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Simplified spectrophotometric method using methylene blue for determining anionic surfactants: Applications to the study of primary biodegradation in aerobic screening tests

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Abstract

In the present work, we propose a simplified spectrophotometric method for determining anionic surfactants, based on the formation of the ionic pair anionic surfactant-methylene blue (AS–MB). This method, in relation to the conventional analytic procedure, considerably reduces not only the quantity of chloroform used in extracting the ionic pair formed, but also the time and the quantity of sample necessary to perform the assay, eliminating the filtration stage. The method has been simplified by displacing the transfer equilibrium of the ionic pair AS–MB towards the organic phase, augmenting the volumetric relationship of chloroform/sample. The method proposed has been applied in the study of primary biodegradation kinetics of linear alkylbenzenesulfonate (LAS).

Keywords: Anionic surfactants; LAS; Primary biodegradation; Analysis; Simplified method; Methylene blue

1. Introduction

Anionic surfactants are currently the types most used, being incorporated in the majority of detergent and cleaning-product formulas in daily use. Linear-chain alkylbenzenesulfonate types, are the most popularly used synthetic anionic surfactants. They have been extensively used for over 30 years with an estimated global consumption of 2.8 million tonnes in 1998 (Ying, 2006). These surfactants pass into sewage-treatment plants, where they are partially aerobically degraded and partially adsorbed to sewage sludge that is applied to land. Finally, they are dumped into the waterways and onto soil, where they constitute some of the main factors affecting the natural ecosystem (McEvoy and Giger, 1985). Therefore, it is important to determine the concentration of anionic surfactants with accuracy and have quick and simple procedures to monitor their biodegradation over time.

Anionic surfactants are usually determined by spectrophotometric methods using methylene blue, this standard method being used to determine the surface agents in tap-water samples (ISO 7875-1, 1996). However, this official method is not only long and tedious but also requires great quantities of chloroform and sample.

The method is based on the formation of an ionic pair between the anionic surfactants, AS, and the methylene blue, MB, according to the reaction:

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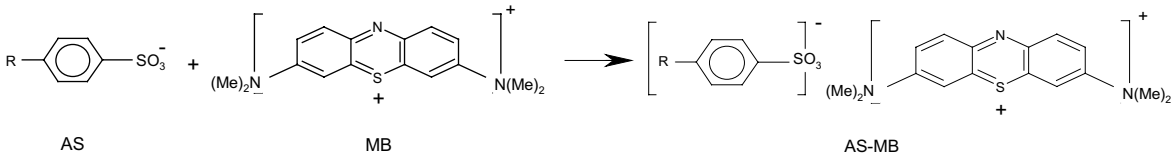

(1)

This requires three successive extractions of AS–MB content in 100 ml of sample previously alkalinized with 15, 10, and 10 ml of chloroform (Fig. 1). The ionic pair is determined by spectrophotometry, measuring the absorbance at 650 nm.

Simplified methods that reduce the quantities of reagent (Chitikela et al., 1995) by using a certain kind of adsorbent (Moskvin et al., 1996) or by reducing the volume of sample and of reagents used, as Koga et al. (1999) have been proposed. However, this method also involves tedious procedures without eliminating the filtration stage. This procedure becomes especially complicated when monitoring the biodegradation of anionic surfactants. For this, it is necessary to make successive determinations at low surfactant concentrations until the biodegradation is complete.

In the present work, we propose a simplification of the spectrophotometric methylene blue method that can be useful for determining anionic surfactants in relatively clean aqueous samples (Fig. 2). This method is applied to the monitoring of the primary biodegradation of LAS.

The primary biodegradation of LAS has been studied by several authors. Yakabe et al. (1992), studying its biodegradation in well water in the presence of acclimated bacteria

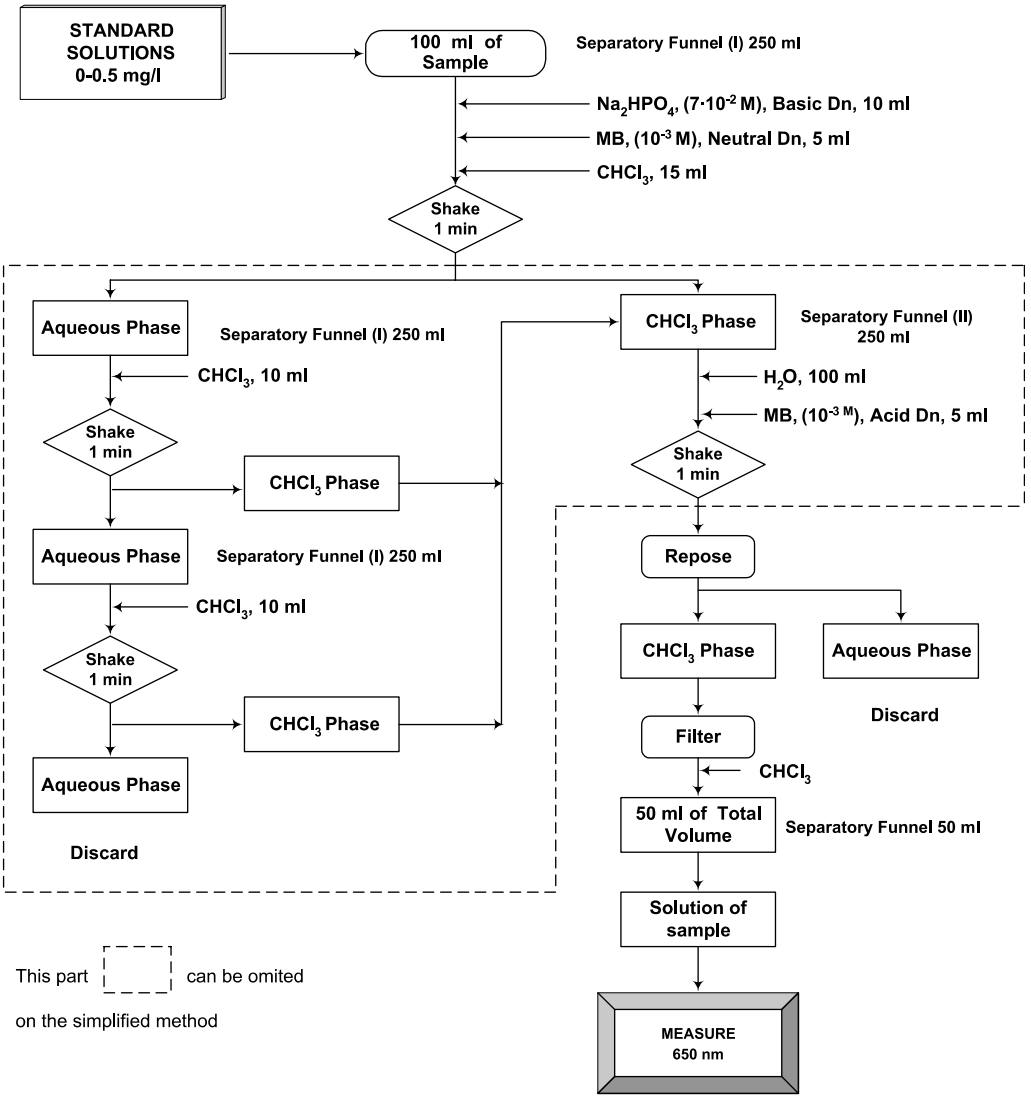

Fig. 1. Scheme of the normalized analytic procedure for determining anionic surfactants.

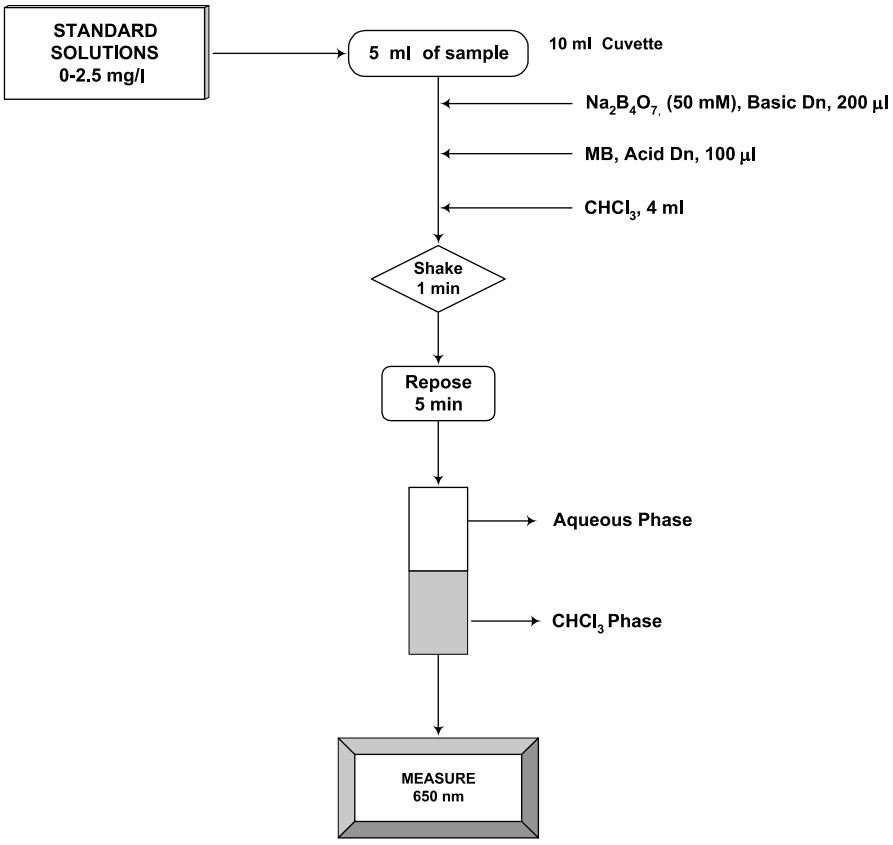

Fig. 2. Scheme of the simplified analytic procedure for determining anionic surfactants.

at an initial concentration of 2.44 mg/l, found that the surfactant concentration fell according to a zero-order kinetic. In seawater, at an initial concentration of 4 mg/l, Quiroga and Sales (1991) proposed a model represented by a second-order polynomial equation. This model shows a good fit of the experimental data and justifies the adaptation period of the microorganisms and the concentration of the residual surfactant. In waste waters, biodegradation studied by Moreno et al. (1990) and Berna et al. (1989) have determined that the concentration of the residual surfactant decreased according to a first-order kinetic.

2. Materials and methods

2.1. Reagents

Commercial dodecylbenzene sulfonate acid at 95%, SULFONAX (purchased by KAO Corporation, SA) was used directly without purification.

Solutions:

- The stock LAS solution is prepared at 1 g/l and pH neutral.
- The standard LAS solution of 10 mg/l is prepared by 1/100 dilution of the stock LAS solution.
- Buffer solution sodium tetraborate ($Na_2B_4O_7$, P.A. by Panreac) 50 mM and pH 10.5.
- Chloroform ($CHCl_3$, P.A. by Panreac).
- Methylene blue reagent stabilized at a slightly acidic pH; 0.1 g of methylene blue is dissolved ($C_{16}H_{18}CIN_3S \cdot xH_2O$, P.A. by Panreac) in 100 ml of borax buffer solution 10 mM and pH 5–6. This solution is kept in a topaz-coloured flask.

2.2. Apparatus

For the absorbance measurements, a double-beam spectrophotometer SPECTRONIC UNICAM UV-V was used. The biodegradation assays were made in a 2-l Erlenmeyer flask, with the orbital stirrer SELECTA which produces rocking with the capacity for six conical matrasses that allow up to 220 rocking motions per min with a 5- to 10-cm sweep.

2.3. Simplified analytic procedure

The following solutions were prepared:

Stock LAS solution of 1 g/l: 1 g of the commercial product dodecylbenzene sulfonate acid was dissolved in 750 ml of distilled water, adjusted to pH 7.0 by addition of NaOH solution, and levelled to 1 l.

Standard LAS solution of 10 mg/l: was prepared for dissolution 1/100 in the above distilled water.

Methylene blue reagent estabilised at a slightly acid pH of g/l (3.13 mM): 0.1 g of methylene blue was dissolved

in 100 ml of tetraborate buffer solution 10 mM. The pH of this solution should be 5.0–6.0. This solution is kept in a topaz-coloured flask.

Sodium tetraborate buffer, 50 mM at pH 10.5: 19 g of sodium decahydrated tetraborate ($Na_2B_4O_7 \cdot 10H_2O$) is dissolved in 850 ml of distilled water. The pH is adjusted to 10.5 and levelled to 1 l.

Phenolphthalein indicator: 1 g of phenolphthalein is dissolved in 50 ml of ethanol (C_2H_5OH, 95% v/v) and, under constant stirring, 50 ml of water are added. Any precipitate is eliminated by filtration.

In a glass test tube (spectrophotometric quality), 5 ml of the sample are added and alkalinized by the addition of 50 mM sodium tetraborate at pH 10.5 to the colour change of the phenolphthalein (pH > 8.3), 200 μl generally being sufficient. Next, 100 μl of stabilized methylene blue are added and homogenized, and 4 ml of chloroform are added. After vigorous stirring for 30 s and then 5 min at rest in the same test tube, without filtering, the solution is measured for absorbance at 650 nm against air.

2.4. Biodegradation test

The static biodegradation tests were carried out according to the OECD 301 E test for ready biodegradability (OECD, 1993a): a solution of the surfactant tested (the only carbon source) in a mineral medium is inoculated and incubated under aerobic conditions in dark light. The procedure consists of introducing 1.2 l of surfactant solution (for which the biodegradability is to be determined) into a 2-l Erlenmeyer flask and inoculating the solution with 0.5 ml of water from a secondary treatment of a sewage-treatment plant (STP) that operates with active sludges. The Erlenmeyer flask is closed with air-permeable hydrophobic cotton and left in darkness in a thermostatically controlled chamber at 25 °C. The constant rocking of the orbital stirrer (125 sweep/min) provides the necessary aeration. The surfactant solution is prepared by dissolving the desired quantity of surfactant in the nutrient solution and adjusting the pH to 7.0. The degradation is followed by the simplified methylene-blue-active-substances method.

The nutrient solution for the biodegradation assays (or diluted water) is prepared by adding 1 ml of the solutions A, B, C and D to each litre of distilled water. Solution A: dissolve 8.5 g of KH_2PO_4, 21.75 g of K_2HPO_4, 33.4 g of $Na_2HPO_4 \cdot 2H_2O$ and 1.7 g of NH_4Cl in distilled water and level to 1 l of final volume. Solution B: 2.5 g of $MgSO_4 \cdot H_2O$ in 1 l of distilled water. Solution C: 36.42 g of $CaCl_2 \cdot 2H_2O$ in 1 l of distilled water. Solution D: 0.25 g of $FeCl_3$ in 1 l of distilled water. All the reagents used for the preparation of the nutrient solution were PA quality from PANREAC.

The biodegradation profiles were determined by measuring the AS concentration during the biodegradation process.

During these biodegradation assays, the number of viable microorganisms was measured by a heterotrophic count in a dish (APHA, 1992), expressing the result as colony-forming units (CFU) per ml. The culture medium, nutritive agar, enables the detection for broad variety of microorganisms. With a sterile pipette, 1 ml of sample is taken from the culture, and a series of dilutions are made: 1:10 in ClNa at 0.9% until reaching a dilution of microorganisms of between 30 and 80 viable cells per ml of test solution. Each dilution is analysed in duplicate.

3. Results and discussion

Koga et al. (1999), studying the equilibrium of the substances AS, MB, and the associated ionic pair AS–MB in the water and the chloroform phases, in addition to the stability of each species of the respective phases, demonstrated that both the AS and the MB molecules alone were never transferred to the chloroform phase but rather were associated, forming the ionic pair AS–MB.

The mass-transfer equilibrium between phases of the ionic pair AS–MB can be represented by the equation:

$$[\text{AS–MB}]_{aq} \rightleftarrows [\text{AS–MB}]_{cl} \tag{2}$$

and its equilibrium constant by the expression:

$$K = \frac{[\text{AS–MB}]_{cl}}{[\text{AS–MB}]_{aq}} \tag{3}$$

where the subindex "cl" indicates the surfactant concentration in the chloroform phase, the subindex "aq" in the aqueous phase.

On the basis of the definition of concentration, the quotient between the number of moles ($n_{AS–MB}$), and the volume of the phase in which is dissolved, the concentration of the ionic pair AS–MB in each of the phases is given in the chloroform phase by:

$$[\text{AS–MB}]_{cl} = \frac{n_{\text{AS–MB}_{cl}}}{V_{cl}} \tag{4}$$

and in the aqueous phase by:

$$[\text{AS–MB}]_{aq} = \frac{n_{\text{AS–MB}_{aq}}}{V_{aq}} \tag{5}$$

In the expression of the equilibrium constant, by replacing and taking into account that the total number of moles of the surfactant is given by:

$$n_t = n_{\text{AS–MB}_{aq}} + n_{\text{AS–MB}_{cl}} \tag{6}$$

we get:

$$K = \frac{n_{\text{AS–MB}_{cl}}}{n_t - n_{\text{AS–MB}_{AQ}}} \cdot \frac{V_{aq}}{V_{cl}} \tag{7}$$

It is possible to make a quantitative extraction in a single stage (Fig. 2) by displacing the transfer equilibrium of the ionic pair AS–MB towards the organic phase, strongly increasing the quantity of chloroform with respect to the quantity of sample.

Different relationships of sample volume/chloroform volume used in the extraction were tested, as well as

different stirring times, with the highest recovery being found when 5 ml of sample previously alkalinized with the colorant methylene blue was placed in contact with 4 ml of chloroform, stirred for 1 min and then left 5 min at rest. The absorbance due to the ionic pair AS–MB in the chloroform phase was measured at 650 nm directly in the test tube without the necessity of filtration (procedure described in Materials and Methods as well as Fig. 2).

3.1. Performance characteristics of the analytical method proposed

The performance characteristics of the analytical method proposed were determined from the calibration curve based on three replicates for each concentration of AS in the range of 0–2.5 mg/l, following the method of Cuadros et al. (1993, 1996). Fig. 3 shows the absorbance data measured at 650 nm against air for the surfactant concentrations of 0, 0.5, 1, 1.5, 2 and 2.5 mg/l.

For the calibration curve (Fig. 3), a linear fit was tested by ordinary least-squares (OLS), which minimizes the sum of the squares of the error (differences between the observed and predicted values for the dependent variable). The calibration curve, as well as the parameters of the regression analysis (standard deviation of the ordinate at the origin S_a, of the slope S_b and of the regression S_r; the correlation coefficient; and determination coefficient R^2) are also shown in Fig. 3.

3.1.1. Testing the hypothesis of the OLS model

The OLS regression model is applicable only if the starting data meet a number of requisites such as: absence of random errors in the independent variable; randomness and independence of the response variable; normal distribution of the errors of the response variable; equality of the variances (homocedasticity); and linearity of the response variable. Generally, the first three requisites are assumed to be fulfilled and only linearity and homocedasticity are tested.

Linearity refers to "in-line" linearity (also termed rectilinearity), which describes the absence of curvature in the calibration line (goodness of fit of the analytical signal values determined experimentally in relation to the analyte concentration). The random distribution of the residuals around the central axis (Fig. 3) as well as the value of $P_{LOF} \geqslant 0.01$ ($P_{LOF} = 0.9335$) of the lack of fit test (LOF) rule out a curved behaviour. In this case, the linear model used in the regression is correct.

The homocedasticity or equality of variances is shown in the residual graph (Fig. 4). The dispersion of points around the central line tends to increase with the concentration value. There is, therefore, evidence of possible heterocedasticity—that is, that the variances are not homogeneous and depend on the concentration. To confirm or rule out this possibility, Cochran's test and Bartlett's test are performed (Massart et al., 1997; Miller and Miller, 2000). Since the p-values are, respectively, 0.37621 and 0.06226, and the smaller of the p-values are greater or equal to 0.05, there is no statistically significant difference among the mean squares at the 95.0% confidence level. The calibration curve is given by the equation:

$$A_{650} = 0.3907 + 0.4385 \cdot [\mathrm{AS}] \quad (8)$$

3.1.2. Linearity

This refers to "on-line" linearity, which indicates the greater or lesser dispersion of data around the calibration line and determines the linear calibration range. The "on-line" linearity can be evaluated from the determination coefficient R^2, the relative standard deviation of the slope (S_b/b) and from the expression: $\mathrm{LIN_{OL}}(\%) =$

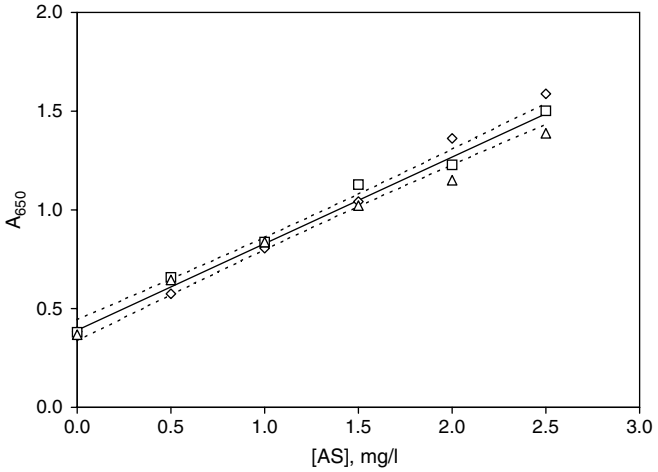

Fig. 3. Calibrated straight line for the anionic surfactant dodecylbenzenesulfonic acid according to the simplified method of substances active to methylene blue. The absorbance was measured at 650 nm and at each concentration the measurements were made in triplicate. Intercept ordinate at the origin (0.3907); slope of the regression line (0.4385); S_a: standard deviation of the ordinate at the origin (0.01657); S_b: standard deviation of the slope (0.02509); S_r: standard deviation of the regression (0.06000); r: correlation coefficient (0.988776), and R^2: determination coefficient (97.7677).

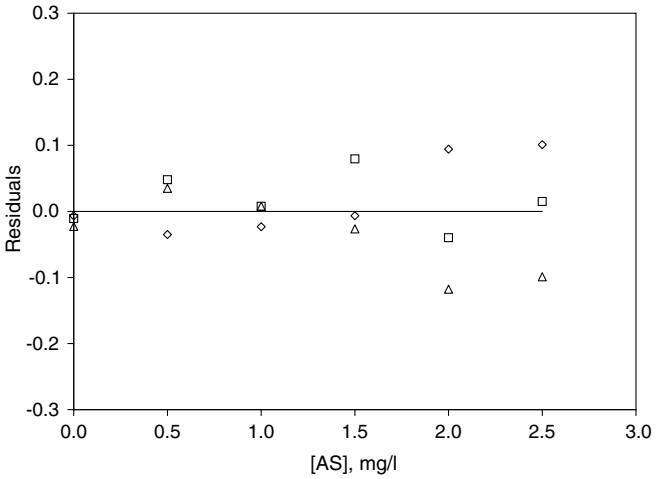

Fig. 4. Residual plot to check homocedasticity.

$100(1 - S_b/b)$. The values found for these parameters are, respectively, 97.7677, 0.059 and $\mathrm{LIN_{OL}} = 94.1\%$.

3.1.3. Sensitivity

There are two ways of expressing sensitivity of an analytical method: calibration sensitivity ($\mathrm{SENS_{cal}}$), which measures the relationship between the instrumental signal and the concentration of the solution analysed; and analytical sensitivity ($\mathrm{SENS_{anal}}$), which is the least variation of concentration that an analytical method is able to discern (analytic sensitivity is also known as resolution sensitivity). The former is evaluated from the calibration slope, b, and the second from the expression S_r/m, where S_r is the standard deviation of the regression. The values found for sensitivity are:

$$\mathrm{SENS_{cal}} = b = 0.4385 \frac{\text{Units of absorbance}}{\text{mg/l}} \quad (9)$$

$$\mathrm{SENS_{anal}} = \frac{S_r}{b} = 0.14 \ \text{mg/l} \quad (10)$$

which indicate, respectively, that an increase of 1 mg/l in the concentration means an increase of 0.4385 units in the signal and that the method distinguishes changes of 0.14 mg/l in concentration.

3.1.4. Precision

Precision measures the degree of uncertainty of an analytical result. It is due to the instrumental signal errors and to the use of values of the slope and intercept estimated from the calibration straight line in order to transform the instrumental signal measured in concentration. From the calibration data, S_c can be determined for each concentration from the expression:

$$S_c = \sqrt{\left(\frac{S_r}{b}\right)^2 \left(\frac{1}{n} + \frac{1}{m}\right) + \left(\frac{S_b}{b}\right)^2 (c - \overline{c})} \quad (11)$$

where n is the number of points of the calibration straight line, m the number of replicates, c the concentration of surfactant, and $\overline{c}$ the mean concentration of surfactant in the interval of concentrations considered. Table 1 lists the values of S_c for each concentration value.

3.1.5. Lower limits

Detection limit (DL). This is the least concentration that can be detected with reasonable certainty by a given analytical procedure. Using the approximation of the IUPAC, the detection limit is calculated from the expression: $\mathrm{DL} = 3 \cdot S_{co}$. The value calculated for the detection limit is 0.22 mg/l.

Quantification limit (QL). This represents the minimum concentration quantifiable by the analytical method. It is calculated from the expression: $\mathrm{QL} = 10 \times S_{co}$. The value calculated for the quantification limit is 0.73 mg/l.

Table 1
Precision values found in the range of 0–2.5 mg/l

AS concentrations (mg/l)	S_c	S_c/c
0.0	0.0726	–
0.5	0.0831	0.166
1.0	0.0924	0.092
1.5	0.1009	0.067
2.0	0.1087	0.054
2.5	0.1160	0.046

$n = 6$, $m = 3$, $c = 1.25$ mg/l. n: number of points of the calibration straight line; m: number of replicates; c: concentration of surfactant; S_c: precision.

3.2. Biodegradation of LAS

In the biodegradation experiments, the surfactant concentration was analysed over time using the simplified methylene-blue-active-substances method. The assays were made at 5, 25, 50 and 100 mg/l, and the results are shown in Fig. 5, where, for comparison, the surfactant concentration is expressed as a percentage of residual surfactant. It was found that during the acclimation period of the microorganisms, the surfactant concentration increased slightly, apparently due to accumulation of surfactant at the interface; the solution was stirred vigorously before sampling to minimize this effect. As the surfactant concentration increased, a greater time was needed to reach the same level of biodegradation. At low concentrations (up to 25 mg/l), after a short adaptation time, primary biodegradation of LAS is rapid and is reached in 2 days, whereas for higher concentrations (50 mg/l) the process was slower and required a longer adaptation time for the organisms in the medium (8 days). In experiments made at the highest concentration (100 mg/l), no biodegradation at all was detected for 9 days.

After the acclimation period of the microorganisms, the kinetic study of the primary degradation profiles registered low concentration values (5 mg/l), the kinetic being on the order of zero, and at higher surfactant concentrations (25 and 50 mg/l), first order. However, to predict the behaviour of the biodegradation of the surfactant, it is necessary to develop models that can be applied throughout the time interval of the biodegradation process and for an interval

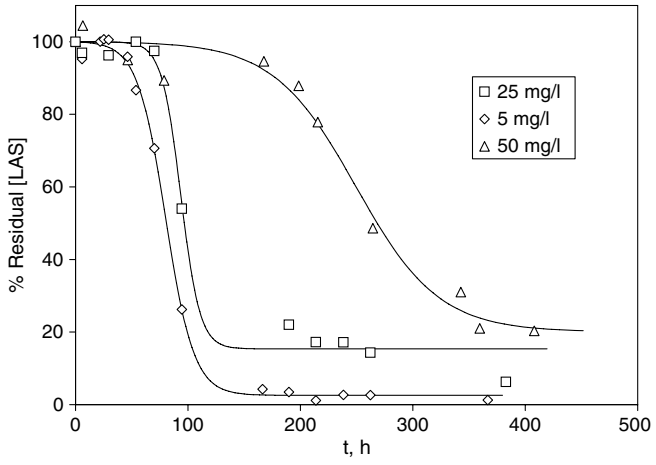

Fig. 5. Biodegradation profiles of LAS at 5, 25, 50 mg/l. Experimental points and results calculated with the model proposed (Eq. (20)).

of concentrations as broad as possible. During the biodegradation process, the growth of the microorganisms involved in the process was monitored in all the experiments performed. It has been tested that biodegradation profiles and the evolution of the growth of the microorganisms expressed as CFU are completely coupled (Fig. 6).

The biodegradation profiles present a sigmoidal shape, and the growth curves a bell shape with the latency or adaptation phase of the microorganisms to the surfactant showing an exponential growth phase, which reaches their maximum just when the degradation stage of the surfactant ends. An exponential decline follows when the surfactant concentration is practically null. All this indicates that the substrate, which is the only source of available carbon, supports the growth of microorganisms, suggesting the use of kinetic models for substrates that support growth (Simkins and Alexander, 1984).

In such cases, the rates of microorganism growth and of the disappearance of organic compound are coupled, allowing the approximate fulfilment of Gaden's equation (Gaden, 1959):

$$\frac{dX}{dt} = -Y_{ap}\frac{dS}{dt} \tag{12}$$

by integration

$$X = X_0 + Y_{ap}(S_0 - S) \tag{13}$$

where S_0 is the initial concentration of the substrate, S the substrate concentration at each point in time, X the initial concentration of biomass, and Y_{ap} the biomass yield in CFU/g substrate.

Taking into account the two resulting profiles, we can relate the biomass concentration (X) to the concentration of the organic compound (S) at each point in time of the biodegradation process.

If Monod's model (Monod, 1949) is used for the growth

$$\frac{1}{X}\frac{dX}{dt} = \mu = \frac{\mu_m S}{K_S + S} \tag{14}$$

the biodegradation rate of the substrate is:

$$\frac{dS}{dt} = -\frac{1}{Y_{ap}}\frac{\mu_m S}{K_S + S}X = -\frac{\mu_m S}{K_S + S}\left(\frac{X_0}{Y_{ap}} + S_0 - S\right) \tag{15}$$

On introducing the conversion of the substrate (x), we get:

$$S = S_0(1 - x) \tag{16}$$

$$\frac{dx}{dt} = \frac{\mu_m(1 - x)}{K_S + S_0(1 - x)}\left(\frac{X_0}{Y_{ap}} + S_0 x\right) \tag{17}$$

In the limit case in which $K_S >> S$, while taking into account the definition of conversion (Lechuga, 2005), we would get

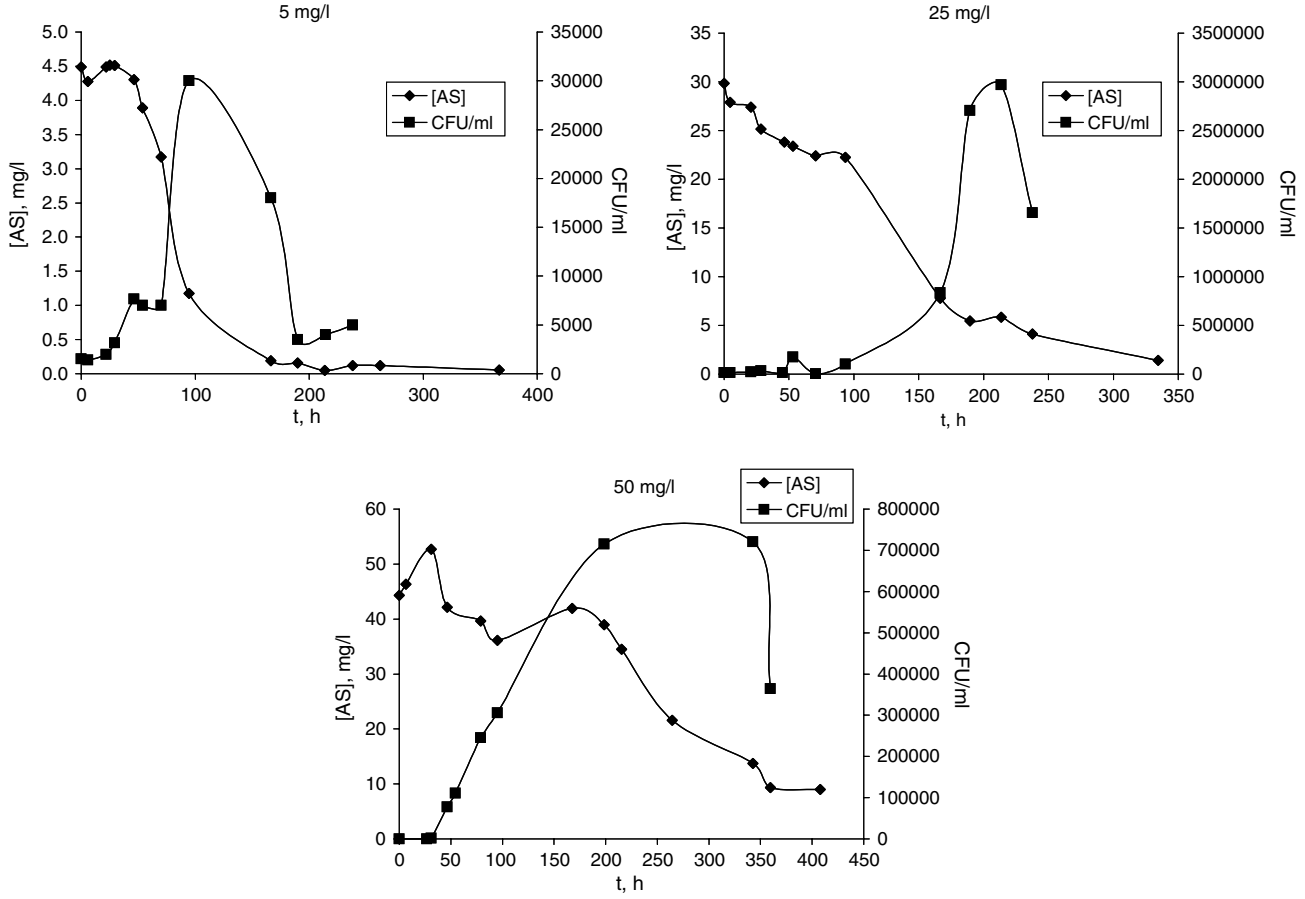

Fig. 6. Biodegradation profiles and Growth curves expressed as CFU of LAS at 5, 25, 50 mg/l.

Table 2
Parameters of the kinetic model for substrates that support growth

S_0 (mg/l)	α_0	β_S	μ_m (h^{-1})	K_S (mg/l)	x_m	r
LAS						
5	1.70×10^{-3}	0.746	0.060	3.73	0.974	0.999
25	1.92×10^{-3}	0.462	0.053	11.55	0.981	0.999
50	9.00×10^{-4}	0.145	0.049	7.26	0.799	0.966
Recalculated parameters						
5	1.70×10^{-3}	0.663	0.054	4.171	0.974	0.999
25	1.91×10^{-3}	0.182	0.054	4.171	0.981	0.990
50	9.11×10^{-4}	0.093	0.054	4.171	0.799	0.966

S_0: initial concentration of the substrate; α_0 and β_S: parameters of the model; μ_m: maximum specific growth rate; K_S: saturation constant; x_m; maximum conversion; r: determination coefficient.

a maximum conversion, a function of the residual substrate concentration:

$$x_m = \frac{S_0 - S_R}{S_0} \tag{18}$$

$$S_R = S_0(1 - x_m) \tag{19}$$

The integrated expression, with the explicit form conversion would be:

$$x = \frac{\alpha_0 x_m \left(1 - \exp\left(-\frac{\mu_m}{\beta_S}(x_m + \alpha_0)t\right)\right)}{x_m \exp\left(-\frac{\mu_m}{\beta_S}(x_m + \alpha_0)t\right) + \alpha_0} \tag{20}$$

with

$$\beta_S = \frac{K_S}{S_0} \quad \alpha_0 = \frac{X_0}{Y_{ap} S_0} \tag{21}$$

The values of the parameters of the model α_0, μ_m, β_S *y* x_m are listed in Table 2 as well as the value of K_S calculated from the relationship established in Eq. (21). The specific growth rate μ_m and the saturation constant K_S are not a function of the substrate concentration used, and thus a mean value was taken and the rest of the parameters were recalculated (Table 2).

Fig. 5 presents the fit of this model (continuous plot). As reflected in the graph, the model shows a good fit to the experimental dependence observed for all the experimental points, including the adaptation period of the microorganisms with a mean relative deviation of 7.42%. The parameters evaluated also justify this model, β_S decreases on increasing the initial surfactant concentration, while μ_m and the maximum conversion attained (x_m) decline on increasing the initial substrate concentration.

4. Conclusions

The performance characteristics found in the simplified method of methylene-blue-active-substances demonstrate that this procedure is adequate for the analysis of anionic surfactants in waters and in biodegradation assays within the range of 0.22–2.5 mg/l. Our proposed method requires only 5 ml of sample, 4 ml of the extraction solvent (chloroform) and one tenth of the analytical time of the official analytical method.

This method has been successfully applied for monitoring the primary biodegradation process of anionic surfactant in aerobic screening biodegradation tests.

A kinetic model for substrates that support the growth of microorganisms taking into account Monod's Model, the equation proposed by Gaden, and a residual concentration of non-biodegradable surfactant has been applied. This model has enabled the evaluation of the specific growth rate μ_m and the saturation constant K_S for LAS. These parameters are necessary to design biodegradation systems that can be applied to surfactant concentrations of up to 50 mg/l, concentrations far higher than normally found in aquatic environments.

References

APHA, AWWA, WPCF, 1992. Standard Methods for the Examination of Water and Wastewater. American Public Health Association. 18th ed.

Berna, J.L., Ferrer, J., Moreno, A., Prats, D., Ruiz, F., 1989. The fate of LAS in the environment. Tenside Surfact. Det. 26, 101–107.

Chitikela, S., Dentel, S.K., Allen, H.E., 1995. Modified method for the analysis of anionic surfactants as methylene blue active substances. Analyst 120, 2001–2004.

Cuadros, L., García, A.M., Jiménez, C., Román, M., 1993. Estimation of performance characteristics of an analytical method using the data set of the calibration experiment. Anal. Lett. 26, 1243–1258.

Cuadros, L., García, A.M., Bosque, J.M., 1996. Statistical estimation of linear calibration range. Anal. Lett. 29, 1231–1239.

Gaden, E., 1959. Fermentation process kinetics. J. Biochem. Microbiol. 1, 63–69.

ISO 7875-1, 1996. Water quality. Determination of Surfactants. Part 1: Determination of Anionic Surfactants by Measurement of the Methylene Blue Index (MBAS). ISO/TC 147.

Koga, M., Yamamichi, Y., Nomoto, Y., Irie, M., 1999. Rapid determination of anionic surfactants by improved spectrophotometric method using methylene blue. Anal. Sci. 15, 563–568.

Lechuga, M., 2005. Biodegradación y toxicidad de tensioactivos comerciales. Ph.D. Thesis, University of Granada, Spain.

Massart, D.L., Vandeginste, B.G.M., Buydens, L.M.C., De Jong, S., Lewi, P.J., Smeyers-Verbeke, J., 1997. Handbook of Chemometrics and Qualimetrics: Part A. Elsevier.

McEvoy, J., Giger, W., 1985. Accumulation of linear alkylbenzenesulfonate surfactants in sewage sludges. Naturwissenschaften 72, 429–431.

Miller, J.N., Miller, J.C., 2000. Statistics and Chemometrics for Analytical Chemistry, fourth ed. Prentice Hall.

Monod, J., 1949. The growth of bacterial cultures. Annu. Rev. Microb. 3, 371–394.

Moreno, A., Ferrer, J., Berna, J., 1990. Biodegradability of LAS in a sewer system. Tenside Surfact. Det. 27, 312–315.

Moskvin, L.N., Nikolaeva, D.N., Mikhailova, N.V., 1996. Determination of anionic surfactants in water with adsorption preconcentration. J. Anal. Chem. 51, 282–285.

OECD, 1993a. OECD Guidelines for the Testing of ChemicalsSection 3: Degradation and Acumulation, vol. 1. OECD, Paris, France.

Quiroga, J.M., Sales, D., 1991. Surfactants biodegradation in sea water. Tenside Surfact. Det. 28, 200–203.

Simkins, S., Alexander, N., 1984. Models for mineralisation kinetics with the variables of substrate concentration and population density. Appl. Environ. Microb. 47, 1299–1305.

Yakabe, Y., Etoh, C., Matsunobo, Y., Katsuura, H., Miura, K., Yoshimuyra, K., 1992. Kinetic study on the biodegradation of linear alkylbenzenesulfonates (LAS) in well-water. Chemosphere 24, 969–977.

Ying, G.-G., 2006. Fate, behaviour and effects of surfactants and their degradation products in the environment. Environ. Int. 32, 417–431.