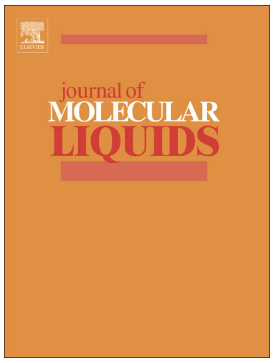

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Benzene ring containing cationic gemini surfactants: Synthesis, surface properties and antibacterial activity

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Abstract

New quaternary ammonium gemini surfactants of the general formula C_nH_{2n+1}-Ph-NHCOCH$_2$N$^+$(CH$_3$)$_2$-(CH$_2$)$_s$-N$^+$(CH$_3$)$_2$CH$_2$CONH-Ph-C_nH_{2n+1} (with n = 8, 10, 12, 14, 16 and s = 2, 4, 6) have been synthesized by an efficient synthetic pathway based on the quaternization of *N*,*N*,*N*′,*N*′-tetramethylalkylenediamine with 2-bromo-N-(4-(alkyloxy) phenyl) acetamides. Their surface properties were investigated by surface tension, electrical conductivity and dynamic light scattering (DLS) measurements. The study shows that the incorporation of a benzene ring in the hydrophobic tail prompts micelle formation which leads to a smaller cmc values in the range of 0.21-0.009 mM compared to analogous geminis bearing alkyl hydrophobic chains. The length of the spacer and the hydrophobic chain has a pronounced effect on the aggregation behavior of surfactants molecules. This was confirmed by the average surfaces occupied by these molecules at the water–air interface calculated from the Gibbs equation. The size of the aggregates was measured by employing dynamic light scattering technique. The antimicrobial activity of investigated surfactants was evaluated against three microorganisms: *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans.*

Keywords: Gemini surfactant, Quaternary ammonium salt, Surface-active properties, aggregates, antimicrobial activity

1. Introduction

Gemini surfactants, also called dimeric surfactants, are composed of two monomeric surfactant molecules linked covalently by a spacer group. Since the pioneer work of Bunton et al. on bisquatarnary ammonium bromide gemini surfactants [1], this class of compounds is gaining increasing attention due to their unique properties. In comparison with conventional surfactants, gemini surfactants are known to be much more efficient in reducing the surface tension of water and the interfacial tension of the oil-water interface [2-3]. Besides, they exhibit extremely lower CMC values, better water solubility, unusual micelle structures and aggregation behavior, and interesting rheological properties [4-6]. Thereby, they have find a wide range of applications in advanced technologic domains such as the fabrication of high-porosity materials [7], enhanced oil recovery process [8], genetic science and pharmaceutical applications, detergents, cosmetics, skin and personal care products manufacturing [9-14].

In the last decade, a variety of geminis have been designed and synthesized in the aim to highlight the effect of structural parameters on the physicochemical properties of related surfactants [15-17]. One of the most studied gemini surfactants are bis(quaternary alkylammonium bromide) surfactants [18]. The effect of the nature and the length of the spacer group and the hydrophobic chain have been systemically investigated. Alkyl chains have been usually used as hydrophobic tail of gemini surfactants whereas common spacers used to control the separation between the head groups are apolar aliphatic or aromatic group [19], polar polyether group [20], short $(CH_2)_2$ [21] or long $(CH_2)_{6\text{-}12}$ methylene group [22], rigid (stilbene [23-24], benzene [25-27], adamantine [28-29]) or flexible (polymethylene) groups [30].

It was shown that the length and the rigidity of the spacer group has a strong influence on different surfactant properties such as cmc, surface tension, water solubility and foaming ability [31]. For instance, Song and Rosen found that geminis with a flexible hydrophilic spacer aggregates more readily than homologous surfactants with a rigid hydrophobic spacer [32]. Similar conclusions were obtained by Wang et al. when comparing the aggregation properties of dicationic quaternary ammonium gemini surfactants bearing diethyl ether, hexyl or p-xylyl spacer group by using electrical conductivity and fluorescence measurements [33]. An opposite behavior was obtained for gemini quaternary ammonium salts bearing a rigid adamantine spacer. These compounds exhibited lower surface tension values as compared to conventional gemini quaternary ammonium salts and were able to pack more tightly at the air-

water interface thereby promoting self-association in the bulk [34]. Zhang et al. showed that the variation in the CMC values reported in the literature for geminis with spacers different in rigidity is mainly caused by changes in the other properties of the spacer such as the hydrophobicity, the chain length and the π–π stacking for phenyl spacers[35].

Besides these, only few studies have been devoted to the hydrophobic tail rigidity effect on the micellization behavior.

In this context, we report in this work a new family of cationic gemini surfactants bearing 4-alkylbenzene group on the hydrophobic chains, connected through an amide function, to the ammonium headgoup, named as Gem n-s-n (n = 8, 10, 12, 14, 16 and s = 2, 4, 6). The amide moiety is considered as a biodegradable linkage suitable for the preparation of eco-friendly surfactants [36]. The aim of this study is to clarify the effect of benzene ring on the surface properties of gemini surfactants and to highlight a structure-property relationship upon variation in different structural parameters such as hydrocarbon chain and spacer lengths. The surface and bulk properties of these surfactants such as critical micellar concentration (cmc), surface tension (γ_{cmc}), surface area occupied by a molecule at the air-water interface (A_{min}), degree of counterion binding (β) and standard free energy of micellization (ΔG^0_{mic}) have been discussed on the basis of surface tension, conductivity and dynamic light scattering measurements. The antibacterial activity against Gram-positve, Gram-negative and fungi microorganisms was also evaluated.

2. Experimental part

2.1. Characterization

Confirmation of the structures of the intermediates and products was obtained by nuclear magnetic resonance (NMR) and High resolution mass spectrometry (HRMS). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 300 MHz spectrometer (Wissembourg, France). Tetramethylsilane was used as an internal reference for chemical shifts. HRMS was carried out using a Finnigan Matt TSQ 7000 mass spectrometer (EIS mode) coupled with liquid chromatography interface.

Conductivity Measurements. Conductivities were determined using a digital conductimeter apparatus (OHAUS conductivity STARTER 300C). The cmc was determined by adding adequate quantity of a concentrated surfactant solution to water in order to change the surfactant concentration from concentrations well below the cmc to up to at least 1 to 2 times

the cmc. The solutions were thermostated in the cell at 25.0 ± 0.1 °C. The CMC values were estimated from the break point on each curve of conductivity versus surfactant concentration.

Surface Tension Measurements. The cmc and surface tensions at the cmc values were determined at 25°C, by the Wilhelmy plate method using a GIBERTINI K100 tensiometer at 25°C. The tensiometer was calibrated using Millipore water.

Dynamic light scattering (DLS) measurements. Particles size and polydispersity were measured at 25°C using a Zetasizer Nano-S model (Malvern Instruments Ltd.) equipped with a He–Ne laser (λ = 633 nm, 4.0 mW). The aqueous solutions were prepared using demineralized water and were filtered through a 0.45 μm filter. The time dependent correlation function of the scattered light intensity was measured at a scattering angle of 173° relative to the laser source (back-scattering detection). The Stokes radius (R_S) of the particles was estimated from their diffusion coefficient (D) using the Stokes–Einstein equation $D=k_BT/6\ \pi\eta R_S$, where k_B is Boltzmann's constant, T is the absolute temperature, and η is the viscosity of the solvent, All measurements were performed at 25.0 ± 0.1℃.

2.2. Materials

Chemicals used for the synthesis: 4-aminophenol, 1-bromooctane (99%), 1-bromodecane (98%), 1-bromododecane (97%), 1-bromotetradecane (97%), 1-bromohexadecane (97%), bromoacetyl chloride (95%) , *N*,*N*,*N*′,*N*′-Tetramethylethylenediamine (99%), *N*,*N*,*N*′,*N*′-Tetramethyl-1,4-butanediamine (98%), *N*,*N*,*N*′,*N*′-Tetramethyl-1,6-hexanediamine (99%), butanone (98%) were purchased from Sigma-Aldrich and used without further purification. All other reagents employed were common laboratory materials. The solvents were of commercial grade quality and were dried and distilled before use.

4-(alkyloxy)benzenamine (**2a-e**) were prepared using a three step procedure from 4-aminophenol according to our previously described procedure [37]. Detailed characterizations can be found in the Supporting information file.

2.2.1. Synthesis of 2-bromo-N-(4-(alkyloxy) phenyl) acetamide (3a-e)

2-bromo-N-(4-(Alkyloxy)phenyl) acetamide **(3a-e)** were synthesized via a slight modifications of the literature procedures [38]. 13 mmol of 4-(alkyloxy)benzenamine (**2a-e**)

were dissolved in 15 mL of dichloromethane then a solution of (2.76 g, 20 mmol) of potassium carbonate K_2CO_3 dissolved in 15 mL of water was added. The solution was cooled to 5 °C then (3.14 g, 20 mmol) of bromoacetyl chloride dissolved in 15 mL of dichloromethane were added dropwise. The reaction mixture was stirred at room temperature for about 3 h. The aqueous solution was separated and extracted two times with dichloromethane. The organic phase was washed with water then dried over anhydrous sodium sulphate and finally concentrated to yield a white product quantitatively. If required, it can be further purified by flash column chromatography using dichloromethane as eluent.

2-bromo-N-(4-(Octyloxy)phenyl)acetamide (3a): white solid, yield (quantitative), m.p.102°C, ^{1}H NMR (300 MHz, $CDCl_3$):δ 0.88 (t, 3H, CH_3, ^3J = 6.88Hz), 1.28 (m, 8H, $C_2H_5(CH_2)_4$), 1.44 (m, 2H, CH_3CH_2-), 1.76 (m, 2H, CH_2CH_2O-), 3.93 (t, 2H, CH_2O-, ^3J = 6.55 Hz), 4 .02 (s, 2H ,$COCH_2Br$), 6.86 (d, 2H, Ar-H , ^3J = 8.97 Hz), 7.39 (d, 2H, Ar-H, ^3J = 8.97Hz), 8.04 (s, 1H, NH).^{13}C NMR (300 MHz, $CHCl_3$): 14.14, 22.68, 26.04, 29.25, 31.83, 68.28, 114.83, 121.97, 129.67, 153.83, 163.15

2-bromo-N-(4-(decyloxy)phenyl)acetamide (3b): white solid, yield (99%), m.p.105°C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (t, 3H, CH_3, ^3J = 6.88Hz), 1.26 (m, 12H, $C_2H_5(CH_2)_6$), 1.44 (m, 2H, CH_3CH_2-), 1.76 (m, 2H, CH_2CH_2O-), 3.93 (t, 2H, CH_2O-, ^3J = 6.55 Hz),4 .01(s, 2H, $COCH_2Br$), 6.86 (d, 2H, Ar-H, ^3J = 8.97Hz),7.39 (d, 2H, Ar, ^3J = 8.97Hz), 8.05 (s,1H, NH).^{13}C NMR (300 MHz, $CDCl_3$): δ 14.16, 22.71,26.04, 29.25, 29.35, 29.58, 31.92,68.28, 114.83,121.97,129.66,156.65, 163.16.

2-bromo-N-(4-(dodecyloxy)phenyl)acetamide (3c): white solid, yield (95%), m.p.112°C,^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, 3H, CH_3, ^3J = 6.88Hz), 1.26 (m, 16H, $C_2H_5(CH_2)_8$),1.44 (m, 2H, CH_3CH_2-), 1.76 (m, 2H, CH_2CH_2O-), 3.93 (t, 2H, CH_2O-,^3J =6.55 Hz), 4.01(s, 2H ,$COCH_2Br$), 6.86 (d, 2H, Ar-H , ^3J = 9.00Hz),7.39 (d, 2H, Ar-H, ^3J = 9.00 Hz), 8.05 (s, 1H, NH).^{13}C NMR (300 MHz, $CDCl_3$): δ 14.15, 22.71, 26.03, 29.25, 29.41, 29.65, 31.93, 68.29, 114.84,121.96,129.69,156.66,163.16.

2-bromo-N-(4-(tetradecyloxy)phenyl)acetamide (3d): white solid, yield (85%),m.p.114°C.

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (t, 3H, CH_3, ^3J = 6.88Hz), 1.25 (m, 20H, $C_2H_5(CH_2)_{10}$), 1.42 (m, 2H, CH_3CH_2-), 1.76 (m, 2H, CH_2CH_2O-), 3.93 (t, 2H, CH_2O-, ^3J = 6.55 Hz), 4.01(s, 2H, $COCH_2Br$), 6.86 (d, 2H, Ar-H, ^3J = 9.0 Hz), 7.39 (d, 2H, Ar-H, ^3J = 9.0Hz), 8.06 (s, 1H, NH).^{13}C NMR (300 MHz, $CDCl_3$): δ 14.18, 22.73, 26.04, 29.25, 29.42, 29.69, 31.95, 68.27, 114.81,121.97,129.64,156.69,163.16.

2-bromo-N-(4-(hexadecyloxy)phenyl)acetamide (3e): white solid, yield (80%),m.p.120°C.

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, 3H, CH_3, ^{3}J = 6.88Hz), 1.25 (m, 24H, $C_2H_5(\underline{CH_2})_{12}$), 1.39 (m, 4H, $CH_3\underline{CH_2}$-), 1.76 (m, 2H, $\underline{CH_2}CH_2O$-), 3.93 (t, 2H, $\underline{CH_2}O$-, ^{3}J = 6.55 Hz), 4 .01(s, 2H, $CO\underline{CH_2}Br$), 6.86 (d, 2H, Ar-H, ^{3}J = 9.00Hz) ,7.39 (d, 2H, Ar-H, ^{3}J = 9.00Hz), 8.02 (s, 1H, N<u>H</u>).^{13}C NMR (300 MHz,$CDCl_3$): δ 14.10, 22.68, 26.02, 29.24, 29.49, 29.68, 31.92, 68.32, 114.88,121.94, 129.71,156.69,163.10.

2.2.2. Synthesis of Gemini surfactants (Gem n-s-n)

In a typical procedure, 20 mmol of 2-bromo-N-(4-(alkyloxy) phenyl) acetamide **(3a-e)** were added to 10 mmol of *N,N,N',N'*-tetramethylalkanediamine dissolved in 35 mL of diethyl ether. The reaction mixture was stirred for 72 hours at 40°C. The progress of the reaction was monitored by thin layer chromatography using a mixture of dichloromethane/ethyl acetate (90:10) as eluent. The obtained solid was filtered, washed several times with diethyl ether then recrystallized from pure ethanol to eliminate the residual conventional surfactant and give the gemini surfactant **(Gem n-s-n)** with 85−99% yield.

Gem 8-2-8: white solid, yield (90%). ^{1}H NMR (300 MHz, DMSO d^6): δ 0.85 (t, 6H, CH_3, ^{3}J = 6.90 Hz), 1.26 (m, 16H, $C_2H_5(\underline{CH_2})_4$), 1.39 (m, 4H, $CH_3\underline{CH_2}$-), 1.68 (m, 4H, $\underline{CH_2}CH_2O$-), 3.39 (s,12H, $(\underline{CH_3})_2N^+$), 3.90 (t, 4H, $\underline{CH_2}O$-, ^{3}J = 6.49Hz), 4.33 (s, 4H, $N^+(\underline{CH_2})_2N^+$), 4.42 (s, 4H, $CO\underline{CH_2}N^+$), 6.87 (d, 4H, Ar-H, ^{3}J = 9.08Hz), 7.46 (d, 4H, Ar-H, ^{3}J = 9.08 Hz), 10.58 (s, 2H, N<u>H</u>). ^{13}C NMR (300 MHz, DMSO d^6): δ 14.94, 22.06, 25.5, 28.66, 28.76, 31.22, 52.5, 55.61, 61.84, 67.50, 114.41,121.36, 130.37, 155.41, 160.91. HRMS (ESI): m/z 320.2458 (calculated), 320.2466 (found), $[M-2Br]^{2+}$.

Gem 10-2-10: white solid, yield (87%). ^{1}H NMR (300 MHz, DMSO d^6): δ 0.85 (t, 6H, ^{3}J = 6.76Hz, CH_3), 1.24 (m, 24H, $C_2H_5(\underline{CH_2})_6$), 1.38 (m, 4H, $CH_3\underline{CH_2}$-), 1.67 (m, 4H, $\underline{CH_2}CH_2O$-), 3.34(s, 12H, $(\underline{CH_3})_2N^+$), 3.89 (t, 4H, $\underline{CH_2}O$-, ^{3}J = 6.45 Hz), 4.31(s, 4H, $N^+(\underline{CH_2})_2N^+$), 4.39 (s, 4H, $CO\underline{CH_2}N^+$), 6.84 (d, 4H, Ar-H, ^{3}J = 9.06 Hz), 7.42 (d, 4H, Ar-H, ^{3}J = 9.04 Hz), 10.54 (s, 2H, N<u>H</u>). ^{13}C NMR (300MHz, DMSO d^6): 13.93, 22.08, 25.50, 28.69, 28.60, 28.96, 29.00, 31.27, 52.52, 55.58, 61.80, 67.49, 114.39, 121.34, 130.38, 155.40,160.90. HRMS (ESI): m/z 348.2771 (calculated), 348.2776 (found), $[M-2Br]^{2+}$.

Gem12-2-12: white solid, yield (88%).^{1}H NMR (300 MHz, DMSO d^6): δ 0.85 (t, 6H, ^{3}J = 6.87Hz, CH_3), 1.24 (m, 32H, $C_2H_5(\underline{CH_2})_8$), 1.38 (m, 4H, $CH_3\underline{CH_2}$-),1.68 (m, 4H, $\underline{CH_2}CH_2O$-), 3.38(s, 12H, $(\underline{CH_3})_2N^+$), 3.90 (t, 4H, $\underline{CH_2}O$-, ^{3}J = 6.48Hz), 4.32(s, 4H, $N^+(\underline{CH_2})_2N^+$), 4.40 (s,

4H, CO$\underline{CH_2}N^+$), 6.83 (d, 4H, Ar-H, ^3J = 9.04 Hz), 7.42 (d, 4H, Ar-H, ^3J = 9.03 Hz), 10.56 (s, 2H, N$\underline{H}$). ^{13}C NMR (300MHz, DMSO d^6):14.42, 22.56, 25.99, 29.18, 29.28, 29.48, 31.76, 53.09, 55.98, 58.46, 68.12, 114.91, 121.91, 130.86, 155.93, 161.50.

Gem14-2-14: white solid, yield (81%), ^{1}H NMR (300 MHz, DMSO d^6):δ 0.86 (t, 6H, ^3J = 6.82Hz, CH_3), 1.26 (m, 40H, $C_2H_5(\underline{CH_2})_{10}$), 1.40 (m, 4H, $CH_3\underline{CH_2}$-), 1.67 (m, 4H, $\underline{CH_2}CH_2O$-), 3.42 (s, 12H, $(\underline{CH_3})_2N^+$), 3.93 (t, 4H, $\underline{CH_2}$O-, ^3J = 6.49Hz), 4.35(s, 4H, $N^+(\underline{CH_2})_2N^+$), 4.45(s, 4H, CO$\underline{CH_2}N^+$), 6.86 (d, 4H, Ar-H, ^3J =8.95Hz),7.46 (d, 4H, Ar-H, ^3J =8.95Hz), 10.60 (s, 2H, N$\underline{H}$). ^{13}C NMR (300MHz, DMSO d^6):13.38, 21.57, 25.08, 28.18, 28.31, 28.50, 30.81, 52.29, 55.60, 62.29, 67.50, 114.32, 121.31, 130.04, 155.38, 164.47

Gem 16-2-16: white solid, yield (85%).^{1}H NMR (300 MHz, DMSO d^6): δ 0.86 (t, 6H, ^3J = 6.50Hz, CH_3), 1.25 (m, 48H, $C_2H_5(\underline{CH_2})_{12}$), 1.40 (m, 4H, $CH_3\underline{CH_2}$-), 1.69 (m, 4H, $\underline{CH_2}CH_2O$-),3.41 (s, 12H, $(\underline{CH_3})_2N^+$), 3.93 (t, 4H, $\underline{CH_2}$O-, ^3J = 6.23Hz), 4.34 (s, 4H, $N^+(\underline{CH_2})_2N^+$), 4.43 (s, 4H, CO$\underline{CH_2}N^+$), 6.86 (d, 4H, Ar-H, ^3J =8.57Hz), 7.45 (d, 4H, Ar-H, ^3J = 8.57Hz), 10.48 (s, 2H, N$\underline{H}$). ^{13}C NMR (300MHz, DMSO d^6): 14.23, 22.43, 25.95, 28.79, 29.04, 29.19, 29.39, 31.68, 53.18, 56.60, 68.45, 115.18, 122.18, 127.02,158.19, 166.17.

***Gem* 8-4-8:** white solid, yield (quantitative).^{1}H NMR (300 MHz, DMSO d^6): δ 0.85 (t, 6H, ^3J = 6.96Hz, CH_3), 1.26 (m, 16H, $C_2H_5(\underline{CH_2})_4$), 1.38 (m, 4H, $CH_3\underline{CH_2}$-),1.68 (m, 4H, $\underline{CH_2}CH_2O$-), 1.80 (m, 4H, $N^+CH_2(\underline{CH_2})_2CH_2N^+$), 3.26 (s, 12H, $(\underline{CH_3})_2N^+$), 3.61(s, 4H, $\underline{CH_2}N^+$), 3.91 (t, 4H, $\underline{CH_2}$O-, ^3J =6.50Hz), 4.32 (s, 4H, CO$\underline{CH_2}N^+$), 6.90 (d, 4H, Ar-H, ^3J = 9.04Hz), 7.50 (d, 4H, Ar-H, ^3J =9.04Hz), 10.59 (s, 2H, N$\underline{H}$). ^{13}C NMR (300MHz, DMSO d^6): 13.94, 19.24, 22.06, 25.48, 28.64, 28.72, 31.21, 51.32, 62.63, 63.47, 67.52, 114.51, 121.26, 130.51, 155.42, 161.24. HRMS (ESI): m/z 334.2615 (calculated), 334.2629 (found), $[M–2Br]^{2+}$.

Gem10-4-10: white solid, yield (100%).^{1}H NMR (300 MHz, DMSO d^6): δ 0.86 (t, 6H, ^3J = 6.34Hz, CH_3), 1.26 (m, 24H, $C_2H_5(\underline{CH_2})_6$), 1.40 (m, 4H, $CH_3\underline{CH_2}$-),1.69 (m, 4H, $\underline{CH_2}CH_2O$-), 1.88 (m, 4H, $N^+CH_2(\underline{CH_2})_2CH_2N^+$), 3.30(s, 12H, $(\underline{CH_3})_2N^+$), 3.66 (s, 4H, $\underline{CH_2}N^+$), 3.93 (t, 4H, $\underline{CH_2}$O-, ^3J = 6 .41Hz), 4.38 (s, 4H, $\underline{CH_2}N^+$), 6.90 (d, 4H, Ar-H, ^3J =8.53Hz), 7.52 (d, 4H, Ar-H, ^3J =8.53Hz), 10.57 (s, 2H, N$\underline{H}$).^{13}C NMR (300MHz, DMSO d^6): 13.88, 19.08, 21.57, 25.05, 28.17, 28.45, 30.81, 51.13, 62.51, 63.71, 67.50, 114.36, 121.67, 130.26, 155.32, 160.82.

Gem12-4-12: white solid, yield (97%).^{1}H NMR (300 MHz, DMSO d^{6}): δ 0.84 (t, 6H, ^{3}J = 6.86Hz, CH_3), 1.23 (m, 32H, $C_2H_5(CH_2)_8$), 1.37 (m, 4H, CH_3CH_2), 1.67 (m, 4H, CH_2CH_2O-), 1.82 (m, 4H, $N^+CH_2(CH_2)_2CH_2N^+$), 3.27(s, 12H, $(CH_3)_2N^+$), 3.63 (s, 4H, CH_2N^+), 3.90 (t, 4H, CH_2O-, ^{3}J = 6.45Hz), 4.36 (s, 4H, CH_2N^+), 6.87 (d, 4H, Ar-H, ^{3}J = 9.02Hz), 7.50 (d, 4H, Ar-H, J=9.02Hz), 10.62 (s, 2H, NH).^{13}C NMR (300MHz, DMSO d^{6}): 13.92, 19.27, 22.09, 25.56, 28.67, 28.79, 31.29, 51.32, 62.36, 63.54, 67.52, 114.45, 121.24, 130.56, 155.41, 161.23.

Gem14-4-14: white solid, yield (90%).^{1}H NMR (300 MHz, DMSO d^{6}): δ 0.84 (t, 6H, ^{3}J = 6.78Hz, CH_3), 1.23 (m, 40H, $C_2H_5(CH_2)_{10}$), 1.37 (m, 4H, CH_3CH_2-),1.67 (m, 4H, CH_2CH_2O-),1.81 (m, 4H, $N^+CH_2(CH_2)_2CH_2N^+$), 3.27 (s, 12H, $(CH_3)_2N^+$), 3.62 (s, 4H, CH_2N^+), 3.90 (t, 4H, CH_2O, ^{3}J=6.43Hz), 4.33 (s, 4H, CH_2N^+), 6.88 (d, 4H, Ar-H, ^{3}J = 8.96Hz), 7.50 (d, 4H, Ar-H, ^{3}J = 8.96Hz), 10.60 (s, 2H, NH).^{13}C NMR (300MHz, DMSO d^{6}): 13.87, 19.19, 22.01, 25.43, 28.64, 28.72, 28.92, 28.99, 31.21, 51.31, 61.12, 62.40, 63.49, 67.49, 114.45, 121.20, 130.50, 155.38, 161.18.

Gem16-4-16: white solid, yield (93%). ^{1}H NMR (300 MHz, DMSO d^{6}): δ 0.85 (t, 6H, ^{3}J = 6.97Hz, CH_3), 1.25 (m, 48H, $C_2H_5(CH_2)_{12}$), 1.40 (m, 4H, CH_3CH_2-),1.69 (m, 4H, CH_2CH_2O-), 1.80 (m, 4H, $N^+CH_2(CH_2)_2CH_2N^+$), 3.28 (s, 12H, $(CH_3)_2N^+$), 3.64(s, 4H, CH_2N^+), 3.93 (t, 4H, CH_2O, ^{3}J =6 .50Hz), 4.33 (s, 4H, $COCH_2N^+$), 6.90 (d, 4H, Ar-H, ^{3}J = 9.03Hz), 7.48 (d, 4H, Ar-H, ^{3}J =9.03Hz), 10.49 (s, 2H, NH).^{13}C NMR (300MHz, DMSO d^{6}):13.46, 19.08, 21.65, 25.13, 28.25, 28.36, 28.59, 30.88, 51.28, 62.53, 63.58, 67.55, 114.43, 121.28, 130.19, 155.41, 160.89.

Gem 8-6-8: white solid, yield (89%).^{1}H NMR (300 MHz, DMSO d^{6}): δ 0.85 (t, 6H, ^{3}J = 6.89Hz, CH_3), 1.25 (m, 16H, $C_2H_5CH_2)_4$), 1.34 (m, 4H, CH_3CH_2),1.34 (m, 4H, $N^+C_2H_4(CH_2)_2C_2H_4N^+$), 1.67 (m, 4H, CH_2CH_2O), 1.80 (m, 4H, $N^+CH_2CH_2$), 3.27 (s, 12H, $(CH_3)_2N^+$), 3.54 (s, 4H, N^+CH_2), 3.90 (t, 4H, CH_2O, ^{3}J = 6.49Hz), 4.36 (s, 4H, $COCH_2N^+$), 6.89 (d, 4H, Ar-H, ^{3}J =9.06 Hz), 7.52 (d, 4H, Ar-H, ^{3}J = 9.05Hz), 10.66 (s, 2H, NH).^{13}C NMR (300 MHz, DMSO d^{6}): 14.40, 21.15, 22.54, 25.57, 25.97, 29.19, 29.21, 31.69, 51.77, 62.53, 64.99, 68.05, 115.01, 121.71, 131.10, 155.93, 161.79. HRMS (ESI): m/z 348.2771 (calculated), 348.2270 (found), $[M-2Br]^{2+}$.

Gem 10-6-10: white solid, yield (89%). ^{1}H NMR (300 MHz, DMSO d^{6}): δ 0.84 (t, 6H, ^{3}J = 6.87Hz, CH_3), 1.24 (m, 24H, $C_2H_5CH_2)_6$), 1.33 (m, 8H, CH_3CH_2 and $N^+C_2H_4(CH_2)_2C_2H_4N^+$), 1.67 (m, 4H, CH_2CH_2O), 1.79 (m, 4H, $N^+CH_2CH_2$), 3.26 (s, 12H, $(CH_3)_2N^+$), 3.54 (m, 4H,

$N^+\underline{CH_2}$), 3.90 (t, 4H, $\underline{CH_2}O$, ^3J =6.45Hz), 4.34 (s, 4H, $CO\underline{CH_2}N^+$),6.88 (d, 4H, Ar-H, ^3J = 9.02Hz), 7.48 (d, 4H, Ar-H, ^3J = 9.02 Hz), 10.62 (s, 2H, N$\underline{H}$).^{13}C NMR (300MHz, DMSO d^6): 13.88, 21.60, 22.02, 25.05, 25.43, 28.60, 28.71, 28.88, 28.93, 31.22, 51.23, 62.10, 64.30, 67.47, 114.96, 121.17, 130.49, 155.37, 161.25.

Gem12-6-12: white solid, yield (88%).^{1}H NMR (300 MHz, DMSO d^6): δ 0.84 (t, 6H, ^3J = 6.86 Hz, CH_3), 1.23 (m, 32H, $C_2H_5\underline{(CH_2)_8}$), 1.33 (m, 8H, $CH_3\underline{CH_2}$ and $N^+C_2H_4\underline{(CH_2)_2}C_2H_4N^+$), 1.67 (m, 4H, $\underline{CH_2}CH_2O$), 1.78 (m, 4H, $N^+CH_2\underline{CH_2}$) , 3.25 (s, 12H, $\underline{(CH_3)_2}N^+$), 3.54(m, 4H, $N^+\underline{CH_2}$), 3.90 (t, 4H, $\underline{CH_2}O$, ^3J = 6.46Hz), 4.30 (s, 4H, $CO\underline{CH_2}N^+$), 6.89 (d, 4H, Ar-H, ^3J = 8.99Hz), 7.48 (d, 4H, Ar-H, ^3J = 8.98Hz), 10.54 (s, 2H, N$\underline{H}$).

Gem14-6-14 : white solid, yield (92%).^{1}H NMR (300 MHz, DMSO d^6): δ 0.85 (t, 6H, ^3J = 6.70Hz, CH_3), 1.25 (m, 40H, $C_2H_5\underline{(CH_2)_{10}}$), 1.37 (m, 8H, $CH_3\underline{CH_2}$ and $N^+C_2H_4\underline{(CH_2)_2}C_2H_4N^+$), 1.68 (m, 4H, $\underline{CH_2}CH_2O$), 1.81 (s, 4H, $N^+CH_2\underline{CH_2}$), 3.26 (s, 12H, $\underline{(CH_3)_2}N^+$), 3.55(s, 4H, $N^+\underline{CH_2}$), 3.92 (t, 4H, $\underline{CH_2}O$, ^3J =5.90 Hz), 4.32 (s, 4H, $CO\underline{CH_2}N^+$), 6.88 (d, 4H, Ar-H, ^3J =8.29Hz), 7.49 (d, 4H, Ar-H, ^3J =8.29Hz), 10.48 (s, 2H, N$\underline{H}$). ^{13}C NMR (300 MHz, DMSO d^6):14.24, 22.19, 22.43, 25.59, 25.91, 29.04, 29.15, 29.37, 31.67, 51.97, 63.09, 65.29, 68.33,115.21,121.99,130.05,156.16,161.76.

Gem16-6-16 : white solid, yield (94%). ^{1}H NMR (300 MHz, DMSO d^6): δ 0.86 (t, 6H, ^3J = 6.73Hz, CH_3), 1.25 (m, 48H, $C_2H_5\underline{(CH_2)_{12}}$), 1.37 (m, 8H, $CH_3\underline{CH_2}$ and $N^+C_2H_4\underline{(CH_2)_2}C_2H_4N^+$), 1.68 (m, 4H, $\underline{CH_2}CH_2O$), 1.81 (s,4H, $N^+CH_2\underline{CH_2}$) , 3.26 (s, 12H, $\underline{(CH_3)_2}N^+$), 3.54 (s, 4H, $N^+\underline{CH_2}$), 3.92 (t, 4H, $\underline{CH_2}O$, ^3J = 6.46Hz), 4.32 (s, 4H, $CO\underline{CH_2}N^+$), 6.89 (d, 4H, Ar-H, ^3J =8.88Hz), 7.50 (d, 4H, Ar-H, ^3J = 8.78Hz), 10.50 (s, 2H, N$\underline{H}$).^{13}C NMR (300 MHz, DMSO d^6): 14.20, 22.21, 22.40, 25.60, 25.90, 29.07, 29.14, 29.34, 31.64, 52.08, 63.19, 65.32, 68.43, 115.31, 122.1, 133.93, 156.09, 161.82

2.3. Antimicrobial activity.

Antibacterial and antifungal activities of Gem n-s-n surfactants were evaluated using measurement of Minimal Inhibitory Concentration (MIC; lowest concentration of compound at which *in vitro* tested microorganism do not show visible growth after incubation [39]) and Minimal Lethal Concentrations (MLC; lowest concentration of antimicrobial agent that will *in vitro* kill microorganism and consequently prevents it's growth after subculture onto antibiotic-free media). Bacterial and mycological nutrient broths where used for bacteria and

fungi respectively. Gram-positive bacteria strain *Staphylococcus aureus* (ATCC 25923); Gram-negative bacteria strain *Escherichia coli* (ATCC 2592 and fungal strain *Candida albicans (*ATCC 10231) were used for this study. Standardized inoculum of bacteria and fungal strains were prepared by incubating the microorganisms in nutrient bacterial (24 h at 37°C) and mycological broths (48h at 25°C) and then diluted to approximately 10^6 cfu/mL. For MIC determination the studied compounds were dissolved in double distilled water and sterilized (filtration, 0,45 µm, Millipore). 1 mL of each concentration (concentration range 2-512 µg/mL) of the Gem n-s-n surfactant was added to 1 mL of sterilized nutrient broth in a hemolysis tube then inoculated with 20 µL of standardized microbial inoculum. A negative control sample was made up without surfactant. Triplicate experiments were carried out for each concentration. The MLC were determined from aliquots taken from MIC tubes by inoculating three PCA (plate count agar) plates with 20 µL of the concentrations above the MIC. Plates were incubated for 24 h at 37°C for antibacterial test and for 72 h at 25°C for antifungal test.

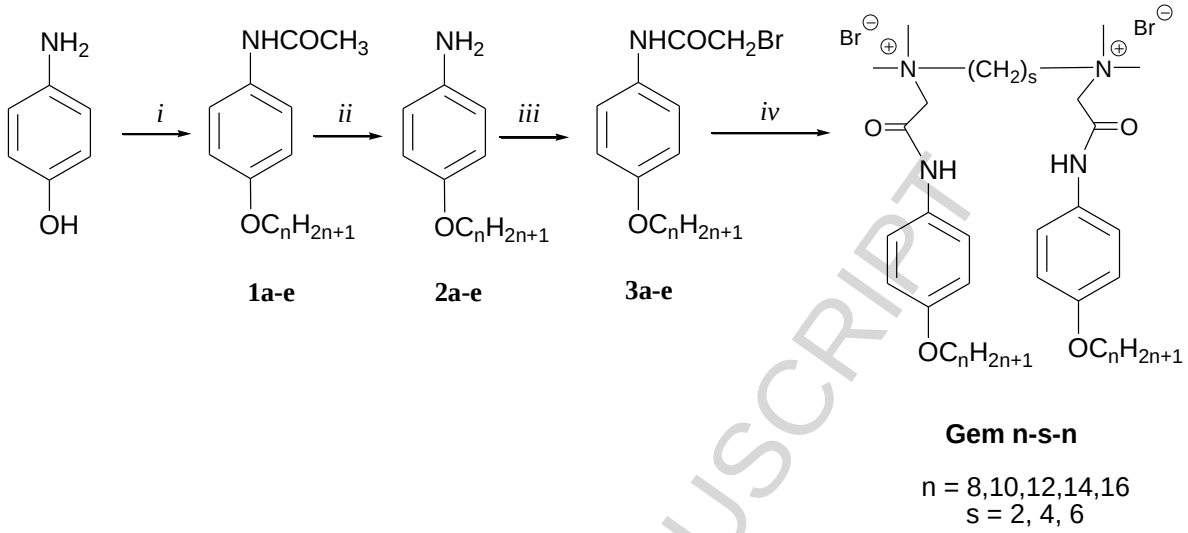
3. Results and discussion.

3.1 Synthesis

Gemini surfactants with different spacer length (s = 2, 4, 6) and hydrophobic chain length (n = 8, 10, 12, 14, 16) were prepared according to the straightforward synthetic procedure illustrated in scheme 1.

The general procedure is based on the quaternization of commercially available *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, *N*,*N*,*N*′,*N*′-tetramethyl-1,4-butanediamine or *N*,*N*,*N*′,*N*′-tetramethyl-1,6-hexanediamine with 2-bromo-N-(4-(alkyloxy) phenyl) acetamides (**3a-e**) under reflux of diethyl ether during 3 days. These latter derivatives were prepared in four-stage process starting from 4-aminophenol. The initial step consists of the reaction of 4-aminophenol with acetic anhydride followed by the etherification with the suitable alkyl bromide compound in the presence of potassium carbonate. The amine function of resulted compounds is then deprotected under reflux of a concentrated hydrochloric acid solution. The reaction of 4-(alkyloxy)benzenamine (**2a-e**) with bromoacetyl chloride in the presence of an aqueous solution of potassium carbonate at room temperature affords the 2-bromo-N-(4-(alkyloxy) phenyl) acetamides intermediates (**3a-e**) with almost quantitative yields.

After quaternization reaction, the gemini surfactants were recovered by filtration then recrystallized from ethanol to give the final products in good yields and high purity. The surfactants were characterized by ^{1}H and ^{13}C NMR and high resolution mass spectrometry.



Scheme 1. Synthetic pathway of gemini surfactants Gem n-s-n 1. *(i)* 1. Acetic anhydride, H_2O, 110°C, 2. $C_nH_{2n+1}Br$ (n= 8, 10, 12, 14, 16), K_2CO_3, butanone, reflux; (ii) HCl, H_2O, reflux; *(iii)* $BrCH_2COCl$, rt; *(iv)* $(CH_3)_2N(CH_2)_sN(CH_3)_2$ with s = 2, 4, 6, Et_2O, reflux.

3.2. Surface activity

In the aim to investigate the surface activity of synthesized gemini surfactants and highlight the effect of their molecular structure on the adsorption behavior, we determine the equilibrium surface tensions as function of surfactant concentrations using the Wilhelmy plate method. Surface tension (γ) *versus* the logarithm of the concentration of Gem n-2-n series plots are shown, as an example, in figure 1. The breakpoint of the curves can be correlated to critical micelle concentrations. The absence of a minimum around these points indicates the purity of surfactants. The cmc and γ_{cmc} values of Gem n-s-n surfactants are collected in table1.

Table 1.Surface activity parameters of gemini surfactants Gem n-s-n

Surfactant	**cmc** [a] **(mol/L)**	γ_{cmc} **(mN/m)**	**cmc**[b] **(mol/L)**	β	ΔG^0_{mic} **(kJ/mol)**	pC_{20}	**cmc/C_{20}**	Γ_{max} **(µmol/m^2)**	A_{min} **(nm^2)**
Gem 8-2-8	2.51 x 10^{-4}	30.4	3.22 x 10^{-4}	0.81	-39.94	4.60	9.99	1.11	1.49
Gem 8-4-8	2.08 x 10^{-4}	32.0	2.13 x 10^{-4}	0.69	-36.95	4.67	9.73	1.14	1.46
Gem 8-6-8	1.09 x 10^{-4}	36.0	1.17 x 10^{-4}	0.70	-38.85	4.92	9.07	0.99	1.66
Gem 10-2-10	4.07 x 10^{-5}	33.1	4.82 x 10^{-5}	0.75	-43.22	5.53	1.38	1.13	1.47
Gem 10-4-10	2.07 x 10^{-5}	41.6	4.37 x 10^{-5}	0.57	-37.22	5.31	4.23	1.06	1.57
Gem 10-6-10	1.09 x 10^{-5}	45.0	2.04 x 10^{-5}	0.50	-38.26	5.02	1.15	1.22	1.36
Gem 12-2-12	3.71 x 10^{-5}	44.6	3.44 x 10^{-5}	0.51	-35.76	4.69	1.82	1.80	0.92
Gem 12-4-12	1.42 x 10^{-5}	44.8	2.16 x 10^{-5}	0.57	-39.09	4.97	1.33	1.65	1.01
Gem 12-6-12	1.04 x 10^{-5}	49.0	8.29 x 10^{-6}	0.53	-40.26	5.06	1.19	2.01	0.83
Gem 14-2-14	2.08 x 10^{-5}	47.0	2.34 x10^{-5}	0.44	-34.04	4.8	1.31	2.36	0.70
Gem 14-4-14	1.18 x 10^{-5}	46.0	2.51 x 10^{-5}	0.51	-36.52	4.97	1.10	1.96	0.84
Gem 14-6-14	2.75 x 10^{-5}	51.1	2.50 x 10^{-5}	0.56	-38.23	4.86	1.99	1.81	0.92
Gem 16-2-16	1.40 x 10^{-5}	46.0	/	/	/	5.35	3.13	0.87	1.91
Gem 16-4-16	9.00 x 10^{-6}	48.0	1.98 x 10^{-5}	0.47	-35.67	5.49	2.78	1.24	1.34
Gem 16-6-16	2.88 x 10^{-5}	45.0	2.34 x 10^{-5}	0.47	-35.42	4.87	2.13	1.72	0.97

[a] cmc from surface tension, [b] cmc from conductivity

As expected, all the investigated bisammonium surfactants displayed a micellization process in water with a very low cmc values in the range of (0.21-0.009 mM) much lower than conventional surfactants as dedecyltrimethyl ammonium bromide DTAB (0.846 mmol [40]) and dodecyl N-ethanamine N,N,N-trimethyl ammonium bromide $C_{12}H_{25}$-$NHCOCH_2N^+(CH_3)_3$ Br^- (2.9 mM [38]). This indicates that gemini surfactants have a stronger tendency to self-assemble in aqueous solution at lower concentrations than single chain monomeric surfactants probably because of the increase of hydrophobic interactions. This trend is in good agreement with those reported for many Gemini surfactants.

The positive effect of introduction of a rigid oxyphenyl group on the terminal hydrophobic chain can be clearly highlighted by comparing the micellization data of previously reported gemini ammonium surfactant analogous bearing amide functions and flexible dodecylalkyl hydrophobic terminal chains $C_{12}H_{25}$-$NHCOCH_2N^+(CH_3)_2(CH_2)_s$-$N^+(CH_3)_2CH_2CONHC_{12}H_{25}$ (CMC = 0.30, 0.23, 0.19, 0.14, 0.11 mM for s = 2, 4, 6, 8 and 12 respectively [38]) with newly synthesized Gemini surfactants Gem 12-2-12, Gem 12-4-12 and Gem 12-6-12 with 4-dodedecylphenyl chains (0.0371, 0.0142, 0.0104 mM). The latter surfactants exhibit 8-10 times lower CMC values indicating their outstanding surface activity.

These results suggest that the inclusion of a phenylene unit on the hydrophobic chain favored micellization and can be explained from several factors. The benzene ring can acts as: a) a hydrophobic unit since it is commonly admitted that a benzene group is roughly equivalent in its effect on the cmc to three and a half methylene groups [41]; b) a rigid bulky spacer connecting the terminal alkyl chain and the ionic head thereby affecting the orientation of the hydrophobic tail and the self-association of surfactant molecules; c) it can also induce π-π interactions among the adjacent molecules which should be a favorable factor for aggregation of surfactant molecules.

This trend is in good agreement with that reported for conventional quaternary ammonium surfactants containing a phenylene unit within a long alkyl hydrophobic chain. De et al. showed that the formation and the structure of micelles are less hindered by a phenyl group located near the head group than in a terminal position of the hydrophobic tail [42]. In this latter case, smaller aggregates with significantly fewer surfactants were formed due the coalescence of the alkylene segments. Similar results were obtained by Li et al. for poly(oxyethylene) glycol alkyl ethers nonionic surfactants with a benzene ring in the hydrophobic chains [43].

A progressive fall in cmc values of investigated gemini surfactants was noted in accordance with the lengthening of the alkyl chain (n) and to lesser extent with the spacer chain (s) (figure 2). This is likely due to the enhancement of hydrophobicity with the number of methylene group in the chains which makes the aggregation easier for surfactant molecules in the bulk solution.

A distinct behavior was noticed for compounds Gem14-6-14 and Gem 16-6-16 bearing the longer spacer chain (s = 6) and hydrophobic chain (n = 14 and 16) lengths. Higher cmc values were reached by these compounds indicating a minimum stability of the surfactant in the micellar state. Nevertheless, the difference between these two gemini surfactants with varying lengths of the hydrophobic chain (tetradecyl and hexadecyl) was small.

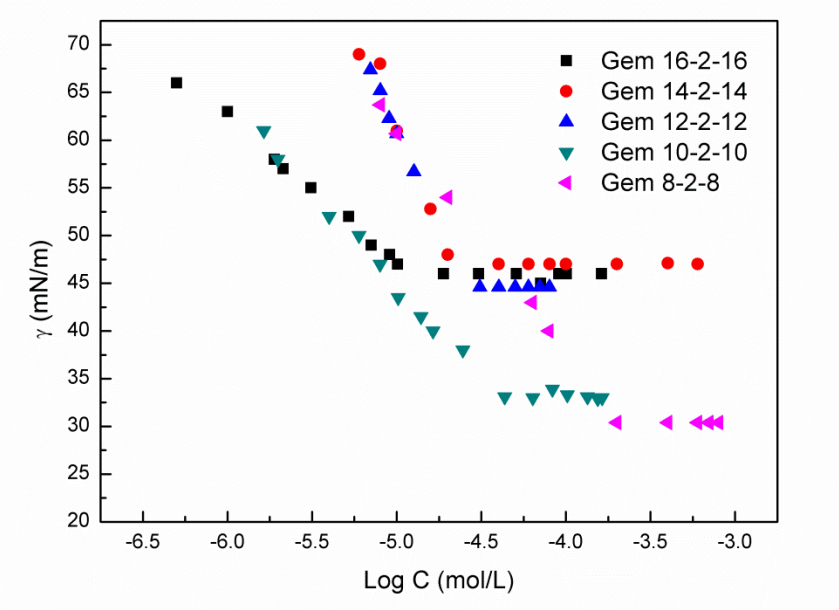


Fig.1 Surface tension curves of Gem n-2-n surfactants with n = 8, 10,12,14,16

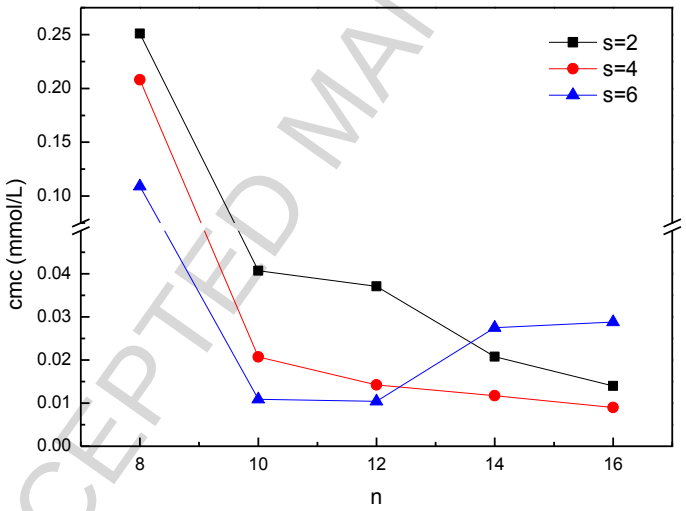


Fig. 2 Variation of the cmc with the hydrocarbon carbon number n for Gem n-2-n, Gem n-4-n and Gem n-6-n surfactants.

An important parameter to evaluate the surface activity of surfactants is the packing density of surfactant molecules at the air-water interface. The maximum surface excess concentration (Γ_{max}) and the minimum surface area per molecule (A_{min}) at the air-water interface were calculated using the Gibbs adsorption equations (1) and (2) [44].

$$\Gamma_{max} = -\left(\frac{1}{n \times 2.30RT}\right)\left(\frac{\partial \gamma}{\partial \log C}\right)_T \quad (1)$$

$$\mathbf{A_{min}} = \frac{\mathbf{10^{16}}}{\Gamma_{max} \times N_A} \qquad \mathbf{(2)}$$

where $\boldsymbol{\gamma}$ is the surface tension, $(\frac{\partial \gamma}{\partial log C})_T$ **:** is the slope of the descending section of the surface tension isotherm at the cmc, **T** is the absolute temperature in K, **R** is the gas constant (R = 8.314 J $mol^{-1}K^{-1}$), N_A is Avogadro's number, The prefactor **n** is related to the number of species at the air-water interface. For gemini surfactant n is generally taken as 3 by considering a divalent surfactant ion and two univalent counterions.

The values of $\boldsymbol{\Gamma}_{max}$ and $\mathbf{A}_{min}$ are shown in table 1. It comes out that regardless the length of the spacer, the $\mathbf{A}_{min}$ values of Gem n-s-n surfactants goes through a minimum for n= 12-14 then increases abruptly for longer chain (figure 3). Most likely, the initial decrease of $\mathbf{A}_{min}$ reflects that gemini surfactants with longer hydrophobic tails have higher packing densities at the air-water interface. It is interesting to notice that the variation of the spacer length does not affect the A_{min} to greater extent. This trend is different than that commonly observed for gemini surfactants [45]. It seems that the presence of a rigid phenyl group on the hydrophobic chain may assist the orientation of the hydrophobic chain toward the air-water interface leading to a more tightly packing of surfactant molecules at the air-water interface. The existence of π-π interaction among the adjacent phenyl groups of Gem n-s-n molecules may be the main reason for the more compact aggregate structure [43].The presence of amide function that links the ammonium headgroup with the alkylphenyl hydrophobic chain should probably also facilities the intermolecular association among surfactant molecules through hydrogen bonding interactions in self-organized assemblies [46].

For longer hydrophobic chain (n > 14 for s = 2, 4 and n >12 for s = 6), A_{min} increases abruptly particularly for shorter spacer length (A_{min} (Gem 14-2-14) =0.70 nm^2, A_{min} (Gem 16-2-16) = 1.91 nm^2). This variation in minimum surface area per molecule values (A_{min}) indicates that too long hydrophobic tail prevents the orientation of hydrophobic chains into the air. It is noteworthy that the values of A_{min} increases only slightly for hexamethylene spacer s = 6 (A_{min} (Gem 14-6-14) = 0.92 nm^2, A_{min} (Gem 16-6-16) = 0.97 nm^2). This result suggests that for hexedecyl spacer, a compact packing of surfactant molecules is maintained at the air-water interface even for long hydrophobic tail. Such "scenario" can be achieved if the spacer adopts a folder conformation toward the air side according to the conformation of surfactant molecules represented in scheme 2.

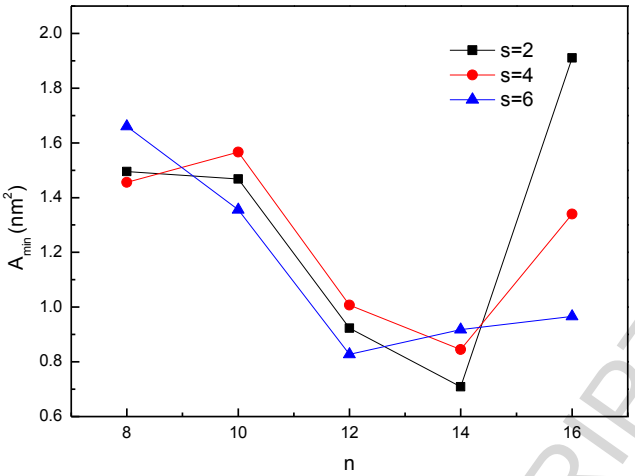
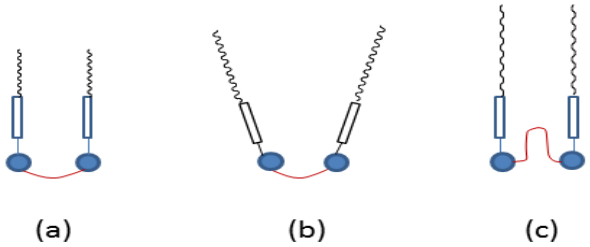


Fig. 3 Variation of A_{min} with the hydrocarbon carbon number n for Gem n-2-n, Gem n-4-n and Gem n-6-n surfactants



Scheme 2. Plausible conformation of gemini surfactants: a) short spacer, n=8-12, b) short spacer, n= 14-16, c) long spacer (s = 6), n = 8-16

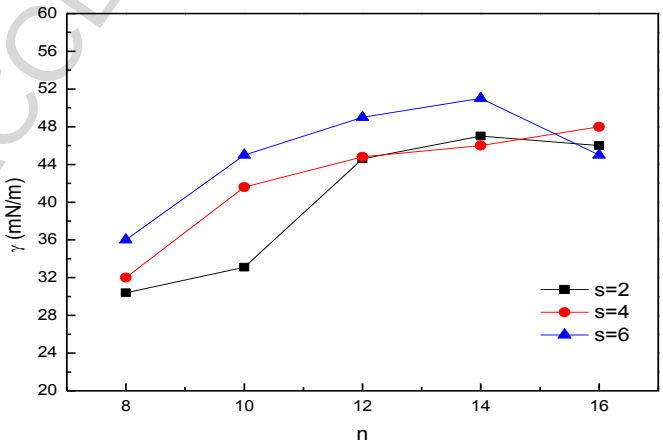


Fig. 4 Variation of γ_{cmc} with the hydrocarbon carbon number n for Gem n-2-n, Gem n-4-n and Gem n-6-n surfactants.

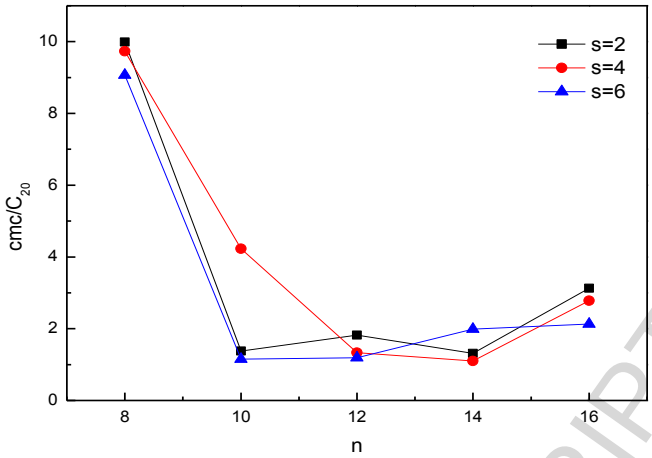


Fig. 5 Variation of cmc/C_{20} ratios with the hydrocarbon carbon number n for Gem n-2-n, Gem n-4-n and Gem n-6-n surfactants.

The performance of a surfactant in lowering the surface tension γ of a solution was investigated in terms of surface tension at cmc (γ_{cmc}) and the adsorption efficiency (pC_{20}) from the surface tension isotherm. The pC_{20} corresponds to the logarithm of the surfactant concentration C_{20} required to produce 20 $mN.m^{-1}$ reduction in surface tension of water. The pC_{20} values were evaluated by using equation **3** [47]:

$$pC_{20} = -\log C_{20} \quad (\mathbf{3})$$

The γ_{cmc} values of gemini surfactants were found to increase with increasing length of the hydrophobic chain (n) and tend to gentle at n = 12-14 (figure 4). The effect of the spacer is less noticeable although with a slight increase of surface tension values with spacer chain length (s).

The cmc/C_{20} ratio is considered as a convenient measure of the relative effects of structural factors on the micellization and adsorption processes. The larger the cmc/C_{20} values, the more efficiently the surfactant is adsorbed at the air-water interface at the expense of the micellization process and the more efficiently it reduces surface tension. The cmc/C_{20} ratios indicate that compounds with octyl alkyl chain have a greater preference for adsorption than for micellization (figure 5).

3.3. Conductivity measurements and thermodynamic of micellization

The critical micellar concentrations have also be evaluated by conductivity measurements. Figure 6 shows an example of the variation in conductivity with concentration for Gem 8-4-8 surfactant. The cmc values were determined from the break points in the curves of specific conductivity (K) versus gemini surfactant concentration (C) and are listed in table 1. One can see that the cmc values obtained by electrical conductivity method are mostly slightly higher than those determined by tensiometry. Nevertheless, similar decreasing trend of cmc values with the elongation of the hydrophobic alkyl chain were obtained with distinct behavior of compounds Gem14-6-14 and Gem 16-6-16 (see figure S1, supplementary file). Several studies have shown that the cmc values may differ significantly when evaluated by different techniques and was attributed to the formation of non-surface-active premicellar aggregates of surfactant in aqueous solutions [44].

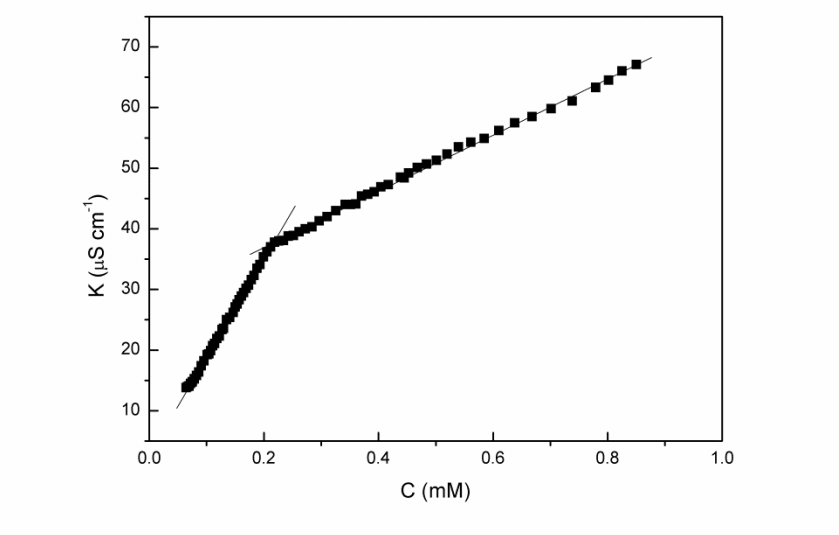


Fig. 6 Specific conductivity as a function of concentration for surfactant Gem 8-4-8

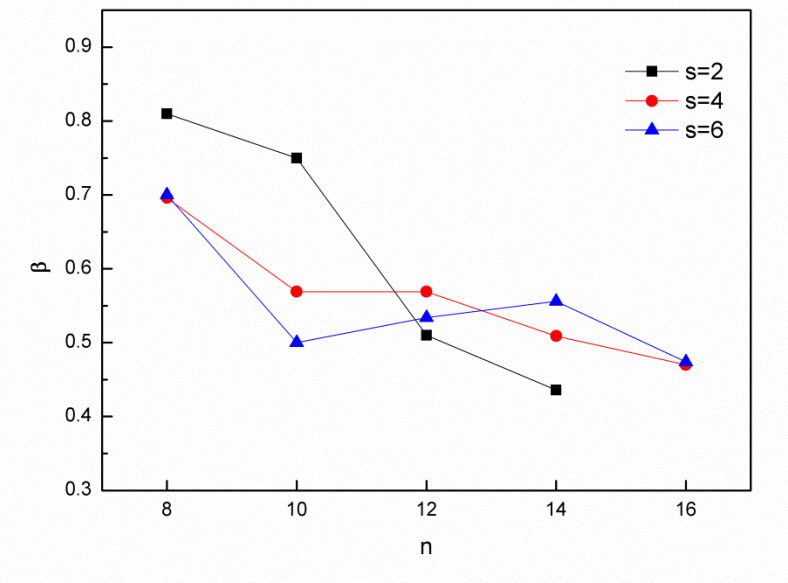


Fig.7 Variation of β values with the hydrocarbon carbon number n for Gem n-2-n, Gem n-4-n and Gem n-6-n surfactants

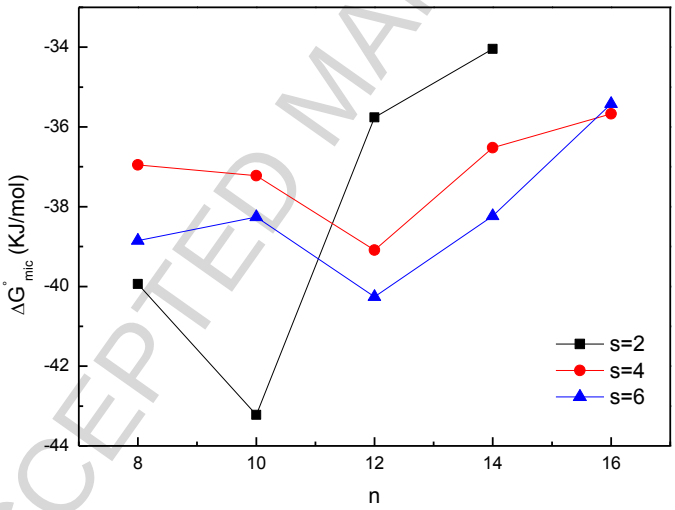


Fig.8 Variation of The Gibbs free energies of micellization (ΔG^0_{mic}) with the hydrocarbon carbon number n for Gem n-2-n, Gem n-4-n and Gem n-6-n surfactants

The degree of counterion binding to micelles (β) is important parameter that governs the stability of micelles. It can be estimated according to the formula α+β = 1 where α is The ionization coefficient obtained from the ratio of the slope values above and below the CMC obtained from the electrical conductivity measurements (conductance vs. concentration). The main approximation underlying this expression is that a micellized surfactant whose electrical charge is not compensated by a bound counterion contributes to the conductivity of the solution with the same amount as if it is free [48].

From table 1 and figure 7, one can be seen that β values decreases with the increasing of hydrophobic chain length (n) particularly for the gemini surfactant series with a short spacer length Gem n-2-n. The decrease in counterion binding indicates a lower surface charge density at the micelle-solution interface for the compounds with longer hydrophobic tails. This variation may be ascribed to a reduction in aggregation number of micelles or the increase of micelle size and/or shape [48]. This trend was evidenced from DLS measurements reported in the subsequent section.

The Gibbs free energy of micellization for gemini surfactants (ΔG^0_{mic}) is associated with the transfer of one mole of surfactant from the aqueous phase to the micellar pseudophase. It was calculated using Equation (**4**) [44]:

$$\Delta G^0_{mic} = RT(0.5 + \beta)\ln(X_{cmc}) \quad (\mathbf{4})$$

Where R is the gaz constant (8.314 J mol^{-1} K^{-1}); T is absolute temperature; β is the degree of counterion binding; X_{cmc} is the cmc molar fraction, X_{cmc} = cmc/55.4 where cmc is in mol L^{-1} and 55.4 comes from 1L of water corresponding to 55.4 mol of water at 25°C.

The values of micellization free energy calculated for the studied gemini surfactants are tabulated in table 1. It can be seen that all ΔG^0_{mic} values are negative indicating that the micellization is a thermodynamically spontaneous process. Compound Gem 10-2-10 exhibit the lowest micellization free energy values reflecting it high tendency to form micelles.

The plot of ΔG^0_{mic} versus the hydrophobic carbon number (n) shows two regimens (Figure 8). ΔG^0_{mic} goes through a minimum in the range of n = 10-12 then increased gradually. This variation in micellization free energies indicates that the increase of hydrophobic chain length promotes the micellar growth for n ≅ 10-12 due to the strengthening on the hydrophobic interaction whereas becomes unfavorable upon further increasing n.

The length of the spacer length seems to affects also the micellization process. Interestingly, Gemini surfactants with ethylene spacer (s=2) are more conducive to micellization for n ≤ 10. The opposite trend was obtained for n = 12 to 16, i.e. the homologous surfactants with longer spacer (s=6) exhibit the lowest ΔG^0_{mic} values.

There are several factors that contribute on micellization process mainly electrostatic repulsion between heads groups, attractive interactions between headgroup and bounded counterion, steric hindrance, hydrophobic effect of chains and spacers and π-π interaction among adjacent phenyl groups. For short spacer and hydrophobic chain, electrostatic repulsion between heads groups and π-π interaction among adjacent phenyl groups must dominate favoring micellization. As n increases, these interactions are weakened in favor of steric hindrance whence the increases of A_{min} previously argued. As a result, the micellar growth is hindered.

A different behavior is expected for long spacer. Because hexamethylene spacer (s=6) is more flexible and hydrophobic in nature, it can adopts a folded conformation and become incorporated into the hydrophobic micelle core; the hydrophobic effect becomes dominant and favor the micellization process. It is noteworthy that for long alkyl chain (n > 12), the orientation of alkyl chain toward water-air interface should be disturbed (A_{min} of Gem n-6-n increases slightly for n varying from 12-16), as a consequence surfactant molecule has more difficulty in aggregation. These findings go well in agreement with the CMC values.

3.4 Size of the aggregates

The size of the aggregates formed by investigated surfactants in aqueous solution has been investigated above the cmc value by dynamic light scattering (DLS) measurements. Figure 9 shows the size distribution of gemini surfactants according to the spacer and hydrocarbon chain lengths. One can be see that the varying terminal alkyl chain length or the spacer length has a systematic effect on the size of studied samples.

Surfactants bearing C_8H_{17}, $C_{10}H_{21}$ and $C_{12}H_{25}$ hydrocarbon tail and a short to middle-length spacer (s = 2, 4) provide mostly a bimodal distribution containing two peaks with an average hydrodynamic diameter of 73.9-151 nm and 4- 19.8 nm, respectively. The aggregate sizes increased with progressive increase of the alkyl chain length and spacer length.

The smallest population is expected to be related to micelle organization while the larger aggregates suggest a spontaneous organization in vesicles. The coexistence of small micelles

with large vesicles was already reported for other gemini surfactants [49]. The introduction of an amide function on the molecular structure of surfactants is known to promote the formation of bilayer vesicles due to the Intermolecular H-bonding between N-H and C=O functions among neighboring molecules [50]. These H-bonding interactions are able to minimize the repulsive interactions among cationic head groups leading to highly stable bilayer organizations.

Compounds Gem 8-2-8 and Gem 10-2-10 show an additional population of bigger aggregates in the range of 337 nm and 436 nm, respectively.

It is interesting to note that the aggregation profile of compounds evolves for longer spacer length (s=6) towards a monomodal distribution with one obvious peak corresponding to an average apparent hydrodynamic radius of about 186-330 nm.

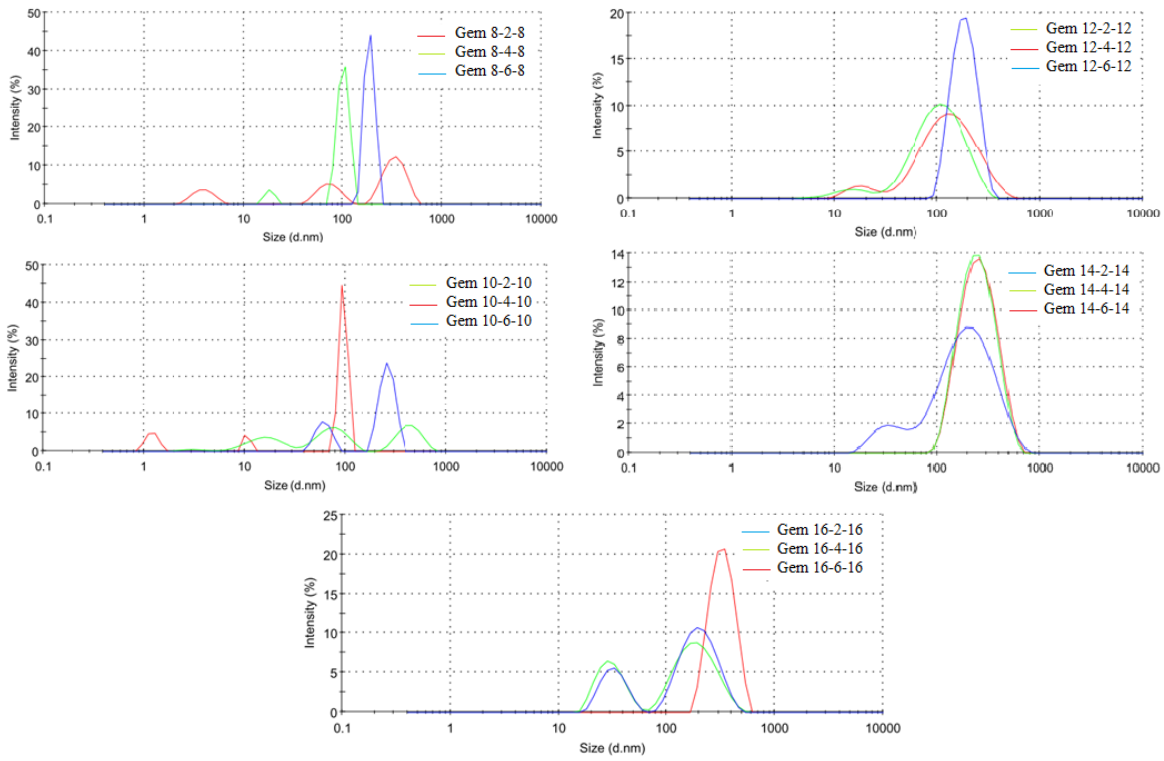


Fig.9 Aggregates size distribution diameter of gemini surfactants from DLS analysis

3.5 Antibacterial and antifungal activity

The antimicrobial activity of Gem n-s-n surfactants was tested against three microorganisms: A Gram-positive bacteria (*Staphylococcus aureus*), a Gram-negative bacteria (*Escherichia coli*) and a yeast (*Candida albicans*). The activity was evaluated by the minimum inhibitory concentrations (MIC) and the minimal lethal concentrations (MLC). The MIC data reported in table 2 show that Gem n-s-n are more active toward Gram-positive tested bacteria strain than for Gram-negative one and the fungal strain. It is generally admitted that the quaternary ammonium surfactants are membrane active agents and that their antimicrobial mechanism is based on cytoplasmic membranes disturbance by combined hydrophobic and electrostatic interactions [51]. In fact, the Gram-positive bacteria layer is composed of porous peptidoglycan (complex polysaccharide) and a single underline phospholipid bilayer, while Gram-negative bacteria have a double phospholipid bilayer sandwiching a thinner peptidoglycan layer. Furthermore, their phospholipid outer bilayer membrane is connected and surrounded by an extra hydrophilic lipopolysaccharide outer membrane which restricts the entrance of lipophilic small molecules and biocides [52]. The cell wall of yeasts shows intermediate Gram-positive and Gram-negative bacteria permeability. Therefore, the low sensibility of Gram-negative and yeast microorganisms to gemini surfactants is related to the composition and the organization of their outer layers.

The surfactants with tetradecyl and hexadecyl chain are inactive on the three tested microorganisms up to the concentration of 512 μg/mL. Antibacterial activity depression may be due to the poor water solubility of Gem 14-s-14 and Gem 16-s-14 derivatives and the formation of viscous solutions that inhibit the transfer through the microbial cell membranes [34,53].

MIC results show that increasing the hydrophobic chain length from 8 to 12 carbon atoms promotes *Staphylococcus aureus* tested strain growth inhibition. This can be caused by the surfactants higher hydrophobic nature, which stimulates interactions with the inner core of the bacterial cell membrane [38, 54]. The role of the spacer on the activity of investigated gemini surfactants is not so obvious. An optimum hydrophilic/hydrophobic balance should be a key parameter that governs the bacterial activity of quaternary ammonium surfactants. Among all of the investigated gemini surfactants, compound Gem 12-4-12 showed the maximal antibacterial activity at 17 μM against *Staphylococcus aureus*. This value is significantly lower than that reported for conventional dodecyltrimethylammonium bromide DTAB (MIC

= 44.8 μM [55]) or for monomeric ammonium surfactant analogous containing an amide function (MIC of dodecyl N-ethanamide N,N,N-trimethylammonium bromide is 43 μM [38]). The presence of two positive charges and two hydrophobic chains in the dimeric surfactants could exacerbate their adsorption on the interface of the negatively charged cell membrane through electrostatic and hydrophobic interactions compared to monomeric quaternary ammonium surfactants [34].

When comparing the MLC values of investigated gemini surfactant, one finds that compound Gem 10-6-10 present the strongest lethal activity against both Gram-positive and Gram-negative bacteria. The majority of here-investigated surfactants have a bacteriostatic activity since they exhibit significant minimal bactericidal concentrations.

Table 2. Antibacterial activities of active gemini surfactants.

Surfactant	MIC (μmol/L)[MLC(μmol/L)][a]		
	S. aureus	*E. coli*	*C. albicans*
Gem 8-2-8	159.8 [-][b]	639.4 [-][b]	159.8 [319.7]
Gem 8-4-8	301.6 [-][b]	301.6 [-][b]	617.8 [-][b]
Gem 8-6-8	37.34 [597.5]	291.8 [-][b]	149.4 [149.4]
Gem 10-2-10	74.69 [597.5]	74.7 [-][b]	597.5 [-][b]
Gem 10-4-10	72.32 [144.6]	144.6 [-][b]	72.3 [-][b]
Gem 10-6-10	70.15 [140.3]	561.2 [561.2]	140.3 [-][b]
Gem 12-4-12	17.0 [544.1]	544.1 [544.1]	544.1 [-][b]

[a] The MIC values of Gem 12-2-12, Gem 12-6-12, Gem 14-s-14 and Gem 16-s-16 with s=2,4,6 are higher than 520 μg/L
[b] The MLC is above the detection limit

4. Conclusion

In summary, a novel series of gemini surfactants bearing 4-alkoxyphenyl hydrophobic chains, amide connector groups linking these tails to the ammonium heads and different methylene spacer lengths have been synthesized and characterized by ^{1}H and ^{13}C NMR and high resolution mass spectrometry (HRMS). These surfactants were investigated with respect to surface active properties and biological activities. The present work revealed the positive contribution of the benzene ring, present in the hydrophobic chain, on the micellization behavior of investigated surfactants. Lower cmc values were reached compared to conventional gemini quaternary ammonium surfactants having alkyl hydrophobic chains and similar spacer length. π-π interactions among benzene rings in hydrophobic chains should assist favorable packing of surfactants molecules. The gemini surfactants with hexamethylene

spacer were found to pack very tightly at the air-water interface. This behavior is most likely due to the tendency of the spacer to adopt a folder conformation towards the hydrophobic phase allowing to maintain the interaction of benzene rings and amide functions of two neighboring molecules to the detriment of the electrostatic repulsion of the cationic head groups. DLS measurements showed that the size of aggregates increases with progressive lengthening of the hydrophobic and spacer chains for investigated amphiphiles. Concerning the biocide properties, only the geminis with octyl, decyl and dodecyl chains (n= 8, 10, 12) showed an activity towards the pathogenic *E. coli*, *S aureus* and *C. albican* microorganisms. An optimum hydrophilic/hydrophobic balance and a good water solubility of surfactants are key parameters that enable a better interaction with bacteria cell surface. Owing to their superior surface properties and good antibacterial activity, such derivatives might be good candidates for wastewater and surface treatments.

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Author contributions: SS-B designed the research, KT synthesized the compounds and performed the charaterizations and the surface properties study, MMB and NB performed the antibacterial study, DLS study was carried out at HG laboratory, SS-B and KT interpreted the experiments and wrote the paper.

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Figures and table captions

Scheme 1. Synthetic pathway of gemini surfactants Gem n-s-n 1. *(i)* 1. Acetic anhydride, H_2O, 110°C, 2. $C_nH_{2n+1}Br$ (n= 8, 10, 12, 14, 16), K_2CO_3, butanone, reflux; (ii) HCl, H_2O, reflux; *(iii)* $BrCH_2COCl$, rt; *(iv)* $(CH_3)_2N(CH_2)_sN(CH_3)_2$ with s = 2, 4, 6, Et_2O, reflux.

Fig.1 Surface tension curves of Gem n-2-n surfactants with n = 8, 10,12,14,16

Fig. 2 Variation of the cmc with the hydrocarbon carbon number n for Gem n-2-n, Gem n-4-n and Gem n-6-n surfactants.

Fig. 3 Variation of A_{min} with the hydrocarbon carbon number n for Gem n-2-n, Gem n-4-n and Gem n-6-n surfactants

Scheme 2. Plausible conformation of gemini surfactants: a) short spacer, n=8-12, b) short spacer, n= 14-16, c) long spacer (s = 6), n = 8-16

Fig. 4 Variation of γ_{cmc} with the hydrocarbon carbon number n for Gem n-2-n, Gem n-4-n and Gem n-6-n surfactants.

Fig. 5 Variation of cmc/C_{20} ratios with the hydrocarbon carbon number n for Gem n-2-n, Gem n-4-n and Gem n-6-n surfactants.

Fig. 6 Specific conductivity as a function of concentration for surfactant Gem 8-4-8

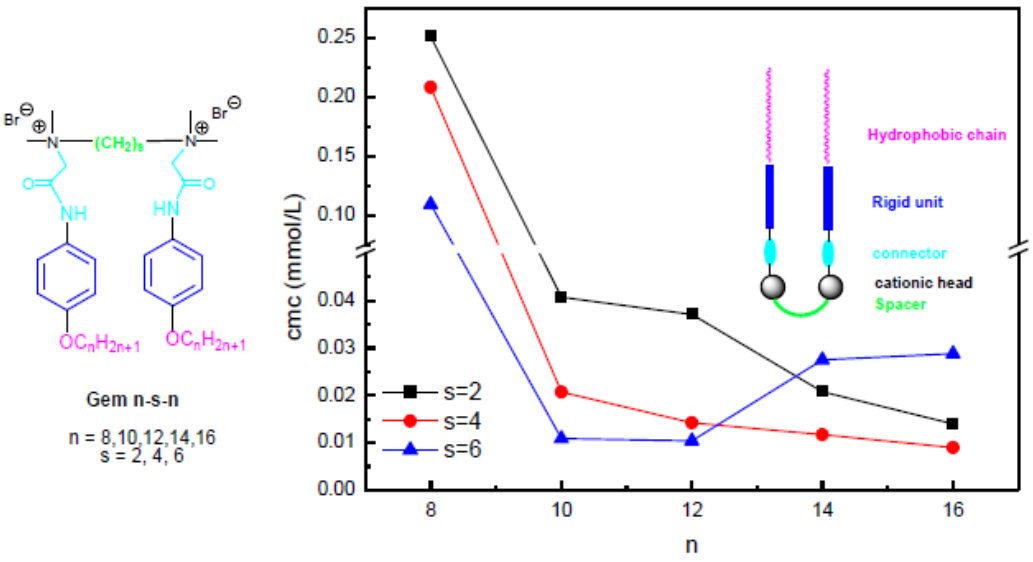
Fig. 7 Variation of β values with the hydrocarbon carbon number n for Gem n-2-n, Gem n-4-n and Gem n-6-n surfactants

Fig. 8 Variation of The Gibbs free energies of micellization (ΔG^0_{mic}) with the hydrocarbon carbon number n for Gem n-2-n, Gem n-4-n and Gem n-6-n surfactants

Fig. 9 Aggregates size distribution diameter of gemini surfactants from DLS analysis

Table 1. Surface activity parameters of gemini surfactants Gem n-s-n

Table 2. Antibacterial activities of active gemini surfactants.



Graphical abstract

Highlights

- We synthesized and characterized novel amide based cationic gemini surfactants.
- We established a structure – surface activity relationship.
- Benzene ring contributes to the micelle formation *via* π-π interactions.
- Conformations of surfactants at micelle-water and water-air interfaces are proposed.
- Theses surfactants possess good antimicrobial activities