# Synthesis and evaluation of Gemini cationic surfactant based on 4-(4-nitrobenzyl)pyridine: surface and biological activities

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#### ABSTRACT

Gemini cationic surfactants were prepared via alkylation of 4-(4-nitrobenzyl)pyridine in acetone in one step with 1,10-dibromodecane, 1,8-dibromooctane, and 1,6-dibromohexane. The chemical structure of prepared Gemini cationic surfactants were confirmed by using <sup>1</sup>H NMR and Fouriertransform infrared spectroscopy. At 25°C and 40°C, surface tension was estimated, in addition surface properties such as critical micelle concentration, effectiveness ( $\pi_{CMC}$ ), minimum surface area ( $A_{min}$ ), efficiency (Pc<sub>20</sub>) and maximum surface excess ( $\Gamma_{max}$ ). The results of the thermodynamic parameters of adsorption and micellization were evaluated and revealed that both processes are spontaneous. It is clear that the prepared Gemini surfactants have a high proclivity for adsorption at surfaces and micellization in the majority of their solutions. The prepared surfactants' antibacterial efficacy against gram-negative, gram-positive, and fungi was studied. In this study, new compounds with anti-bacterial and anti-fungal properties were prepared, and their properties were improved by lengthening the carbon chain. By increasing the hydrophobicity and spacer carbon length of the Gemini surfactants, the antibacterial specialized features of these compounds were increased.

Keywords: Gemini surfactants; Surface properties; Antimicrobial activities

#### 1. Introduction

In Gemini surfactants, a stiff or flexible spacer connects two hydrophilic head chains and two hydrophobic tail. Since the Bunton et al. [1] creation of bisquaternary ammonium bromide Gemini surfactants, these surfactants have attracted a lot of attention. Gemini surfactants have a lower critical micelle concentration (CMC), a similar accumulation performance, greater water solubility and when compared to ordinary surfactants, it has better antibacterial activity. As a result, Gemini surfactants have many applications in a variety of applications disinfectants [2], inhibitors of erosion [3], clothing attire [4], adsorption of dye [5], permeable ingredients [6], improved oil recapture [7] and other disciplines. Cationic Gemini surfactants are known as Gemini surfactants and are the most commonly researched Gemini surfactants [8,9]. Gemini surfactants are thought to have increased surface activity as compared to monomeric surfactants. They have good rheological properties, a low CMC, and a potent capacity to lower surface tension [10–15]. Through the introducing of various functional groups or altering the hydrophobic chain lengths and spacer lengths [16] characteristics can be radically altered [17,18]. For example, the addition of hydroxyl improved the cationic Gemini surfactants' water solubility [19], and the addition of ester, Si–O–Si, and amide groups increased the ability Gemini surfactants' to degrade [20–22]. Variations in spacer group and extents

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of the hydrophobic series also contributed to the antibacterial activity, foam possessions, and CMC of Gemini surfactants [23,24]. Gemini surfactants better solubilization, foaming, and wetting capabilities than a traditional monomeric amphiphilic one. Gemini surfactants' unique chemical makeup increases their propensity to create a wide range of aggregate morphologies in solution [25,26]. Because surfactants are so widely used, these substances' natural side effects are becoming increasingly worrisome. Because cationic Gemini surfactants include hydrophobic chains and positively charged nitrogen atoms, they are more effectively absorbed by sewage and enter water [27]. Furthermore, crustaceans, ciliated protozoa, algae, and bacteria are all poisoned by cationic surfactants [28,29]. As a result, developing a type of best cationic Gemini surfactant is really appealing. Three Gemini surfactants comprising nitro and N<sup>+</sup> collections with varied extents of hydrophobic chain and spacer were prepared in this study using a one-step procedure. Sound effects of spacer and hydrophobic series extents on foaming characteristics, electrolyte tolerance, and surface activity of these surfactants were discussed. Chemical hydrolysis determined the best characteristics of Gemini surfactants Furthermore, these surfactants have antiseptic action in contradiction of both gram-positive and gram-negative bacteria.

#### 2. Materials and methods

# 2.1. Materials

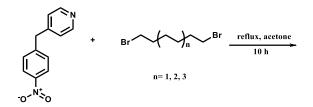
Merck Company (USA) provided the 4-(4-nitrobenzyl) pyridine purity 99.9%. Aldrich Chemical Company provided analytical degrees of 1,10-dibromodecane, 1,8-dibromooctane and 1,6-dibromohexane (Germany) purity 99.9%. Without further refining, Biochem, Egypt provided solvents and reagents were used as it is purity 99.9%.

#### 2.2. Instrumentation

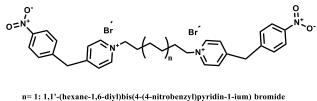
A Fourier-transform infrared spectrophotometer (FTIR) used to test the produced surfactants for the KBr disc method as well as <sup>1</sup>H NMR spectra reported using a Varian NMR-300 Mercury 300 MHz spectrometer with trimethylsilane (TMS) as an internal standard and DMSO-D6 as a solvent were used to ensure the structures of the surfactants produced (Fig. 1).

# 3. Methodology

The Gemini cationic surfactants were made by reacting dibromo alkanes (0.1 mol), such as 1,10-dibromodecane, 1,8-dibromooctane and 1,6-dibromohexane with (0.2 mol) of 4-(4-nitrobenzyl)pyridine in 50 mL acetone for 12 h. The components were refluxed then left-handed to complete the reaction, which resulted in the precipitation of cationic compounds. To make the necessary Gemini cationic surfactants, the obtained Gemini From acetone, quaternary ammonium compounds were separated and crystallised three times (G6, G8 and G10) [30]. The Gemini cationic surfactants' synthesis method is shown in Fig. 1.



4-(4-nitrobenzyl)pyridine



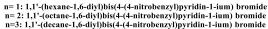


Fig. 1. Chemical structures of the prepared Gemini surfactants.

# 4. Measurements

### 4.1. Surface tension

The Krüss K6 tensiometer was used to measure surface tension using the ring method approach. At 25°C, Gemini cationic surfactant surface tension measurements in aqueous solution with concentrations ranging from 0.05 to 0.00000166 M/L were investigated. To ensure stability and complete adsorption at the solution's surface, the solutions were poured into an ideal Teflon cup and allowed to sit for 2 h. At least three tests were performed on the surface tension data, with the listed values serving as the average. Surface tension profile used to determine CMC and surface characteristics [31].

#### 4.2. Antimicrobial studies

The new Gemini surfactants (G6, G8 and G10) were estimated Just and erythromycin/metronidazole were used as a standard for their antibacterial action against a widespread variety of pathogenic bacteria and fungi. EPRI, Cairo, Egypt, provided the numerous types of investigated organisms. The accompanying media utilized in the antibacterial action of produced compounds are nutrient agar for bacteria and Czapek's dox agar for fungal mould. Beef extract is used to make nutrient agar (3.0 g/L), sodium chloride (3.0 g/L), peptone (5.0 g/L), and agar (20.0 g/L), then it is diluted to 1 L, heated to boil, and autoclaved to sanitize instruments. Sucrose (20.0 g/L), magnesium sulfate (0.5 g/L), potassium chloride (0.5 g/L), agar (20.0 g/L), Sodium nitrate (2.0 g/L) and ferrous sulphate (0.01 g/L) make up Czapek's dox agar at that point full to 1 L then warmed mix to boil furthermore clean media via autoclave [32]. Rising of microbes. An evaluation is performed to determine whether an antibiotic has the ability to kill or inhibit the growth of living bacteria, Filter-paper discagar diffusion is the system that is used (Kirby-Bauer). Bacterial and fungal rinsing were grown and compared to reference standards [33]. After 24-48 h

at 35°C-37°C (for bacteria) and 3-4 d at 25°C-27°C (for yeast and fungi) incubation period at 28°C, the widths of restraint regions were measured after filtering to remove mycelia remains before using the solution containing the microorganisms for vaccine. To determine opposition and capability for disc syntheses and immunization, 1.0 mL of inocula was combined with 50 mL of agar media at 40°C. 120 mm Petri plates were filled with agar after which it was allowed to cool to ambient temperature before being used. Using appropriate disinfected tubes, wells (6 mm in diameter) were cut in agar plates. After that, a 0.1 mL solution of produced compounds consisting 1 mg surfactants in 1 mL of DMSO (dimethyl sulfoxide) (DMSO has trivial effect on development of microorganisms) was included in the agar. Plates were placed on a smooth surface and hatched for 24 h at 30°C for bacteria, after which the width of restraint zones was calculated. The biocidal action of produced compounds was assessed by the repression sector shaped by these chemicals against certain test bacteria. Every sample's sector of development restraint was calculated by taking the average of three replicates [34]. Fungi, gram-negative bacteria, and gram-positive bacteria were all used.

### 5. Results and discussion

#### 5.1. Confirmation of chemical structure

FTIR and <sup>1</sup>H NMR were used to describe the forms (G6, G8, and G10) surfactants: FTIR spectra  $v = 3,416 \text{ cm}^{-1}$  (CH<sub>2</sub>) <sup>as'</sup> 2,856 cm<sup>-1</sup> (CH<sub>2</sub>), 2,612 cm<sup>-1</sup> (-N<sup>+</sup>), 1,465 cm<sup>-1</sup> (CH<sub>2</sub>)<sub>n'</sub> 1,346 cm<sup>-1</sup> (NO<sub>2</sub>) (Fig. 2).

<sup>1</sup>H NMR spectra  $\delta$  = 1.45 ppm (m, nH, CH<sub>2</sub>), 1.89 ppm (m, 2H, CH<sub>2</sub>CH<sub>2</sub>N<sup>+</sup>), 3.48 ppm (t, 2H, CH<sub>2</sub>CH<sub>2</sub>N<sup>+</sup>), 4.48 ppm (t, 2H, NO<sub>2</sub>CHCHCCH<sub>2</sub>), 7.67 ppm (t, H, NCHCH), 7.69 ppm (t, H, NO<sub>2</sub>CHCH), 8.14 ppm (t, H, NO<sub>2</sub>CHCH<sub>2</sub>) and 8.44 ppm (t, H, NCHCH<sub>2</sub>) (Fig. 3).

#### 5.2. Surface action of prepared surfactants

#### 5.2.1. Surface properties

Figs. 4 and 5 show a range of surface tensions vs log C of surfactants (G6 as a typical molecule) at 25°C. The surface tension outline was depicted by raising surface - active accumulation in solution followed by large reductions in surface tension, indicating that increasing the concentration causes surfactant particles to collect at the air/water interface, then, at a particular concentration known as CMC, the curves ruptured [35]. According to the surface tension profile, surface tension values decreased steadily by increased of spacer chain's length from 1,6-dibromohexane (G6) to 1,10-dibromodecane (G10). The movement of surfactant particles to the air/water interface may be caused by an increase in the common repulsion between hydrophobic chains in nonpolar medium (nonpolar medium) and polar medium (water). Furthermore, the CMC values of generated Gemini cationic surfactant particles declined considerably as the length of the spacer chain rose; the bigger spacer chain and the aqueous phase experienced greater repulsion, hence CMC esteem decreased dramatically, Table 1.

# 5.2.2. Effectiveness ( $\gamma_{CMC}$ ) and efficiency ( $Pc_{20}$ )

The efficiency of produced surfactants is determined by comparing the surface tension of the surfactant solution at critical micelle concentration ( $\gamma_{CMC}$ ) to the surface tension of distilled water ( $\gamma_0$ ), as shown in Table 1 [36,37].

$$\pi_{\rm CMC} = \gamma_0 - \gamma_{\rm CMC} \tag{1}$$

A sequence of surfactant fits the lower efficiency values to the lower surface action compounds, and vice versa. The surface activity of 1,8-dibromooctane and 1,10-dibromodecane derivatives (G8 and G10) is higher than the surface activity of 1,6-dibromohexane derivative (G6) as shown in Table 1. (Pc<sub>20</sub>) refers to surfactant concentrations that can lower a solution's surface tension by 20 mN/m. Table 1 of contents the use of integrated Gemini cationic surfactants improves efficiency. As the amount of methylene groups (–CH<sub>2</sub>–) along spacer chains rises, molecules become more hydrophobic. As a result, water hydrophobic cooperations increase, lowering surface tension and decreasing efficiency Pc<sub>20</sub>. Maximum surface excess ( $\Gamma_{max}$ ) and minimum surface area ( $A_{min}$ ).

Greatest surface overabundance of prepared surfactants,  $\Gamma_{max'}$  displays the accumulation of surfactant particles at the air/water interface, and Gibb's equation can be used to calculate it [38].

$$\Gamma_{\max} = \frac{1}{2.303 n R T} \left( \frac{\partial \gamma}{\partial \text{Log} C} \right)$$
(2)

where R = gas constant (8.314 J/mol·K) and T = t + 273 (°K), and The number n represents the number of ionic species whose concentration at the interface varies with the concentration of surfactant in the solution, in the case of Gemini surfactants, this is equal to 3. The maximal values of generated surfactants were found at various temperatures and are presented in Table 1. The quantity of molecules of surfactant near the interface decreases as the length of the spacer chain is lengthened, as seen in Table 1. The next equation describes the normal region in which each surfactant molecule that has been adsorbed is involved [39].

$$A_{\min} = \frac{10^{14}}{N_A \Gamma_{\max}} \tag{3}$$

where  $N_{A}$  is Avogadro's number.

As the spacer chain length increases, a larger area at the interface typically becomes available for each surfactant molecule. G6 surfactant occupied the biggest area at the interface at 25°C, which was 41.22 Å, whereas G10 surfactant occupied the smallest area, which was 43.48 Å.

#### 5.2.3. Emulsification ability

Is determined by how quickly 9 mL of pure water can be separated from an emulsion created by a surfactant solution (0.1%). Utilizing weight percent and paraffin oil, the emulsification competency of the generated surfactants was calculated (10 mL:10 mL). Emulsification power of produced

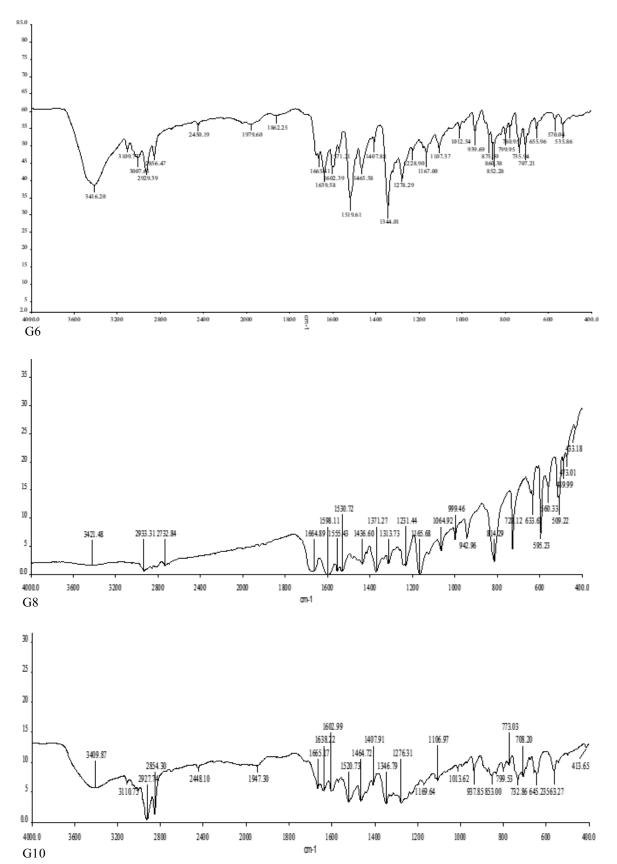


Fig. 2. FTIR spectrum of the surfactants (G6, G8 and G10).

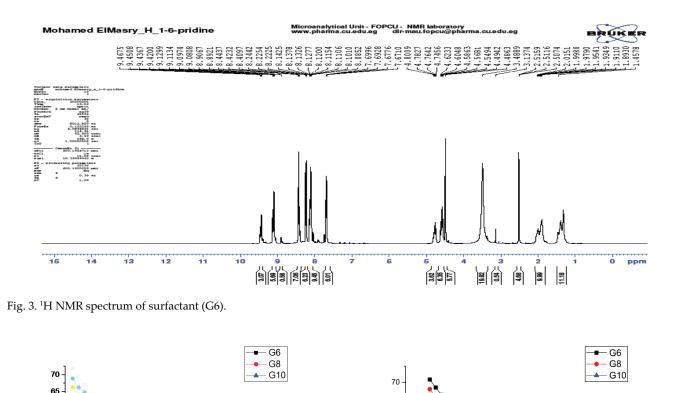


Fig. 4. Surface tension against  $-\log$  concentration of surfactants (G6, G8 and G10) at 25°C.

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surfactant such as a function of time at 25°C is shown in Fig. 5. The stability of the emulsion produced by synthetic surfactants improves with time, and the reverse is also true. The power of emulsification is related to the length of the spacer chain. Evidently, the length of the spacer chain affects how well the produced surfactants emulsify.

G8's emulsification power is increased to 300 s by lengthening the spacer chain to eight methylene groups. A short spacer chain containing sex methylene groups (G6) has the lowest emulsification propensity at 150 s. For 600 s, the longest spacer chain (G10) provides a moderately stable oil/ solution emulsion. As a result, there is a noticeable decrease in surface tension and saturation concentration of various surfactants at the air–solution interface. Due to the emulsification estimates, produced surfactants are unable to combine with oil to form an emulsion, and their applicability in oil field is projected without the ability to produce a stable emulsion with water, as shown in Fig. 6.

Fig. 5. Surface tension against –log concentration of surfactants (G6, G8 and G10) at  $40^{\circ}$ C.

4

-LogC,M/L

3

2

#### 5.2.4. Powerful foaming

6

5

Surface Tension, mN.m<sup>-</sup>

60

50

40

30

20

Foam is a serious concern in the oil business, for example, increasing the pressing factor of the framework and allowing high-pressing-factor liquids to cause pipeline explosions. Accordingly, after vigorously shaking 100 mL of 0.1% surfactant solution in a stoppered graduated 250 mL cylinder at 25°C, the power of foaming of the produced surfactants was determined. Was determined. Synthetic Gemini cationic surfactant has a slight inclination to foam generation, as shown in the foaming power data in Fig. 7, and can thus be used in a variety of applications. Additives for oilfield applications or laundry in washing machines as Fig. 7.

#### 5.2.5. Micellization and thermodynamic

At 25°C, 45°C and 55°C, the rendering to Gibbs equations of Gemini cationic surfactant was determined [40], and information are brief in Table 2:

Surface Tension mN.m

55 50

45 40

35 30

25 20

Table 1 Surface parameters of the surfactants at 25°C and 40°C

Compound	Temp.	CMC (mM)	$\pi_{_{\mathrm{CMC}}}$ (mN/m)	Pc <sub>20</sub> (M/L)	$\Gamma_{\rm max} \times 10^{-10}$	$A_{\min}$ (Å)
G6	25	0.473	32	4.776	4.029	41.22
G6	40	0.567	31	4.948	4.243	39.13
G8	25	0.459	33	5.003	3.890	42.69
G8	40	0.532	32	5.061	4.077	40.73
G10	25	0.437	34	5.117	3.819	43.48
G10	40	0.510	33	5.201	3.929	42.26

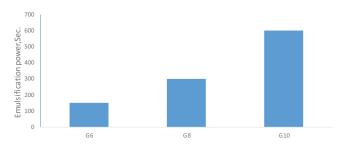


Fig. 6. Emulsification power is affected by the length of the carbon chain in the spacer.

$$\Delta G_{\rm mic}^{\circ} = nRT\log \rm CMC \tag{4}$$

$$\Delta G_{\rm ads}^{\circ} = \Delta G_{\rm mic}^{\circ} - \left(0.06\pi_{\rm cmc}A_{\rm min}\right) \tag{5}$$

$$-\Delta S_{\rm mic}^{\circ} = \frac{\Delta G^{\circ} {\rm mic}}{\Delta T}$$
(6)

$$-\Delta S_{\rm ads}^{\circ} = \frac{\Delta G^{\circ} a ds}{\Delta T}$$
<sup>(7)</sup>

$$\Delta H_{\rm mic}^{\circ} = \Delta G_{\rm mic}^{\circ} + T \Delta S_{\rm mic}^{\circ} \tag{8}$$

$$\Delta H_{\rm ads}^{\circ} = \Delta G_{\rm ads}^{\circ} + T \Delta S_{\rm ads}^{\circ} \tag{9}$$

where *n* is the number of ionic species in solution (3), *R* is the gas constant (8.314 J/mol·K), *T* is the absolute temperature, CMC is the effectiveness, and  $A_{min}$  is the smallest surface area.

Adsorption and micellization are spontaneous processes, according to the negative results for both for the produced Gemini cationic surfactants (G6, G8, and G10). The length of the spacer chain lengthens the negative free energies of micellization and adsorption. High negative  $\Delta G_{ads}$  values showed that adsorption predominates over micellization because adsorption is controlled by the thermodynamic stability of molecules at the air/water interface. Because adsorbed and micellized surfactant molecules are more stable than those that are scattered freely in the aqueous phase, the negative of free energy increases as the temperature rises from 25°C to 40°C, as shown in Table 2. The orderly nature of the diquaternary ammonium salt molecules engaged in the micellar phase is suggested by the fact that the micellization entropy changes ( $\Delta S_{mic}^{\circ}$ ) are minimal. While the hydrophobic alkyl chains wrap around

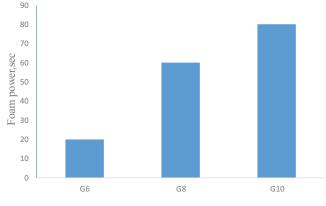


Fig. 7. Foam height is affected by the length of the carbon chain in the spacer.

the micellar core with great similarity, causing an apparent minimization of molecules, the hydrophilic head positive nitrogen group directs to the aqueous phase. Micelle stability results from this arrangement's reduction of conflict in the surfactant-aqueous phase system. According to Table 2, which shows the sequence of enthalpy changes ( $\Delta H^{\circ}_{ads/mic}$ ) for the micellization and adsorption processes, adsorption is thermodynamically superior to micellization.

# 5.2.6. Gemini's generated cationic surfactants' antimicrobial activity

Antimicrobials and pesticides use a variety of cationic surfactants [41-43]. In order to examine the microbiological activity of cationic surfactants, gram-positive (Bacillus subtilis and Staphylococcus aureus), gram-negative (Pseudomonas aeruginosa and Escherichia coli), and dangerous fungi (Aspergillus niger and Candida albicans) were used. The antibacterial activity of the Gemini cationic surfactant generated appears to be primarily affected by the length of the hydrophobic chain, hence antimicrobial activity rises as spacer chain length increases, according to records analysis in Fig. 8 and Table 3. 1,1'-(decane-1,10-diyl)bis (4(4nitrobenzyl) pyridin-1-ium) bromide (G10) presented most extreme antimicrobial actions gram-negative and gram-positive microorganisms were tested. This could be due to a link between antibacterial action and prepared compound surface activities. By lengthening the spacer chain, the tendency of generated antimicrobial chemicals to adsorb on bacterial membrane surfaces rises. As a

Compound	<i>T</i> (K)	$\Delta G_{\rm ads}^{\circ}$ (kJ/mol)	$\Delta G_{\rm mic}^{\circ}$ (kJ/mol)	$\Delta S^{\circ}_{ m ads}$ (kJ/mol)	$\Delta S^{\circ}_{ m mic}$ (kJ/mol)	$\Delta H_{\mathrm{ads}}^{\mathrm{o}}$	$\Delta H_{\rm mic}^{\rm o}$
G6	298	-25.5062	-24.7148	_	_	-	-
G6	313	-26.0698	-25.342	1.73798	1.68946		
G8	298	-25.5802	-24.8118	-	-	-	-
G8	313	-25.9631	-25.2544	-1.66421	1.61696		
G10	298	-25.9226	-25.2182	-	-	-	-
G10	313	-26.5171	-25.6577	-1.6478	1.60385		

Table 2 Thermodynamic parameters of the surfactants at 25°C and 40°C

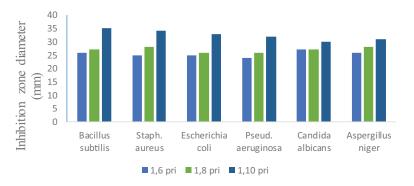


Fig. 8. Efficiency of the prepared surfactants (G6, G8 and G10) as antimicrobial

result of the studied chemicals' high population at cellular membranes, their potent activity has increased [44,45]. In addition, the antibacterial activity of Gemini surfactants generated was found to be stronger against gram-positive bacteria than gram-negative bacteria. The cell membrane architecture of two distinct bacterial species demonstrates this. Gram-negative bacteria are more resistant to biocides and amphiphilic compounds than gram-positive bacteria because their outer membranes are virtually entirely made of proteins and lipopolysaccharides [46]. The most important factor in the antibacterial effect is the length of the alkyl chain. It has been shown that the antibacterial activity is inversely correlated with the alkyl chain length. The lengthening of the hydrocarbon spacer between the two ammonium groups is another structural characteristic. Gemini surfactants with a 12-carbon spacer have greater action than those with six or eight carbons, as seen in Table 3. The results of the biological investigation also revealed promising features of the diquaternary ammonium salts' mode of action as biocides against pathogenic fungus strains (Candida albicans and Aspergillus niger) (Candida albicans and Aspergillus niger). This could be interpreted as follows: gram-positive bacteria's lipoteichonic acid layer, which is characterised by its charged composition and molecular capacity to bind with quaternary with a positive charge nitrogen, is where adsorption takes place. The positively charged biocide targets the extremely hydrophobic lipid layer of gram-negative bacteria. Because biocide adsorption has a considerable disturbing impact on biological responses within the cells, the selective penetrability of cellar membranes may be of concern. When counter ions, such as chloride atoms (Cl<sup>-</sup>), are existing in the cellar membranes, their influence increases [46,47].

# 6. Conclusion

Gemini surfactants are used in a variety of applications because they may be customised and are designer chemicals. As a result, a novel Gemini surfactant-based surfactant known as 4-(4-nitrobenzyl)pyridine was created. Their interest was aroused by the low CMC and high efficiency a group of surfactants in reducing water's surface tension, which was controlled by chemical compositions and the spacer chain's length. The study shows that, like the homogeneous sequence of Gemini surfactant, the propensity to micellize rises while CMC falls in lockstep with the alkyl chain length spacer attached to the polar head group. As a result, it seems that the primary mechanism for these surfactants to micellize is the hydrophobic interaction between their alkyl chains. The surfactant of Gemini that was investigated was found to have antibacterial properties. How effective the spacers are against bacteria depends on the length of the alkyl chain's carbon chain. The action against bacteria and fungus is boosted by lengthening the spacer's carbon chain. In contrast, the surfactant with ten the spacer chain's carbon atoms appears to have significant antibacterial action. The study of antibacterial properties in connection to Gemini surfactant surface action indicates that log CMC can be used to predict the effectiveness of antimicrobial resistance by using a valid biomarker of hydrophobicity. These surfactants of Gemini possess a somewhat stronger surface action than typical cationic surfactants like alkyl trimethylammonium compounds. Gemini surfactants demonstrated antiseptic action in contradiction of gram-positive bacteria that was on par with or superior to cetyltrimethylammonium chloride, a common

Test organism Compound	Bacillus subtilis	Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa	Candida albicans	Aspergillus niger
G6	26	25	25	24	27	26
G8	27	28	26	26	27	28
G10	35	34	33	32	30	31
Reference	29	30	27	28	26	29

Table 3 Antimicrobial properties of the established substances measured by (mm)

antibacterial substance. The improved understanding of the structural factors affecting the biological action of the surface qualities long chain (12 carbon atoms) defined in this study is anticipated to benefit the development of surfactants and better selection of their enhanced physicochemical and organic possessions for modern medicinal and engineering-oil field requests.

The antimicrobial activity of the prepared Gemini surfactants compounds was tested using the agar diffusion technique [48].

The tested the compounds were assessed against, gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 35556), gram-negative bacteria (*Escherichia coli* ATCC 23282 and *Pseudomonas aeruginosa* ATCC 10145), yeast (*Candida albicans* IMRU 3669) and filamentous fungus (*Aspergillus niger* ATCC 16404). The fungus was cultivated on Czapek's Dox agar medium, whereas the bacteria and yeast were grown on nutrient agar. The tested substances were assessed in the concentration of 5,000 ppm.

Controlling the positive was Erythromycin for bacteria, Nalidixic acid for yeast and Metronidazole fungus.

All tests were repeated twice, and data was calculated as the average of the obtained results.

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