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Preparation and antibacterial activity of quaternized chitosan with iodine

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ABSTRACT

Chitosan (CTS) is a natural polymer with active groups such as $-NH_2$ which can be functionalized to introduce new positively charged N-atoms and protonated amino group for better use. In this study, to improve the stability of iodine, a novel complex (CTS–CTA–I_2) was prepared by mixing N-(2-hydroxy) propyl-3-trimethylammonium chitosan chloride (CTS–CTA) with iodine in ethanol solution. The CTS–CTA–I_2 was characterized by Fourier transform infrared spectra (FTIR), Ultraviolet and visible (UV–vis) spectra and thermal gravimetric analysis (TG). Besides, the interaction of iodine with CTS–CTA was also studied. The mole ratio of CTS–CTA with iodine was measured by iodometric titration method and the max mole ratio of CTS–CTA with iodine was 1:1.33. The antimicrobial activity of CTS, CTS–CTA and CTS–CTS–I_2 complexes was investigated against *Escherichia coli* and *Staphylococcus aureus* and the antibacterial property of CTS–CTA–I_2 was superior to CTS–CTA.

1. Introduction

Iodine is the heaviest essential element known to be needed by all living organisms. Iodate, iodide, molecular iodine and organic iodine are the major species of iodine. Iodate and iodide are added to the table salt and food for supplementing the daily intake of iodine [1,2]. Molecular iodine is a powerful antimicrobial agent, and it has been widely used for antimicrobial applications [3,4]. However, the utilization of its volatile nature is limited which can be decreased by attaching iodine to functionalized polymers [5]. Therefore, several researchers have synthesized polymer–iodine complexes from both natural and synthetic polymers [6–8]. According to the report [4,9,10], polyvinylpyrrolidone–iodine complex which is a combination of iodine with polyvinylpyrrolidone (PVP) is the most extensively studied polymer–iodine complex. Though much works have been done on the study of interaction of iodine with functionalized natural polymer, no one has reported the synthesis of chitosan (CTS) based on iodine complex.

As one of the most important and abundant natural polysaccharides next to cellulose and starch, chitosan (CTS) has advantages of non-toxic, biocompatible, biodegradable and antimicrobial activity [11,12], which mainly attribute to the active hydroxyl and amino groups. Due to its unique physicochemical characteristics and biological activities, CTS and its derivatives have been widely investigated for applications in biomedical, biotechnological and pharmaceutical fields [13,14]. However, CTS shows some limitations because of its poor solubility [15]. CTS is a weak base, and a certain amount of acid is required to transform the glucosamine units into the positively charged, water-soluble form. Consequently, at a neutral pH, most CTS molecules will lose their charge and precipitate from solution. According to the reports [16,17], the introduction of substituting groups will decrease the intermolecular hydrogen bonding, and it will be helpful to increase the antibacterial activity and the solubility of CTS. Among the derivatives of CTS, quaternized CTS has attracted much attention, due to the properties of retaining cationic charges at neutral pH, good water solubility and antibacterial activity [18–20]. The original amino group of CTS is replaced by methyl, and the positively charged N-atoms and protonated amino group can be obtained subsequently. It is reported that molecular iodine shows a strong tendency to form charge transfer complexes even with the weak electron donors [21]. Thus, it is more possible to easily combine the quaternized CTS with iodine. In addition, the reagents used in the preparation of quaternized CTS are of low cost, and the processing is relatively simple.

This study is to develop an iodine complex (CTS–CTA–I_2) based on quaternized CTS without a significant loss of germicidal efficacy of iodine which has advantages of its easy availability and low cost. Besides, the thermal stability and the mole rate of quaternized CTS with iodine have been studied in detail.

2. Materials and methods

2.1. Materials

Chitosan (degree of deacetylation > 92% and molecular weight calculated from the gel permeation chromatography (GPC) method $\approx 2.82 \times 10^5$) was purchased from Zhejiang Yuhuan Ocean Biochemistry Co., Ltd. (Zhejiang, China). 3-Chloro-2-hydroxy propyl trimethyl ammonium chloride was purchased from Tokyo Chem. Ind., Japan. All other reagents were of analytical grade.

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Scheme 1. Synthesis scheme of CTS–CTA.

2.2. Preparation of CTS–CTA

The CTS–CTA was prepared by a modified method proposed by the literature [22]. The reaction scheme for synthesizing CTS–CTA was presented in Scheme 1. Briefly, 5 g of CTS was dissolved in a 2% (wt%) acetic acid solution, and then 40% (wt%) sodium hydroxide solution was added into the solution until pH reaches 8–9. The CTS precipitation was put into a 250 mL flask, as well as isopropanol, stirring the solution at room temperature for 1 h. The CTA was then added into the solution and reacted at 60 °C for 7 h. The pH was adjusted to 7 with 10% HCl (wt%) subsequently, the solution was evaporated, and 200 mL of 95% (wt%) ethyl alcohol was added to precipitate the production, the solid was filtered and rinsed with 95% (wt%) ethyl alcohol for three times, then vacuum dried at 60 °C for 24 h. The weight of the product was about 7.4 g.

To measure the degree of substitution (DS) of CTS–CTA, a certain quality of CTS–CTA was dissolved in deionized water, and DS was determined by titrating the amount of Cl^- ions on the CTS–CTA with $AgNO_3$ solution according to the literature [23]. DS of the quaternary ammonium salt groups was calculated using the following equation:

$$DS\% = \frac{VM}{VM + (W - VM \times 314)/161}$$

where W is the weight of CTS–CTA in grams, and V (mL) and M (mol/L) are the volume and concentration of silver nitrate solution used for titration, respectively. The numbers 314 and 161 corresponded to the molecular weight of the repeat structural unit of CTS–CTA and CTS. When the hydrogen atoms of $-NH_2$ on CTS were fully substituted, the DS of CTS–CTA was 200%, since there were two hydrogen atoms on each amino group which could be substituted by the quaternary ammonium salt groups. And the measured DS of CTS–CTA was 151%, which indicated that 49% of the hydrogen atoms of $-NH_2$ on CTS.

2.3. Iodination of CTS–CTA

1.0 g of CTS–CTA power was mixed with 100 mL of different concentrations of molecular iodine solution prepared in ethyl alcohol (0.4, 1, 1.4, 1.8 and 2.2, w/v) in a 250 mL round bottom flask. The heterogeneous mixtures were stirred at room temperature for 5 h. The reddish brown solid was filtered and thoroughly rinsed with 95% (wt%) ethyl alcohol to remove the loosely bound iodine, then vacuum dried at room temperature for 24 h.

2.4. Characterization

Infrared (IR) spectra of CTS, CTS–CTA and CTS–CTA–I_2 were measured with KBr pellets on FT/IR-370 plus Fourier transform infrared spectrometer (Thermo Nicolet Company, USA). The Ultraviolet and visible (UV–vis) spectra of aqueous iodine (I_2) solution and iodine released from CTS–CTA–I_2 powder at pH 6 were measured by using UV-1601PC Ultraviolet spectrometer. Thermal gravimetric (TG) and derivative thermogravimetric (DTG) analyses were implemented using Universal DSCPT-10 thermal analyzer. The analysis was performed under continuous flow of dry nitrogen gas at a heating rate of 20 °C/min.

2.5. Iodometric titration

1.0 g of CTS–CTA–I_2 complex was immersed at room temperature for 4 h which was added into 100 mL calibrated sodium thiosulfate solution for further use. Then the supernatant fluid was taken for determining excess Sodium thiosulfate via iodometry.

2.6. Antibacterial activity test

Antibacterial activities were investigated using agar diffusion method [24]. The activity was determined by measuring the diameter of the

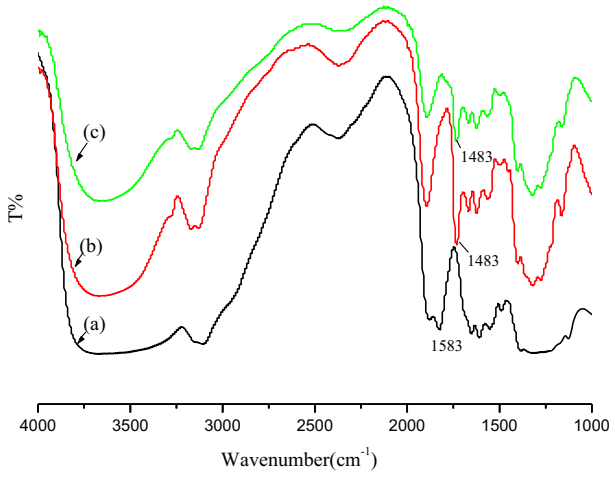

Fig. 1. IR spectra of CTS (a), CTS–CTA (b), and CTS–CTA–I_2 (c).

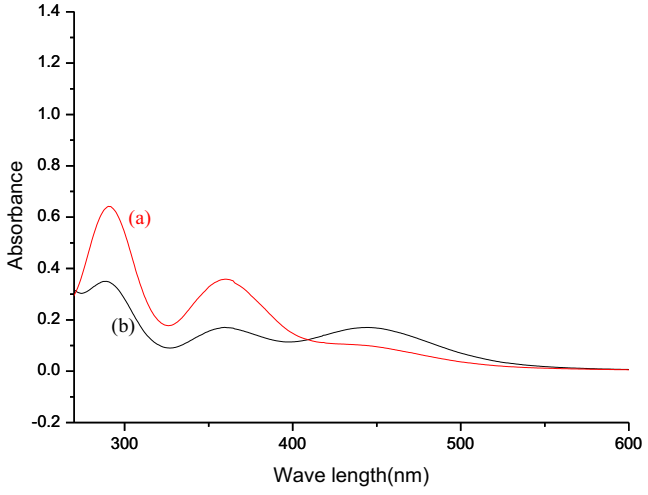

Fig. 2. UV–vis spectra of aqueous iodine (I_2) solution (b) and iodine released from CTS–CTA–I_2 complex (a) at pH 6.