Preparation and Characterization of Chitosan Nanofibers Containing Silver Nanoparticles

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Abstract: Chitosan (CS) nanofibers containing silver nanoparticles (AgNPs) were prepared by in-situ reducing method. A water soluble carboxymethyl chitosan (CMCT) was applied for the preparation of AgNPs. The impact factor such as the concentration of CMCT, silver nitrate ($AgNO_3$) content, temperature and the heating time during the preparation of AgNPs were studied. The result showed that the proper value of the concentration of CMCT, $AgNO_3$ content, temperature and the heating time were set as 0.1%, 20 μL $AgNO_3$ (1.7 mol/L), 90° and 3 h, separately and the maximum concentration of AgNPs could be acquired. To solve the spinnability of chitosan nanofiber, a super high molecular weight polyethylene oxide (PEO) was introduced to the system, and a new mixed solvent system was prepared by adding acetic acid, dimethyl sulfoxide (DMSO) and several drops of Triton X-100™ to distilled water. CS/PEO (80/20) with the concentration of 3% was dissolved in the mixed solvent to prepare electrospinning solution for CS/PEO (80/20) nanofiber fabrication. The CS containing AgNPs electrospun solution could be prepared by replacing the distilled water to silver nanoparticle solution during the preparation of mixed solvent. Ultraviolet visible (UV-Vis) spectra and transmission electron microscope (TEM) results showed that silver nanoparticles were prepared successfully. CS membranes with and without AgNPs were acquired via a traditional electrospinning equipment. These two nanofiber membranes were characterized by scanning electron microscope (SEM) images and mechanical testing. It could be noticed from the SEM images that there was a good morphology and random distribution for the nanofibers with an average fiber diameter of 180 nm. The mechanical property results showed that the addition of AgNPs decreased the mechanical strength significantly but the mechanical strength could still support wound dressing application.

Key words*: *chitosan (CS); silver nanoparticles (AgNPs); electrospinning; wound dressing
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Introduction

Silver nanoparticles (AgNPs) have been raised a lot of concern these years because of its antibacterial property applying in biomedical engineering[1-6] especially in wound dressing field[7-15]. *In-situ* reducing method is one of the most widely used methods for the preparation of AgNPs[16]. Carboxymethyl chitosan (CMCT) is a good reducing agent which can be applied for the preparation of AgNPs. Here, the CMCT plays the roles of reducing agent and antibacterial agent. It is clearly that AgNPs own the antibacterial ability, and the addition of CMCT is expected to improve this effect furthermore.

Chitosan (CS) is a natural polymer extracted from animal shells and can be used as the raw material of wound dressing. Owing to its good antibacterial property, CS is often applied in the biomedical field. Electrospinning technique is an excellent processing method which can be utilized for manufacturing nanofibers. The basic electrospinning equipment consists of a voltage supplier, a syringe pump and a receiver. The nanofibers produced by electrospinning have masses of strengths such as high surface-to-volume ratio, high porosity, adjustable pore size and morphological similarity to the extracellular matrix[17]. However, many natural polymers, especially CS, are not easy for electrospinning. It is hard to acquire an electrospun CS membrane without any beads or drawbacks. Thus, many studies pay attention to the electrospinning process of CS and it is expected to acquire CS membranes with large CS percentage and good surface morphology.

In this study, a water soluble CMCT was applied for the preparation of AgNPs. The impact factor such as the concentration of CMCT, $AgNO_3$ content, temperature and the heating time during the preparation of AgNPs were studied. A mixed solvent system consisted of distilled water, dimethyl sulfoxide (DMSO), acetic acid and Triton X-100™ was reported as the solvent for the electrospinning process of chitosan[18-19]. A new solvent system which reduced the amount of Triton X-100™ was developed on the basis of reported results. Considering the morphology of nanofiber, a certain amount of high molecular polyethylene oxide (PEO) was added to the system and CS/PEO blends were dissolved in the prepared solvent system. The CS membranes were acquired via the electrospinning process. To introduce the AgNPs to electrospun AgNPs membranes, the distilled water was replaced by silver nanoparticle solution when preparing the mixed solvent. And then the CS electrospun membranes with AgNPs could also be produced. The silver nanoparticle solution was characterized by ultraviolet and visible spectrophotometer and transmission electron microscopy (TEM) images. Meanwhile, the prepared nanofiber was characterized by scanning electron microscope (SEM) images and mechanical strength, and the properties of nanofiber could be studied ulteriorly for wound dressing application.

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1 Experimental

1.1 Materials

CS with a degree of deacetylation of 80.0% – 95.0%, Triton X-100^{TM}, DMSO, acetic acid were provided by Sinopharm Chemical Reagent Co., Ltd. Super high molecular polyethylene oxide (PEO, $M_w > 5\ 000\ 000$) was supplied by Alfa Aesar Co., UK. All the reagents were used as received unless specified.

1.2 Preparation of AgNPs

The NaOH was added to the distilled water and pH was adjusted to the range of 11.5 to 12. The CMCT (10 mL) with a concentration of 0.1% was prepared utilizing the NaOH solution. The prepared solution was then transferred to a transparent glass bottle and the bottle was put onto a magnetic stirring apparatus with the temperature of 70 ℃ and the stirring speed of 700 r/min. Different volumes (12.5, 15, 20, 22.5, 25 μL) of $AgNO_3$ solution with a concentration of 1.7 mol/L was to heel added dropwise to the glass bottle and terminated until the color of the solution turned into shallow yellow. The reaction process lasted for 1 h under the same condition. At the end of the day, the solution containing AgNPs was prepared successfully and the surface of the bottle was covered with an aluminum foil so that the AgNPs could be dispersed evenly into the solution and existed at a relative steady state for a long time.

1.3 Scaffolds fabrication

1.3.1 Solution preparation

A mixed solvent of acetic acid and DMSO was to start with prepared before the preparation of electrospinning solution. Distilled water, DMSO, acetic acid and Triton were mixed together with the mass of 17.584, 2.356, 0.544 and 0.06 g, separately. Considering the CS is hard to be electrospun, a high molecular PEO was introduced to the system. The ratio of CS/PEO (80/20) was proved to be easily electrospun during the experiment. Consequently, the CS/PEO (80/20) with a total concentration of 3% were dissolved in the mixed solvent and stirred for 10 h to get the electrospinning solution. Meanwhile, the AgNPs could be added to the electrospinning solution by replacing the distilled water into the diluted silver nanoparticle solution.

1.3.2 Electrospinning

The electrospinning process was carried out in traditional equipment based on a voltage supplier, a syringe pump and a grounded receiver. Considering that nanofibers with a relative smooth surface and good morphology can only be acquired at above 40 ℃, a self-controlled heated equipment was utilized during the electrospinning process. The parameters of electrospinning such as voltage, flow rate and distance between needle tips to the receiver were adjusted to 15 kV, 1.2 mL/h and 20 cm, respectively. The electrospun membranes were then crosslinked by glutaraldehyde vapor for about 12 h. Finally, the scaffolds were saved in a vacuum drying apparatus for wiping out the residual solvent over 7 d.

1.4 Characterization method of AgNPs

1.4.1 UV-Vis characterization

The silver nanoparticle solution was prepared. CMCT plays the role of reducing agent and stabilizer in the preparation of AgNPs, the maximum absorption peak of UV can be detected at around 410 nm, which indicates the existence of AgNPs. To illustrate the successful preparation of AgNPs, the solution was diluted with distilled water and tested in an ultraviolet and visible spectrophotometer[20-24] (Jingke Industrial Co., Ltd., Shanghai, China). And the scanning range of wavelength is about 300 to 600 nm.

1.4.2 TEM images

To visualize the AgNPs in further experiments, TEM images were tested in JEM2000 (Japan). The silver nanoparticle solution was dropped on a copper grid and then the copper grid was put into a vacuum oven at 60° over 1 d.

1.5 Characterization of electrospun scaffolds

1.5.1 Fiber morphology

The morphology of electrospun nanofiber was characterized by SEM (TM100, Hitachi, Japan). The membranes were sputter-coated with gold for 30 with an acceleration voltage of 10 kV and a current of 5 mA.

1.5.2 Mechanical properties

The mechanical properties of nanofiber were tested using a universal materials testing machine (H5K-S, Hounsfield, UK). All samples were tailored with a uniform size of 50 mm × 10 mm and tested for over 3 times with different parts of fiber. The drawing process was carried out with a cross-head speed of 10 mm/min until breakage.

1.5.3 TEM images

To identify the existence of AgNPs in electrospun CS/PEO (80/20) nanofibers containing AgNPs, TEM images were tested in JEM2000 (Japan). The nanofiber was received on a copper grid and then the copper grid was put into a vacuum oven at 60 ℃ over 1 d.

1.5.4 X-ray diffraction (XRD) profiles

To identify the existence of AgNPs in electrospun CS/PEO (80/20) nanofibers containing AgNPs, XRD was carried out viaD/max-2550 PC-XRD equipment (Rigaku, Japan, Cu Kα, $\lambda = 0.154$ nm)

1.6 Statistical analysis

All the data were expressed as means ± standard deviation (SD) and checked by normality tests. Origin 8.0 (Origin Lab Inc., USA) was applied for statistics analysis. All the data were analyzed applying one way analysis of variance (ANOVA) with Tukey's test. Statistical differences were considered significance at $p < 0.05$.

2 Results and Discussion

2.1 Characterization and analysis of AgNPs

2.1.1 UV-Vis analysis for AgNPs

The formation of silver nanoparticle solution was influenced by many parameters such as the concentration of CMCT, the amount of $AgNO_3$, the heating time and the heating temperature.

2.1.1.1 Different concentrations of CMCT

It could be concluded from Fig. 1 that the concentration of AgNPs increased with the increase of CMCT while decreased after the optimal concentration of CMCT. The CMCT plays the role of reducing agent. With the concentration of CMCT increasing from 0.05% to 0.10%, the amount of AgNPs originated from Ag^+ increased with the increase of CMCT, while the amount of AgNPs decreased with the concentration of CMCT increasing from 0.10% to 0.15%. This might be because the excess amount of CMCT was readily covered the $AgNO_3$ thus creating agglomeration and the reduction reaction was hindered. There was a significant agglomeration for CMCT with the concentration of 0.20% and 0.25%, so the data were abandoned. And the optimal concentration of CMCT was thus affirmed as 0.10%.

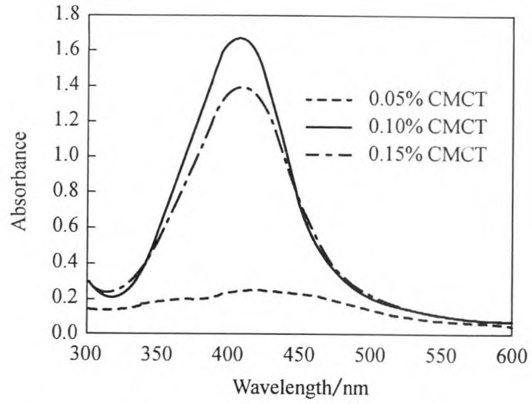
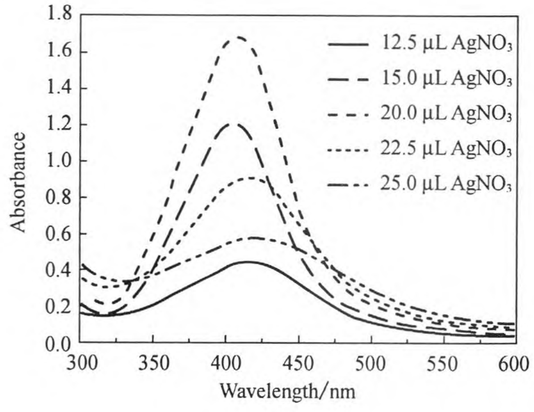


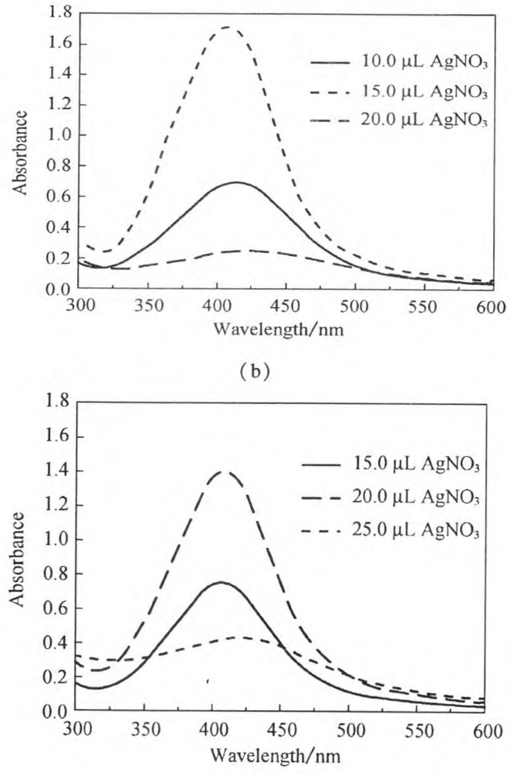
Fig. 1 UV-Vis Spectra of silver nanoparticle solution with different contents (0. 05%, 0. 10%, and 0.15%) of CMCT

2.1.1.2 Different concentrations of $AgNO_3$

Figure 2(a) showed the UV-Vis spectra of silver nanoparticle solution with different volumes (12. 5, 15. 0, 20.0, 22.5, 25.0 μL) of $AgNO_3$ (1.7 mol/L), it could be seen from Fig. 2 (a) that the amount of silver nanoparticle increased with the volume of $AgNO_3$ increasing from 12.5 μL to 20 μL while the content of silver nanoparticle decreased after increasing the volume of $AgNO_3$ from 20.0 μL to 25.0 μL (Fig. 2(a)). On the basis of acquiring AgNPs as many as possible, the optimal volume of $AgNO_3$ is 20 μL (the concentration of CMCT was fixed as 0.1%). Consequently, there was an optimal volume of $AgNO_3$ to acquire a relatively highest amount of AgNPs. To identify this result, the concentration of CMCT was changed to 0.05% (Fig. 2(b)) and 0. 15% (Fig. 2(c)), it could be concluded from Fig. 2(b) that the amount of silver nanoparticle increasesd with the volume of $AgNO_3$ increasing from 10 μL to 15 μL while the content of silver nanoparticle decreased after increasing the volume of $AgNO_3$ from 15 μL to 20 μL (Fig. 2(b)), so the optimal volume of $AgNO_3$ was 15 μL when the concentration of CMCT was fixed as 0.05%. From Fig. 2 (c), it could be noticed that the amount of silver nanoparticle increased with the volume of $AgNO_3$ increasing from 15 μL to 20 μL while the content of silver nanoparticle decreased after increasing the volume of $AgNO_3$ from 20 μL to 25 μL (Fig. 2(c)), so the optimal volume of $AgNO_3$ was 20 μL when the concentration of carboxymethyl chitosan was fixed as 0.15%.



(a)



(b)

(c)

Fig. 2 UV-Vis spectra of silver nanoparticle solution with different volumes (12.5, 15.0, 20.0, 22.5, 25.0 μL) of $AgNO_3$ (1.7 mol/L), the concentrations of CMCT as (a) 0.10%; (b) 0.05%; (c) 0.15%

2.1.1.3 Different heating time

The UV-Vis spectra of silver nanoparticle solution with different heating time (0.5, 1.0, 2.0, 3.0 h) were exhibited in Fig. 3, from which could be concluded that the concentration of AgNPs increased with the heating time increased until 3.0 h. The concentration of CMCT and the volume of $AgNO_3$ (1.7 mol/L) were fixed as 0.1% and 20.0 μL separately.

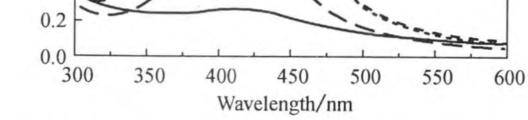


Fig. 3 UV-Vis spectra of silver nanoparticle solution with different heating time

2.1.1.4 Different heating temperatures

The UV-Vis spectra of silver nanoparticle solution with different heating temperatures (50, 70, 90 ℃) were shown in Fig. 4 (the concentration of CMCT and the volume of $AgNO_3$ (1.7 mol/L) were fixed as 0.1% and 20.0 μL separately), it could be seen from Fig. 4 that the concentration of AgNPs has a

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positive correlation with the heating temperature. This might be because the relatively higher temperature contributes to the reduction reaction process. It should be noticed that there was almost no AgNPs appeared at 50 ℃, so the heating temperature should be adjusted much higher. Meanwhile, the boiling point of water was 100 ℃, so the heating temperature could not be adjusted to over 90 ℃. In a word, the heating temperature could be adjusted as high as possible at the temperature range of 70 ℃ to 90 ℃.

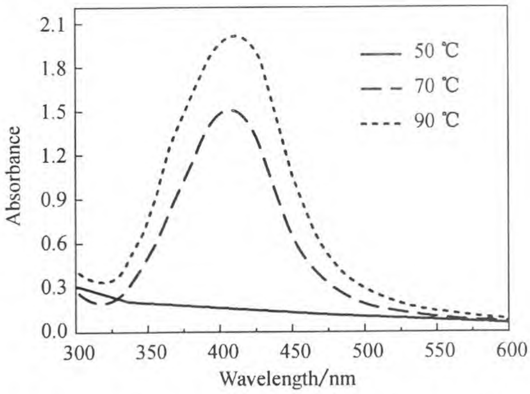
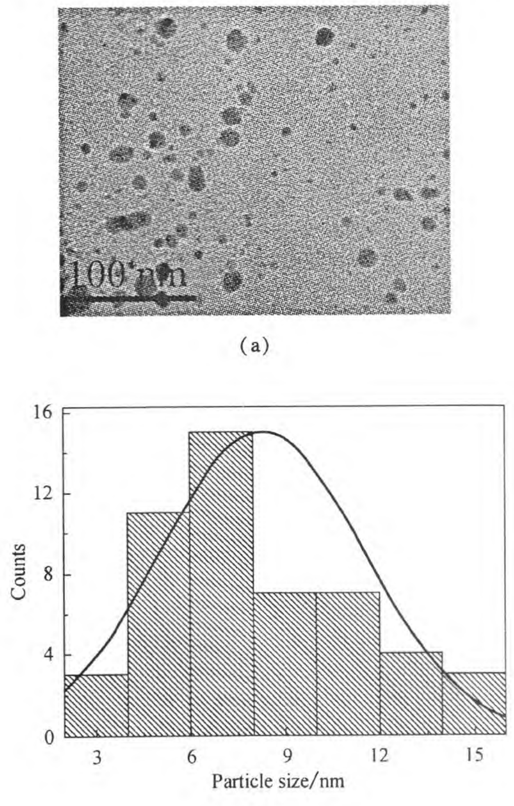


Fig. 4 UV-Vis spectra of silver nanoparticle solution with different heating temperatures

2.1.2 TEM images

The TEM image of AgNPs was shown in Fig. 5, and it could be noticed that the AgNPS were spread evenly. The size distribution of AgNPs was recorded in Fig. 5(b) and it could be concluded that the average size of AgNPs was less than 10 nm. TEM results identify the existence of AgNPs further on.



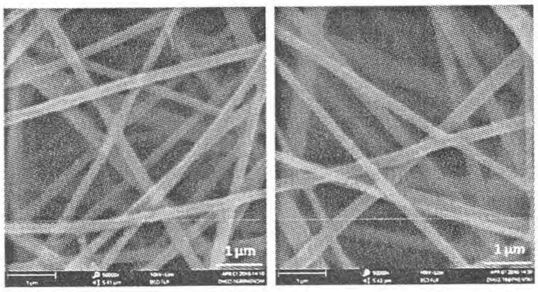
(a)

(b)

Fig. 5 AgNPs' characterization of (a) TEM images; (b) size distribution

2.2 Morphology of CS nanofibers

CS/PEO scaffolds with and without AgNPs were prepared by electrospinning. It could be noticed from Fig. 6 that the addition of AgNPs had no significant impact on the fiber morphology. It could be noticed from the SEM images that there was a good morphology and random distribution for the nanofibers with an average fiber diameter of 180 nm. The morphology of nanofibers with AgNPs did not change after doping with AgNPs, and the structures of the nanofibers with AgNPs were smooth and uniform. TEM image of CS/PEO nanofiber containing AgNPs was shown in Fig. 7 (the scale bar is 1 μm), from which could be seen that AgNPs existed in the hybrid nanofiber.



(a) (b)

Fig. 6 SEM images of electrospun CS/PEO (80/20) nanofibers: (a) without containing AgNPs; (b) containing AgNPs

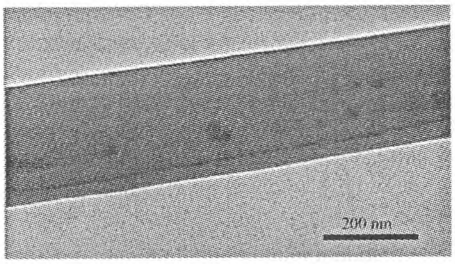


Fig. 7 TEM images of electrospun CS/PEO (80/20) nanofibers containing AgNPs

2.3 XRD profiles of CS nanofibers

To identify the existence of silver in electrospun CS/PEO (80/20) nanofibers containing AgNPs, the XRD was carried out. It could be concluded from Fig. 8 that the XRD profile of CS nanofibers containing AgNPs (Fig. 8(b)) owned the peak at $2\theta = 38.1$, 44.3 corresponding to the lattice planes of (111) and (200) for AgNPs [25]. While the same peak especially the strong peak at $2\theta = 38.1$ could not be found at the XRD profile of CS nanofibers without containing AgNPs (Fig. 8(a)). Notably, the content of silver was relatively low, and the relationship between silver content in nanofibers and the antibacterial property of nanofibers could be studied in the future research.

2.4 Stress-strain curves of AgNPs

The mechanical property of electrospun membranes with different CS/PEO ratio (70/30, 80/20, 80/20 with AgNPs) was shown in Fig. 9, from which could be concluded that the addition of AgNPs decreased the mechanical strength significantly. And the mechanical strength increases with the decrease of CS's content. It could be noticed from Table 1 that the tensile strength and Young's Modulus of CS membranes containing AgNPs decreased significantly while the elongation at break increased to some extent compared with CS membranes.

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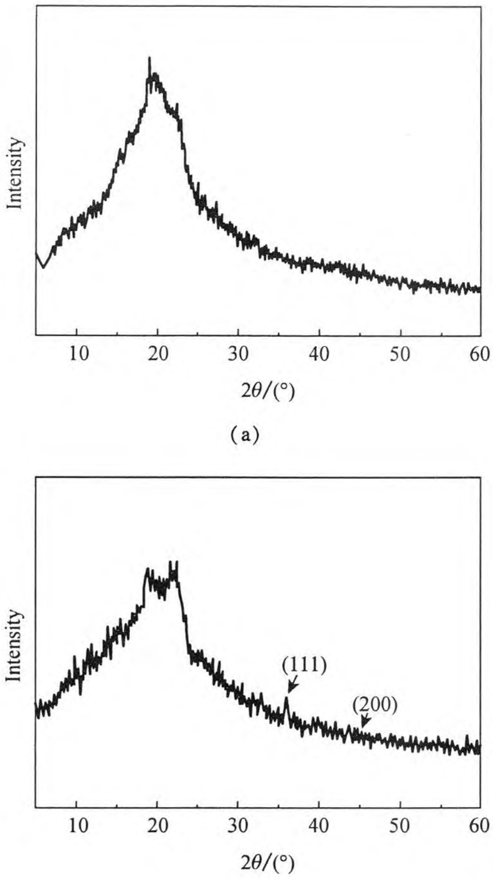

(a)

(b)

Fig. 8 XRD profiles of electrospun CS/PEO (80/20) nanofibers (a) without containing AgNPs and (b) containing AgNPs

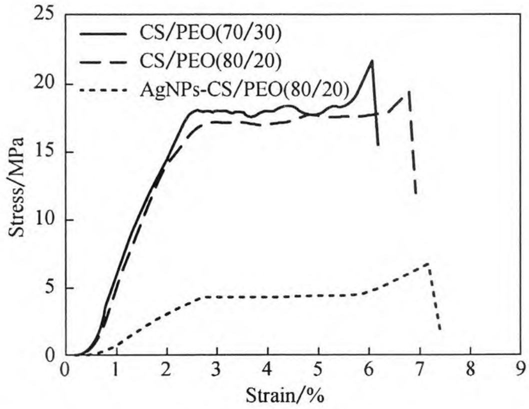

Fig. 9 Stress-strain curves of electrospun membranes with different CS/PEO ratios (70/30, 80/20, 80/20 with AgNPs)

Table 1 Mechanical properties of electrospun membranes with different CS/PEO ratios

Material	Tensile strength/ MPa	Young's modulus/MPa	Elongation at break/%
CS/PEO (70/30)	21.71 ± 0.15	1 015.04 ± 10.55	6.07 ± 0.08
CS/PEO (80/20)	19.53 ± 0.28	1 072.29 ± 6.18	6.79 ± 0.26
AgNPs-CS/PEO (80/20)	6.69 ± 0.12	224.97 ± 4.01	7.18 ± 0.13

3 Conclusions

Silver nanoparticle solution was prepared via the *in-situ* reducing method. A water soluble CMCT was applied for the preparation of AgNPs. The impact factors such as the concentration of CMCT, $AgNO_3$ content, temperature and the heating time during the preparation of AgNPs were studied. To obtain as many as AgNPs and keep the particle size spreading evenly, the concentration of CMCT was chosen as 0.1%; the volume of $AgNO_3$ (1.7 mol/L) was set as 20 μL; the heating time and heating temperature were set as 3 h and 90 ℃ separately. It could be concluded from TEM result that the average size of AgNPs was less than 10 nm. It could be noticed from the SEM images that there was a good morphology and random distribution for the nanofibers with an average fiber diameter of 180 nm. The mechanical property results showed that the addition of AgNPs decreased the mechanical strength significantly but still had enough mechanical strength for its potential application in wound dressing. The comprehensive properties of chitosan membranes containing AgNPs could be studied ulteriorly for wound dressing application.

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