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Synthesis, Surface and Antimicrobial Activities of Cationic Gemini Surfactants with Semi-Rigid Spacers

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Abstract Four cationic gemini surfactants featuring semi-rigid spacers were synthesized via a two-step process. The surface-active properties of these surfactants were investigated through surface tension and electrical conductivity measurement. The thermodynamic parameters of micellization were evaluated from electrical conductivity measurements at temperatures ranging from 293 to 313 K. The aggregation behavior of these synthesized gemini surfactants in water were investigated using dynamic light scattering and transmission electron microscopy. Further, the antimicrobial activities of these synthesized gemini surfactants against both Gram-positive and Gram-negative bacteria were also investigated.

Keywords Gemini surfactants · Semi-rigid spacer · Critical micelle concentration · Thermodynamic parameter · Aggregation behavior · Antimicrobial activity

Introduction

Gemini or dimeric surfactants have attracted attention among several industrial and academic research groups [1– 4]. These surfactants, generally made up of two hydrocarbon chains and two head groups linked by a spacer, possess better physicochemical properties, such as lower critical micelle concentration (CMC) values, higher solubility power, unique rheological behavior, and enhanced

Hai-lin Zhu zhuhailin99@126.com; zhuhailin@nuc.edu.cn wetting and foaming properties, than their conventional counterparts [5–8]. Many studies have been focused on new surfactants with tailored structures or functionalities in order to obtain surfactants with specific physicochemical properties for targeted applications.

The physicochemical properties of gemini surfactants are primarily dependent on their molecular structures, such as the charge of head groups, the length of the hydrophobic tails and the nature of the spacers. The effect of the hydrophobic chain length on the surface and bulk properties has been systematically investigated [9–11], whereas the investigation of the spacer effect is less common. The spacer connecting the two head groups can be flexible, rigid, or semi-rigid [12]. Previous studies have shown that the aggregation behavior of gemini surfacants is affected by not only the length but also the rigidity of the spacer. Additionally, various studies have reported the effects of flexible [6, 8, 13] or rigid spacers [14-16] on the properties of gemini surfactants, while relatively few studies have reported on gemini surfactants with semi-rigid spacers [12, 17].

Currently, the most commonly studied gemini surfactant type is alkanediyl- α - ω -bis(alkyl dimethyl ammonium) dibromide, which is referred to as m-s-m, where m and s stand for the carbon atom number in the tail alkyl chain and in the methylene spacer, respectively. Previous studies have shown that 12-s-12 series surfactants with short spacers ($2 \le s \le 12$) tend to form spherical or elongated micelles, while 12-16-12 and 12-20-12 form vesicles [18].

Zhang et al. [17] studied the aggregation behavior of two gemini surfactants, differing only in space rigidity, 1-dodecanaminium, N,N'-[[(2E)-1,4-dioxo-2-butene-1,4diyl] bis(oxy-2,1-ethanediyl)]bis[N,N- dimethyl-,bromide] (12-fo-12) and 1-dodecanaminium, N,N'-[(1,4-dioxo-1,4butanediyl)bis(oxy-2,1-ethanediyl)] bis[N,Ndimethyl-

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,bromide] (12-su-12). They found that 12-su-12 with flexible spacer formed solely micelles in aqueous solutions, while 12-fo-12 featuring a semi-rigid spacer could form micelles and vesicles, depending on the concentration. Zhu et al. [12] found that gemini surfactants with semi-rigid spacers self-assembled into the mixture of micelles and vesicles at low surfactant concentration and formed vesicles at high surfactant concentration, whereas gemini surfactants with long fully rigid spacers preferred to form vesicles.

Cationic surfactants have been proven to have antimicrobial activities. Cationic gemini surfacants, which consist of two symmetric quaternary ammonium groups, exhibit a much better antimicrobial potency [19, 20]. The pathogenic bacterial cell membrane is predominantly negatively charged as compared with eukaryotic cells. Cationic surfactants have a unique ability to adsorb at the interface of the cell membrane. The lipophilic component then diffuses through the cell wall and the polar head binds to the cell membrane, disrupting the cytoplasmic materials and killing the bacteria. Although there are many reports discussing the antimicrobial activity of cationic gemini surfactants, their structure–activity relationship still remains elusive.

In this work, four cationic gemini surfactants, with the spacer containing a rigid benzene ring and two ester groups, were synthesized utilizing a two-step process. The effects of different lengths of alkyl chain and spacer chain on the aggregation and antimicrobial activities were systematically investigated.

Experimental

Materials

N,N-Dimethyldodecylamine (97 %), *N,N*-Dimethyltetradecylamine (95 %), hydroquinone (99.5 %), 1,4-Benzenedimethanol (99 %), chloroacetyl chloride (98 %), triethylamine (98 %) were purchased from J&K Scientific (Beijing) and used without further purification. All other chemicals used were of reagent grade. Ultra-pure water (ASINO, USA) was used to prepare the solutions for all experiments.

Synthesis of the Surfactants Featuring Semi-Rigid Spacers

The synthetic route is shown in Scheme 1.

The synthesis route of the intermediate is similar to the one reported in the literature [21]. Hydroquinone or 1,4-Benzenedimethanol (0.1 mol) was added to 100 mL anhydrous dioxane. Temperature was maintained at 0-5 °C. Triethylamine (0.3 mol) was added into the



Scheme 1 The synthesized route of cationinc gemini surfactants

mixture, and then chloroacetyl chloride (0.25 mol) was added dropwise after complete dissolution of the mixture. The solution was next heated to 30 °C for 4 h. The mixture was then filtered after the completion of the reaction. The filtrate was concentrated under vacuum to obtain a brown solid, which was then washed with absolute methanol to obtain the resulting white solid (1).

This product (1) (10 mmol) was dissolved in 40 mL anhydrous dioxane, then N,N-Dimethyldodecylamine or N,N-Dimethyltetradecylamine (25 mmol) was added into the mixture. The mixture was stirred under refluxing for 48 h. When the reaction was completed, the mixture was cooled to room temperature and the white precipitate obtained was collected by filtration. The crude product was recrystallized from acetone at least three times to produce the final products as a white solid (2).

The structure was confirmed by FT-IR, ¹HNMR, ESI–MS spectra. The FT-IR spectra were recorded in FTIR-8400S instruments, and ¹HNMR spectra were recorded in a chloroform (CDCl₃) solution with a Bruker 300 MHz NMR Spectrometer, and ESI–MS spectra were recorded in a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer.

Spectral Characteristics of Synthesized Surfactants

I: ¹H NMR (*CDCl*₃, ppm) 0.865(t,6H,2**CH**₃), 1.240-1.344(m,36H,2CH₃(**CH**₂)₉), 1.817(m,4H,2CH₃(CH₂)₉**CH**₂), 3.625(s,12H,2 N(**CH**₃)₂), 3.846(m,4H,2CH₃(CH₂)₉**CH**₂), **CH**₂), 5.977(s,4H,2 N(CH₃)₂**CH**₂COO), 6.849(m,4H, **ArH**). ESI–MS(positive): m/z 653.50289 [M-Cl]⁺, 309.26623 [M-2Cl]²⁺/2. FT-IR (KBr, cm⁻¹): 2917, 2848, 1766, 1502, 1463, 1243, 1180, 1155, 833, 719.

II: ¹H NMR (*CDCl*₃, ppm) 0.852-0.893(t,6H,2**CH**₃), 1.248-1.349(m,44H,2CH₃(**CH**₂)₁₁), 1.816(m,4H,2CH₃ (CH₂)₁₁**CH**₂), 3.636(s,12H,2 N(**CH**₃)₂), 3.853(m,4H,2CH₃ (CH₂)₁₁**CH**₂**CH**₂), 6.005(s,4H,2 N(**CH**₃)₂**CH**₂COO), 7.272 (m,4H,**ArH**). ESI–MS(positive): m/z 709.56446 [M-Cl]⁺, 337.29753 [M-2Cl]²⁺/2. FT-IR (KBr, cm⁻¹): 2917, 2848, 1762, 1502, 1465, 1240, 1182, 1157, 835, 719.

III: ¹H NMR (*CDCl*₃, ppm) 0.874-0.916(t,6H,2**CH**₃), 1.269-1.341(m,36H,2CH₃(**CH**₂)₉), 1.720(m,4H,2CH₃(CH₂)₉) **CH**₂), 3.545(s,12H,2 N(**CH**₃)₂), 3.838(m,4H,2CH₃(CH₂)₉) CH₂**CH**₂), 5.158(s,4H,2 N(CH₃)₂**CH**₂COO), 5.330(m,4H, **CH**₂Ar**CH**₂), 7.346(m,4H,Ar**H**). ESI–MS(positive): m/z 681.53316 [M-Cl]⁺, 323.28188 [M-2Cl]²⁺/2. FT-IR (KBr, cm⁻¹): 2917, 2850, 1747, 1517, 1465, 1241, 1191, 813, 719.

IV: ¹H NMR (*CDCl*₃, ppm) 0.852-0.891(t,6H,2**CH**₃), 1.247-1.323(m,44H,2CH₃(**CH**₂)₁₁), 1.719(m,4H,2CH₃ (CH₂)₁₁**CH**₂), 3.531(s,12H,2 N(**CH**₃)₂), 3.814(m,4H,2CH₃ (CH₂)₉CH₂**CH**₂), 5.125(s,4H,2 N(CH₃)₂**CH**₂COO), 5.354 (m,4H,**CH**₂Ar**CH**₂), 7.268-7.306(m,4H,**ArH**). ESI–MS (positive): m/z 737.59576 [M-Cl]⁺, 351.31318 [M-2Cl]²⁺/2. FT-IR (KBr, cm⁻¹): 2915, 2848, 1747, 1519, 1467, 1243, 1189, 811, 719.

Surface Tension Measurements

The surface tensions of the aqueous surfactant solutions were measured on a K100 automatic tensiometer (Krüss, Germany) with a du Nouy ring. Measurements were performed at 20 ± 0.1 °C until successive values agreed to within 0.2 mN m⁻¹.

Krafft Temperature

The Krafft temperature($T_{\rm K}$) was determined by heating the surfactant solution until a clear solution was obtained [22]. All solution concentrations of these surfactants were 1.0 wt% (at least twice the CMC of the studied gemini surfactant).

Electrical Conductivity Measurements

The conductivity of the surfactant solutions was measured by using a low-frequency conductivity analyzer (DDS-307; Shanghai Precision & Scientific Instruments, China). A super-thermostatic cistern (temperature fluctuation: ± 0.5 °C; SX-CH1015; Beijing Heng Odd Instruments, China) was used to control the temperature. The uncertainty of the measurement was found to be $\pm 0.01 \ \mu\text{S cm}^{-1}$.

Dynamic Light Scattering (DLS) Measurements

DLS measurements were performed using the BrookHaven 90 Plus DLS instrument at 25 °C with 660 nm laser light, and the light collection at 90°. All solutions were went through ultrasonic treatment and filtered throught a 0.2- μ m Millipore filter into the cylindrical scattering cells before the experiments. The data obtained in each case were the average of 3 runs. The particle size was calculated from the NNLS method.

Transmission Electron Microscopy (TEM)

TEM measurements were performed using a JEM-1011 TEM instrument. The samples were prepared by the negative staining method. An amount of 2 % (wt) phosphotungstic acid was used as the staining agent. A drop of surfactant solution was placed on a copper grid, and the excess liquid was absorbed by a piece of filter paper. The copper grid was then placed onto a drop of phosphotungstic acid solution, and the sample was dried at room temperature before measurement.

Determination of the Antimicrobial Activity

The antimicrobial activity of the synthesized surfactants was tested against reference strains of Gram-positive (*Bacillus subtilis* ATCC 6633) and Gram-negative (*Escherichia coli* ATCC 8099). The organisms were incubated by a streak culture method and then refrigerated.

The bacterial strains were put into incubator for 4 h for activation, and were then scraped into sterile water by inoculating loops. The organisms were shaken at 34 °C for 24 h to incubate them. The concentrations of the bacteria suspensions were approximately 10^7 cfu/mL.

To assess the biocidal activity, a nutrient medium agar was used, composed of $(g L^{-1}) 10.0 g$ peptone, 5.0 g beef extract, 5.0 g sodium chloride and 20.0 g agar. The pH was adjusted to 7.0–7.5. The agar medium was poured, while hot, into a Petri dish (diameter 9 cm) after autoclaving.

The target gemini surfactants were dissolved in sterile water after sterilization, and the solution was then added into 10 mL bacteria suspensions and incubated by shaking at 34 °C for 1 h. The test organisms were coated on the Petri dish by the ten times dilution method and were put in an incubator at 34 °C for 24 h. Bacteria suspensions without surfactants were also incubated for comparison. The antibacterial rate was calculated from the following equation:

$$\eta(\%) = \frac{I - II}{I} \times 100 \tag{1}$$

where, I is the average clump count without surfactant and II is the average clump count with surfactant. The lowest concentration of the antibacterial agent with an antibacterial rate above 90 % was determined to be the MIC.

Results and Discussion

Krafft Temperature

The Krafft temperatures $(T_{\rm K})$ were all determined by visual observation of the clarification of 1.0 wt% aqueous

Surfactant	T_{Krafft} (°C)	$\gamma_{\rm CMC}~({\rm mN}~{\rm m}^{-1})$	$CMC_s \pmod{L^{-1}}$	pC ₂₀	$\Gamma_{\rm max} \ (10^{-10} \ {\rm mol} \ {\rm cm}^{-2})$	$A_{\min} (\mathrm{nm}^2)$
I	<0	37.8	0.65	3.19	1.09	1.51
II	<0	37.7	0.10	4.04	1.27	1.31
III	<0	41.1	0.58	3.24	0.89	1.86
IV	<0	40.9	0.09	3.99	1.24	1.34
DTAB		39 ^a	16 ^a	2.1 ^a	_	0.66 ^b
TTAB		37.8 ^a	3.7 ^a	2.9 ^a	_	0.62 ^b

Table 1 Physico-chemical properties of synthesized surfactants

^a Data taken from Ref. [24]

^b Data taken from Ref. [25]



Fig. 1 Dependence of surface tension on surfactant concentration at 20 $^{\circ}\mathrm{C}$

solutions of these surfactants [22]. It can be seen from Table 1 that the Krafft points of the four synthesized gemini surfactants featuring semi-rigid spacers are all below 0 °C. Such low Krafft temperatures permit the use of these surfactants in cold water.

Surface Activity

The surface tension measurement is a classical method of studying the critical micelle concentration (CMC) of surfactants. The relationships between surface tensions (γ) and the concentrations of synthesized surfactants at 20 °C were determined. Figure 1 shows that γ first decreases with the increase in the concentration of the surfactant and then becomes almost constant after the concentration reaches a certain value. There, the intersection point is taken as the critical micelle concentration (CMC). Table 1 provides the values of the CMC measured via the surface-tension method (CMC_s), the surface tension corresponding to CMC_s (γ_{CMC}), the maximum surface excess (Γ_{max}), the minimum surface area per surfactant (A_{min}), and the

concentration of the surfactant solution required to suppress surface tension by 20 mN $m^{-1}~(pC_{20})$ for the surfactants.

 Γ_{max} , A_{min} , and pC₂₀ were calculated by using the Gibbs equations, as follows [19]:

$$\Gamma_{\max} = \frac{-1}{2.303nRT} \left(\frac{\partial\gamma}{\partial \log c}\right)_{T,P},\tag{2}$$

$$A_{\min} = \left(N_A \times \Gamma_{\max}\right)^{-1} \times 10^{14},\tag{3}$$

$$pC_{20} = \frac{\gamma_0 - 20 - \gamma_{\rm CMC}}{2.303 n R T \Gamma_{\rm max}} - \log c, \tag{4}$$

where *R* is the gas constant (8.314 J mol⁻¹ K⁻¹), *T* is the absolute temperature, *c* is the surfactant concentration, N_A is Avogadro's number (6.02 × 10²³ mol⁻¹), and γ_0 is the surface tension of water. For a gemini surfactant, the value of *n* is rather complex. The values n = 2 and 3 have been used in many published documents. Nevertheless, if the value used for *n* affects the way in which Γ and A_{\min} vary, then it has no bearing on the way in which Γ and A_{\min} vary with the tail carbon number. Therefore, the use of a different value of *n* (2 or 3) does not affect the conclusion inferred from these variations [23]. Here, the value of *n* is taken as 3.

Table 1 shows the CMC values obtained for surfactants I, II, III and IV were 0.65, 0.10, 0.58 and 0.09 mmol L⁻¹, respectively. Similar to conventional ionic surfactants, the CMC values decreased with increasing alkyl chain length from 12 to 14. This is attributed to the hydrophobicity of surfactant molecules increasing with increasing the number of methylene groups in the chains, which demonstrates the increasing of the mutual repulsion between polar water molecules and nonpolar hydrophobic chains. All the synthesized gemini surfactants have lower CMC values than their corresponding single-tail surfactant [24], suggesting excellent micelle-forming ability in water. Among these synthesized gemini surfactants, with an increase of the spacer length by two methylene units, the CMC values

obviously, showing slightly better micelle-forming ability and weaker surface tension-lowering ability.

The A_{\min} value of the synthesized gemini surfactant is larger than that of its corresponding single-tail surfactant [25], suggesting that the presence of the spacer group increases the value of A_{\min} . This may be attributed to the fact that the benzene ring is rigid and hydrophobic, and that it prefers to lay on the surface when it is adsorbed at the water-air interface. The A_{\min} value increases with increasing the spacer length. This is thought to be because the spacer becomes more flexible and more hydrophobic with increasing the two methylene units to the spacer, resulting in weaker adsorption at the air-water interface.

The pC₂₀ value measures the efficiency of the surfactant in lowering surface tension [26]. The higher pC₂₀ value indicates that the surfactant adsorbs more efficiently at the interface and reduces the interface tension more efficiently. Comparing the pC₂₀ values of these synthesized gemini surfactants and their corresponding single-tail surfactants, it is hypothesized that the synthesized surfactants are more surface active. Increasing the length of hydrophobic chains, pC_{20} value increases, showing a higher adsorption efficiency. The pC_{20} values decrease slightly with the increase in the number of methylene groups along the spacer.

Thermodynamics of Micellization

The electrical conductivity (κ) of the synthesized surfactants in aqueous solution against c at different temperatures is shown in Fig. 2. As the figure shows, electrical conductivity increased with increasing the temperature and the surfactant concentration. As the surfactant concentration increased, electric conductivity increased rapidly, until a certain concentration point where it became gradual. The concentration at the break point was the CMC of the surfactant. Here, we used the Carpena method [27] in order to determine the CMC values and the degree of counter ion association (β). This method was based on the fitting of the κ -c curve to the integral of a Boltzmann-type sigmoidal equation:



Fig. 2 Specific conductivity (κ) versus surfactant concentration (c) at different temperature

$$\kappa(c) = \kappa(0) + A_1 c + \Delta c (A_2 - A_1) \ln\left(\frac{1 + e^{(c-c_0)/\Delta c}}{1 + e^{-c_0/\Delta c}}\right),$$
(5)

 β were calculated from the following equation:

$$\beta = 1 - A_2/A_1,\tag{6}$$

where $\kappa(0)$, A_1 , A_2 , Δc , and c_0 are the values of κ in pure water, the pre-micellar slope, the post-micellar slope, the width of the transition, and the central point of the transition region (CMC), respectively. The values of the CMC and β in different temperatures enabled us to analyze the thermodynamic energetics of micelle formation. For a gemini surfactant, the free energy of micellization ($\Delta_{\rm mic}G^0$) was given by Zana [28]:

$$\Delta_{\rm mic}G^0 = RT(0.5 + \beta)\ln(\rm CMC), \tag{7}$$

where CMC is in mole fraction units. The enthalpy of micellization $(\Delta_{mic}H^0)$ can be determined by using the Gibbs-Helmholtz equation:

$$\Delta_{\rm mic} H^0 = -T^2 \frac{(\partial (\Delta_{\rm mic} G^0/T))}{\partial T}$$

= $-RT^2 (0.5 + \beta) \frac{\partial \ln \rm CMC}{\partial T},$ (8)

The standard entropy of micellization $(\Delta_{\rm mic}S^0)$ is determined by using the following equation:

 $\Delta_{\rm mic}S^0 = (\Delta_{\rm mic}H^0 - \Delta_{\rm mic}G^0)/T,$ (9)

The thermodynamic parameters of micellization for synthesized surfactants at different temperatures are listed in Table 2. It is clear that, for synthesized gemini surfactants, the free energies ($\Delta_{\rm mic}G^0$) of micellization are all negative, indicating that the micellization processes are all spontaneous. With increasing alkyl chain length from 12 to 14, the free energies of micellization $(\Delta_{\rm mic}G^0)$ become more negative, which implies that micellization is favored due to the increase in the hydrophobic interactions between the hydrophobic chains. When the spacer increases by two methylene units, the free energies of micellization also become more negative. This may be attributed to an increase of hydrophobic interaction resulting in a stronger tendency for aggregation. The values of $\Delta_{\rm mic} H^0$ were all negative, which suggests that micellization was exothermic at the experimental temperature, and the dispersion forces play a significant role in the micellization process [29]. For all the synthesized gemini surfactants, the values of the standard entropy of micellization $(T\Delta_{mic}S^0)$ are larger than the values of $-\Delta_{\rm mic}H^0$, which indicates that the micellization is entropy-driven. With the increase of temperature, $T\Delta_{\rm mic}S^0$ decreases while $-\Delta_{\rm mic}H^0$ increases, suggesting that the entropy-driven becomes weaker and the enthalpy-driven becomes stronger, and that the enthalpyentropy compensation phenomenon may exist.

Table 2 Influence oftemperature on the parametersof micellization for synthesized	Surfactant	t (°C)	CMC (mmol L ⁻¹)	β	$ \begin{array}{c} \triangle_{\rm mic} G^0 \\ ({\rm KJ} \ {\rm mol}^{-1}) \end{array} $		$T \triangle_{\rm mic} S^0$ (KJ mol ⁻¹)
surfactants	Ι	20	1.067	0.452	-25.19	-10.34	14.85
		25	1.308	0.443	-24.90	-10.18	14.73
		30	1.425	0.434	-24.88	-10.85	14.03
		35	1.512	0.422	-24.82	-11.06	13.76
		40	1.635	0.410	-24.71	-11.28	13.44
	Π	20	0.197	0.639	-34.83	-13.92	20.91
		25	0.249	0.618	-34.12	-14.13	19.99
		30	0.285	0.610	-34.07	-14.50	19.57
		35	0.301	0.591	-33.88	-14.73	19.16
		40	0.325	0.578	-33.81	-15.03	18.78
	III	20	0.946	0.613	-29.78	-12.09	17.69
		25	1.131	0.578	-28.86	-12.11	16.75
		30	1.273	0.567	-28.72	-12.39	16.33
		35	1.378	0.556	-28.68	-12.67	16.01
		40	1.419	0.540	-28.63	-12.89	15.74
	IV	20	0.178	0.666	-35.94	-13.25	22.70
		25	0.190	0.651	-35.90	-13.53	22.38
		30	0.203	0.635	-35.81	-13.79	22.02
		35	0.229	0.629	-35.86	-14.17	21.68
		40	0.238	0.593	-35.17	-14.17	21.00



Fig. 3 Hydrodynamic radius (R_h) distributions of the aggregates of the surfactants. **a** Surfactants at 10 times the CMC, **b** surfactants at 10 times the CMC with 0.1 M Na Br addition

Size of Aggregation

The aggregation state of the four synthesized gemini surfactants in aqueous solution was first studied by DLS. The concentrations were about 10 times the CMC, with the size distribution by DLS shown in Fig. 3. From Fig. 3a, one can see that there are obvious peaks with the hydrodynamic radius (R_h) of about 50–200 nm, reflecting the typical size of large aggregates. The effect of salt on the aggregation was also investigated, and the results (Fig. 3b) show that the peaks with a R_h value of 50–200 nm disappear, leaving micelles peaks with a R_h value around 3 nm. This result agrees with the observation by Zhang et al., for gemini surfactants with diethylammonium headgroups and a diamido spacer [23].

To identify the morphologies of the aggregates, we performed the TEM measurement. Figure 4 exhibits the TEM images of the aggregates of the four synthesized gemini surfactants. Through TEM observation, vesicles were observed in the aqueous solutions of II and III, while for I and IV only large aggregates were found. As shown in Fig. 4, the size of aggregates corresponded to the large size distribution in the DLS plots as shown in Fig. 3a.

Antimicrobial Activity

The antimicrobial activities of these synthesized surfactants were tested against reference strains of *Bacillus subtilis* and *Escherichia coli*. Figure 5 shows the relation curve of antibacterial rate and concentration of gemini surfactants. From Fig. 5, one can see that these target gemini surfactants exhibit antimicrobial activities against both Gram-positive and Gram-negative bacteria, showing broad bactericidal performance. Antibacterial rate increases with an increase in the concentration of the gemini surfactants for any given bacteria. Bacterial cell surfaces are usually negatively charged; cationic surfactants can easily disturb bacterial membranes due to the positively charged polar head. By increasing the concentration of the surfactant, the positive charge density of the head group increases, resulting in an increase in sterilization.

The MIC is defined as the lowest concentration of the antibacterial agent where the antibacterial rate is above 90 %, which is used as a measurement of antibacterial activity. The lower the MIC, the higher the antibacterial activity of the reagent. Table 3 lists the MIC values for synthesized gemini surfactants. Upon comparison with benzalkonium chloride (1227) [30], these gemini surfactants are more efficient biocides. Due to the existence of spacers, these gemini surfactants contribute more positive charge, thereby facilitating more electrostatic interaction at the membrane, and exhibiting higher antibacterial activities [31]. Electrostatic and hydrophobic interactions help to facilitate adsorption onto cationic surfactants. The MIC values decrease with the addition of two methylene units to the spacer, which is in accordance with the surface activities. When comparing the MIC values of I, II and III, and IV, one finds that the MIC value shows an increase with increasing the alkyl chain length. This demonstrates that antibacterial activities decrease when increasing the surface activities of the reagents, which is opposite of the hypothesis. It is well known that the antibacterial activities are strongly dependent on the alkyl chain length, and that there is an optimal chain length for the maximum antibacterial activities [32]. Since most phospholipid molecules in cells contain 12 carbon atoms, the surfactants containing 12 carbon atoms are most similar to the phospholipid molecule in physical and chemical properties, and require minimal energy. Another strong explanation is that the antibacterial activity depends on the hydrophilic-hydrophobic balance of the cationic surfactant. The hydrophobic character increases with further increasing the alkyl chain length (n > 12), which may be too hydrophobic







◄ Fig. 5 Bactericidal rate curve of four synthesized reagents. a Scherichia coli, b Bacillus subtilis

Table 3 The MIC values of synthesized surfactants against *Escherichia coli* and *Bacillus subtilis* (mg/L)

Microorganisms	Ι	II	III	IV	1227
E. coli	18	39	17	26	50 ^a
B. subtilis	20	34	11	22	

^a Data taken from Ref. [30]

to facilitate transport through the bacterial cell membrane [33]. Additionally, further increasing the alkyl chain length decreases the solubility of water, leading to lower antibacterial activity.

Conclusion

The prepared gemini surfactants featuring semi-rigid spacers are expected to have good surface activity. The CMC values decrease with increases in the length of alkyl chain of the surfactants, while $\gamma_{\rm CMC}$ values increase with increases in the spacer length. The thermodynamic parameters of micellization derived from electrical conductivity indicate that the micellization is spontaneous and

driven by entropy. It was found that these synthesized gemini surfactants featuring a semi-rigid spacer formed large aggregates in aqueous solution. The results of antibacterial activity test show that these synthesized gemini surfactants display excellent antimicrobial activities both against *B. subtilis* and *E. coli*, and the antibacterial rate increases with an increase in the concentration of the gemini surfactants. Surfactants with the hydrophobic chains containing 12 carbon atoms were found to have higher activity among the synthesized gemini homologues.

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