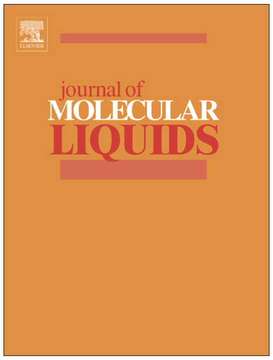

Aggregation and antimicrobial properties of gemini surfactants with mono- and di-(2-hydroxypropyl)ammonium head-groups: Effect of the spacer length and computational studies

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Aggregation and Antimicrobial Properties of Gemini Surfactants with Mono- and Di-(2-Hydroxypropyl)ammonium Head-Groups: Effect of the Spacer Length and Computational Studies

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Abstract

Cationic gemini surfactants, alkanediyl-α,ω-bis[(2-hydroxypropyl)dodecylammonium] dibromide (abbreviated as C_{12}-s-C_{12}[iso-Pr(OH)] and C_{12}-s-C_{12}[iso-Pr(OH)]$_2$, with s = 2, 3, 4 and 5) have been synthesized, and their aggregation properties in aqueous solution have been studied by surface tension, electrical conductivity and dynamic light scattering methods. On the basis of the results of studying aqueous solutions of the synthesized gemini surfactants by tensiometric and conductometric methods, their principal surfactivity parameters, such as the degree of counterion binding (β), critical micelle concentration (CMC), effectiveness of surface tension reduction (π_{CMC}), surface excess concentration (Γ_{max}), area per molecule at the interface (A_{min}), changes of Gibbs free energies of adsorption (ΔG_{ad}) and micellization (ΔG_{mic}) have been calculated. For both classes of these gemini surfactants, a character of the surfactivity parameters change with an elongation of the spacer chain was defined and comparative analysis with other ammonium-type gemini surfactants having different head-groups was performed. Using dynamic light scattering method, regularities of variation of the aggregates sizes were studied depending on the number of 2-hydroxypropyl groups and the spacer chain length was investigated. Meanwhile, it was revealed that the mentioned gemini surfactants possess antimicrobial properties. The mechanism of the epoxide ring opening and salt formation reactions were calculated by using Kohn–Sham DFT with the B3LYP functional. The epoxide ring opening via primary (dodecylamine) and secondary

(**INT2**) amines was determined as exergonic (13.4 and 4.4 kcal/mol) reactions as observed experimentally.

Key words: gemini surfactant, surface tension, micellization, adsorption, antimicrobial properties.

1. Introduction

Because of the elevating demand, researches on gemini surfactants are expanding. The gemini surfactants are bearing very peculiar features (e.g., high surfactivity, low Krafft point, smaller CMC compared to conventional surfactants [1,2]). These particularities boost the effectiveness of the applicability of gemini surfactants [3,4]. Due to these circumstances, synthesis of such surfactants by more rational ways and their study attract more and more interest. As known, physico-chemical properties of traditional surfactants depend on alkyl chain length and nature of head-group [5]. In the case of gemini surfactants, in addition to these factors, spacer chain length and nature are also important [6]. The most studied gemini surfactants are of alkanediyl-α,ω-bis(dialkyl-alkylammonium bromide) class [7,8]. For these surfactants, variation of properties depending on nature of alkyl chain, head-group and spacer was studied in a wider form. It was established that lengthening of hydrophobic alkyl chain, replacement of the methyl group linked to nitrogen atom by ethyl, propyl and butyl groups and elongation of spacer chain cause a change in CMC and Krafft point [9]. But this variation is not monotonic with elongation of chains and is characterized by presence of maximum or minimum. In the case of the gemini surfactants having amide group in hydrophilic part, with an increase of the spacer-chain length (alk=12, s=2-12), the CMC decreases [10]. When elongating the spacer chain in the gemini surfactants with imidazolium head-group ($[C_{12}$–s–C_{12}im]Br_2, s=2, 4, 6), the CMC increases [11]. In the case of 1,10-(alkane-1,5-diyl)-bis(1-dodecyl pyrrolidinium)bromide (C12–Cs–C12PB, s = 3, 4, 6, 8, 10, 12, 14, and 16) gemini surfactants, the CMC rises when the spacer chain is lengthened from C_3 to C_4, but then the CMC is lowered with a subsequent elongation of the alkylene chain [12]. M. Borse and coauthors have determined that, the methyl group bonded to the nitrogen atom of the cationic gemini surfactant is substituted by ethylol group, the CMC diminishes [13,14]. They also established that, in the case of alkanediyl-α,ω-bis[methyl(2-hydroxylethyl) dodecylammonium] dibromide (abbreviated as 12-*s*-12 (OH), with *s* = 4, 6, 8 and 10 methylenes) class surfactants, increasing the spacer chain from C_4 to C_6 brings about a rise in the CMC. However, with a further chain elongation, the CMC value starts to decrease. Average degree of ionization of micelles (α_{ave}) becomes increased with lengthening the spacer chain. In the alkanediyl-α,ω-bis[methyl(2-hydroxylethyl) hexadecylammonium] dibromide (abbreviated as 16-*s*-16 (OH), with *s* = 2, 3 and 4 methylenes) class surfactants, elongation of the methylene-based chain causes a rise in the CMC [15]. In the gemini surfactants, where both methyl groups in the head-group are replaced by ethylol group, for example, in the gemini surfactants with dihydroxyethylamino head-groups (abbreviated as 12-*s*-12 $(OH)_2$ with *s* = 4, 6, 8 and 10 methylenes), an increase in the spacer chain length leads to a decrease in the CMC. When the ethylol group connected to the nitrogen atom in the head-group is substituted by 2-hydroxypropyl group, the value of CMC decreases [16]. Therefore, obtainment and study of the gemini surfactants containing 2-hydroxypropyl group are of a large interest.

The submitted article is devoted to synthesis of the new gemini surfactants of the C_{12}-s-C_{12}[iso-Pr(OH)] and C_{12}-s-C_{12}[iso-Pr(OH)]$_2$ class as well as a study of the regularities of variation of properties of these surfactants in dependence, on the spacer chain size. Meanwhile, a relationship between antimicrobial properties of the obtained surfactants and the spacer nature has been investigated.

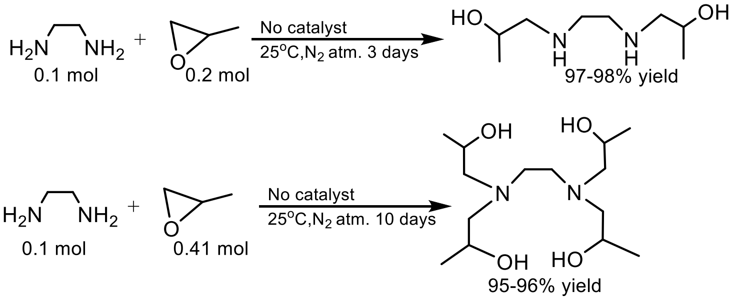
2. Experimental

2.1. Materials and instruments

Propylene oxide (PO) was used as a product (99.97–99.98% purity) of "Organic Synthesis" factory (Sumgayit, Azerbaijan). Ethylenediamine (purity ≥99% Alfa Aesar®, Great Britain), 1-dodecyl amine (purity>98% Alfa Aesar GmbH & Co KG, Germany), 1-bromododecane (purity>98% Alfa Aesar, England), 1,3-dibromopropane (purity>97% Sigma-Aldrich), 1,4-dibromobutane (purity>99% Sigma-Aldrich, China) and 1,5-dibromopentane (purity>97% Sigma-Aldrich) of analytical grade were taken. ^{1}H NMR and ^{13}C NMR spectra were registered using Bruker TOP SPIN spectrometer 300.13 MHz and 75.46 MHz) with chemical shifts (δ in ppm) downfield from TMS using such solvents as D_2O, $CDCl_3$ and DMSO-d_6. IR spectra were registered using ALPHA FT-IR spectrometer (Bruker) using KBr disks. The elemental analyses (C, H, and N) of gemini surfactants were carried out on a LECO TruSpec CHN Analyzer (Chicago).

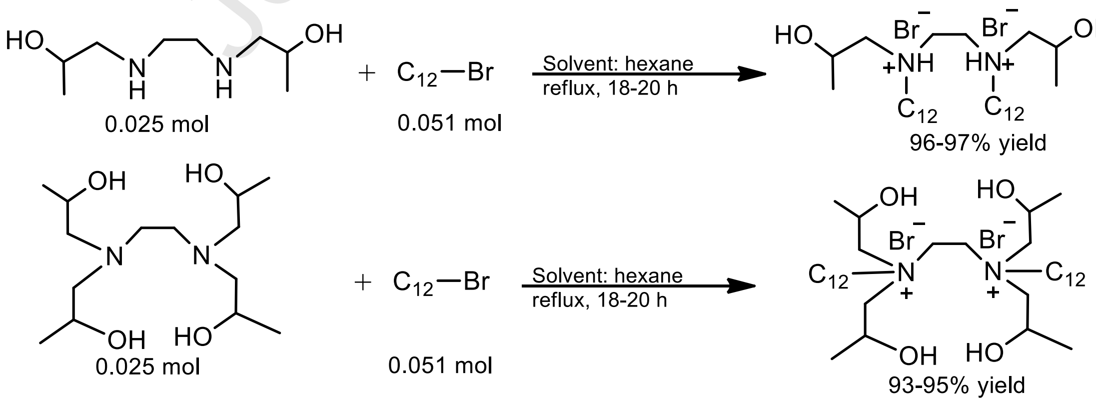
2.2. Synthesis of C_{12}-2-C_{12}[iso-Pr(OH)] and C_{12}-2-C_{12}[iso-Pr(OH)]$_2$ gemini surfactants

To synthesize the gemini surfactants with spacer C_2, in the 1st step, ethylenediamine was propoxylated with PO at the mole ratios 1:2 and 1:4. The reactions occur according to the following scheme (Scheme



Scheme 1. The reactions of synthesis of N,N'-bis(2-hydroxypropyl)ethylenediamine and N,N,N',N'-tetrakis(2-hydroxypropyl)ethylenediamine.

To synthesize N,N'-bis(2-hydroxypropyl)ethylenediamine, 0.1 mol of ethylenediamine was introduced into a round-bottom flask and 0.2 mol of PO was added. The reaction was carried out at room temperature by mixing on the magnetic mixer, under the nitrogen atmosphere. As the reaction is exothermic, the flask was placed into a water bath and, in this way, a temperature rise was prevented and formation of side-products was avoided. The yield of the reaction product was 97-98%. It is a white, paste-like substance. The product dissolves well in water, ethanol, acetone, ethyl acetate and CCl_4, partially – in hexane. The structure and composition of the synthesized N,N'-bis(2-hydroxypropyl)ethylenediamine were confirmed by methods of NMR- and IR-spectroscopy [17]. N,N,N',N'-tetrakis(2-hydroxypropyl)ethylenediamine was synthesized in accordance with the above-mentioned method taking 0.1 mol ethylenediamine and 0.41 mol PO. The yield of the reaction product was 95-96%. The structure and composition of N,N,N',N'-tetrakis(2-hydroxypropyl)ethylenediamine were confirmed by identification using NMR- and IR-spectra [18]. At the second stage, N,N'-bis(2-hydroxypropyl)ethylenediamine and N,N,N',N'-tetrakis(2-hydroxypropyl)ethylenediamine were subjected to quaternization reaction by interaction with dodecyl bromide (Scheme 2):



Scheme 2. Reaction of synthesis of cationic gemini surfactants

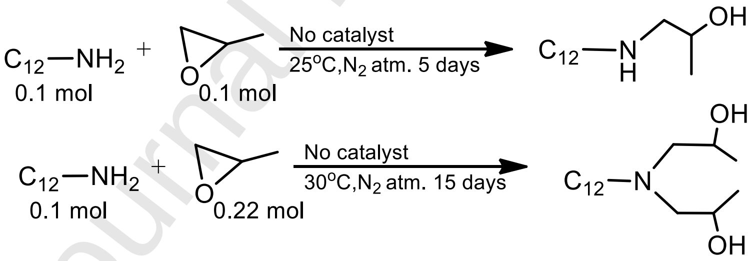
0.025 mol N,N'-bis(2-hydroxypropyl)ethylenediamine and 25 mL hexane were placed into a flat-bottom two-neck flask. After transition of the mixture to fully-homogeneous state, 0.052 mol of 1-

bromododecane was added to it. The reaction was conducted in the mentioned flask equipped with magnetic stirrer, reflux condenser and heater, for 18-20 hours, at the temperature of boiling of the mixture.

To isolate the synthesized C_{12}-2-C_{12}[iso-Pr(OH)] gemini surfactant from the reaction mixture, a vacuum distillation was performed. By this procedure, the gemini surfactant was separated from the solvent and unreacted 1-bromalkane. For purification of the gemini surfactant, it was crystallized in acetone thrice. The yield of C_{12}-2-C_{12}[iso-Pr(OH)] gemini surfactant was 96-97%. To synthesize the C_{12}-2-C_{12}[iso-Pr(OH)]$_2$ gemini surfactant, by the same method, the reaction of N,N,N',N'-tetrakis(2-hydroxypropyl)ethylenediamine with 1-bromdodecane was carried out. The yield of the C_{12}-2-C_{12}[iso-Pr(OH)]$_2$ gemini surfactant was 93-95%. The obtained gemini surfactants dissolve well in ethanol, acetone, ethyl acetate, partially – in water. The degree of their purity was established by methods of NMR- and IR-spectroscopy [17,18].

2.3. Synthesis of the gemini surfactants C_{12}-s-C_{12}[iso-Pr(OH)] and C_{12}-s-C_{12}[iso-Pr(OH)]$_2$ with s=3, 4 and 5

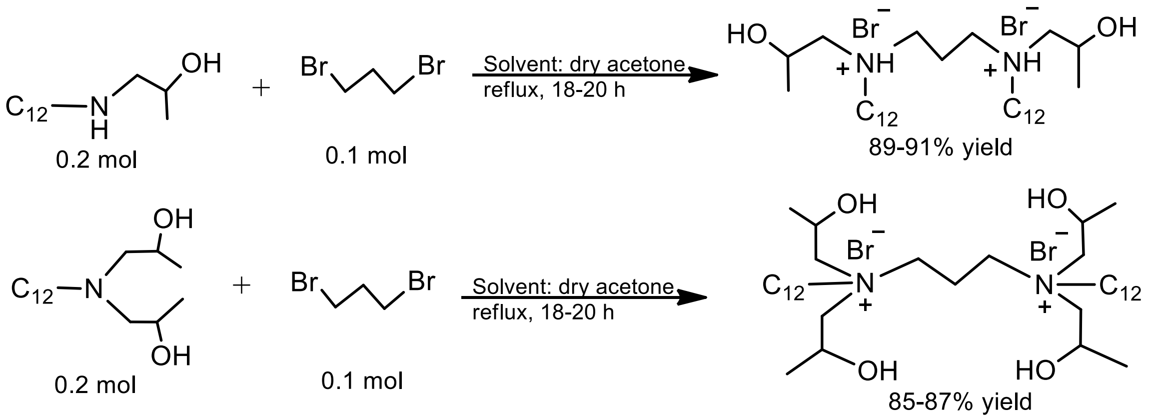
For synthesis of the gemini surfactants with the spacer chain length C_2, C_3, C_4 and C_5, at the first stage, dodecylamine was propoxylated with PO at molar ratios 1:1 and 1:2 (Scheme 3):



Scheme 3. Reaction scheme of the synthesis of mono- and di-(2-hidroxypropyldodecylamines).

To synthesize alkyl di-(2-hidroxypropyldodecylamines), 0.1 moles of dodecyl amine and 0.22 moles of PO are charged into a flask. To prevent oxidation of dodecyl amines, the reaction is made in the atmosphere of nitrogen [19].

In the second stage, the synthesized mono- and di-(2-hydroxypropyldodecylamines) were reacted with 1,3-dibromopropane (1,2-dibromoethane, 1,4-dibromobutane and 1,5-dibromopentane) according to the following scheme (Scheme 4):



Scheme 4. Reaction scheme of the synthesis of gemini surfactants based on dibromoalkanes (C_3)

Samples of 37.5 g (0.2 mol) of mono-2-hydroxypropyldodecylamine and 23.0 g (0.1 mol) of 1,3-dibromopropane (or respective amount of 1,2-dibromoethane, 1,4-dibromobutane or 1,5-dibromopentane in mol) were refluxed with 60 mL of dry acetone at 75 °C for 18-20 h. After completion of the reaction, the crude white solid thus obtained was washed with a hexane/ethyl acetate mixture and then recrystallized four to five times from an acetone/methanol mixture; the salt was then dried in desiccator for 15 h. A white solid product was obtained in 85-91 % yield [20]. But, in the reaction with 1,2-dibromethane, the yield of the obtained surfactant turned out to be 20-40%. That is why synthesis of the gemini surfactants with a spacer s=2 according to the reactions given in Schemes 1 and 2 is more convenient. The structure of the synthesized gemini surfactants is confirmed by methods of IR- and NMR-spectroscopy (IR- and NMR-spectra of the gemini surfactants of s=3 and s=4 are given in Supporting İnformation whereas the spectra of the gemini surfactant of s=5 are contained in Reference 20). The elemental analysis of the synthesized gemini surfactants show purity of these compounds as represented in Table 1.

2.4. *Computational Details*

The Gaussian 16 package [21] was utilized for the reaction profile calculations. The calculations were carried out by using DFT with the B3LYP functional and 6-311+G* [22] basis sets for all atoms (H, C, N, O and Br). The reaction path was calculated for experimental reaction condition (1atm, 298.15 K). Since the reactions are solvent-free, the calculations were conducted for gas phase. All transition states and intermediates are optimized without geometry constraints with the above- mentioned method. We applied IRC (Intrinsic Reaction Coordinate) search for determination of intermediate structures connected with saddle points. Optimized Cartesian coordinates, total energies, Gibbs energies, and enthalpies of all structures are provided in SI.

2.5. Determination of surfactivity and electroconductivity of the synthesized salts

Surface tension values of the synthesized gemini surfactants were measured by means of Du Nouy tensiometer [23]. 0.00025-4.0 % wt. aqueous solutions of the gemini surfactants were prepared and, after storing them for one day, their surface tensions were determined at 25 °C. For preparing these solutions, bidistillate was used. The surface tension of the used bidistillate at the border with air at 25 °C equalled 72.0 mN/m. The respective graphs were built using the results of the experiment with an error less than ±0.2 mN/m.

Specific electroconductivities – κ of the aqueous solutions of the obtained gemini surfactants were evaluated using "ANION-402" conductometer (Russian Federation) [23]. For conducting the measurements, first, 0.001-4.0% wt. aqueous solutions were prepared and, after two days, their specific electroconductivities were measured at 25 °C. The value of this quantity at 25 °C for the bidistillate used for preparing the solutions was equal to 1.5-2.5 μS. The respective plots were drawn using the results of the measurements with an error of the conductometer within ±0.3 μS/cm.

2.6. Dynamic Light Scattering (DLS)

The size distribution of the aggregates formed in the aqueous solutions of the synthesized C_{12}-s-C_{12}[iso-Pr(OH)] and C_{12}-s-C_{12}[iso-Pr(OH)]$_2$ gemini surfactants was determined using a Particle Size Analyzer (HORIBA LB-550, Japan) instrument at 298.15 K. The light source was a 650-nm laser diode of power 5 mW. The interval of size measurements was from 1 nm up to 6 μm. The concentration of samples was 0.1% and 0.3%. Each sample was measured at least three times. The distribution of diffusion coefficients (D) of the solutes was obtained by analyzing the correlation function of scattering data via the CONTIN method. Then, the apparent equivalent hydrodynamic diameter (D_h) was determined using the Stokes−Einstein equation [24] $D_h = kT/3\pi\eta D$, where k is the Boltzmann constant, T is the absolute temperature, and η is the solvent viscosity.

2.7. Antimicrobial properties of gemini surfactants

To study antimicrobial properties of the synthesized gemini surfactants, one gram-positive (*Staphylococcus aureus*- ATCC 6538) bacterium, two gram-negative (*Pseudomonas aeruginosa*- ATCC 9027*, Escherichia coli*-ATCC 10536) bacteria and fungus (*Candida albicans*- ATCC 14037*)* laboratory strains were used. Those bacteria were cultivated in meaty-peptone agar and the yeast fungus - in Sabouraud medium. The antibacterial activity of the synthesized gemini surfactants was studied by the disc - diffusion method. According to this method, from 1 day cultures of the microorganisms suspensions were prepared in the physiological solution provided that 1 mL of the suspension must contain 1 bln microbial cells. Each microorganism suspension was spread on the surface of the respective feeding media using tampons. 2, 4, and 8 mg/mL concentration solutions of the gemini surfactants were impregnated onto sterile paper discs of 6 mm diameter and placed on the surface of the feeding media with the inoculated microorganisms. These samples of bacteria were thermostated for 24 h at 37°C and those of fungus - for 48 h at 30°C. Then, the measurements were carried out.

The antimicrobial activity was estimated by measuring the inhibition zone diameter (mm). Triplicates were maintained and the inhibition experiment was repeated thrice. For each replicate, the measurements were taken in three different fixed directions. Furthermore, sterile water was used as a negative control.

2.8. Bactericidal effectiveness activity against sulfate-reducing bacteria (SRB)

The bactericide effectiveness of the synthesized gemini surfactants against SRB growth was studied in accordance with the serial dilution method. The used water was put to microbial inhibition test. This test was performed according to ASTM D4412-84 [25]. The growth of about 1,000,000 bacteria cells in 1 g of water was reached. The synthesized gemini surfactants were tested as a bactericide against SRB at the doses of 15, 75 and 150 mg/l. Incubation of the system was made at contact time of 3.0 h; all of the systems were cultured in specific media for SRB during 21 days at temperature 303 K.

3. Results and Discussion

3.1. Synthesis of the Gemini surfactants

To synthesize the gemini surfactants having different spacers, a two-step reaction was conducted. At the first step, dodecylamine was propoxylated by PO. Here, depending on the dodecylamine:PO mol ratio (1:1 or 1:2), mono- or di-2-hydroxypropyldodecylamine are obtained. At the second step, the obtained aminoalcohols via the quaternization reaction with dibromoalkanes are converted to salts. For synthesis of the gemini surfactants with s=2, the synthesized two aminoalcohols were reacted with 1,2-dibromoethane. In these reactions, the yield of the gemini surfactants was 20-40%. Its reason was creation of sterical effect by 2-hydroxypropyl groups. As a result, one side of the reacted 1,2-dibromoethane hinders entrance of the second side into the reaction. In order to obtain the gemini surfactant with s=2 in a high yield, N,N'-bis(2-hydroxypropyl)ethylenediamine and N,N,N',N'-tetrakis(2-hydroxypropyl)etilenediamine were reacted with dodecyl bromide.

3.2. Impact of spacer length on colloidal-chemical parameters of the gemini surfactants

3.2.1. Critical micelle concentration (CMC)

According to the determined values of the surface tension of the aqueous solutions of the synthesized gemini surfactants, surface tension isotherms for the surfactants of the C_{12}-s-C_{12}[iso-Pr(OH)] and C_{12}-s-C_{12}[iso-Pr(OH)]$_2$ classes were built (Figs. 1,2). As is seen from these figures, with an increase of the gemini surfactants concentration, the values of the surface tension of their aqueous solutions at the border with air sharply decreases. After a certain concentration, stabilization of the surface tension starts. This concentration is critical micellization concentration (CMC). In the case of C_{12}-5-C_{12}[iso-Pr(OH)] and C_{12}-s-C_{12}[iso-Pr(OH)]$_2$ surfactants with s=3, 4 and 5, with an increase of the concentration, an abrupt diminution of the surface tension is observed and, after some concentration, is replaced by weaker lowering of γ. This concentration is referred to as critical premicellar aggregation (CPC). The values of CMC and CPC of the synthesized gemini surfactants are presented in Table 2. As is evident from the table, in the case of the gemini surfactants of the C_{12}-s-C_{12}[iso-Pr(OH)] class, with lengthening the spacer chain from C_2 to C_3, the CMC value diminishes, but with an elongation from C_3 to C_5, the CMC rises. Regarding the C_{12}-s-C_{12}[iso-Pr(OH)]$_2$ class gemini surfactants, as the spacer chain length increases from C_2 to C_4, the CMC decreases but, at s=5, its value starts to augment. In alkanedyil-α-ω-bis (dimethyldodecylammonium)bromide-C_{12}-s-C_{12}(Me)$_2$ [26], 1,1′-(alkane-1, 5-diyl)bis(1-dodecyl pyrrolidinium) bromide C_{12}-s-C_{12}(PB) [12] class gemini surfactants, with an elongation of the spacer chain, the CMC rises but, after s=4-5, it starts to diminish. In the case of 1,1′-(alkane-1,5-diyl)bis(1-dodecyl imidazolium)bromide C_{12}-s-C_{12}(IM) [11] class gemini surfactants, the CMC increases when the spacer chain length augments from C_2 to C_6 but in alkanediyl-α,ω-bis[di(2-hydroxylethyl) dodecylammonium] dibromide C_{12}-s-C_{12}(OH)$_2$ class gemini surfactants, the CMC becomes lowered with lengthening the spacer chain from C_4 to C_{10}. Regarding alkanedyil-α-ω-bis (diethyldodecylammonium) bromide-C_{12}-s-C_{12}(Et)$_2$ and alkanedyil-α-ω-bis(methylhydroxyethyldodecylammonium) bromide-C_{12}-s-C_{12}(MeEtOH)$_2$ [27] class gemini surfactants, with increasing the spacer chain from C_4 to C_6, the CMC rises but it decreases when the mentioned chain is further elongated from C_6 to C_{10} [28]. As is seen, the nature of head-group in gemini surfactants influences the change of the CMC value depending on the size of their spacer.

3.2.2. Surface pressure

The surface pressure - π_{CMC} values of the synthesized gemini surfactants were calculated using the following formula [29]:

$$\pi_{CMC} = \gamma_0 - \gamma_{CMC} \qquad (3)$$

where γ_0 is the surface tension at the water-air border in the lack of surfactant, γ_{CMC} is the surface tension value at CMC. In Table 2, the values of the surface pressure of the synthesized gemini surfactants are given. As is noticed from the table, in the C_{12}-s-C_{12}[iso-Pr(OH)] class gemini surfactants, the CMC value are lowered as the spacer chain lengthens, but, in C_{12}-s-C_{12}[iso-Pr(OH)]$_2$ class gemini surfactants, oppositely, with an elongation of the spacer chain, the π_{CMC} rises. As is evident from the obtained results, the variation of the surface pressure with an increase of the spacer chain length depends on the nature of head-group in gemini surfactants. In C_{12}-s-C_{12}(Me)$_2$, [26,30], C_{12}-s-C_{12}(IM) [11] class gemini surfactants, when the spacer chain lengthens, the π_{CMC} value decreases but in bis(monoglyceride-1-hydroxymethyl-2-fatty acid ester)-α,ω-alkanediether) [31] and other type cationic gemini amphiphiles [32], the π_{CMC} value augments.

3.2.3. Study of the gemini surfactants by electroconductometric method

Specific electroconductivity of the synthesized cationic gemini surfactants at various concentrations (C) was studied at 25 °C. In accordance with the obtained values, κ vs. C plots were built (Figs. 3,4). Based on the drawn graphs, a dissociation degree – α and a binding degree - β of the counterion of the gemini surfactants were computed by means of the formula given below:

$$\beta=1-\alpha=1-S_2/S_1 \qquad (5)$$

where S_1 and S_2 are respectively the slopes dκ/dC before and after the CMC.

The obtained values are set in Table 2. It is obvious from the table that, in C_{12}-s-C_{12}[iso-Pr(OH)] and C_{12}-s-C_{12}[iso-Pr(OH)]$_2$ class gemini surfactants, β decreases with lengthening the spacer chain. Its reason is related with a change in the charge density on the surface of the micelles. In the case of these gemini surfactants, two positively-charged cationic groups are linked by covalent bond. With elongating the spacer chain, positively charged groups become apart from each other and the charge density decreases. As a result, dissosiation of the counterion increases and β diminishes. But, in C_{12}-5-C_{12}[iso-Pr(OH)]$_2$ gemini surfactant, a deviation is observed. It is caused by a high capability of this surfactant for premicellar aggregation in distinction from the other surfactants.

3.3. Dependence of free Gibbs energy of the gemini surfactants micellization process on the spacer length

A change of free Gibbs energy of the micellization process (ΔG_{mic}) in the aqueous solutions of the synthesized gemini surfactants was computed using the formula given below [30]:

$$\Delta G_{mic} = RT(0.5+\beta)lnX_{CMC} - 0.5RTln2 \qquad (9)$$

$$\Delta G_{agg} = RT(0.5+\beta)lnX_{CPC} - 0.5RTln2 \qquad (10)$$

where R is universal gas constant (8.314 J/mol·K), T is absolute temperature (K), X_{CMC} is the CMC in molar fraction, X_{CMC} = CMC/55.4, where CMC is in mol/L, 55.5 originates from 1 L of water corresponding to 55.5 mol of water at 25 °C, β is the binding degree of counterion. In Table 2, the values of ΔG_{mic} for the gemini surfactants are presented. As is seen, for both classes gemini surfactants, the values of ΔG_{mic} are negative. Therefore, the micelle formation in the case of these gemini surfactants is a spontaneous process. In the C_{12}-s-C_{12}[iso-Pr(OH)] class gemini surfactants, with an elongation of the spacer chain from C_2 to C_3, ΔG_{mic} diminishes, then starts to rise. But, in the C_{12}-s-C_{12}[iso-Pr(OH)]$_2$ class gemini surfactants, when the spacer chain lengthens from s=2 to s=4, ΔG_{mic} increases. Then, when passing from C_4 to C_5, the value of ΔG_{mic} becomes lowered.

3.4. DLS measurements

In Figs.5-10, the graphs of size distribution determined by DLS method for the aggregates formed by the gemini surfactants with the alkyl chain C_{12} are depicted. As is seen from Fig.5, a diameter of the aggregates of C_{12}-2-C_{12}[iso-Pr(OH)] gemini surfactant in aqueous solution is equal to 130-150 nm and variation of concentration does not influence the diameter of these aggregates. But, in the case of the aggregates of C_{12}-3-C_{12}[iso-Pr(OH)] gemini surfactant, the diameter increases when the concentration rises. Thus, with a rise of concentration thrice, the diameter of the aggregates increases from 10 to 40 nm (Fig.6). For C_{12}-5-C_{12}[iso-Pr(OH)] gemini surfactant, a change of the aggregate diameter with a concentration rise is more abrupt (Fig 7). For example, when increasing the concentration 2.5 times, the diameter of the aggregates of this surfactant augments from 6.6 nm to 3400 nm. As is seen from Fig.8, the diameter of the aggregates of C_{12}-2-C_{12}[iso-Pr(OH)]$_2$ gemini surfactant in the water solution varies in the range 30-60 nm. When rising the concentration thrice, the diameter of its aggregates increases partially. The diameter of the aggregates of C_{12}-3-C_{12}[iso-Pr(OH)]$_2$ gemini surfactant is nearly 10 nm. But three-fold rise of its concentration does not change the diameter of the aggregates (Fig.9). Regarding the aqueous solutions of C_{12}-5-C_{12}[iso-Pr(OH)]$_2$ gemini surfactant, the diameter of the aggregates equals 10-13 nm. This diameter remains relatively constant with increasing the concentration (Fig 10). As is clear from Figs. 5-7, in the case of C_{12}-s-C_{12}[iso-Pr(OH)] class gemini surfactants, with an elongation of the spacer chain, an impact of the concentration on the aggregate diameter is strengthened. Thus, in the gemini surfactant with s=2, the concentration weakly influences the diameter of the aggregates, but, in the case of the surfactant with s=5, this impact is felt sharply. Regarding C_{12}-s-C_{12}[iso-Pr(OH)]$_2$ gemini surfactants, with lengthening the spacer, the impact of concentration on the aggregates diameter weakens

(Fig.8-10). For the both classes of the gemini surfactants, at low concentrations of the aqueous solutions, the aggregate diameter diminishes as the spacer is elongated.

3.5. Study of antibacterial properties of the gemini surfactants

Some authors [4,32,33] mention that antimicrobial properties of cationic surfactants change depending on the alkyl chain length. Thus, with an elongation of these surfactants hydrophobic chain their antimicrobial properties are enhanced. This dependence is called “cutoff effect”. As hydrophobic chain of surfactants lengthens, their CMC values diminish. That is why, in some literature sources, it is noted that these antimicrobial properties depend also on the CMC value, in addition to the alkyl chain size. In the case of cationic surfactants, with a decrease of the CMC value, their antimicrobial properties become improved [34,35].

In Table 3, the results of studies of antibacterial properties of the synthesized gemini surfactants are demonstrated. As is seen from the table, C_{12}-5-C_{12}[iso-Pr(OH)] and C_{12}-5-C_{12}[iso-Pr(OH)]$_2$ surfactants exhibit more effective antimicrobial properties against *S.aureus* bacterium, C_{12}-5-C_{12}[iso-Pr(OH)] and C_{12}-2-C_{12}[iso-Pr(OH)] surfactants - against *E.coli* bacterium, C_{12}-2-C_{12}[iso-Pr(OH)] and C_{12}-3-C_{12}[iso-Pr(OH)] surfactants – against *P.aeruginosa* bacterium, C_{12}-5-C_{12}[iso-Pr(OH)] and C_{12}-5-C_{12}[iso-Pr(OH)]$_2$ surfactants – against *C.albicans* fungus. In comparison with Gram-positive bacteria, Gram-negative bacteria are usually more stable against antimicrobial means. The reason is a different structure of the cell membrane of these bacteria. In the case of the Gram-negative bacteria, the external layer in the outer membrane consists mainly of lipopolysaccharides and proteins. This circumstance stipulates hindrances for penetration of biocides and amphiphiles [36]. A specific range of the HLB is required for entrance through the mentioned outer membrane.

From the comparison of the results regarding the antimicrobial properties of the synthesized gemini surfactants, it is noticeable that the surfactants with the spacer chain length s=5 possess more effectiveness as an antimicrobial agent. The antibacterial activities are considerably influenced by the hydrophobic chain length spacer and CMC of gemini surfactants. As is seen from Table 2, sometimes, elongation of the spacer chain does not cause lowering of the CMC but it brings about a decrease of the value of the critical premicellar concentration (CPC). Therefore, in the case of the synthesized gemini surfactants, the antimicrobial effect is related both to spacer chain length and their CPC value.

In Table 4, the results of the study of antimicrobial properties of the synthesized gemini surfactants against SRB are set. It is noticed from the table that C_{12}-2-C_{12}[iso-Pr(OH)] and C_{12}-4-C_{12}[iso-Pr(OH)] gemini surfactants demonstrate a higher effect as compared with the other surfactants. C_{12}-s-C_{12}[iso-Pr(OH)]$_2$ class gemini surfactants, C_{12}-4-C_{12}[iso-Pr(OH)]$_2$ and C_{12}-5-C_{12}[iso-Pr(OH)]$_2$ gemini surfactants have a stronger antibacterial effect.

The results on biocidal activity against sulfate-reducing bacteria given in Table 4 reveal that the activity was enhanced by increasing the spacer chain length between two ammonium head group for series C_{12}-s-C_{12}[iso-Pr(OH)]$_2$ gemini surfactants. Elongation of the spacer chain increases the adsorption tendency of the synthesized surfactant molecules on surfaces of microorganism's membranes. Hence, the potent action of the tested compounds is increased due to their high population at the cellular membrane [37]. In the case of C_{12}-s-C_{12}[iso-Pr(OH)] class gemini surfactants, with lengthening of the spacer chain the sizes of the aggregates in aqueous solution augment. So enhancement in antibacterial effect against sulfate-reducing bacteria is not observed [38].

3.6. Mechanistic studies

We turn to computation for rationalizing the reaction mechanism and experimentally observed higher conversion of the reactants for both epoxide opening and salt formation routes. Reactions mentioned in Scheme 3 and 4 are taken for calculations. Fig. 11 shows both epoxide opening and salt formation paths. Dodecylamine and PO interaction is calculated to have a high barrier (54.2 kcal/mol) and yields mono-(2-hydroxypropyl)dodecylamine (**INT2**). Further reaction profile is divided into two routes: **INT2+PO** (blue) and **INT2 + Br-(CH$_2$)$_3$-Br** (red) interactions. According to the blue route, activation energy for di-(2-hydroxypropyl)dodecylamine (**INT4-q**) formation is calculated 4.9 kcal higher in energy than **INT2** formation. It clearly shows conversion of dodecylamine into secondary amine (**INT2**) via epoxide opening makes a little difficulty (steric hindrance) as a nucleophile attacking to the second PO molecule. Direct salt (**INT4-t**) formation (**INT2 + Br-(CH$_2$)$_3$-Br**) activation energy is 41.7 kcal/mol which is not much different than tertiary amine (**INT4-q + Br-(CH$_2$)$_3$-Br**) conversion to salt in activation energy (43.7 kcal/mol). This fact supports experimental observation in regard to the same yield of tertiary and quaternary gemini surfactants (see Scheme 4). Overall quaternary salt (**PRO-q**) formation reaction is 22.8 kcal/mol (endergonic), whereas tertiary salt (**PRO-t**) formation is 2.2 kcal/mol (endergonic). Optimized structures of the final products are described in Fig. 12.

As seen from the **PRO-q** structure, the closest Br^- and N^+ (quaternary) electrostatic interaction distance is 4.06 Å. Br^- ions are located closer to the tertiary NH^+ at the **PRO-t** structure: Electrostatic interaction becomes possible via the middle H atom (N-H-Br) with bond lengths 1.08 and 2.06 Å.

The epoxide opening (**TS1**) and salt formation (**TS3-q**) transition state structures are shown in Fig. 13. As seen in the **TS1** structure, nucleophilic attack of dodecylamine to an external PO carbon atom is possible via 2.24 Å bond length. At the same time, the external epoxide ring C-O bond is elongated to 2.07 Å and the amine hydrogen is attracted by the PO oxygen with 1.80 Å distance. The quaternary salt formation transition state (**TS3-q**) is also described in Fig. 13. The nucleophilic attack of **INT4-q** to 1,3-dibromopropane (**INT5-q**) occurs via **TS3-q** with 43.7 kcal/mol energy barrier. Bromine extrusion (Br-C) and C-N bond formation distances are calculated as 2.69 and 2.03 Å respectively. Energy barriers of the rate limiting epoxide ring opening steps are bigger. The similar barrier was calculated previously for a catalyst-free reaction [39].

4. Conclusion

Cationic gemini surfactants containing the fragments (C_{12}-s-C_{12}[iso-Pr(OH)], C_{12}-s-C_{12}[iso-Pr(OH)]$_2$) with mono-and di-2-hydroxypropyl groups and the spacer length s=2-5 were synthesized. The micellization capabilities of the synthesized gemini surfactants were evaluated by tensiometric and conductometric methods. It was determined that, in the case of the both classes gemini surfactants, with an elongation of the spacer chain, CMC, at first, decreases, then rises whereas β becomes lowered. The surface pressure in C_{12}-s-C_{12}[iso-Pr(OH)] class surfactants diminishes with lengthening the spacer while in the case of C_{12}-s-C_{12}[iso-Pr(OH)]$_2$ class surfactants, it rises. C_{12}-s-C_{12}[iso-Pr(OH)]$_2$ class gemini surfactants have a higher tendency to premicellar aggregation. An elongation at the spacer facilitates formation of premicellar aggregates still more. By DLS method, it was revealed that, in C_{12}-s-C_{12}[iso-Pr(OH)] class gemini surfactants, the dependence of the aggregate diameter on concentration is intensified with lengthening the spacer. But, in C_{12}-s-C_{12}[iso-Pr(OH)]$_2$ class gemini surfactants, this dependence weakens with an elongation of the spacer chain. The antimicrobial properties of the synthesized gemini surfactants weakly depend on the number of 2-hydroxypropyl groups but strongly – on the length of the alkyl chain. The surfactants containing C_{12}-chain possess stronger antimicrobial properties. The reaction profile was calculated for the quaternary (**PRO-q**) and tertiary (**PRO-t**) gemini surfactants formation. It was identified that **PRO-t** and **PRO-q** formation reactions are both endergonic. Epoxide opening reactions via primary (dodecylamine) and secondary (**INT2**) amines were determined as exergonic via big energy barriers (54.2 and 59.1 kcal/mol).

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Table 1. Element analyses of the synthesized gemini surfactants

Gemini surfactants	Mr	Found			Calculated		
		C, %	H, %	N, %	C, %	H, %	N, %
C_{12}-2-C_{12}[iso-Pr(OH)]	674,72	56.89	10.52	4.19	56.96	10.46	4.15
C_{12}-3-C_{12}[iso-Pr(OH)]	688,75	57.70	10.29	4.08	57.55	10.54	4.07
C_{12}-4-C_{12}[iso-Pr(OH)]	702,77	58.20	10.49	4.04	58.11	10.61	3.99
C_{12}-5-C_{12}[iso-Pr(OH)]	716,80	58.72	10.60	3.90	58.65	10.69	3.91
C_{12}-2-C_{12}[iso-Pr(OH)]$_2$	790,88	57.70	10.38	3.51	57.71	10.45	3.54
C_{12}-3-C_{12}[iso-Pr(OH)]$_2$	804,91	58.21	10.45	3.48	58.20	10.52	3.48
C_{12}-4-C_{12}[iso-Pr(OH)]$_2$	818,93	58.68	10.51	3.45	58.67	10.58	3.42
C_{12}-5-C_{12}[iso-Pr(OH)]$_2$	832,96	59.13	10.58	3.37	59.12	10.65	3.36

Table 2. Surface activity parameters of the synthesized gemini surfactants (25 °C)[a]

Surfactants	CPC[b]×10^4, mol·dm^{-3}	CMC[b]×10^4, mol·dm^{-3}	CMC[c]×10^4, mol·dm^{-3}	γ_{CMC}, mN·m^{-1}	π_{CMC}, mN·m^{-1}	β	ΔG_{mic}, kJ·mol^{-1}	ΔG_{ad}, kJ·mol^{-1}
C_{12}-2-C_{12}[iso-Pr(OH)]	-	6.66	6.67	28.6	43,4	0,480	-55.01	-59.14
C_{12}-3-C_{12}[iso-Pr(OH)]	-	1.45	1.49	29.3	42,7	0,474	-62.03	-64.88
C_{12}-4-C_{12}[iso-Pr(OH)]	-	7.11	7.32	30.9	41,1	0,469	-54.08	-58.36
C_{12}-5-C_{12}[iso-Pr(OH)]	3.49	13.95	14.35	31.5	40,5	0,462	-50.48	-53.47
C_{12}-2-C_{12}[iso-Pr(OH)]$_2$	-	3.16	3.25	33.1	38,9	0,420	-55.04	-59.60
C_{12}-3-C_{12}[iso-Pr(OH)]$_2$	1.24	3.10	3.19	28.8	43,2	0,406	-54.29	-57.68
C_{12}-4-C_{12}[iso-Pr(OH)]$_2$	1.22	3.05	3.14	28.5	43,5	0,386	-53.17	-56.88
C_{12}-5-C_{12}[iso-Pr(OH)]$_2$	0.89	6.60	7.20	27.9	44,1	0,549	-58.93	-62.29

[a] The standard uncertainties-u are u(T) = 0.1 K and u(p) = 20 kPa. The combined expanded uncertainties U_c are $U_c(\Gamma_{max})$ = 0.01 mol/cm^2, $U_c(A_{min})$ = 0.5 Å^2, U_c(CMC) =10^{-6} mol/dm^3, $U_c(\pi)$ = 0.1 mN/m, $U_c(\gamma)$ = 0.1 mN/m and $U_c(pC_{20})$ = 0.002 (0.68 level of confidence).

[b] The CMC is determined by surface tension method.

[c] The CMC is determined by electroconductivity method.

Table 3. Results of study of antimicrobial properties of gemini surfactants by disk-diffusion method

Surfactants	Diameter of inhibition zone (mm)				
	Concentration, mg/ml	*S.aureus*	*E.coli*	*P.aeruginosa*	*Candida albicans*
C_{12}-2-C_{12}[iso-Pr(OH)]	8	14	17	15	-
	4	12	11	10	-
	2	-	8	-	-
C_{12}-3-C_{12}[iso-Pr(OH)]	8	17	15	14	14
	4	-	8	-	12
	2	-	-	-	-
C_{12}-4-C_{12}[iso-Pr(OH)]	8	14	12	10	9
	4	11	9	-	-
	2	8	-	-	-
C_{12}-5-C_{12}[iso-Pr(OH)]	8	36	21	11	44
	4	29	18	-	37
	2	21	9	-	26
C_{12}-2-C_{12}[iso-Pr(OH)]$_2$	8	22	15	10	10
	4	12	13	8	10
	2	10	-	-	-
C_{12}-3-C_{12}[iso-Pr(OH)]$_2$	8	11	12	10	18
	4	10	-	-	13
	2	-	-	-	9
C_{12}-4-C_{12}[iso-Pr(OH)]$_2$	8	16	12	16	20
	4	14	10	11	15
	2	12	-	-	-
C_{12}-5-C_{12}[iso-Pr(OH)]$_2$	8	41	15	10	32
	4	25	8	8	29
	2	22	8	-	15

Uncertainty-diameter of inhibition zone ±0.5 mm.

Table 4. Results of study of antibacterial effect of the synthesized gemini surfactants against SRB

Gemini surfactants	Concentration of gemini surfactants, mg/kg		
	15	75	150
C_{12}-2-C_{12}[iso-Pr(OH)]	10^3	Nil	Nil
C_{12}-3-C_{12}[iso-Pr(OH)]	10^4	10^3	10^1
C_{12}-4-C_{12}[iso-Pr(OH)]	10^2	10^1	10^1
C_{12}-5-C_{12}[iso-Pr(OH)]	10^5	10^4	10^2
C_{12}-2-C_{12}[iso-Pr(OH)]$_2$	10^4	10^3	10^2
C_{12}-3-C_{12}[iso-Pr(OH)]$_2$	10^5	10^4	10^1
C_{12}-4-C_{12}[iso-Pr(OH)]$_2$	10^4	10^2	10^1
C_{12}-5-C_{12}[iso-Pr(OH)]$_2$	10^2	10^1	10^1

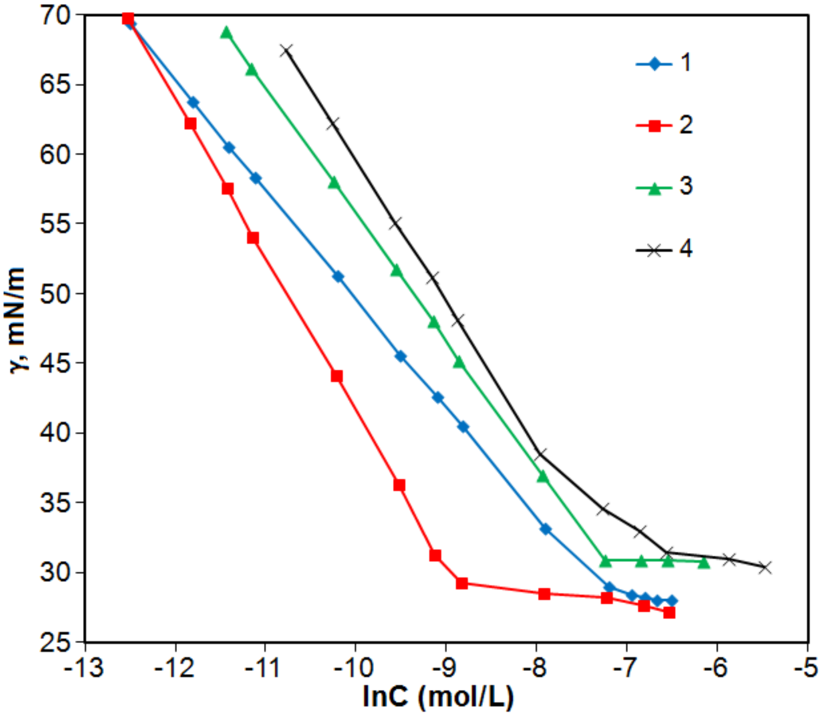


Fig. 1. Surface tension vs natural logarithmic concentration plot of C_{12}-s-C_{12}[iso-Pr(OH)]. 1. s=2; 2. s=3; 3. s=4; 4. s=5

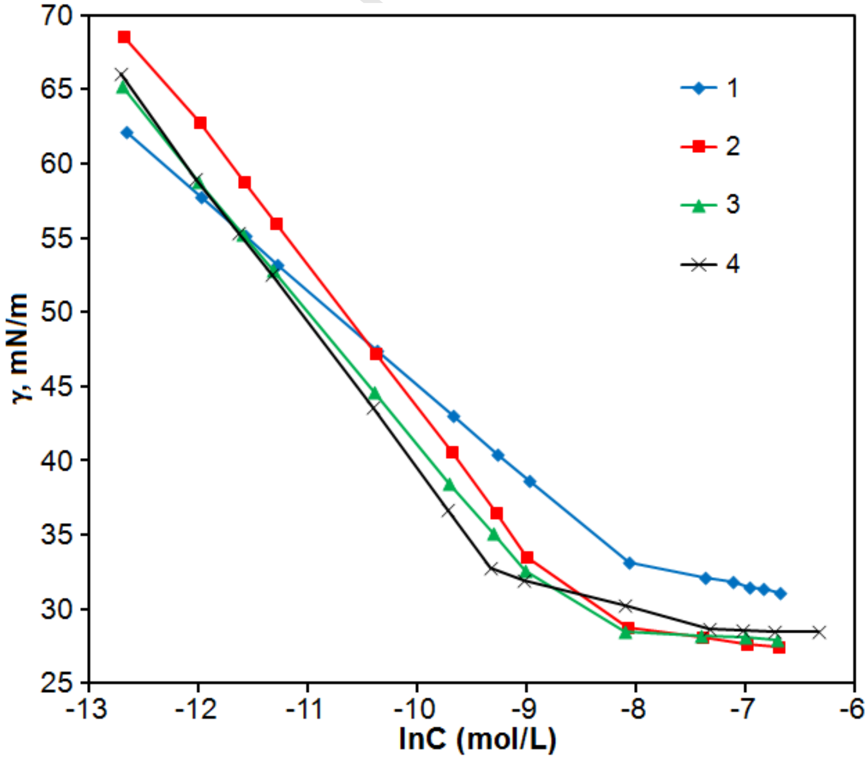


Fig. 2. Surface tension vs natural logarithmic concentration plot of C_{12}-s-C_{12}[iso-Pr(OH)]$_2$. 1. s=2; 2. s=3; 3. s=4; 4. s=5

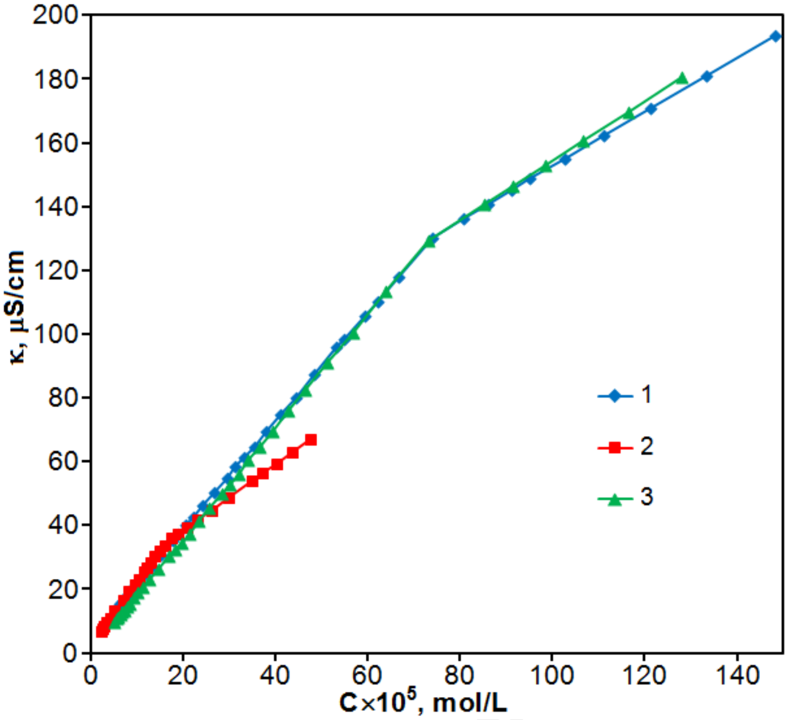


Fig. 3a. Variations of the specific conductivity **κ** vs the gemini surfactant concentration **C** for C_{12}-s-C_{12}[iso-Pr(OH)]. 1. s=2. 2. s=3. 3. s=4

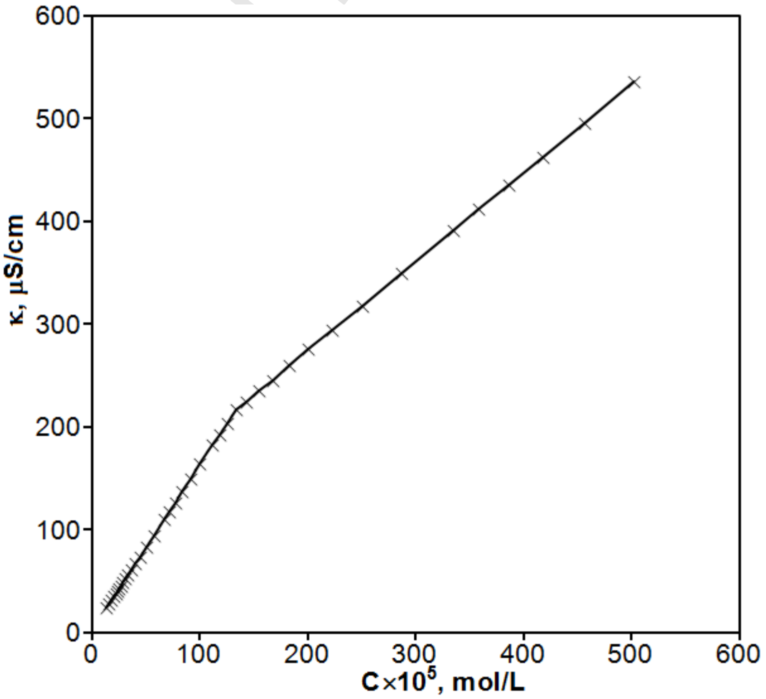


Fig. 3b. Variations of the specific conductivity **κ** vs the gemini surfactant concentration **C** for C_{12}-5-C_{12}[iso-Pr(OH)].

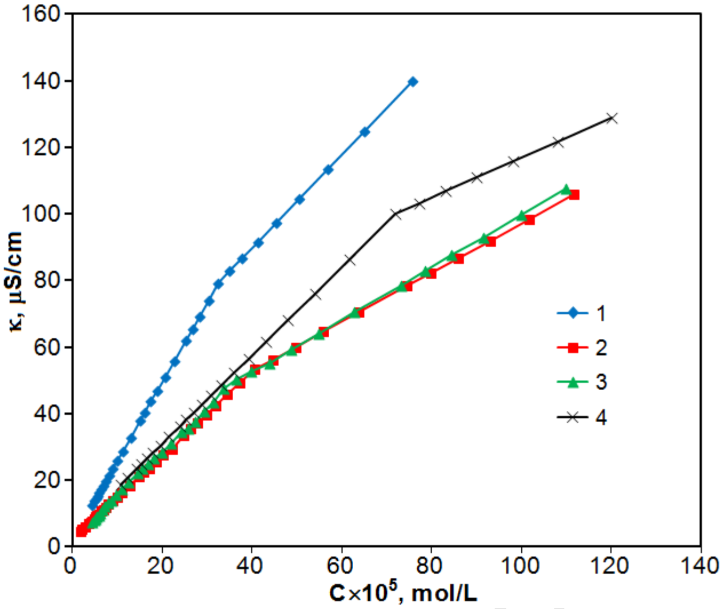


Fig. 4. Variations of the specific conductivity **κ** vs the gemini surfactant concentration **C** for C_{12}-s-C_{12}[iso-Pr(OH)]. 1. s=2. 2. s=3. 3. s=4; 4. s=5

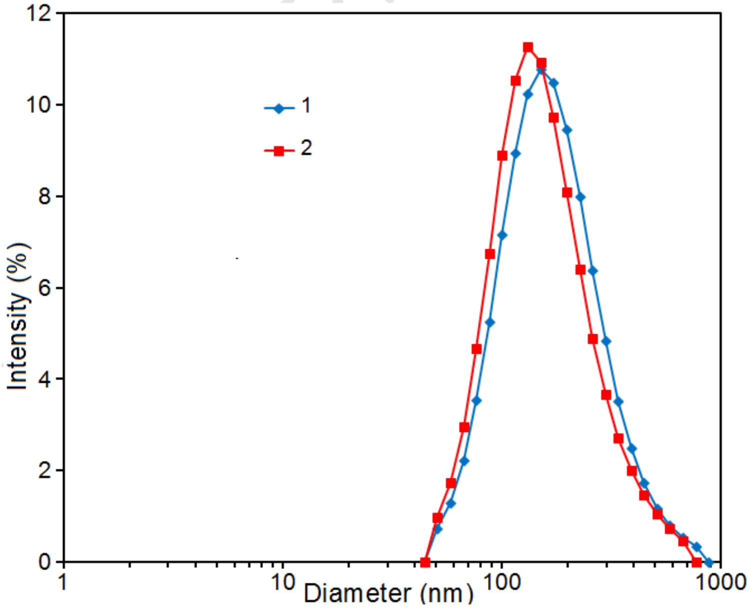


Fig. 5. DLS measurements of the size distributions for C_{12}-2-C_{12}[iso-Pr(OH)] at different concentrations. Concentration, % wt.: 1. 0.1; 2. 0.3

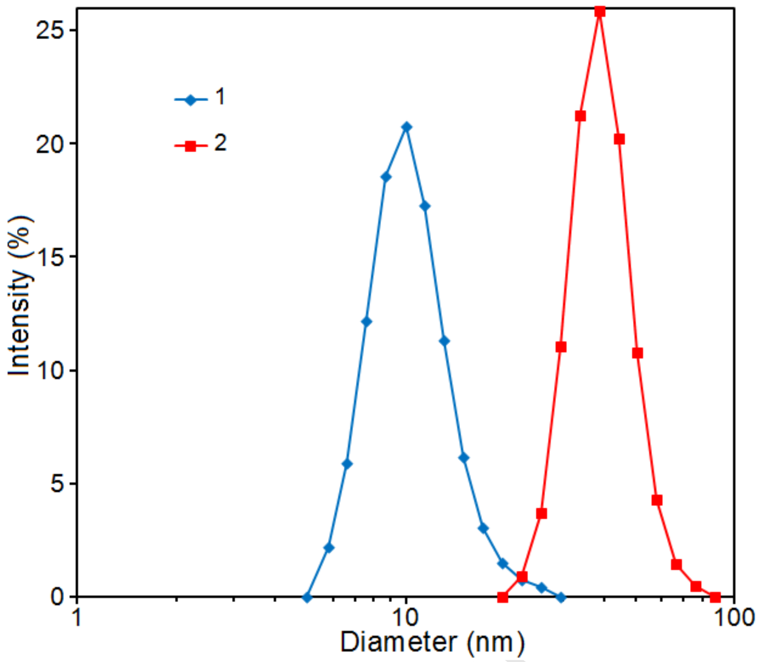


Fig. 6. DLS measurements of the size distributions for C_{12}-3-C_{12}[iso-Pr(OH)] at different concentrations. Concentration, % wt.: 1. 0.1; 2. 0.3

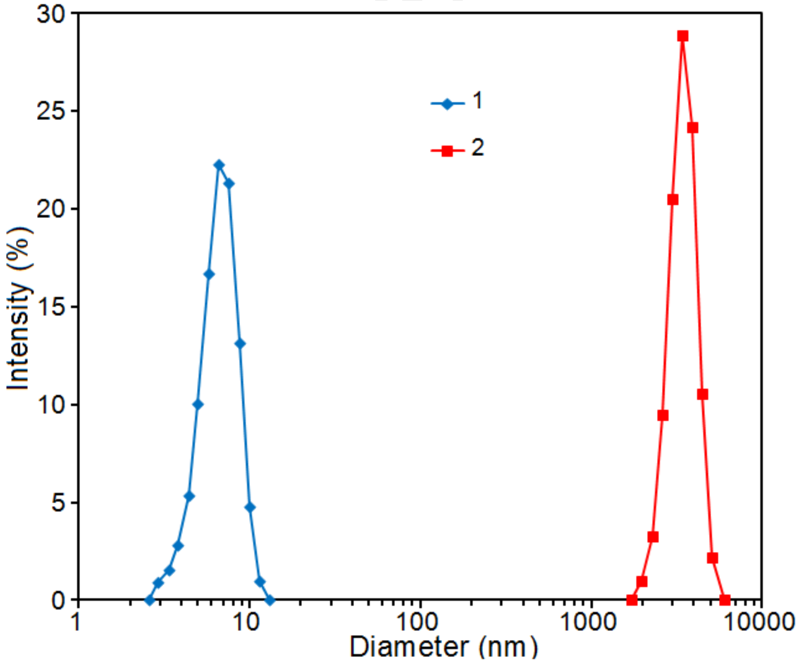


Fig. 7. DLS measurements of the size distributions for C_{12}-5-C_{12}[iso-Pr(OH)] at different concentrations. Concentration, % wt.: 1. 0.4; 2. 1.0

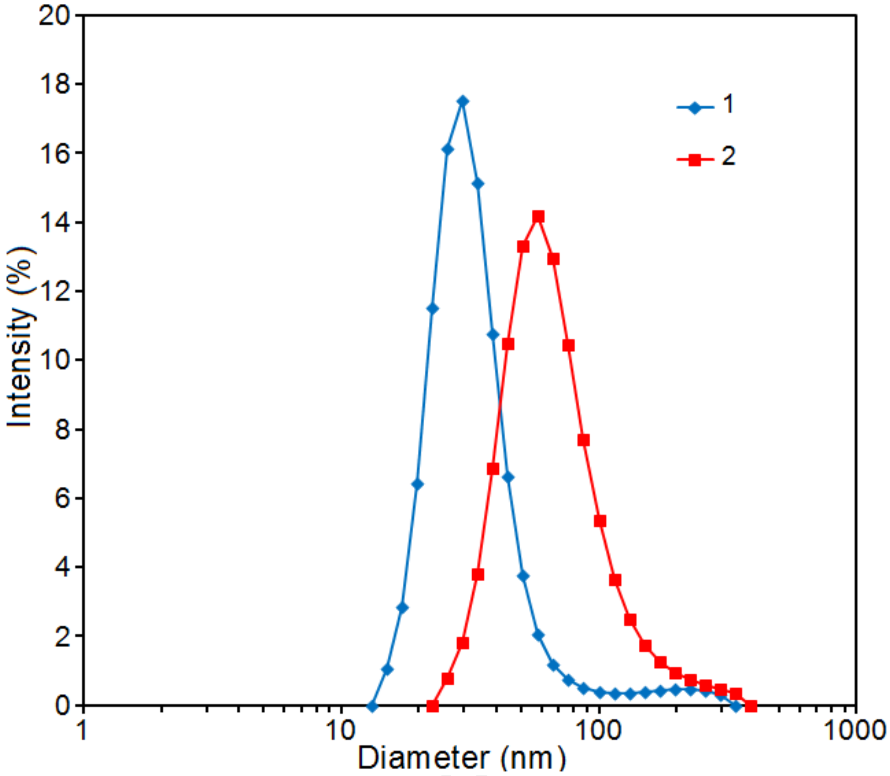


Fig. 8. DLS measurements of the size distributions for C_{12}-2-C_{12}[iso-Pr(OH)]$_2$ at different concentrations. Concentration, % wt.: 1. 0.1; 2. 0.3

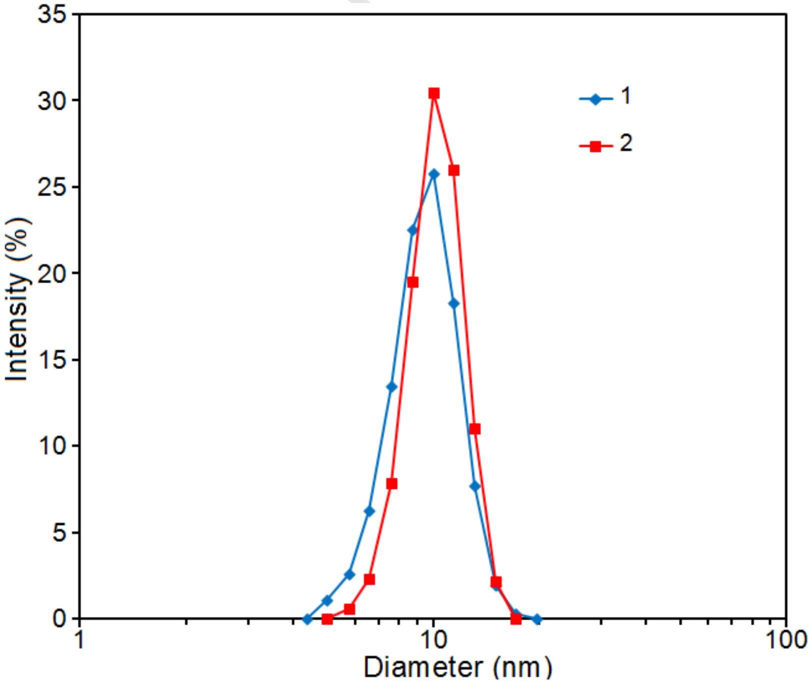


Fig. 9. DLS measurements of the size distributions for C_{12}-3-C_{12}[iso-Pr(OH)]$_2$ at different concentrations. Concentration, % wt.: 1. 0.1; 2. 0.3

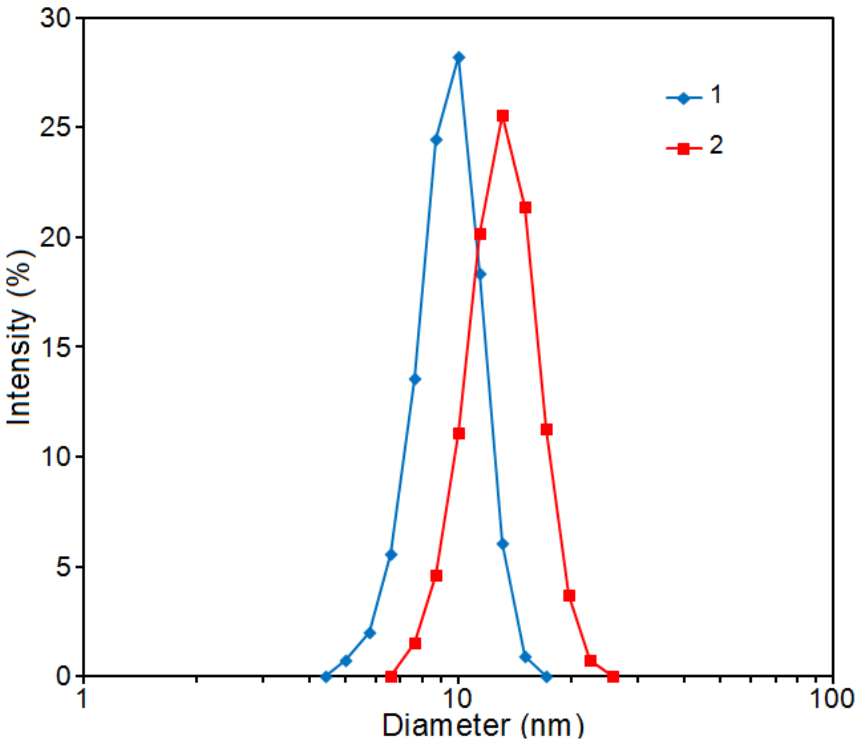


Fig. 10. DLS measurements of the size distributions for C_{12}-5-C_{12}[iso-Pr(OH)]$_2$ at different concentrations. Concentration, % wt.: 1. 0.12; 2. 0.3

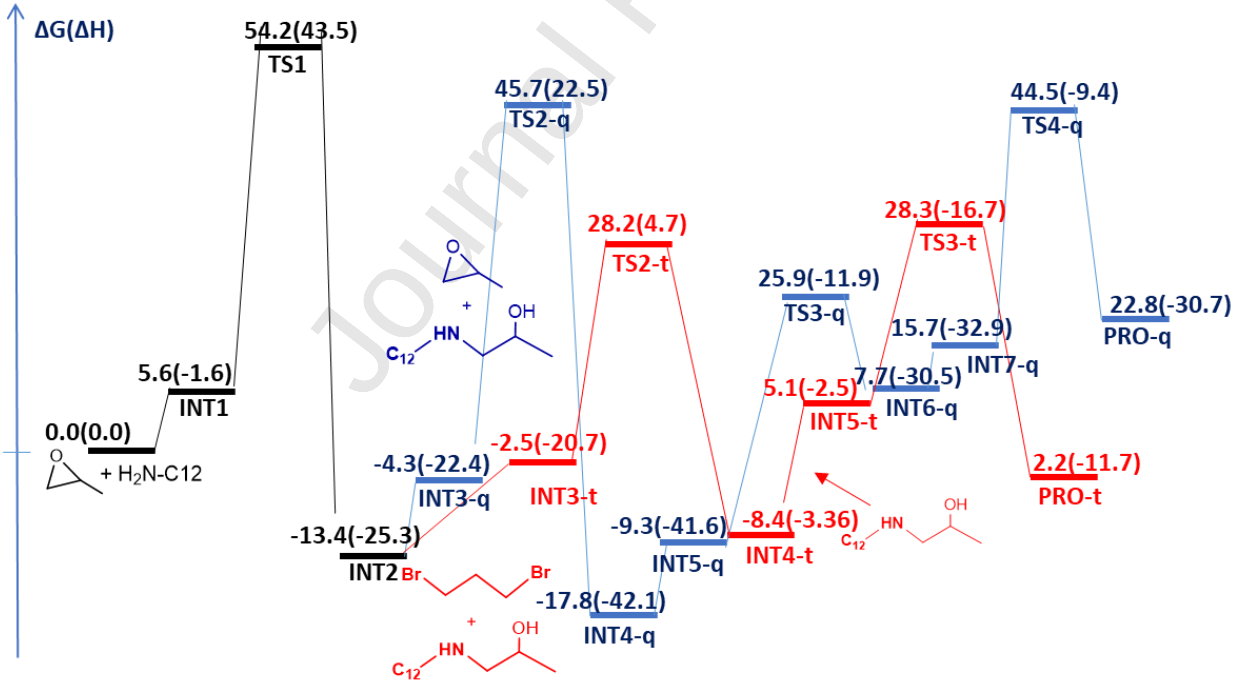


Fig. 11. Reaction profile for the formation of tertiary (**PRO-t**=C12-3-C12[iso-PrOH]) and quaternary (**PRO-q**=C12-3-C12[iso-PrOH]$_2$) gemini surfactants.

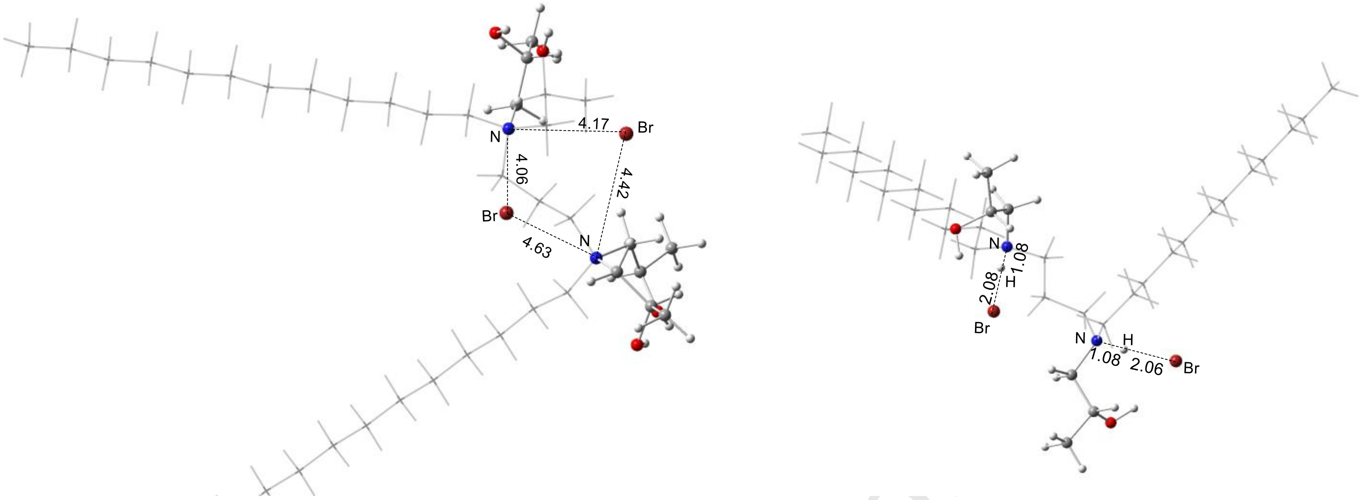
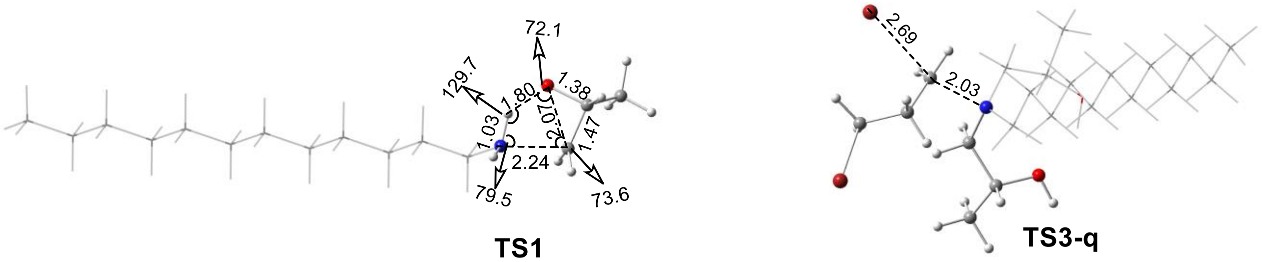


Fig. 12. Optimized structures with important bond lengths (Å) of **PRO-q** (left), **PRO-t** (right). Carbon chains are omitted for clarity.



TS1

TS3-q

Fig. 13. Optimized TS1 and TS3-q structures with important geometry parameters (Å and degree). Carbon chains are omitted for clarity.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

The authors declare with certain confidence that no financial interests, personal relationships or anything of similar nature that can have any kind of influence on the work presented in the current paper are known to them.

Highlights

- Gemini surfactants with $(CH_2)_n$ spacer groups and different head group have been synthesized.
- Self-assembly of gemini surfactants with different spacers is investigated.
- These cationic gemini surfactants exhibit a high antibacterial capacity.