Cationic gemini surfactants containing an O-substituted spacer and hydroxyethyl moiety in the polar heads: self-assembly, biodegradability and aquatic toxicity

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ABSTRACT

Micellization and some ecological properties of new cationic gemini surfactants with oxygen-substituted spacer and hydroxyethyl groups connected to the polar heads have been studied. The incorporation of a hydroxyethyl group in the polar head favors self-aggregation whereas the presence of the oxygen in the spacer increases critical micelle concentration. Surfactants investigated are not biodegradable due to their toxic effect on microorganisms responsible for biodegradation. Aquatic toxicity increases with the hydrophobic chain and decreases by increasing the hydrophilicity of spacer and polar heads. New gemini surfactants are less toxic to the aquatic environment than monomeric surfactants and non-oxygen-containing gemini surfactants.

Keywords

Gemini surfactants, O-containing spacer, self-aggregation, biodegradation, aquatic toxicity.

1. Introduction

Gemini surfactants, dimeric quaternary ammonium salts, represent a new class of cationic surfactants. These compounds consist of two monomeric moieties, a hydrophilic head group (positively charged nitrogen atom) and a hydrophobic part (long alkyl chain) [1,2], connected by a flexible or rigid spacer [3]. Gemini surfactants exhibit superior surface, interfacial and biological properties than their conventional monomeric analogues [4,5]. Critical micelle concentration (cmc) of gemini surfactants is much lower compared to the cmc values of monomeric surfactants [6]. Lower critical micelle concentration means that the same effect can be reached by using lower amount of the gemini surfactant [7]. Elongating the alkyl chain decreases gemini surfactants cmc [8–10] while adding heteroatoms or multiple bonds into the spacer increases the cmc value [3,9,11]. On the other hand, an increase in the spacer length can either lead to an increase or decrease in the cmc depending on its length, flexibility and the nature of groups it incorporates [12–16].

Because of their unique properties, gemini surfactants are used in different areas such as household, medicine, pharmacy or petrochemistry [17–19] as biocides [20], corrosion inhibitors [21,22], detergents [7], emulsifiers [23], gene delivery agents [24], in micellar catalysis [25,26] and much more. The use of gemini surfactants is increasing and consequently their potential risk to the aquatic environment also increases. Compounds which are biodegradable can be easily mineralized by microorganisms present in the environment, unfortunately, gemini surfactants are hardly biodegradable [4]. In addition, although they are less toxic than their monomeric analogues, dimeric surfactants are harmful to water organisms [27]. However, by the modification of their chemical structure as adding ester bonds or hydrophilic substitutes [22,27,28], it is possible to favor their biodegradability and lower their

aquatic toxicity. As the potential application of gemini surfactants is developing, examinations of their ecological properties need more attention.

In the present work, the self-aggregation, biodegradability and aquatic toxicity of two cationic gemini surfactants with C12 or C18 alkyl chains, an oxygen in the spacer and a hydroxyethyl group connected to the positively charged nitrogen atoms are investigated.

2. Materials and Methods

2.1 Materials

The gemini surfactants, 3-oxa-1,5-tetramethylene-bis(*N*-dodecyl-*N*-hydroxyethyl-*N*-methylammonium) dichloride (12-MOH-O-MOH-12) and 3-oxa-1,5-tetramethylene-bis(*N*-octadecyl-*N*-hydroxyethyl-*N*-methylammonium) dichloride (18-MOH-O-MOH-18), were obtained according to Scheme 1 by reaction of 1 equivalent of bis(2-chloroethyl)ether (Fluka, purity $\geq 99\%$) with 3.3 equivalents of the corresponding *N*-alkyl-*N*-hydroxyethyl-*N*-methylamine in *n*-propanol (POCH, 99.5%) under reflux for 30 h. The crude products were washed with diethyl ether (POCH, 99.5%). 12-MOH-O-MOH-12 and 18-MOH-O-MOH-18 were obtained with a 31 and 42 % yield, respectively. *N*-alkyl-*N*-hydroxyethyl-*N*-methylamines were synthesized by reaction of 1 equivalent of *N*-methyl ethanolamine (Merck, $\geq 98\%$) with 1 equivalent of 1-bromododecane (Aldrich, 97%) or 1-bromooctadecane (Merck, $\geq 97\%$) in acetonitrile (VWR, HPLC grade 99.7%) under reflux for 20 h in presence of sodium carbonate (Eurochem, 99.6%)[29].

The chemical structure of the synthesized cationic gemini surfactants was characterized by ^1H NMR (Varian 400 Hz).

12-MOH-O-MOH-12 (CDCl_3): 0.88 ppm (t, 6H, $-\text{CH}_2\text{CH}_3$), 1.26-1.35 ppm (m, 36H, $-\text{CH}_2(\text{CH}_2)_9\text{CH}_3$), 1.72 ppm (m, 4H, $-\text{NCH}_2\text{CH}_2-$), 3.38 ppm (s, 6H, $-\text{N}(\text{CH}_3)$), 3.59 ppm (t, 4H,

-NCH₂CH₂-), 3.78 ppm (t, 4H, -NCH₂CH₂O-), 3.96 ppm (t, 4H, -NCH₂CH₂OH), 4.07 ppm (t, 4H, -NCH₂CH₂O-), 4.23 ppm (t, 4H, -NCH₂CH₂OH), 5.61 ppm (s, 2H, -CH₂OH).

18-MOH-O-MOH-18 (CDCl₃): 0.88 ppm (t, 6H, -CH₂CH₃), 1.26-1.37 ppm (m, 60H, -CH₂(CH₂)₁₅CH₃), 1.72 ppm (m, 4H, -NCH₂CH₂-), 3.38 ppm (s, 6H, -N(CH₃), 3.61 ppm (t, 4H, -NCH₂CH₂-), 3.78 ppm (t, 4H, -NCH₂CH₂O-), 3.97 ppm (t, 4H, -NCH₂CH₂OH), 4.07 ppm (t, 4H, -NCH₂CH₂O-), 4.23 ppm (t, 4H, -NCH₂CH₂OH), 5.59 ppm (s, 2H, -CH₂OH).

Scheme 1. Synthesis route and structure of the gemini surfactants.

2.2. Methods

2.2.1. Conductometry

Conductivity was measured using an epoxy/graphite Orion Conductivity Cell 013005MD with a cell constant of 0.475 cm⁻¹ in conjunction with a Thermo Scientific Orion VersaStar multiparameter instrument. All solutions were prepared in Milli-Q® ultrapure water (Type I) with a specific conductivity of 2-3 μS.cm⁻¹. The measurements were performed at 20 and 35 °C in a thermostated glass vessel. The method to obtain the cmc values of the gemini surfactants was based on differentiation of conductivity data against surfactant concentration

and fitting the first derivative plot to Boltzmann type sigmoid by using Origin® v.8 graphing and analysis software.

2.2.2. *Surface activity*

Surface tension measurements were made at 20 °C by the Wilhelmy method using a Krüss K12 tensiometer with a platinum plate. Glass containers and the platinum plate were cleaned with chromic acid mixture, thoroughly rinsed with water and dried to the flame prior to the measurements. The value of surface tension at the equilibrium was taken when the standard deviation did not exceed 0.10 mN/m for five consecutive measurements.

2.2.3. *Fluorimetry*

Steady-state fluorescence measurements were performed using a Shimadzu RF 540 spectrofluorometer equipped with a thermostated cell holder at 35 °C. Both excitation and emission band slits were fixed at 2 nm. All data were acquired using quartz cells with 1 cm path length. Pyrene was used as the fluorescent probe. The two gemini surfactants tested were prepared at different concentrations in aqueous solution containing 1×10^{-6} M of pyrene. The fluorescence emission spectra of pyrene dissolved in aqueous solutions of gemini surfactants were recorded from 340 to 450 nm after excitation at 332 nm. Pyrene exhibits fine structure in 370-400 nm region of the steady-state fluorescence emission spectra. The nature and intensity of peaks are dependent on the polarity of the environment. The intensity ratio of the first ($\lambda = 374$ nm) to the third ($\lambda = 384$ nm) vibronic peaks, i.e., I_1/I_3 , shows the greatest solvent dependency, and hence, can be used to obtain the cmc of the gemini surfactants in water [30]. In this work, the cmc was determined as the central point of the transition by fitting the ratio I_1/I_3 against surfactant concentration to a Boltzmann type sigmoid (Origin® software).

A fluorescence quenching experiment was carried out for 12-MOH-O-MOH-12 to determine the aggregation number of the micelles. The measurements were made with the same spectrofluorometer and conditions above described. Pyrene was used as fluorescent probe and

cetyl pyridinium chloride as quencher. To avoid the formation of pyrene excimers, the pyrene concentration was maintained at low level (1×10^{-6} M) and concentration of the probe and quencher was adjusted to maintain Poisson distribution [31].

2.2.4. *Dynamic light scattering (DLS)*

Dynamic light scattering (DLS) measurements were conducted at 35 °C using a DLS apparatus (Zetasizer, Nano-ZS Malvern Instruments) with a He-Ne laser (633 nm, 4 mW) equipped with a built-in temperature controller. Thirty repeated measurements were performed for each sample. The data evaluation of DLS measurements was performed with the CONTIN algorithm and data are reported with an uncertainty of less than 7%.

2.2.5. *CO₂ headspace biodegradation test*

The CO₂ headspace test [32,33] was applied to evaluate the ready biodegradability of 12-MOH-O-MOH-12 and 18-MOH-O-MOH-18 under aerobic conditions. This method allows the evaluation of the ultimate aerobic biodegradation (mineralization to carbon dioxide) of an organic compound in aqueous medium by measuring the net increase in total inorganic carbon over time with respect to unamended blanks. Gemini surfactants were tested at a concentration of 12 mg C/L. Samples were inoculated with activated sludge collected from a municipal wastewater treatment plant and incubated in the dark at 20 ± 1 °C in sealed vessels (120 mL) with an air headspace/liquid volume ratio of 1:2. Sodium n-dodecyl sulfate (SDS) was used as reference substance and tested at 20 mg C/L. Two replicates of surfactants, blank and reference substance were performed for each sampling day except for the last day, when four replicates were performed. Inhibition tests (SDS biodegradation inhibition) were also carried out for 12-MOH-O-MOH-12 and 18-MOH-O-MOH-18 (SDS at 20 mg C/L+ tested surfactant at 12 mg C/L in each bottle). The tests run for 28 days. Every sampling day, after injecting a sodium hydroxide solution to the vessels, shaking and allowing settling, suitable volumes were withdrawn by syringe from the liquid phase of each vessel and kept in small beakers carefully

filled to the brim and covered with a cap to prevent CO₂ exchange with the air. The concentration of inorganic carbon was determined in a carbon analyzer (Shimadzu TOC-5050). The extent of biodegradation was expressed as a percentage of the theoretical amount of inorganic carbon based on the initial amount of the tested surfactant. A surfactant is considered as ready biodegradable when the extent of ultimate biodegradation is greater than 60% at the end of the test period.

2.2.6. Daphnia magna immobilization test

Daphnia magna, laboratory bred, not more than 24 h old, were used in this aquatic toxicity test [34], where the swimming capability inhibition is the end point. The pH of the medium was 8.0 and the total hardness was 250 mg/L (as CaCO₃), with a Ca/Mg ratio of 4/1. For the gemini surfactants, ten concentrations in a geometric series were tested in a concentration range first established in a preliminary test, 0.4-4 mg/L and 0.05-0.5 mg/L for 12-MOH-O-MOH-12 and 18-MOH-O-MOH-18, respectively. Twenty daphnia, divided into four batches of five animals each, were used at each test concentration. Tests were performed in the dark at 20 °C. The immobility percentages at 48 h were plotted against concentration on logarithmic-probability paper and a linear relationship was obtained. The Probit method was employed as statistical procedure to determine the IC₅₀ (the estimated concentration to immobilize 50% of the daphnia after 48h exposure) and the corresponding 95% confidence interval (CI).

3. Results and Discussion

3.1. Conductometry

The variation of the specific conductivity (κ) in aqueous solutions of the synthesized surfactants as a function of the concentration was determined at 20 and 35 °C. The graphs obtained at 35 °C are shown in Fig. 1.

Fig. 1. Specific conductivity (κ) and differential conductivity ($d\kappa/dc$) versus surfactant concentration in water at 20 °C (a) and 35 °C (b) for 12-MOH-O-MOH-12 and 18-MOH-O-MOH-18.

The conductivity (κ) values fit into two straight lines of different slope in pre- and post-micellization regions. The abrupt change in the slope is because of the binding of some counterions to the micelle and to the lower mobility of micelles compared to monomers. The

break point of the slopes corresponds to the critical micelle concentration (cmc) value. Sometimes the intersection point is not obvious and to avoid errors in the estimation of cmc another method, the analysis of the plot of differential conductivity ($d\kappa/dC$) versus surfactant concentration was applied. Data were fitted to a Boltzmann-type sigmoid [35,36].

The slope ratios α of linear regressions in pre- and post- micellization regions gives the counterion binding parameter, β , as $\beta = 1-\alpha$. The standard Gibbs energy of micellization (ΔG°_{mic}) for gemini ionic surfactants was calculated by equation (1) [37]:

$$\Delta G^\circ_{mic} = 2RT(1/2 + \beta)\ln cmc - RT\ln 2 \quad (1)$$

Where R is the gas constant, T is the temperature (K) and the cmc is expressed in mol/L. The values of cmc, β and ΔG°_{mic} are presented in Table 1.

Table 1

Aggregation parameters of tested gemini surfactants at 20 and 35 °C: Critical micelle concentration (cmc), counter binding parameter (β) and Gibbs free energy of micellization (ΔG°_{mic}) determined by conductometry.

Surfactant	T	cmc	β	ΔG°_{mic}
	(°C)	(mM)		(kJ/mol)
12-MOH-O-MOH-12	20	1.46	0.49	-31.5
	35	1.59	0.46	-31.8
18-MOH-O-MOH-18	20	0.030	0.53	-52.1
	35	0.031	0.47	-51.9

The cmc values of the gemini surfactants investigated are lower than the values of corresponding monomeric ones. Thus, the cmc value of 12-MOH-O-MOH-12 (Table 1) is one order of magnitude lower than the values of 16 mM and 21 mM reported for *N*-dodecyl-*N*-hydroxyethyl-*N,N*-dimethylammonium bromide [38] and for *N*-dodecyl-*N,N,N*-trimethylammonium chloride [39], respectively.

Elongating the hydrocarbon chains from C12 to C18 results in lowering the gemini surfactant cmc value by two orders of magnitude (Table 1). This is consistent with works previously published about the effect of the chain length on the cmc [8,9,40–42]. Therefore, lengthening the hydrophobic alkyl chain promotes self-assembly. Surfactants with long alkyl chains exhibit greater tendency to form micelles due to their more hydrophobic character.

Increasing the temperature from 20 to 35 °C leads to slight increase in the cmc value for both gemini homologues. For gemini ionic surfactant it has been described that when increasing temperature a reduction on critical micelle concentration is initially produced until a minimum is achieved and then the cmc increases with increasing temperature [41,43,44]. First, when temperature increases, the degree of hydration of the ionic head group domain decreases, leading to the increase of hydrophobicity of surfactants and favoring the aggregation process. On the other hand, with the increase of temperature, the structure of water around the hydrophobic domain would be partially broken disfavoring the self-assembly process.

The hydrophilic character of the surfactants also affects the cmc value. The replacement of two methylene groups by an oxygen atom in the gemini surfactant spacer increases its hydrophilicity what causes a better solubility in water [9]. The higher hydrophilicity of the spacer leads to an increase of surfactant cmc. It was already reported for other gemini surfactants [45,46] and can be observed when comparing the cmc values of 12-MOH-O-MOH-12 and 18-MOH-O-MOH-18 (Table 1) with the values previously obtained for 1-6-hexamethylene-bis(*N*-dodecyl-*N*-hydroxyethyl-*N*-metylammonium) dibromide (G6-MOH-12)

and 1-6-hexamethylene-bis(*N*-octadecyl-*N*-hydroxyethyl-*N*-metylammonium) dibromide (G6-MOH-18), 1.05 and 0.026 mM, respectively, at the same temperature (35 °C) [9]. On the other hand, the cmc value decreases by replacing a methyl by a hydroxyethyl group in the polar heads as observed by comparing the values for 12-MOH-O-MOH-12 and 18-MOH-O-MOH-18 (Table 1) with those reported at 35 °C for 3-oxa-1,5-tetramethylene-bis(*N*-dodecyl-*N,N*-dimethylammonium) dichloride (12-O-12) and 3-oxa-1,5-tetramethylene-bis(*N*-octadecyl-*N,N*-dimethylammonium) dichloride (18-O-18), 1.72 mM and 0.044 mM, respectively [9].

With regard to the degree of counterion binding β , the values slightly increase on going from C12 to C18 homologue (Table 1). It means that the counterion is more tightly bound to the aggregates as the alkyl chain becomes longer. These results agree with those reported for similar cationic gemini surfactants [9] and indicate the formation of more compact micelles in the case of C18 homologue as a consequence of increased shielding of the electrostatic interactions between the ionic head groups.

The values of the standard free energy of micellization ($\Delta G^\circ_{\text{mic}}$) for the tested gemini surfactants are negative (Table 1) indicating the process of forming micelles is spontaneous. Increasing the hydrophobic character of the surfactants leads to more negative values of $\Delta G^\circ_{\text{mic}}$, which promotes the micellization process. Besides, the values of Gibbs energy for monomeric surfactants are less negative than for gemini indicating that the micelle formation by dimeric cationic surfactants is more favorable [47].

3.2. Surface activity

Critical micelle concentration (cmc), surface tension at cmc (γ_{cmc}), surface pressure at cmc (π_{cmc}), maximum surface excess concentration (Γ_{max}), minimum surface area per molecule adsorbed at the air-water interface (A_{min}), surfactant efficiency (pC_{20}) and Gibbs free energy of adsorption (ΔG^0_{ad}) for tested gemini surfactants are given in Table 2. The values were obtained

from the graphs of surface tension vs. logarithm of surfactant concentration (Fig. 2). Because of the minimum in the surface tension plot for 12-MOH-O-MOH-12, only an estimation of the parameter values could be made.

Table 2

Surface tension parameters for tested gemini surfactants (T = 20 °C).

Surfactant	cmc	γ_{cmc}	π_{cmc}	Γ_{max}	A_{min}	pC_{20}	ΔG_{ad}^0
	(mM)	(mN/m)	(mN/m)	(mol/cm ²)	(Å ²)		(KJ/mol)
12-MOH-O-MOH-12	1.80*	36.8*	35.2*	$1.22 \cdot 10^{-10} *$	137*	4.26*	-60.4
18-MOH-O-MOH-18	0.030	39.0	33.0	$1.47 \cdot 10^{-10}$	113	5.02	-74.5

(*) Rough values estimated.

Fig. 2. Surface tension vs. logarithm of surfactant concentration at 20 °C.

Surface tension decreases with increasing the concentration of tested gemini surfactants before reaching a nearly constant value, which indicates that the water-air interface is saturated by the cationic surfactant molecules and micelles start forming. However, the surface tension minimum appearing for 12-MOH-O-MOH-12 makes difficult to obtain reliable values for the

surface activity parameters. In these cases, the real cmc value is usually located after the minimum and close to the concentration where the surface tension attains the plateau [48] and it has been the criterion adopted. The cmc value (Table 2) decreases with increasing the alkyl chain from 12 to 18 carbon atoms because of the hydrophobicity increase. These cmc values are in agreement with those obtained by conductometry. 12-MOH-O-MOH-12 and 18-MOH-O-MOH-18 decrease the surface tension of water from 72 mN/m to 36.8 and 39.0 mN/, respectively.

Surface pressure at cmc was calculated by equation (2):

$$\pi_{cmc} = \gamma - \gamma_{cmc} \quad (2)$$

Where γ is the surface tension of pure water and γ_{cmc} is the surface tension at cmc. The higher the π_{cmc} value, the more effective the surfactant is. Therefore, C12 homologue is more effective decreasing the surface tension than the C18 homologue. Another parameter providing information about the surfactant adsorption at the water-air interface is the surfactant efficiency (pC_{20}) defined as $-\log C_{20}$, where C_{20} is the concentration of surfactant that reduces the surface tension of pure solvent by 20 mN/m. A higher pC_{20} value reflects more efficiency in reducing surface tension. Therefore, C18 homologue is more efficient than C12 homologue (Table 2). On the other hand, comparing 12-MOH-O-MOH-12 (Table 2) with a monomeric analogue, *N*-dodecyl-*N,N,N*-trimethylammonium bromide, $pC_{20} = 2,23$ [49], the pC_{20} value of gemini surfactant is two times higher, confirming the dimeric quaternary ammonium salts are more active surface agents than monomeric compounds.

The Gibbs adsorption equation was applied to estimate the maximum surface excess concentration (Γ_{max}). It should be noted that few years ago some authors questioned the validity of this equation [50–52]. Whereas according to Gibbs the saturation is already attained at the

beginning of the abrupt linear decrease of surface tension as the concentration increases, other authors consider that the decline of surface tension is caused by the continuously increasing of the adsorption at the interface. Despite this controversy, the Gibbs equation continues being widely applied and allows comparison with published data. Thus, according to Gibbs adsorption equation (3), $\Gamma_{\max}$ value is directly related to the slope ($\Delta\gamma/\Delta\log C$) of the straight line in the plot of surface tension vs. logarithm of surfactant concentration in the region below cmc:

$$\Gamma_{\max} = (-1/2.303nRT) (\Delta\gamma/\Delta\log C) \quad (3)$$

where R is the gas constant, T is the temperature (K) and n is the number of ionic species at the interface (for gemini surfactants $n = 3$) [53]. Whether γ is expressed in mN/m, $\Gamma_{\max}$ results to be in mmol/m². The minimum surface area per molecule ($A_{\min}$) expressed in Å² was calculated from equation (4):

$$A_{\min} = 10^{16}/N_A \cdot \Gamma_{\max} \quad (4)$$

Where N_A is the Avogadro number and $\Gamma_{\max}$ is expressed in mol/cm².

Different trends have been reported about the effect of the alkyl chain length on $A_{\min}$ in a homologue series. Whereas an $A_{\min}$ increase related to a bigger size of the molecules is reported by some authors [44,54] in other cases an $A_{\min}$ reduction is attributed to a more tight packing because of hydrophobic interactions between the larger alkyl chains [44]. However, in many cases a regular tendency is not produced [55,56]. In the present study, the $A_{\min}$ decreases with increasing the alkyl chain length. However, because only two surfactants have been investigated and $A_{\min}$ for 12-MOH-O-MOH-12 is a rough value, no precise conclusions can be drawn.

The standard free energy of adsorption (ΔG_{ads}^0), energy of transfer one mol of surfactant from the bulk solution to the air-liquid interface, was calculated according to equation (5):

$$\Delta G_{ads}^0 = \Delta G_{mic}^0 - \pi/\Gamma_{max} \quad (5)$$

The negative values of ΔG_{ads}^0 obtained for the two surfactants (Table 2) mean that the adsorption at the interface is a spontaneous process being slightly favored by the alkyl chain elongation. The much larger values of $|\Delta G_{ad}^0|$ respect to $|\Delta G_{mic}^0|$ (Table 1) reveal a predominant adsorption at the liquid-air interface over micellization in the bulk solution for the gemini surfactants investigated.

3.3. Fluorimetry

Micelle aggregation behavior of tested gemini surfactants was studied by steady-state fluorescence measurements using pyrene as the probe. The intensity ratio of the first to the third vibronic peak of pyrene (I_1/I_3) was measured as a function of the surfactant concentration. Graphs for 12-MOH-O-MOH-12 and 18-MOH-O-MOH-18 are presented in Fig. 3. The plots show a typical sigmoidal decrease around cmc. At concentrations below cmc, I_1/I_3 ratio values correspond to a locating of pyrene molecules in a polar environment. Increasing the concentration of the surfactant, the I_1/I_3 ratio decreases indicating the pyrene is located in a more hydrophobic environment, inside the micelles. After reaching the cmc, the ratio becomes constant due to preferential residence of the probe in the hydrophobic core of micelles. The cmc values were estimated from the midpoint of the transition [57].

Fig. 3. Variation of the I_1/I_3 ratio with the surfactant concentration in aqueous solution at 35 °C for tested gemini surfactants.

The cmc value derived from the fluorescence measurements for C12 gemini surfactant, 1.48 mM, is in good agreement with that obtained by conductometry (Table 1) at the same temperature (35 °C). The value obtained for C18 homologue, 0.064 mM, although is of the same order of magnitude, is higher than that obtained by conductometry. However, taking into account that critical micelle concentration is not one point but a concentration range [58], it can be said that results obtained by the three different techniques are in good agreement.

The static fluorescence quenching method was applied to determine the micelle aggregation number of the C12 gemini surfactant. To calculate the aggregation number (N_{agg}) equation (6) was used [59]:

$$\ln(I_0/I) = (N_{agg}C_Q)/(C_S - cmc) \quad (6)$$

Where I and I_0 are the fluorescence intensity at $\lambda = 374$ nm with and without quencher, respectively, and C_S and C_Q are the concentrations of the gemini surfactant and the quencher,

respectively. The method could not be applied to the C18 gemini surfactant because it forms vesicle-like aggregates instead of micelles above cmc (see Section 3.4. Dynamic light scattering (DLS)). From the slope of the linear plot between $\ln(I_0/I)$ and C_Q in micelle solutions of 12-MOH-O-MOH-12 (Fig. 4), the aggregation number was determined, $N_{agg} = 23$. The low aggregation number suggests the formation of spherical micelles. The result obtained is consistent with the values reported for some similar gemini surfactants [9,60]. When the N_{agg} of 12-MOH-O-MOH-12 is compared with the values obtained for more hydrophobic gemini surfactant analogues [9] a slight increase in the number of surfactant molecules forming the micelles with increasing the hydrophilicity of the surfactant is corroborated.

Fig. 4. Variation of $\ln(I_0/I)$ as a function of quencher concentration at 35 °C for 12-MOH-O-MOH-12.

3.4. Dynamic light scattering (DLS).

Estimation of aggregates size of gemini surfactants in aqueous solution was performed by dynamic light scattering (DLS). Fig. 5 shows the size distribution of the aggregates formed by 12-MOH-O-MOH-12 and 18-MOH-O-MOH-18 above the critical micelle concentration. Hydrodynamic diameter (D_h) of the C12 gemini surfactant, 2-3 nm, suggests the formation of

spherical micelle aggregates. Gemini surfactant with C18 alkyl chains forms aggregates with $D_h = 60$ nm, therefore, this C18 gemini surfactant likely forms vesicle-like aggregates when self-assembly occurs.

Fig. 5. Size distribution of the aggregates for 12-MOH-O-MOH-12 and 18-MOH-O-MOH-18 above critical micelle concentration.

3.5. Aerobic biodegradability

Aerobic ready biodegradability of synthesized gemini surfactants was evaluated by the CO_2 headspace test [32,33]. This standard method is the reference test method adopted by the European Union for testing surfactant ready biodegradability [61]. In the test, the microbial transformation of the parent molecule into inorganic final products of the degradation process (CO_2 and H_2O) was determined.

The biodegradation results are presented in Table 3. The surfactants, 12-MOH-O-MOH-12 and 18-MOH-O-MOH-18, are not ready biodegradable as they underwent less than 2% of biodegradation after 28 days. Elongating the alkyl chain from C12 to C18 has not changed the biodegradation percentage. Biodegradation results are consistent with data reported

in literature about the low or null mineralization of this type of cationic gemini surfactants [27,62–64].

Table 3

Biodegradation percentages of tested gemini surfactants and reference substance (SDS) determined by the CO₂ headspace test (95% CI of 28-day results calculated from 4 replicates).

Surfactant	Biodegradation (%)			
	7 days	14 days	21 days	28 days
SDS	70	87	94	92 ± 4.2
12-MOH-O-MOH-12	0	0	0	0 ± 1.4
18-MOH-O-MOH-18	0	0	0	0 ± 1.3

The high antimicrobial activity of the cationic surfactants could be the reason of the low biodegradation. Quaternary ammonium salts are known as an effective biocide agents and gemini surfactants are more active than monomeric ones [4,63].

The cationic compounds studied strongly inhibit the inoculum activity at the concentration tested (12 mg C/L), as SDS degradation inhibition percentages of 30% and 68% were obtained for mixtures of SDS with 12-MOH-O-MOH-12 or with 18-MOH-O-MOH-18, respectively. Therefore, the toxicity to the microorganisms responsible for aerobic biodegradation increases with the alkyl chain length. The values obtained are clearly higher than the limit commonly accepted to consider serious inhibitory effects ($\geq 25\%$). From the results obtained, the failure in the biodegradation test can be attributed to the toxic effects of these surfactants on the microorganisms responsible for aerobic biodegradation under the standard test conditions as it was concluded for analogue compounds [27]. Comparing the result for 12-MOH-O-MOH-12 (30%) with the values of 37% and 56% obtained, respectively, for

similar surfactants as 12-O-12 (3-oxa-1,5-tetramethylene-bis(*N*-dodecyl-*N,N*-dimethylammonium) dichloride) and G6-MOH-12 (1-6-hexamethylene-bis(*N*-dodecyl-*N*-hydroxyethyl-*N*-methylammonium) dibromide) [27], it can be observed that the increase of the compound hydrophilicity decreases the inhibitory effect. In addition, it seems more important the substitution of two methylene groups by an oxygen atom in the spacer than replacing a methyl by an hydroxyethyl group in the polar heads for reducing the surfactant toxicity to the aerobic microorganisms of the test inoculum.

The results here obtained confirm that cationic gemini surfactants are hardly biodegradable. However, some approaches as different modifications of their structure can improve the biodegradation [21,22,28]. Likewise, as described for monomeric cationic surfactants, adsorption on biomass can decrease inhibition on the inoculum activity and thus enhance surfactant biodegradation [65]. The use of immobilized consortium of microorganisms could also favor the degradation process [66].

3.6. Aquatic toxicity

The acute toxicity test on fresh water crustacea (*Daphnia magna*) was carried out to assess the aquatic toxicity of the cationic gemini surfactants. *Daphnia magna* was selected as model organism due to its high sensitivity to pollution and wide use for evaluating toxicity of surfactants [27,67–70].

The plots of percentage of immobilized daphnia at 48 hours versus surfactant concentration for 12-MOH-O-MOH-12 and 18-MOH-O-MOH-18 are presented in Fig. 6. The estimated concentration to immobilize 50% of daphnia after 48 hours of exposure and the corresponding 95% confidence interval, IC₅₀ (95% CI), were 1.2 (0.98-1.3) mg/L and 0.19 (0.12-0.25) mg/L for 12-MOH-O-MOH-12 and 18-MOH-O-MOH-18, respectively. Therefore, 18-MOH-O-MOH-18 presents higher toxicity to daphnia than 12-MOH-O-MOH-12.

Elongating the hydrophobic alkyl chains of the cationic gemini surfactants increases the aquatic toxicity. These results about the effect of the alkyl chain length on the aquatic toxicity are in agreement with those reported for monomeric cationic surfactants [65,69]. Based on the IC_{50} values and the Ecotoxicity Hazard classes proposed by the OECD [71], 12-MOH-O-MOH-12 belongs to the class of chemicals considered as toxic to aquatic life (Acute Toxicity II) and 18-MOH-O-MOH-18 to the class considered as very toxic (Acute Toxicity I).

Fig. 6. Percentages of immobilized daphnia at 48 hours as a function of surfactant concentration for 12-MOH-O-MOH-12 and 18-MOH-O-MOH-18.

Comparing the acute toxicity to daphnia of the tested C12 surfactant ($IC_{50} = 1.2$ mg/L) with the value of $IC_{50} = 0.38$ mg/L for the monomeric surfactant *N*-dodecyl-*N,N,N*-trimethylammonium bromide [69], 12-MOH-O-MOH-12 has lower aquatic toxicity. The IC_{50} value for the dimeric surfactant is three times higher indicating the use of gemini surfactants seems to be less hazardous for the aquatic environment, which is important from the ecological point of view. The results here obtained are in agreement with those previously reported by the authors [27], showing that quaternary ammonium-based gemini surfactants are less toxic to the aquatic life than monomeric analogues.

On the other hand, according to our previous work [27], comparing the result of 12-MOH-O-MOH-12 ($IC_{50} = 1.2$ (0.98-1.3) mg/L) with the results for similar gemini surfactants as 12-O-12 ($IC_{50} = 1.1$ (0.99-1.2) mg/L) and G6-MOH-12 ($IC_{50} = 1.1$ (0.95-1.3) mg/L), the IC_{50} value has slightly increased from 1.1 to 1.2 mg/L. However, the increase is so small that the IC_{50} value of 12-MOH-O-MOH-12 is included in the 95% CI of 12-O-12 and G6-MOH-12 and, therefore, there are not significant differences among the surfactants regarding their toxicity to daphnia. A higher IC_{50} value for 12-MOH-O-MOH-12 was expected taking into account that for the most hydrophobic analogue of this gemini surfactant series, 12-6-12 (1,6-hexamethylene-bis(*N*-dodecyl-*N,N*-dimethylammonium) dibromide), the IC_{50} was 0.65 mg/L [27] and the replacement of two methylene groups by an oxygen atom in the spacer (12-O-12) or a methyl group by a hydroxyethyl moiety in the polar head group (G6-MOH-12) resulted in significantly less toxic compounds to the aquatic life [27].

Conclusions

New long-chain (C12 and C18) cationic gemini surfactants containing an oxygen-substituted spacer and hydroxyethyl groups connected to the positively charged nitrogen atoms have been prepared. The hydroxyethyl group in the polar head favors self-aggregation whereas the presence of the oxygen atom in the spacer increases the critical micelle concentration. Small aggregates corresponding to spherical micelles are formed by the C12 homologue whereas the C18 homologue forms vesicle-like aggregates. Cationic gemini surfactants synthesized are not readily biodegradable and show inhibitory effects on the activity of microorganisms responsible for the biodegradation process that increase with the alkyl chain length. Aquatic toxicity increases with the hydrophobic chain and decreases by increasing the hydrophilic nature of the spacer and polar heads. Gemini surfactants investigated are less toxic to the aquatic environment than monomeric surfactants and non-oxygen-containing gemini surfactants. This

research provides information about the micellization process and some ecological properties of new gemini surfactants, which is of great interest for developing surfactants more efficient and less toxic to the aquatic environment.

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