Synthesis and Evaluation of Chitosan-Vitamin C Complexes

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ABSTRACT: Chitosan (CS) is a biocompatible, biodegradable, and nontoxic polysaccharide polymer. It dissolves in water only if the pH is lower than 6.5. To extend its range of application, many water-soluble derivatives have, therefore, been prepared. In this research, chitosan-vitamin C complexes (CSVC) were synthesized and characterized with FTIR, DSC, and ^{1}H-NMR. The solubility of CSVC in distilled water was greatly improved. The $\bullet O_2^-$ scavenging activity of CSVC was compared with CS and vitamin C (VC) by measuring the auto-oxidation rate of pyrogallic acid. Results showed that the scavenging activity on $\bullet O_2^-$ by CSVC was stronger than that by CS. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 114: 2986–2991, 2009

Key words: biopolymers; chitosan; vitamin C; FTIR; antioxidation

INTRODUCTION

Chitosan (CS), a partially deacetylated form of chitin, is an amino-containing basic polysaccharide formed primarily of repeating units of β-(1→4)-2-amino-2-deoxy-D-glucose. With a pKa of 5.5–6.5, chitosan is soluble and forms the cationic polymer in aqueous acidic medium below pH 6.5 in the presence of a small amount of acids such as inorganic acid (HCl and HNO_3) and organic acid (AcOH and lactic acid) because the amino groups dispersed on the structure are protonated. It is insoluble in water and precipitates above pH 6.5 by the addition of alkali solution.[1]

Because of its favorable physicochemical and biological properties such as being gel-formable at low pH ranges, antibacterial, biodegradability, biocompatible, epithelial permeation enhancement, mucoadhesive and nontoxic, chitosan is considered as an ideal excipient that can potentially be used as a polymeric carrier in targeted drug delivery and sustained release system.[2] The application of chitosan in pharmaceutical respect includes gel formulations, gastric float formulations, bioadhesive formulations, and colon-targeted formulations, etc.[3,4]

The application of chitosan in biomedical fields is limited owing to the poor solubility in physiological media. Through chemical modification, chitosan derivatives of specific functions (i.e., improved solubility and bioactivity) can be obtained by introducing active groups on to the hydroxyl (C3 and C6) and amino groups (C2) of chitosan molecules. Studies suggest that amino groups on the surface of chitosan chains are reactive enough to react with a number of acid chlorides, acid anhydrides, and aldehydes.[5]

In biological systems, vitamin C (VC) plays the role of an effective antioxidant due to the presence of the enediol moiety. It also serves as a cofactor in hydroxylation reactions and scavenges reactive oxygen species. The biochemical functions of VC, especially its functions in antivirus and antitumor are of increasing interests.[6] However, the use of VC is limited by its physical and chemical instability. VC is a six-carbon keto-lactone, which contains four hydroxyls and a lactone. It is highly unstable and very easy to get oxidized and changes to dehydroascorbic acid when exposed to light, air, and elevated temperature. To increase the stability of VC, various derivatives have been synthesized, including the metal salts (Na, Ca salts), ethers, esters, and the polysaccharide derivatives.[7]

Chitosan contains amino groups, which are protonatable in the acidic media, whereas VC contains acidic hydroxyl functionality, thus allowing the formation of complexeses through ionic interaction.[8] In this research, the synthesis of chitosan-vitamin C complexes (CSVC) was investigated, which was further characterized by FTIR, DSC, and ^{1}H-NMR. The apparent solubility and $\bullet O_2^-$ scavenging activity between CS and CSVC were also compared.

EXPERIMENTAL

Materials

Chitosan was purchased from Zhejiang Golden Shell Biochemical (Yuhuan, Zhijiang, China) and used

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without further purification. The viscosity molecular weight was 8.56×10^5 Da, and the degree of deacetylation was 84% as measured on an acid-base titration method. Vitamin C was from Northeast Pharmaceutical Group (Shenyang, Liaoning, China). The double-distilled water was used for all experiments. Pyrogallic acid was from Tian Jin Bodi Chemicals Company (Tianjin, China). Isopropyl alcohol was of the analytical grade.

Synthesis of chitosan-vitamin C complexes (CSVC)

A 250-mL, three-necked round bottomed flask was charged with 6.0 g of CS and 60 mL of isopropyl alcohol, and the flask was fitted with a subsurface nitrogen feed to remove dissolved oxygen from solutions. While stirring the slurry under nitrogen for 1 h, a solution of 6.62 g VC in 25 mL of distilled water was prepared. This solution was then added to the CS slurry by syringe, and after 30 min, a solution of 26 mL of isopropyl alcohol and 11 mL of distilled water was added. After stirring two additional hours, the polymer was recovered under nitrogen. The polymer was washed under nitrogen once with a solution of 160 mL of isopropyl alcohol and 40 mL of water, and once with 200 mL of isopropyl alcohol. The light tan solid was brought to near dryness under a nitrogen stream. Finally, the product was dried at 40°C under vacuum for 24 h.

Characterization

The FTIR spectra were obtained using a Bruker FTIR spectrophotometer over the wave number range between 4000 and 400 cm^{-1}. All powder samples were compressed into KBr disks for the FTIR measurements.

Thermal analysis was carried out using DSC60 Differential Scanning Calorimeter (Shimadzu, Japan). Approximately 2 mg of samples were hermetically sealed in an aluminum pan under a nitrogen atmosphere and heated from 30 to 300°C at a scanning rate of 10°C/min.

The ^{1}H-NMR spectra of CS and CSVC were obtained from 1% solution in 1% CF_3COOH/D_2O and D_2O, respectively, using a Bruker ARX-300 MHz spectrometer. The ^{1}H-NMR spectrum of VC was obtained using D_2O as the solvent. Chemical shifts were reported in parts per million.

Apparent solubility testing

According to the General Notices of Chinese Pharmacopeia (2005 Edition), 0.1 g of CSVC was added into 1 mL and 10 mL of different solvents, respectively, and stirred vigorously at 25 ± 2°C for 24 h to observe its apparent solubility based on the transparency of solutions. Solvents used were distilled water, DMSO, DMF, methanol, ethanol, acetonitrile, and chloroform.

Antioxidation activity testing[9–11]

The $\bullet O_2^-$ scavenging capability of different samples was assayed by measuring the inhibition of auto-oxidation rate of pyrogallic acid. The blank solution was prepared as follows: Tris-HCl buffer (0.05 mol/L, 4.5 mL, pH 7.2) was added into a 10-mL test tube. 1.1 mL of deionized water was then added and vortexed. The UV-vis absorbance of solutions containing pyrogallic acid without and with the test samples added were determined according to the following procedure: Tris-HCl buffer (0.05 mol/L, 4.5 mL, pH 7.2) was added into a 10-mL test tube. 1 mL deionized water or 1 mL test sample solution at different concentrations was added under vortex. Then pyrogallic acid (10 mmol/L, 0.1 mL) was added. The mixture was vortexed evenly and the absorbance at 325 nm was measured immediately against the blank solution at 60-s intervals for 4 min. The auto-oxidation rate constant of pyrogallic acid before and after the addition of test sample (k_0 and k_1, respectively) was calculated from the slope of curve of absorbance verses time. The antioxidation rate of test samples was calculated according to the following equation: Antioxidation rate $= \frac{k_0 - k_1}{k_0} * 100\%$.

The measurements were carried out in triplicate. Statistical comparisons were made using Microsoft Excel and the level of significance was taken as $P \leq 0.05$.

RESULTS AND DISCUSSION

Mechanism in the formation of CSVC

Complexes are formed by noncovelent bonds, such as electrostatic attractions, H-bonds, salt bonds, hydrophobic bonds, etc. In this study, the term "chitosan-vitamin C complexes" is different from "chitosan-vitamin C mixture" in that no protonation of chitosan occurs in the latter.[12] VC presents several electrophilic groups. It contains four hydroxyl groups in positions 2, 3, 5, and 6 with different acidities allowing acid–base reactions. The —OH in position 3 is the more acidic one (pKa = 4.2), whereas the pKa values of hydroxyls in positions 2, 5, and 6 are 11.6, 17, and 16, respectively.[13] According to the Lewis acid–base theory, the acidic hydroxyl in position 3 of VC was expected to react with the amino group of chitosan. The possible complexes formation mechanism was shown in Figure 1.

VC also presents a lactone structure, which can be subject to ring opening in the presence of amine. Comparison of the UV–VIS absorption spectrum of VC with that of CSVC indicated quite similar

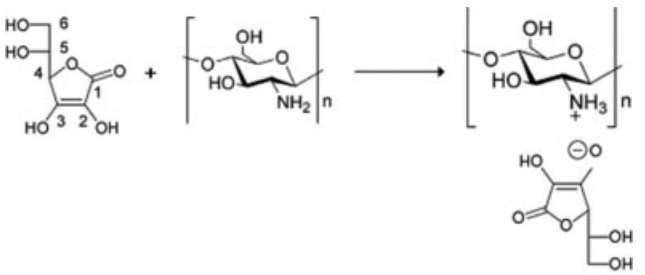

Figure 1 The possible reaction mechanism between chitosan and vitamin C.

behavior at λmax = 242 nm, clearly demonstrating the presence of VC moiety in the polymeric chains of CSVC. The ring opening reaction did not occur in the formation of complexes.

FTIR spectra

The FTIR spectra of chitosan, vitamin C, chitosan-vitamin C mixture, and chitosan-vitamin C complexes were shown in Figure 2. In Figure 2(a), a characteristic strong peak at 3417.5 cm^{-1} was attributed to overlapping stretch vibrations of $-NH_2$ and $-OH$ groups, and the peak for amide I (C=O) at 1643.7 cm^{-1} was seen. The aliphatic C—H stretching vibration of the polymer backbone occurred at 2923.6 cm^{-1}.[14] The weak deformation vibration of $C-CH_3$ appeared at 1383.8 cm^{-1}, indicating a higher degree of deacetylation of chitosan used in this experiment.[15] The four peaks in Figure 2(b) from 3524 cm^{-1} to 3214 cm^{-1} were attributed to the four $-OH$ groups at C_6, C_3, C_5, C_2, respectively. The stretch vibration of lactone C=O forming intramolecular H-bond occured at 1754.4 cm^{-1} and that of lactone C=O forming intermolecular H-bond occured at 1673.8 cm^{-1}.[16] Figure 2(d,c) showed quite different FTIR spectra from each other, demonstrating the formation of complexes between chitosan and vitamin C. The peak at 1754.4 cm^{-1}, which was the stretch vibration of lactone C=O forming intramolecular H-bond in vitamin C, was shifted to 1720.8 cm^{-1} at a reduced intensity. It could be seen that new absorption band characteristic of bending vibration of $-NH_3^+$ appeared at 1616.1 cm^{-1}. This result suggested that the $-NH_2$ groups on the chitosan chains were protonated by the H^+ supplied by

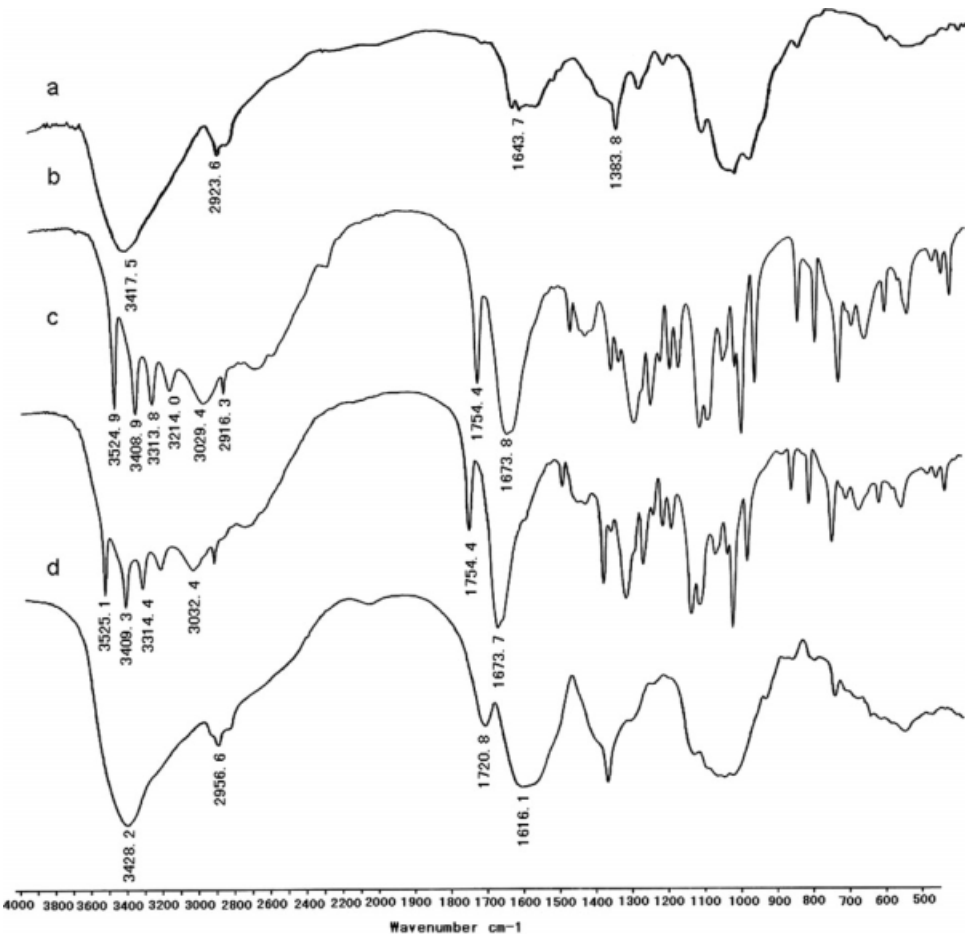

Figure 2 The FTIR spectra of chitosan (a), vitamin C (b), chitosan-vitamin C mixture (c), and chitosan-vitamin C complexes (d).

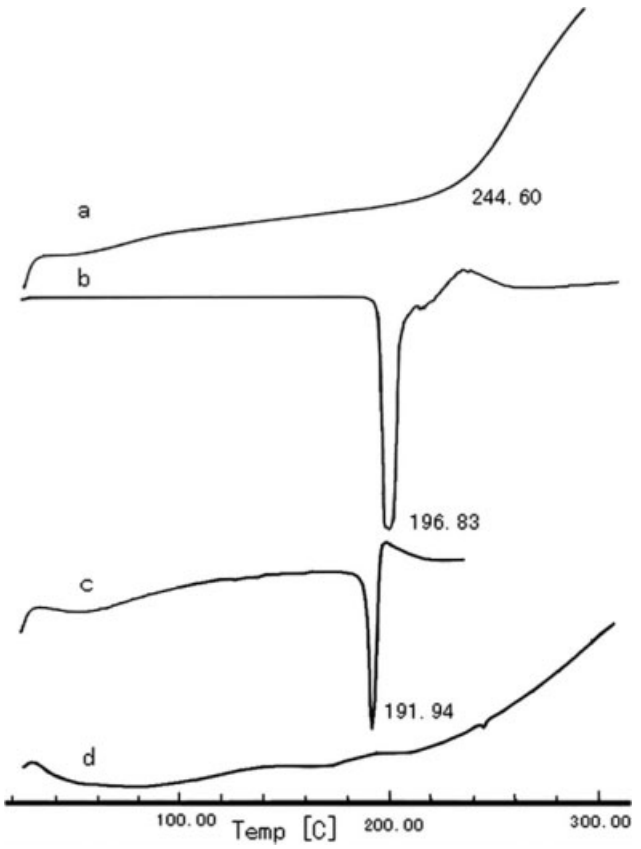

Figure 3 The DSC thermographs of chitosan (a), vitamin C (b), chitosan-vitamin C mixture (c), and chitosan-vitamin C complexes (d).

vitamin C.[17] The decrease of peak at 3428.2 cm^{-1} in Figure 2(d) indicated the reduction of free —NH_2 groups after the formation of CSVC.[18]

DSC analysis

The DSC measurements were performed to investigate changes in the physical state of materials. As shown in Figure 3(a), the temperature at about 244.6°C was the onset temperature of decomposition for chitosan.[19] In Figure 3(b,c), Vitamin C showed the melting point at 196.83°C, which was decreased to 191.94°C in the chitosan-vitamin C mixture (2 : 1, g : g). However, in CSVC [Fig. 3(d)], the endothermic peak of vitamin C could not be seen. Therefore, DSC testing results indicated the formation of complexes between chitosan and vitamin C through the ionic interaction.

NMR

The ^{1}H-NMR spectrum of chitosan [Fig. 4(a)] exhibited typical peaks, including the methyl protons from the survival *N*-acetylglucosamine unit at 1.9577 ppm (—$COCH_3$) and the proton on the carbon bearing amino groups at 3.0726 ppm (H-2). The signals at 3.6082–3.7950 were corresponding to the ring methenyl protons of chitosan backbones (H-3, H-4, H-5, H-6).[20,21] The chemical shifts in the ^{1}H-NMR spectrum of VC [Fig. 4(b)] were assigned as follows: 4.94 ppm (CH-ring), 4.02–4.07 ppm (—CH) and 3.72–3.74 ppm (—CH_2).[22,23] In Figure 4(c), the ^{1}H-NMR spectrum of CSVC showed the characteristic peaks of CS and VC with certain chemical shifts because of the ionic interaction. The signal at 4.55 ppm was characteristic of the hydrogen at the C4 position of the VC ring, which appeared at 4.94 before the formation of CSVC.

Apparent solubility testing

Although the polymer backbone consists of hydrophilic functional groups, chitosan is normally insoluble in water and most common organic solvents (e.g., DMSO, DMF, NMP, alcohols, and pyridine). The insolubility is a result of its extensive intramolecular and intermolecular H-bonding between the chains and sheets of chitosan as shown in Figure 5.[24] Our tests showed that CSVC was also insoluble in common organic solvents, such as DMSO, DMF, methanol, ethanol, acetonitrile, and chloroform. However, the solubility of CSVC in distilled water was greatly improved. Solubility tests showed that 0.1 g of the complexes formed viscous and transparent hydrogel in 1 mL of distilled water. A viscous and transparent solution was formed when 0.1 g of the complexes were added into 10 mL of distilled water. This is because the intermolecular H-bonding formed between —OH and —NH_2 groups among chitosan molecules broke down when complexes were formed.

Superoxide radical scavenging activity

Superoxide anion is a reduced form of molecular oxygen created by receiving one electron. It is an initial free radical formed from mitochondrial electrontransport systems. Superoxide anion radical is produced by a number of cellular reactions and plays an important role in the formation of other cell-damaging free radicals and molecules.[25]

Pyrogallic acid can auto-oxidize in the alkaline condition to produce colored intermediate and $\bullet O_2^-$. The colored intermediate showed maximum UV-absorption at 325 nm with a relatively constant production velocity within 4 min. The rate constant of this auto-oxidation reaction was dependent on $\bullet O_2^-$ concentration because $\bullet O_2^-$ could catalyze this auto-oxidation reaction. If samples with scavenging capacity on $\bullet O_2^-$ were added into the system, the production of colored intermediate would be decreased, showing lowered UV absorption. The

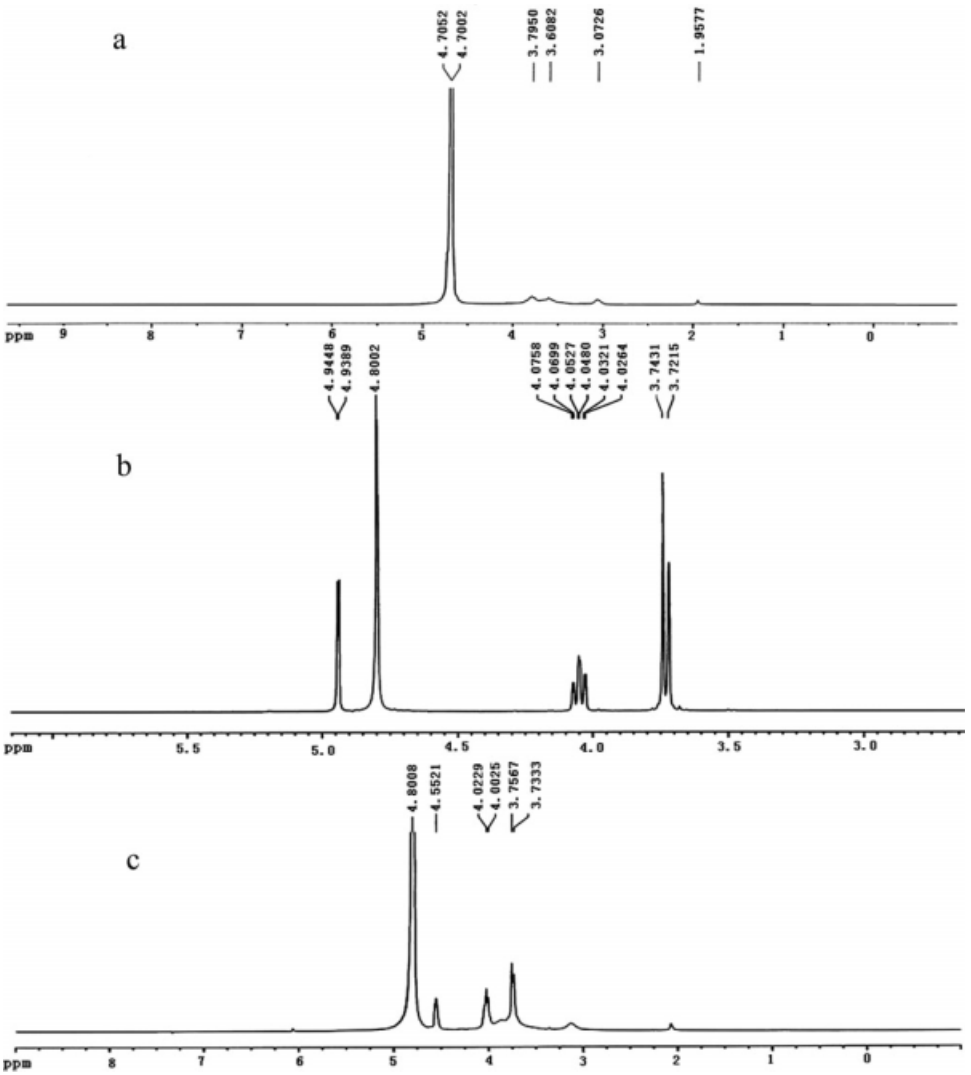

Figure 4 ^{1}H-NMR spectra of chitosan (a), vitamin C (b), and chitosan-vitamin C complexes (c).

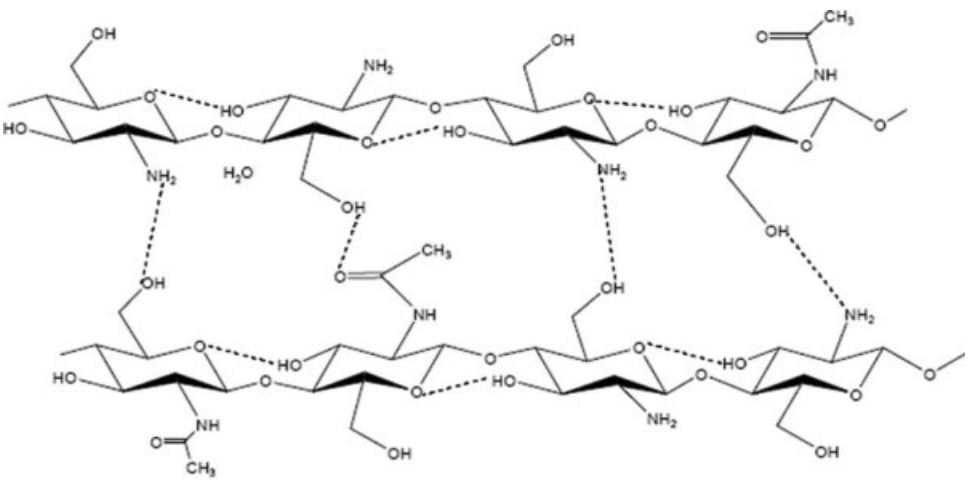

Figure 5 The crystalline structure of chitosan.

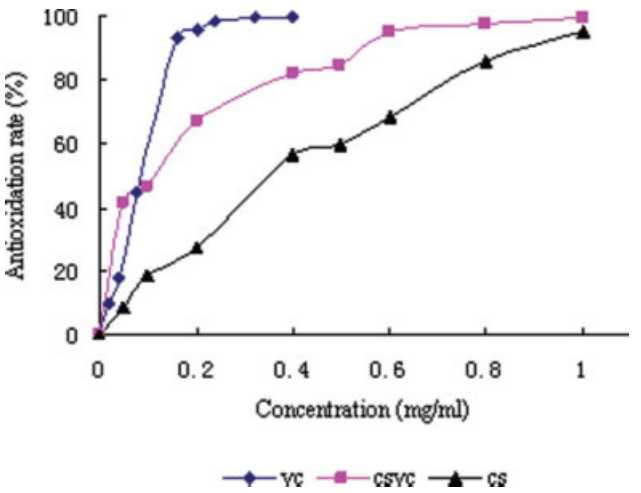

Figure 6 The antioxidation rates of CS, CSVC, and VC. [Color figure can be viewed in the online issue which is available at www.interscience.wiley.com.]

lower the absorbance, the better the scavenging effect on $\bullet O_2^-$.

Recently, the antioxidation activity of CS, and its derivatives has attracted much attention. The active hydroxyl and amino groups in the polymer chains are the origin of the scavenging ability of CS.[26]

Figure 6 showed that the inhibitory effect of samples were concentration related, which increased as the sample concentrations increased. The scavenging activity on superoxide radical by CSVC was stronger than that by CS, which was attributed to the synergic actions of vitamin C moietys present in the polymer chains. At low concentrations (<0.05 mg/mL), the scavenging activity of CSVC was stronger than that of VC, but after certain concentrations (>0.1 mg/mL), its scavenging activity was lower than that of VC.

CONCLUSIONS

In this study, water soluble chitosan-ascorbic acid complexes were synthesized by ionic interaction in the heterogeneous condition. Characterizations made by FTIR, DSC, and ^{1}H-NMR confirmed the formation of complexes. Compared with chitosan, the complexes showed good water solubility and increased $\bullet O_2^-$ scavenging capability. Pharmaceutical applications of the complexes, especially its use as a drug carrier in microspheres, nanoparticles, and other dosage forms, need our further research.

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