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Synthesis, Surface and Antimicrobial Activity of Piperazine-Based Cationic Oligomeric Surfactants

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Abstract

Humans have a basic need for better health, yet the accelerating spread of dangerous microorganisms and their detrimental impact on human health present a tremendous challenge to modern science. In this study, piperazine-based cationic oligomeric surfactants (dimeric and tetrameric quaternary ammonium salts) were synthesized. Elucidation of chemical structures of the synthesized cationic surfactants took place using Fourier Transform Infrared, Proton and Carbon Nuclear Magnetic Resonance Spectra. Also, their surface parameters as critical micelle concentration, effectiveness, maximum surface excess and minimum surface area were determined. The tested compounds were evaluated against Gram (+) bacteria as *Staphylococcus aureus* and *Bacillus cereus*; Gram (-) bacteria as *Escherichia coli* and *Pseudomonas aeuroginosa*. The antimicrobial efficiency was measured via inhibition of the bacterial growth represented as the diameter of the inhibitory zone (mm/mg sample) in contrast to Kanamycin for (G+) bacteria and Ampicillin for (G-) bacteria as standards. The results showed that the prepared cationic oligomeric surfactants can adsorb and orient themselves efficiently at the air/water interface and they have considerable antimicrobial activity against tested species.

Keywords: Cationic oligomeric surfactants; Antimicrobial agents;Amidation reaction; Michael addition reaction; Piperazine

1. Introduction

Oligomeric surfactants are characteristic class of surfactants, whose structures have attracted both academic and industrial interests. They show an improved property profile and give remarkable properties compared to typical surfactants. Oligomeric surfactants are those surfactants which contain multiple hydrophobic and hydrophilic groups in a molecule. Depending on degree of oligomerization (n), different oligomeric surfactants can be prepared as dimeric, trimeric, tetrameric…etc. Also, the shapes of oligomers can be altered from linear to star-like or cyclic shape. The surfactant fragments can be linked together either through hydrophilic head groups (head type) or through hydrophobic tail groups (tail-end type). The presence of these different architectures of the oligomeric surfactants play an important role in controlling their solution properties and aggregate morphologies, leading to a possible access to a broader range of applications [1].

Numerous sectors that are connected to human health as hospitals and dental equipments, food storage and packaging, water purification methods, and residential sanitation, are highly concerned about contamination by microbes [2]. A wide range of infections and diseases have been caused by the dangerous microbes that exist in these places. Rapid antibiotic resistance development makes things even more difficult [3, 4].Therefore, it is important to keep these regions clean and free of pollution. At the same time, attempts were made to create novel compounds with structural modifications and antimicrobial characteristics [5].

Antimicrobial agents are substances that have the ability to kill or prevent the growth of bacteria on a surface or in the environment. Conventional cationicsurfactantscontaining quaternary ammonium

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headgroups exhibit considerable bactericidal potential by directly breaking the bacterial cell wall.

The simplest oligomeric surfactants are called dimeric or gemini surfactants (contain two hydrophobic chains and two hydrophilic head groups). They have remarkable and unique properties [6], as they have more antimicrobial action than their equivalent monomeric surfactants.Biological activity of oligomeric surfactants can be increased by increasing their degree of oligomerization [7]. Therefore, cationic oligomeric surfactants which contain three or more amphiphilic moieties connected by spacer groups, and with a lower critical micelle concentration (CMC), are anticipated to have significant antibacterial action. Recent studies have also revealed that the presence of amide group within surfactant structure is favorable for increasing their biocompatibility and antimicrobial activity [8].

Based on all mentioned above, this study aimed to prepare new cationic oligomeric surfactants containing quaternary ammonium head groupsand bearing amide linkage within their spacers, determine the properties of their surface, and then assess them as antibacterial agents.

2 Methodologies

2.1 Materials

Materials used in this work (piperazine, methyl meth acrylate, dodecyl bromide, manganous chloride) were obtained from Acros organics, while the used solvents (methyl alcohol and acetone) were obtained from ADWIC Company.

2.2 Synthetic procedure

Preparation of cationic oligomeric surfactants

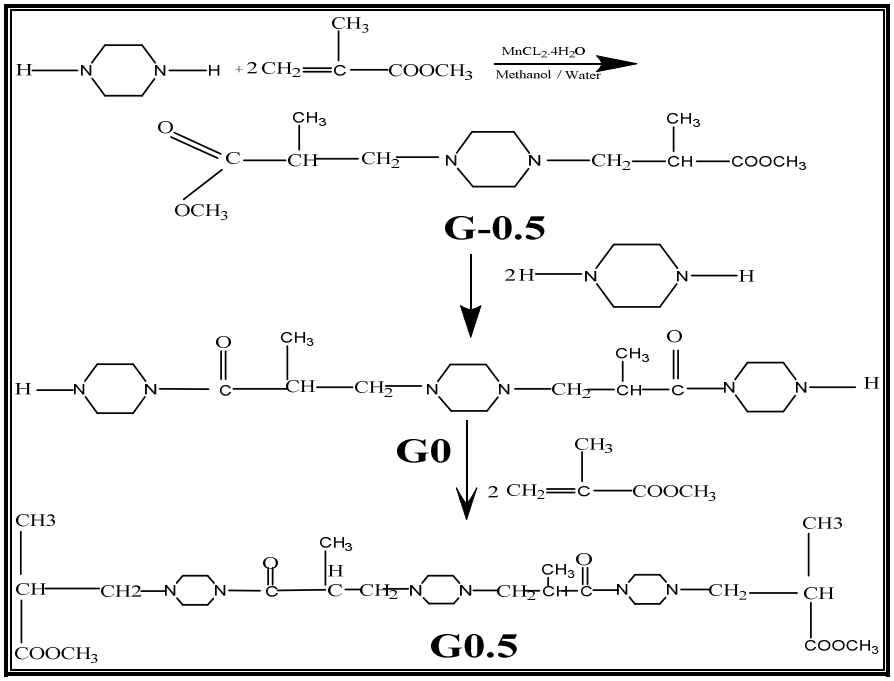
Cationic oligomeric surfactants based on piperazinewere prepared through successive steps using Michael addition and amidation reactions, respectively, followed by quaternization reaction step**.** Michael addition reaction included the preparation of methyl ester end products ($G_{-0.5}$, $G_{0.5}$), while amine end product (G_0) was obtained from the amidation step as shown in (Scheme 1). Quaternization of each of G_0 and $G_{0.5}$ was carried out to obtain G_0C_{12} and $G_{0.5}C_{12}$, respectively.

2.2.1Preparation of ($G_{-0.5}$) via Michael addition reaction

Compound ($G_{-0.5}$) was prepared as mentioned previously in our work [9, 10].

2.2.2 Preparation of (G_0) via amidation reaction

G_0 was prepared via amidation reaction as follows: In a bath of ice, gradually add $G_{-0.5}$ solution (1.0 mole / 100 ml methyl alcohol) to piperazine solution (2.0 moles / 300 ml methyl alcohol) with stirring at 0ºC. It is important to carefully operate the rate of addition to avoid the reaction's temperature from rising. The reaction mixture was removed from the ice bath and stirred using magnetic stirrer (at room temperature) for 72 hours to finish the amidation reaction. Excess methanol was removed by placing the mixture in rotary evaporator using decreased pressure at 45 ºC [11]. Product G_0 was obtained as shown in scheme (1).



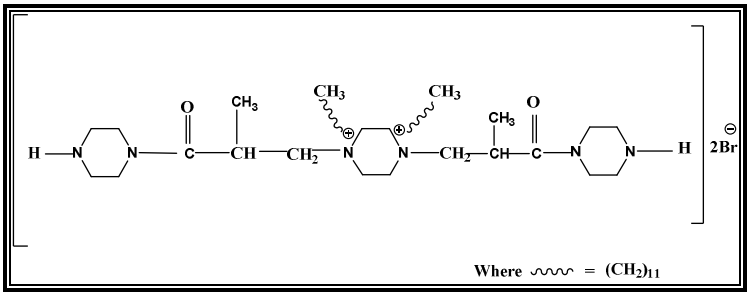
Scheme 1

2.2.3Preparation of ($G_{0.5}$) via Michael addition reaction

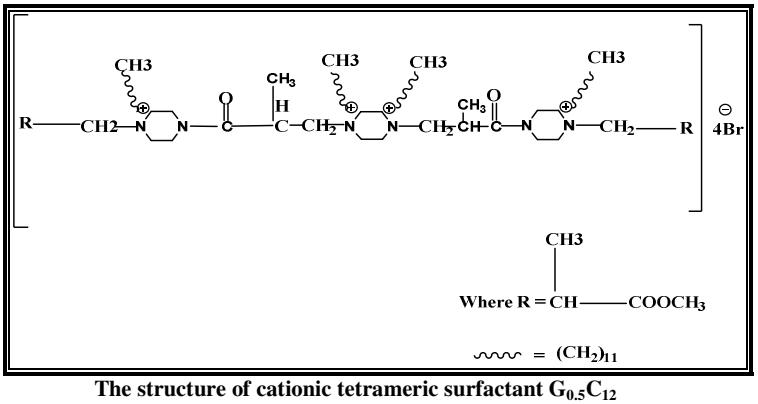
Preparation of compound ($G_{0.5}$) was carried out by addition of methyl methacrylate (2.035 mol) to 0.2L of methanol which was cooled in a bath of ice. 0.83 mol of (G_0) was dissolved in methyl alcohol (200 ml) then gradually added to the above solution, while being continuously stirred for two hours. After that, the mixture's temperature was raised to 25 ºC and stirred for 48 hrs. The excess methylmethacrylate and methyl alcohol were then removed using rotary evaporator with decreased pressure at 45ºC [10].

2.2.4 Quaternization of[G_0 and $G_{0.5}$] to prepare cationic oligomeric surfactants [G_0C_{12} and $G_{0.5}C_{12}$]

Dodecyl bromide was refluxed with stoichiometric quantities of G_0 and of $G_{0.5}$ in a methanol/acetone mixture for 24 hours at 70 to 80 °C [12]. Then, Evaporation of the used solvents took place, the excess alkyl halide was then removed from the generated compounds by dissolving them in methyl alcohol and extracting them with hexane. The resulted products [G_0C_{12} and $G_{0.5}C_{12}$] were obtained as shown in (Scheme 2).



The structure of cationic dimeric surfactant G_0C_{12}



The structure of cationic tetrameric surfactant $G_{0.5}C_{12}$

Scheme 2

2.3 Structural confirmation of the prepared cationic oligomeric surfactants

Chemical structures of the prepared cationic oligomeric surfactants were investigated using various spectroscopic techniques: (a) Fourier Transform Infrared Spectroscopy: measured using Mattson ATI genesis FTIR spectrometer. A resolution of 2 cm^{-1} was used for all spectra with an incidence angle of 80^o, and (b) Nuclear Magnetic Resonance (^1H and ^{13}C NMR) spectra. The prepared compounds were estimated in dimethyl sulfoxide using NMR spectrometer (Jeol ECA, Japan).

2.4 Surface parameters of the prepared cationic oligomeric surfactants

Determination of the critical micellization concentration (CMC) and surface activity is the basic step in evaluating the property profile of surfactants. The CMC values of the substances under investigation were detected via surface tension technique using Attention Theta Optical Tensiometer (Biolin Scientific Company, Finland).Different surface characteristics of the prepared cationic oligomeric surfactants were detected includingmaximum surface excess, effectiveness, minimum surface area and standard free energies of micellization and adsorption.

2.5 Biological activity by Agar disc-diffusion Method

Antimicrobial activity of the studied compounds [G_0C_{12} and $G_{0.5}C_{12}$] was determined at the Micro analytical center of the Faculty of Science at Cairo University using agar disc-diffusion method.

Agar disc-diffusion method is the certified method in several clinical microbiology laboratories for routine antimicrobial susceptibility testing [13]. This methodcan be used by the Clinical and Laboratory Standards Institute (CLSI) for drug discovery

prediction of therapeutic testing against bacteria, fungi, and yeasts.
The substances under investigation were screened for their in vitro anti-bacterial activity. The primary benefits are reproducibility, simplicity, ease of modifying antimicrobial discs and the ability to use as a screening test against many isolates [14].Dimethyl sulfoxide (DMSO) was used to prepare the stock solutions, since it had no effect on the microorganisms in the studied concentrations. Mueller-Hinton agar plates are inoculated with a standardised inoculum of the test microorganism G+ and G- bacterial species of the tested bacterial species; *Pseudomonas aeruginosa* (ATCC 9027) and *Escherichia coli* (ATCC 8739) as G- but *Staphylococcus aureus* (ATCC 6538) and *Bacillus cereus* (ATCC 14579) as G+ bacterial standard isolates (corresponding to 0.5 McFarland turbidity standard) [15].

Evaluation of antibacterial activity by disc diffusion assay. All bacterial pathogens were inoculated, and the suspension was adjusted to 1.5 $x10^8$ colony forming units (CFU) (0.5 Mcfarland scale) and finally diluted to $1x10^8$ CFU [16].

Commercially manufactured paper discs (approximated diameter of 8 mm) with desired concentration of the tested compound positioned on surface of the inoculated agar plate. The incubation of agar plates occurred under proper circumstances, typically for 16-24 h at 35-37°C for bacterial species [17].

The diameter of the growth inhibition zone (which is the area of no growth around the disc, also known as “Zone of inhibition” or" Clear zone") around each disc impregnated with tested compound is measured in millimeters and the diameter of the disc is included in the result. This is done manually with a ruler held on the back of the inverted agar plate (Lalitha, 2004). DMSO was used as negative control, whereas Ampicillin (for G(-)bacteria) and Kanamycin (for G (+) bacteria) were used as positive controls for antimicrobial activity.

Accordingtothe disc diffusion test, the bacterial susceptibility classified as susceptible, intermediate, or resistant and provides qualitative results [18].

3 Results and discussion

3.1 Preparation of cationic oligomeric surfactants

FTIR spectra of the synthesized materials obtained from Michael addition steps ($G_{-0.5}$ and $G_{0.5}$) exhibited a characteristic band that related to carbonyl group in wavelength value ranged 1729.03-1736.30 cm^{-1} indicating the presence of methyl ester. This peak disappeared from the spectra of the product G_0as a result ofamidation reaction, and the corresponding CO group shifted to 1634.36 cm^{-1} because of appearance of amide group. Also, the terminal amine group leads to appearance of N–H band at 3396.85 cm^{-1}.

Chemical structures of[G_0C_{12} and $G_{0.5}C_{12}$] were characterized using ^{1}H-NMR and ^{13}C-NMR spectroscopy as follows:
(G_0 C_{12}): ^{1}H-NMR spectra (300 MHz, DMSO) δ =(0.83) ppm for [$(CH_2)_n$-$\underline{CH_3}$] group, δ =(1.08) ppm for [NH] group, δ =(1.22) ppm for [$(\underline{CH_2})_n$-CH_3] groups, δ =(1.52) ppm for[$\underline{CH_2}$- CH_2-N^+] group, δ =(3.14) ppm for [CH_2-$\underline{CH}(CH_3)$-CO] group, , δ =(3.22) ppm for [$\underline{CH_2}$-N^+] group, δ =(2.97) ppm for [$\underline{CH_2}$-NH] group and δ = (3.35) ppm for [cyclic $\underline{CH_2}$-N^+] group after quaternization step.^{13}C NMR (75.5 MHz, DMSO) δ = (14.02) ppm for [$(CH_2)_n$-$\underline{CH_3}$] group, δ =(15.73) ppm for [CH_2-$CH(\underline{CH_3})$-CO] group , δ = 22.14 ppm for [$\underline{CH_2}$-CH_3] group , δ = (28.76) ppm for [$(\underline{CH_2})_n$-CH_3] group, δ = (31.34) ppm for [CH_2-$\underline{CH}(CH_3)$-CO] group , δ = (48.72) ppm for [$\underline{CH_2}$-NH] group, δ = (50.63) ppm for [cyclic $\underline{CH_2}$-N-CO] group , δ =(59.01) ppm for [cyclic $\underline{CH_2}$-N^+] group , δ = (175.91) ppm for CO group .

($G_{0.5}$ C_{12}): ^{1}H-NMR spectra (300 MHz, DMSO) δ =(0.83) ppm for [$(CH_2)_n$-$\underline{CH_3}$] group, δ =(1.20) ppm for [$(\underline{CH_2})_n$-CH_3] groups, δ =(3.03) ppm for [CH_2-$\underline{CH}$-$COOCH_3$] group , δ =(3.13) ppm for [CH_2-$\underline{CH}(CH_3)$-CO] group, δ =(3.21) ppm for [$\underline{CH_2}$-N^+] group, δ = (3.36) ppm for [cyclic $\underline{CH_2}$-N^+] group, δ = (3.59) ppm for [$COO\underline{CH_3}$] group. ^{13}C NMR (75.5 MHz, DMSO) δ= (14.07) ppm for [$(CH_2)_n$-$\underline{CH_3}$] group, (15.38) ppm for [CH_2-$CH(\underline{CH_3})$-CO] group, δ = (22.21) ppm for [$\underline{CH_2}$-CH_3] group , δ = (28.92) ppm for [$(\underline{CH_2})_n$-CH_3] group , δ = (31.41) ppm for [CH_2-$\underline{CH}(CH_3)$-CO] group, δ = (48.6) ppm for [cyclic $\underline{CH_2}$-N-CO] group, , δ =(59.01) ppm for [Aliphatic $\underline{CH_2}$-N^+] group, δ = (60.46) ppm for [$COCH(CH3)\underline{CH_2}$-$N^+$] group , δ = (175.94) ppm for CO group .
The results were found to be consistent with the expected structures.

3.2 Surface activities of cationic oligomeric surfactants

The surface-active properties of the investigated surfactants were evaluated in an aqueous solution utilizing conventional techniques.

3.2.1 Critical Micelle Concentration

Surfactant aggregation is extensively researched for a variety of industrial and research purposes [19]. Surfactants have numerous applications, they can act as solubilizers, emulsifiers, detergents, and models of

various biochemical and pharmacological systems [20, 21]. Micelles, vesicles, bilayers, and other nanostructures may develop at a specific concentration of amphiphile molecules in solution [22]. Critical micelle concentration, maximum surface excess, minimum surface area, thermodynamic values, and other micellization parameters play significant roles in a variety of applications [23].

Fig. (1) illustrates the change of surface tension of the two prepared cationic oligomeric surfactants (G_0C_{12} and $G_{0.5}C_{12}$) against -log molar concentration at 40°C. The curves shown in Fig. 1 present typical behavior of surfactants, where the change in surface tension is significant at lower concentrations and small at higher one. CMC can be determined by taking the intercept of the two linear parts of the curve. CMC of G_0C_{12} (which contains 2 hydrophobic chains) and $G_{0.5}$ C_{12} (contains 4 hydrophobic chains) exhibited lower CMC values than CMC of the conventional cationic dodecyl trimethyl ammonium bromide surfactant DTAB (16.38 ± 0.04 mmol/L at 40°C) as stated before by others [24]. This signifies that the investigated cationic oligomeric surfactants have high capability to produce micelles even more than theconventional surfactant. Also, surfactant $G_{0.5}$ C_{12} which has 4 cationic quaternary nitrogen, and 4 alkyl chains revealed the lowest CMC value. This shows that CMC of the cationic oligomeric surfactants depends on the degree of oligomerization [1].

The surface tension for G_0C_{12} and $G_{0.5}C_{12}$ at their respective CMCs (γ_{CMC}) are given in Table1, where $G_{0.5}$ C_{12}shows lower surface tension (γ_{CMC}) value than G_0C_{12}.

Table (1): Critical micelle concentration and surface parameters of the prepared cationic oligomers at 40 °C

Surfactants	CMCx10^2 (mol / l)	γ_{CMC} (mN/m)	Π_{CMC} (mN/m)	Γ_{max}x10^{11} (mol/cm^2)	A_{min} (nm^2)	ΔG^oads kJ/mol	ΔG^omic kJ/mol
G_0 C_{12}	0.123	30.65	38.34	9.723	1.71	-17.5	-17.43
$G_{0.5}$ C_{12}	0.029	28.69	40.30	4.08	4.07	-21.2	-21.13

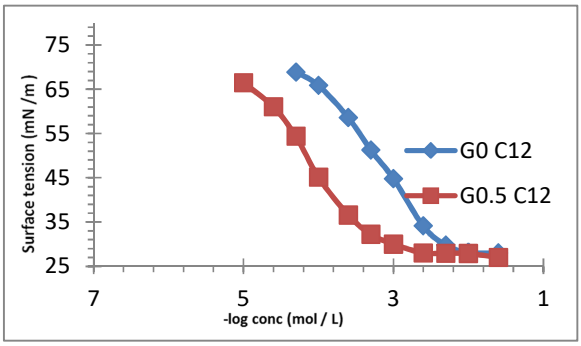


Figure 1: Variation of the surface tension with -log concentrationsfor G_0C_{12} and $G_{0.5}C_{12}$ at 40 °C

3.2.2 Effectiveness (π_{cmc})

(π_{CMC}) represents a surfactant's effectiveness in lowering surface tension, and it can be determined using equation:

$$\pi_{cmc} = \gamma_o - \gamma_{cmc}$$

where γ_o represents the surface tension of pure water, while γ_{cmc} represents surface tension at CMC [25]. The most effective surfactant has the lowest surface tension at the (CMC).

From Table 1, we can conclude that $G_{0.5}C_{12}$ has the greatest drop in surface tension at its CMC.

3.2.3 Maximum surface excess (Γ_{max})

(Γ_{max}) of surfactant ions can be determinedfrom slope of the straight line ($\delta\gamma$ /δ log c) of the surface tension plot, using Gibbs adsorption equation [26]

$$\Gamma_{max} = - (\delta\gamma/\delta \log c)_T / 2.303\ n\ RT$$

where T: absolute temperature, ($\delta\gamma$ /δ log c): the slope of the surface tension versus log concentration curve [27, 28].

The adsorption capacity of surfactants has a vital role in determining applications of surfactants at different interfaces. The difference in surfactants surface activity can be attributed to differences in their packing density [29].

Concerning oligomeric surfactants, (n) indicates how many species are present at the interface. Its value is determined from the degree of ionic surfactants dissociation, which is not acknowledged for all surfactants. There exist two hypotheses for dissociation of surfactant ions, the first that there is no dissociation, and the surfactant can be treated as a molecule. The second considers a complete dissociation. Many oligomeric surfactants have unknown degrees of dissociation, however within the same series of surfactants, it might vary depending on the spacer length or different degrees of oligomerization.

So, if we considered complete dissociation in solution, we supposed that n=3 for surfactant $G_0C_{12}$and n=5 for surfactant $G_{0.5}C_{12}$.

As shown in Table 1, G_0 C_{12} surfactant has higher maximum surface excess value than $G_{0.5}C_{12}$.

3.2.4 Minimum surface area (A_{min})

It is the area per molecule at the interface, from which we can know information about orientation of the adsorbed molecule and its degree of packing.

The average minimum surface area occupied by each molecule adsorbed at the interface [30] can be calculated through the following equation:

$$A_{min} = 10^{16} / N_A \cdot \Gamma_{max}$$

N_A represents Avogadro's number. Decreasing of Γ_{max} indicates that fewer molecules are being adsorbed at the interface. Therefore, the available area at interface for each surfactant molecule increase as clarified in the values of A_{min} (Table 1).

3.2.5 Standard free energies

Micellization and adsorption are phase transformation processes since the surfactant can present freely in solution, can adsorb at interface (adsorption), or can form micelles (micellization).

(ΔG^omic, ΔG^oads) were determined using Gibbs adsorption equations [31]:

$$\Delta G^o mic = RT \ln CMC$$

$$\Delta G^o ads = \Delta G^o mic - 6.023\times 10^{-1} \times \pi_{cmc} \times A_{min}$$

where T: the absolute temperature.

According to Table 1, the values of $\Delta G^o_{mic}$and ΔG^o_{ads} of the cationic oligomeric surfactants are negative, demonstrating the spontaneity of the two processes.

3.3 Antimicrobial activity

Antibiotic-resistant microorganisms have become an increasingly serious hazard to public health [32]. Surfactants are extensively used as antibacterial agents due to their ability to solubilize lipids. Quaternary ammonium salts (QAS) are cationic surfactants that can be employed in a variety of industries, including the chemical industry and home chemicals, due to their good water solubility, low toxicity, high surface activity, and good bactericidal action. Their great biocidal activity is due to presence of positive charge that can be adsorbed on the negatively charged microbial surface, changing the permeability of the cell membrane, and killing microorganisms [33].

In this study, the prepared cationic oligomeric surfactants with several hydrophobic chains were examined against several harmful bacteria. The antimicrobial efficiency was measured by the bacterial growth inhibition expressed as inhibition zone diameter (mm/mg sample) in comparison to Kanamycin for (G+) bacteria and Ampicillin for (G-) bacteria as standard antibacterial agents. The results showed that both $G_0C_{12}$and $G_{0.5}C_{12}$ has considerable antimicrobial activity towards the tested species as clarified in Table 2. Electrostatic interaction is the initial interaction between the investigated molecules and the negatively charged bacterial membranes. In addition, the hydrophobic tail of the cationic surfactant integrates into the hydrophobic membrane core of the bacteria, where it denatures structural proteins and enzymes, leading to cell lysis and death [7].

Also, it was recognized that $G_{0.5}C_{12}$ (which contains four cationic head groups and four hydrophobic chains) has a slight increase in inhibition zone more than that of G_0C_{12} (two head and two hydrophobic groups). This means that oligomeric surfactants with a high cationic charge number and multiple hydrophobic chains interact strongly with the cell membrane.

Table (2): Antimicrobial activity of the prepared cationic oligomers against different pathogenic bacteria

Sample		Inhibition zone diameter (mm / mg Sample)			
		G+		G-	
		Bacillus cereus	***Staphylococcus aureus***	***Escherichia coli***	***Pseudomonas aeruginosa***
Control: DMSO		0.0	0.0	0.0	0.0
Standard: Antibacterial agent	**Kanamycin(G+)**	27	25	--	--
	Ampicillin (G-)	--	--	25	26
G0 C12		17	16	16	18
G0.5 C12		19	18	19	21

4 Conclusions

- The studied cationic oligomeric quaternary ammonium salts have been adsorbed and located themselves efficiently at the air/aqueous interface.
- The prepared cationic oligomeric surfactants have highcapability to produce micelles even more than the conventional surfactant. Also, surfactant $G_{0.5}C_{12}$ which has 4 cationic quaternary nitrogen, and 4 alkyl chains revealed the lowest CMC value. This shows that CMC of the cationic oligomeric surfactants depends on the degree of oligomerization.
- The values of $\Delta G^{o}_{mic}$and ΔG^{o}_{ads} of the cationic oligomeric surfactants are always negative, indicating that both processes are spontaneous.
- G_0C_{12} and $G_{0.5}C_{12}$ has considerable antibacterialeffectivenessagainst the investigated species.

Interest-based Conflicts: There are none to report.

Acknowledgement

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