Preparation and Antibacterial Activity of Low Molecular Weight Quaternized Chitosan Gelatin Film with Iodine

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Abstract

The chitosan was degraded to the low molecular weight chitosan (CTS') by oxidation, and the low molecular weight quaternized chitosan (QCTS') was prepared by CTS' with 3-chloro-2-hydroxypropyl three methyl ammonium chloride (CTA). The stable complex (QCTS'-I_2) was obtained using QCTS' and iodine, and the complex was mainly composed of uniform nanoparticles with the diameter of about 1000nm. The CTS-[QCTS'-I_2]-PVP gelatin film was prepared by QCTS' and chitosan with polyvinylpyrrolidone (PVP). The scanning electron microscopy (SEM) showed that the complex uniformly distributed on gelatin films. Approximately 6.5% of iodine was released in about 45mins in the phosphate buffer (pH 7.4). The antibacterial ring diameter of CTS-[QCTS'-I_2]-PVP gelatin film for E. coli and S. aureus is greater than 15 mm.

Keywords: Low molecular weight chitosan; Quaternization; Iodine; Antibacterial; Iodometric titration

1. Introduction

Chitosan (CTS), the deacetylated version of chitin and the most abundant naturally occurring amino-polysaccharide, has attracted worldwide attention due to its unique physicochemical characteristics and biological activities(Hejazi & Amiji, 2003; Shimono, Takatori, Ueda, Mori, Higashi & Nakamura, 2002), CTS and its derivatives have been widely investigated for applications in biomedical, biotechnological and pharmaceutical fields(Ravi Kumar, 2000; Rinaudo, 2006). However, natural CTS can only be dissolved in acid solution of above pH 6.5(Fei Liu, Lin Guan, Zhi Yang, Li & De Yao, 2001), which limits its widespread applications. In addition, CTS of high molecular weight can therefore only be dissolved in acid solution. Solutions made from CTS in dilute acid solution often need repeated washing to neutralize the acid, which restricts its applications, thus to prepare the low molecular weight chitosan and modify CTS is desirable for improving its water-solubility.

In recent years, low molecular weight chitosan (CTS') have shown great potential in the applications of drug delivery and gene delivery (Csaba, Köping-Höggård & Alonso, 2009; Lavertu, Méthot, Tran-Khanh & Buschmann, 2006). Compared with high molecular weight CTS, CTS' shows better solubility, biocompatibility (Chae, Jang & Nah, 2005; Lee, Nah, Kwon, Koh, Ko & Kim, 2001; Liu, Song, Li, Li & Yao, 2007). Existing methods for preparing CTS' typically include degradation by acids, yeasts, oxidation and other degradation methods such as irradiation using microwaves and ultrasound. Among various methods developed to prepare CTS', oxidization degradation has attracted considerable attention since it causes rapid degradation and is a simple, inexpensive process without by-products (Huang, Wu, Chen & Lian, 2008). As an efficient solution to improve its antibacterial activity in aqueous water, a number of modifications of CTS have been investigated, such as alkylation(Yang, Chou & Li, 2005), quaternization(Thatte, 2004) and carboxymethylation(Belalia, Grelier, Benaissa & Coma, 2008).

Quaternization is a promising kind of modification due to its positive charged N-atoms and protonated amino group (Rúnarsson et al., 2007). The antibacterial activity of quaternized chitosan is therefore superior to CTS (Sajomsang, Tantayanon, Tangpasuthadol & Daly, 2008; Xu, Xin, Li, Huang & Zhou, 2010). In addition, the reagents used in the prepared of quaternized chitosan were of low cost, and the processing was relatively simple. The method has been widely used in the production of chitosan derivatives (Chiellini, Cinelli, Chiellini & Imam, 2004; Qin et al., 2004).

Iodine is one of the necessary elements for human life, it can promote biological oxidation, regulate protein synthesis and oxidation. Also, it has strong bactericidal properties(Ellis & Van Vree, 1989). However, iodine can be easily sublimated and thus some complex compounds with iodine is unstable with poor dispersibility, and iodine can be easily absorbed by human body, but excessive iodine intake may harm the health(Siggia, 1957). For instance, excessive iodine intake can lead to high iodine goiter, hyperthyroidism and memory decline.

The interaction of small molecules with functionalized natural polymer have been widely investigated, and a number of groups have synthesized polymer-iodine complexes using synthetic and natural polymers using molecular and ionic iodine(Chen & Wang, 2001; Rendleman Jr, 2003), none of which so far has reported the synthesis of quaternized chitosan based iodine complex.

The objective of this study is to synthesis the low molecular weight quaternized chitosan for better iodine absorbency, because positive charge of quaternary ammonium salt has a larger attraction, and to investigate the antibacterial activity of gelatin films against a gram-positive bacterium Staphylococcus aureus and a gram-negative bacterium Escherichia coli respectively. The amount of available free iodine released in the phosphate buffer has also been measured by titrimetric method.

2. Materials and Methods

2.1 Materials

Chitosan [degree of deacetylation>92% and molecular weight calculated from the gel permeation chromatography (GPC) method ≈ 2.82×10^5) 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 analytic grade.

2.2 Sample Preparation and Measurement

2.2.1Preparation of CTS'

The CTS' was prepared according to previous research(Huang, Wu, Chen & Lian, 2008). Briefly, added into 300mL of 5% (wt%)H_2O_2 solution, 24g of CTS was stirred at 70℃ in thermostatic bath for 1h. Then the solution was evaporated until it was a third volume of the original solution, and 200mL of 95%(wt%) ethyl alcohol was added to precipitate the production, following that the solid was filtered and rinsed in 95%(wt%) ethyl alcohol for three times, then vacuum dried at 60℃ for 24h. The weight of the product was 16g, and the molecular weight was 5500-6000.

2.2.2 Preparation of QCTS'

The QCTS' was synthesized according to previous research, with proper modifications(Yu, Song, Shi, Xu & Bin, 2011). Briefly, added into 10g of 40 % (wt %) Sodium hydroxide solution, 24g of CTA was stirred at room temperature for 30 minutes. Dispersed in 50mL of Isopropyl alcohol, 4g of CTS' was stirred at room temperature for 1h. Then the CTS' isopropyl alcohol solution was transferred into a 250mL flask, adding the alkaline CTA solution slowly, and then reacted at 50℃ for 6h. The pH was adjusted to 7 with 10% HCl (wt %) subsequently, the solution was evaporated, and 200mL of 95% (wt %) ethyl alcohol was added to precipitate the production, following that the solid was filtered and rinsed in 95% (wt %) ethyl alcohol for three times, and then vacuum dried at 60℃ for 24h. The weight of the product was 3.1g, and the degree of substitution was 0.92.

2.2.3 Preparation of the QCTS'-I_2 Solution

8.6g of potassium iodide was dissolved in 60mL of 2% (wt %) Acetic acid solution, and then the solution was stirred at room temperature at the speed of 300r/min for 1h, followed by adding 0.05g of sodium dodecyl sulfate and 80g of tertiary butyl alcohol. Then added 1.4g QCTS', the solution was stirred at 55℃ for 2h. The QCTS'-I_2 solution was then obtained.

2.2.4 Preparation of Gelatin Films

0.76g of gelatin was dissolved in 200mL of 2 % (wt %) acetic acid solution, and then the solution was stirred at room temperature for 1h, followed by adding 3.6g of CTS and 0.73g of PVP. Subsequently, while stirring at 50℃ for 1h, the QCTS'-I_2 solution of different volume (0, 1, 3, 5, 8, 10mL) was added to the solution, respectively. Finally, the solution was moved into the mould and dried at room temperature for 48 h.

2.2.5 Characterization

IR spectra of CTS', QCTS' and gelatin films were measured with KBr pellets on FT/IR-370 plus fourier transform infrared spectrometer (Thermo Nicolet company, USA). The CTS'-CTA-I_2 solution was measured on Laser particle size analyzer (Beck Kumaner Company, USA). The gelatin films and the QCTS'-I_2 solution were analyzed by scanning electron microscope (SEM, Hitachi, Japan).

2.2.6 Antibacterial Activity Test

Antibacterial activities were investigated using agar well diffusion method. The activity was determined by measuring the diameter of the inhibition zone (in mm). Escherichia coli(as Gram negative bacteria) and Staphylococcus aureus (as Gram positive bacteria) were dispersed into the medium, Centrifuged pellets of bacteria from a 24 h old culture containing approximately 10^4–10^6 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 %, 1000mL of distilled water, pH =7.0), which was autoclaved under 121℃ for at least 20 min. Wells were created in medium with the help of a sterile metallic bores and then cooled down to 45℃. The bacteria concentration of about 1.08×10^5 cells / L, 30mL of the solution taken diluted 10-fold mounted to a petri dish of 12 cm diameter in the cooled and solidified. The CTS-PVP gelatin film and the CTS-[QCTS'-I_2]-PVP gelatin film having a diameter of 10 mm of the wafer, UV sterilized 30 min, respectively, attached to different locations of the dish, the plates were kept for incubation at 37℃for 24 h and then examined for the formation of zone of inhibition. Each inhibition zone was measured three times by caliper to get an average value and recorded inhibition zone diameter.

2.2.7 Iodometric Titration

Added into 100mLof calibrated Sodium thiosulfate solution, 1.0g of CTS-[QCTS'-I_2]-PVP gelatin film (5ml of CTS'-CTA-I_2 solution) was immersed at room temperature for 4h. Then took the supernatant fluid, the solution was determined via iodometry(Ahmad, Mazumdar & Kumar, 2013).

2.2.8 Iodine Releasing of CTS-[QCTS'-I_2]-PVP Gelatin Film

Iodine releasing of CTS-[QCTS'-I_2]-PVP gelatin film (5ml of CTS'-CTA-I_2 solution) was determined as follows. The CTS-[QCTS'-I_2]-PVP gelatin film (5ml of CTS'-CTA-I_2 solution) was placed into a beaker with 250mL phosphate buffer (pH 7.4), while stirring at 37℃ in a water bath. At appropriate time intervals, the solution (3mL) was withdrawn and determined by a UV spectrophotometer at 231 nm. At the same time, 3mL of phosphate buffer (pH 7.4) was added into the beaker.

3. Results and Discussion

3.1 SEM Image and Particle Size Distribution of the QCTS'-I_2 Solution

Fig.1 and Fig.2 shows SEM image and Particle size distribution of the QCTS'-I_2 solution respectively. The complex in QCTS'-I_2 solution appeared in a regulate structure. It can be seen clearly in Fig.2 that the diameter of the complex was about 1000nm and the shape of the I_2 was no longer available in the form of spherical shape but of polyhedron, which was mainly due to the combination of the positive change of the quaternary ammonium salt and I_2 which can be deformed easily. Also the particle diameter at about 80nm in Fig.1was attributed to I^-_n(n=3,5,7,9…) which was not complexed with QCTS' (Miyajima et al., 2010).

3.2 IR Spectra Analysis

Fig.3 shows IR spectra of CTS' and QCTS'. There are three characteristic peaks of CTS' at 3284 cm^{-1} (O-H stretch), 2867 cm^{-1} (C-H stretch), 1583 cm^{-1} (N-H bend)(Lim & Hudson, 2004; Wang, Zhu, Xue & Wu, 2012). Compared with CTS', QCTS' shows that the characteristic peak (1538 cm^{-1}) was weakened and a new peak positioned at 1464 cm^{-1} appeared, which attributed to the methyl groups of the ammonium(Caiqin, Ling, Yumin, Xiaowen & Jiawei, 2002; Zhao, Wu, Guo, Du, Yu & Tang, 2010).

The spectrum of CTS-PVP gelatin film (Fig.3(c)) is different from that of CTS' (Fig. 3(a)) due to the addition of PVP. 3284cm^{-1} (O-H stretch)and 2867cm^{-1} (C-H stretch)(Anjali Devi, Smitha, Sridhar & Aminabhavi, 2006)in CTS-PVP gelatin film shifted to 3310 cm^{-1} and 2933cm^{-1} respectively, and a new peak of 1649cm^{-1} (amide Ⅰ) appeared. This shows that PVP was blended with CTS. Compared with CTS-PVP gelatin film (Fig.3(c)), the intensity of all peaks in CTS-[QCTS'-I_2]-PVP gelatin film (Fig. 3(d)) became stronger obviously, and they did not shift. We suppose that the non-polar iodine molecule do not produce vibration and rotation among molecules, which shows that the molecular structure of the iodine is not damaged.

3.3 SEM Image of Gelatin Films

Fig.4 shows SEM image of CTS-PVP and CTS-[QCTS'-I_2]-PVP gelatin film. Compared with CTS-PVP gelatin film (Fig.4(a)), CTS-[QCTS'-I_2]-PVP gelatin film (Fig.4(b))shows that QCTS'-I_2 complex distributed evenly, and the shape and size were uniform, which was the same as the result of Fig.1.

3.4 Iodometric Titration

Fig.5 shows that the iodine content in the CTS-[QCTS'-I_2]-PVP gelatin film. The iodine content in the CTS-[QCTS'-I_2]-PVP gelatin film increased with the increase of volume of QCTS'-I_2 solution. This could be ascribed to the fact that the positive charge quaternary ammonium groups adsorbed iodine molecules firmly, which made the iodine molecule stable.

3.5 Iodine Releasing of CTS-[QCTS'-I_2]-PVP Gelatin Film

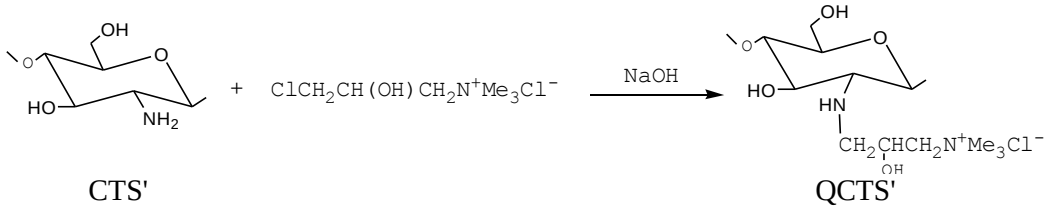
It can be seen in Fig.6 that the iodine releasing of CTS-[QCTS'-I_2]-PVP gelatin film. It was apparent that iodine release in vitro showed a very rapid within initial 25min, and then followed by a slow iodine release after about 20min. About 6.5% of iodine was released in about 45min in the phosphate buffer (pH 7.4). The first release of 5.6% is due to the iodine desorption from the blend film surface, which easily diffuses in the initial incubation time (Xu, Du, Huang & Gao, 2003). Then the reversed release occurred in about 20min, due to iodine resorption onto gelatin film surface again (Zambaux, Bonneaux, Gref, Dellacherie & Vigneron, 2001). Finally, a constant sustained release of the iodine for the following hours occurred, resulting from the diffusion of the iodine dispersed in the phosphate buffer.

3.6 Antibacterial Activity

Fig.7 shows that the antibacterial efficacies of the gelatin film against E. coli and S. aureus by the sample. According the agar well diffusion method, the antibacterial ring diameter is less than 10 mm which is slightly sensitive; the antibacterial ring diameter is ranging from 10 to 15 nm which is generally sensitive; the antibacterial ring diameter is greater than 15mm which is highly sensitive. It can be seen that CTS-PVP gelatin film has no antibacterial activity, and CTS-[QCTS'-I_2]-PVP gelatin film shows a strong antibacterial activity against two bacteria of E. coli and S. aureus, which is highly sensitive, suggesting that it can be used for relevant applications.

4. Conclusions

The CTS was degraded to the CTS' by oxidation, and the QCTS' was prepared by the CTS' with CTA, which made the iodine molecule stable. The complex in QCTS'-I_2 solution appeared in a regulate structure, and the diameter of the complex was about 1000nm, and QCTS'-I_2 complex distributed more evenly and dispersedly. CTS-[QCTS'-I_2]-PVP gelatin film shows strong antibacterial activity against two bacteria of *E. coli* and *S. aureus*. The results suggested that the CTS-[QCTS'-I_2]-PVP gelatin film has become a promising material for potential applications in biomedical fields, such as drug delivery and wound dressing.



CTS' QCTS'

Scheme.1: Synthesis Scheme of QCTS'

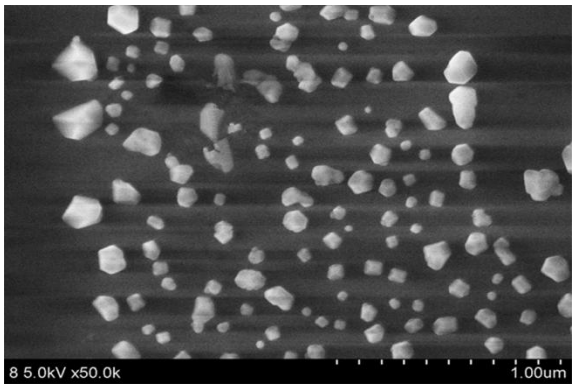


Fig.1: SEM Image of QCTS'-I_2 Solution

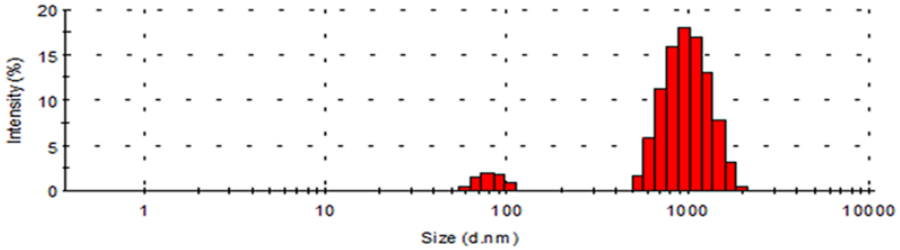


Fig.2: Particle Size Distribution of QCTS'-I_2 Solution

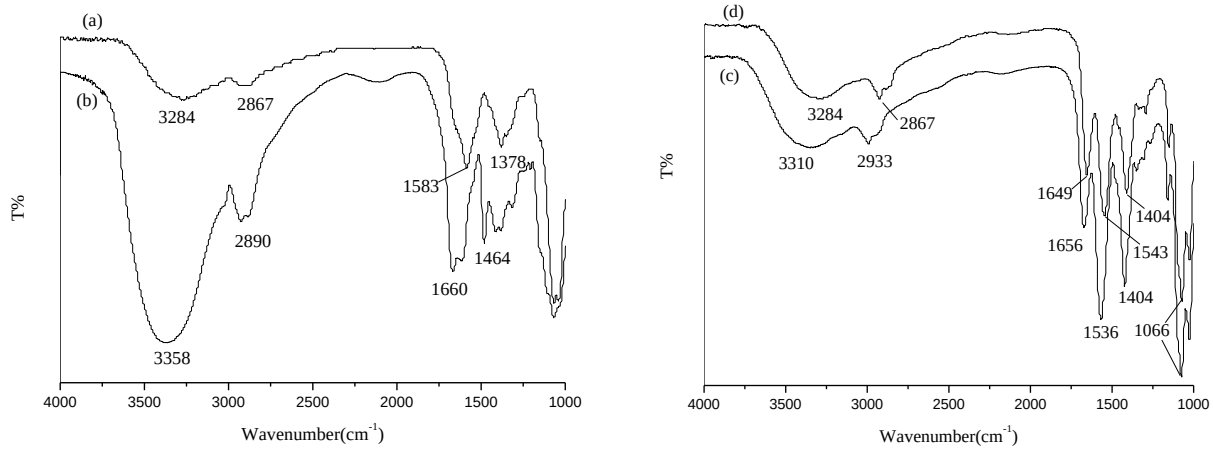


Fig.3: IR spectra of CTS'(a), QCTS'(b), CTS-PVP Gelatin Film (c), CTS-[QCTS'-I_2]-PVP Gelatin Film (d)

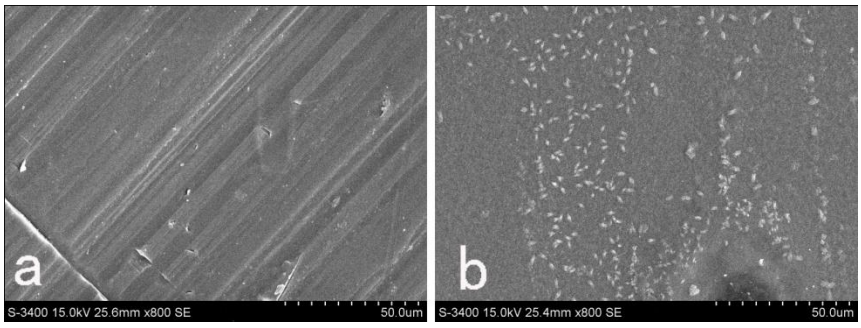


Fig.4: SEM Image of CTS-PVP Gelatin Film (a) and CTS-[QCTS'-I_2]-PVP Gelatin Film (b)

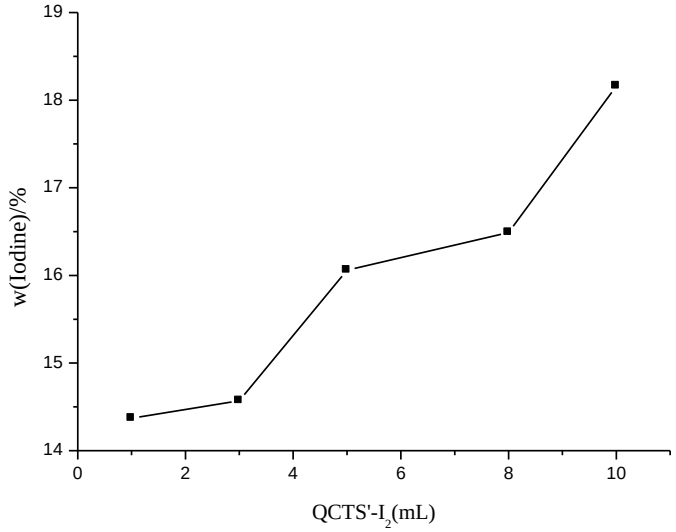


Fig.5: The Iodine Content in the CTS-[QCTS'-I_2]-PVP Gelatin Film

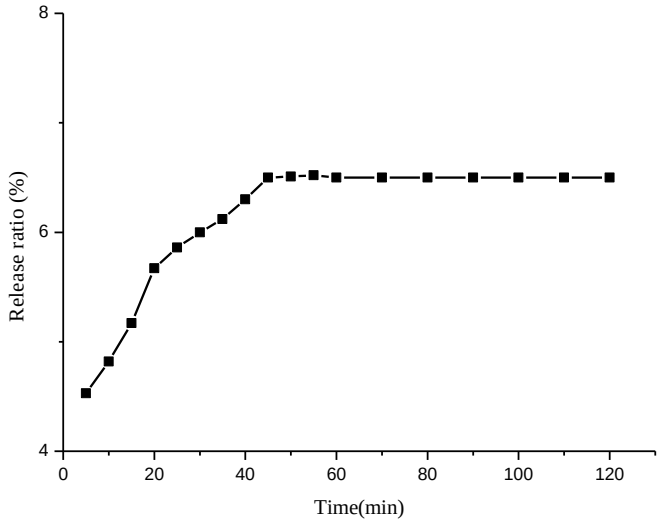


Fig.6: Iodine Releasing of CTS-[QCTS'-I_2]-PVP Gelatin Film

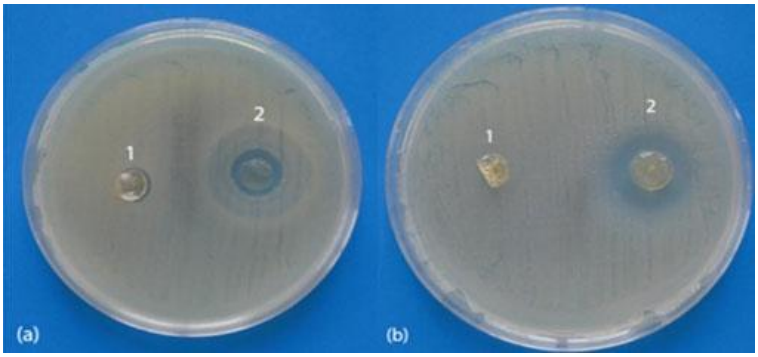


Fig.7: Inhibitory effect on *E. coli*(a) and *S. aureus* (b) by CTS-PVP Gelatin Film (1), CTS-[QCTS'-I_2]-PVP Gelatin Film (2)

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