

Preparation, Surface-Active Properties and Antimicrobial Activities of Gemini Surfactants based on Morpholine and Piperidine

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ABSTRACT

A novel class of ester functionalized cationic gemini surfactants 4,4'-((ethane-1,2-diylbis(oxy))bis(2-oxoethane-2,1-diyl))bis(4-(alkoxy-2-hydroxypropyl)morpholin-4-ium) dibromide 5a,b and 1,1'-((ethane-1,2-diylbis(oxy))bis(2-oxoethane-2,1-diyl))bis(1-(alkoxy-2-hydroxypropyl)piperidin-1-ium) dibromide 6a,b were synthesized and characterized by FT-IR, ¹HNMR and elemental analysis. The surface activity property of prepared surfactants was investigated by surface tension method. A series of surface active parameters, including CMC, Γ_{max} , pC₂₀, CMC/C₂₀, A_{min}, γ CMC and n were obtained from surface measurement. The surface properties of these piperidinium and morpholinium surfactants have been determined by surface tension method. The CMC values of these surfactants decreases with increase of hydrocarbon chain length. For an identical hydrocarbon chain length, the CMC values obtained for piperidinium (6a,6b) series are smaller than those obtained for morpholinium surfactants (5a,5b). These compounds exhibited antibacterial activity against both Gram-positive and Gram-negative bacteria as well as fungi.

Keywords: Antimicrobial activities, Critical micelle concentration, Gemini surfactant, Ester functionalized cationic gemini surfactants, Morpholine piperidine surfactants, Surface active property

INTRODUCTION

Gemini surfactant is a type of surfactants with more than one hydrophilic head group and hydrophobic tail group linked by a spacer at or near the head groups. Unique properties of gemini surfactants, such as low critical micelle concentration, good water solubility, unusual micelle structures and aggregation behavior, high efficiency in reducing oil/water interfacial tension, and interesting rheological properties have attracted the attention of academic researchers and field experts [1-7].

In recent years, much attention has been focused on the development of cationic gemini surfactants [8], because of their unique ability to form a complex with a variety of negatively charged molecules/particles and have been widely used as antistatic agents [9], softeners, and oil-well drilling agents [8]. Moreover, the use of gemini amphiphilic aggregates in gene delivery [10,11] antimicrobial activity [12,13] and the synthesis of mesoporous material [14,15] have developed.

Many new categories of cationic gemini surfactants have been developed and investigated recently [16-20]. Among all the novel surfactants, gemini surfactants which contain morpholin moieties as hydrophilic head group have several advantages. For example, they could display strong inhibition efficiency, which is owing to the presence of nitrogen and oxygen atoms in the same ring [21]. Up to now, there are many investigations on cationic morpholine surfactants about their and possible use in several applications. Asadov et al. [22] proved the morpholine amphiphile has Petro-collecting and dispersing properties. Aiad et al. [23] synthesized a series of metallosurfactants, from the reaction of fatty acids (I_{a-d}) with morpholine then investigated their surface properties and antimicrobial activity. Also, Bhadani et al. synthesized a new series of ester functionalized cationic gemini surfactants having different cationic head groups (i.e., piperidinium, pyrrolidinium, morpholinium and quaternary ammonium). These new gemini surfactants were

investigated for their micellization and viscosity properties using surface tension, conductivity, fluorescence and rheology techniques. The physicochemical properties of the aqueous surfactant system were influenced by polarity, size and the nature of cationic head groups [24].

In this present study, a series of a new type of ester functionalized cationic gemini surfactant based on morpholine or piperidine, was prepared by introducing hydroxyl and ether groups in the lipophilic part and ester group in spacer. Their surface properties as well as antimicrobial activity were measured and remarked that the different cationic head groups have an appreciable impact on the solution properties of the surfactants.

EXPERIMENTAL PROCEDURES

Materials

Octanol and dodecanol were purchased from Aldrich and used without further purification. Bromoacetic acid, ethylene glycol. All solvents used throughout this study were of chemically pure grade and were fractionally distilled just before use.

General methods

Melting points were determined with an Electro thermal IA 9100 apparatus and were uncorrected. Infrared spectra were recorded with a Bruker Vector 22 Spectrometer. ^1H nuclear magnetic resonance (^1H NMR) spectra were obtained with Varian 300 MHz spectrometer with TMS as an internal standard and mass spectra were recorded on 5988 HB dido-array spectrometer.

Synthetic procedure of surfactants

Synthesis of alkyl glycidyl ether GE-n 1a,b

Octanol (13.02 g, 0.1 mol) was heated in the reaction vessel to 40°C. Sodium hydroxide (6.02 g, 0.15 mol) and TBAB (0.20 g, 0.63 mmol) were added, and the mixture was stirred for 30 min at the same temperature. Epichlorohydrin (18.50 g, 0.20 mol) was added, and the temperature was kept between 38 and 42°C. The reaction was completed after 4hrs. The product was extracted twice with 25 mL of n-hexane and was purified by distillation (50-51°C at 0.041 mm Hg).

GE-12 was synthesized by the method of Najem et al [25]. GE-12 was purified by distillation (97-98°C at 0.060 mm Hg for GE-12).

IR (KBr, cm^{-1}) 3440, 2930, 1118. ^1H NMR (300 MHz, CDCl_3) **1a** δ 3.73 (d, 2H), 3.54 (t, 2H), 3.19 (m, 1H), 2.86 (m, 2H), 1.29-1.41 (m, 12H), 0.88 (t, 3H). **MS m/z (parent ions):** 186.162 (M^{+1}). IR (KBr, cm^{-1}) 3436, 2928, 1119. ^1H NMR of **1b** δ 3.69 (d, 2H), 3.41 (t, 2H), 3.14 (m, 1H), 2.81 (m, 2H), 1.25-1.39 (m, 20H), 0.88 (t, 3H) **MS m/z (parent ions):** 242.40 (M^{+1}).

Synthesis of N (alkyloxy-2-hydroxypropyl) morpholine 3a,b or piperidine 4a,b

N(alkyloxy-2-hydroxypropyl) morpholin **3a,b** or piperidin **4a,b**. Alkyl groups are octyl, and dodecyl and their derivatives are **3a,b** and **4a,b**, respectively (Scheme 1). Alkyl glycidyl ether (octyl, dodecyl) 0.010 mol was stirred magnetically with base 0.0125 mol (morpholine or piperidine) in the presence of catalytic amount of zinc perchlorate hexahydrate at 80°C for 1 h under solvent free conditions. The progress of reaction was monitored by thin layer chromatography [Silica gel G coated (0.25 mm thick) glass plates, using hexane:ethyl acetate (in a ratio of 90:10 or 85:15) as the mobile phase; the spots were visualised by iodine]. The products were purified by column chromatography using silica gel (60-120 mesh) as stationary phase and eluting with hexane:ethyl acetate (95:5) solvent system. The isolated yield of pure product is 75-80%. All the N(alkyloxy-2-hydroxypropyl) morpholine **3a,b** or piperidine **4a,b** were synthesized by the general synthetic method. IR (KBr, cm^{-1}) 3466, 2924, 1458, 1296, 1120, 868, 637. ^1H NMR of **3a**: 3.69 [m, 4H] 3.47 [m, 1H] 3.39-3.52 [m, 4H], 3.26 [s, 1H], 2.35-2.67 [m, 4H], 1.47 [m, 2H] 1.27 [s, 12H] and δ 0.94 [t, 3H]. **MS m/z (parent ions):** 273.41 (M^{+1}).

IR (KBr, cm^{-1}) 3463, 2921, 1453, 1293, 1119, 865, 635. ^1H NMR of **3b**: 3.67 [m, 4H] 3.46 [m, 1H] 3.37-3.55 [m, 4H] 3.25 [s, 1H], 2.33-2.65 [m, 4H], 1.48 [m, 2H] 1.27 [s, 20H] and δ 0.94 [t,3H]. **MS m/z (parent ions):** 329.52 (M^{+1}).

IR (KBr, cm^{-1}) 3465, 2921, 1453, 1295, 1123, 869, 639. ^1H NMR of **4a**: δ 3.27-3.51 [m, 4H] 3.23 [m, 1H], 3.19 [s, 1H], 2.26 [m, 2H], 1.7-2.52 [m, 8H], 1.37 [m, 2H], 1.24 [s, 12H] and [0.92 [t,3H]. **MS m/z (parent ions):** 271.44 (M^{+1}). IR (KBr, cm^{-1}) 3464, 2922, 1456, 1294, 1117, 864, 633. ^1H NMR of **4b**: δ 3.29-3.55 [m, 4H] 3.26 [m, 1H],

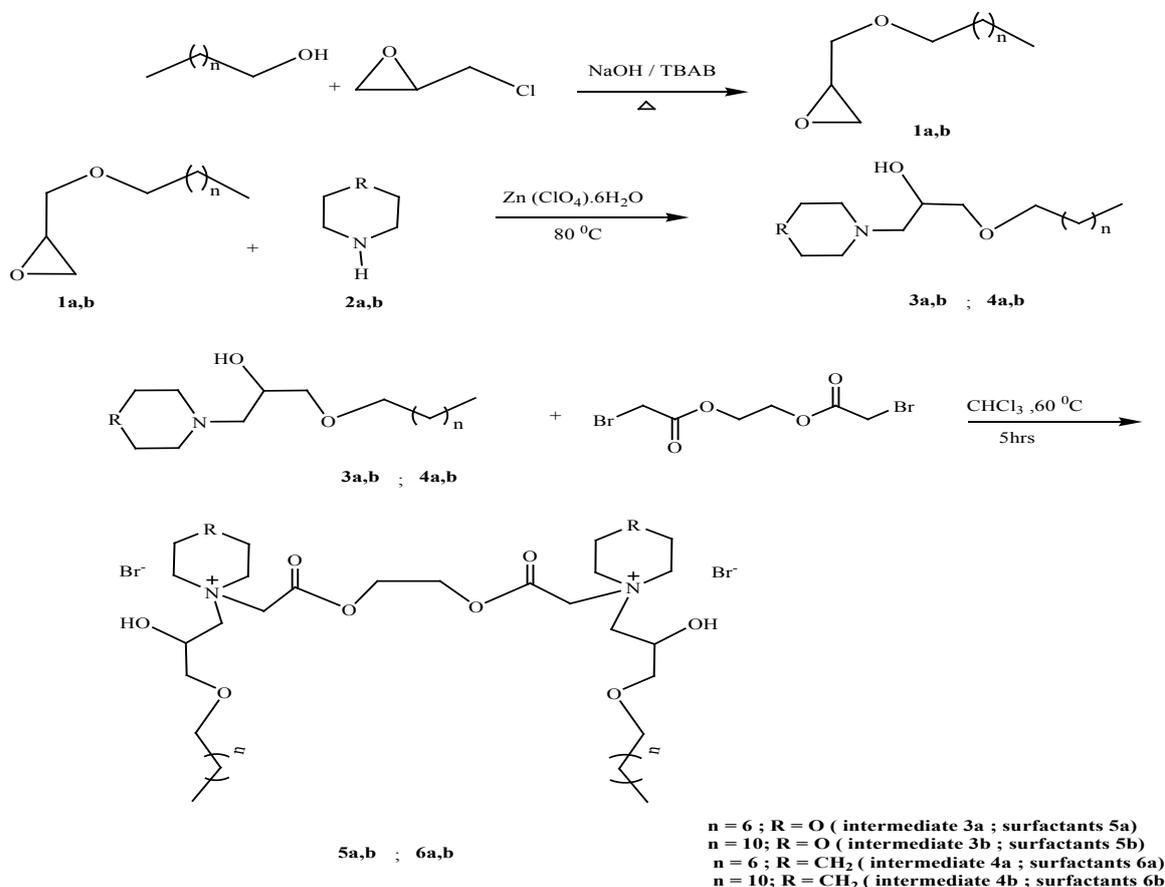
3.13 [brs, 1H], 2.29 [m, 2H], 1.7-2.52 [m, 8H], 1.37 [m, 2H], 1.24 [s, 12H] and [0.92 [t, 3H]. MS m/z (parent ions): 327.55 ($M^{+}+1$).

Synthesis of 4,4'-((ethane-1,2-diylbis(oxy))bis(2-oxoethane-2,1-diyl))bis(4-(alkoxy-2-hydroxypropyl)morpholin-4-ium) dibromide 5a,b and 1,1'-((ethane-1,2-diylbis(oxy))bis(2-oxoethane-2,1-diyl))bis(1-(alkoxy-2-hydroxypropyl)piperidin-1-ium) dibromide 6a,b

Ethane-1,2-diyl bis(2-bromoacetate) was synthesized by reacting ethylene glycol (12.41 g, 0.20 mol) and bromoacetic acid (55.58 g, 0.40 mol) in the presence of p-toluene sulfonic acid monohydrate (1.90 g, 0.01 mol) for 8 h at 130°C. The crude reaction mixture was washed twice with 200 ml of deionized water followed by 100 ml of aqueous methanol (80:20 water/methanol). Thereafter, it was dissolved in chloroform and dried using Na_2SO_4 . The solvent was removed under reduced pressure in a rotary flash evaporator at 60°C to get ethane-1,2-diyl bis(2-bromoacetate) in 55% isolated yield.

N(octyloxy-2-hydroxypropyl) morpholine 3a (27.30 g, 0.1 mol) or N(dodecyloxy-2-hydroxypropyl) morpholine 3b (32.90, 0.1 mol) or N(octyloxy-2-hydroxypropyl) piperidine 4a (27.10 g, 0.1 mol) or N(octyloxy-2-hydroxypropyl) piperidine 4b (32.70 g, 0.1 mol) was dissolved in 50 ml of dry chloroform at room temperature and ethane-1,2-diyl bis(2-bromoacetate) (14.59 g, 0.048 mol) was slowly added to the stirred solution. After addition, the reaction mixture was stirred for 5 h at 60°C. The crude reaction mixture after the removal of chloroform under reduced pressure was subsequently washed twice with 100 ml of diethyl ether and cold precipitated in acetone under inert nitrogen atmosphere. The precipitates after removal of solvent upon drying in a rotary flash evaporator at 70°C for 3 h give pure corresponding gemini surfactants in 65-79% isolated yield (Scheme 1).

4,4'-((ethane-1,2-diylbis(oxy))bis(2-oxoethane-2,1-diyl))bis(4-(octyloxy-2-hydroxypropyl)morpholin-4-ium) dibromide **5a** slight yellowish waxy (65%); IR(KBr, cm^{-1}) 3465, 2924, 1728, 1120. ^1H NMR (300 MHz, CDCl_3) δ 5.43 (s, 4H), 4.71 (s, 4H), 4.49-4.54 (m, 2H), 4.36-4.34 (m, 8H), 4.05-4.02 (m, 4H), 3.95-4.00 (m, 4H), 3.65 (s, 2H), 3.39-3.50 (m, 8H), 2.85 (d, 4H), 1.35-1.24 (m, 24H), 0.88 (t, 6H). Elemental analysis calculated for $\text{C}_{36}\text{H}_{70}\text{N}_2\text{O}_{10}\text{Br}_2$: C, 50.82; H, 8.29; N, 3.29. Measured: C, 50.77; H, 8.23; N, 3.29.



Scheme 1: Synthesis of ester functionalized cationic gemini surfactants

4,4'-((ethane-1,2-diylbis(oxy))bis(2-oxoethane-2,1-diyl))bis(4-(dodecyloxy-2-hydroxypropyl)morpholin-4-ium) dibromide **5b** White solid (67.5%); IR(KBr, cm^{-1}) 3461, 2934, 1725, 1118, ^1H NMR (300 MHz, CDCl_3) δ 5.22 (s, 4H), 4.61 (s, 4H), 4.38-4.44 (m, 2H), 4.26-4.31 (m, 8H), 4.02-4.0 (m, 4H), 3.91-3.95 (m, 4H), 3.61 (s, 2H), 3.37-3.40 (m, 8H), 2.81 (d, 4H), 1.21-1.33 (m, 40H), 0.88 (t, 6H). Elemental analysis calculated for $\text{C}_{44}\text{H}_{86}\text{N}_2\text{O}_{10}\text{Br}_2$: C, 54.88; H, 9.00; N, 2.91. Measured: C, 54.83; H, 8.93; N, 2.90.

1,1'-((ethane-1,2-diylbis(oxy))bis(2-oxoethane-2,1-diyl))bis(1-(octyloxy-2-hydroxypropyl)piperidin-1-ium) dibromide **6a**: viscous brown (73.0%); IR(KBr, cm^{-1}) 3449, 2938, 1734, 1117. ^1H NMR (300 MHz, CDCl_3) δ 5.17 (s, 4H), 4.57 (s, 4H), 4.38-4.44 (m, 2H), 4.24-4.28 (m, 4H), 3.64-3.88 (m, 4H), 3.45 (s, 2H), 3.29-3.33 (m, 8H), 2.09 (m, 4H), 1.74-1.89 (m, 12H), 1.36-1.25 (m, 40H), 0.88 (t, 6H). Elemental analysis calculated for $\text{C}_{38}\text{H}_{74}\text{N}_2\text{O}_8\text{Br}_2$: C, 53.90; H, 8.81; N, 3.31. Measured: C, 53.77; H, 8.73; N, 3.29.

1,1'-((ethane-1,2-diylbis(oxy))bis(2-oxoethane-2,1-diyl))bis(1-(dodecyloxy-2-hydroxypropyl)piperidin-1-ium) dibromide **6b**. White solid (79.0%); IR (KBr, cm^{-1}) 3443, 2933, 1731, 1115. ^1H NMR (300 MHz, CDCl_3) δ 5.13 (s, 4H), 4.52 (s, 4H), 4.35-4.41 (m, 2H), 4.21-4.25 (m, 4H), 3.54-3.68 (m, 4H), 3.41 (s, 2H), 3.26-3.31 (m, 8H), 2.05 (m, 4H), 1.71-1.85 (m, 12H), 1.36-1.25 (m, 24H), 0.88 (t, 6H). Elemental analysis calculated for $\text{C}_{46}\text{H}_{90}\text{N}_2\text{O}_8\text{Br}_2$: C, 57.61; H, 9.46; N, 2.93. Measured: C, 57.55; H, 9.38; N, 2.89.

Surface tension measurement

Surface-tension measurements were done at $25 \pm 0.2^\circ\text{C}$ using a Du Nouy platinum ring method with a Krüss K 20 Tensiometer. Those values which remained constant for a period of 30 min were taken as the surface tension of the solution. The critical micelle concentration (CMC) and the surface tension at the CMC were determined as the values of the break point of surface tension vs. concentration plots (semilogarithmic scale).

Measurement of antimicrobial activities

The antimicrobial activity of synthesized gemini-compounds was tested against the reference bacteria and fungi strains: *B. subtilis* NCIB-3610, *S. aureus* NCTC-7447, *P. aeruginosa* NCIB-9016, *E. coli* NCTC-10416, *C. albicans* IMRU-3669 and *S. niger* IMI-31276. The minimum inhibitory concentrations (MIC, $\mu\text{g}/\text{ml}$) were determined by a tube standard two fold dilution method [26] using gram positive, gram negative bacteria and fungi. The lowest concentration of synthesized surfactants (**5a,b**; **6a,b**) at which there was no visible growth was determined as the MIC. The test was repeated three times.

RESULTS AND DISCUSSION

Synthesis and characterization

Cationic gemini surfactants containing ester group in the spacer and bearing morpholine, piperidine head groups have been synthesized via nucleophilic substitution reaction using zinc perchlorate as catalyst (Scheme 1). Alkyl glycidyl ether (octyl and dodecyl) **1a,b** on reaction with morpholine **2a** or piperidine **2b** gave the corresponding N(alkyloxy-2-hydroxypropyl) morpholine **3a,b** or piperidine **4a,b**. Subsequent quaternization of respective heterocyclic alkoxy hydroxypropyl amines with ethane-1,2-diyl bis(2-bromoacetate) gave corresponding gemini surfactants: 4,4'-((ethane-1,2-diylbis(oxy))bis(2-oxoethane-2,1-diyl))bis(4-(octyloxy-2-hydroxypropyl)morpholin-4-ium)dibromide **5a**, 4,4'-((ethane-1,2-diylbis(oxy))bis(2-oxoethane-2,1-diyl))bis(4-(dodecyloxy-2-hydroxypropyl)morpholin-4-ium) dibromide **5b**, 1,1'-((ethane-1,2-diylbis(oxy))bis(2-oxoethane-2,1-diyl))bis(1-(octyloxy-2-hydroxypropyl)piperidin-1-ium) dibromide **6a** and 1,1'-((ethane-1,2-diylbis(oxy))bis(2-oxoethane-2,1-diyl))bis(1-(dodecyloxy-2-hydroxypropyl)piperidin-1-ium) dibromide **6b**.

The chemical structures of the new cationic gemini surfactants were established by ^1H NMR and elemental analysis. The chemical shifts for methylene protons of the spacer unit in between two ester functionality are observed as a singlet between δ 5.43, 5.22, 5.17 and 5.13 ppm for, **5a**, **5b**, **6a** and **6b** respectively. The resonance for the protons in between the positively charged quaternary nitrogen and the ester functional group was observed as a singlet at δ 4.72-4.52 ppm for the gemini surfactants. A characteristic resonance signal for the protons directly attached to the ether carbon, which are part of the hydrophobic alkyl chain length, was observed between δ 4.05-4.28 ppm for the gemini surfactants. The signals for the methylene protons adjacent to positively charged nitrogen, which are part of hydrophobic alkyl chain length, were appeared between δ 2.09 and 2.85 ppm for the gemini surfactants. The MS data obtained for these new gemini surfactants further helped to establish their chemical structure.

Surface activity

Surface properties of the new ester functionalized cationic gemini surfactant based on morpholine and piperidine were investigated by surface tension measurements. CMC corresponds to the point on the curve at which a sharp change of the slope occurs. The fitting lines after the change in the slope and the fitting lines of the slope demonstrating a decrease in surface tension intersect at a certain point, which denotes CMC values of the surfactants (Figure 1). It was clear that the chemical structure of these gemini surfactants had significant influence on their surface activities. The CMC values of these gemini surfactants were found to be dependent on the length of hydrophobic chain and the nature of the cationic head group. From the results listed in Table 1 increasing hydrophobic chain length (nonpolar tail) decreases the CMC of the investigated surfactants at 25°C. At identical hydrocarbon chain length, CMC values obtained for piperidinium series were smaller than those obtained for morpholinium series studied herein. However, the CMC values of these new ester based gemini surfactants are comparable to other heterocyclic cationic gemini surfactants, i.e., pyridinium, imidazolium, pyrrolidinium surfactants as well as conventional quaternary ammonium gemini surfactants and these gemini surfactants are equivalently good as other recently developed gemini surfactants [27-37].

γ_{CMC} is the maximum ability of the surfactant to reduce surface tension of the water close to the CMC value. The surfactant monomers adsorb at the air–water interface and reduce the surface tension of the water. At certain point the entire interface is completely occupied by the surfactant monomers and further increasing the surfactant concentration does not bring further decrease in the surface tension of the aqueous system. At this particular point the slope of the surface tension curve suddenly changes and becomes constant and the corresponding point is referred to as γ_{min} . Values of γ_{CMC} for piperidinium series **6a** and **6b** were observed as 40.25, 37.20 mN/m while, for morpholinium series **5a**, **5b** as 39.30, 35.95 mN/m, respectively. The slight differences in γ_{CMC} values for both series can be attributed to the nature of headgroup.

The other surface properties, i.e., surface excess concentration (Γ_{max}), surface area occupied by molecules at the air/water interface (A_{min}), efficiency in surface tension reduction by 20 mN m⁻¹ (C_{20}) were deduced from the surface tension plot of the gemini surfactants (Table 1). The maximum surface excess concentration at the air/water interface, Γ_{max} , is calculated by applying the Gibbs adsorption isotherm equation:

$$\Gamma_{\text{max}} = -(1/2.304nRT) (d\gamma/d \log C)_T \quad (1)$$

Here, γ is the surface tension (mNm⁻¹), R the gas constant (8.31 J mol⁻¹ K⁻¹), T the absolute temperature, C the surfactant concentration and $(d\gamma/d \log C)$ the slope below the CMC in the surface tension plots. The n is a constant, which depends on the number of individual ions comprising the surfactant that are adsorbed at the interface. For gemini surfactants the value of n is 3, therefore the value of n is taken as 3 [38]. The continual adsorption of the surfactant monomers at the air-water interface with increasing surfactant concentration causes the surface tension of the aqueous system to gradually decrease. The constant adsorption of the surfactants results in active accumulation at

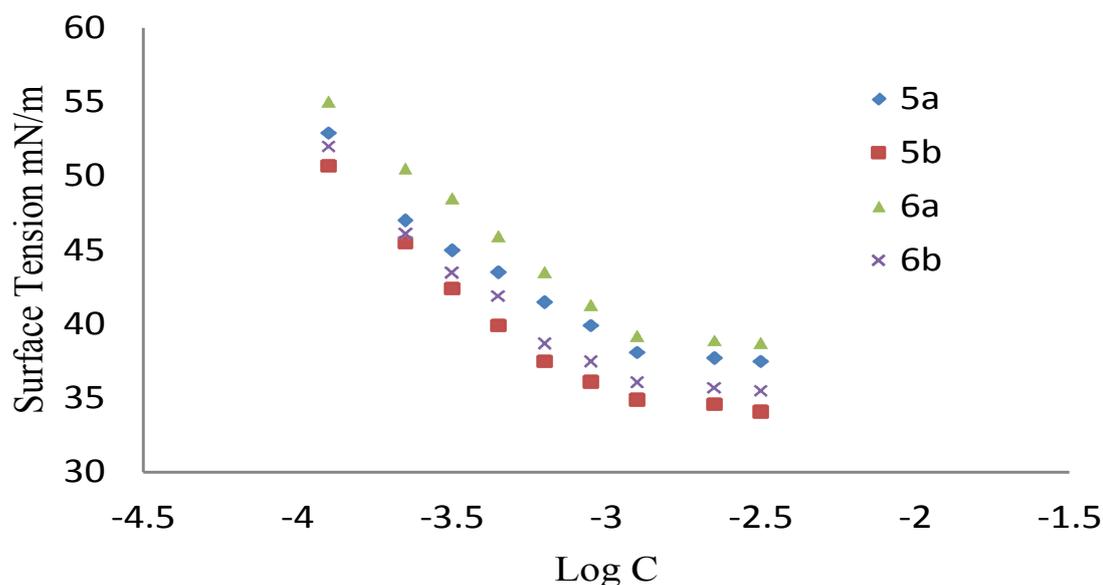


Figure 1: Variation in surface tension of surfactants 5a,b; 6a,6b versus concentration at 25°C, for structures see Scheme 1

Surfactant ^a	CMC mM	γ_{CMC} mN/m	Π_{cmc} mN/m	$10^4\Gamma_{max}$ mol/m ²	A_{min} nm ²	pC_{20}	CMC/ C ₂₀
5a	0.95	40.25	32.15	1.21	1.37	3.52	3.41
5b	0.81	37.20	35.20	1.48	1.12	3.63	3.46
6a	0.74	39.30	33.10	1.24	1.33	3.55	2.66
6b	0.62	35.95	36.45	1.50	1.10	3.67	2.92

Table 1: Surface-active properties of gemini surfactants 4,4'-((ethane-1,2-diylbis(oxy))bis(2-oxoethane-2,1-diyl))bis(4-(alkoxy-2-hydroxypropyl)morpholin-4-ium) dibromide **5a,b** and 1,1'-((ethane-1,2-diylbis(oxy))bis(2-oxoethane-2,1-diyl))bis(1-(alkoxy-2-hydroxypropyl)piperidin-1-ium) dibromide **6a,b**

^aFor structures see Scheme 1. CMC: Critical Micelle Concentration; γ_{cmc} : Surface Tension at CMC; Π_{cmc} : Surface Pressure at CMC; Γ_{max} : Maximum Surface Excess Concentration; A_{min} : Minimum Area Occupied per Molecule

the interface and the concentration of the surfactant monomers become more concentrated at the interface compared to bulk solution. Γ_{max} signifies the area associated with the concentration of the gemini surfactant at the air–water interface and its value depends on the length of hydrophobic chain and the nature of the cationic head groups.

The minimum area occupied per surfactant molecule (A_{min}) at the air-water interface has been obtained by using the equation 2

$$A_{min} = 1/N \Gamma_{max} \quad (2)$$

Where N is Avogadro's number and A_{min} is in nm² (Table 1). All the surfactants (**5a,b**; **6a,b**) synthesized in present work have been found to be having resemble A_{min} values as compared to previously reported morpholinium and piperidinium ionic liquids [24]. A greater value of Γ_{max} and a smaller value of A_{min} means a denser arrangement of surfactant molecules at the surface of the solution. It is clearly seen from Table 1 that series **6a,6b** has a significantly large Γ_{max} value and a correspondingly smaller A_{min} value than morpholinium series **5a,5b** indicating a denser arrangement of piperidinium molecules **6a,6b** at the air-water interface compared with morpholinium **5a,5b**. The difference between A_{min} values in both series, suggests that variations of slopes are essentially due to the nature of head group. It is well establish that the hydrophilic head group size is a dominant factor affecting the Γ_{max} and A_{min} values of surfactants.

Other important surface active parameter, such as adsorption efficiency (pC_{20}) and effectiveness of surface tension reduction (Π_{CMC}) can also be obtained from surface tension curve. pC_{20} is obtained by using equation 3:

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

In the equation, C is the molar concentration of surfactant and C_{20} represents the surfactant concentration at which the surface tension of pure solvent is reduced by 20 mN m⁻¹. C_{20} is the minimum concentration which leads to a saturation of the surface adsorption [39]. Therefore, C_{20} can be a measurement for the adsorption efficiency of surfactant molecules at air/water interface. So the greater the value of pC_{20} is, the higher the adsorption efficiency of surfactant Π_{CMC} is the surface pressure at CMC, this is defined by using the equation. (4):

$$\Pi_{CMC} = \gamma_0 - \gamma_{CMC} \quad (4)$$

where γ_0 is the surface tension of pure solvent and γ_{CMC} is the surface tension of solution at CMC. Π_{CMC} is an indication of the maximum reduction of surface tension, and it can be used to evaluate the effectiveness of surfactant to lower the surface tension of water [39]. So the larger the value of Π_{CMC} is, the higher the effectiveness of surfactant is. The values of pC_{20} and Π_{CMC} were listed in Table 1. The variation of pC_{20} and Π_{CMC} indicated that both the pC_{20} and the CMC increased with the increase of hydrocarbon chain increasing. The value of CMC/ C_{20} ratio is to determine structural factors in the adsorption and micellization process. The surfactant with larger CMC/ C_{20} ratio has greater tendency to adsorb at the interface than to form micelles in solution. From Table 1, it can be seen that the surfactant with the increase of hydrophobic chain length was easier to adsorb at the air/water interface than self-assemble in solution. The result was in accordance with the conclusion from the value of pC_{20} .

Antimicrobial activity

The antimicrobial activity of the synthesized gemini surfactants on various microorganisms was evaluated by determining the minimal inhibitory concentration ($\mu\text{g/ml}$). The minimum concentrations of the tested compounds

Test organism Compound	Gram positive bacteria		Gram negative bacteria		Yeast	Molds
	<i>B. subtilis</i> NCIB-3610	<i>S. aureus</i> NCTC-7447	<i>E. coli</i> NCTC-10416	<i>P. aeruginosa</i> NCIB-9016	<i>C. albicans</i> IMRU-3669	<i>S. niger</i> IMI-31276
5a	256	256	>512	>512	512	512
5b	256	256	512	256	256	256
6a	256	256	512	512	512	512
6b	128	128	128	128	256	256

Table 2: Minimal inhibitory concentration ($\mu\text{g/ml}$) values of gemini surfactants based on morpholine and piperidine (5a-6b)

which inhibit the growth of microorganisms were shown in Table 2. The tested gemini surfactants were active toward almost microorganisms. However, Gram-positive bacteria were more sensitive than Gram-negative toward the tested compounds. This rule is also confirmed by results obtained previously [40], where Gram-negative strains were less sensitive when comparing to Gram-positive *Staphylococcus aureus*. Differences in the bacterial sensitivity to surfactants may result from the construction of their cell walls, which in Gram-negative bacteria contain an outer membrane providing an additional barrier [41]. The results also showed that test compound with 12 carbon atoms within hydrophobic chains and piperidine head group (**6b**) inhibited growth of both Gram-positive and Gram-negative bacteria (*E. coli*) on similar level (MIC 128 $\mu\text{g/ml}$). On the other hand, the activity of compound with 8 carbon atoms within hydrophobic chains and morpholine headgroup (**5a**) was weaker against *E. coli* NCTC-10416 and *P. aeruginosa* NCIB-9016 (MIC > 512 $\mu\text{g/ml}$).

The MIC values for the majority of compounds investigated, in this case, were 256 $\mu\text{g/mL}$. Therefore, compounds under study were considered practically toxic toward selected bacterial species and fungal strains.

CONCLUSION

The ester functionality combined with polar ethylene oxide units as hydrophilic spacer investigated in the present work offers more versatility to quaternary ammonium gemini surfactants compared to conventional quaternary ammonium gemini surfactants. The CMC values of these gemini surfactants have been found to be lower or comparable to conventional quaternary ammonium gemini surfactants and other category of heterocyclic gemini surfactants reported in the literature. My results show that the physicochemical properties of the gemini surfactants can be varied by not only the change in the hydrophobic alkyl length or the spacer length but also by varying the type of cationic headgroup. The surface properties and the viscosity of the aqueous solutions of the gemini surfactants can be tuned to desired values by just varying their headgroup and keeping the length of the spacer and the hydrophobic tail constant. These results will be very helpful for developing many new surfactant molecules for industrial applications.

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