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Research on the Preparation and Antibacterial Properties of 2-*N*-Thiosemicarbazide-6-O-Hydroxypropyl Chitosan Membranes with Iodine

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ABSTRACT: The 2-*N*-thiosemicarbazide-6-O-hydroxypropyl chitosan (ATU-HPCS) was prepared by chitosan grafted hydroxypropyl and thiosemicarbazide through the method of "amino protection-graft-deprotection," while the ATU-HPCS gel membranes were obtained from gelatin and polyvinyl pyrrolidone as additives, and the ATU-HPCS membranes with iodine (ATU-HPCS-I_2-M) were prepared by adding the ethanol solution of iodine in the ATU-HPCS gel membranes. The ATU-HPCS-I_2-M were characterized to evaluate their potential applications as antibacterial materials. The iodine releasing rule of ATU-HPCS-I_2-M showed a sustained-release effect of iodine, the maximum emission was approximately 0.80%. The inhibition zone diameters of ATU-HPCS-I_2-M against *Staphylococcus aureus* (as Gram-positive bacteria) and *Escherichia coli* (as Gram-negative bacteria) were both greater than 15 mm, it demonstrated significant antibacterial activity compared with the ATU-HPCS gel membranes. The double effects of the biocompatibility of chitosan and the sustained-release of iodine provided an ideal healing environment for wound surface. These properties have made ATU-HPCS-I_2-M highly potential as a novel natural macromolecule antimicrobial material preventing the bacteria from burns, surgery wounds, etc. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40535.

KEYWORDS: antimicrobial material; biomaterials; biomedical applications; membranes with iodine; sustained-release of iodine

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INTRODUCTION

Chitosan (CS), obtained by alkaline deacetylation of chitin, is linear and partly acetylated (1-4)-2-amino-2-deoxy-β-D-glucan. As the only basic polysaccharide in nature, it has several distinctive biomedical properties such as nontoxicity, negligible cytotoxicity, biocompatibility, biodegradability, bioadhesion, and easier modified ability than various synthetic polymers.[1–3] These properties have made CS highly prospective in the applications of antimicrobial materials, biomedical materials, etc.[4–10]

The antimicrobial activity of chitosan and its derivatives have been investigated widely,[11–16] however, no obvious bacteriostatic effect has been demonstrated so far, when they were prepared into antimicrobial material (e.g. CS membranes).[17] Recently, much attention of CS has been paid to the binding of metal ions and metal oxides, a series of studies on CS compounded with Au^{2+}, Zn^{2+}, Cu^{2+}, Ag+, ZnO, TiO_2 have been done to improve the antimicrobial activity of CS antimicrobial material,[18–22] but the antimicrobial activity of CS-metal complexes were not desirable because of the dosage of metal, while some metals like gold and silver were expensive. However, only little attention has been devoted to their adsorption of halogens. By contrast, the broad-spectrum antibacterial agent-iodine was cheap and have excellent antibacterial and antiviral effect[23,24], the iodine was therefore used to combine with CS by some groups in some studies.[25,26]

According to our current experimental results and the research results of the combined forms between CS and iodine molecules of Shigeno et al.,[26] the CS had only small adsorbing ability of iodine, the binding force between amino groups of CS and iodine molecules was performed by the poor behavior of charge-transfers,[26] thus the CS-iodine adducts obtained is instability and cannot have a persistently antibacterial effects. Therefore the thiosemicarbazide was tried to graft onto the CS to improve the effect of charge-transfers and enhance its ability of adsorbing iodine, which was corresponded to the actions of increased lone pair electrons of sulfur and nitrogen element on thiosemicarbazide. In addition, the hydroxypropyl was grafted onto the CS to enhance its water solubility.[27,28]

In this study, the 2-*N*-thiosemicarbazide-6-O-hydroxypropyl chitosan (ATU-HPCS) were synthesized through "amino

protection-graft-deprotection," which was realized by hydroxypropyl positioning grafted on the hydroxyl groups after the CS was amino-protected with benzaldehyde, then the thiosemicarbazide was positioning grafted on the amino groups to obtain the ATU-HPCS after the deprotection of amino groups. This derivative is an excellent biological material with desirable water solubility, membranes-forming behavior and strong complexing (adsorption) of iodine, which could be prepared into ATU-HPCS gel membranes and ATU-HPCS membranes with iodine (ATU-HPCS-I_2-M). The properties were characterized by FT-IR, UV, SEM, XRD, TGA, the iodine content, releasing regulation of iodine and the bacteriostatic effect, etc. These results demonstrated the potential applications of ATU-HPCS-I_2-M in antimicrobial membrane materials.

EXPERIMENTAL

Materials

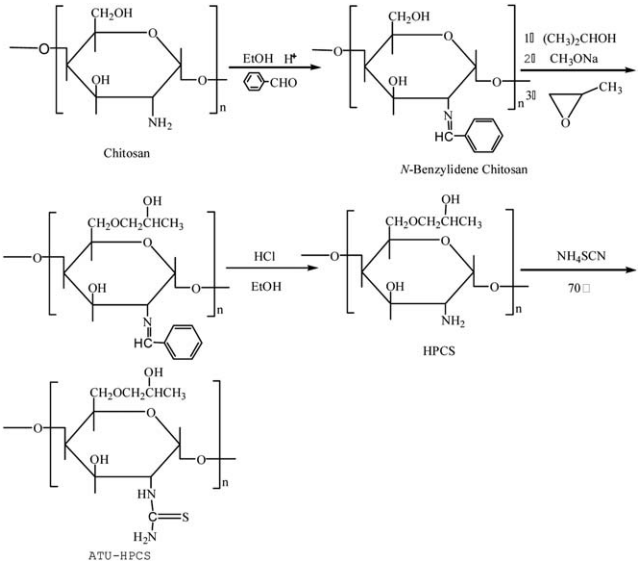
Chitosan (Medical grade, with a degree of deacetylation of 90% and molecular weight of 1.32×10^5, was purchased from Zhejiang Golden-Shell Biochemical (China), all other chemicals were at least analytical grade and used as received without further purification. The crop-threatening bacteria (*S. aureus* and *E. coli*) and nutrient broth etc. used for the antimicrobial assay were provided by Central Laboratory and Hematology, Tongji Hospital, affiliated with Tongji University.

Preparation of 2-*N*-Thiosemicarbazide-6-O-Hydroxypropyl Chitosan (ATU-HPCS)

Firstly, CS (3.0 g, 18.0 mmol of pyranose ring) was dissolved in 100 mL acetic acid solution (1 wt %) in a three-necked flask, a certain amount of ethanol was added at room temperature to increase the solubility of the benzaldehyde groups. After then 10 mL of benzaldehyde was dropped into the solution, the solution was stirred until a milky white solution was obtained. A certain amount of sodium hydroxide solution (5 mol/L) was added to adjust the pH of the homogeneous solution to neutral for the protection of amino groups, and a white precipitate was prepared. The precipitate was collected by filtration and then washed completely by Soxhlet extraction with ethanol for 6 h to remove unreacted benzaldehyde, finally, a white Benzaldehyde Schiff base-Chitosan (B-CS) powder was extracted after vacuum dried at 40°C for 6 h.[29]

Secondly, B-CS (3.0 g) was basified with 2.0 g of sodium hydroxide aqueous solution (33 wt %) in isopropanol solution (20 mL), the mixture was sealed overnight. Then 1 mL of tetramethylammonium hydroxide aqueous solution (25 wt %) as catalyst and 20 mL of propylene oxide was added, the mixture was stirred at room temperature for 1 h, and then was heated to 60°C for 8 h in water bath under 400 r/min, after that the mixture was adjusted to neutral by adding hydrochloric acid (10 wt %), cooled down and soaked repeatedly in acetone. Afterwards the mixture was filtered and soaked in hydrochloric acid/ethanol solution for 24 h. Followed by filtration and vacuum dried at 60°C for 6 h, a white hydroxypropyl chitosan (HPCS) powder was obtained.[27,28]

Finally, to a solution of ammonium thiocyanate (2.8 g, 36.8 mmol) in 25 mL anhydrous ethanol, HPCS (3.2 g) was added,



Scheme 1. Synthetic route of 2-*N*-thiosemicarbazide-6-O-hydroxypropyl chitosan(ATU-HPCS).

the mixture was stirred and refluxed for 5 h at 70°C. After the mixture was cooled down, filtered and soxhlet extracted with ethanol for 4 h, a yellow 2-*N*-thiosemicarbazide-6-O-hydroxypropyl chitosan (ATU-HPCS) powder was obtained after vacuum dried at 60°C for 6 h. The synthetic route is illustrated in Scheme 1.

Preparation of ATU-HPCS Gel Membranes and ATU-HPCS-I_2-M

The ATU-HPCS aqueous solution (1 wt %) was prepared by dissolving ATU-HPCS (1.4 g) in 100 mL d.i. water in water bath at 60°C. After 20 mL of gelatin aqueous solution (5 wt %) and 20 mL of polyvinyl pyrrolidone (PVP) aqueous solution (5 wt %) were added, the mixture was stirred under 400 r/min until an uniform phase was reached. The blend was poured into the mold to dry out for 24 to 36 h at room temperature to obtain the ATU-HPCS gel membranes.[30,31]

Repeated the steps above, 5 mL, 10 mL, 15 mL, 20 mL, 25 mL iodine in ethanol solution with I_2 % (mass fraction) = 5 were added in the blend solutions obtained above, respectively, stirred for 1 h under 800 r/min, the blend was poured into the mold to dry out for 24 to 36 h at room temperature to obtain the ATU-HPCS-I_2-M.

Measurements

The characteristic peaks of CS, ATU-HPCS, ATU-HPCS gel membranes, and ATU-HPCS-I_2-M with I_2 % (mass fraction) = 21.09 were confirmed with AVATAR370 FTIR infrared spectrometer (USA Thermo Nicolet Company). Samples were ground enough to make KBr pellets under hydraulic pressure of 400 kg/cm^2 and spectra were recorded in the range of 500 to 4000 cm^{-1}. During each scan, the amount of samples and KBr were kept constant in order to observe the changes of the characteristic peaks between different samples.

UV scanning (UV-1601PC UV spectrometer, Shimadzu, Japan) of ATU-HPCS gel membranes and ATU-HPCS-I_2-M with I_2 %

(mass fraction) = 21.09 were then carried out under UV irradiation by using a medium pressure mercury arc lamp from PrimarcTM UV Technology which provided multiple wavelengths from 200 to 800 nm. The total exposure energy was 2.5 J/cm^2.

Scanning electron microscopy (SEM) (HITACHI S-3400, Japan) observations of the ATU-HPCS gel membranes and ATU-HPCS-I_2-M with I_2 % (mass fraction) = 21.09 were carried out as follows: The dry samples were spread on a double sided conducting adhesive tape, pasted on a metallic stub, coated with a gold layer of 100 μm thickness using an ion sputter coating unit (HITACHI E-1010 ION) for 2 min and observed with a Electron Probe Microanalyzer. All the SEM photomicrographs were obtained under an accelerating voltage of 35.0 to 45.0 kV and at a magnification of ×2000.

The X-ray diffraction (XRD) patterns of ATU-HPCS gel membranes and ATU-HPCS-I_2-M with I_2 % (mass fraction) = 21.09 were taken on a BRUKER D2 PHASER X-ray diffractometer with Cu-Kα radiation combined with nickel filter operating at 30 kV and 10 mA. The samples were maintained stationary while scattering angles from 5° to 80° were scanned in the reflection mode at a scanning rate of 4°/min.

The thermogravimetric analysis of ATU-HPCS gel membranes and ATU-HPCS-I_2-M with I_2 % (mass fraction) = 21.09 were carried out by TGA (STA PT-1000, Linseis, Germany). A certain amount of sample (about 10 mg) was placed in a platinum pan and scanned from 25°C to 600°C at a heating rate of 20°C/min under nitrogen atmosphere, with a gas flow rate of 20 mL/min.

Solubility determination of ATU-HPCS: excess samples were stirred at room temperature for 1 h in 25 mL of each of the following solvents: distilled water, acetic acid solution (1% v/v), HCl (1% v/v), DMSO, DMF, C_2H_5OH, acetone, sodium hydroxide aqueous solution (1 wt %). Let it sit for overnight, draw a certain volume of supernatant with a pipette, placed in a weighing bottle and weighed as W1, and then heated to evaporate the solvent and weighed as W_2. The solubility was calculated by the following equation: Solubility = $[W_2/(W_1 - W_2)] \times 100$, where W_1 is the weight of the supernatant that included ATU-HPCS and solvent, while W_2 is the weight of the oven dried ATU-HPCS.

The iodine content of ATU-HPCS-I_2-M was tested by iodometry, 1.0 g ATU-HPCS-I_2-M of different mass fraction were quantitatively weighed in iodine flasks, away from light and stirred until the ATU-HPCS-I_2-M were transparent at room temperature after 100 mL calibrated $Na_2S_2O_3$ solution was added. The supernatant was transferred into the basic burette to titrate 25 mL calibrated KIO_3 solution until transparent, with 5 mL KI solution and 5 mL 0.5 mol/L sulfuric acid solution was added, starch as indicator. Formula of iodine content was as below:

$$I_2\% = \frac{(C_1 - C_2) \times 0.1}{2\mathrm{m}} \times 254 \times 100\%,$$

$$C_{Na_2S_2O_3} = \frac{0.025 \times C_{KIO_3} \times 6}{V} \quad C_2 = C_{Na_2S_2O_3}$$

where C_1 is calibrated concentration of sodium thiosulfate (mol/L), V is sodium thiosulfate consumption (mL), m is sample quality.

The releasing regulation of iodine of ATU-HPCS-I_2-M: the samples were cut into square of 4 × 4 cm and immersed in 500 mL pH = 7 simulation exudate solution, stirred slowly at 37°C, 3 mL supernatant was selected at regular intervals for absorbance at 231 nm with the simulation exudate solution as blank sample, and another 3 mL analog exudate was added after each test. The characteristic absorption wavelength of UV–visible spectrophotometer of iodine was tested at 231 nm in simulated exudate with pH = 7. The iodine standard curve was drawn after the absorbance of iodine in ethanol solution of different concentrations tested at 231nm. The regression equation was obtained at $C = (0.12984 + 12198.17353\ A) \times 10^{-6}$, where C is the concentration of iodine in ethanol solution, A is the absorbance.

$$\text{Formula release } (\%) = \frac{\mathrm{M}}{0.0372} \times 100\%,$$

$$\text{release mass } (M) = C \times 0.25 \times 254$$

Antibacterial activities were investigated using agar well diffusion method, the activity was determined by measuring the diameter of the inhibition zone (in mm). *Staphylococcus aureus* (*S. aureus*, as Gram-positive bacteria) and *Escherichia coli* (*E. coli*, as Gram-negative bacteria) were dispersed into the medium, Centrifuged pellets of bacteria from a 24 h old culture containing approximately 104–106 CFU (colony forming unit) per mL were spread on the surface of nutrient agar (tryptone 1%, yeast extract 0.5%, NaCl 0.5%, agar 1%, 1000 mL of distilled water, pH = 7.0), which was autoclaved under 121°C for at least 20 min. Wells were created in medium with the help of a sterile metallic bores and then cooled down to 45°C. The bacteria concentration of about 1.08 × 10^5 cells/L, 30 mL of the solution diluted 10-fold mounted to a petri dish of 12 cm diameter in the cooled and solidified. The CS, ATU-HPCS, ATU-HPCS gel membranes, and the ATU-HPCS-I_2-M with different mass fraction of iodine having a diameter of 10 mm of the wafer, UV sterilized 30 min, respectively, attached to different locations in the dish, the plates were kept for incubation at 37°C for 24 h and then the plates were checked for the formation of zone of inhibition. Each inhibition zone was measured three times by caliper to get an average value. Inhibition zone diameter was recorded afterwards.[32]

RESULTS AND DISCUSSION

FT-IR Characterization of CS and ATU-HPCS

FT-IR spectroscopy was employed to detect the structural changes of CS and ATU-HPCS (Figure 1). The FT-IR spectrum of CS [Figure 1(a)] showed four strong absorption peaks at 1130, 1084, and 887 cm^{-1} which were characteristic peaks of the saccharide structure. The very strong broad absorption peak around 3444 cm^{-1} should be assigned to the stretching vibration of OH and NH, and the intermolecular hydrogen bonds of the polysaccharide. There were weak absorption peaks at 1651 and 1595 cm^{-1} corresponding to amide I and amide II, respectively, which indicated that chitosan had a high deacetylation degree.[32] Besides, the absorption peak at 2875 cm^{-1} was due to -CH_2- stretching vibration.[33]

In comparison with CS, the grafting of hydroxypropyl on CS [Figure 1(b)] made the absorption peak at 3427 cm^{-1} due to

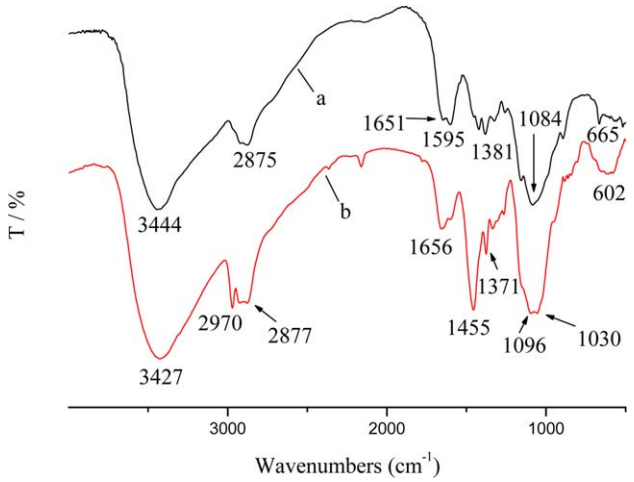


Figure 1. IR characterization of (a) CS and (b) ATU-HPCS. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

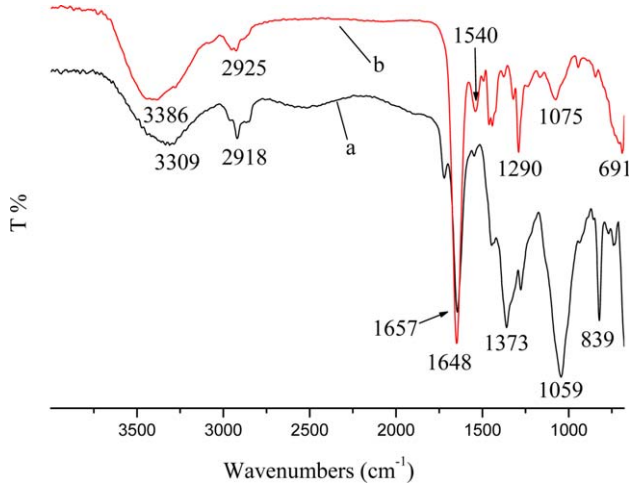


Figure 2. IR characterization of (a) ATU-HPCS gel membranes and (b) ATU-HPCS-I_2-M. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

OH and NH stretching vibration and 2877 cm^{-1} corresponding to -CH_2- stretching vibration enhancement. The new absorption peak appeared at 2970 cm^{-1} could assigned to the characteristic peaks of -CH_3, and the C-O absorption peak became stronger and moved to 1096 cm^{-1}. These evidences indicated that the hydroxylpropyl has been grafted onto CS.[27,28]

In addition, in virtue of the grafting of thiosemicarbazide on CS, the C = S groups made the absorption peak at 1084 cm^{-1} split into two strong peaks at 1095 and 1030 cm^{-1}. The new absorption peak occurred at 1455 cm^{-1} could assigned to the characteristic absorption peak of thiosemicarbazide, and the C—N absorption peak became stronger and moved to 1371 cm^{-1}. These results indicated that the thiosemicarbazide had been grafted onto CS, all above confirmed the structure of the ATU-HPCS.

FT-IR Characterization of ATU-HPCS Gel Membranes and ATU-HPCS-I_2-M

Figure 2 shows a comparison of the transmission FT-IR spectra for ATU-HPCS-I_2-M [Figure 2(b)] with ATU-HPCS gel membranes [Figure 2(a)]. First, the stretching vibration absorption peak of OH and NH red shifted from 3312 to 3386 cm^{-1} as a result of the adduct of iodine, the characteristic broad absorption band around 1657 cm^{-1} assigned the overlap of C=O, NH (secondary amide), and C=C (phenyl) showed no changes.[32] Secondly, the absorption peaks at 1373 cm^{-1} which due to the C-H plane bending vibration of CS and PVP disappeared, and the characteristic absorption peaks at 1059 and 838 cm^{-1} of saccharide structure were weakened and disappeared, respectively. These changes were due to the complexation (adsorption) of iodine that had an impact on the molecular structure of the copolymer molecules and the spatial structure of the membranes.[23]

UV Spectra of ATU-HPCS Gel Membranes and ATU-HPCS-I_2-M

The UV transmittance of ATU-HPCS gel membranes [Figure 3(a)] increased along with the increase of wavelength, there was a sharp rise after 320 nm, buttends to balance after 450 nm, the maximum transmittance was about 73%. Compared with the ATU-HPCS gel membranes, there was no UV transmittance of ATU-HPCS-I_2-M [Figure 3(b)] before 570 nm, after that was a rocketing upwards, the negligible transmittance was probably due to the absorption of iodine to UV-visible. The UV results were consistent with the SEM, indicated that the ATU-HPCS-I_2-M has an excellent effect of adsorbing iodine.

SEM Observation of ATU-HPCS Gel Membranes and ATU-HPCS-I_2-M

Microstructures of the ATU-HPCS gel membranes surface and ATU-HPCS-I_2-M surface were investigated by scanning electron microscopy as showed in Figure.4. The surface of ATU-HPCS gel membranes [Figure 4(a)] showed crystallinity in some extent, which was caused by the copolymer repulsion between the molecular chains because of the existence of hydrophobic groups in the process of graft copolymerization reaction.

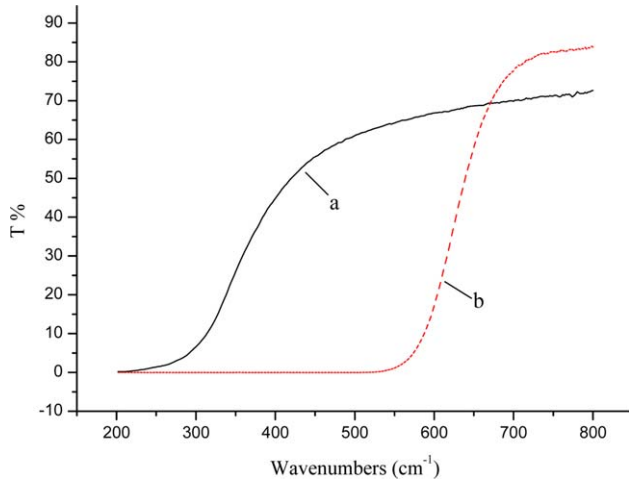


Figure 3. UV spectra of (a) ATU-HPCS gel membranes and (b) ATU-HPCS-I_2-M. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

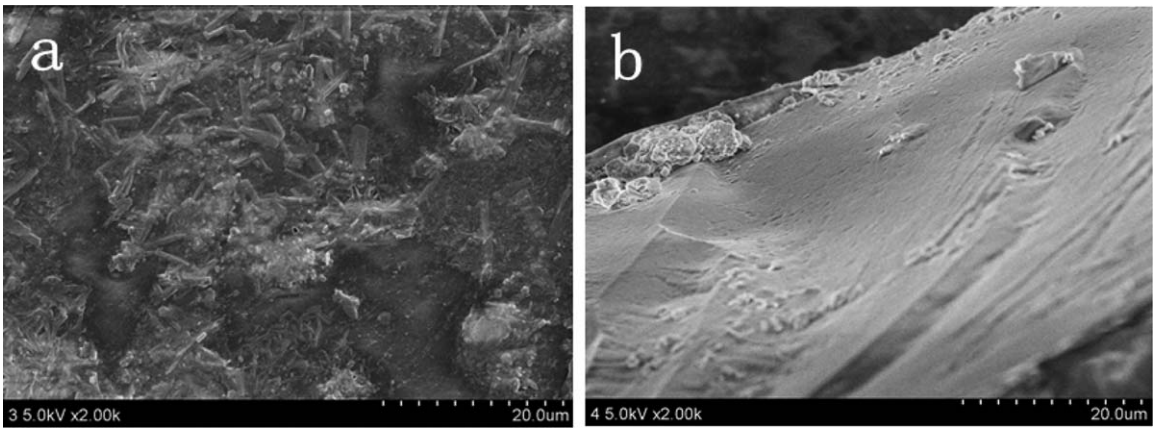


Figure 4. The 2000 times of scanning electron microscopy of (a) ATU-HPCS gel membranes and (b) ATU-HPCS-I_2-M.

The ATU-HPCS-I_2-M surface [Figure 4(b)] tended to blend flat compared with ATU-HPCS gel membranes surface, showed no crystalline pattern, which was probably due to the excellent complexation (adsorption) ability of iodine on the ATU-HPCS, indicated a good compatibility between each component of ATU-HPCS-I_2-M.

XRD Patterns of ATU-HPCS Gel Membranes and ATU-HPCS-I_2-M

The XRD patterns of ATU-HPCS gel membranes and ATU-HPCS-I_2-M were shown in Figure 5. The two peaks in the diffractograms of ATU-HPCS gel membranes (Figure 5a) at 2θ values of 12.4° and 21.5° corresponding to characteristic peaks of chitosan and PVP. Two strong peaks at 2θ values of 27.6° and 29.8° may assigned to the grafted of thiosemicarbazide on CS, the XRD patterns of ATU-HPCS gel membranes indicated some extent degree of crystallinity, consistent the same results with the SEM.

According to the XRD patterns of ATU-HPCS-I_2-M [Figure 5(b)], the characteristic peaks at 2θ values of 21.5° of chitosan and PVP got stronger and shifted to 19.8°, while other original peaks of ATU-HPCS gel membranes were decreased and disappeared because of the complexation(adsorption) of iodine. Meanwhile the characteristic peaks of iodine appeared at 2θ values of 24.9°, 28.6°, and 35.7° corresponding with the diffraction intension of crystal face of iodine (JCPDS 06–0183) were (111), (112), (004). All above indicated the great impacts on molecular arrangement, structure and crystallinity due to the adsorption of iodine.[34]

Thermal Stability Analysis

The TGA and derivative thermogravimetry (DTG) curves of ATU-HPCS gel membranes and ATU-HPCS-I_2-M were shown in Figures 6 and 7, a significant mass loss was occurred at 280°C, it could be seen from the TG, DTG curves of ATU-HPCS gel membranes [Figures 6(a) and 7(a)], which was assigned to the dehydration of the saccharide rings and the decomposition of ATU-HPCS gel membranes. The mass loss occurred at 100°C was due to the water elimination which was adsorbed physically to the membranes, while the mass loss of water evaporation of ATU-HPCS-I_2-M (Figure 7) moved from 100°C to 90°C, probably because the complexation (adsorption) of iodine led to the destruction of hydrogen bonds of water molecules.[23]

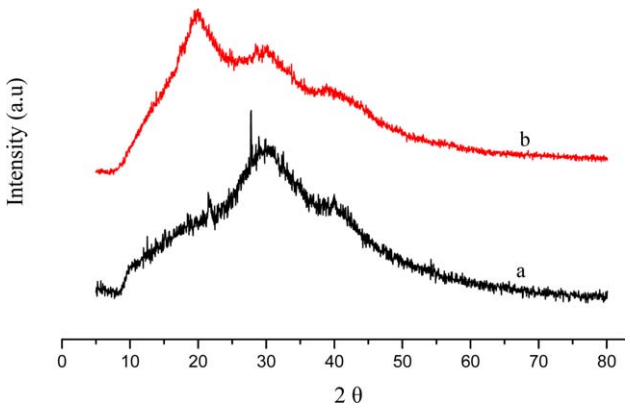


Figure 5. XRD patterns of (a) ATU-HPCS gel membrane and (b) ATU-HPCS-I_2-M. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

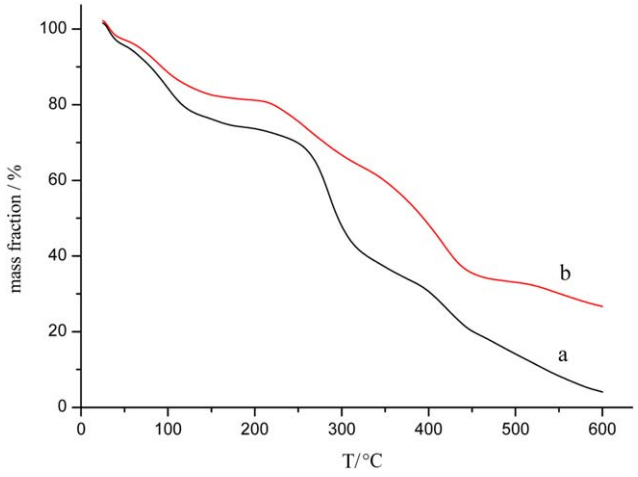


Figure 6. The TG curves of (a) ATU-HPCS gel membranes and (b) ATU-HPCS-I_2-M. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

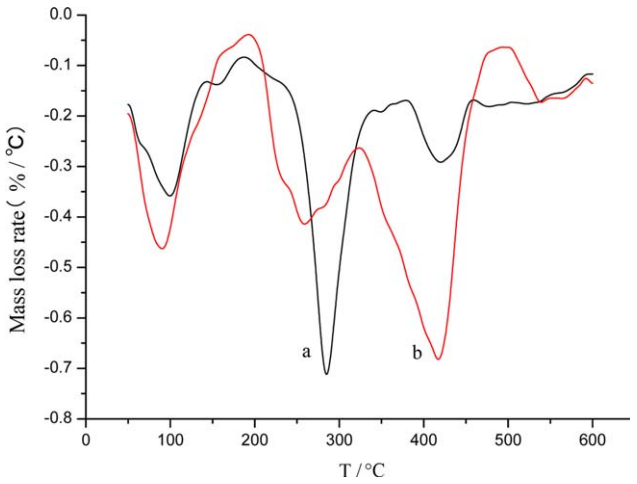


Figure 7. The DTG curves of (a) ATU-HPCS gel membranes and (b) ATU-HPCS-I_2-M. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

In addition, the ATU-HPCS-I_2-M had more residues than ATU-HPCS gel membranes when the temperature was 600°C (Figure 6), the main-chains pyrolysis of ATU-HPCS-I_2-M has two degradation steps seen from Figure 7(b), it was only partially decomposed at 260°C, and the relatively larger mass loss moved to about 430°C. It was probably due to the intermolecular force of ATU-HPCS-I_2-M were more stronger, and the compatibility of different constituents were better after the complexation (adsorption) of iodine, indicated a higher thermal stability of ATU-HPCS-I_2-M compared with ATU-HPCS gel membranes.

Solubility Tests of ATU-HPCS

The solubility were determined according to the following criteria: 1 g $< S <$ 10 g dissolve; 0.01 g $< S <$ 1 g slightly soluble; $S <$ 0.01 g insoluble, where S is solubility. The solubility tests of ATU-HPCS showed good solubility in acidic aqueous solution (1% v/v), HCl (1% v/v) and water, slightly soluble in DMSO and DMF, insoluble in ethanol and acetone and w(NaOH) = 1% aqueous solution seen from Table I. Besides, the good solubility in water also indicated the success of grafting the hydroxypropyl, consistent the same results with the IR.

Iodine Content of ATU-HPCS-I_2-M

The iodine content of ATU-HPCS-I_2-M increased along with the added volumes of iodine in ethanol solution seen from Table II, the mass fraction of iodine was 21.09% when the volumes of iodine in ethanol solution was 25 mL, which indicated that the ATU-HPCS had a good adsorption of iodine. It was corresponded to the action of increased lone pair electrons of sulfur and nitrogen element on thiosemicarbazide.

Iodine Release of ATU-HPCS-I_2-M

The releasing rule of ATU-HPCS-I_2-M (Figure 8) in simulated exudate showed that there was a rapid release within a short time, after reaching a certain concentration, the release of iodine reached a balance. This was due to the release of iodine firstly occurred on the surface of the membrane, then inside the membrane, thus the release rate decreased and remained steady.[24]

Table I. Solubility of ATU-HPCS in Various Solvents

Solvent	Temperature (°C)	Solubility	Soluble fraction
H_2O	25	4	Dissolve
Acetic acid solution (1% v/v)	25	6	Dissolve
HCl (1% v/v)	25	7	Dissolve
DMSO	25	0.5	Slightly soluble
DMF	25	0.3	Slightly soluble
Ethanol	25	0.005	Insoluble
Acetone	25	0.001	Insoluble
w(NaOH) = 1 % aqueous solution	25	0.008	Insoluble

In addition, the emissions of iodine was maintained within a certain range and kept at a stable leveling out for a long time, there was still no downtrends at 200 min. And the release of iodine increased along with the increase of mass fraction of ATU-HPCS, the maximum release of iodine remained at about 0.80% when the mass fraction of ATU-HPCS-I_2-M was remained at 21.09%. These results indicated that the ATU-HPCS-I_2-M had a sustained-release effect of iodine and a stable emissions,[24,35] which could reduce the skin irritation when using to the antimicrobial materials.

Antibacterial Property Analysis of ATU-HPCS Gel Membranes and ATU-HPCS-I_2-M

According to the standard of antibiotic sensitivity: when the inhibition zone diameter <10 mm, the drug sensitivity is resistance; 10 to 15 mm, is moderately sensitive; >15 mm, is highly sensitive. The antibacterial activity of the ATU-HPCS gel membranes and ATU-HPCS-I_2-M with different mass fraction of iodine were tested by the method of measuring the diameter of the inhibition zone (Table III). The inhibition zone diameters of CS against *S. aureus* and *E. coli* were both (10 ± 1) mm, the inhibition zone diameters of ATU-HPCS against *S. aureus* and *E. coli* were (12 ± 1) mm and (11 ± 1) mm, respectively, the ATU-HPCS gel membrane had the same inhibition zone diameters with the ATU-HPCS, all of them showed little bacteriostasis effect. The inhibition zone diameters of ATU-HPCS-I_2-M with different mass fraction of iodine against S. aureus and *E. coli* were both greater than 15 mm, which indicated a palpable antibacterial activity. Besides, the bacteriostatic level of ATU-HPCS-I_2-M against *S. aureus* and *E. coli* increased along with the increase of iodine content, and the antimicrobial effects had no obvious growth after it reaches I_2 % (mass fraction) >11.42.

Table II. I_2 Content Determination Results of ATU-HPCS-I_2-M

The volume added of iodine in ethanol solution	5 mL	10 mL	15 mL	20 mL	25 mL
I_2 (%)	2.67	8.82	11.42	17.19	21.09

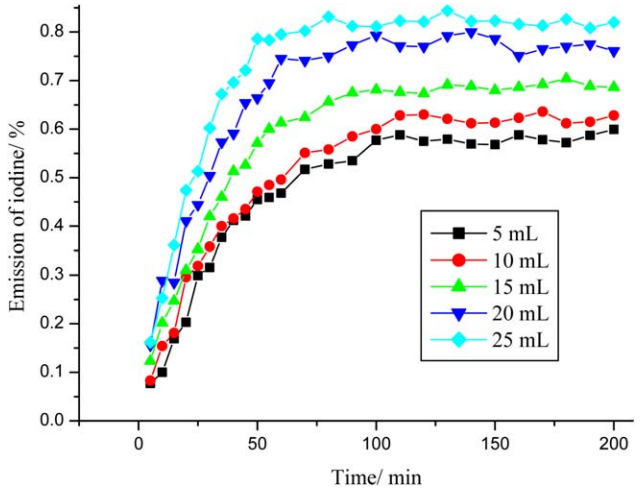


Figure 8. The iodine release of ATU-HPCS-I_2-M. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Compared with the antibacterial activity of CS, the antibacterial activity of ATU-HPCS and ATU-HPCS-I_2-M have different degrees of increase, and the ATU-HPCS-I_2-M's improvement was observable.

Moreover, the ATU-HPCS-I_2-M were more active against the *S. aureus* than against the *E. coli*, and the inhibition zone diameters of ATU-HPCS-I_2-M against *S. aureus* and *E. coli* were (32 ± 1) mm and (30 ± 1) mm, respectively, when the iodine mass fraction of the ATU-HPCS-I_2-M was 21.09%. The different inhibition zone diameters of *S. aureus* and *E. coli* could be attributed to their different cell wall. The cell wall of Gram-positive bacteria (e.g. *S. aureus*) is fully composed of peptide polyglycogen. The peptidoglycan layer is composed of net works with plenty of pores, which allow foreign molecules to come into the cell without difficulty and more rapid absorption of ions into the cell. But the cell wall of the Gram-negative bacteria (e.g. *E. coli*) is made up of a thin membrane of peptide polyglycogen and an outer membrane constituted of lipopolysaccharide, lipoprotein and phospholipids. Because of the complicated bilayer cell structure, the outer membrane is a potential barrier against foreign molecules with high molecular weight.[32] Therefore, the ATU-HPCS-I_2-M have different effects on the two kinds of bacteria.

Table III. Inhibition Indices of CS, ATU-HPCS, ATU-HPCS Gel Membrane and ATU-HPCS-I_2-M with Different Iodine Content Against *S. aureus* and *E. coli*

	Inhibition zone (mm)	
	Tested microorganisms	
Samples	*S. aureus*	*E. coli*
CS	10 ± 1	10 ± 1
ATU-HPCS	12 ± 1	11 ± 1
ATU-HPCS gel membranes	12 ± 1	11 ± 1
I_2 % (mass fraction) = 2.67 ATU-HPCS-I_2-M	18 ± 1	16 ± 1
I_2 % (mass fraction) = 8.82 ATU-HPCS-I_2-M	23 ± 1	21 ± 1
I_2 % (mass fraction) = 11.42 ATU-HPCS-I_2-M	28 ± 1	25 ± 1
I_2 % (mass fraction) = 17.19 ATU-HPCS-I_2-M	30 ± 1	28 ± 1
I_2 % (mass fraction) = 21.09 ATU-HPCS-I_2-M	32 ± 1	30 ± 1

The various antibacterial tests done above prove the excellent antimicrobial activity of the prepared ATU-HPCS-I_2-M,[23–26] made ATU-HPCS-I_2-M hold highly potential as bacteriostatic material that prevent the most bacteria.

CONCLUSIONS

The ATU-HPCS was prepared by CS grafted hydroxypropyl after the amino groups were protected with benzaldehyde, and the thiosemicarbazide was grafted after the deprotection of amino groups. Contrastive analysis of the characteristics of the ATU-HPCS gel membranes and the ATU-HPCS-I_2-M: the ATU-HPCS-I_2-M had better thermal stability, and the iodine content of ATU-HPCS-I_2-M increased along with the added volume of iodine in ethanol solution, which indicated the ATU-HPCS has a good adsorption effect on iodine. The iodine releasing of ATU-HPCS-I_2-M showed a stable leveling out for a long time after reached a certain concentration, indicated a sustained-release effect of iodine, the maximum emission of iodine was approximately 0.80%. The antimicrobial test result showed that the antibacterial diameters of against *Staphylococcus aureus* and *E. coli* of the ATU-HPCS-I_2-M were both greater than 15 mm. The ATU-HPCS-I_2-M could be made into various forms of antibacterial, anti-inflammatory medical membranes based on different parts of the human body needs. Due to the double effects of sterilization, anti-inflammation and tissue repaired capabilities of CS on human trauma, and the sustained-release of iodine, which could reduce the stimulation of iodine on the wound. The ATU-HPCS-I_2-M provided an ideal healing environment for wound surface as a low-cost second skin, demonstrated the potential applications in antimicrobial membrane materials.

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