



Preparation and antibacterial properties of polystyrene-supported polyethylene glycols for iodine

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ABSTRACT

A series of polystyrene-supported polyethylene glycols (PS-PEG), as a new kind of water insoluble antibacterial resins, were obtained through graft-polymerization between chloromethylated polystyrene (CMPS) and PEG. The resins after adsorbing iodine showed excellent antibiotic activities on *Escherichia coli* and *Staphylococcus aureus* by adopting plate counting method. The antibiotic abilities were directly influenced by iodine adsorption capacity and the molecular weight of PEG. The resins under the wet state show more efficiency than those under dry state. Among these resins, PS-4-PEG800 has the strongest bactericidal ability under the wet state, which could inhibit 99.3% and 93.8% on the *E. coli* and *S. aureus*, respectively. Furthermore, it is also a kind of environment-friendly material and can be easily separated from the sterilized water.

Keywords: Polystyrene; Polyethylene glycol; Iodine; Antibacterial property

1. Introduction

Antibacterial materials such as quaternary ammonium compounds [1], metal ions/oxides [2], antibiotics [3] and antimicrobial peptides [4] are becoming more and more important in our life [5–7]. Because they can effectively protect against numerous kinds of pollutants [8–10]. Although these materials achieved great success, they face various disadvantages, for example, organic antibacterial materials [11–13] have strong bactericidal abilities and abundant resource, but they suffer from some drawbacks such as poor thermal resistance and easy hydrolyzation. Chitin micropowder that was extracted and purified from natural plants and animals is a kind of natural antimicrobial material [14,15], but its heat-resistant performance is poor. Among the various

antibiotic materials, iodine adsorbing materials, which could effectively remove the bacteria, viruses, and parasites [16,17] from water, are widely used as bactericides and viricides. Furthermore, the materials are economical and environment friendly since it can be re-used for several times. With these advantages, in the last decades, many investigations have been carried out in order to obtain high-performance iodine adsorption material. In 2012, Klimaviciute et al. [18] prepared cationic cross-linked starch-iodine complexes with different contents of cationic quaternary ammonium groups and iodine to evaluate their antimicrobial properties. Singhal and Ray [19] imparted the antibacterial property to nylon-6 by adsorption of iodine. Aoki et al. [20] successfully synthesized the antimicrobial fabric by radiation-induced graft polymerization of N-vinyl pyrrolidone onto polyolefin nonwoven fabric and subsequent adsorption of iodine.

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Meanwhile, the water-insoluble bactericidal materials to conduct water disinfection became a new emerging sterilization technique [21], which can be used on daily drinking water treatment, the disinfection of aquaculture, the sterilization of industrial water, and so on [22]. For example, the water-insoluble antibacterial materials with surface-grafted material PSt/SiO₂ have been prepared and the test results showed that the materials' antibacterial ability is closely related to their structure [23]. Meanwhile, Zuo et al. [24] successfully synthesized poly[2-(tert-butylamino)ethyl methacrylate] by solution blending and solvent evaporation methods.

On the other hand, because of ready availability, facile functionalization, low cost and chemical inertness, polystyrene (PS) are widely used in organic synthetic chemistry [25]. Nevertheless, the applications of PS are limited for its wettability, brittleness, and relative low glass transition temperature [26,27]. In order to improve its physical and chemical properties, increasing number of researchers has devoted to the chemical modification of PS [28,29]. Because polyethylene glycols (PEG) own various advantages, such as non-toxicity, water-solubility, they can be widely used in foodstuffs, cosmetics, and pharmaceutical products [30–32]. Recently, PS modified with PEG has been prepared to improve the performances by solid-phase peptide synthesis [33,34], and chromatographic packing [35–37].

Based on above studies, we try to synthesize a new water insoluble antibacterial PS-PEG resin with the high specific surface area, excellent mechanical properties, and thermal stability. For this kind of material, PS provided the basic matrix, which was not soluble in the water, and PEG provided oxygen atoms for absorption of iodine. The bactericidal properties of the resins after adsorbing iodine against *E. coli* and *S. aureus* were studied by adopting colony count method.

2. Experimental setup

2.1. Materials and instruments

Nutrient agar was obtained from Luqiao Limited Liability Company (Beijing, China). Tryptone was an analytical reagent purchased from Aoboxing Biotechnology Limited Company (Beijing, China). Yeast extracts were obtained from Damao Chemical Reagent Factory (Tianjin, China). Sodium chloride and sodium hydroxide were analytical reagents provided by Ruijinte Chemical Limited Company (Tianjin, China).

The original bacterium was incubated in a SPX-250B-Z biochemical incubator.

2.2. Preparation of polystyrene-supported polyethylene glycols resins

2.2.1. Chloromethylation reaction of PS resins

The PS-supported polyethylene glycols (PS-PEG) resins were obtained according to the previous report [38]. 24 g of PS was dried under vacuum for 12 h at 50°C and swollen in 144 mL chloromethyl ether for 12 h. The mixture was stirred on the oil bath at 38°C and 14.4 g of zinc chloride was put in (Fig. 1). Then the flask was transferred to ice water bath, the mixture was washed by 95% ethanol. The products

Table 1
Elemental analysis of CMPS^a

Resins	Time (h)	Cl (W%)	Cl (mmol g ⁻¹)
CMPS-1	0.2	2.8	0.79
CMPS-2	0.4	5.3	1.49
CMPS-3	0.9	9.8	2.76
CMPS-4	3	13.2	3.72

^a Cited from reference [38].

were extracted in a Soxhlet extractor for 12 h with 95% ethyl alcohol. Finally, the products were dried under vacuum at 50°C for 48 h and then four kinds of products were obtained named CMPS-1, CMPS-2, CMPS-3, and CMPS-4 through controlling the reaction time (0.2, 0.4, 0.9, and 3 h). Table 1 shows the elemental analysis of CMPS, the results supported and confirmed an increase in the content of chlorine with the extension of reaction time.

2.2.2. Graft polymerization of polyethylene glycols onto polystyrene

6 g CMPS was swollen in 150 mL dioxane which was dried by NaOH for 0.5 h. Then 5.16 g of NaH and 90 g of PEG400 (PEG600 132 g and PEG800 180 g) were added to the mixture under nitrogen. After refluxing at 97°C for 48 h, the mixture was cooled to room temperature. 30 mL methanol was added to decompose the unreacted NaH and then the mixture was filtered, washed by water, dioxane, and ethyl alcohol separately (Fig. 1). The products were extracted with ethanol for 8 h finally. The 12 PS-PEG resins were synthesized and denoted as PS-1-PEG400, PS-1-PEG600, PS-1-PEG800, PS-2-PEG400, PS-2-PEG600, PS-2-PEG800, PS-3-PEG400, PS-3-PEG600, PS-3-PEG800, PS-4-PEG400, PS-4-PEG600, and PS-4-PEG800, respectively. Taking PS-4-PEG800 as an example, scanning electron microscopy (SEM) micrographs of CMPS-4 and PS-4-PEG800 resins were taken by using the SEM to characterize the structure of PS-4-PEG800. The differential scanning calorimetry (DSC) of CMPS-4 and PS-4-PEG800 resins was carried out using DSCQ20 instrument.

2.3. Preparation of polystyrene-supported polyethylene glycols for iodine

The PS-supported polyethylene glycols for iodine (PS-PEG-I₂) were obtained by placing (20 ± 1) mg PS-PEG resins in 100 mL conical flask containing 50 mL iodine solution with initial concentration of 0.1 mmol·L⁻¹. The flask was placed in a thermostatic shaker and shaken at 20°C for 45 h. Thermogravimetric analysis (TGA) was taken using thermal gravimetric analyzer to test the thermostability of PS-PEG-I₂.

2.4. Measuring antibacterial ability of PS-PEG-I₂ against *E. coli*, *S. aureus*, and *B. subtilis*

2.4.1. Evaluating antibacterial activity of PS-PEG-I₂ with different dosages

1 mL of original cell suspension *E. coli*, *S. aureus*, and *B. subtilis* with bacterium age of 14 h and concentration of

about 10^9 CFU/mL was added into several clean tubes, respectively, in which 46 mL of water and different masses of PS-PEG- I_2 had been added. These mixtures were shaken for 10 min and then these suspensions were allowed to stand for 3 min until PS-PEG- I_2 settled. 1 mL of these supernatants were taken, diluted with standard serial dilution method, respectively, and the plate counting was carried out at different grades, so that the concentrations of viable cell for the different supernatant samples were determined as CFU. The antibacterial ratio was still calculated according to the following equation:

$$\text{Antibacterial ratio \%} = (\text{number of original cell} - \text{number of viable cell}) / \text{number of original cell} \times 100\% \quad (1)$$

2.4.2. Evaluating antibacterial activity of PS-PEG- I_2 in different contact time periods

1 mL of original cell suspension *E. coli* and *S. aureus* with bacterium age of 14 h and concentration of about 10^9 CFU/mL was added into several clean tubes, respectively, in which 46 mL of water and 0.06 g of PS-PEG- I_2 had been added. These mixtures were shaken, and after contacting for different times, these suspensions were allowed to stand for 3 min until PS-PEG- I_2 settled. 0.1 mL of these supernatants was taken and diluted continuously, spread on plates and the concentrations of viable cell for the different supernatant samples were determined as CFU. The antibacterial ratio was still calculated according to Eq. (1).

2.5. Experiments on the bactericidal ability of dry and wet state to *E. coli* and *S. aureus*

The experiments on the bactericidal ability of dry and wet state to *E. coli* and *S. aureus* were performed in the following procedure: 64 mg of PS-PEG resins were prepared after adsorbing iodine. Part of materials, which are called wet PS-PEG- I_2 functional particles, were kept in solution and could be used directly after washed with distilled water. Other part of materials, which are called dry PS-PEG- I_2 functional particles, were dried for 7 d after filtration.

4 mL of the bacterial suspension (10^9 CFU/mL) was diluted to 50 mL with sterile water and mixed with PS-PEG- I_2 . The mixture was shaken in a shaking table for 28 h. 0.1 mL of these supernatants were taken and diluted continuously, spread on plates and the concentrations of viable cell for the different supernatant samples were determined as CFU. The antibacterial ratio was still calculated according to Eq. (1). The bactericidal ability of dry and wet state to *E. coli* and *S. aureus* was tested finally.

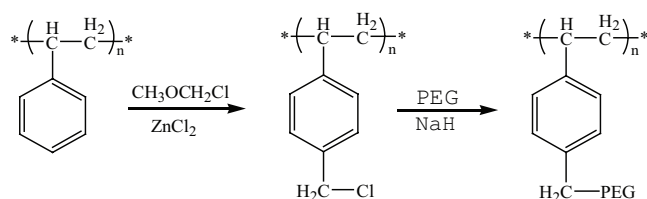


Fig. 1. Schematic representation of the synthesis of polystyrene-supported polyethylene glycols resins (PS-PEG).

3. Results and discussion

3.1. SEM studies

Fig. 2 shows SEM images of CMPS (a) and PS-PEG800 (b). It is clearly seen that an obvious change in the surface was observed after PEG800 grafted. The layer surface became coarse possibly due to the introduction of PEG800, which means the PEG800 has been successfully grafted on the CMPS.

3.2. DSC studies

Fig. 3 shows the DSC traces of the pure CMPS and PS-PEG800. It is clearly shown that the glass transition

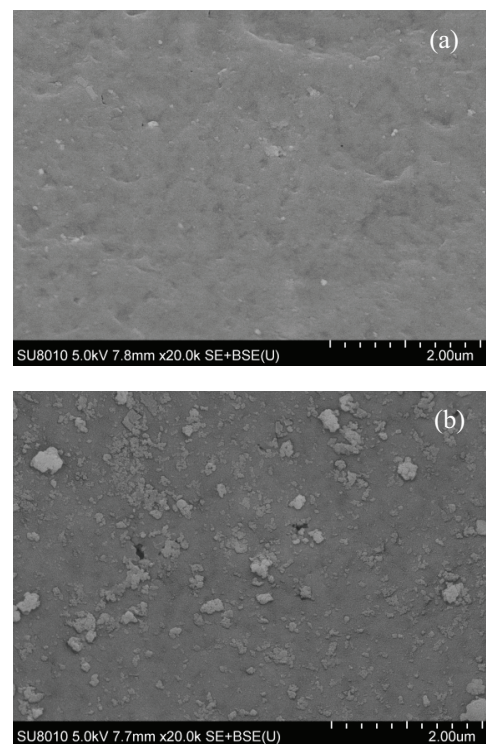


Fig. 2. SEM images of CMPS (a) and PS-PEG800 (b).

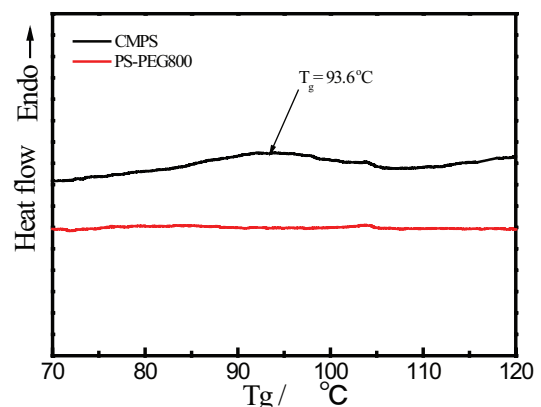


Fig. 3. Differential scanning calorimeter traces of the CMPS and PS-PEG800.

temperature (T_g) of the pure CMPS polymer was 93.6°C. Due to the introduction of PEG, the movement of the CMPS segment increased, leading to the slowdown of the glass transition temperature, which was beyond the scope of the test. In view of the above, the transition temperature of the PS-PEG800 is not obvious.

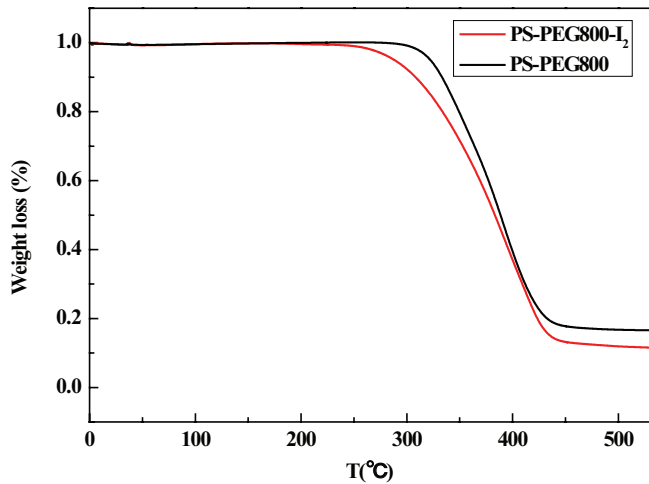


Fig. 4. TGA curves of PS-PEG800 and PS-PEG800-I₂.

3.3. TGA studies

The thermal stability of PS-PEG800 and PS-PEG800-I₂ evaluated by TGA is shown in Fig. 4. It can be seen that PS-PEG800 displayed the weight loss of 8.16% in the range of 100°C–600°C. Meanwhile, PS-PEG800-I₂ showed that the weight loss was about 14.82% at the same ranges. Indeed, there was an ascending tendency after adsorbing iodine, but the change was limited, which implied that the introduction of iodine has little effect on the thermal stability of the resins.

3.4. Antibacterial activity of PS-PEG-I₂ with different dosages to *E. coli*, *S. aureus*, and *B. subtilis*

Figs. 5 and 6 show the variation of the antibacterial ratio of S-PEG-I₂ to *E. coli* and *S. aureus*, respectively. With the increasing of the materials, the antibacterial ratio increased rapidly. The amount of iodine adsorption increased with the increasing of the chlorine content. The main reasons are as follows: (1) the increasing of the chlorine content also led to the increase in the amount of PEG; (2) the adsorption amount of PS-PEG for iodine increased with the number of oxyethylene units. At the same time, the higher molecular weight of PEG, the higher the bactericidal ratio is appeared. The antibacterial ratio of the *E. coli* and *S. aureus* can reach up to 90%

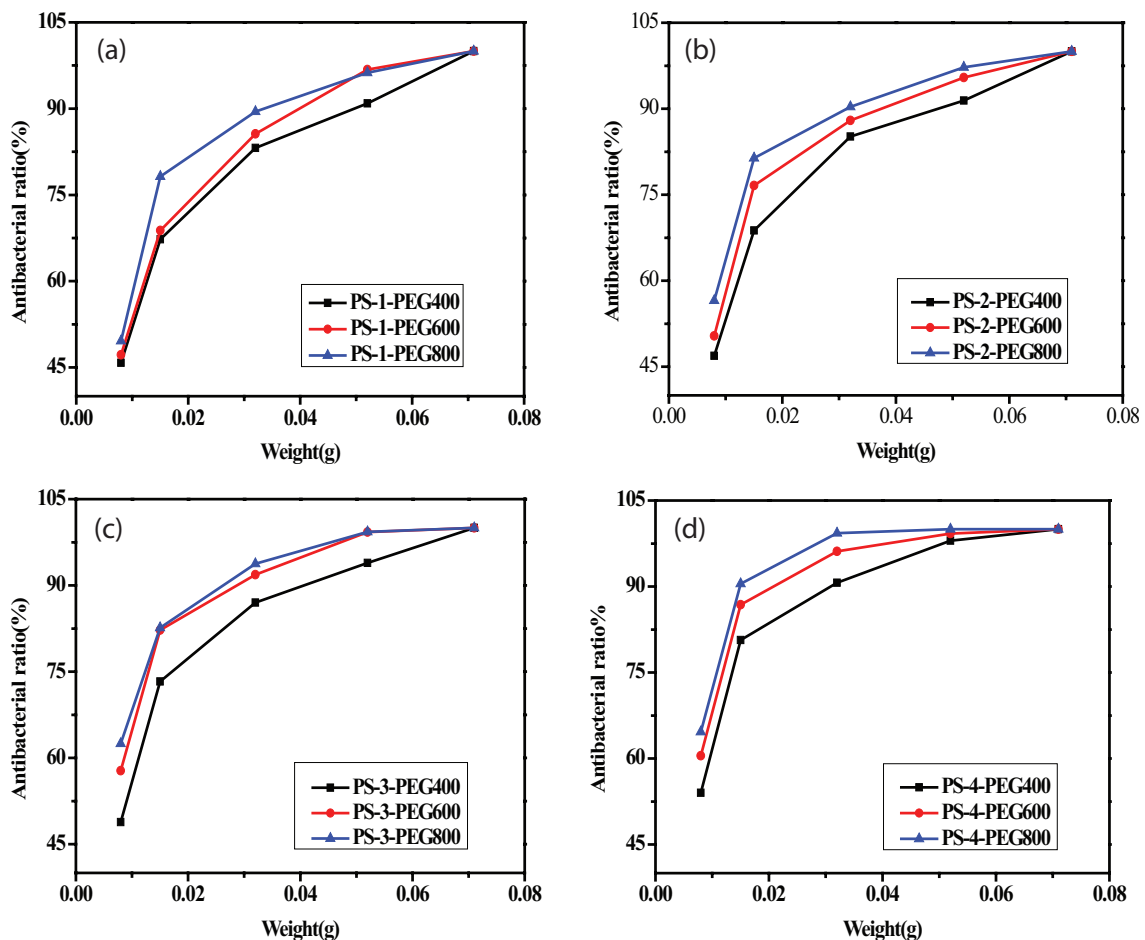


Fig. 5. Variation of the antibacterial ratio of PS-PEG-I₂ against *E. coli* with dosage (28 h).

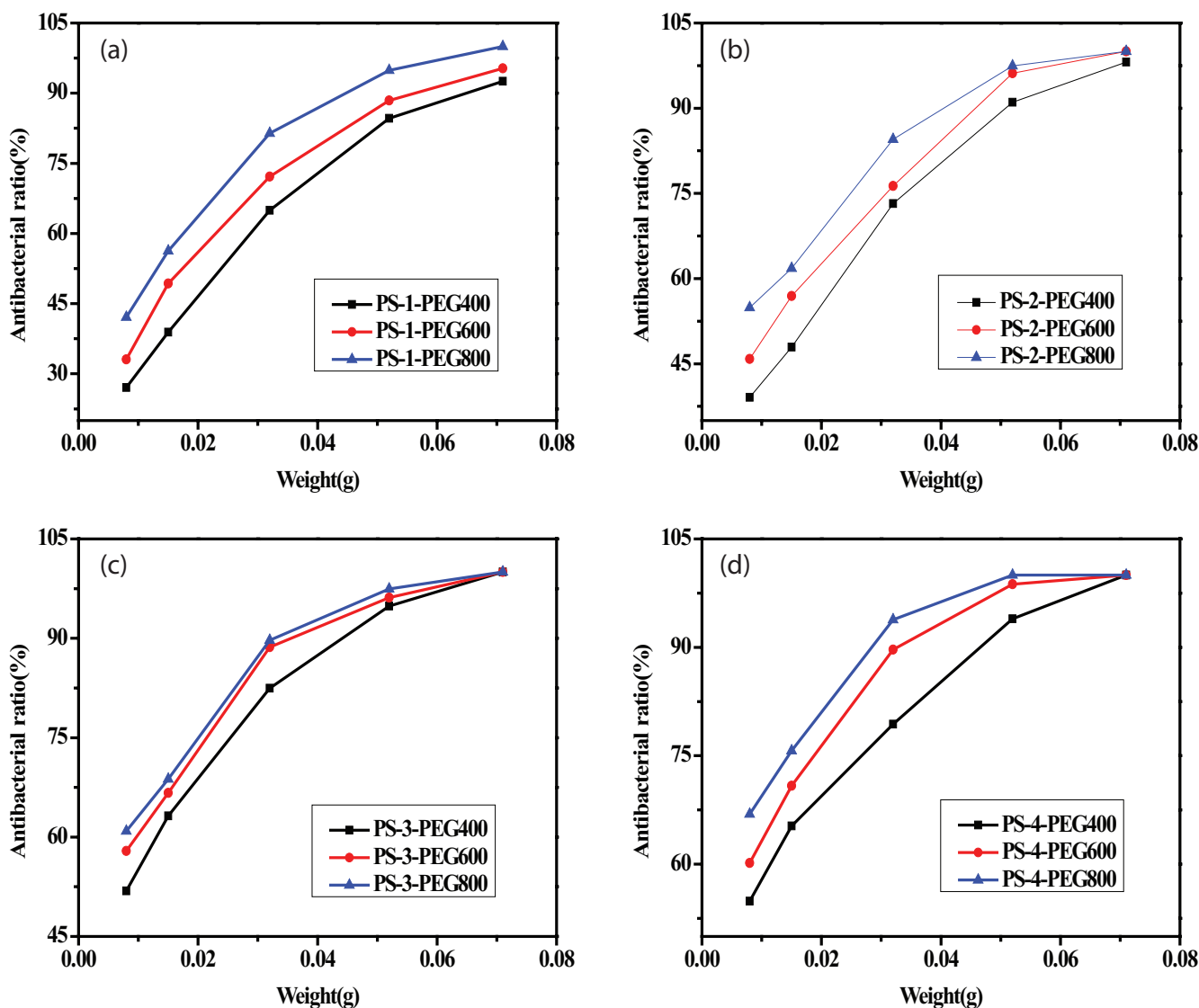


Fig. 6. Variation of the antibacterial ratio of PS-PEG- I_2 against *S. aureus* with dosage (28 h).

when the amount of the PS-PEG- I_2 is about 50 mg. 100% of the antibacterial ratio can be achieved when we use about 70 mg of the PS-PEG- I_2 . We can also find that PS-4-PEG800 has the best bactericidal result and the antibacterial ratio can reach 100% when 50 mg of the PS-4-PEG800 is used. The bactericidal effect of *E. coli* is better than that of *S. aureus* at the same test conditions.

The variation of the antibacterial ratio of PS-PEG- I_2 against *B. subtilis* is shown in Table 2. And the results showed that this material did not exhibit a significant effect on the sterilization ability for *B. subtilis*.

3.5. Antibacterial activity of PS-PEG- I_2 with different contact time to *E. coli* and *S. aureus*

The variation of antibacterial ratio of PS-PEG- I_2 against *E. coli* and *S. aureus* with the dosage is shown in Figs. 7 and 8, respectively. With increasing time, the sterilization effect is gradually raised. For the same sterilization

Table 2
Variation of the concentration of bacteria of CMPS-PEG- I_2 against *B. subtilis* with dosage (28 h)

Volume/mg	8	16	32	48	72
Concentration of bacteria before sterilizing/ 6.25×10^5 CFU/mL	180	180	180	180	180
Concentration of bacteria after sterilizing/ 6.25×10^5 CFU/mL	175	165	177	164	159

time, the antibacterial ratio increased with the increasing of chlorine content and the molecular weight of PEG. With the raising of the amount of iodine adsorbed on the resin, the antibacterial ratio also showed an increasing trend which is mainly due to more iodine releasing in the same time. The antibacterial ratio can reach up to 50% when the sterilization time is about 6 h. With the consumption of iodine during the sterilization process, the curves become flat. The antibacterial ratio can reach 100% at about 28 h.

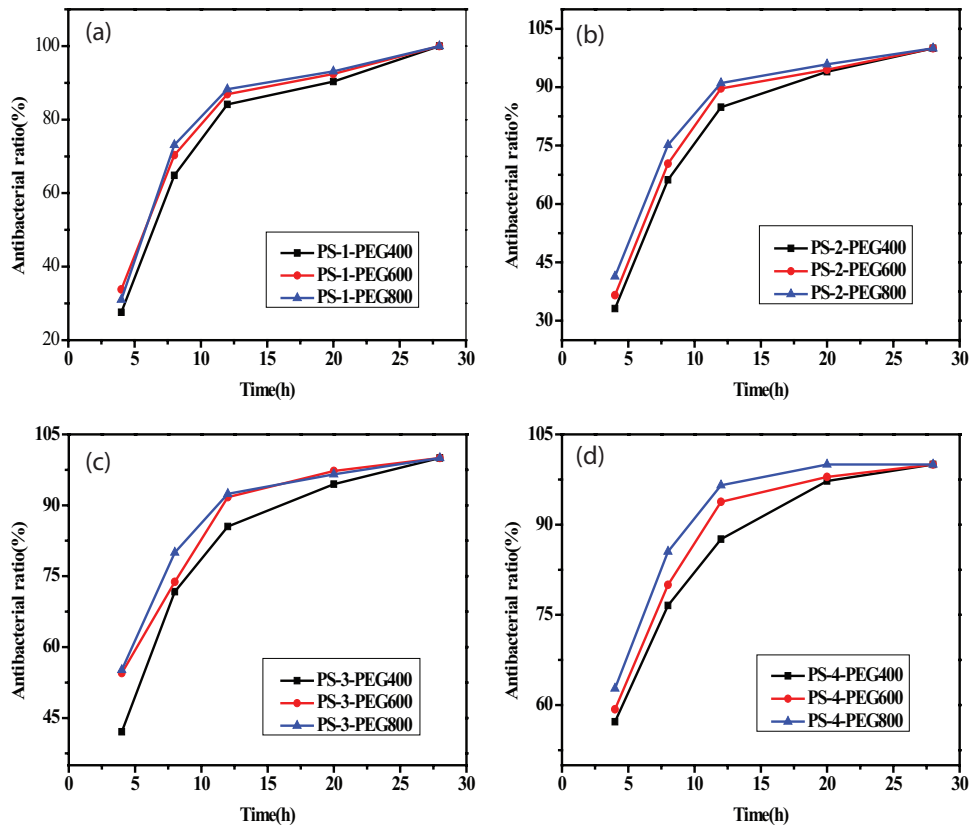


Fig. 7. Variation of antibacterial ratio of CMPS-PEG-I₂ against *E. coli* with contact time.

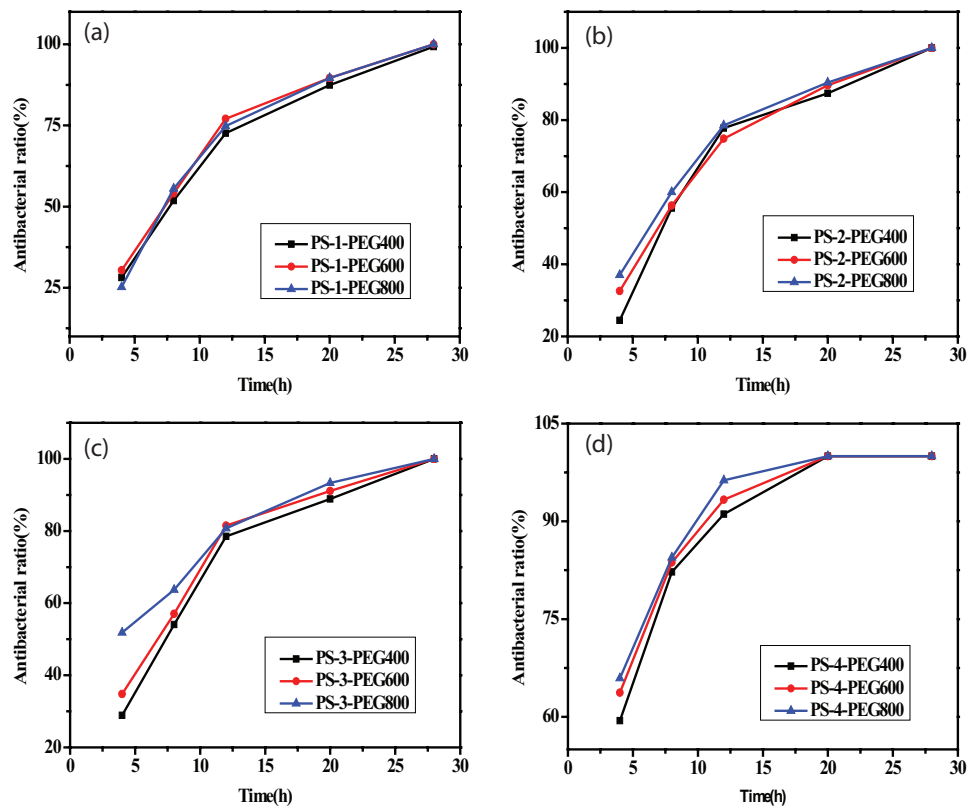


Fig. 8. Variation of antibacterial ratio of PS-PEG-I₂ against *S. aureus* with contact time.

Table 3
Comparison of the antibacterial activities of CMPS-PEG-I₂ with dry and wet state

Resins	<i>E. coli</i>		<i>S. aureus</i>	
	Inhibition (%) ^a	Inhibition (%) ^b	Inhibition (%) ^a	Inhibition (%) ^b
PS-1-PEG400	24.76	83.18	21.9	64.95
PS-2-PEG400	21.9	85.6	23.81	73.2
PS-3-PEG400	24.76	89.47	28.57	82.47
PS-4-PEG400	19.05	85.15	28.57	79.38
PS-1-PEG600	25.71	87.94	25.71	72.16
PS-2-PEG600	29.52	90.34	25.71	76.29
PS-3-PEG600	23.81	87.02	29.52	88.66
PS-4-PEG600	27.62	91.85	33.33	89.69
PS-1-PEG800	31.43	93.75	28.57	81.44
PS-2-PEG800	29.52	90.67	27.62	84.54
PS-3-PEG800	31.43	96.12	34.29	89.69
PS-4-PEG800	28.57	99.32	28.57	93.81

^aUnder dry state.

^bUnder wet state.

3.6. Comparison of the antibacterial activities of PS-PEG-I₂ with dry and wet state

As shown in Table 3, the antibacterial ratio of the dry state is about 1/3 of the wet, which is possibly caused by iodine volatilizing during the process of drying.

4. Conclusions

PS-PEG-I₂ resin, a kind of water insoluble antibacterial with excellent mechanical properties and thermal stability, were successfully synthesized. For this kind of material, PS provided the basic matrix, and PEG provided oxygen atoms for adsorption of I₂. More important, the materials showed excellent antibacterial properties for *E. coli* and *S. aureus*. And the relationship of the antibacterial effect with structure was also studied, and we found that the molecular weight of PEG has a positive correlation to the antibacterial effect possibly more oxygen atoms could absorb more iodine. Furthermore, the materials are economical and environment friendly since it can be re-used for several times and easily separated from the sterilized water by simple filtration.

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