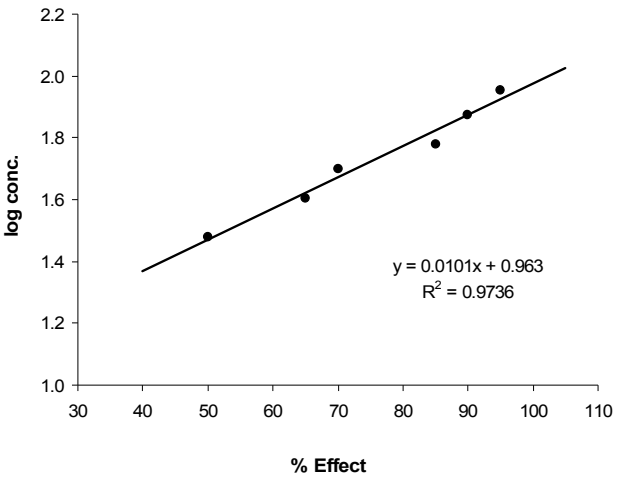

Figure 3: Linear relationship between the % effect and concentration for the tests with *Daphnia magna.*

Table 3: Acute toxicity data for AKYPO® and LAS for the tests with *Daphnia magna* (values of IC_{50} in mg/L).

Surfactant	IC_{50}
AKYPO® RLM-25	3.478
LAS	10.69
AKYPO® RLM-100	18.74
AKYPO® LF2	120.95

EC_{50} values for the tests with microalgae were calculated using linear regression analysis based on the dose-response curves (Figure 4). Table 4 shows (in decreasing order of toxicity) the EC_{50} values for the tests with microalgae, for the different surfactants tested.

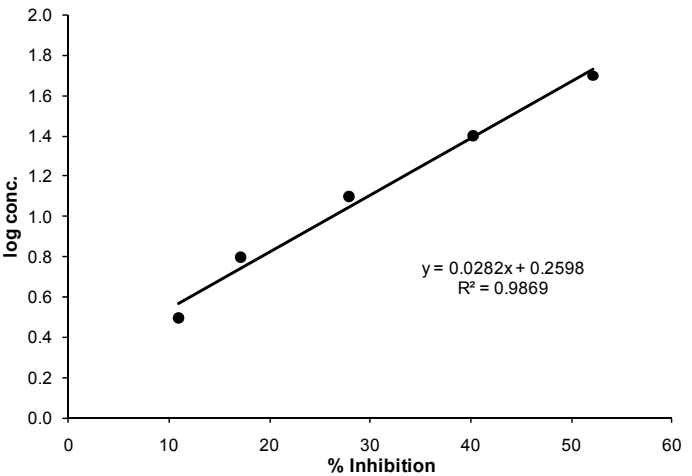

Figure 4: Linear relationship between the % inhibition and concentration for the tests with microalgae.

Table 4: Acute toxicity data for AKYPO® and LAS for the tests with microalgae (values of EC_{50} in mg/L).

Surfactant	EC_{50}
AKYPO® RLM-25	7.08
AKYPO® RLM-100	26.01
AKYPO® LF2	76.26
LAS	151.07

The results presented in Tables 2, 3, and 4, and in the graphs in Figure 5 show that *Vibrio fischeri* was more sensitive to toxic effects from AKYPO® and LAS than was *Daphnia magna* and microalgae, according to the results shown by García et al. [9] for non ionic surfactants alkylglucosides.

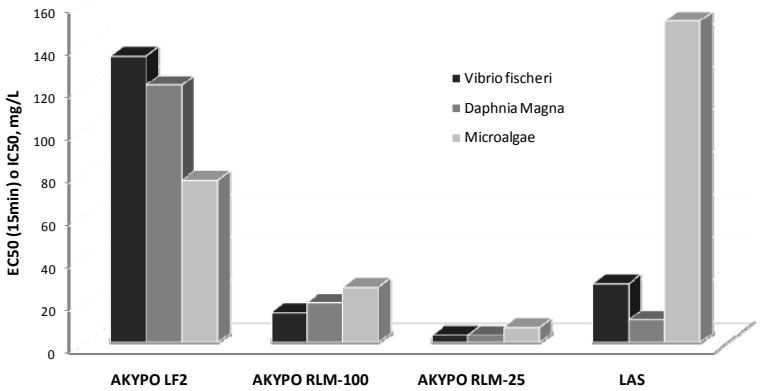

Figure 5: Determination of AKYPO® and LAS toxicity using *Daphnia magna, Vibrio fischeri*, and microalgae.

The results show that the toxicity is lower for the AKYPO® with the shortest alkyl chain. The degree of ethoxylation (n) has the reverse effect: the bigger degree of ethoxylation the smaller toxicity. The relation between LAS toxicity with AKYPO® toxicity depends on the test assayed. Using the bioluminescent bacteria *Vibrio fischeri* only the AKYPO® LF2 results less toxic than the LAS. With *Daphnia magna* AKYPO® LF2 and AKYPO® RLM100 are less toxic than the surfactant LAS. However, according to microalgae test, LAS presents the smallest toxicity. In fact, as may be seen in Figure 5, LAS is the surfactant with the more dispersal toxicity values.

Guilhermino et al. [10] indicate 0.22 mg/L as border value for *Daphnia magna* test. So, in view of the results (Table 3), all the surfactants tested present acceptable toxicity.

4 Conclusions

Toxicity values of three ether carboxylic derivatives anionic surfactants with commercial name AKYPO® and the well known anionic surfactant LAS have been determined using three methods: 24-h immobilization test with *Daphnia magna*, the LumiStox®300 test with the luminescent bacterium strain *Vibrio fischeri* and the 72-h algal growth-inhibition test with the microalgae *Selenastrum capricornutum*. The results obtained show that *Vibrio fischeri* is more sensitive to toxic effects from AKYPO® and LAS than *Daphnia magna* and microalgae. The influence of AKYPO® structure is in the sense that the toxicity

is lower for the AKYPO with the shortest alkyl chain, and the bigger degree of ethoxylation the smaller toxicity. The relation between LAS toxicity with AKYPO® toxicity depends on the test assayed because LAS is the surfactant with the more dispersal toxicity values. However, according with Guilhermino et al. [10] and the results obtained for *Daphnia magna* test, all the surfactants tested present acceptable toxicity.

References

[1] Steber J., Guhl W., Steker N. and Schröder F. (1995) Alkyl poliglycosides-ecological evaluation of a new generation of nonionic surfactants. *Tenside Surfactants Detergents*, 32 (6), 515-521.

[2] Cserháti T., Forgács E., and Oros G. (2002) Biological activity and environmental impact of anionic surfactants. *Environment International,* 28, 337– 348.

[3] Farré M., García M., Tirapu L., Ginebreda A. and Barceló D. (2001). Wastewater toxicity screening of non-ionic surfactants by Toxalert® and Microtox® bioluminiscence inhibition assays. *Analytica Chimica Acta*, 427 (2), 181-189.

[4] Sandbacka M., Christianson I. and Isomaa B. (2000). The acute toxicity of surfactants on fish cells, *Daphnia magna* and fish - A comparative study. *Toxicology in Vitro*, 14, 61-68.

[5] Rand G. (1995). Aquatic Toxicology: Effects, Environmental fate and Risk Assessment. Gary M. Rand (Ed). 1083 p.

[6] UNE EN ISO 11348-2 (NORMA UNE EN ISO 11348-2). Determinación del efecto inhibidor de muestras de agua sobre la luminiscencia de *Vibrio fischeri* (Ensayo de bacterias luminiscentes).

[7] UNE-EN ISO 6341 (NORMA UNE EN ISO 6341). Determinación de la inhibición de la movilidad de Daphnia magna Straus (Cladocera, Crustacea) (Ensayo de toxicidad aguda).

[8] OECD Guideline for Testing of Chemicals. (1984). Alga, growth inhibition test.

[9] García M., Ribosa I., Campos E. and Sánchez-leal J. (1997). Ecological properties of alkylglucosides. *Chemosphere*, 35 (3), 545-556.

[10] Guilhermino L., Diamantino T., Silva M.C., and Soares A. M. V. M. (2000) Acute Toxicity Test with *Daphnia magna:* An Alternative to Mammals in the Prescreening of Chemical Toxicity? *Ecotoxicology and Environmental Safety Environmental Research* Section B, 46, 357-362.