Preparation and characterization of chitosan amino acid salts

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Abstract. Chitosan has many unique properties, such as the extensive sources, renewability, excellent biocompatibility and biodegradability. To improve the water solubility of chitosan is an effective approach to overcome the limitations of its application. In this paper, three water-soluble chitosan acid salts were prepared via a simple and green process by using chitosan and amino acids as the original materials. The water solubility and the moisture absorption of the prepared chitosan salts were tested. Meanwhile, three chitosan amino acid salts were characterized by Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD) and potentiometric titration. The results showed that the water solubility of three chitosan salts was greatly improved and the moisture absorption was more than 15.3%-32.8% higher than that of chitosan. Structural analysis indicated that the formation of chitosan amino acid salts were via protonation reaction rather than simple physical adsorption. Relative to chitosan, the crystallinity of chitosan amino acid salts were decreased. The structural change of chitosan has greatly improved its performance and expanded its application fields.

1. Introduction
Chitosan, the only basic polysaccharide found in nature so far, is of non-toxicity, biocompatibility, biodegradability, and has many biological functional properties, such as bio-adsorption, antibacterial, hemostasis and wound healing [1-3]. However, chitosan has poor water solubility in alkaline and neutral media, which greatly limits its application. It is one of the main research points of chitosan chemistry to improve the solubility of chitosan by chemical modification [4]. As a result, a variety of chitosan derivatives have been synthesized. Nevertheless, these synthetic products were either prepared with harsh conditions or introduced into new groups. The introduction of new groups not only affected the biological properties of chitosan, but even reduced its safety [5-6]. The powdery chitosan salt is a protonated product of chitosan. It has good water solubility and stability, is easy to transport and use, and has many unique physiological activities and functional properties [7-9].

Amino acids, the basic units of protein composition, have excellent biocompatibility, affinity and special functionality [10-12]. They were widely applied in food, medicine, additives and cosmetics industries, especially in the pharmaceutical industry. For example, 4-hydroxyproline was very effective in treating chronic hepatitis and preventing cirrhosis. And S-carbamyl-L-cysteine could achieve the antitumor effects as an amino acid structural analogue needed by tumor cells. Due to the amphoteric nature of amino acids, it is easy to introduce amino acids into chitosan to obtain chitosan salts with multiple biological activities.
In this paper, water-soluble chitosan amino acid salts were successfully prepared in water medium and purified by alcohol precipitation. Meanwhile, the target products were characterized and tested so as to attract extensive attention in the application fields.

2. Materials and methods

2.1. Materials
Chitosan (molecular weight 200 kDa, deacetylation degree ≥90%) was purchased from Zhejiang Aoxing Biotechnology Co., Ltd. Amino acids (including L-glutamic acid, L-aspartic acid and L-threonine) were purchased from Nanjing Songguan Biotechnology Co., Ltd. The other reagents were of analytical grade and used without further purification.

2.2. Preparation of chitosan amino acid salts
Chitosan (1.0 g) was added into a beaker containing 30 mL deionized water and swollen overnight. Then amino acids (0.5 g) were slowly added while stirring. The mixture was stirred for 24 hours at room temperature and half of the solvent was removed under the reduced pressure. Anhydrous ethanol was slowly added into the solutions until no fibrous precipitate appeared. The suspension was centrifuged at 3500 rpm for 5 minutes and the precipitate residue was washed with ethanol for twice. After vacuum drying, the off-white powders were obtained.

2.3. Determination of water solubility
The dried chitosan amino acid salt samples (0.1 g) were weighed accurately and dissolved in 20 mL distilled water. The absorbance of the solution at 420 nm was measured and the distilled water was used as a blank control.

2.4. Determination of water absorption
The dried samples of chitosan amino acid salts were accurately weighed (M₀) and put into a bottle. Then the total mass (M₁) of the samples and the bottle was weighed. The bottle was then placed in a closed environment with a relative humidity of 43% (saturated sodium carbonate solution). After 24 h, the mass (M₂) of the bottle was weighed again. The moisture absorption percent of the samples was calculated according to the following formula:

\[
\text{Moisture absorption (\%)} = \frac{(M_2 - M_1)}{M_0} \times 100
\]

Where \( M_1 \) and \( M_2 \) were the total mass of the samples and the bottle before and after moisture absorption, respectively, in g; \( M_0 \) was the mass of the dried samples, in g.

2.5. Potentiometric titration
Potentiometric titration was carried out by using the 0.1 mol·L⁻¹ sodium hydroxide and hydrochloric acid as the titrants in forward and backward titrations, respectively. Samples of chitosan amino acid salts (0.2 g) were added into 20 mL distilled water and stirred until completely dissolved. Then sodium hydroxide was added with a micrometer syringe while magnetically stirred. The values of pH were recorded per 3 min after addition of alkali. The pH-NaOH volume titration curve was made to analyze the chemical structure of samples.

2.6. FTIR characterization
FTIR spectra were recorded by using an IR spectrometer (Spectrum 100, PerkinElmer, MA, USA). Samples were prepared using the method of compressed KBr discs. About 2 mg powders were used for testing with a wave number range from 4000 to 450 cm⁻¹ at a resolution of 4.0 cm⁻¹.
2.7. **XRD analysis**
The crystal structure of samples was characterized by using an X-ray powder diffractometer (D8 Advance, Bruker, Germany) with a Cu Kα radiation, a voltage of 40 kV and a current of 100 mA. The scanning range was from 5° to 50° with a scanning speed of 5°/min. And the X-ray diffraction curve of the powdery samples was recorded.

3. **Results and discussion**

3.1. **Preparation of chitosan amino acid salts**
Three chitosan amino acid salts including chitosan glutamic acid salt (CGS), chitosan aspartic acid salt (CAS) and chitosan threonine salt (CTS) were successfully prepared via a simple and green preparation process. The yields were 112.5%, 121.7% and 116.3%, respectively. The appearance of the solid powders was loose and off-white.

3.2. **Water solubility of chitosan amino acid salts**
The water solubility of chitosan amino acid salts was investigated by UV absorption method. The results were shown in Table 1. The absorbance of three salts was close to that of distilled water, indicating that three chitosan amino acid salts had good water solubility, especially acidic amino acid salts (CAS and CGS). This might be related to the isoelectric points of amino acids, because the isoelectric points of aspartic acid (2.77) and glutamic acid (3.22) were lower than that of threonine (6.16), resulting in the higher ionization degree of chitosan amino groups. Chitosan is insoluble in neutral and alkaline aqueous solutions and the water solubility of these salts was greatly improved compared with chitosan.

| Samples | CGS  | CAS  | CTS  |
|---------|------|------|------|
| OD\textsubscript{420} | 0.042 | 0.035 | 0.068 |

3.3. **Moisture absorption of chitosan amino acid salts**
The moisture absorption of the materials has a promoting effect on the healing of the wound surface. The moisture absorption of three chitosan amino acid salts was investigated and the results were shown in Figure 1. Compared to chitosan, the moisture absorption of three chitosan amino acid salts was increased by 20.4% (CGS), 32.8% (CAS) and 15.3% (CTS), respectively. The moisture absorption of a compound is related to its own hydrophilic groups, polarity and chemical structure. For the salts, on the one hand, the hydrophilicity of the amino group was enhanced after protonation; on the other hand, the crystal structures of the solid chitosan salts were changed obviously.
3.4. Potentiometric titration

Potentiometric titration curves of chitosan amino acid salts had been determined for the aqueous solutions, which were subject to the protonation degree of amino groups by a change in pH. And the results were shown in Figure 2. Two titration jumps occurred on the titration curve of CGS and CAS. The first jump was attributed to the free amino acids adsorbed by chitosan, and the second jump was related to the protonated chitosan (\(-\text{NH}_3^+\)). Whereas, there was only one jump on the titration curve of CTS, probably because threonine was a neutral amino acid so that the adsorbed free amino acids and \(-\text{NH}_3^+\) could not be titrated grade. Additionally, it could also be seen that the aqueous solutions of the chitosan salts had a strong buffering effect, thereby expanding their dissolution ranges.

![Figure 1. Moisture absorption of chitosan amino acid salts.](image1)

![Figure 2. The titration curves of chitosan acid salts: (a) CGS; (b) CTS; (c) CAS.](image2)
3.5. FTIR characterization
As can be seen from Figure 3(a), chitosan presented a broad absorption band at about 3400 cm\(^{-1}\), corresponding to \(-\text{OH}\) and \(-\text{NH}\) stretching vibrations. The characteristic absorption peaks of amide I (C=O) and II (-\text{NH}_2) bands appeared at about 1654 and 1593 cm\(^{-1}\), respectively [13]. From the spectra of chitosan amino acid salts (Figure 3 (b-d)), the broad absorption band at about 3400 cm\(^{-1}\) became wider and weaker than that of chitosan, which indicated the formation of -\text{NH}_3^+ in the molecule. In the spectra of CGS (Figure 3b) and CAS (Figure 3d), the absorption peaks of free amino groups at 1593 cm\(^{-1}\) disappeared, showing that the interaction between -\text{COOH} of amino acids and -\text{NH}_2 of chitosan had occurred. In a word, FTIR spectra indicated that chitosan amino acid salts formed successfully by protonation reaction rather than simple physical adsorption.

![FTIR spectra](image)

**Figure 3.** FTIR spectra of: (a) chitosan; (b) CGS; (c) CTS; (d) CAS.

3.6. XRD analysis
Figure 4 showed the XRD patterns of chitosan and its amino acid salts. The pattern of chitosan gave the characteristic diffraction peaks at 10° and 20° (2θ). For three chitosan amino acid salts, the peak at around 20° became lower and broader than that of the pure chitosan. On the other hand, three chitosan salts exhibited the patterns which lacked the characteristic peaks at 10° and appeared some small sharp peaks. These indicated that the polysaccharide chains of chitosan acid salts were partly destroyed, resulting in the decrease of crystallinity.
4. Conclusions
Three chitosan amino acid salts were successfully obtained by a simple and green approach. And their structures were characterized by FTIR spectroscopy, XRD and potentiometric titration, confirming that the formation of chitosan amino acid salts were via protonation reaction rather than simple physical adsorption. Compared with chitosan, the properties of chitosan amino acid salts obtained were greatly improved, which provided a broader prospect for the application.

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Author contributions
Zhang Hu designed the experiments and wrote the paper; Ya Qi Qin and Jie Guang performed the experiments; And Ya Qi Qin analyzed the data; Ying Cai contributed the experimental materials and proofread the paper.

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