Preparation and Antimicrobial Properties of Gemini Surfactant-Supported Triiodide Complex System

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ABSTRACT: Iodine is an effective, simple, and inexpensive bactericide in disinfection. However, the poor solubility and stability of iodine in water limit its applications. In addition, the active iodine content in the commercial iodophors is quite low, and the reported triiodide complex is unstable. In this work, a long-term stable triiodide complex antimicrobial system was prepared by mixing iodine and a cationic gemini surfactant into lauryldimethylamine oxide (LDAO) aqueous solution, and its stability was examined by means of UV−vis spectrophotometry. It was found that the content of LDAO, cationic gemini surfactant and H_2SO_4 played crucial roles in stabilizing antimicrobial system, and the active iodine (i.e., triiodide complex) content of the optimum formulation can remain stable for 150 days, as iodine is encapsulated by the mixed vesicles assembled by the protonated LDAO and the added gemini surfactant. However, the active iodine reduced rapidly when NaCl was added or the pH was increased in the environment. Furthermore, the antimicrobial efficacy of the optimized formulation was studied against *Candida albicans*, and more than 4 log reduction in viable cell after 5 min of contact was obtained.

KEYWORDS: fungicide, triiodide complex, gemini surfactant, long-term stability, mixed micelles, cryo-TEM
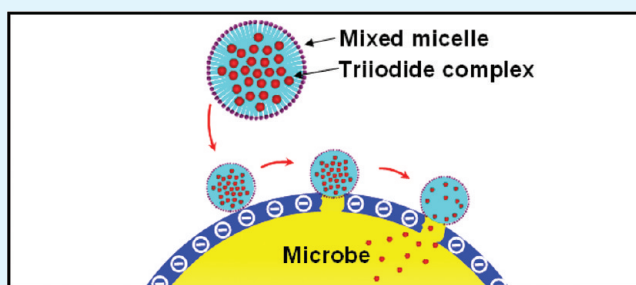
1. INTRODUCTION

It is well-recognized that iodine is an effective, simple, and inexpensive disinfectant due to its quick reactions with living microorganisms.[1] Because of its wide spectra of action and low toxicity, iodine has been used extensively for the antibacterial treatment in medicine, health, water processing, and environmental protection.[2] The form of antimicrobial iodine contains molecular iodine I_2, hypoiodous acid HOI, iodine cation H_2OI^+, triiodide anion I_3^-, and iodate anion IO_3^-.[3−5] Among them, I_2 and HOI have strong germicidal properties, but H_2OI^+ does not function in disinfection processes because its concentration is too low; I_3^- is a moderate oxidant and shows an inferior antibacterial activity compared to I_2; IO_3^- is an oxidant only at pH values lower than 4 and therefore has no effect in the actual conditions where disinfections normally are carried out at about pH 6−8. During the First World War,[6] iodine was employed to disinfect drinking water for troops. Since the 1950s, iodine-based disinfection has been widely applied in water and hospital disinfection,[7−9] and even used by NASA in space flights.[10] In recent years, the frequent hits of SARS,[11] A(H1N1)v,[12] earthquakes, and tsunamis have influenced scientists' efforts in seeking applicable disinfectants to cure the world. Effective but less expensive disinfectors such as iodine-based antibacter-icides may find applications in the public disinfection during the reconstruction course.

However, iodine is poorly soluble in water, and its solubility in water is only 0.334 g/L at 25 °C.[4] To increase the concentration of iodine in the formulation, various recipes were developed to introduce KI (or NaI) into water, ethyl alcohol, and glycerol, or in mixtures of these solvents. For instance, in Lugol's solution, KI, or NaI can react with iodine to form potassium (or sodium) triiodide which is water-soluble.[13] Nevertheless, the high content of free molecular iodine (e.g., in Lugol's solution: $[I_2]$ = 170 mg/L) generates the disadvantages of staining and irritation of living tissues and is unstable for the high iodine vapor pressure.[4]

An effective way to overcome these deficiencies is to reduce the content of free molecular iodine in the solutions. A typical example is iodophor in which iodine is combined with carriers of water-soluble polymers that release free iodine to kill microorganism. Compared with Lugol's solution, iodophors show less inflammation. The well-known commodity iodophor is povidone-iodine (PVP-I),[14] in which triiodide anion is fixed via a hydrogen bond between two carbonyl groups of the 1-vinyl-2-pyrrolidinone (PVP) and triiodide anion, thus the free iodine is largely reduced.[14,15] It was reported that such an iodine-based antibacterial system possesses a rapid bactericidal action against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*[16] and HIV.[17] Nevertheless, the high-molecular-weight PVP polymer conjugating iodine is less degradable, rendering potential risk to environment.[18] In addition, the active iodine

content in iodophor products is normally less than 0.75 wt %, too low for economic considerations.

Alternatively, small-molecule compounds such as cyclodextrins[19,20] and amine oxides[21,22] are employed as complexing agent to increase the active iodine content. Because there are some cavities in their molecules, cyclodextrins can capture iodine to produce inclusion complexes,[19] whereas amine oxide can react with iodine in water to form triiodide complex.[22] Because of their poor solubility in water, the long-term stability of these iodine-based antibacterial aqueous systems is rather weak.[19] Obviously, if iodine and iodine-based compounds precipitate in aqueous solution rapidly or lose through iodine vapor, the antimicrobial activity will be significantly reduced. Thus, it is very essential to stabilize the active iodine for disinfection in the antimicrobial environment.

To achieve such a goal, it is crucial to choose a suitable stabilizer that could enhance the long-term stability of active iodine. As a new class of amphiphilies, gemini surfactants that have two hydrophobic tails and two cationic head groups linked by a spacer show improved excellent chemical stability and dispersion stabilization.[23-25] Such unique properties may furnish gemini surfactants to enhance the long-term stability of active iodine. Furthermore, cationic gemini surfactants themselves are also bactericides,[24,26] which could bring synergistic antimicrobial effect along with active iodine.

Here in this study, an attempt has been made to prepare a long-term stable triiodide complex antimicrobial system supported with a cationic gemini surfactant, dimethylene-1,2-bis(dodecyl dimethylammonium bromide) (12−2−12). The preparation parameters affecting the long-term stability and iodine loading of antimicrobial system were examined, and the antimicrobial activity of the optimal formulation was tested against *Candida albicans* that is a common fungus and a causal agent of opportunistic infection in animals, and is more difficult to be killed than other bacterium, such as *Escherichia coli*.[27-29] Moreover, cryo-TEM observation was employed to elucidate the mechanism of the improved stability of iodine-based antimicrobial system by the cationic gemini surfactant.
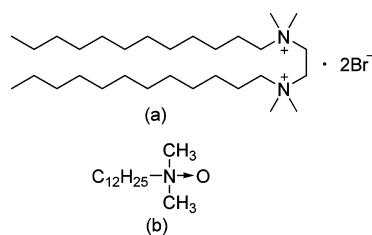
Figure 1. Chemical structures of (a) the gemini surfactant 12−2−12 and (b) zwitterionic surfactant LDAO used in this work.

2. EXPERIMENTAL SECTION

2.1. Materials. The cationic gemini surfactant, dimethylene-1,2-bis(dodecyl dimethylammonium bromide) (12−2−12, Figure 1a) with purity higher than 98% was synthesized according to a previously reported procedure.[30,31] Lauryldimethylamine oxide (LDAO, Figure 1b) with 30 wt % active content in water was purchased from Kehongda Co., Ltd. (Chengdu, China). The iodine with analytical grade and H_2SO_4 (98 wt %) were obtained from Guanghua Chemicals

Co., Ltd. (Guangzhou, China) and used as received. Starch indicator was obtained from Kelong Chemical Reagent Factory (Chengdu, China). The water used was triply distilled by a quartz water-purification system.

2.2. Preparation of Triiodide Complex Antimicrobial Systems. The triiodide complex antimicrobial solutions were prepared by mixing designed amount of iodine, gemini surfactant 12−2−12 and H_2SO_4 into the 30 wt % LDAO aqueous solution at desired temperatures. After gentle mechanical agitation for 60 min, the solutions were then kept at room temperature overnight to get the final samples. The antimicrobial formulations with various feed ratios were listed in Table 1.

Table 1. Feed Composition and Preparation Conditions of the Antimicrobial Systems

sample code	I_2 (g)	LDAO (g)	T (°C)	12−2−12 (g)	H_2SO_4 (g)	stability
L1	2	10	60	5	5	poor
L2	2	30	60	5	5	poor
L3	2	50	60	5	5	good
T1	2	50	20	5	5	poor
T2	2	50	40	5	5	poor
T3	2	50	60	5	5	good
C1	2	50	60	0	5	good
C2	2	50	60	5	5	good
S1	2	50	60	5	0	poor
S2	2	50	60	5	5	good

2.3. Determination of Active Iodine Content. When H_2SO_4 is added in our system, the pH value is 0.67 measured by pH Electrode, and the content of HOI is so low in the antimicrobial system that could be neglected.[4] Starch indicator was used to determine the free iodine, and the pure iodine solution was tested as a control. We prepared iodine solution by putting iodine powder into distilled water with a high-speed magnetic stirrer in a lightproof box at room temperature. After 5 h, we can obtain the fresh iodine solution. In the antimicrobial system prepared in this work, the total content of the active iodine is equal to the sum of triiodide complex and free iodine.

The concentrations of triiodide complex in the fungicides were determined by an ultraviolet−visible (UV−vis) spectrophotometer (UNICO UV-4802) operated on the Spectra Manager software. All measurements were carried out at 25 ± 0.5 °C with a standard 1 cm thick quartz cell. Since the absorption peak of triiodide appeared at 361 nm,[19] the changes in the intensity of absorption at this wavelength were recorded with prescribed time. The conversion of spectral data to triiodide complex concentration was carried out with a calibration curve prepared beforehand.

2.4. Stability Study of Antimicrobial Systems. To test the stability of the antimicrobial systems at simulated application environment, the antimicrobial formulations were diluted with distilled water to 1000 times, and the salinity or pH of the diluted solutions was adjusted by adding NaCl or NaOH powder. Then the solutions were distributed into a series of 100 mL glass bottles that were sealed with a cover and placed into a lightproof box at room temperature. At consecutive time intervals, the samples were taken out for active iodine content monitoring. For each test, the result was an average value of three runs of test.

2.5. Antimicrobial Activity Test. The antimicrobial activity was tested at West China School of Pubic Health of Sichuan University with the following procedures.

2.5.1. Pretreatment of the Fungicides. Antimicrobial systems (samples S1 and S2 in Table 1) were diluted with water to obtain diluted solution with 100 $\mu g/mL$ of active iodine, and 5000 $\mu g/mL$ cationic gemini surfactant (12−2−12) solution was used as a control.

2.5.2. Preparation of Fungi Cell. *Candida albicans* ATCC 1023 (China General Microbiological Culture Collection Center) was used as a test organism to examine the antimicrobial efficacy of the fungicides, and the cationic gemini surfactant 12−2−12 was employed
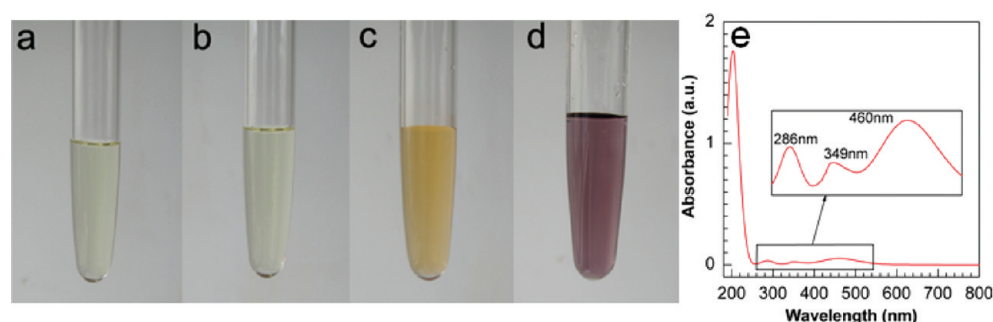
Figure 2. Appearances of antimicrobial systems and iodine solutions: (a) diluted S2 solution, (b) diluted S2 solution with starch indicator, (c) iodine solution, (d) iodine solution with starch indicator, and (e) UV−vis absorption spectrum of iodine solution.

as a reference. Fungal strain was cultivated during 4 to 6 days on sabouraud dextrose agar (SDA) at 25 °C until sufficient spores were formed. The spores were then harvested by adding 5 mL of M9GY medium (minimal phosphate medium) at pH 5.0 to the SDA slant, which were gently scraped to suspend the microorganisms. The fungal solution was adjusted with a counting cell under microscope, 1×10^6 to 1×10^7 spores per milliliter by adding M9GY if necessary.

2.5.3. Antimicrobial Tests. All the antimicrobial activity tests were run at 20 °C. First, 0.5 mL of the suspension and 4.5 mL of the antimicrobial solutions were mixed in sterilized test tubes for 5 min. One-half a milliliter of the mixed solutions was added in a primary subculture tube containing 20 mL of neutralizer for 30 min to stabilize the survived cells. Then the numbers of the viable bacteria were determined by plate counting technique on SDA plates after a serial dilution. Each test was carried out for three times and the average values were taken as the final results. The sterile neutralizer solution was freshly prepared by adding 1% (v/v) 0.1 M $Na_2S_2O_3$ to 600 mL of 0.85% (w/v) NaCl solution containing 0.1% (v/v) Tween 80.[32−34]

2.6. Surface Tension Measurement. Surface tensions of 12−2−12, LDAO and their mixed solution with H_2SO_4 were measured with Wilhelmy plate technique with an automatic surface tensiometer (BZY-1, Shanghai Hengping Instrument, China) as described previously.[31,35] Measurements were taken at 25 ± 0.5 °C until a constant surface tension was reached. Solutions of 12−2−12, LDAO and their mixed solution with H_2SO_4 were diluted continuously with pure water, respectively, and the surface tension at each concentration was obtained by averaging three runs of measurement. The critical micelle concentration (cmc) was taken at the intersection of the linear portions of the surface tension plots against the logarithm of the surfactant concentration.

2.7. Cryo-TEM Observation. The cryo-TEM observation was carried out in a controlled-environment vitrification system as described previously.[36,37] The chamber temperature was 25−28 °C, and the relative humidity was kept close to saturation to prevent evaporation during sample preparation. The diluted S2 (Table 1) solution in antimicrobial test and the original concentrated S2 solution were respectively placed on microperforated cryo-TEM grids, and blotted with a special filter paper to obtain a thin liquid film on the grids. The grids were refrigerated rapidly in liquid ethane at −180 °C and then transferred into a JEM2010 cryo-microscope with a Gatan 626 cryo-holder and its workstation. The acceleration voltage was 200 kV, and the working temperature was kept below −170 °C. The images were recorded digitally with a charge-coupled-device camera (Gatan 832) under low-dose conditions with an underfocus of approximately 3 μm.

3. RESULTS AND DISCUSSION

3.1. Form of Active Iodine in Antimicrobial System. As mentioned earlier, the total concentration of the active iodine equals to the sum of the triiodide complex and free iodine in the antimicrobial systems. In order to check if free iodine presents in antimicrobial systems, starch indicator was added to the diluted solutio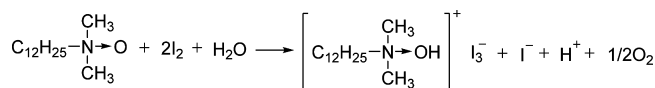n of S2 in Table 1. It is well-known that amylose in starch is responsible for the formation of a deep blue color in the presence of free iodine,[38] and thus this method was used in this work to determine the presence or absence of free iodine.

Before the addition of starch indicator, the color of the diluted S2 solution is faintly yellow (Figure 2a), and remains unchanged after the starch indicator was introduced (Figure 2b), implying that no free iodine presents. On the contrary, the color of the iodine solution changes abruptly from yellow (Figure 2c) to deep blue (Figure 2d) after the addition of starch indicator, indicative of the presence of free iodine, which can also been proved by a peak at 460 nm in UV−vis absorption spectrum (Figure 2e) that attributes to the presence of molecular iodine.[39] These results show that the form of active iodine in the antimicrobial system is triiodide complex; in other words, the content of active iodine in the antimicrobial system just amounts to the concentration of triiodide complex.

3.2. Effect of Feed Ratio and Preparation Parameters on Stability. In this work, the antimicrobial systems are composed of I_2, LDAO, 12−2−12 and H_2SO_4. The compositions of antimicrobial systems are quite complicated, so that it is hard to obtain a one-phase stable system. As shown in Table 1, for the target of the optimal preparation condition, these parameters can be ascribed to four aspects for the preparation of antimicrobial systems, i.e., the preparation temperature, LDAO, cationic gemini surfactant 12−2−12, H_2SO_4. These parameters will be examined respectively in the following sections.

3.2.1. Effect of LDAO Content on Stability. It is reported[40] that LDAO is readily biodegradable and shows the very low aquatic toxicity, and in the current antimicrobial systems it plays the same role as KI or NaI in Lugol's solution. It can react with iodine in water to form triiodide complex (Scheme 1).[22]

Scheme 1. Reaction of LDAO with Iodine to Form Triiodide Complex in Water

$$C_{12}H_{25}-\overset{CH_3}{\underset{CH_3}{N}}{\rightarrow}O \ + \ 2I_2 \ + \ H_2O \longrightarrow \left[C_{12}H_{25}-\overset{CH_3}{\underset{CH_3}{N}}{\rightarrow}OH \right]^+ \ I_3^- \ + \ I^- \ + \ H^+ + \ 1/2O_2$$

When the amount of LDAO was increased from 10 to 50 g, a stable homogeneous solution was obtained (Samples L1, L2 and L3 in Table 1). As illustrated in Scheme 1, the stoichiometric mole ratio of LDAO to I_2 is 1:2. However, the mole ratio of the stable homogeneous sample L3 in Table 1 is 8.3:1, which is much higher than stoichiometry. This indicates that the excessive LDAO may assemble into micelles or vesicles to improve the solubility of triiodide complex in this system.

3.2.2. Effect of Temperature on Stability. Temperature also plays an important role in the preparation of antimicrobial systems. To clarify the effect of the reaction temperature on the stability of antimicrobial systems, we carried out comparative experiments at different temperatures. As exhibited in Table 1, when the temperature was increased from 20 to 60 °C, stable homogeneous solutions (T1, T2 and T3) were obtained. This means that 60 °C is the optimum temperature for the preparation of the stable antimicrobial system.

3.2.3. Effect of 12−2−12 on Stability. Gemini surfactants are composed of two hydrophobic tails and two cationic head groups connected by a spacer group. The unique molecular structure endows with low cmc and excellent dispersion stabilization. In this work, 12−2−12 is adopted as a cationic dispersion agent to improve the stability of the antimicrobial system.

Compared in Figure 3 are UV−vis absorption spectra between 190 and 800 nm for antimicrobial system C1 without
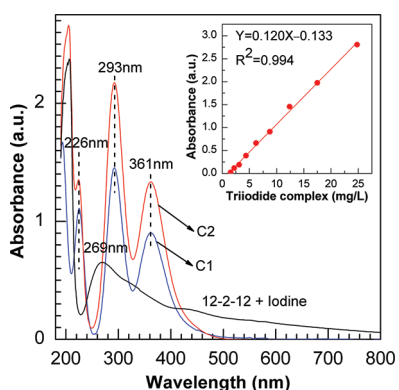
Figure 3. UV−vis absorption spectrum of antimicrobial system C1 (without 12−2−12), C2 (with 12−2−12), and the mixed solution of gemini surfactant and iodine. Inset: Calibration curve of triiodide complex.

12−2−12 and C2 with 12−2−12. One can find that both C1 and C2 have two absorbance maxima at 293 and 361 nm that attribute to I_3^-.[41] However, the absorbance peaks at 293 and 361 nm of C1 are lower than those of C2, suggesting that the iodine loading in C2 is higher than C1. According to the calibration curve of triiodide complex (inset of Figure 3), the active iodine (i.e., triiodide complex) content in C2 is 1.24 wt %, higher than the 0.86 wt % content in C1. Obviously, the difference in the iodine content should be related to the feed ratio of the two formulations. Comparing the composition of C1 and C2 (Table 1), one can immediately attribute the high iodine content in C2 to the presence of 12−2−12. Compared with the corresponding single-chain counterparts, gemini surfactants are more efficient in lowering surface tension and have much lower critical micelle concentration (cmc). Due to their higher surface activity, they have improved dispersion stability.[23−25] Therefore, 12−2−12 can greatly stabilize the antimicrobial system. In addition, no signal of free iodine was detected at 460 nm in C1 and C2,[19] in good agreement with the measurement results from starch indicator. Additionally, it is well-known that iodine reacts with halides to form complex iodohalides.[42,43] To check if I_2Br^- is present in this system, we put iodine into the gemini surfactant (12−2−12) solution and stirred for more than 5 h to form complex iodohalide. As shown in Figure 3, a new peak at 269 nm appears in the mixed

solution of gemini surfactant and iodine, which is attributed to I_2Br^-.[44] However, there is no peak at 269 nm in C2, implying that complex iodohalide (I_2Br^-) was not formed by the 12−2−12 and iodine in the antimicrobial system.

3.2.4. Effect of H_2SO_4 on Stability. LDAO is an amphiphile that may exist either in a neutral or cationic protonated form depending on the pH of aqueous solutions,[45] and these two forms could have different roles on the stability of antimicrobial system. Thus it is necessary to check the effect of acidity on the stability. Here the acid used in this work is H_2SO_4 that is strong enough to protonate LDAO.

For the samples S1 without H_2SO_4 and S2 with H_2SO_4, it is difficult to identify which one is stable as both systems are homogeneous solutions and their appearances look similar. To gain insight into the effect of H_2SO_4 on the stability, the UV−vis absorbance evolution of the diluted antimicrobial system was further monitored. Both S1 (Figure 4) and S2 (Figure 5)
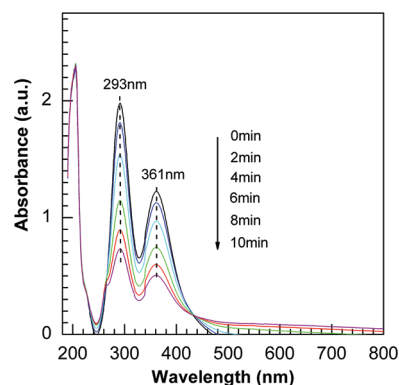
Figure 4. UV−vis absorbance evolution of diluted antimicrobial system S1 from 0 to 10 min.
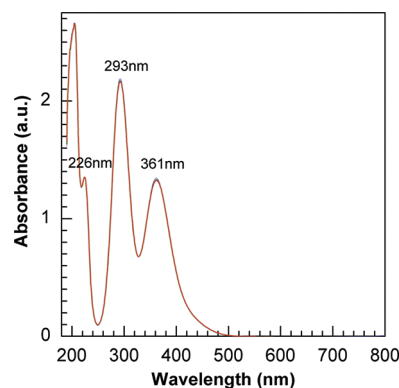

Figure 5. UV−vis curves overlapped of diluted antimicrobial system S2 from 0 to 10 min.

show two peaks at 293 and 361 nm that corresponding to the presence of I_3^-.[41] However, the absorbance peaks particularly those at 293 and 361 nm in S1 curves decrease gradually when the monitoring time is extended (Figure 4), indicating the a reduction of the active iodine content in the antimicrobial system. On the contrary, the UV−vis absorption curve of S2 remains unchanged during the same time scale (Figure 5), implying no decay of active iodine in the antimicrobial system.

With the calibration curve shown in the inset of Figure 3, the active iodine content could be gained by the conversion of the spectral data. The long-term stability of the active iodine in antimicrobial systems is particularly vital in practical

applications. Compared in Figure 6 is the variation of the active iodine content with storage time in S1 and S2. S1 is not stable
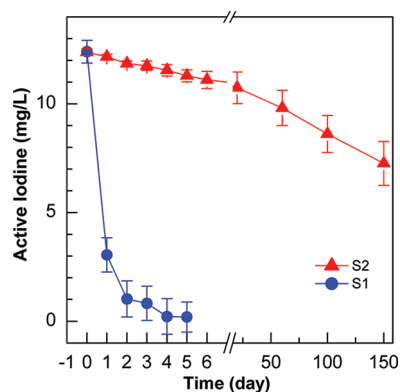

Figure 6. Concentration of active iodine remaining in diluted antimicrobial system S1 and S2 for different exposure times.

and displays a great reduction after 5 days, and the yellow precipitate of triiodide complex can be found in the solution accounting for the decrease of active iodine. However, the active iodine in S2 can be maintained for a relatively long time. For example, 9.81, 8.61, and 7.26 mg/L active iodine were retained in 60, 100, and 150 days of storage, respectively, which demonstrates that S2 possesses better long-term stability. These results indicate that the stability of active iodine gains a substantial enhancement by adding H_2SO_4. Such a phenomenon could be explained by the protonation of superfluous LDAO in the system. It is commonly recognized that LDAO will exist in a cationic protonated form in acidic condition.[45] Thus when H_2SO_4 is added, the protonated cationic LDAO could assemble into mixed micelles or vesicles with the cationic gemini surfactant 12−2−12. It is postulated that such mixed micelles or vesicles may stabilize active iodine in the system, which was confirmed by the cryo-TEM observations in the later section.

Based on the above experimental findings, the optimum condition to prepare stable antimicrobial system (S2) could be obtained: 2 g of I_2, 50 g of LDAO solution, 5 g of 12−2−12, 5 g of H_2SO_4, and the reaction temperature 60 °C. With such a formulation, the final active iodine content is 1.24 wt %, higher than 0.75 wt % in commercial PVP-I formulation.[46] Additionally, the system can maintain active iodine for 150 days, which suggests that it is a long-term stable fungicide. As the sample S2 possesses the optimal formulation and fairly good long-term stability, it will be used in the subsequent study.

3.3. Effects of Environmental Salinity and pH on Stability. To ensure good antimicrobial efficacy, it is indispensable to stabilize the active iodine in the antimicrobial environment where inorganic salt such as NaCl is usually present, and acid produced by some bacteria also exists, which influence the antimicrobial activity of antibactericides.[47] To check whether S2 has potential long-term stability in salt solution, we monitored the effect of different NaCl levels on the stability of triiodide complex continuously for 2 weeks. As shown in Figure 7, the active iodine content decreases upon the addition of NaCl, and the higher the NaCl content is, the more the iodine content decreases. When the concentration of NaCl is as low as 0.01% or 0.1%, antimicrobial system is still stable after 2 weeks, and the corresponding contents of the active iodine remain as 9.99 and 8.84 mg/L, respectively. However, the active iodine reduces remarkably to 2.35 mg/L after 2
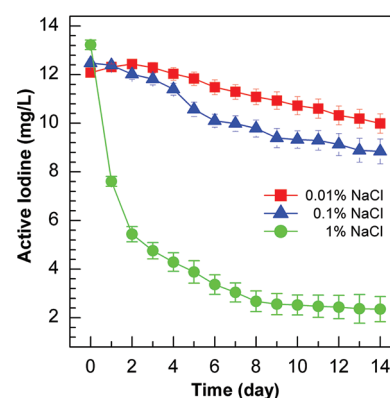
Figure 7. Influence of NaCl content on the stability of antimicrobial system.

weeks when the NaCl concentration is increased to 1%. This means that the antimicrobial system is unstable at high salinity, and could be explained that halogen anions particularly at high concentrations may exchange triiodide anion,[48] thus the free triiodide anions exchanged by halogen anions are less stable than those bound to LDAO.

Figure 8 shows the pH dependence of the stability of antimicrobial system. The content of active iodine decreases
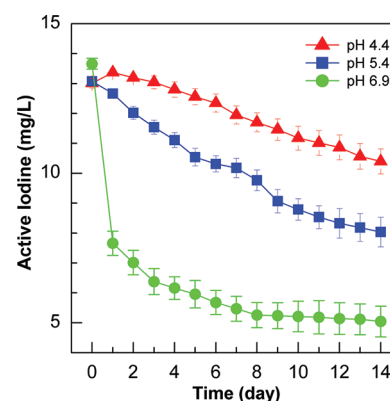

Figure 8. Effect of pH on the stability of antimicrobial system.

slightly under acidic conditions. For example, the antimicrobial systems at pH 4.4 and 5.4 remain 10.40 and 8.03 mg/L of active iodine after 2 weeks, respectively. However, active iodine decreases remarkably in neutral condition; the concentration at pH 6.9 only remains 5.04 mg/L under the same condition. When antimicrobial system is in alkaline environment, active iodine could react with base and hence results in reduction of antimicrobial efficacy.

3.4. Antimicrobial Efficacy. Based on the above results from the optimized feed ratio and the preparation parameters of antimicrobial systems, we have obtained the optimal formulation S2, and it was tested for fungi *Candida albicans*, along with cationic gemini surfactant 12−2−12 as a control which is also a bactericide.[24,26]

Compared in Figure 9 is the viable fungal cell after 5 min of contact with antimicrobial systems and 12−2−12. The results show that 12−2−12 has little antimicrobial activity against *Candida albicans*, and gives only 2 log reduction in viable cell after 5 min of contact (Figure 9a), whereas both S1 and S2 exhibit more pronounced antimicrobial ability, 4 log reduction in viable cell is obtained for S1, and more than 4 log reduction
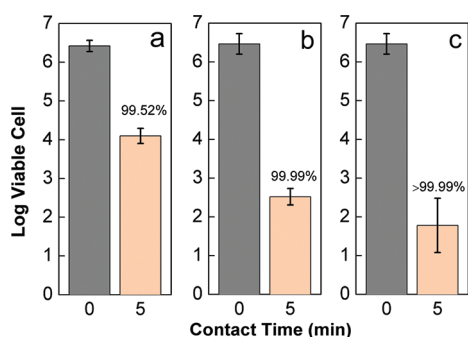
Figure 9. Survived *Candida albicans* cells after 5 min contact with (a) 12−2−12, (b) antimicrobial system S1, and (c) antimicrobial system S2. The results are averaged from three replicates.

for S2 (Figure 9b, c). Because the stability of the active iodine in antimicrobial systems can affect the antimicrobial efficacy, the weak stability of the active iodine in S1 debases the antimicrobial efficacy of S1. However, the enhanced stability of the active iodine in S2 imparts better antimicrobial efficacy than in S1. To find out why S2 has enhanced stability and antimicrobial activity, we investigate the aggregation behavior of 12−2−12 and protonated LDAO in S2 solution.

Although the conventional cationic surfactant and cationic gemini surfactant themselves can assemble into mixed micelles,[49,50] the mixed micelles assembled by cationic gemini surfactant and protonated LDAO were less documented. To check if 12−2−12 and protonated LDAO could assemble into mixed micelles, tested their mixed solutions were tested with surface tensiometer and observed by cryo-TEM measurements.

The surface tension of the mixed solution at pH 3, which agrees with pH value in S2 was measured. For comparison, the surface tension of 12−2−12 and LDAO were measured at pH 7. As shown in Figure 10, the surface tension of all the three
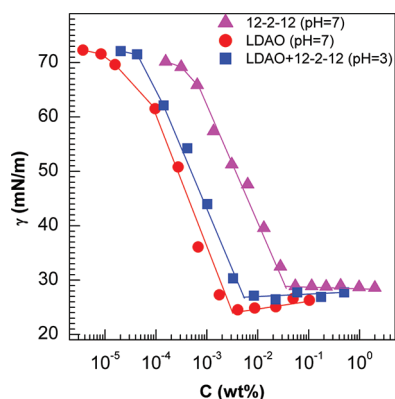
Figure 10. Variation in surface tension with surfactant concentration for 12−2−12, LDAO, and their mixed solution at 25 °C.

samples decreases with increasing their concentrations and then reaches clear break points, which are taken as their cmc. The cmc of LDAO is 0.0021 wt %, while the cmc of 12−2−12 is 0.044 wt % that is consistent with the previous report.[51] The mixed solution of protonated LDAO and cationic gemini surfactant has only one plateau, indicating that the protonated LDAO and 12−2−12 assemble into mixed micelles. Because of its poor water solubility, the triiodide complex prefers to go into hydrophobic cores of the mixed micelles and hence improves the antimicrobial efficacy in S2.

Cryo-TEM is an appropriate tool to observe aqueous assemblies of amphiphilic molecules due to the rapid vitrification process which examines the micellar structures without potentially disruptive processes such as solvent evaporation.[52] Figure 11a shows a typical cryo-TEM image of the original S2 solution. Spherical vesicle is formed in the solution, and the diameter is more than 120 nm. Usually, the edge of a vesicle is darker than the central region in cryo-TEM images;[35,53,54] however, it is worth noting that the interior of the vesicle in S2 is dark. Such a discrepancy probably results from the active iodine confined in hydrophobic interior and its high electron density[52] causes dark region of the vesicle in cryo-TEM image. In a word, vesicles assembled by protonated LDAO and 12−2−12 enhance the solubility of active iodine.

When an antimicrobial test is carried out, the antimicrobial system must be diluted, which could cause disruption of assemblies. To check the change of vesicles in the system after dilution, the diluted S2 was also observed by cryo-TEM. As shown in Figure 11b, lots of spherical micelles are evidenced, and the diameter of the micelles is only in the range of 15−20 nm. The dark cores of mixed micelles assembled by protonated LDAO and 12−2−12 indicate that the active iodine goes into hydrophobic cores of micelles, which should be attributed to the enhanced stability of the active iodine in S2.

3.5. Antimicrobial Mechanism. On the basis of what was studied above, we proposed the antimicrobial mechanism of active iodine in antimicrobial system (Figure 12). Because the outer of microbial membranes contains abundant negatively charged lipids, the micellar shells consisting of cationic head groups of protonated LDAO and 12−2−12 can be actively "electrophoresed" into the negatively charged cellular membranes of a microbial pathogen, like most cationic surfactants.[55−57] Then hydrophobic tails of cationic gemini surfactant could insert into lipid bilayer of cellular membranes due to hydrophobic interaction. The strong interaction of cellular membranes and cationic gemini surfactant disrupts cellular membranes and results in the formation of the holes on the cell surface.[58] Furthermore, triiodide complex in mixed micelles diffuses rapidly into the cell through these holes and kills the microbial pathogen. Triiodide complex, protonated LDAO and 12−2−12 produce synergistic effect in the antimicrobial process, thus S2 has better antimicrobial activity than gemini surfactant alone. From the results of the antimicrobial efficacy tests and stability, it can be concluded that the antimicrobial system is a quickly effective and long-term stable fungicide.

4. CONCLUSIONS

In this paper, a long-term stable antimicrobial system was developed by mixing iodine and cationic gemini surfactant into LDAO solution. The cationic gemini surfactant can enhance evidently the active iodine loading in antimicrobial system due to the vesicles assembled by cationic gemini surfactant and protonated LDAO. The 1.24 wt % active iodine content in the optimum antimicrobial formulation is higher than that in the commercial PVP-I formulation. The antimicrobial system shows a long-term stability that maintains active iodine for at least 150 days, but the active iodine reduced rapidly when NaCl is added or the pH is increased in the environment. The antimicrobial system also shows a rapid antimicrobial activity against *Candida albicans* with more than a 4 log reduction, which resulted from the formation of the mixed micelles containing active iodine verified by cryo-TEM observation. It can be concluded that the antimicrobial system is a long-term
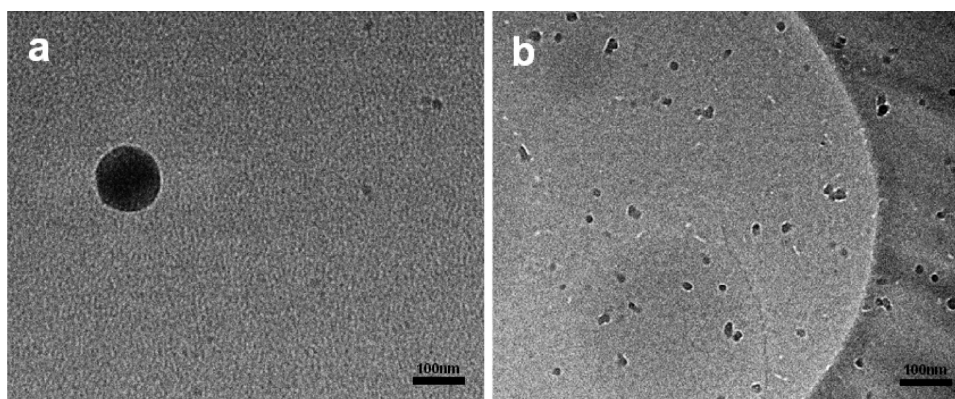
Figure 11. Cryo-TEM images of antimicrobial system at different concentrations. (a) original concentrated S2 solution; (b) diluted S2 solution in antimicrobial test.
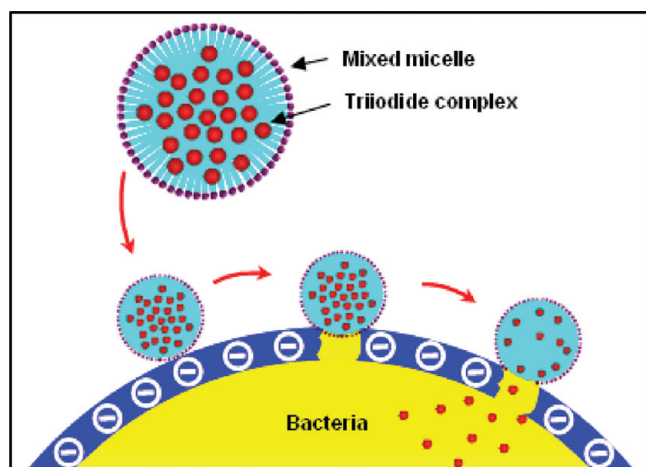
Figure 12. Schematic representation of antimicrobial mechanism of triiodide complex antimicrobial system.

stable and quickly effective fungicide, and which may be a good candidate to replace PVP-I and find potential applications in aquaculture areas.

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Notes
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