Preparation and Characterization of Novel Ce(III)-Gelatin Complex

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ABSTRACT: A new complex of cerium(III) [Ce(III)] with gelatin has been prepared in an aqueous solution. The FTIR, UV–vis, and XRD studies of the Ce(III)-gelatin complex revealed that the carboxyl groups of gelatin coordinated to Ce(III). The thermal property of the complex was investigated by thermo gravimetric analysis. It was discovered that the thermally decomposed rate of the complex decreased obviously. In addition, the antimicrobial activity of the complex to *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherachia coli* was measured and found that its minimal inhibitory concentration (MIC) to the growth of bacterium was 350 μg/mL, which was almost the same as Ce(III) ion. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 108: 3804–3807, 2008

Key words: Ce(III)-gelatin complex; preparation; thermal properties; FTIR; UV–vis spectroscopy; X-ray diffraction; antimicrobial activity

INTRODUCTION

It has been found that rare earth elements (REEs) have important biological effects and pharmacological actions,[1–3] besides being widely used in electronic and glass-manufacturing industries.[4] From the end of 20th century, many people directed their researches to the complexation processes between the amino acids and different rare earth metal ions. The studies consisted of the phase equilibrium of rare earth salts with amino acids in water, characterization and thermochemical properties for solid complex.[5,6] And many researches showed that these complexes can be used as pesticide, fertilizer, bactericide, and so on. Especially, because the REE can promote or inhibit the process of metabolism, the research on these complexes is one of the most essential fields of biochemistry.

Gelatin, standing for a family of proteins, is a proteinaceous material. It is obtained by denaturation and partial hydrolysis of fibrous collagen, which are the most abundant structural protein of animals and by far the main organic component of skin and bone of vertebrates. As a natural polymer, gelatin is widely used in pharmaceutical, cosmetic, photographic, and food industries.[7–9] In addition, recently, because of its low antigenity, nontoxic, biodegradable and other special advantages, its use is expanding to new applications as an important biomedical material, such as in the drug delivery system[10–12] and tissue engineering.[13,14]

Gelatin modifications not only maintain inherent biological activities of gelatin, but also gain a lot of new proprieties and functions. As aforementioned, much research interest has been focused on the amino acids and its rare earth complexes. However, there is little research on the interaction between rare earth ions and proteinaceous macromolecule. But the potential arises from the fact that the amino acids of gelatin have active groups that can also interact with rare earth ions. So the main purpose of this work is to study the preparation and some properties of the cerium(III)-gelatin complex, which may help to provide a new biomedical material.

MATERIALS AND METHODS

Raw materials

Gelatin powder type B (bloom value 274) was purchased from Rousselot Co. (Guangdong, China) and used as received. The cerium(III) nitrate hexahydrate was supplied by the QinXi Chemical Co. (Shanghai, China). Nutrient agar was supplied by Luqiao, Beijing. Other reagents were all Analysis purity.

Staphylococcus aureus, *Pseudomonas aeruginosa*, and *Escherachia coli* were supplied by Beijing Centers for disaster control prevention.

Preparation of Ce(III)-gelatin complex

One gram of powder gelatin was soaked in 20 mL deionized water and heated to 60°C to get a homo-

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geneous solution (its pH was adjusted to 4.6 with 0.5 mol/L HCl). Then, 0.1 mol/L cerium nitrate hexahydrate solution was added to the gelatin solution and the system was placed in a water-bath controlled at a certain temperature. The obtained mixture was concentrated in the Rotary Evaporator and cooled; the deposition was separated and then washed by ethanol. It was desiccated in the desiccator.

Characterization of the Ce(III)-gelatin complex

FTIR and UV

FTIR spectra of gelatin and Ce(III)-gelatin were recorded using a spectrophotometer Nicolet Nexus 670. The spectra were recorded by absorption mode at 4 cm^{-1} interval and 16-times scamming. It was in the wavelength range of 4000–400 cm^{-1} wavenumbers.

The gelatin and Ce(III)-gelatin were also examined by a UV–vis spectrophotometer (CINTRA20) at a predetermined time interval from 190 to 400 cm^{-1} wavenumbers.

X-ray diffraction

X-ray diffraction (XRD) patterns were recorded at 10°/min on a Japan Rigaku D/max 2500 diffractometer using Cu-Kα radiation (λ = 0.154 nm) at a generator voltage of 40 kV and a generator current of 100 mA. The scattering angle was ranged from 5° to 90°.

Thermo gravimetric analysis

The thermo gravimetric measurements were performed on a Netzsch TG 209 instrument under nitrogen atmosphere at a heating rate of 10°C/min.

Antibacterial activity

The antibacterial activity of the Ce(III)-gelatin was determined by the minimal inhibitory concentration (MIC; μg/mL), as usual. *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* were inoculated to the nutrient agar in the Petri dish. They were tested using serial dilutions ranging from 5.5 to 2800 μg/mL. Test isolates were maintained as frozen stocks which were thawed and adjusted to inoculum density of $\sim 5 \times 10^5$ to 5×10^6 cfu/mL. Following inoculation, microtitre assay plates were incubated at 36°C for 24 h. The minimum inhibitory concentration (MIC) was then determined as the lowest concentration of the Ce(III)-gelatin that inhibited visible growth of the test isolates.

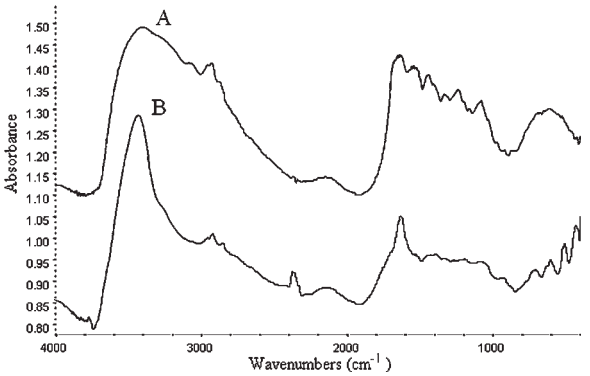


Figure 1 FTIR spectra of (A) gelatin and (B) Ce-gelatin.

RESULTS AND DISCUSSION

FTIR for Ce(III)-gelatin

Previous researches indicate that there are three probable coordinate sides in gelatin: the carboxylic oxygen in side chains, the amino nitrogen, and the acyl nitrogen. Because the pH of this system was about 4.5, the isolated carboxyl group may present, which can be react with Ce(III). The reaction between gelatin and Ce(III) was distributed at 1800 to 1200 cm^{-1}. Figure 1 shows the wide scan FTIR spectra for Ce(III)-gelatin sample and gelatin. It can be obviously seen that the FTIR spectra of the complex is different from that of the gelatin, which means some change happened in gelatin. From Figure 1(A), we can observe the characteristic absorption band of gelatin: the amide I band (antisymmetric stretching vibration of carboxyl group or C=O vibration) having major peak at 1634.39 cm^{-1} and the amide II band (*N*—H bending vibration) having major peak at 1542 cm^{-1}. But in Figure 1(B), the amide I band shifted to 1639.22 cm^{-1}, the amideII band not having obviously changes. Moreover, in Figure 1(B), we can find that the absorption band of C—O symmetric stretching vibration at 1243 cm^{-1} in Figure 1(A) disappeared. So, we can conclude that there is a directly reaction between Ce(III) and carboxyl group in side chains of gelatin.

UV for Ce(III)-gelatin

The UV–vis absorption spectra of the gelatin and Ce(III)-gelatin are shown in Figure 2. It was found that the gelatin [Fig. 2(B)] displayed a distinct absorption peak at 230 nm and a weak one at 280 nm. In contrast, the absorption spectra of Ce(III)-gelatin [Fig. 2(A)] showed a sharper and better-defined absorption at 250 nm, which might be attributed to the reaction between gelatin and Ce.

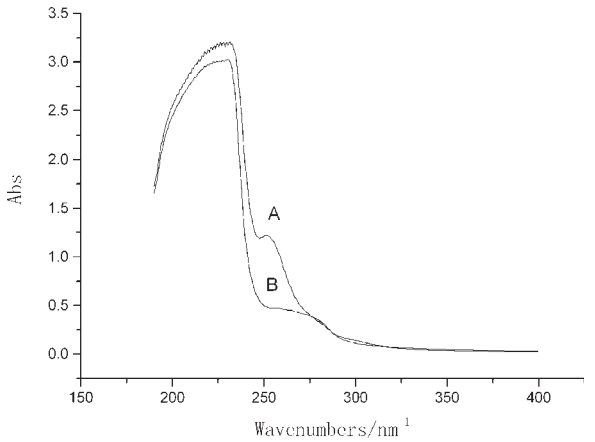
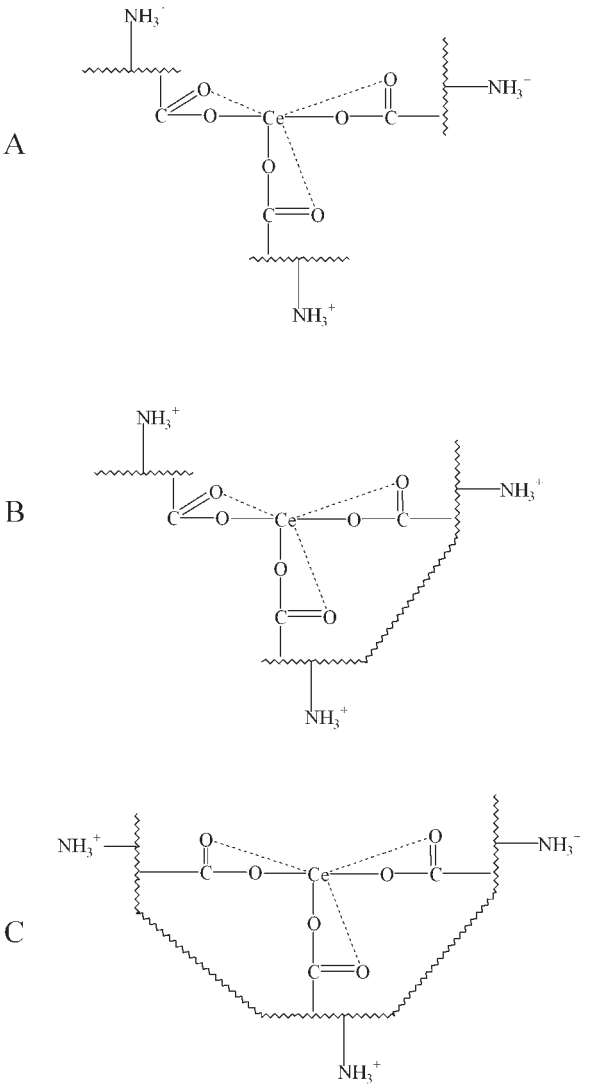


Figure 2 UV–vis spectra of (A) Ce(III)-gelatin and (B) gelatin.

X-ray diffraction

Wide angle XRD of gelatin [Fig. 3(B)] displays characteristic XRD pattern, in the sample considered here, much of the scattered intensity was present in all samples as a broad isotropic amorphous peak at 20°, which was also observed from Ce(III)-gelatin [Fig. 3(A)]. There was another peak, not as same as the pinnacle of pure $Ce(NO_3)_3$, in the Ce(III)-gelatin sample at 41.233°, which was indicative of reaction between gelatin and Ce(III), but not the simple blending. The plots also revealed that the Ce(III)-gelatin was amorphous as well as the gelatin.

On the basis of the above analytical data, we can conclude that the carboxyl group in side chain of gelatin can react with Ce(III) to form a coordinated complex having the proposed structure as shown in Scheme 1. The complex may be both intramolecular and intermolecular.



Scheme 1 Structure of the Ce(III)-gelatin.

Thermal properties

The TGA curves of Figure 4 revealed that the onset thermal-decomposed temperature of Ce(III)-gelatin is 332°C, while that of gelatin is 283.3°C, and the thermal decomposed rate is obviously reduced. Moreover, from the DTG curves of Figure 5, it can be

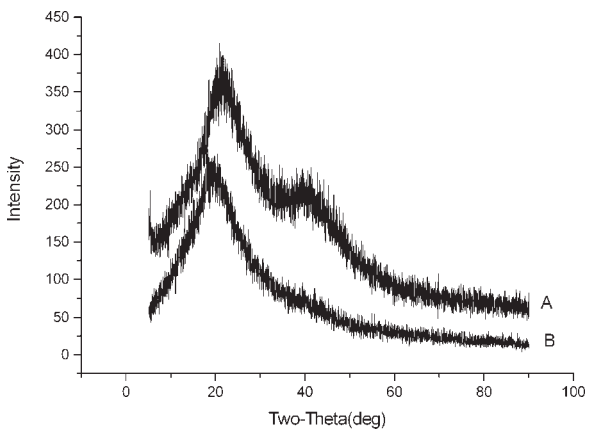


Figure 3 XRD patterns of (A) Ce(III)-gelatin and (B) gelatin.

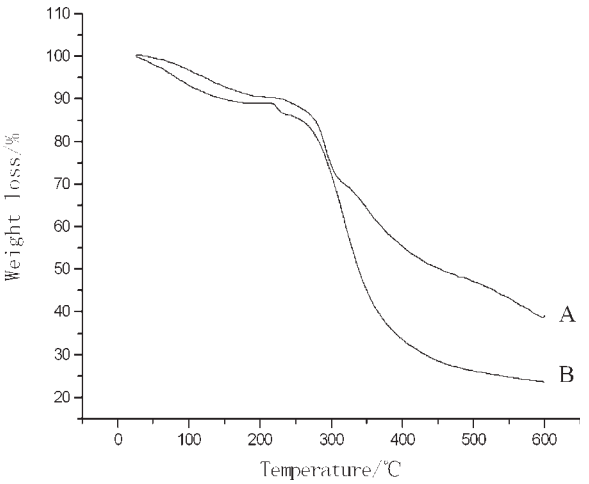


Figure 4 TGA curves of (A) Ce(III)-gelatin and (B) gelatin.

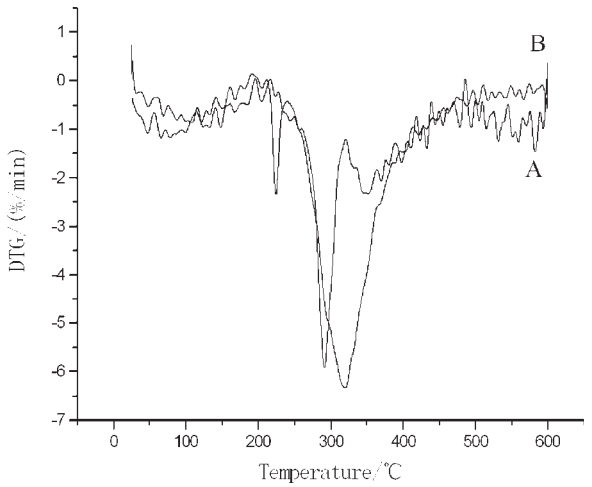


Figure 5 DTG curves of (A) Ce(III)-gelatin and (B) gelatin.

seen that the maximum thermal decomposed rate and T_{max} of gelatin are 6.3%/min and 320.3°C, while the corresponding ones of the complex are 5.9%/min and 291.7°C, respectively. Therefore, the thermal decomposed rate of Ce(III)-gelatin drops to a large extent. We find that compared with gelatin, the thermal stability of Ce(III)-gelatin was improved.

Effect of antimicrobial activity

Because Ce(III) showed antimicrobial activity, we also tested the effect of the Ce(III)-gelatin on three bacteria. Table I showed the antimicrobial activity of the complex to *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherachia coli*.

Antimicrobial activities of the complex and the coordinators are listed in Table I, as estimated by the MIC (μg/mL). MICs of the complex are all 350 μg/mL for *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherachia coli*. However, these of gelatin showed no activity. As previously found, the Ce(III) ion, as aqueous $Ce(NO_3)_3$ showed remarkable activities against Gram-negative bacteria (such as *Escherachia coli*) and Gram-positive bacteria (*Staphylococcus aureus*). These results strongly suggest that the antimicrobial activity of the complex is attributed to the cerium's property.

TABLE I
The MIC of Ce(III)-Gelatin Complex and Complex Coordinators

	MIC (μg/mL)		
Organism	Ce (III)-gelatin	Ce (III)	Gelatin
Escherachia coli	350	350	n.i.[a]
Pseudomonad aeruginusa	350	350	n.i.[a]
Staphylococcus aureus	350	350	n.i.[a]

[a] n.i., no inhibition.

CONCLUSIONS

A new complex of Ce(III)-gelatin was first prepared in an aqueous solution. The IR, UV–vis, and XRD provided information related to the proposed structure of the complex. As showed in Figures 1–3, the Ce(III) could react with gelatin to form a complex. And the thermal property of the complex is different from that of gelatin.

Interestingly, it was found that the complex has the same antimicrobial activity as Ce(III) and would be an important biomedical material because it has the nutrition property of gelatin as well as the bacteriostic activity of Ce(III).

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