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# Colloidal and biological properties of cationic single-chain and dimeric surfactants

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ABSTRACT

The colloidal and biological properties of the two single-chain surfactants *N*-benzyl-*N*,*N*-dimethyl-*N*-(1-dodecyl)ammonium bromide (PH12) and *N*-cyclohexylmethyl-*N*,*N*-dimethyl-*N*-(1-dodecyl)ammonium bromide (CH12) and their two dimeric counterparts *N*,*N*'-(1,3-phenylenebis(methylene))bis(*N*,*N*-dimethyl-*N*-(1-dodecyl)ammonium dibromide (12PH12) and *N*,*N*'-(cyclohexane-1,3-diylbis(methylene))bis(*N*,*N*-dimethyl-*N*-(1-dodecyl)ammonium dibromide (12CH12) were investigated. The thermodynamic functions of the self-aggregation process were estimated by using calorimetric measurements and the micellization enthalpy values, $\Delta H_M$, were examined considering the different enthalpic contributions to $\Delta H_M$. In the investigation of the structure-property relationship, it was found that the surfactant structure does not influence practically the foamability of the surfactants, but it plays a key role in their solubilization capacity, antimicrobial activity and biodegradability.

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## 1. Introduction

Surfactants are amongst the most common chemical products. They contain a hydrophilic head group attached to a large hydrophobic part. Typically, the hydrophobic part is an alkyl group with at least eight $CH_2$ units. The head group can be cationic, anionic, zwitterionic, or non ionic [1]. Surfactants are employed in large quantities every day on a worldwide scale as constituents of many different products. In particular, quaternary ammonium cationic surfactants can be used as ingredients of cosmetic products [2], in emulsion polymerization [3], in textile processing [4], etc. They can also be used in medicine as gene delivery agents [5]. In order to improve the physicochemical and biological properties of cationic surfactants, as well as their specific applications in modern technologies, it is necessary to develop new molecules and to investigate the structure–property relationship in surfactant systems. So far a significant number of quaternary ammonium surfactants with novel chemical structures have been prepared. Examples are surfactants with more than one head group connected to a single hydrocarbon tail [6], dimeric, trimeric and tetrameric surfactants [7,8], or bola-form surfactants [9,10].

Recently the preparation and physicochemical characterization of the single chain surfactants *N*-benzyl-*N*,*N*-dimethyl-*N*-(1-dodecyl)ammonium bromide (PH12) and *N*-cyclohexylmethyl-*N*,*N*-dimethyl-*N*-(1-dodecyl)ammonium bromide (CH12) and their two dimeric counterparts *N*,*N*'-(1,3-phenylenebis(methylene))bis(*N*,*N*-dimethyl-*N*-(1-dodecyl) ammonium dibromide (12PH12) and *N*,*N*'-(cyclohexane-1,3-diylbis(methylene))bis(*N*,*N*-dimethyl-*N*-(1-dodecyl)ammonium dibromide (12CH12) was carried out [11]. Results indicated that the surfactant structure influences their surface activity as well as the characteristics of the micellar aggregates formed in aqueous solution. The surfactant structure also plays an important role in micelle morphology. The study of how the surfactant structure affects colloidal and biological properties such as their solubilization capacity, foamability, antimicrobial activity and biodegradability is of interest. It is known the industrial importance of the surfactant solubilization capacity since many of the surfactant applications are based on their ability to solubilize hydrophobic compounds [12,13]. Foamability of surfactants is also worth studying given that they are used in formulations such as shampoos, detergents, etc. as well as in other industrial applications [14]. On the other hand,

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most of the quaternary ammonium-based surfactants are known to possess a significant antimicrobial activity [15], which is important in relation to their possible biomedical applications [16]. However, the extensive use of surfactants in practical applications may cause their accumulation in the environment. Since quaternary ammonium surfactants may provoke toxicity to ecological targets, the environmental impact of these chemicals, as related to their biodegradability, is of concern [17]. Consequently, information about the biodegradability of the surfactants is essential in order to establish structure/activity relationships since the environmental impact of surfactants has become almost as important as their functional performance [18].

In this work the foamability, solubilization capacity, antimicrobial activity and biodegradability of PH12, CH12, 12PH12, and 12CH12 were investigated. Prior to these studies, their self-aggregation process was examined by means of isothermal titration calorimetry in order to complete their physicochemical characterization.

## 2. Experimental

### 2.1. Materials

Reagents were purchased from Aldrich or Fluka and used as received.

The syntheses of the single-chain and the dimeric surfactants (see Scheme 1) were done as described in Ref. [11].

### 2.2. Microorganisms

Seven bacteria were used. Gram-negative bacteria included *Escherichia coli* CECT 101, *Klebsiella pneumoniae* CECT 143, and *Pseudomonas fluorescens* CECT 378. Gram-positive bacteria included *Enterococcus faecalis* CECT 481, *Micrococcus luteus* CECT 245, *Mycobacterium phlei* CECT 3009, and *Staphylococcus epidermidis* CECT 231. These reference strains were obtained from the CECT (Spanish Culture Collection of Type Cultures, University of Valencia, Spain).

### 2.3. Calorimetric measurements

A multichannel thermal activity monitor (TAM) isothermal heat conduction microcalorimeter (Thermometric AB 2277/201, Järfälla, Sweden) was used for measuring directly the critical micellar concentration, cmc, and the enthalpy of micelle formation of the surfactant solutions. The calorimeter was connected to an external water circulator (Heto) and the whole system was placed in a room in which the temperature was kept constant within ±0.5 K. The sample cell of the calorimeter was initially loaded with 0.9 mL of pure water, at 303 K. A surfactant solution of a concentration ~15-fold the cmc was injected in small aliquots to the stirred sample (250 rpm) cell using a 250 μL Hamilton syringe, which was positioned in a computer controlled syringe pump (Hamilton Microlab M). Each aliquot was 10 μL, with a 240 s interval between injections, and addition of the concentrated solution continued until the desired range of concentration had been covered. The experiments were computer controlled using Digitam 4.1 software (Thermometric); the same program was used for data analysis. The calorimeter was electrically calibrated before each titration experiment. Nevertheless, in order to check the method, the micellization enthalpy and the cmc of butanedyil-bis(dimethyldodecylammonium) bromide, 12-4-12,2Br$^-$, were determined. Their values were – 9.7 kJ mol$^{-1}$ and $1.1 \times 10^{-3}$ mol dm$^{-3}$, respectively, in agreement with literature data [19].

### 2.4. Foamability

Foamability was determined by inverting and uprighting 10 times a 50-mL burette containing the surfactant solution at a concentration 3 times higher than its critical micelle concentration and then measuring the height of the resulting foam [20].

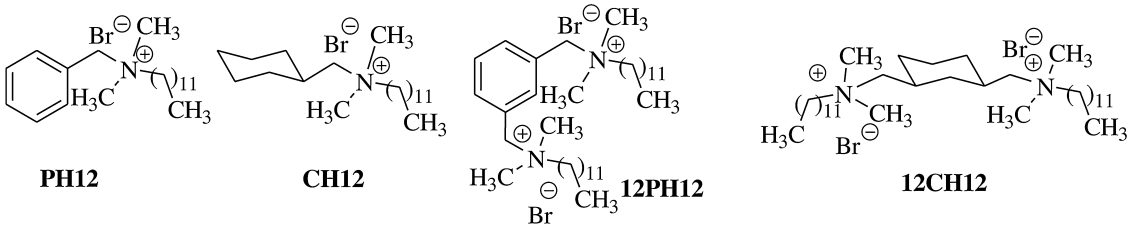
### 2.5. Solubilization capacity

Excess amounts of tetramethylsilane, TMS, and 1,3,5-trimethylbenzene, TMB, in 0.04 mol dm$^{-3}$ surfactant in $D_2O$ were left in an ultrasonic bath for 2 h, followed by $^1$H NMR analysis of the solubilized material in $D_2O$ after the layers had separated (see Fig. 1A and Fig. 2A, Supplementary material). The NMR experiments were recorded on a Bruker Avance 500 spectrometer (500.2 MHz for $^1$H) equipped with a 5 mm inverse probe and a Great 1/10 pulsed-gradient unit capable of producing magnetic field gradients in the *z* direction of about 50 G cm$^{-1}$. The apparatus is in the General Research Services of the University of Seville (CITIUS). The chemical shifts in the $^1$H NMR spectra were referenced to the residual HDO signal.

An excess amount of dispersed red in aqueous surfactant solutions, at several surfactant concentrations, was left in an ultrasonic bath for two hours. The resultant solution was filtered, using a standard gravity filtration method, and the dye concentration was determined spectrophotometrically at 390 nm. The absorbance of the dye solutions was measured in a Hitachi UV-3900 spectrophotometer. The temperature was maintained at 303 ± 0.1 K by using a water-jacketed cell compartment. High concentrations of 12PH12 could not be investigated because of solubility problems.

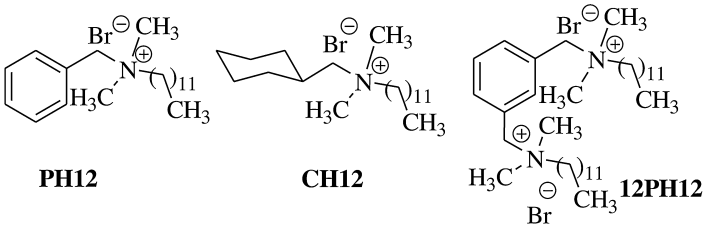
### 2.6. Antimicrobial activity

Antimicrobial activities were determined *in vitro* on the basis of the minimal inhibitory concentration (MIC) values, defined as the lowest concentration of an antimicrobial compound required to inhibit or kill a microorganism; that is, the minimal concentration without visible growth. The experiments were performed in duplicate using a two-fold serial antimicrobial macro-broth dilution method [21] and they were reproducible within a precision better than 8%. The antimicrobial compounds tested were solved in




**Scheme 1.** Molecular structure of single chain and dimeric surfactants.

Mueller-Hinton broth, purchased from Difco (prepared according to the manufacturer's instructions), to twice the highest final concentration desired and then serially two-fold diluted. The final concentrations of the antimicrobial compound in this test are half those of the initial dilution series because of the addition of an equal concentration of inoculum in broth. The inoculum of each bacterial strain was prepared by adjusting the turbidity of a broth culture incubated for a short time to match the McFarland 0.5 turbidity standard [22] and then further diluting it 1:200 in broth. The final bacterial concentration achieved in each test tube was of ca. $5 \times 10^4$ to $5 \times 10^5$ CFU mL$^{-1}$. The cultures were incubated at 310 K for 16 to 20 h. Mueller–Hinton broth without the antimicrobial compound but inoculated served as a control. The growth of the microorganisms was determined visually after incubation. A very faint haziness or a small clump of possible growth was disregarded, whereas a large cluster of growth or definite turbidity was considered evidence that the compound had failed to inhibit growth completely at that concentration. The lowest concentration of antimicrobial compound resulting in complete inhibition of visible growth was taken as the MIC [21].



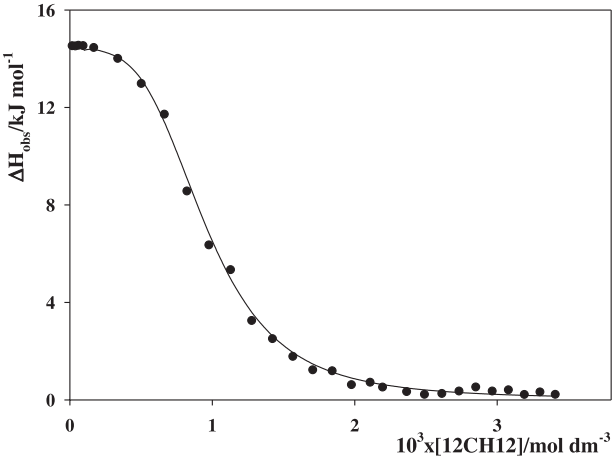
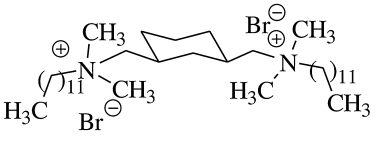




**Fig. 1.** Typical calorimetric curve of the variation of $\Delta H_{obs}$ with surfactant concentration for 12CH12 at 303 K.




### 2.7. Biodegradability

The biodegradability of the surfactants was assessed with the ready biodegradability test (OECD method 301A). The method uses the loss of dissolved organic carbon, DOC, as an estimate of biodegradation [23]. A sample from a wastewater treatment plant (WTP) was used as inoculum. The sample was taken from an aerobic biological reactor in Fundación Centro de las Nuevas Tecnologías del Agua (CENTA), Carrión de los Céspedes (Sevilla, Spain). The inoculum was incubated during 5 days at 150 rpm and 295 K before biodegradation tests. The surfactant solutions were placed in duplicate 250 mL Erlenmeyer flasks, and mixed with an inorganic salts solution (pH 7.4) and 10 mL of inoculum ($10^7$ cells mL$^{-1}$), to give a final DOC of 20–25 mg mL$^{-1}$ and a total volume of 100 mL. Two controls were run, one without surfactant and another one with 100 mg mL$^{-1}$ acetate, to monitor possible toxicity effects in the inoculum. The flasks were incubated at 295 K on an orbital shaker operating at 150 rpm. Periodically, 15 mL-samples were removed from the flasks and filtered (Acrodisc syringe filter, 0.2 μm). The concentration of DOC in the samples was then determined as total organic carbon (TOC) in solution with a Shimadzu TOC-V sch analyzer.

The results were expressed as percentages of degradation ($P_t$) and were analyzed with the following equation:

$$P_t = P_{max}(1 - \exp(-kt)) \quad (1)$$

The values of $P_{max}$ (maximum extent of biodegradation, %) and $k$ (first-order rate constant, h$^{-1}$) were obtained by minimizing the cumulative squared residuals between experimental and calculated values of $P_t$ with time ($t$). Experimental $P_t$ values were corrected for TOC losses in the control without chemical amendment. The software used for the minimization was Microsoft Excel 97 (Solver option). The half lives of the surfactants ($t_{1/2}$) were calculated as $t_{1/2} = 0.693/k$, and the maximum rate of biodegradation was calculated as $P_{max} \times k$.

## 3. Results and discussion

### 3.1. Thermodynamic functions of micellization

Data in Table 1 provides relevant information about the aqueous PH12, CH12, 12PH12, and 12CH12 micellar solutions.

The differential molar enthalpies of dilution were determined by using isothermal titration calorimetry. A typical calorimetric curve of the observed enthalpy variation with surfactant concentration is shown in Fig. 1. The titration process was found to be endothermic for the four surfactants, which indicates that the enthalpies of micelle formation, $\Delta H_M$, are negative. As has been previously discussed [24,25], when the final concentration is in the premicellar region, the added micellar aggregates dissociate into monomers and the monomers are then diluted. When the final concentration is above the cmc, the added micellar aggregates are only diluted. Therefore $\Delta H_M$ was obtained from the difference between the observed enthalpies of the two linear segments of the plots. $T\Delta S_M$ was estimated from the $\Delta H_M$ values and those of $\Delta G_M$ listed in Table 1. $\Delta H_M$ and $-T\Delta S_M$ values are listed in Table 2, together with the cmc values obtained by calorimetric measurements. The cmc values agree with those obtained by conductivity measurements (see Table 1). The enthalpies of micellization are all negative and the entropies of micellization are all positive. The self-aggregation of the surfactants investigated is mainly driven by entropy.

There are several contributions to the enthalpy of micellization, $\Delta H_M$; some of them are present for both the single-chain and the dimeric surfactants, but the contributions associated with the spacer are specific of the dimeric surfactants [26]. The most probable contributions to $\Delta H_M$, common to all the surfactants studied, are: (i) The hydrophobic contribution, $\Delta H_{M,chain\ transfer}$, related to the transfer of the surfactant hydrophobic tails from the aqueous phase into the micellar core. It is the main contribution to $\Delta H_M$ and has a negative sign. This transfer is accompanied by the release of most of the water molecules that surround the hydrophobic tails in the aqueous phase, which are structured differently from those

**Table 1**
Critical micelle concentration, cmc, micellar ionization degree, $\alpha$, average aggregation number, $N_{agg}$, and Gibbs energy of micellization, $\Delta G_M$, for the single-chain and dimeric surfactants studied at 303 K (data taken from Ref. [11]).

| Surfactant | $10^3 \times$ cmc (mol dm$^{-3}$) | $\alpha$ | $N_{agg}$ | $\Delta G_M$ (kJ mol$^{-1}$) |
|---|---|---|---|---|
| PH12 | 5.8 ± 0.2 | 0.30 ± 0.02 | 32 ± 2[a] (37 ± 2)[b] | −39 ± 2 |
| CH12 | 4.7 ± 0.2 | 0.36 ± 0.03 | 27 ± 2[a] (34 ± 2)[b] | −39 ± 2 |
| 12PH12 | 0.97 ± 0.05 | 0.25 ± 0.02 | 18 ± 1[a] (25 ± 1)[b] | −69 ± 3 |
| 12CH12 | 1.00 ± 0.04 | 0.32 ± 0.02 | 16 ± 1[a] (22 ± 1)[b] | −65 ± 3 |

Experimental results are expressed as the mean ±SD ($n$ = 3).
[a] Obtained by steady-state fluorescence quenching, SSFQ.
[b] Obtained by time-resolved fluorescence quenching, TRFQ.

in the bulk phase [1]. (ii) The electrostatic contribution due to the association of the counterions to the charged head groups, $\Delta H_{M,elec}$. (iii) The repulsion between the charged head groups and between the condensed counterions, $\Delta H_{M,repul}$. $\Delta H_{M,elec}$ and $\Delta H_{M,repul}$ are negative and positive, respectively, if only electrostatic interactions are considered [27] and the former is usually smaller (in absolute value) than the latter.

For dimeric surfactants three additional contributions have to be taken into account. First, the contribution related to the transfer of the spacer from the aqueous phase to the micelle, $\Delta H_{M,spacer\ transfer}$. Either if the spacer remains principally at the micelle surface or it is partly embedded in the micelle interior, water molecules are released and, therefore, this contribution is exothermic. For short spacers remaining at the micelle surface $\Delta H_{M,spacer\ transfer}$ is small. The second and third contributions are associated with the conformational change of the spacer, $\Delta H_{M,spacer\ conf}$, and the steric hindrance between the two hydrophobic tails, $\Delta H_{M,spacer\ steric}$ [26]. The sign of $\Delta H_{M,spacer\ conf}$ is unknown, but $\Delta H_{M,spacer\ conf}$ is a positive contribution.

One can see in Table 2 that the $\Delta H_M$ values are substantially more exothermic for the dimeric surfactants than for the single-chain surfactants. A similar result was previously found by Bai et al. [19] in the study of the aggregation process of alkenedyil bis(dodecyldimethylammonium bromide), 12-*s*-12,2Br$^-$, dimeric surfactants and their single-chain counterparts. This experimental observation can be explained by considering the hydrophobic contribution, $\Delta H_{M,chain\ transfer}$, which is expected to be more exothermic for a surfactant with two alkyl tails than for a single-chain surfactant.

The enthalpy of micellization of dodecyltrimethylammonium bromide, DTAB, at 303 K is 3.4 kJ mol$^{-1}$ [28]. This value can be compared to those corresponding to CH12 and PH12. The hydrophobic contribution related to the transfer of the dodecyl chain from water into the micelles is the same for the three surfactants. However, for PH12 and CH12 there is an additional exothermic hydrophobic contribution to $\Delta H_M$, corresponding to the transfer of the rings from water to the micelles. It could be appreciable since NMR measurements show that the rings are bent towards the micelle interior [11]. In relation to the contributions $\Delta H_{M,repul}$ and $\Delta H_{M,elec}$, the micellar ionization degrees of DTAB, PH12, and CH12 are 0.27 [29], 0.30 [11], and 0.36 [11], respectively. Therefore, the endothermic $\Delta H_{M,repul}$ term will follow the trend $\Delta H_{M,repul}(DTAB) < \Delta H_{M,repul}(PH12) < \Delta H_{M,repul}(CH12)$

**Table 2**
Cmc values and thermodynamic parameters for the surfactants investigated ($T$ = 303 K).

| Surfactant | $10^3 \times$ cmc[a] (mol dm$^{-3}$) | $\Delta H_M$ (kJ mol$^{-1}$) | $-T\Delta S_M$ (kJ mol$^{-1}$) |
|---|---|---|---|
| PH12 | 5.9 ± 0.5 | −7.8 ± 0.7 | −47 ± 4 |
| CH12 | 4.5 ± 0.4 | −2.9 ± 0.3 | −42 ± 4 |
| 12PH12 | 1.03 ± 0.09 | −19 ± 2 | −88 ± 8 |
| 12CH12 | 1.14 ± 0.09 | −14 ± 1 | −79 ± 6 |

The experimental values are expressed as the mean ±SD ($n$ = 3).
[a] Calorimetric measurements.

and that of the exothermic contribution related to the association of the counterions to the head groups will be $|\Delta H_{M,elec}(DTAB)| > |\Delta H_{M,elec}(PH12)| > |\Delta H_{M,elec}(CH12)|$. That is, the terms $\Delta H_{M,repul}$ and $\Delta H_{M,elec}$ would make $\Delta H_M$ of PH12 and CH12 less exothermic than that of DTAB. The enthalpy of micellization values show that $\Delta H_M(DTAB) \sim \Delta H_M(CH12)$ and $\Delta H_M(DTAB)$ is substantially less exothermic than $\Delta H_M(PH12)$. Beyer et al. found that $\Delta H_M$(cetyltrimethylammonium chloride, CTAC) = −0.55 kJ mol$^{-1}$ [28], which can be compared to $\Delta H_M$(cetyldimethylbenzylammonium chloride, CBAC) = −4.56 kJ mol$^{-1}$ [30], at 298 K. The approximately 4 kJ mol$^{-1}$ difference between these two values should be due to the presence of the benzyl ring in the head group. The discrepancy between $\Delta H_M(DTAB)$ and $\Delta H_M(PH12)$ is 4.4 kJ mol$^{-1}$ and it could be assigned to the influence of the presence of the aromatic ring on the different contributions to $\Delta H_M$. No data corresponding to surfactants with cyclohexyl rings in the head group were found in the literature. However, comparison between $\Delta H_M(DTAB)$ and $\Delta H_M(CH12)$ seems to indicate that the substitution of a $-CH_3$ by a $-CH_2-C_6H_{12}$ in the head group does not substantially influence the enthalpy of micellization. This could be the result of various enthalpic contributions varying in opposite ways.

The enthalpies of micellization obtained in this work for the dimeric surfactants are similar to those estimated by Bai et al. for 12-*s*-12,2Br$^-$ surfactants with $8 < s < 12$ [19]. In respect to the influence of the ring nature on the enthalpy of micellization, $\Delta H_M(12PH12)$ is substantially more exothermic than $\Delta H_M(12CH12)$. As in the case of the single-chain surfactants, the substitution of the cyclohexyl ring by a phenyl ring results in the enthalpy of micellization being more exothermic.

### 3.2. Foamability

A pure liquid does not produce foam, but the addition of surfactants decreases the surface tension and allows foam production. Foam (*bubbly liquid*) is formed when a non-equilibrium dispersion of gas bubbles in a relatively small volume of liquid containing surfactants exits [14]. The surfactant molecules adsorb at the gas/liquid interface and are responsible for how easily foam is formed. The foamability is a measure of the foam generating power of a surfactant solution. It is interesting to point out that the amount of foam formed depends on the machinery (or method) responsible for its production [14]. Table 3 summarizes the information about the foamability of the surfactants investigated. In order to check the method, the foamability of DTAB was also determined, its value being 1.8 cm, in good agreement with literature data [31].

Foamability has been found to depend on the total surfactant concentration (maximum foamability for [surfactant] > cmc), the cmc, and the adsorption rate of the surfactant molecules at the air/liquid interface [32]. Para et al. [33] recently investigated the mechanism of adsorption at the air/solution interface of 12-*s*-12,2Br$^-$ dimeric surfactants, together with that corresponding to DTAB and didodecyltrimethylammonium bromide, DDAB. They found that the surface activity of the dimeric surfactants was much

**Table 3**
Surface excess concentration at the air/water interface, $\Gamma_{exc}$, and foam height for the surfactants investigated.

| Surfactant | $10^6 \times \Gamma_{exc}$[a] (mol m$^{-2}$) | Foam height[b] (cm) |
|---|---|---|
| PH12 | 3.2 ± 0.2 | 2.1 ± 0.3 |
| CH12 | 2.2 ± 0.1 | 1.9 ± 0.2 |
| 12PH12 | 1.84 ± 0.09 | 2.0 ± 0.3 |
| 12CH12 | 1.36 ± 0.08 | 1.8 ± 0.2 |

The experimental values are expressed as the mean ±SD ($n$ = 3).
[a] Data taken from Ref. [11] at 303 K.
[b] [Surfactant] = 3 × cmc.

**Table 4**
Concentration of solubilized tetramethylsilane, TMS, and 1,3,5-trimethylbenzene, TMB, in 0.04 mol dm$^{-3}$ $D_2O$ solutions.

| Surfactant | $10^3 \times$ [TMS] (mol dm$^{-3}$) | $10^3 \times$ [TMB] (mol dm$^{-3}$) |
|---|---|---|
| PH12 | 1.8 ± 0.1 | 28 ± 1 |
| CH12 | 1.7 ± 0.1 | 25 ± 1 |
| 12PH12 | –[a] | 65 ± 3 |
| 12CH12 | 5.5 ± 0.3 | 65 ± 3 |

The experimental values are expressed as the mean ±SD ($n$ = 3).
[a] Experiments precluded due to solubility problems.

lower than that of DDAB, particularly for $s \leq 6$, and not much different from that of DTAB. Pinazo et al. [34] investigated the foamability of a single-chain monomeric surfactant, $N^{\alpha}$-lauroyl arginine methyl ester, LAM, and three related dimeric surfactants, $C_n(LA)_2$, with $n$ = 3,6, and 9-methylene groups in the spacer. The authors found that the key parameter determining the surfactants foamability is the cmc. The lower the cmc the better the foamability. It is interesting to point out that the cmc of LAM is more than three orders of magnitude higher than those of $C_n(LA)_2$. Tan et al. [35] determined the foamability of polypropylene glycol surfactants, PPGs. They found that although high molecular weight PPGs have higher surface activity, foamability decreases with increasing PPG molecular weight because of the slower diffusion to, or at the air–solution interface. Other authors investigating the relationship foamability–surfactant structure did not find a general trend because of the several factors operating on foamability [14,31,33–41]. With regard to the surfactants investigated in this work, an increase in the number of hydrophobic chains would affect foamability in two opposite ways. On the one hand, the decrease in the cmc favors the generation of foam. On the other hand, the surface excess concentration, $\Gamma_{exc}$, is higher for the single-chain surfactants than for the dimeric surfactants. As a consequence, dimeric surfactants formed a less packed monolayer at the air/solution interface (higher surface tension at the cmc [11]), which would make the foam generation more difficult. The similar foam generating power of the surfactants investigated could be the result of these two factors operating in opposite directions, although others factors not considered could also be operative.

### 3.3. Solubilization capacity

The industrial importance of solubilization of hydrophobic species by surfactants was mentioned in the introduction section. Therefore, it is interesting to obtain information about the solubilization properties of the new surfactants prepared. However, given that the solubilization properties are solubilizate-dependent, three different solubilizates (see Scheme 2) were investigated: (i) tetramethylsilane, TMS, is a lipophilic compound with no dipole moment; (ii) 1,3,5-trimethylbenzene o mesytilene, TMB, is an aromatic compound; (iii) Dispersed Red 19 is a polar dye. The solubilization of TMS and TMB was investigated in the presence of a surfactant concentration equal to 0.04 mol dm$^{-3}$, well above their cmc', by using $^1$H NMR measurements. The results are summarized

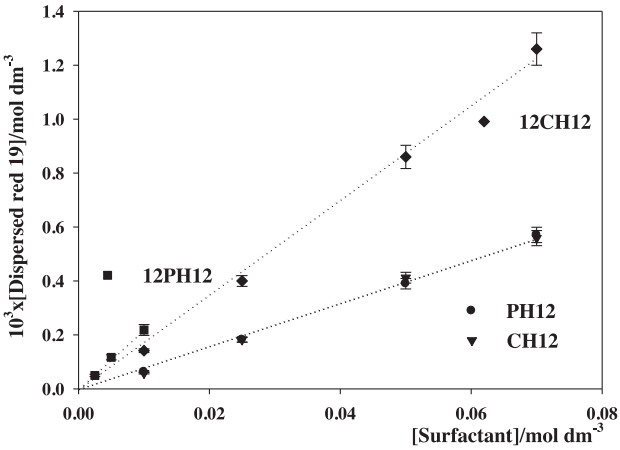


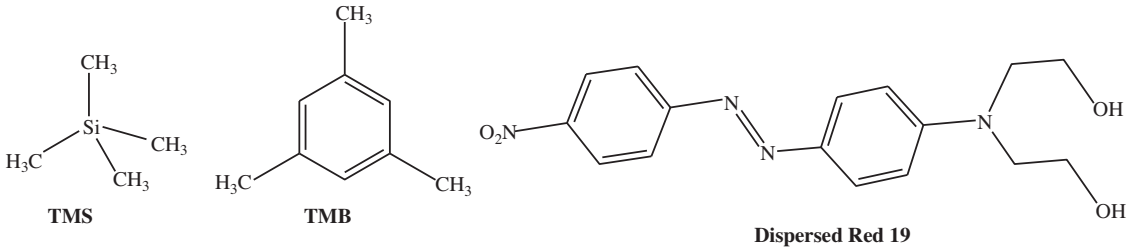

**Fig. 2.** Dispersed red solubilization in water as a function of surfactant concentration (lines are presented as visual guides). The experimental values are expressed as the mean ±SD ($n$ = 3).

in Table 4. Fig. 2 shows the concentration of solubilized Dispersed Red, determined by UV–visible spectroscopy, as a function of surfactant concentration for the four surfactants investigated. [12PH12] > 0.01 M could not be studied because of solubility problems. The solubilization capacity of DTAB was also investigated. The results were $1 \times 10^{-3}$ mol dm$^{-3}$ and $12 \times 10^{-3}$ mol dm$^{-3}$ for TMS and TMB, respectively. The surfactants studied show a clear preference for solubilizing the aromatic TMB over TMS, which could be due to ion-dipole interactions [42]. With regard to Dispersed Red, the solubilization capacities of the single chain surfactants are between those of TTAB and DTAB. Data in Table 4 and Fig. 2 show that the dimeric surfactants have a higher solubilization capacity than their single-chain counterparts. The increase in the solubilization capacity of surfactants by increasing the number of hydrophobic chains was previously found by other authors [31,41–47] and it was explained by considering the tendency of the dimeric surfactants to form elongated aggregates [41–47]. No influence of the ring nature in the solubilization capacity of either the single-chain or the dimeric surfactants was observed.

### 3.4. Antimicrobial activity

Cationic surfactants can easily disturb bacterial membranes and, in general, have antimicrobial activity [48]. Bacterial cell surface is usually negatively charged and the adsorption of cationic amphiphiles onto the negatively charged cell surface is facilitated by electrostatic interactions, along with hydrophobic interactions since the alkyl surfactant tails can penetrate into the hydrophobic part of the membrane [49]. The antimicrobial activity of the surfactants investigated against Gram-positive and Gram-negative bacteria was expressed as the minimum inhibitory concentration, MIC, which is defined as the lowest concentration of the surfactant needed to inhibit visible growth after 24 h of incubation at 310 K [21]. MIC values were determined by the broth dilution method and are listed in Table 5. This table also includes the MIC values corresponding to DTAB, a classical antimicrobial surfactant.

Table 5 shows that the four surfactants studied show antimicrobial activity for all the bacteria investigated. As expected, the MIC values are higher for Gram-negative than for Gram-positive bacteria. Gram-positive bacteria have a rather simple cell wall composed of a peptidoglycan layer, which permits the penetration of amphiphiles without difficulty [17]. The external layer of the outer membrane of Gram-negative bacteria is almost completely composed of lipopolysaccharides and proteins, which make difficult the entrance of biocides [16]. In most cases, the dimeric surfactants




**Scheme 2.** Molecular structure of the solubilizates studied.

present a stronger antimicrobial activity than their single-chain counterparts. A similar result was previously found by other authors [16,50–53] and it was explained by considering that the presence of two positively charged head groups and two hydrophobic chains strengthens the electrostatic as well as the hydrophobic interactions with the surface cells. It was found that the antimicrobial activity of alkanedyil-bis(dimethylalkylammonium bromide) dimeric surfactants [47], *m–s–m*, strongly depends on m and s, the surfactant 12–2–12 presenting the optimal biocidal activity. Pérez et al. [16] found that the MIC values against *E. coli* of various dimeric surfactants derived from lysine are within the range 42–176 μM. Table 5 shows that 12PH12 and 12CH12 MIC values are low when compared to literature values corresponding to dimeric surfactants. Data in Table 5 also show that, in most cases, the antimicrobial activity of PH12 and CH12 is stronger than that of DTAB. That is, substitution of a methyl group by either $-CH_2C_6H_6$ or $-CH_2C_6H_{12}$ in the head group increases the biocidal character of the surfactant. The MIC values summarized in Table 5 indicate that the ring nature does not practically influence the antimicrobial activity of the dimeric surfactants, although the substitution of the phenyl ring by the cyclohexyl ring in the single-chain surfactants results in a MIC diminution for Gram-positive bacteria.

### 3.5. Biodegradability

The biodegradability of a synthetic chemical is probably one of the most important factors for predicting its environmental behavior [54,55]. Indeed, the biodegradability of surfactants has become of importance because the possibility of their rapid biodegradation under environmentally relevant conditions will allow their widespread use, whereas the absence of biodegradation will result in restriction or even complete banning. The biodegradability of the surfactants was investigated by using the ready biodegradability test (method 301A) proposed by the Organization for Economic Cooperation and Development (OECD), who has traditionally assumed the responsibility for developing normalized tests to assess biodegradability. A sample from a WTP was used because it represents the first point of discharge of many anthropogenic organic chemicals, before their introduction into natural

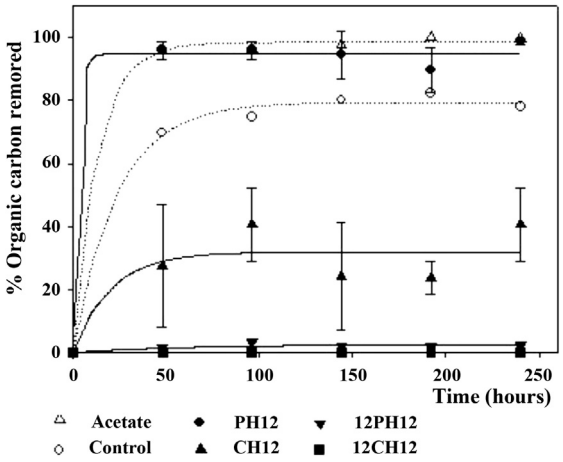



**Fig. 3.** Biodegradation of the surfactants investigated. The experimental values are expressed as the mean ±SD ($n = 2$).

environments. The test gave positive results with the surfactants PH12 and CH12, whereas 12PH12 and 12CH12 were not biodegraded (see Table 6 and Fig. 3). Biodegradation occurred without a lag phase with the single chain surfactants, but PH12 disappeared fastest and to a greater extent than CH12, with half lives of 1.5 h and 4.8 h, respectively. These values are substantially smaller than the half life of TOC in the control without amendment (16.5 h) and with acetate (9.2 h). The extensive biodegradation (as evidenced by $P_{max}$) of PH12 agrees with the general consideration of cationic surfactants as a group of easily biodegradable surfactants, with a significant mineralization reported for some of them [56]. The lower $P_{max}$ observed with CH12 suggests, however, that the cyclohexyl ring posseses some restrictions to microorganisms, as compared with the phenyl ring, that may cause an incomplete biodegradation. The exact cause remains uncertain, and its clarification requires a detailed study of possible metabolites formed. However, these differences in biodegradability agree with the higher recalcitrance of the cyclohexane structure, what has already been observed in a study focused on the biodegradability of

**Table 5**
Minimum inhibitory concentration, MIC, for the surfactants investigated and DTAB, expressed as μg/mL. Values in parentheses indicate MIC' in μM. Experiments were performed in duplicate and the mean MIC value was reported. They were reproducible within a precision better than 8%.

| MIC | | | | | |
|---|---|---|---|---|---|
| Microorganism | PH12 | CH12 | 12PH12 | 12CH12 | DTAB |
| Gram-positive | | | | | |
| *Micrococcus luteus* CECT 245 | 0.19 (0.49) | 0.095(0.24) | 0.76 (1.10) | 0.76 (1.07) | 0.095 (0.29) |
| *Staphylococcus epidermidis* CECT 231 | 1.52 (3.92) | 1.52 (3.84) | 1.52 (2.20) | 1.52 (2.14) | 6.08 (19.39) |
| *Enterococcus faecalis* CECT 481 | 12.16 (31.36) | 6.08 (15.36) | 3.04 (4.40) | 0.76 (1.07) | 24.32 (77.57) |
| *Mycobacterium phlei* CECT 3009 | 12.16 (31.36) | 3.04 (7.68) | 1.52 (2.20) | 1.52 (2.14) | 24.32 (77.57) |
| Gram-negative | | | | | |
| *Escherichia coli* CECT 101 | 12.16 (31.36) | 12.16 (30.72) | 3.04 (4.40) | 1.52 (2.14) | 97.28 (310.3) |
| *Klebsiella pneumoniae* CECT 143 | 12.16 (31.36) | 12.16 (30.72) | 6.08 (6.60) | 6.08 (8.56) | 3.04 (9.70) |
| *Pseudomonas fluorescens* CECT 378 | 0.76 (1.96) | 0.76 (1.92) | 0.76 (1.10) | 076 (1.07) | 3.04 (9.70) |

**Table 6**
Maximum percentages of degradation, $P_{max}$, maximum rate, and half lives, $t_{1/2}$, for the biodegradation process of the surfactants investigated. The experimental values are expressed as the mean ±SD ($n = 2$).

| | Control | Acetate | Surfactant | |
|---|---|---|---|---|
| | | | PH12 | CH12 |
| $P_{max}$(%) | 79.4 | 98.4 | 91 ± 8 | 36 ± 20 |
| Maximum rate (mg $L^{-1}$ $h^{-1}$) | 0.60 | 8.20 | 2.7 ± 0.2 | 0.6 ± 0.9 |
| $t_{1/2}$ (h) | 16.5 | 9.20 | 1.50 ± 0.02 | 5 ± 4 |

gasoline components [57]. Indeed, that study determined substantially higher biodegradation half lives for this cyclic alkane than for monoaromatic hydrocarbons, such as benzene and toluene. These results agree with those found by Tehrani-Bhaga et al. [40] with cationic ester-containing surfactants and it can be rationalized by considering that, generally, the presence of the phenyl ring, either in the head group of the single-chain surfactants or in the spacer of the dimeric surfactants, increases the biodegradability of these compounds. Besides, this could also be attributed to their higher MIC values.

The levels of TOC remained constant during the whole experimental period (672 h) in the presence of 12PH12 and 12CH12, which not only indicates that the dimeric surfactants persisted in the medium, but also that they inhibited the biodegradation of the organic matter already present in the wastewater treatment plant sample. These results may therefore reflect the higher toxicity of the dimeric surfactants against microorganisms, as compared with the single-chain forms, already shown in Table 5.

### 3.6. Concluding remarks

This work combines the study of colloidal and biological properties of cationic single-chain and dimeric quaternary ammonium surfactants with phenyl and cyclohexyl rings, either in the head group or in the spacer, after a complete physicochemical characterization. It provides information about structure-property relationships. The thermodynamic functions of micellization show that the self-aggregation processes of the surfactants are entropy driven. Micellization is more exothermic for the dimeric surfactants than for the single-chain surfactants mainly due to the hydrophobic contribution to $\Delta H_M$, associated with the transfer of the alkyl chains from the aqueous phase into the micelles. Besides, the presence of the phenyl ring results in a more exothermic $\Delta H_M$. The surfactants investigated present similar capacity to generate foam; that is, no relationship between surfactant structure and foamability was found. This was not the case for the solubilization capacity of the surfactants, which is substantially higher for the dimeric than for the single-chain surfactants, although no influence of the ring nature was found. Surfactant structure plays a key role in their biological properties. All the surfactants show a substantial antimicrobial activity against both Gram-positive and Gram-negative bacteria. An increase in the number of hydrophobic chains results in an increment in the biocidal character of the surfactants. The ring nature does not practically influence the antimicrobial activity of the dimeric surfactants, but the substitution of the phenyl ring by a cyclohexyl ring results in a diminution of the MIC values corresponding to Gram-positive bacteria for the single-chain surfactants. The evaluation of the biodegradability of the surfactants shows that PH12 was readily biodegradable ($P_{max}$ = 91%) and CH12 reaches a biodegradation level of 36%. However, the two dimeric surfactants are non biodegradable. That is, the increase in the number of hydrophobic chains provokes a strong diminution of the biodegradability of the surfactants. Besides, the substitution of the cyclohexyl ring by the phenyl ring in the head group of single-chain surfactants makes the surfactants more easily biodegradable. These results could be attributed, on the one side, to the higher recalcitrance of the cyclohexyl ring, and, on the other side, to the inhibitory effects on the bacterial degraders due to the higher antimicrobial activity.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.colsurfb.2013.10.017.

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