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Ester-Gemini and Monomeric Cationic Surfactants; Synthesis, Characterization, Surface Parameters and Biological Activity

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Abstract

Gemini cationic surfactant is a promising generation of surfactants characterized by their unique surface properties and biological activities. Herein, a series of three Gemini cationic surfactants containing ester bond with different alkyl chain length and their corresponding monomeric structures have been synthesized. The monomeric surfactant were synthesized via simple esterification of different fatty acids (stearic, myrisitic, lauric acid) with 2-dimethyl amino ethanol, followed by quaternization with 1-bromohexane; while, and 1,6-dibromohexane was used to prepare the corresponding Gemini cationic surfactants. The structures of the synthesized compounds were elucidated using ^{1}H-NMR, FTIR spectroscopy. The biological activities and surface properties have been assayed and it was found that the prepared Gemini surfactants have higher antimicrobial efficiencies than the corresponding monomeric structure.

"Keywords: Gemini cationic surfactant; bis-quaternary, surface activity; biological efficiency"

1. Introduction

Lowering the free energy of the boundary phase reduces surface and interface tension, which is the driving force behind amphiphilic adsorption. This fundamental properties of amphiphiles are the foundation for their wide range of functional applications [1]. The surfactants market is predicted to achieve a growth rate of 4.5% from USD 42.1 billion in 2020 to USD 52.4 billion in 2025. The market's expansion is fuelled by the world's rising population and urbanisation. Furthermore, as a result of COVID-19, there is an increasing awareness of products such as hand sanitizer, which is driving demand [2]. Gemini surfactants are more effective at lowering interfacial tension and forming micelles at very low critical micellar concentrations than monomeric surfactants. The cationic structures of the surfactant formed from hydrophobic units covalently bounded and hydrophilic groups, as well as different spacers to minimize the concentration of surfactant, is a promising strategy in this regard [2, 3]. They also have better wetting properties, as well as unusual rheological and aggregation properties. [4, 5]. Gemini surfactants have a wide range of applications due to their unique properties, including enhanced oil recovery [6], transfection of genes [7], RNA-delivery [8] Inhibition of iron corrosion [9, 10], and environmental protection [11-13]. Environmental friendly products to replace traditional surfactants can become a major trend as a result of rising governmental pressure and environmental protection. One way to increase biodegradability is to incorporate weak bonds, such as ester bonds, into surfactants. For instance, Geo *et-al* reported synthesis of gemini surfactants with ester bond on the spacer unit based on 1,2-bis-chloroacetoxy-ethane which exhibited good foaming properties and emulsifying power [14]. Several studies on the biodegradability of

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surfactants with ester bonds have shown that these surfactants are biodegradable [15-18]. Over the last half-century, many lives have been saved due to antibacterial agents, which have also helped in the development of modern medicine. The increasing efficacy of these life-saving therapies is being restricted by bacterial immunity and drug resistance. [19]. To avoid cross-resistance Antibacterial drugs with a novel target and molecular structure have been developed. Herein, biodegradable ester-bonded monomeric and Gemini surfactants were synthesized and their chemical structures have been characterized. The effect of chain length on their surface parameters and biological activities were discussed.

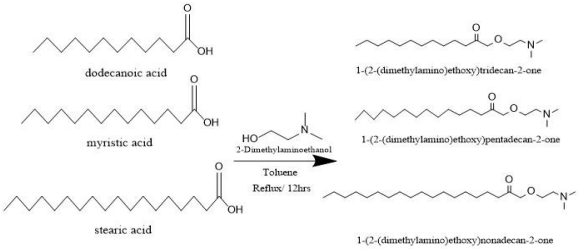
2. Materials and experimental methods

2.1. Materials

Chemicals used to synthesize the Gemini-cationic surfactants were analytical grade and were used without purification. Dodecanoic, myrisitic acid, stearic acid, N,N-dimethyl ethanolamine, 1-bromohexane, 1,6-dibromohexane and p-toluene sulphonic acid were obtained from Sigma-Aldrich Chemicals Co., Inc. El-Gomhoria Chemical Co., Egypt, provided high-quality ethyl alcohol absolute, toluene, and diethyl ether.

2.2 Synthesis of monomeric and Gemini cationic surfactants

N,N-Dimethyl ethanolamine (0.2 mole) was esterified by dodecanoic, myrisitic, stearic acid (0.2) mole under reflux conditions in toluene (250 mL) as a solvent, and 0.01 percent p-toluene sulphonic acid as a dehydrating agent. The reaction was stopped when the azeotropic volume of water (0.2 mol., 3.6 mL) was obtained using dean stark connection. Then, the solvent was removed from the reaction medium using a vacuum rotary evaporator, and the product was recrystallized with petroleum ether, as illustrated in ***Scheme 1***.



Scheme 1. Synthesis route of the fatty ester derivatives

The prepared N,N-dimethyl ethanolamine ester derivatives (0.1 mol) were refluxed with hexyl bromide (0.1 mol) and 1,6-dibromohexane (0.05 mol), individually, in 100 mL absolute ethanol as a solvent for 5 h, then the solvent was evaporated under vacuum at 50 ^{o}C. The products were recrystallized and precipitated in diethyl ether (200 mL) and dried under vacuum at room temperature. The monomeric cationic surfactants were designated as: L12, M14, S18, and the Gemini cationic surfactant as GL12, GM14, and GS18, corresponded to the dodecanoic, myrisitic and stearic chains, respectively ***(Scheme 2)***.

2.3. Characterization:

FTIR ^{1}H-NMR and elemental analysis were used to validate the chemical compositions of L12, M14, S18, GL12, GM14, GS18 surfactants. FTIR analysis was pointed out using ATI Mattsonm Infinity Series™. ^{1}H-NMR was done using GEMINI 200 (1H 400MHz) in DMSO, Bench top 961 was supported by Win FirstTM V2.01 Software. Elemental analysis of carbon, hydrogen and nitrogen was carried out using CHNS-932 (LECO) Vario Elemental Analyzer.

2.4. Measurements of Surface Tension

The surface tension values of freshly prepared monomeric and Gemini cationic surfactants solutions were determined with detached ring method using a K6 Processor tensiometer (Kruss Company, Germany) at 25, 40, and 65 °C. Before each experiment, purified water was used for ring calibration, and the readings were considered as average of three repetitions at each concentration [20, 21]. The critical micelle concentrations were pointed out from the surface tension (γ) versus [log c] plots of the synthesized surfactants from the intersection between the regression straight line of the linearly dependent region and the straight line passing through the plateau [22, 23].

The effectiveness (π_{CMC}) was determined from the difference in the surface tension values of water (γ_o) and that at critical micelle concentration (γ_{CMC}), while the efficiency (C_{20}) is the concentration of surfactant needed to reduce the surface tension by 20 dyne/cm for the synthesized surfactants (***Eq. 1***) [24]:

$$\pi_{CMC} = \gamma_o - \gamma_{CMC} \quad (1)$$

The maximum surface excess (Γ_{max}) for the two types of the synthesized surfactants was expressed as

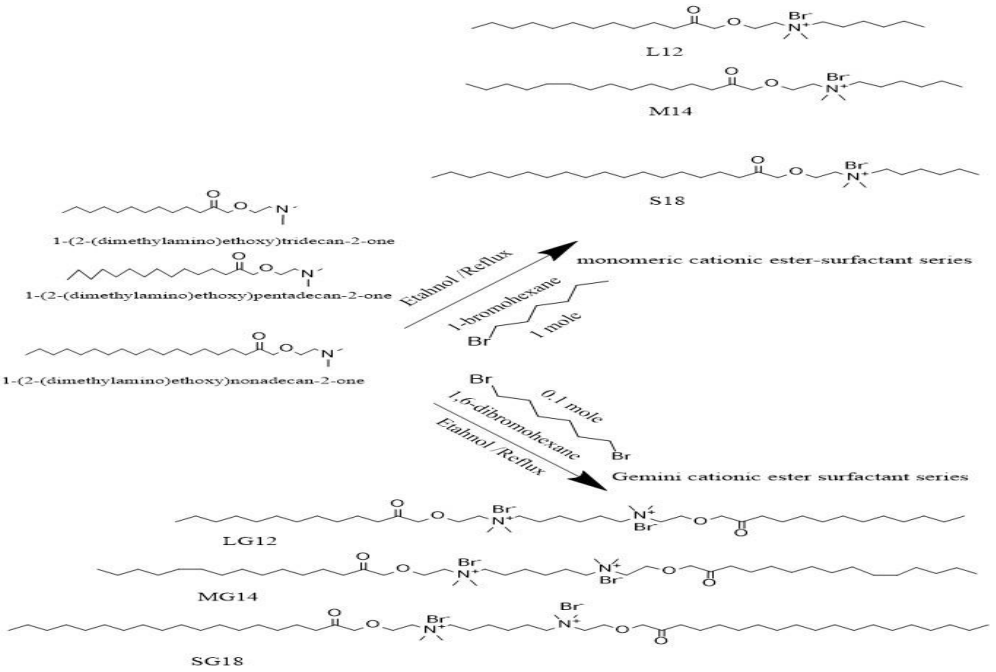
the concentration of the surfactant at the interface per unit area, measured using Gibb's adsorption equation (***Eq. 2***) [25, 26].

$$\Gamma_{max} = \left(\frac{1}{2.303\, nRT}\right)\left(\frac{\delta \gamma}{\delta \log c}\right)_T \qquad (2)$$

δγ/δlog c: Slope of the pre-micellar region, T: temperature (°K), n: number of active species in solution (2 for monomeric, 3 for Gemini surfactants), R: universal gas constant.

The minimum surface area (A_{min}) is the average area (A^2) occupied at the interface by surfactants molecules (**Eq. 3**), where N: 6.02×10^{23} molecule/mole [13].

$$A_{min} = \frac{10^{16}}{\Gamma_{max} N} \qquad (3)$$



Scheme 2: synthetic routes of mono and Gemini Ester cationic surfactants.

2.5. The Antimicrobial Activity Test

A diverse set of Gram positive, Gram negative bacteria and fungi species were obtained from the Microbiology department of the Faculty of Medicine at AL-Azhar University in Cairo. Two-fold serial dilutions were prepared (1000 µg, 500 µg, 250 µg, 125 µg, 62.5 µg, 31.25 µg until reach 1.56 µg) of the test substances, as well as one quality control (QC) antibiotic of Penicillin G, Ciproflxacin and Flucnazole, in a micro dilution plate. Take a few colonies from an agar plate with a sterile swab to make the inoculum, prepare overnight broth, then from the broth prepare a McFarland standard (half McFarland with Optical Density 0.1 at 580 nm). The McFarland standard is also being diluted in the media. Incubate the micro dilution plate with the inoculum and serially diluted test compounds for 18 h, then the micro-dilution plate was recorded by ELISA reader [27]. Plate a portion of the well that shows no eyed-detectable growth on a suitable agar medium, incubate the agar, and search for colonies to evaluate the MBC value. The prepared surfactants were tested against *Bacillus pumilis* (MTCC-2296) and *Streptococcus faecalis* (MTCC-0459) Gram-positive bacteria, *Escherichia coli* (ATCC-25955) and *Enterobacter cloacae* (ATCC-23355) are Gram-negative bacteria and *Aspergillus niger* (MTCC-1881) and fungi (*Candida albicans*, ATCC-10231).

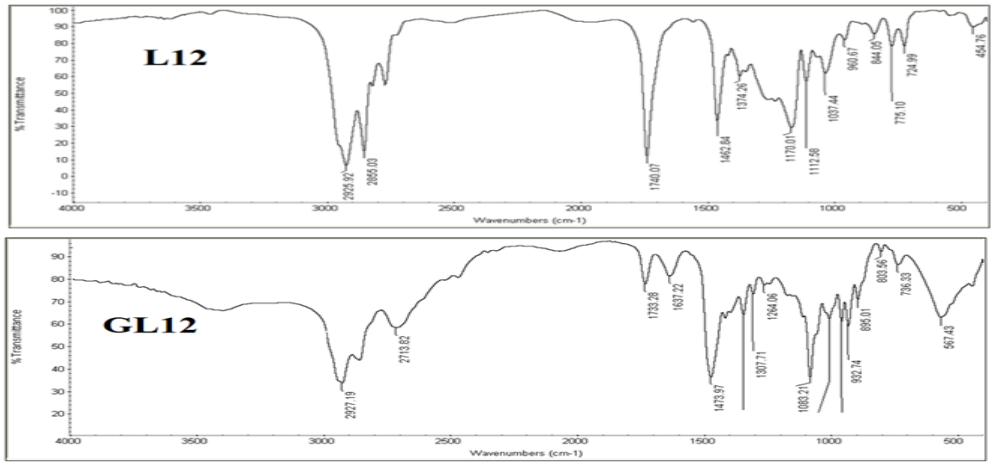


Fig.1. FTIR spectra of the synthesized surfactants L12 and GL12

3. Results and Discussion

3.1. Structure confirmation

FTIR and ^{1}H-NMR spectroscopy and elemental analysis are used to validate the chemical composition of the synthesized cationic surfactants, ***Scheme 1-2***. FTIR spectra of compound L12 (***Figure 1***) (as representative for M14 and S18) displays the distinguishing bands at: 2926 cm^{-1}, 2855 cm^{-1} for asymmetric and symmetric C-H stretching, 1740 cm^{-1} band for the stretching of C=O of the ester group, 1463 cm^{-1} for C-H symmetric bending of methylene groups, 1374 cm^{-1} for C-H symmetric bending of gem dimethyl. The absorption bands at 1170 cm^{-1} referred to the C-O stretching band. Based on the similarity of the chemical function groups of both monomeric and Gemini surfactants (GL12, GM14, GS18), ***Figure 1***, FTIR spectra of the Gemini surfactants were identical to the monomeric surfactants.

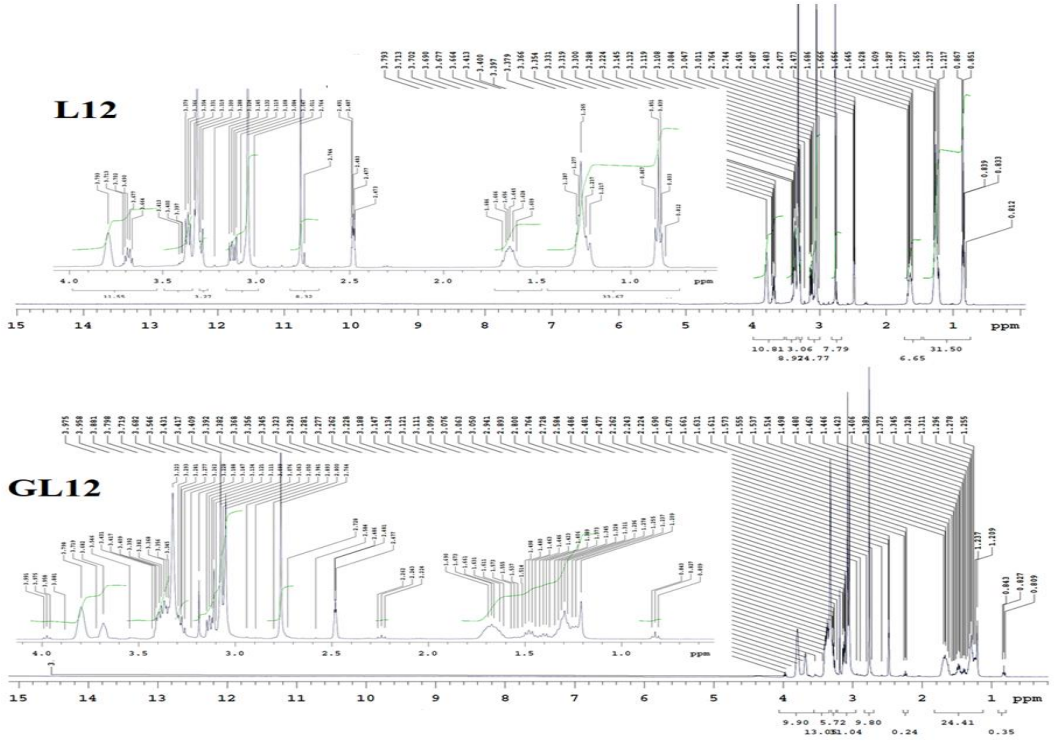


Fig. 2 ^{1}-HNMR spectra of L12 and GL12 compounds

^{1}H-NMR spectrum of L12 (***Figure 2***) (as representative for M14 and S18) exhibited signals at: δ = 0.85 ppm (t, 6H, $CH_2\underline{\mathbf{CH}}_3$), δ = 1.277 ppm (m, 22H, $\underline{\mathbf{CH}}_2$), δ = 1.66 ppm (m, 4H, $\underline{\mathbf{CH}}_2CH_2COO$ and $N^+CH_2\underline{\mathbf{CH}}_2$---$CH_3$), δ = 2.74 ppm (t, 2H, $\underline{CH}_2COOCH_2$), δ = 3.23-3.41 ppm (t, 4H, $\underline{\mathbf{CH}}_2N^+\underline{\mathbf{CH}}_2$), δ = 3.37 ppm (S, 6H, N^+ ($\underline{\mathbf{CH}}_3$)$_2$), δ = 3.68 ppm (t, 2H, O=C-O-$\underline{\mathbf{CH}}_2$). While, the synthesized Gemini surfactants had the same proton distribution except those for methylene protons of hydrocarbon chain. ^{1}H-NMR spectrum of GL12 (***Figure 2***) (as representative for GM14 and GS18) showed signals at δ = 0.84 ppm (t, 6H, C$\underline{\mathbf{H}}_3$), δ = 1.21-1.54 ppm (m , 62H , C-$\underline{\mathbf{CH}}_2$ -C), δ = 1.69 ppm (t , 4H, $\underline{\mathbf{CH}}_2$ CH_2C=O), δ = 2.26 ppm (t , 4H , $CH_2\underline{\mathbf{CH}}_2C$=O), δ = 3.14 ppm (t, 8H, $N^+\underline{\mathbf{CH}}_2$), δ = 3.31 ppm (S, 12H, $N^+\underline{\mathbf{CH}}_3$), δ = 3.99 ppm (t, 4H, O=C-O-$\underline{\mathbf{CH}}_2$).

Elemental analysis provided additional structural confirmation of the prepared surfactants. Tables 1 show the results of the elemental analysis. The results indicate that the theoretical of C, H, and N percentage values are in agreement with the found values.

Table (1): Elemental analysis for the prepared gemini surfactants and their monomeric structure

Product (molecular formula)	*M.wt*	*C%*		*H%*		*N%*	
		Calc.	Found	Calc.	Found	Calc.	Found
L12 ($C_{22}H_{46}BrNO_2$)	436.51	60.53	60.8	10.62	10.8	3.21	3.4
M14 ($C_{24}H_{50}BrNO_2$)	464.56	62.05	62.3	10.85	10.2	3.02	3.2
S18 ($C_{28}H_{58}BrNO_2$)	520.67	64.59	63.7	11.23	10.8	2.69	2.9
GL12 ($C_{38}H_{78}Br_2N_2O_4$)	786.84	58.00	57.7	9.99	10.3	3.56	3.9
GM14 ($C_{42}H_{86}Br_2N_2O_4$)	842.95	59.84	60.1	10.28	9.8	3.32	3.7
GS18 ($C_{50}H_{102}Br_2N_2O_4$)	955.16	62.87	62.4	10.76	10.9	2.93	3.4

3.2. Surface activity (effect of hydrophobicity and temperature)

CMCs of the obtained surfactants were determined at 25, 45, 65 ^{o}C. CMCs refer to the surface tension break point -log[C] curves ***(Figures 3-6)***. Analyzing CMCs data of monomeric and Gemini surfactants in ***Table 2*** revealed that the rise in methylene groups in the tails of the different surfactants increases their hydrophobicity for GL12, GM14, GS18 than L12, M14, and S18 which damage the water molecules arrangement at the surface. This raises the free energy of the aqueous, increasing its affinity for micelle formation at lower concentrations. Obviously, CMCs values at 25 ^{o}C for GL12, GM14 and GS18 pointed at 1.02, 0.98, and 0.79 mM, which were lower than L12, M14, and S18 which were 1.29, 1.07 and 1.0 mM.

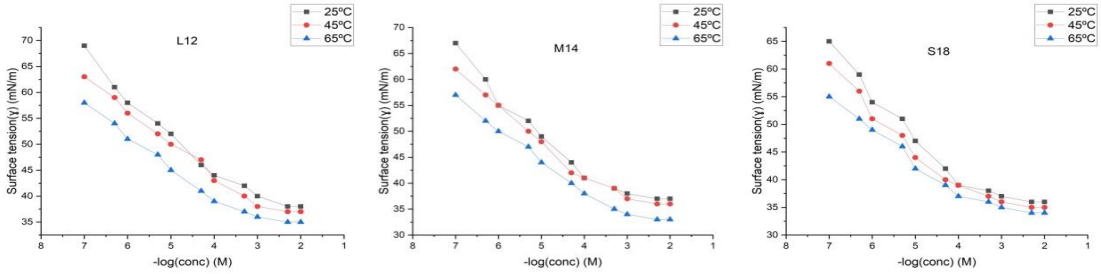


Fig 3. Plots surface tension vs. −log concentration of monomeric ester surfactant L12, M14 and S18 at different temperatures 25, 45, 65

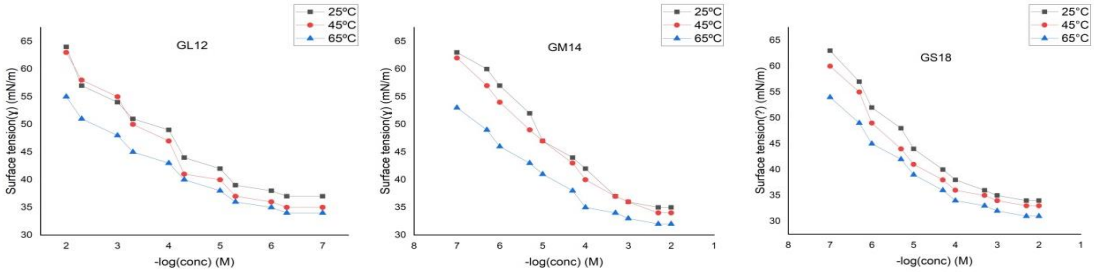


Fig 4. Plots surface tension vs. −log concentration of gemini ester surfactant GL12, GM14 and GS18 at different temperatures 25, 45, 65 ^{o}C.

The direct effect of raising the hydrophobic chain of surfactants on their surface properties can be observed from the values of efficiency and effectiveness as shown in ***Table 2***. The efficiency values upsurge as the length of tails increases. Effectiveness (π_{CMC}) data show that the surfactant with the longest hydrophobic chain length lowers the surface tension the most at CMC. π_{CMC} of GL12, GM14, GS18 at 25 ^{o}C were 35.5, 37.5, 38.5 mNm^{-1} respectively, and L12, M14, S18 were 34.5, 35.5, 36.5 mNm^{-1} respectively.

Table (2): The surface parameters of the synthesized ester cationic monomeric surfactant and their gemini structures at various temperatures

Comp.	*Temp. ^{o}C*	*CMC/ (mM.L^{-1})*	*C_{20} *10^{-5} (mol.L^{-1})*	*π_{CMC} (mN m^{-1})*	*Γ_{max} *10^{-10} (mol.cm^{-2})*	*A_{min}/ A^{2}*	*A min nm^{2}*
L12	25	1.28825	3.55	34.50	0.41	407.30	4.07
	45	1.122019	3.16	32.00	0.38	437.20	4.37
	65	0.870964	2.00	30.00	0.28	588.67	5.89
GL12	25	1.023293	0.69	35.50	0.24	689.94	6.90
	45	0.870964	0.63	33.00	0.23	720.30	7.20
	65	0.707946	0.56	31.00	0.17	1004.49	10.04
M14	25	1.071519	0.71	35.50	0.37	443.31	4.43
	45	1.00	0.68	33.00	0.33	501.90	5.02
	65	0.776247	0.60	32.00	0.31	535.27	5.35
GM14	25	0.977237	0.52	37.50	0.28	591.30	5.91
	45	0.776247	0.43	35.00	0.24	678.49	6.78
	65	0.724436	0.28	33.00	0.16	1008.79	10.09
S18	25	1.00	0.51	36.50	0.36	464.60	4.65
	45	0.776247	0.35	34.00	0.28	582.93	5.83
	65	0.691831	0.32	31.00	0.25	675.42	6.75
GS18	25	0.794328	0.22	38.50	0.22	744.23	7.44
	45	0.676083	0.19	36.00	0.16	1006.49	10.06
	65	0.60256	0.17	34.00	0.16	1069.75	10.70

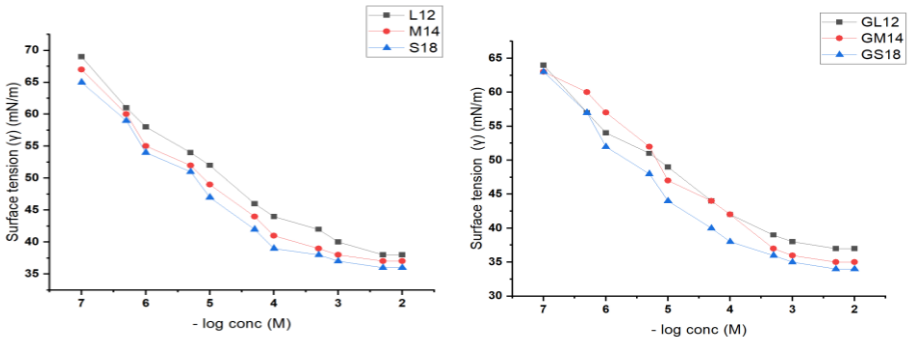


Fig 5. Plots surface tension versus. −log concentration of the synthesized surfactants at 25^{O}C**.**

Table 2 shows the recorded A_{min} of GL12, GM14, GS18, L12, M14, and S18 surfactants. It is clear that surfactants with longer tails had higher A_{min} values. In addition to the consideration of the Gemini surfactants had two tails, it is expected that A_{min} values equal double of that corresponded to the monomeric surfactant molecules. But, A_{min} values were lower than the expected values (***Table 2***). The proposed reason for this behavior is that the longer chains tend to bend in the solution to overcome the repulsion occurred due to their interaction with the polar aqueous medium. This behavior is consistent with the general behavior of the Gemini cationic surfactant [13, 25, 28][29, 30].

The influence of solution temperature on the surface activities of the synthesized Gemini surfactant and their monomeric were studied at 45 and 65 ^{o}C. At higher temperatures, the interaction between the surfactant molecules in the aqueous medium and the water molecules is increased due to the differences in

the polarity between these two phases. That changes the behavior of the surfactant solution than that at lower temperatures.

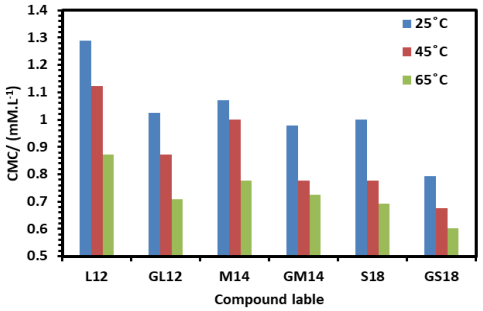


Fig. 6 Effect of solution temperature and length of hydrocarbon chain length on the critical micelle concentration

Increasing the temperature forced the surfactant molecules to decrease their interaction with water molecules. That can be performed by formation of micelles which leads to decrease CMC values, as a result of escaping the molecules to solution bulk. Consequently, surface tension at CMC and efficiency are increased. The decrease of the surfactant molecules at the interface to a decrease in the maximum surface excess and, consequently, the average area available for the molecules at the interface is consequently increased [13, 31]. The variation in the surface activity and surface properties can be monitored in ***Table 2***.

3.3. Biological activity of the synthesized surfactants

Owing to the researcher's emphasis on multi-drug resistant bacteria, various methods for testing the anti-bacterial activity of the prepared compounds were used, including agar well diffusion and disc diffusion methods. Both methods depend on the ability of compounds to diffuse from high to low gradient agar concentration to achieve anti-microbial activity. These methods have the following drawbacks. First, they are inadequate in distinguishing between bactericidal and bacteriostatic effects since bacterial growth inhibition does not indicate bacterial death; second, they are unsuccessful in deciding the minimum inhibitory concentration (MIC); finally, it is difficult to establish the quantity of tested compound diffused through the agar medium [32, 33]. The most appropriate procedure for assessing MIC is a technique called the dilution method. This procedure has been developed by the Clinical Laboratory Standard Institute (CLSI), but it has a drawback in that slight turbidity can be misinterpreted as MIC, leading to incorrect results. [32], this method was corrected by the European committee on antimicrobial susceptibility testing (EUCAST) which is characterized by its accurate results for determination of MIC and MBC by using spectrophotometric assay rather than visual assay as in (CLSI) [34].

Table 3. Inhibition efficiency of serial dilution of 1000 ***µL*** from the synthesized surfactants against gram +ve bacteria (*B. pumilis* MTCC – 2296, *S. faecalis* MTCC – 0459)

	Efficiency, % , against B.PUMILIS						***Efficiency, % , against S.faecalis%***					
Comp / ***Conc.µL..***	***L12***	***GL12***	***M14***	***GM14***	***S18***	***GS18***	***L12***	***GL12***	***M14***	***GM14***	***S18***	***GS18***
1000	90.31	93.02	88.37	89.53	89.53	93.69	93.28	95.60	95.83	96.52	96.99	97.45
500	83.33	91.47	86.43	87.59	81.39	86.43	91.2	92.36	94.67	95.13	93.75	95.60
250	78.29	89.53	84.10	84.88	79.06	82.55	87.96	90.27	93.28	93.75	94.21	94.44
125	75.96	82.945	82.94	83.33	60.46	78.29	84.25	88.42	91.43	92.12	90.04	90.97
62.5	69.37	80.23	77.90	78.68	51.93	62.01	75.92	79.86	88.65	90.27	86.57	89.58
31.3	60.46	67.40	75.96	77.13	34.88	58.91	64.3	76.38	85.87	88.65	78.01	82.63
15.63	44.18	62.79	65.50	67.82	26.74	31.00	58.10	61.34	84.95	85.64	68.98	78.01
7.81	31.78	44.96	56.97	61.24	22.09	23.25	45.37	51.85	78.47	80.09	55.13	72.91
3.91	20.54	23.64	31.78	36.82	9.68	17.82	33.10	43.28	71.52	77.31	24.30	47.91
1.95	3.48	8.91	23.25	28.29	1.937	1.16	18.75	23.84	43.05	44.21	8.31	21.52

This technique is based on Beers-lambert law, which involves 2 fold of serial dilution of the tested compound in liquid medium using 96 microplate wells, and then each well is inoculated with standardized bacterial suspension of 0.5 McFarland. Following well mixing, the inoculated well is allowed to incubate in good condition for the appropriate time and temperature based on the type of

microorganisms. The following equation can be used to calculate bacterial cell inhibition and cell reduction

$$\textbf{Efficiency \% =1 - (}\frac{A24\ t - A0t}{A24b - A0b}\textbf{) ×100}$$

A_{24t}, A_{0t}, A_{24b}, A_{0b} are the absorbance of the medium: after incubation, of positive test at zero hour, of positive control after incubation, and of positive control at zero hour [35], respectively.

The inhibition efficiency of the synthesized surfactants at serial diluted concentration of 1000 µL against Gram +ve bacteria (*B. pumilis MTCC – 2296*, *S. faecalis MTCC – 0459*), Gram -ve bacteria (*E. coli ATCC 25955*, *E. cloacae ATCC 23355*) and chosen fungi (*A. niger MTCC-1881*, *C. albicans (ATCC 10231*) were listed in ***Tables 3-5***.

Table 4. Inhibition efficiency of serial dilution of 1000 µL from the synthesized surfactants against gram -ve bacteria (*E. coli* ATCC 25955, *E. cloacae* ATCC 23355)

	Efficiency, % , against E. cloacae						*Efficiency, % , against E. coli %*					
Comp Conc.µL..	*L12*	*GL12*	*M14*	*GM14*	*S18*	*GS18*	*L12*	*GL12*	*M14*	*GM14*	*S18*	*GS18*
1000	95.82	96.78	96.31	96.95	96.63	97.43	93.80	94.24	94.69	95.57	92.47	93.80
500	94.54	95.66	95.50	95.98	94.86	95.66	92.47	93.36	91.59	92.92	90.26	91.59
250	90.36	92.93	93.41	94.22	93.73	94.54	87.61	90.70	89.82	91.59	88.49	89.82
125	88.12	89.72	91.01	92.29	87.64	88.60	85.84	86.72	85.39	86.28	87.16	88.05
62.5	85.87	86.51	88.12	90.36	79.45	81.38	79.20	83.18	77.43	80.53	83.18	86.72
31.3	81.54	82.34	82.18	85.39	75.28	76.40	76.99	78.31	71.24	78.31	81.41	82.74
15.63	76.24	81.54	70.14	75.12	60.03	66.45	71.68	73.83	66.81	76.54	78.76	80.08
7.81	67.73	74.63	59.23	63.72	48.47	55.85	64.6	69.03	64.15	69.46	75.22	76.10
3.91	48.31	57.14	7.54	23.59	26.48	44.14	43.80	52.21	31.85	39.38	46.01	46.90
1.95	16.53	20.06	0.642	4.17	5.45	28.08	22.56	33.18	5.75	16.81	8.84	44.69

Table 5. Inhibition efficiency of serial dilution of 1000 µL from the synthesized surfactants against chosen fungi (*A. niger* MTCC -1881, *C. albicans* (ATCC 10231)

	Efficiency, % , against A. niger						*Efficiency, % , against C. albicans %*					
Comp Conc.µL..	*L12*	*GL12*	*M14*	*GM14*	*S18*	*GS18*	*L12*	*GL12*	*M14*	*GM14*	*S18*	*GS18*
1000	*95.49*	*96.47*	*96.95*	*97.93*	*96.35*	*97.68*	*97.03*	*97.71*	*97.03*	*98.52*	*95.15*	*97.71*
500	*94.76*	*95.37*	*95.25*	*95.98*	*93.43*	*93.43*	*95.7*	*95.69*	*96.09*	*97.57*	*91.92*	*96.36*
250	*87.83*	*90.87*	*94.64*	*95.74*	*84.30*	*84.67*	*90.71*	*93.40*	*92.59*	*94.21*	*89.23*	*90.57*
125	*82.84*	*84.54*	*93.79*	*95.13*	*77.98*	*78.22*	*83.71*	*87.08*	*91.25*	*93.40*	*85.33*	*87.75*
62.5	*76.27*	*79.56*	*92.09*	*93.55*	*71.65*	*76.27*	*80.34*	*82.63*	*90.17*	*92.73*	*79.41*	*83.18*
31.3	*68.6*	*71.654*	*91.24*	*91.61*	*63.75*	*67.40*	*78.07*	*78.87*	*88.83*	*91.79*	*69.45*	*77.79*
15.63	*59.73*	*61.43*	*83.69*	*88.44*	*58.03*	*60.46*	*66.75*	*69.18*	*77.79*	*84.25*	*65.01*	*69.85*
7.81	*47.32*	*53.89*	*71.53*	*75.91*	*33.82*	*48.29*	*52.35*	*61.77*	*33.37*	*41.99*	*50.20*	*55.99*
3.91	*31.38*	*44.76*	*31.38*	*43.18*	*20.55*	*36.98*	*11.44*	*56.66*	*6.06*	*21.39*	*37.14*	*39.30*
1.95	*4.37*	*16.18*	*2.55*	*4.14*	*16.90*	*30.41*	*4.17*	*43.34*	*3.23*	*4.57*	*4.30*	*20.18*

The cell membrane of microorganisms is made up of a bilayer of lipids (building blocks) and protein layers, which gives them their hydrophobic properties. The key function of such a lipoprotein membrane is to regulate the biochemical reactions that occur in the cell, which is reflected by its permeability. Any factor that affects selective permeability in the cell membrane has a negative impact on microorganisms. Cationic surfactants have a strong tendency to adsorb at the cell membrane's primarily negatively charged interface. As monomeric synthesized ester surfactants are compared to corresponding geminis, the latter introduces more positive charge, allowing for greater electrostatic interaction at the membrane. Adsorption at the cell membrane interface raises the hydrophobicity and permeability of the membrane. As a consequence, biochemical processes in the cytoplasm of the cell are disordered, and the cell is killed [36]. As a result, Gemini surfactants in this

way have better surface active properties and consequently higher biocidal activity against microorganisms than monomeric surfactants.

Tables 3-5 showed that monomeric derivatives had adequate antimicrobial activity in contradiction of the tested pathogenic bacteria and fungi, whereas the analogous Geminis had improved antimicrobial activity.

Table 6 designates MIC, MBC, and MFC: minimum inhibitory concentration, minimum bactericidal concentration, and minimum fungicidal concentration of Gemini and monomeric surfactants against various standard microbial strains. It was revealed that GS18 had the highest inhibition efficiency of 55.85/66.45, 76.10/80.08, and 55.99/69.85 percent in contradiction of *E. cloacae*, *E. coli*, and *C. albicans*, respectively; with lowest MIC/MBC and MIC/MFC of 7.81/15.63 ppm (***Table 6***). On the other hand, GM14 exhibits the highest inhibition efficiency of 67.82/77.13, 77.31/80.09, and 75.91/88.44% with lower MIC/MBC and MIC/MFC of 15.63/31.3, 3.91/7.81, 7.81/15.63 ppm against *B. pumilis*, *S. faecalis*, and *A. niger*, respectively. By comparison, inhibition efficiencies of the monomeric series L12, M14 and S18, the antimicrobial activity was increased by increasing the alkyl chains from 12 to 14 methylene groups; however, antimicrobial activity was decreased by a further increase to 18 methylene group [37].

Table 6 The minimum inhibitory concentration (MIC), the minimum bactericidal concentration (MBC), and the minimum fungicidal concentration (MFC) of the synthesized gemini and monomeric cationic surfactants against different standard microbial strains

Sample code	MIC (ppm/ml)				MBC (ppm /ml)				MIC (ppm/ml)		MFC (ppm /ml	
	B. pumilis	*S. faecalis*	*E. cloacae*	*E. coli*	*B. pumilis*	*S. faecalis*	*E. cloacae*	*E. coli*	*A. niger*	*C. albicans*	*A. niger*	*C. albicans*
L12	31.3	31.3	15.63	7.81	62.5	62.5	31.3	15.63	31.3	15.63	62.5	31.3
GL12	15.63	15.63	7.81	7.81	31.3	31.3	15.63	15.63	15.63	15.63	31.3	31.3
M14	15.63	3.91	15.63	15.6	31.3	7.81	31.3	31.3	15.63	15.63	31.3	31.3
GM14	15.63	3.91	7.81	7.81	31.3	7.81	15.63	15.63	7.81	15.63	15.63	31.3
S18	62.5	15.63	15.63	7.81	125	31.3	31.3	15.63	31.3	15.63	62.5	31.3
GS18	31.3	7.81	7.81	7.81	62.5	15.63	15.63	15.63	15.63	7.81	31.3	15.63

4. Conclusion

A new series of monomeric ester-cationic surfactants and their Gemini homologous were successfully synthesized. The chemical structures of the prepared surfactants (L12 - GS18) were confirmed by FTIR and ^{1}HNMR spectroscopy and elemental analysis. The surface parameters of the prepared surfactants at different temperatures were established and revealed that the long hydrophobic derivative exhibits higher adsorption at the solution interface and more affinity to construct micelles. Furthermore, all the synthesized Gemini cationic surfactants have exhibited nonspecific (broad-spectrum) antimicrobial activities than the monomeric derivatives. The highest activity was attributed to the GS18 at the lower MIC/MBC and MIC/MFC concentrations compared to the GL12 and the GM14. Moreover, the Gemini cationic surfactants GL12, GM14, and GS18 have displayed higher biological activities against gram+ve bacteria, gram–ve bacteria and fungi species than the corresponding monomeric structure.

5. Conflicts of interest

"There are no conflicts to declare".

6. Acknowledgments

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7. References

[1] Multifunctional Gemini Surfactants: Structure, Synthesis, Properties and Applications, (2017).

[2] L.Y. Zakharova, T.N. Pashirova, S. Doktorovova, A.R. Fernandes, E. Sanchez-Lopez, A.M. Silva, S.B. Souto, E.B. Souto, Cationic Surfactants: Self-Assembly, Structure-Activity Correlation and Their Biological Applications, *International Journal of Molecular Sciences*, **20** (2019).

[3] S.M. Tawfik, A.A. Abd-Elaal, S.M. Shaban, A.A. Roshdy, Surface, thermodynamic and biological activities of some synthesized Gemini quaternary ammonium salts based on polyethylene glycol, *Journal of Industrial and Engineering Chemistry*, **30** (2015) 112-119.

[4] M.S. Kamal, A review of gemini surfactants: potential application in enhanced oil recovery, *Journal of Surfactants and Detergents*, **19** (2016) 223-236.

[5] F.M. Menger, J.S. Keiper, Gemini surfactants, *Angewandte Chemie International Edition*, **39** (2000) 1906-1920.

[6] N. Pal, A. Verma, K. Ojha, A. Mandal, Nanoparticle-modified gemini surfactant foams as efficient displacing fluids for enhanced oil recovery, *Journal of Molecular Liquids*, **310** (2020) 113193.

[7] K.-X. Zhang, A.-X. Ding, Z.-L. Tan, Y.-D. Shi, Z.-L. Lu, L. He, Tetraphenylethylene-based gemini surfactant as nonviral gene delivery system: DNA complexation, gene transfection

and cellular tracking, *Journal of Photochemistry and Photobiology A: Chemistry*, **355** (2018) 338-349.

[8] C. Costa, I.S. Oliveira, J.P.N. Silva, S.G. Silva, C. Botelho, M.L.C. do Vale, M.E.C.D. Real Oliveira, A.C. Gomes, E.F. Marques, Effective cytocompatible nanovectors based on serine-derived gemini surfactants and monoolein for small interfering RNA delivery, *Journal of Colloid and Interface Science*, **584** (2021) 34-44.

[9] A.A. Abd-Elaal, S.M. Shaban, S.M. Tawfik, Three Gemini cationic surfactants based on polyethylene glycol as effective corrosion inhibitor for mild steel in acidic environment, *Journal of the Association of Arab Universities for Basic and Applied Sciences*, **24** (2017) 54-65.

[10] S.M. Tawfik, A.A. Abd-Elaal, I. Aiad, Three gemini cationic surfactants as biodegradable corrosion inhibitors for carbon steel in HCl solution, *Research on Chemical Intermediates*, **42** (2016) 1101-1123.

[11] Y. Li, L. Lai, P. Mei, W.-X. Zhang, Y.-H. Li, L. Cheng, Y.-Q. Wang, Y. Liu, Surface properties and solubility enhancement of Gemini/conventional surfactant mixtures based on sulfonate Gemini surfactant, *Journal of Molecular Liquids*, **276** (2019) 488-496.

[12] W. Luo, Q. Huang, X. Zhang, P. Antwi, Y. Mu, M. Zhang, J. Xing, H. Chen, S. Ren, Lanthanum/Gemini surfactant-modified montmorillonite for simultaneous removal of phosphate and nitrate from aqueous solution, *Journal of Water Process Engineering*, **33** (2020) 101036.

[13] S.M. Shaban, A.A. Abd-Elaal, Studying the silver nanoparticles influence on thermodynamic behavior and antimicrobial activities of novel amide Gemini cationic surfactants, *Materials Science and Engineering: C*, **76** (2017) 871-885.

[14] Z. Gao, S. Tai, Q. Zhang, Y. Zhao, B. Lü, Y. Ge, L. Huang, X. Tang, Synthesis and surface activity of biquaternary ammonium salt gemini surfactants with ester bond, *Wuhan University Journal of Natural Sciences*, **13** (2008) 227-231.

[15] M.T. Garcia, I. Ribosa, I. Kowalczyk, M. Pakiet, B. Brycki, Biodegradability and aquatic toxicity of new cleavable betainate cationic oligomeric surfactants, *Journal of Hazardous Materials*, **371** (2019) 108-114.

[16] M. Stjerndahl, K. Holmberg, Synthesis and chemical hydrolysis of surface-active esters, *Journal of Surfactants and Detergents*, **6** (2003) 311-318.

[17] M. Stjerndahl, C.G. van Ginkel, K. Holmberg, Hydrolysis and biodegradation studies of surface-active esters, *Journal of Surfactants and Detergents*, **6** (2003) 319-324.

[18] F.E. Friedli, R. Keys, C. Joe Toney, O. Portwood, D. Whittlinger, M. Doerr, Novel new ester quaternaries for improved performance benefits as rinse cycle fabric softeners, *Journal of Surfactants and Detergents*, **4** (2001) 401-405.

[19] R.J. Fair, Y. Tor, Antibiotics and bacterial resistance in the 21st century, *Perspectives in medicinal chemistry*, **6** (2014) PMC. S14459.

[20] S. Chavda, P. Bahadur, V.K. Aswal, Interaction Between Nonionic and Gemini (Cationic) Surfactants: Effect of Spacer Chain Length, *Journal of Surfactants and Detergents*, **14** (2011) 353-362.

[21] I. Aiad, M.M. El-Sukkary, A. El-Deeb, M.Y. El-Awady, S.M. Shaban, Surface Properties, Thermodynamic Aspects and Antimicrobial Activity of Some Novel Iminium Surfactants, *Journal of Surfactants and Detergents*, **15** (2012) 359-366.

[22] N.A. Negm, M.F. Zaki, Corrosion inhibition efficiency of nonionic Schiff base amphiphiles of p-aminobenzoic acid for aluminum in 4N HCL, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **322** (2008) 97-102.

[23] S.M. Tawfik, A. Sayed, I. Aiad, Corrosion inhibition by some cationic surfactants in oil fields, *Journal of Surfactants and Detergents*, **15** (2012) 577-585.

[24] I. Aiad, M.I. Marzouk, S.A. Shaker, N.E. Ebrahim, A.A. Abd-Elaal, S.M. Tawfik, Antipyrine cationic surfactants capping silver nanoparticles as potent antimicrobial agents against pathogenic bacteria and fungi, *Journal of Molecular Liquids*, **243** (2017) 572-583.

[25] N.A. Negm, A.S. Mohamed, Surface and thermodynamic properties of diquaternary bola-form amphiphiles containing an aromatic spacer, *Journal of surfactants and detergents*, **7** (2004) 23-30.

[26] A.A. Abd-Elaal, S. Tawfik, S. Shaban, Simple one step synthesis of nonionic dithiol surfactants and their self-assembling with silver nanoparticles: Characterization, surface properties, biological activity, *Applied Surface Science*, **342** (2015).

[27] E.E.C.f.A.S.T. Subcommittee on Antifungal Susceptibility Testing of the, J.L. Rodríguez-Tudela, F. Barchiesi, J. Bille, E. Chryssanthou, M. Cuenca-Estrella, D. Denning, J.P. Donnelly, B. Dupont, W. Fegeler, C. Moore, M. Richardson, P.E. Verweij, Method for the determination of minimum inhibitory concentration (MIC) by broth dilution of fermentative yeasts, *Clinical Microbiology and Infection*, **9** (2003) i-viii.

[28] Q. Zhang, Z. Gao, F. Xu, S. Tai, X. Liu, S. Mo, F. Niu, Surface Tension and Aggregation Properties of Novel Cationic Gemini Surfactants with Diethylammonium Headgroups and a Diamido Spacer, *Langmuir*, **28** (2012) 11979-11987.

[29] L. Zhou, X. Jiang, Y. Li, Z. Chen, X. Hu, Synthesis and Properties of a Novel Class of Gemini Pyridinium Surfactants, *Langmuir*, **23** (2007) 11404-11408.

[30] B. Gao, M.M. Sharma, A family of alkyl sulfate gemini surfactants. 1. Characterization of surface properties, *Journal of Colloid and Interface Science*, **404** (2013) 80-84.

[31] S. Chauhan, M. Kaur, K. Kumar, M.S. Chauhan, Study of the effect of electrolyte and temperature on the critical micelle concentration of dodecyltrimethylammonium bromide in aqueous medium, *The Journal of Chemical Thermodynamics*, **78** (2014) 175-181.

[32] S. Chand, I. Lusunzi, D. Veal, L. Williams, P. Karuso, Rapid screening of the antimicrobial activity of extracts and natural products, *The journal of antibiotics*, **47** (1994) 1295-1304.

[33] E.S. Lestari, J.A. Severin, P.M. Filius, K. Kuntaman, D. Offra Duerink, U. Hadi, H. Wahjono, H.A. Verbrugh, P. Antimicrobial Resistance in Indonesia, Prevention, Comparison of the accuracy of disk diffusion zone diameters obtained by manual zone measurements to that by automated zone measurements to determine antimicrobial susceptibility, *Journal of microbiological methods*, **75** (2008) 177-181.

[34] S. Arikan, Current status of antifungal susceptibility testing methods, *Medical mycology*, **45** (2007) 569-587.

[35] P.A. Akinduti, B. Motayo, O.M. Idowu, P.O. Isibor, G.I. Olasehinde, Y.D. Obafemi, H.U. Ugboko, J.O. Oyewale, A. Oluwadun, G.A. Adeyemi, Suitability of spectrophotometric assay for determination of honey microbial inhibition, *Journal of Physics: Conference Series*, **1299** (2019) 012131.

[36] K. Kuperkar, J. Modi, K. Patel, Surface-Active Properties and Antimicrobial Study of Conventional Cationic and Synthesized Symmetrical Gemini Surfactants, *Journal of Surfactants and Detergents*, **15** (2012) 107-115.

[37] S.M. Tawfik, Synthesis, surface, biological activity and mixed micellar phase properties of some biodegradable gemini cationic surfactants containing oxycarbonyl groups in the lipophilic part, *Journal of Industrial and Engineering Chemistry*, **28** (2015) 171-183.