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# Synthesis, surface adsorption, micellization behavior and antibacterial activity of novel gemini surfactants with morpholinium headgroup and benzene-based spacer



## Le-Chi Zheng, Qing-Xiao Tong \*

Department of Chemistry and Key Laboratory for Preparation and Application of Ordered Structural Material of Guangdong Province, Shantou University, Guangdong 515063, PR China

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#### $A \hspace{0.1in} B \hspace{0.1in} S \hspace{0.1in} T \hspace{0.1in} R \hspace{0.1in} A \hspace{0.1in} C \hspace{0.1in} T$

Two series of novel gemini surfactants with morpholinium headgroup and benzene-based rigid spacers (abbreviated as (Mor)m-P-m and (Mor)m-BP-m, where m represents the carbon numbers of hydrophobic chains) were synthesized and characterized by <sup>1</sup>H NMR, ESI-MS, and FT-IR spectra. The effect of lengths of rigid spacer and hydrocarbon chain on their solution properties and antibacterial activities were systematically investigated. Due to the existence of morpholinium, they have a superior surface activity to the classic gemini surfactants. (Mor)m-BP-m possess lower CMC and  $\gamma_{cmc}$  than (Mor)m-P-m, which is assumed to be related to the conformation change of spacer. The possible arrangement models for the adsorptions of (Mor)m-P-m and (Mor)m-BP-m at the airwater interface were proposed by comparing their surface parameters with those of other similar surfactants. The pre-micellar associations occur in aqueous solutions of (Mor)16-P-16, (Mor)14-BP-14 and (Mor)16-BP-16. The thermodynamic parameters indicate the micellar processes are spontaneous, and (Mor)m-BP-m have stronger aggregation tendency than (Mor)m-P-m. Dynamic light scattering (DLS) and transmission electron microscopy (TEM) show they spontaneously self-assemble into vesicle, and the aggregate size of (Mor)m-BP-m is larger than (Mor)m-P-m. (Mor)m-P-m, (Mor)m-BP-m exhibit a higher activity.

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#### 1. Introduction

Gemini surfactant is a new class of surfactant, which consists of two hydrophilic headgroups and two hydrophobic chains connected covalently by a space near, or at the level of the headgroups [1,2]. Compared with traditional surfactants with only one hydrophobic chain and one hydrophilic group, gemini surfactants have many advantages such as higher surface activity, lower critical micelle concentration (CMC) and stronger adsorption capacity at the interface, which are widely employed in tertiary oil recovery, metal anticorrosion and sterilization [3–6].

The softness and length of spacer have a profound impact on the solution properties of gemini surfactants [7]. The previous reports show that gemini surfactants with flexible spacers tend to form spherical or elongated micelles [8–10]. But gemini surfactants with rigid spacers are more likely to form pre-micelles and larger aggregations than micelles, and their tails lie flat at the interface [11–13]. Zhao et al. [14] studied the aggregation behavior of gemini surfactants containing rigid spacers of different lengths. They found the molecule with a short spacer (p-benzenedio) showed rather normal aggregation behavior, but for that with a long spacer (p-dibenzenediol), its hydrophobic chains adopted an unusual conformation and unexpected network structure aggregates were obtained in the solution.

Biphenyl is a common rigid group. Antimicrobial preparations based on biphenyl derivatives have a wide range of medicine and agriculture applications, but their low water solubility hinders the wide practical application [15]. Quaternary ammonium compounds are commonly used as bactericides owing to their bactericidal activities. However, after long-term use of the known antimicrobial agents, microbial strains are prone to produce resistance, which necessitates the constant search for new compounds with antibacterial properties. Gemini surfactants containing biphenyl group, which have good water solubility and thermo-stability, could be effective antimicrobial agents in agriculture and industry.

Most studies about gemini surfactants have been focused on the cationic category alkanediyl-a- $\beta$ -bis(alkyl dimethyl ammonium) dibromide, denoted as m-s-m, where m and s stand for the number of carbon atoms in the hydrophobic alkyl chain and methylene spacer respectively [11]. To further improve the physicochemical properties of m-s-m, researchers have made a lot of attempts and efforts, which brings about some novel surfactants known as the heterocyclic (such

<sup>\*</sup> Corresponding author. E-mail address: qxtong@stu.edu.cn (Q.-X. Tong).

as pyridinium [16–20], pyrrolidinium [21–24], imidazolium [25–28] and morpholinium [29,30]) gemini surfactants. Compared with classic m-s-m type surfactants, these gemini surfactants have higher surface activity and stronger aggregation ability due to the introduction of heterocycles [21,26,30,31]. Among the heterocycles, morpholine is distinctive because of the existence of an oxygen atom [30]. For one thing, the oxygen atom in morpholine can change the positive charge distribution of the headgroup, which has a certain effect on the interaction between the headgroups. For another, the presence of oxygen atom can result in some weak interaction (such as hydrogen bond) between surfactant and water. Morpholinium gemini surfactants with a flexible spacer were reported [30,32], but there is no work about morpholinium gemini surfactants with a rigid spacer.

In this work, we synthesized two series of novel morpholinium gemini surfactants with phenylene (short) and biphenylene (long) rigid spacer (Scheme 1). The effect of the lengths of spacer and hydrocarbon chain on their surface activities, thermodynamic properties, aggregation behaviors and antibacterial properties were investigated, which is conducive to extend their potential applications such as wastewater treatments [33], the synthesis of nanoparticles, nanorods and mesoporous materials [11,34]. The surface parameters were also compared with those of the similar gemini surfactants m-P-m [35] and (Mor)m-6-m [32] to illustrate the influence of the head group and spacer flexibility, which is of fundamental importance in understanding the structureproperty relationship of gemini surfactants.

#### 2. Experimental

#### 2.1. Materials

1-bromodecane, 1-bromododecane, 1-bromotetradecane, 1bromohexadecane, morpholine, 1,4-bis(chloromethyl)-benzene, and 4,4'-bis(chloromethyl)-1,1'-biphenyl were purchased from Energy Chemical. Acetone, ethanol and ethyl acetate are all analytical grades. Both the solvent and the reagents were used as received. Pyrene (Energy Chemical 98%) was recrystallized from ethanol three times before experiments.

#### 2.2. Surface tension measurement

The surface tension was carried out on an automatic A101 tensiometer (Fangrui, China) by the Du Nouy ring method at  $25 \pm 0.1$  °C. Before the experiment, all the surfactant solutions were kept for 24 h to ensure equilibrium status.

#### 2.3. Electrical conductivity measurement

The electrical conductivities of the surfactant solutions were measured by a low-frequency conductivity analyzer (model DDS-307A, Shanghai INESA Scientific Instrument Co., Ltd) at 15  $\pm$  0.1 °C, 20  $\pm$  0.1 °C, 25  $\pm$  0.1 °C, 30  $\pm$  0.1 °C and 35  $\pm$  0.1 °C respectively.

#### 2.4. Steady-state fluorescence measurement

The fluorescence spectra of the surfactant solutions were carried out by a Shimadzu RF-5300PC spectrofluorophotometer. The concentration of fluorescence probe pyrene is  $1 \times 10^{-6}$  mol/L. The surfactant solutions were excited at 335 nm wavelength and the emission spectra were scanned from 350 to 450 nm wavelength. The excitation and emission slit widths were 5 nm and 2 nm, respectively.

#### 2.5. Dynamic light scattering (DLS) measurement

The DLS measurements were performed on a Malvern zeta sizer Nano-zs 90 (Malvern, UK) with a scattering angle of 173° and He—Ne laser ( $\lambda = 632.8$  nm) at room temperature. Every sample was measured three times. The autocorrelation function was analyzed by the CONTIN method to get a hydrodynamic radius (R<sub>b</sub>).

#### 2.6. Transmission electron microscopy (TEM)

The TEM micrographs were obtained using a JEM-F200 transmission electron microscope (JEOL Ltd., Japan). All the samples were prepared by the negative-staining method. Phosphotungstic acid (2%) was used as the staining agent.



**Scheme 1.** Synthesis routes for (Mor)m-P-m and (Mor)m-BP-m (m = 10, 12, 14, 16)



Fig. 1. The curves of the surface tension of (Mor)m-P-m (a) and (Mor)m-BP-m (b) at different concentrations.

Table 1CMC values of the surfactants at 25 °C.

Surfactant	CMC (mM/L)	Surfactant	CMC (mM/L)	Surfactant	CMC (mM/L)	Surfactant	CMC (mM/L)	Surfactant	CMC (mM/L)
		10-P-10	7.27	(Mor)10-6-10	3.1	(Mor)10-P-10	6.49	(Mor)10-BP-10	2.86
(Mor)12	21.0	12-P-12	1.2	(Mor)12-6-12	1.0	(Mor)12-P-12	1.2	(Mor)12-BP-12	0.548
		14-P-14	0.92			(Mor)14-P-14	0.17	(Mor)14-BP-14	0.153
(Mor)16	0.38	16-P-16	0.48			(Mor)16-P-16	0.051	(Mor)16-BP-16	0.046

#### 2.7. Thermal stability

The thermaldynamic properties of the synthesized surfactants were measured by thermal gravimetric analysis (TGA) under the nitrogen atmosphere at a heating rate of 10  $^{\circ}$ C/min.

#### 2.8. Antibacterial activity

Gram-negative *Escherichia coli* (ATCC8739) and gram-positive *Bacillus subtilis* (ATCC6633) were used as the indicative bacteria to characterize the antibacterial activity of the target surfactants by a plate coating method.

In a sterile environment, the bacterial strains were scraped into a sterile liquid medium by an inoculation loop and the organisms were shaken at 37 °C for 24 h to obtain the bacterial suspensions.

The synthesized surfactants were sterilized and dissolved in sterile water to get the antibacterial solution. The antibacterial solution was added to the bacterial suspensions and the mixed solution was incubated by shaking at 37 °C for 1 h (the concentrations of bacteria were approximately  $1 \times 10^7$ – $9 \times 10^7$  cfu/mL). The test organisms were coated on sterile solid medium in the Petri dishes by 10 times dilution method and cultured in an incubator at 37 °C for 24 h. Bacteria suspensions without surfactants were also incubated for comparison. Two parallel samples were measured for every organism. The average numbers of bacterial colonies with and without surfactants were determined.

#### Table 2

Surface paraments of (Mor)m-P-m and (Mor)m-BP-m at 25 °C.

surfactant	CMC (mmol/L)	γ <sub>cmc</sub> (mN/M)	pC <sub>20</sub>	Γ (µmol/m²)	Amin (nm <sup>2</sup> )
(Mor)10-P-10	6.487	39.90	2.56	1.63	1.02
(Mor)12-P-12	1.312	38.24	3.35	1.31	1.26
(Mor)14-P-14	0.171	42.45	4.18	1.29	1.29
(Mor)16-P-16	0.051	49.06	4.49	0.94	1.77
(Mor)10-BP-10	2.859	35.99	3.30	1.32	1.26
(Mor)12-BP-12	0.548	35.40	4.29	0.99	1.68
(Mor)14-BP-14	0.153	41.67	4.53	0.86	1.93
(Mor)16-BP-16	0.046	35.96	5.70	0.68	2.44

The antibacterial rate  $\eta$  (%) was calculated from the following Eq. [36]:

$$\eta(\%) = \frac{A - B}{A} \times 100 \tag{1}$$

where A and B were the average numbers of bacterial colonies without and with surfactants respectively. Minimum inhibitory concentration (MIC) was determined as the lowest concentration of the antibacterial agent with an antibacterial rate above 90% [36].

#### 3. Results and discussion

#### 3.1. Surface activity

The surface tension curves of (Mor)m-P-m and (Mor)m-BP-m aqueous solutions are shown in Fig. 1. The CMC values of them and



Fig. 2. Values of Amin versus m of the gemini surfactants.

the reference substances are listed in Table 1. (Mor)12-P-12 and (Mor)16-P-16 have lower CMC values than the corresponding morpholinium surfactants with a single hydrophobic chain [37,38] denoted as (Mor)12 and (Mor)16, indicating the prominent aggregation capacities of gemini surfactants. (Mor)m-P-m possess smaller CMC than m-P-m, especially for m = 14 and 16, the CMC are about one order of magnitude smaller, suggesting the surface activity was improved by introducing morpholinium.

We abbreviate the morpholinium gemini surfactant with nhexanediyl [32] as (Mor)m-6-m. The length of n-hexanediyl is almost equal to that of phenylenediyl (5.5–6.0 methylene units [3]). The CMC of (Mor)m-P-m are bigger than (Mor)m-6-m, which may be related to the flexibility of the spacer. For the spacers with almost equivalent length, the flexibility of n-hexanediyl makes the spacers easy to locate at the aggregate-water interface, while the rigidity and steric hindrance of phenylenediyl lead the spacers difficult to combine with the hydrophilic outer layer of the micelle [13], resulting in the stronger aggregation tendency of (Mor)m-6-m. It seems that the rigid spacer is unfavorable for the aggregation of gemini surfactants in aqueous solution. However, that is not the case for (Mor)m-BP-m. In Table 1, the CMC of (Mor)m-BP-m are smaller than (Mor)m-P-m and (Mor)m-6-m, especially for m = 10 and 12, indicating the best aggregation ability of (Mor)m-BP-m in aqueous solution.

To better demonstrate the surface activities of (Mor)m-P-m and (Mor)m-BP-m,  $A_{min}$  and  $\Gamma_{max}$  are calculated.  $A_{min}$  is defined as the area occupied by a single molecule of surfactant at the air-water interface, reflecting the stacking density of surfactant molecules.  $A_{min}$  can be obtained from the maximum surface excess concentration  $\Gamma_{max}$ , which can be deduced from the Gibbs adsorption equation:

$$\Gamma_{max} = -\frac{1}{2.303 \text{nRT}} \left(\frac{d\gamma}{d \log C}\right)_T \tag{2}$$

$$A_{min} = \frac{1}{N_A \Gamma_{max}} \times 10^{24} \tag{3}$$

where  $\gamma$  is the surface tension in mN/m;  $\Gamma_{max}$  is the surface excess in  $\mu$ mol/m<sup>2</sup>; R is the gas constant (8.314 J mol<sup>-1</sup> K<sup>-1</sup>); T is the absolute



Fig. 3. Schematic representations for the adsorption at the air-water interface (Mor)m-P-m (a) and (Mor)m-BP-m (b).



Fig. 4. The curves of specific conductivity versus concentration of (Mor)10-P-10 (a), (Mor)12-P-12 (b), (Mor)14-P-14 (c) and (Mor)16-P-16 (d) at different temperatures.

temperature; C is the concentration of surfactant in mol/L;  $d\gamma/dlogC$  is the slope of the linear part of the  $\gamma$ -logC curve where the concentration of surfactant is lower than CMC. For gemini surfactant in aqueous solutions without addition counterion, n takes as 3. N<sub>A</sub> is the Avogadro's constant ( $6.02 \times 10^{23} \text{ mol}^{-1}$ ), and A<sub>min</sub> is in nm<sup>2</sup>.

In Table 2, the CMC values of (Mor)m-P-m and (Mor)m-BP-m decrease obviously with m increasing from 10 to 16, indicating the stronger aggregation ability for longer hydrophobic chains. Long alkyl chains prefer to bend, leading to an increase of  $A_{min}$  and a decrease of  $\Gamma_{max}$ . For the same m, (Mor)m-BP-m have bigger  $A_{min}$  and smaller  $\Gamma_{max}$ . The length of biphenylene spacer is longer than phenylene spacer, which leads to the further distance between two hydrophobic chains, bringing about bigger  $A_{min}$  and smaller  $\Gamma_{max}$ .

From the  $\gamma$ -logC curve, we can also get two other parameters pC20 and  $\gamma_{CMC}$ . pC20 is defined as the negative logarithm of the surfactant concentration required for reducing the surface tension value of water by 20 mN/m. The larger the pC20 is, the higher the efficiency to reduce surface tension will be.  $\gamma_{CMC}$  refers to the lowest surface tension of water that the surfactant can reduce to. The smaller the  $\gamma_{CMC}$  are, the stronger the ability to reduce the surface tension will be. The hydrophobicities of (Mor)m-P-m and (Mor)m-BP-m are enhanced with increasing m, thus pC20 show an increasing trend. The  $\gamma_{CMC}$  of (Mor)m-BP-m are smaller than (Mor)m-P-m, indicating the stronger ability of (Mor) m-BP-m to reduce the surface tension, which may be related to the arrangement of hydrophobic chains at the interface.

When the adsorption of surfactant at the air-water interface is saturated, a nonpolar layer consists of hydrocarbon chains could be obtained. A higher density of hydrophobic groups in the layer leads to the smaller  $\gamma_{CMC}$ , and vice versa.  $\Gamma_{max}$  of (Mor)m-P-m is bigger than that of (Mor)m-BP-m, which means that the higher stacking density of (Mor)m-P-m at the interface. If both the hydrophobic chains of

(Mor)m-P-m orientate to the air, the chain density of (Mor)m-P-m would be higher than (Mor)m-BP-m in the adsorption layer, and the  $\gamma_{CMC}$  should be smaller. However, the results are contrary, which might be ascribed to the structure of the spacer. We could infer the arrangement of the hydrophobic chains of (Mor)m-P-m and (Mor)m-BP-m at the air-water interface by comparing their A<sub>min</sub> with those of other similar gemini surfactants.

Fig. 2 shows the effect of the carbon atoms number (m) in the hydrophobic chain on  $A_{min}$  (n = 3). The  $A_{min}$  of (Mor)10-6-10 and (Mor)12-6-12 are 0.593 and 0.611 nm<sup>2</sup> respectively (black dots) [32]. The  $A_{min}$  of (Mor)10-P-10 and (Mor)12-P-12 are 1.02 and 1.26 nm<sup>2</sup> (pink dots), which are about double those of (Mor)10-6-10 and (Mor)12-6-12 respectively. Such significant differences in the  $A_{min}$  among these gemini surfactants having a similar spacer length proves that both tails of (Mor)m-P-m are unlikely toward the air.

Ge et al. [13] reported a series of gemini surfactants with pxylylene spacer (denoted as m-PX-m, m = 10, 12, 14, 16) and the A<sub>min</sub> values of them are 1.28, 1.77, 2.29 and 3.4 nm<sup>2</sup> respectively (red dots). Wang et al. [11] synthesized gemini surfactants containing *n*-phenylacetamide in the spacer (regarded as m-PAM-m, m = 12, 14,16) and their A<sub>min</sub> values are 1.78, 2.03 and 2.56 nm<sup>2</sup> respectively (blue dots). They found the presence of benzene increases the rigidity of the spacer and hinders the change in the spacer conformation, making the hydrophobic chains of m-PX-m and m-PAM-m lie at the interface. Fig. 2 demonstrates that there is little diversity in Amin between m-PX-m and m-PAM-m. Thus, Amin values of gemini surfactants with a rigid spacer are relatively big, and their hydrophobic chains tend to lie at the air-water interface. In Fig. 2, Amin of (Mor)m-P-m (pink dots) are far smaller than m-PX-m (red dots) and m-PAM-m (blue dots), indicating that both tails of (Mor)m-P-m are impossible to lie at the interface.



Fig. 5. The curves of specific conductivity versus concentration of (Mor)10-BP-10 (a), (Mor)12-BP-12 (b), (Mor)14-BP-14 (c) and (Mor)16-BP-16 (d) at different temperatures.

Based on the above analysis, for the saturated adsorption of gemini surfactants at the interface, their  $A_{min}$  values are relatively small when the tails are oriented to the air and relatively big when the tails lie at the interface. From Fig. 2, the  $A_{min}$  of (Mor)m-P-m are between these two cases. Therefore, the hydrophobic chains of (Mor)m-P-m are probable to locate at the air-water interface adopting a mixed arrangement of being orientated to the air and lying at the surface due to the rigidity and steric effect of phenylene spacers (shown in Fig. 3a).

The spacer lengths of (Mor)m-BP-m (biphenylene) are longer than m-PX-m and m-PAM-m. If the tails of (Mor)m-BP-m lie at the interface,  $A_{min}$  of (Mor)m-BP-m will be bigger than m-PX-m and m-PAM-m. Nevertheless, the  $A_{min}$  values are even slightly smaller (green dots in Fig. 2), indicating that the tails should not lie flat at the interface. The C—C single bond between two benzene rings of biphenylene is expected to enable these benzene rings to rotate. What is more, the longer spacer can weaken the interaction between two alkyl chains [7]. Therefore, both the tails of (Mor)m-BP-m would be unfolded and orientated to the air through the conformation change of spacer, leading to the higher density of hydrophobic chains in the adsorption layer (as shown in Fig. 3b). As a result, the  $\gamma_{CMC}$  of (Mor)m-BP-m is lower than (Mor)m-P-m.

#### 3.2. Thermodynamics of micellization

The micellization thermodynamic parameters of surfactant solutions can be determined by measuring the curves of conductivity at different temperatures. In Fig. 4 and Fig. 5, there are two linear relationships between the conductivity and concentration for each temperature, and the turning point shows the CMC. The counterion binding ( $\beta$ ) can be calculated through the following formula:

$$\beta = 1 - k_2 / k_1 \tag{4}$$

where  $k_1$  and  $k_2$  are the pre-micellar slope and post-micellar slope, respectively.

The values of  $\beta$  and CMC obtained by the conductivity method are listed in Table 3. The counterions are combined on the surface of micelles by the electrostatic attraction. The electrostatic attraction is weak at a high temperature, thus the  $\beta$  values decrease with increasing temperatures [39]. The impacts of temperature on CMC mainly reflect in the following two aspects. On one side, the increase of temperature could weaken the hydration of the hydrophilic groups, which is conducive to the formation of micelles. On the other side, the structured water molecules surrounding the hydrophobic tail would be broken down with increasing the temperature, which is in disfavor of the formation of micelles. These combined effects determine the variations of CMC. Here, the CMC increase at a high temperature, indicating the influence on hydrophobic interaction of tails plays a dominant role for (Mor)m-P-m and (Mor)m-BP-m among the investigated temperatures.

The standard Gibbs free energy in the process of micellization  $\Delta G_{mic}^{o}$  can be calculated by the following formula [25,40]:

$$\Delta G_{\rm mic}^o = \operatorname{RT}(0.5 + \beta) \ln X_{\rm CMC} \tag{5}$$

where  $X_{CMC} = CMC/55.4$  and 55.4 stems from 1 L of water corresponding to 55.4 mol of water at 25 °C. The standard enthalpy change  $\Delta H_{mic}^{o}$ 

#### Table 3

The values of CMC, $eta$ and thermodynamic parameters of (Mor)m-P-m and (Mor)m-BP-m at different temperati	ures
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Surfactant	T (K)	CMC(mmol/L)	β	$\Delta G_{mic} (kJ/mol)$	$\Delta G_{ads} (kJ/mol)$	$\Delta H_{mic} (kJ/mol)$	-T $\Delta S_{mic}$ (kJ/mol)
(Mor)10-P-10	288.15	10.411	0.3618	-17.71		-8.05	-9.66
	293.15	10.504	0.3581	-17.93		-8.81	-9.12
	298.15	10.768	0.3530	-18.07	-50.16	-9.58	-8.49
	303.15	11.020	0.3509	-18.28		-10.4	-7.88
	308.15	11.284	0.3379	-18.25		-11.2	-7.05
(Mor)12-P-12	288.15	2.159	0.5716	-26.06		-48.36	22.30
	293.15	2.254	0.5376	-25.56		-48.54	22.98
	298.15	2.228	0.4871	-24.71	-58.46	-48.83	24.12
	303.15	2.317	0.4602	-24.40		-49.18	24.79
	308.15	2.349	0.4565	-24.67		-49.58	24.91
(Mor)14-P-14	288.15	0.330	0.4575	-27.60		-69.65	42.26
	293.15	0.360	0.4322	-27.14		-70.03	42.89
	298.15	0.370	0.3491	-25.08	-54.62	-70.33	45.25
	303.15	0.380	0.3286	-24.83		-71.11	46.28
	308.15	0.400	0.3252	-25.03		-71.82	46.79
(Mor)16-P-16	288.15	0.119	0.4591	-29.99		-103.20	73.21
	293.15	0.126	0.3835	-27.98		-104.92	76.94
	298.15	0.129	0.3814	-28.34	-51.27	-107.42	79.08
	303.15	0.137	0.3465	-27.54		-109.45	81.91
	308.15	0.139	0.2107	-23.48		-111.49	88.01
(Mor)10-BP-10	288.15	5.530	0.5528	-23.23		-6.93	-16.30
	293.15	5.560	0.5525	-23.62		-8.51	-15.11
	298.15	5.804	0.5456	-23.75	-50.97	-10.13	-13.63
	303.15	5.830	0.5409	-24.03		-11.86	-12.17
	308.15	6.160	0.5367	-24.18		-13.65	-10.53
(Mor)12-BP-12	288.15	0.900	0.6351	-29.99		-45.62	15.64
	293.15	0.910	0.5751	-28.87		-47.40	18.53
	298.15	0.930	0.5412	-28.38	-65.30	-49.20	20.82
	303.15	0.942	0.5446	-28.92		-51.16	22.24
	308.15	0.964	0.5071	-28.28		-52.99	24.71
(Mor)14-BP-14	288.15	0.334	0.5899	-31.39		-114.02	82.63
	293.15	0.353	0.4592	-27.97		-116.15	88.18
	298.15	0.358	0.4345	-27.68	-62.99	-119.35	91.67
	303.15	0.381	0.3386	-25.13		-121.76	96.63
	308.15	0.388	0.3272	-25.15		-125.07	99.92
(Mor)16-BP-16	288.15	0.089	0.6492	-36.73		-98.38	61.65
	293.15	0.091	0.5474	-34.01		-100.78	66.77
	298.15	0.091	0.5050	-33.17	-86.06	-103.44	70.27
	303.15	0.092	0.4579	-32.14		-106.18	74.05
	308.15	0.092	0.4340	-31.84		-109.02	77.17

and standard entropy change  $\Delta S_{mic}^{o}$  of micellization can be obtained through the following relations:

$$\Delta H_{\rm mic}^{0} = \left[\frac{\partial \left(\Delta G_{\rm mic}^{0}/T\right)}{\partial (1/T)}\right] \tag{6}$$

$$\Delta S_{\rm mic}^{\rm o} = \left(\Delta H_{\rm mic}^{\rm o} - \Delta G_{\rm mic}^{\rm o}\right)/T \tag{7}$$

Besides, the standard Gibbs free energy in the process of adsorption  $\Delta G_{ads}^{o}$  are from the following equation [41,42]:

$$\Delta G_{\rm ads}^{\rm o} = \Delta G_{\rm mic}^{\rm o} - \Pi_{\rm CMC} / \Gamma_{\rm max} \tag{8}$$

$$\Pi_{\rm CMC} = \gamma_0 - \gamma_{\rm CMC} \tag{9}$$

where  $\Pi_{CMC}$  and  $\gamma_0$  represent the surface pressure of surfactant at CMC and the surface tension of water at 25 °C, respectively.

In Table 3, the values of  $\Delta G^o_{ads}$  and  $\Delta G^o_{mic}$  are negative, illustrating the adsorption and aggregation processes are spontaneous [25,42]. The  $\Delta G_{mic}^{o}$  of (Mor)m-P-m and (Mor)m-BP-m decrease with the increase of m, indicating that the stronger aggregation ability with longer tails. The absolute  $\Delta G^o_{mic}$  values of (Mor)m-BP-m are bigger than (Mor)m-P-m, suggesting that the stronger aggregation trend of (Mor)m-BP-m in aqueous. The negative values of  $\Delta H^o_{mic}$  showing their micellization process is exothermic.

Gemini surfactants may form pre-micellar at a concentration lower than CMC, which is a specific aggregation behavior different from traditional surfactants [28,43,44]. Zana et al. proposed that the existence of a maximum value in the curve of molar conductivity versus the square root of concentration ( $C^{0.5}$ ) is a typical sign of the formation of pre-micelle [45]. The molar conductivity  $\Lambda$  can be calculated by the following equation:

$$\Lambda = \frac{\kappa - \kappa_0}{C} \tag{11}$$

where *K* is the conductivity of the surfactant solution,  $K_0$  is the conductivity of secondary distilled water, and C is the concentration of surfactant. A values of (Mor)16-P-16 (Fig. 6 d), (Mor)14-BP-14 (Fig. 7 c) and (Mor)16-BP-16 (Fig. 7 d) pass through a maximum while those of other surfactants decline with the increase of C<sup>0.5</sup>, proving the existence of pre-micelles. Generally, there are pre-micelles in the aqueous solution of gemini surfactants containing alkyl chains with at least 14 carbon atoms due to the strong hydrophobic interaction between the long tails [13,38,46–48].

#### 3.3. Micropolarity of aggregates

In the fluorescence emission spectrum, the ratio ( $I_1/I_3$ ) of fluorescence intensity at the first peak (373 nm) and the third peak (384 nm) is sensitively dependent on the polarity of the microenvironment in which pyrene is located [49,50]. When the surfactants form micelles, pyrene will locate in the nonpolar region of micellar core, leading to a sharp drop in  $I_1/I_3$ . Zara et al. [51] proposed that the concentration corresponding to the midpoint of the descent curve between two platforms can be regarded as the CMC. The CMC values measured by fluorescence method are  $6.75 \times 10^{-3}$ ,  $1.058 \times 10^{-3}$ ,  $2.36 \times 10^{-4}$  and  $5.3 \times 10^{-5}$  mol/L for (Mor)m-P-m (Fig. 8a), and  $2.614 \times 10^{-3}$ ,  $6.94 \times 10^{-4}$ ,  $1.85 \times 10^{-4}$  and  $5.1 \times 10^{-5}$  mol/L for (Mor)m-BP-m (Fig. 8b), corresponding to m = 10, 12, 14 and 16 respectively, which is approximately consistent with the results obtained by surface tension and conductivity method. Besides, the minimum  $I_1/I_3$  values of (Mor)m-P-m and (Mor)m-BP-m are close, showing that the length of rigid spacers has little effect on the micropolarity of aggregates.



Fig. 6. Variation of the molar conductivity  $\Lambda$  with C<sup>0.5</sup> for (Mor)m-P-m at different temperatures.



Fig. 7. Variation of the molar conductivity  $\Lambda$  with C<sup>0.5</sup> for (Mor)m-BP-m at different temperatures.

#### 3.4. Size and morphology of aggregates

#### 3.4.1. Dynamic light scattering (DLS)

The result obtained by dynamic light scattering (DLS) is the average hydration diameter of the surfactant aggregates, which helps determine the type of aggregates. In Fig. 9, the peaks at less than 1 nm in the curves are caused by the interaction of multiple headgroups in gemini surfactants and do not indicate the size of aggregates [52]. The hydration diameters of (Mor)m-P-m aggregates are about 20–40 nm and 70–250 nm, and those of (Mor)m-BP-m aggregates are about 30–50 nm and 80–400 nm. The results show that these surfactants

form vesicles in the aqueous solution and the sizes of vesicles formed by (Mor)m-BP-m are slightly larger. To further identify the aggregation morphologies, we also carry out TEM experiments.

### 3.4.2. Transmission electron microscopy (TEM)

Fig. 10 and Fig. 11 show that the synthesized surfactants form vesicles with different sizes in aqueous solutions, which conform to the results from DLS. Compared with (Mor)m-P-m, (Mor)m-BP-m are inclined to form larger vesicles. The possible reason is that the biphenylene spacer is longer and more rigid than the phenylene spacer, which prevents the tails from getting close to each other. Thus the tails



Fig. 8. Pyrene fluorescence intensity ratio  $I_1/I_3$  vs C for (Mor)m-P-m (a) and (Mor)m-BP-m (b).



Fig. 9. The size distributions of (Mor)m-P-m (a) and (Mor)m-BP-m (b) aggregates at 10 times CMC.



Fig. 10. TEM images of (Mor)m-P-m at a concentration of 10 times the CMC [(Mor)10-P-10 (a), (Mor)12-P-12 (b), (Mor)14-P-14 (c), (Mor)16-P-16 (d)].



Fig. 11. TEM images of (Mor)m-BP-m at a concentration of 10 times the CMC [(Mor)10-BP-10 (a), (Mor)12-BP-12 (b), (Mor)14-BP-14 (c), (Mor)16-BP-16 (d)].

of (Mor)m-BP-m occupy more volumes in the aggregates, bringing about the larger size.

#### 3.5. Thermaldynamic properties

The decomposition temperature (T<sub>d</sub>) of each target surfactant is revealed by TGA. As shown in Fig. 12, all surfactants displayed excellent thermal stability. T<sub>d</sub> are recorded at 225, 237, 233, 239 °C for (Mor)m-P-m, and 217, 202, 208, 216 °C for (Mor)m-BP-m, corresponding to m = 10, 12, 14, 16 respectively, which exhibited better thermal stability than the classic type surfactants m-s-m reported, such as 14-2-14 (T<sub>d</sub> = 173.7 °C) and 16-2-16 (T<sub>d</sub> = 172.8 °C) [53].

#### 3.6. Antibacterial property

The antibacterial mechanism [54–56] of cationic surfactants is generally considered as follows. For one thing, the headgroups of cationic surfactant adsorb on the cell wall of bacteria, which brings about a steric effect and changes the permeability of the cell wall. For another, alkane chains insert into the lipid layer of cells by hydrophobic interaction, leading to the deactivation of enzymes and the denaturation of the protein. As a result, the bacteria die. The minimum inhibitory concentration (MIC) is one of the parameters to measure the antibacterial property of an antimicrobial agent. The lower the MIC is, the better the antibacterial property will be. The antibacterial properties of target surfactants against *Escherichia coli* and *Bacillus subtilis* were studied by the spread plate method. The MIC values were investigated and shown in Table 4.

The MIC values of (Mor)m-P-m and (Mor)m-BP-m are smaller than traditional bactericide 1227 for both bacteria, indicating the better antibacterial performance of gemini surfactant. Compared with 1227, the synthesized surfactants possess higher densities of positive charges and hydrophobic chains, bringing about stronger electrostatic adsorption on the cell membrane of bacteria and more prominent hydrophobic effects with the lipoid layers of cells [36,57]. The antibacterial activity of the surfactant varies with the alkyl chain length and reaches the best with an optimal length [58]. Since the MIC values of (Mor)m-P-m and (Mor)m-BP-m exhibit a similar order with the change of hydrophobic chain length, we take (Mor)m-P-m homologues as examples to discuss. The cell membrane of bacteria is mainly composed of phospholipid bilayers and most phospholipid molecules contain 12 carbon atoms. The physicochemical properties of surfactants containing 12 carbon atoms are most similar to the lipid layer, and the energy needed to combine them is minimum [36,58]. Therefore, (Mor)12-P-12 possesses the best



Fig. 12. TGA results of (Mor)m-P-m (a) and (Mor)m-BP-m (b).

 Table 4

 The MIC values of synthesized surfactants against E. coli and B. subtilis (mg/L).

surfactant	Escherichia coli	Bacillus subtilis		
1227	50 [57]	-		
(Mor)10-P-10	15	15		
(Mor)12-P-12	12	11		
(Mor)14-P-14	39	35		
(Mor)16-P-16	43	40		
(Mor)10-BP-10	8	7		
(Mor)12-BP-12	5	3		
(Mor)14-BP-14	30	23		
(Mor)16-BP-16	35	30		

antibacterial performance among the (Mor)m-P-m homologues. Compared with (Mor)12-P-12, (Mor)10-P-10 has weaker hydrophobicity because of the shorter tail, leading to the slightly inadequate binding ability with the lipid layer. Thus, the antibacterial property of (Mor) 10-P-10 is a little inferior. The antibacterial performances of (Mor)14-P-14 and (Mor)16-P-16 are relatively poor, which may be related to the hydrophilic-hydrophobic balance of the surfactant molecules [59]. They are too hydrophobic to penetrate the cell membrane effectively. Moreover, long tails will lead to poor water solubility [36,60], which may be another reason for their low antibacterial activities.

It is noteworthy that the MIC values of (Mor)m-BP-m are smaller than (Mor)m-P-m for both bacteria, suggesting the better antibacterial properties of (Mor)m-BP-m. It could be due to two reasons. For one thing, the steric effect of biphenylene is more prominent than that of phenylene, causing the lower permeability of the cell membrane. For another, conformation changes of biphenylene strengthen the hydrophobic binding between the tails and the lipid layers of cells.

#### 4. Conclusion

In summary, two series of morpholinium gemini surfactants with rigid spacer of different lengths were synthesized. They exhibited superior surface activity to the similar conventional gemini surfactant. Their CMC values decreased with increasing the length of hydrophobic chains. Compared with (Mor)m-P-m, (Mor)m-BP-m possess lower  $\gamma_{\rm cmc}$  and CMC, which could be related to the spacer structures. The thermodynamic parameters showed their micellization processes are spontaneous and exothermic. There were pre-micelles in the micellization processes of (Mor)16-P-16, (Mor)14-BP-14 and (Mor)16-BP-16. The fluorescence measurement indicated that the lengths of rigid spacers have little effect on the micropolarity of aggregates. Vesicles were found in all surfactants aqueous solutions and the sizes of vesicles formed by (Mor)m-BP-m were slightly larger than (Mor)m-P-m. Their antibacterial activities against *E. coli* and *B. subtilis* are excellent,

among which (Mor)m-BP-m displayed a higher activity. Compounds containing 12 carbon atoms in the tails exhibited optimal antibacterial property among the synthesized gemini homologues.

#### Author contributions

Qing-Xiao Tong: Funding acquisition; Project administration; Supervision; Writing - review & editing. Le-Chi Zheng: Data curation; Formal analysis; Investigation; Methodology; Writing - original draft.

#### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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