Preparation and Characterization of Vanillin Cross-linked Chitosan Microspheres Modified by Silver Nanoparticles

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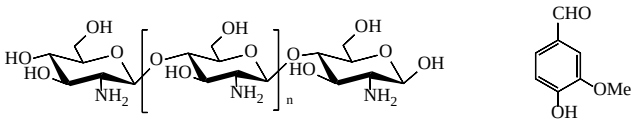
In this work, the vanillin cross-linked chitosan microspheres were firstly prepared by emulsion chemical cross-linking method with vanillin as a cross-linker. Subsequently, with polyvinylpyrrolidone as a nanoparticle stabilizer and dispersant, silver nanoparticles were modified onto the surface of vanillin cross-linked chitosan microspheres by in situ reduction with ascorbic acid to obtain silver nanoparticles modified microspheres. The chitosan and two kinds of microspheres were characterized by SEM, FTIR and XRD. The SEM images showed that the two kinds of microspheres had clear and well-defined spherical shapes with an average particle size of 4.2 μm and the silver nanoparticles were then evenly dispersed on the surface. The FTIR spectra revealed that chitosan was successfully cross-linked by vanillin through Schiff base reaction and hydrogen bond interactions. The X-ray diffraction pattern of vanillin cross-linked chitosan microspheres modified with silver nanoparticles indicated that the silver nanoparticles had a face-centred cubic structure with an average particle size of 44.4 nm confirmed by SEM, which proved the successful synthesis of target composite microspheres.

Keywords: emulsion chemical cross-linking method, chitosan, vanillin, microspheres, silver nanoparticles.

1. INTRODUCTION

Chitosan (CS), also known as de-N-acetylated chitin, is the product of deacetylation of chitin existing widely in nature. Chitosan has a variety of physiological functions such as biodegradability, biocompatibility, non-toxicity and bacteriostatics, and it is the only basic amino polysaccharide found in natural polysaccharides [1, 2]. A large amount of alcoholic hydroxyl (-OH) and amino (-NH_2) groups in chitosan molecules can be acylated [3], hydroxylated, alkylated or react with other chemicals to obtain multiple chitosan derivatives with special functions [4]. The chemical structure of chitosan is shown in Fig. 1.

Vanillin (VA) is an organic compound extracted from vanilla beans of the plants in the Rutaceae family and has a strong vanilla aroma and rich milk fragrance. Moreover, VA is also a natural plant ingredient, generally recognized as a relatively safe food additive and plays an important role in bacteriostasis and bacterial killing [5]. The chemical name of VA is 4-hydroxy-3-methoxy benzaldehyde and the corresponding chemical structure is also shown in Fig. 1.



Chitosan Vanillin

Fig. 1. Chemical structures of chitosan and vanillin

Kong [6] et al. prepared chitosan microspheres by emulsion chemical cross-linking method with smooth surfaces and an average diameter of about 124 μm, and investigated the interface contacting inhibition behaviors of chitosan against bacteria in the dispersing state. The results concluded that the highest antimicrobial activity was observed with a deacetylation degree of 63.6 %. Jia [7] et al. prepared chitosan derivatives by a Schiff base reaction with methyl iodide and studied the antimicrobial activity of chitosan derivatives against Escherichia coli. The results showed that chitosan derivatives had better antimicrobial activity against Escherichia coli and the antibacterial activity of N-N-propyl-N, N-dimethyl chitosan is 20 times that of chitosan. Sanpui [8] et al. prepared a chitosan-Ag-nanoparticle composite by reducing $AgNO_3$ with chitosan under alkaline conditions and it showed significant antimicrobial activity against Escherichia coli.

Chitosan with rigid backbones can be modified into branched polysaccharides by cross-linking chitosan with vanillin (CS-g-VA), which still has good comprehensive properties similar to chitosan and vanillin with bacteriostatic and bactericidal properties [9]. The reaction mechanism of chitosan cross-linked with vanillin is shown in Fig. 2 and the reaction of the amino groups (-NH_2) of chitosan and the carbonyl groups (-C=O) of vanillin results in the formation of C=N bonds and hydrogen bonds via a Schiff base reaction.

As shown in Fig. 3, chitosan-based microspheres can be prepared by the emulsion chemical cross-linking method. In the preparation process in previous works, chitosan was dissolved into a certain concentration of an acetic acid solution, and then the mixture was dripped into an oily solution containing a small amount of emulsifier with a syringe. After a stable water-in-oil (W/O) emulsion system

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was formed, aldehydes were used as cross-linkers for cross-linking curing.

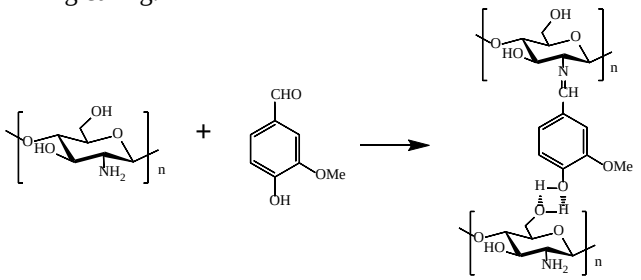

Fig. 2. Reaction mechanism of vanillin cross-linked chitosan

At last, the chitosan microspheres were obtained by centrifugation and filtration, then washed and dried under ambient conditions [10, 11].

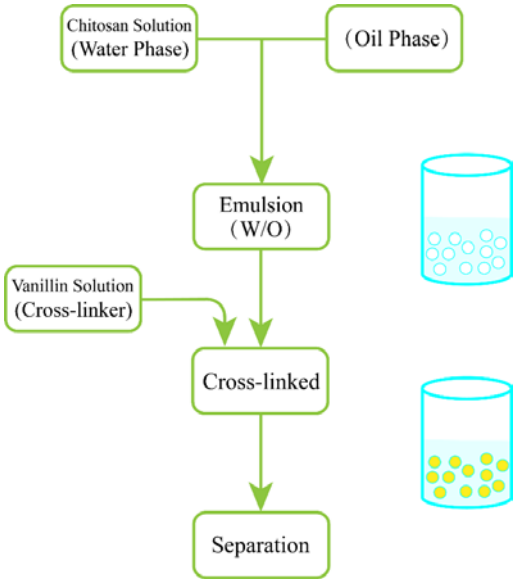

Fig. 3. Schematic diagram of the preparation of chitosan microspheres by emulsion chemical cross-linking method

Generally speaking, if the CS-g-VA is spherical it can increase the specific surface area and provide a venue for the subsequent loading of metal nanoparticles such as silver nanoparticles (AgNPs) to realize some special functions. As is known to all, AgNPs are biocompatible and have low toxicity and durable bactericidal properties, which endow them with potential applications in biotechnology and biomedical science [12, 13].

Thus, based on previous researches, the main focus of this work is to prepare CS-g-VA microspheres and then introduce AgNPs onto the surface of microspheres to achieve AgNPs modified CS-g-VA (AgNPs/CS-g-VA) microspheres. The composite microspheres with embedded AgNPs would make them easy to use and separate with good functional performances [14 – 16].

2. EXPERIMENTAL DETAILS

2.1. Materials

Chitosan (BR), with 80 – 95 % deacetylation degree, was purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Vanillin (AR), liquid paraffin (CP), silver nitrate (AR), ascorbic acid (AR) and span-80 (AR) were all purchased from Shanghai Adamas Reagent Co., Ltd. (China). Polyvinylpyrrolidone (PVP) was purchased from TCI (Shanghai) Development Co., Ltd., petroleum ether (AR), acetone (AR) and anhydrous ethanol (AR) were purchased from Sinopharm Chemical Reagents Co., Ltd. (China) and used as received without further purification.

2.2. Preparation of various samples

A schematic diagram of the preparation of CS-g-VA microspheres is shown in Fig. 4. First, 2 mL span-80 and 100 mL liquid paraffin were mixed under constant magnetic stirring at a speed of 1000 rpm overnight at 30 °C until well-distributed, then the mixture was used as an oil phase. Secondly, 2 wt.% chitosan solution was prepared by dissolving chitosan in 2 wt.% acetic acid aqueous solution and stirred until well-distributed to obtain a water phase. Subsequently, the 5 mL water phase was gradually added into the oil phase with a medical syringe and the mixture was continuously stirred at 1000 rpm at 30 °C to finally form a W/O system with micro-emulsion droplets after about 15 min. When the system was stabilized, 1 mL acetone solution of vanillin was added dropwise into the W/O system with a medical syringe under constant stirring at a speed of 1000 rpm at 30 °C for 5 h. Finally, the microspheres were collected by centrifugation (5 min, 10000 rpm), filtration and washed with petroleum ether, acetone once and deionized water. Finally, the microspheres were dried under a vacuum at 40 °C for 48 h and kept in a desiccator for further analysis.

Fig. 4. Schematic diagram of the preparation of CS-g-VA microspheres

The preparation process of AgNPs/CS-g-VA microspheres can be seen in Fig. 5.

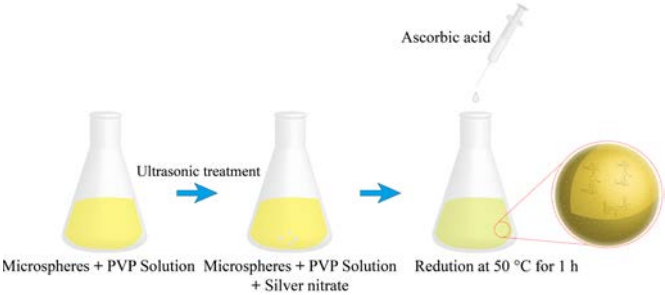

Fig. 5. Schematic diagram of the preparation of AgNPs/CS-g-VA microspheres

Firstly, the as-prepared 0.1 g CS-g-VA and an appropriate amount of PVP were dispersed in 30 mL deionized water by ultrasonic treatment for 30 min to form

a homogeneous solution. Then 5 mg silver nitrate ($AgNO_3$) was added to the above aqueous solution in sequence. After that, 1 mL ascorbic acid aqueous solution was slowly added to the mixed solution with a medical syringe and stirred for 1 h at 50 °C. At last, the product was collected by centrifugation (5 min, 10000 rpm) and washed with anhydrous ethanol and deionized water, then the separated composite microspheres were dried under a vacuum at 40 °C for 12 h.

2.3. Characterization

The morphological features of all samples were examined by a scanning electron microscope (SEM) (Hitachi S-4800, Japan) at 5 kV voltage. After drying down, samples were mounted onto the sample stage with double-sided carbon conductive adhesive and sprayed with gold for 20 s. The average particle size and the particle size distributions of microspheres or nanoparticles were measured and estimated using ImageJ software.

FTIR experiments were conducted using a Fourier transform infrared spectrometer (Shimadzu IRPrestige-21, Japan) with the KBr pellet method in the wavenumber range of 400 – 4000 cm^{-1} with a 2 cm^{-1} resolution.

X-ray diffraction (XRD) experiments were performed with an X-ray diffractometer (Bruker D8 Advance, Germany) by using Cu-Kα radiation at 0.15418 nm at a scanning rate of $0.02°\cdot s^{-1}$ in the 2θ range from 5° to 90°.

3. RESULTS AND DISCUSSION

3.1. SEM analysis

Fig. 6. shows the morphologies of CS-g-VA and AgNPs/CS-g-VA microspheres with similar particle size and roundness but different smoothness, and the particle size distributions of the two kinds of microspheres are given in Fig. 7.

As can be seen from Fig. 6 a, b and c, SEM images confirm the successful preparation of CS-g-VA microspheres and the microspheres are well-defined spherical shapes with an average particle size of 4.2 μm (see Fig. 7 a). From Fig. 6 d, e and f, it can be seen that AgNPs/CS-g-VA microspheres are successfully prepared by in situ reducing silver ions with ascorbic acid and the agglomeration of AgNPs can be reduced due to the addition of PVP [17], and the average particle size of AgNPs embedded on the surface is about 44.4 nm (see Fig. 7 b).

In addition, the surface of AgNPs/CS-g-VA microspheres is rougher after modification by AgNPs and the grooves embedding AgNPs may increase the specific surface area of the microspheres.

3.2. FTIR analysis

Fig. 8 shows the FTIR spectra of CS, CS-g-VA, AgNPs/CS-g-VA microspheres, respectively. As can be seen from the spectrum of CS, a broad absorption peak at around 3421 cm^{-1} is observed and assigned to the -OH stretching vibration absorption formed by hydrogen bond association and $-NH_2$ stretching vibration absorption, in which the -OH stretching vibration is overlapped by N-H stretching [18]. The absorption vibration of C-H stretching of methyl of methylene groups of chitosan is at 2877 cm^{-1} and the absorption band at 1633 cm^{-1} corresponds to the C=O stretching mode (or amide I band). The peak at 1384 cm^{-1} is assigned to the characteristic absorption peak of C-N stretching vibration and the absorption peak of C-O stretching vibration in $-CH_2$-OH is at about 1083 cm^{-1}.

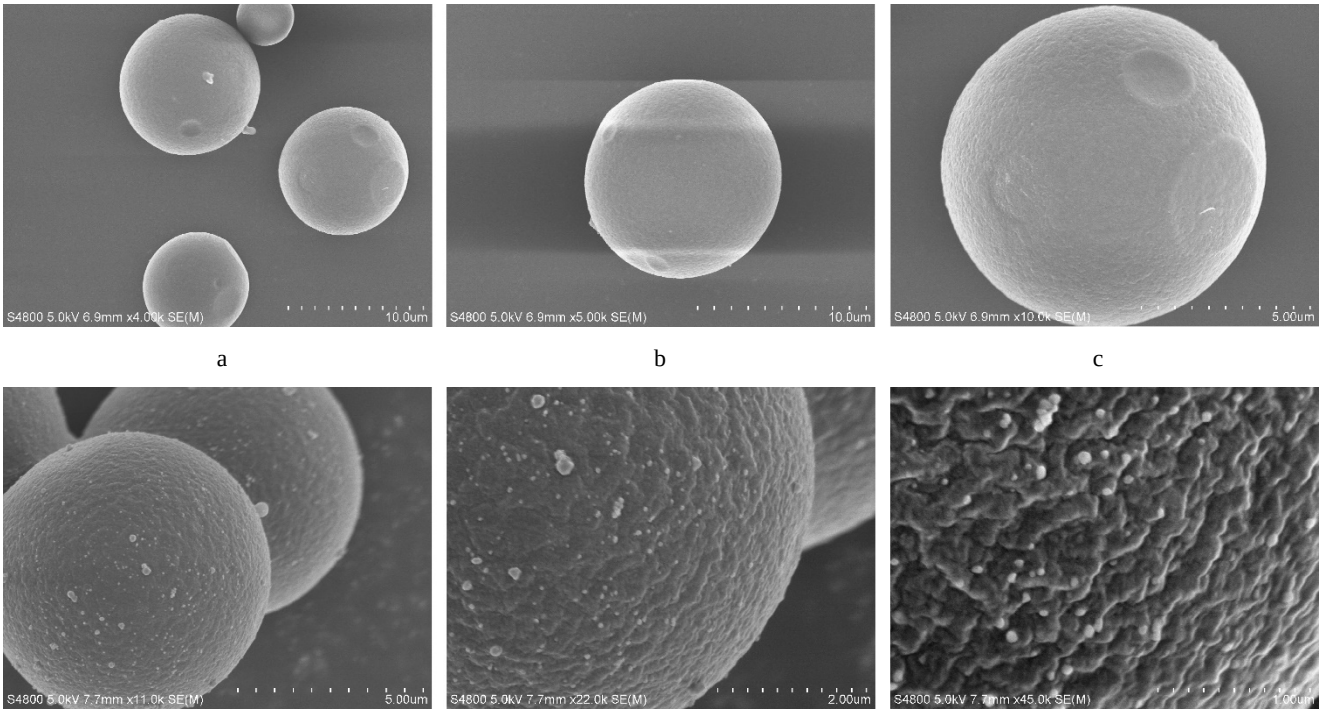

Fig. 6. a, b, c – SEM images of CS-g-VA microspheres (×4.00k, ×5.00k, ×10.0k, respectively); d, e, f – SEM images of AgNPs/CS-g-VA microspheres (×11.00k, ×22.00k, ×45.0k, respectively)

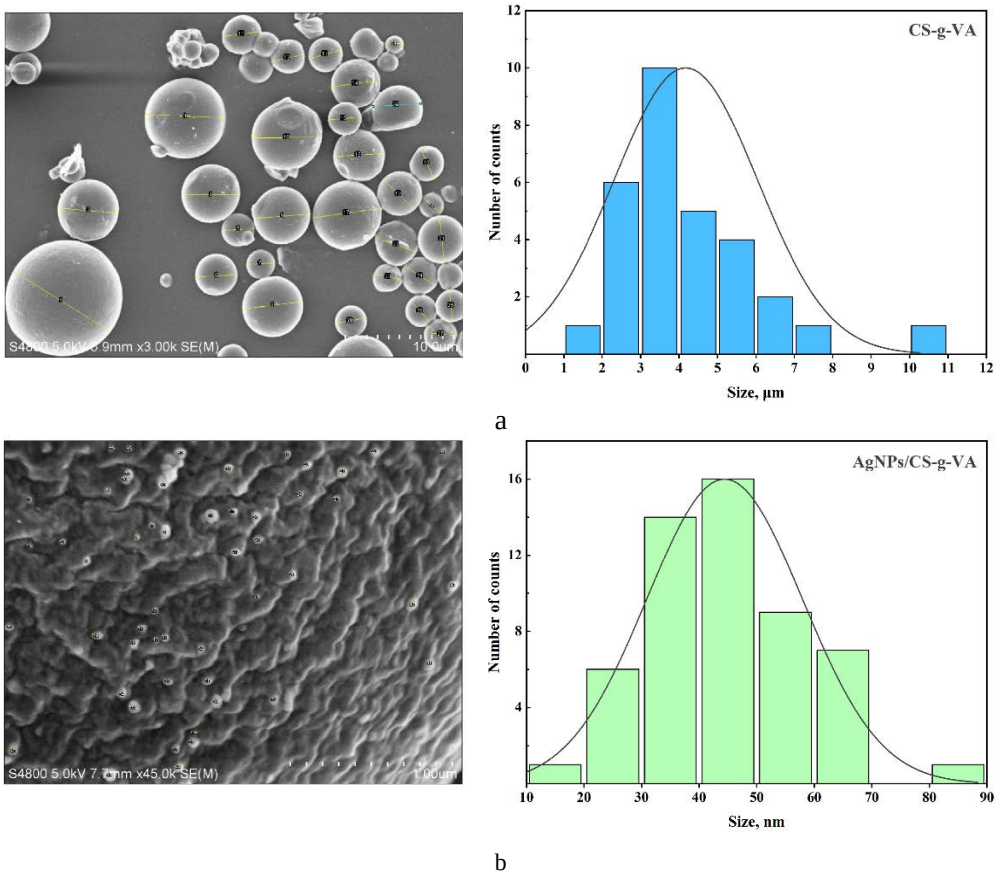

Fig. 7. a – SEM image of CS-g-VA microspheres (left) and the particle size distribution (right) with an average particle size of 4.2 μm; b – SEM image of AgNPs on an AgNPs/CS-g-VA microsphere surface (left) and the particle size distribution (right) with an average particle size of about 44.4 nm

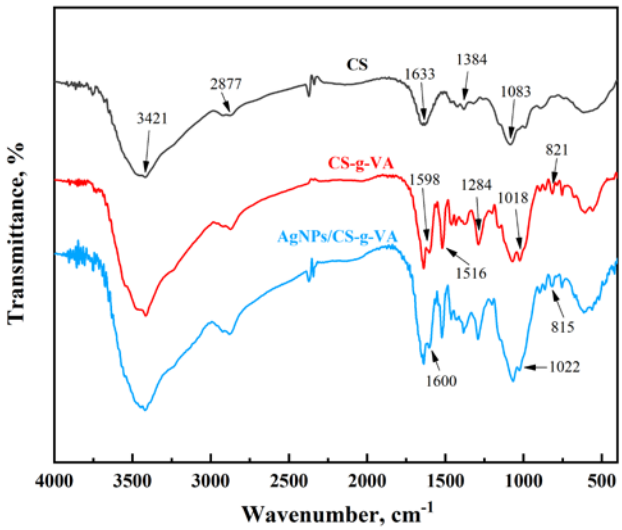

Fig. 8. FTIR spectra of CS, CS-g-VA and AgNPs/CS-g-VA

Compared with CS, in the FTIR spectrum of CS-g-VA microspheres, a new absorption peak centered at 1598 cm^{-1} corresponds to the C=N stretching vibration, which is generated by a Schiff base reaction between -C=O groups in vanillin and -NH_2 groups in chitosan [19]. Moreover, the C-O stretching vibration peak at 1083 cm^{-1} is split into two peaks, one of which is located at 1018 cm^{-1}, indicating the formation of hydrogen bonds between the phenolic hydroxyl groups of vanillin and hydroxyl groups of chitosan. Additionally, the multiple vibration peaks at around 1516 cm^{-1}, 1284 cm^{-1} and 821 cm^{-1} are assigned to vibration absorption of the benzene rings, suggesting that the benzene rings of vanillin have been introduced after chemical cross-linking. Based on the above analysis, chitosan has been successfully cross-linked by vanillin through Schiff base reaction and hydrogen bond interaction [20].

For AgNPs/CS-g-VA, although its FTIR spectrum is quite similar to that of CS-g-VA, some characteristic absorption peaks at 1600 cm^{-1}, 1020 cm^{-1} and 815 cm^{-1} seem to be attenuated due to the interaction between AgNPs and CS-g-VA, which also reveals the successful preparation of AgNPs/CS-g-VA microspheres.

3.3. XRD analysis

The composition of CS, CS-g-VA, and AgNPs/CS-g-VA microspheres were characterized by XRD and presented in Fig. 9. When comparing the XRD patterns of CS-g-VA, AgNPs/CS-g-VA with those of CS-g-VA and CS, the pattern of AgNPs/CS-g-VA shows four different characteristic diffraction peaks observed at 2θ values of 38.1°, 44.3°, 64.5° and 77.6°, respectively.

Since the four diffraction peaks representing the planes of (111), (200), (220) and (311) are well in accordance with JCPDS file 04-0783 corresponding to the

face-centered cubic crystal structure of silver nanoparticles, it indicates that the AgNPs have been successfully loaded on the microsphere surface [21].

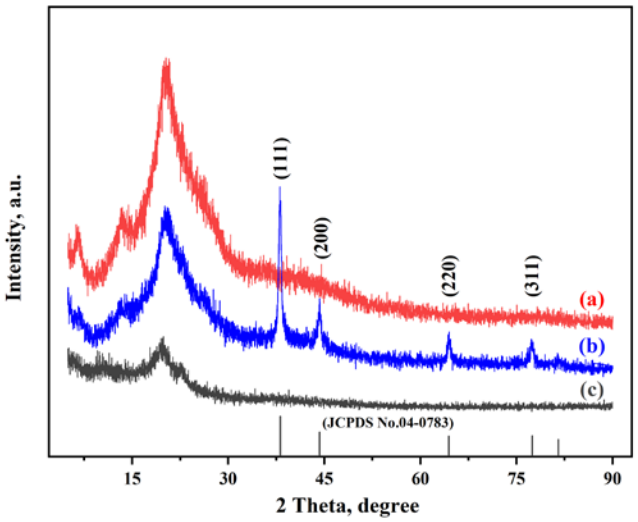

Fig. 9. a – XRD pattern of CS-g-VA; b – XRD pattern of AgNPs/CS-g-VA; c–XRD pattern of CS

4. CONCLUSIONS

In summary, the chitosan-based microspheres with micron size were successfully prepared from renewable chitosan with natural vanillin as a non-toxic cross-linker by the emulsion chemical cross-linking method. On this basis, silver nanoparticles were loaded on the surface of cross-linked microspheres by in situ reduction to achieve silver composite microspheres.

Since silver nanoparticles play an important role in the activity modulation of antimicrobial, antiviral, antiparasitic, anticancer and other biomedical systems, the ternary composite microspheres prepared in this work should have the advantages of low toxicity, easy preparation, low cost, easy separation and easy recycling. In this sense, the chitosan-based silver composite microspheres are expected to be potentially used in but not limited to the fields of broad-spectrum antibacterial agents, antibacterial materials, drug carriers, industrial catalysis, food packaging and preservation, etc.

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