

Carboxymethylchitosan nanofibers containing silver nanoparticles: Preparation, Characterization and Antibacterial activity

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ABSTRACT

Herein we prepare antibacterial composite from carboxymethylchitosan (CMCS) nanofibers and silver nanoparticles (AgNPs) by using poly (vinyl alcohol) (PVA) as reducing, capping and fiber aiding material and investigated to be used in biomedical applications. The AgNPs has spherical shapes and its diameter ranged from 15 to 25 nm and distributed within the prepared nanofibers. The electrospinning parameters from the effect of the CMCS and PVA mass ratio, extrusion rate and field were studied. The optimum condition for electrospinning were 7% for CMCS and 8% from PVA. UV-vis, TEM and XRD used to characterize AgNPs whereas FTIR and SEM used to characterize nanofibers. Results showed that ultra-fine fibers were generated after addition of PVA to CMCS in different mass ratios to from 8 wt. % concentration solutions. Electrospun PVA (AgNPs)/CMCS nanofibers showed good antibacterial effects towards Gram-positive and Gram-negative bacteria. Antibacterial activities of electrospun nanofibers increased by increasing both CMCS and AgNPs content in the electrospun nanofibers.

INTRODUCTION

Chitosan polymer composed of glucosamine and 2-acetyl glucosamine units and it is one of the most few nitrogen containing natural polysaccharides that has antibacterial properties (Rinaudo, 2006). It is soluble in acids, so that it exhibit antibacterial activity appears in an acidic medium only. In addition its electrospun nanofibers produced from acetic acid, formic acid and trifluoroacetic acid (Duan *et al.*, 2004; Ohkawa *et al.*, 2004; Park *et al.*, 2004; Bhattarai *et al.*, 2005; Li and Hsieh, 2006; Jia *et al.*, 2007; Du and Hsieh, 2008). The produced electrospun fibers contains traces of acids or organic solvents which are harmful, reduced its biocompatibility and increase its toxicity. To overcome these problems, carboxymethylchitosan was produced as water-soluble chitosan derivative to obtain

nanofibers from water and then increase its biomedical and biological applications (Chen *et al.*, 2006; Abou-Zeid *et al.*, 2013). Silver metal and its ions can interact with the thiol groups of enzyme and proteins and transport vital substances outside bacterial membrane without affect the human cell. So that it has wide range of bactericidal activity towards Gram-positive and Gram-negative bacteria. The surface area of silver nanoparticles higher than that of silver metal so that it has higher antibacterial activity than silver metal. (Maneerung *et al.*, 2008; Yang *et al.*, 2012). Glucose is a soft reducing agent used to reduce Silver nitrate to silver nanoparticles and it is widely used in biomedical processes.

PVA is a safe poly reducing agent for silver ions and fiber aiding material for carboxymethylchitosan due to it is non-toxic and stable towards chemicals (Acatay *et al.*, 2004; Yang *et al.*, 2004; Zhang *et al.*, 2007). Fiber formation from polymer solutions via electrospinning process depends on electrical high voltage, the distance from tip-to-collector and the rate of extrusion of polymer solution (Deitzel *et al.*, 2001; Frenot and Chronakis, 2003; Sun and Li, 2011).

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Electrospinning is a very effective, simple and inexpensive method of producing nanofibers from its polymer solution compared to other methods such as self-assembly and phase separation and has the potential for large-scale manufacturing (Jayaraman *et al.*, 2004; Zhang *et al.*, 2007). The nanofibers have very large surface area so that it is compatible with human cells' size and suitable to form products for filtration of sub-particles, adsorption of biological and chemical warfare gases, medical industry as artificial blood vessels and organs, sutures, surgical facemask, wound dressing, and drug delivery systems (Mohan, 2003; Bhattarai *et al.*, 2005).

The aim of this work to prepare colloidal silver nanoparticles by using PVA/glucose redox system and then impregnated these nanoparticles within CMCS nanofibers by using electrospinning technique to produce antibacterial nanofibers. UV absorption, XRD and TEM micrograph used to investigate the prepared silver nanoparticles (AgNPs). FTIR and SEM micrograph were used to characterize the produced nanofibers. Finally evaluate its antibacterial activity to be used in biomedical applications.

MATERIALS AND METHODS

Chitosan (CS) (Aldrich, USA, viscosity 1860cps, degree of deacetylation 79.0%). PVA (Fine Chemicals Pvt. Ltd, India, MW 88,000, degree of deacetylation 89% and viscosity 35-50 cps for 4% solution at 20 °C). AgNO₃ (Sigma Chemical Co., USA) All other chemicals and reagents were of analytical grade, and used without further purification

Staphylococcus aureus (*S. aureus*) as Gram-positive bacteria and *Escherichia coli* (*E. coli*) as Gram-negative bacteria used for the antibacterial assay were provided from Antibacterial lab, Faculty of Girls for Arts, Science and Education, Ain Shams University, Cairo, Egypt.

The carboxymethylchitosan (CMCS) was prepared as reported method mentioned elsewhere (Abou-Zeid *et al.*, 2013; Ibrahim *et al.*, 2015b).

Silver nanoparticles (AgNPs) were prepared by the reduction of silver nitrate by using PVA/glucose as reducing agent (Mbhele *et al.*, 2003). Briefly, AgNO₃ samples (0.1 M, 0.01 M and 0.001 M) were added to PVA solution (1, 3, 6 wt. %) in the presence of glucose as reducing agent and to prevent the oxidation of AgNO₃ to Ag₂O. Formation of clear yellow to brownish yellow colour indicated that AgNPs formed and ready to use.

Polymer solutions used for electrospinning were prepared as follow: CMCS solution (7 wt. %), was prepared by dissolving 0.7g of CMCS in 10 ml distilled water under continues stirring for 1 hour. PVA solution containing Ag NPs(8 wt.%) was prepared by dissolving 0.8 g of PVA in 10 ml distilled water with moderate stirring at 60 °C for 2 hr., then add to this solution different concentration of AgNO₃ (0.001, 0.01 and 0.1 M) in the presence of glucose (0.15 gm/10 ml) for 72 hrs. The optimum condition for forming nanofibers is 8 wt. % PVA and 7 wt. % CMCS solution

from 8 wt. % solution, obtained from our previous work (Ibrahim *et al.*, 2015a).

The electrospinning device used consists mainly of extrusion system (syringe pump), collecting electrode, and high voltage supply as shown in Figure 1. Experimental design of the electrospinning process of CMC containing AgNPs are shown in Table 1.



Fig. 1: Electrospinning equipment

Table 1: electrospinning Design of PVA/AgNPs/CMCS.

PVA/AgNPs/C MCS,W/W	Potential, kv	Distance, cm	Field, kv/cm	Extrusion Rate, ml/hr
100/00	30	6, 10	3, 5	1, 3
75/25	30	6, 10	3, 5	1, 3
50/50	30	6, 10	3, 5	1, 3
25/75	30	6, 10	3, 5	1, 3
00/100	30	6, 10	3, 5	1, 3

The morphology and particle size of Ag-NPs were investigated by using, transmission electron microscope (TEM), JEOL JEM 2100 F electron microscope at 200kv. The surface morphology of the electrospun nanofibers were investigated by scanning electron microscope (SEM) examination by mounting the samples on stub with double stick adhesive tape and coated with gold in a S150A sputter coater unit (Edwards, UK). The gold film thickness was 150Å. The samples were then viewed in a JEOL JXA-950 electron probe micro analyzer, Japan. UV-Vis absorption spectra of AgNPs were recorded by using a Spectronic UV-Vis spectrometer (Model: Genesys 2, USA). Fourier transform infrared (FT-IR) spectra of the all samples were obtained using a Nexus 670 FT-IR spectrophotometer (Lelet Co., USA). The spectra of sample in the range of 4000-800 cm⁻¹ are investigated. X-ray diffraction studies were conducted using an X-ray diffractometer (D2 Phaser, Bruker AXS, Germany) operating at 30 kV and 10 mA. The diffraction patterns were recorded using Cu-K radiation and the film samples were analyzed at a 2 range, 1-50.

The antibacterial activity of the prepared nanofibers were determined by disk diffusion method on an agar plate (Abou-Zeid *et al.*, 2011; Ibrahim *et al.*, 2015a). In the antibacterial assay, all data were the means from at least three parallel experiments that the discrepancies among them were less than 5%.

RESULTS AND DISCUSSION

Preparation of silver nanoparticles

On using PVA in the presence of glucose as reducing agent of silver nitrate to form silver nanoparticles large clusters disappears and clear spherical particles formed so that PVA used as capping agent and reducing agent at the same time.

Silver nitrate reduced into silver nanoparticles in aqueous solution and these nano particles have UV-vis absorption peak at 430 nm due to free electrons excitation(Temgire and Joshi, 2004). When AgNO_3 is mixed with PVA solution, the electropositivity of Ag^+ ions allow it to bind with PVA through electrostatic forces, which change the colour of PVA solution from colourless to yellow and brownish yellow in the presence of glucose(Nguyen *et al.*, 2010).

Silver ions/PVA undergo redox system where; the OH groups of PVA converted into C=O groups at the same time Ag^+ converted into Ag^0 . Also, glucose acts as co-reducing agent with PVA through its free aldehydic groups as mentioned elsewhere in previous work for natural biopolymers (Hebeish *et al.*, 2010; Abdel-Halim and Al-Deyab, 2011).

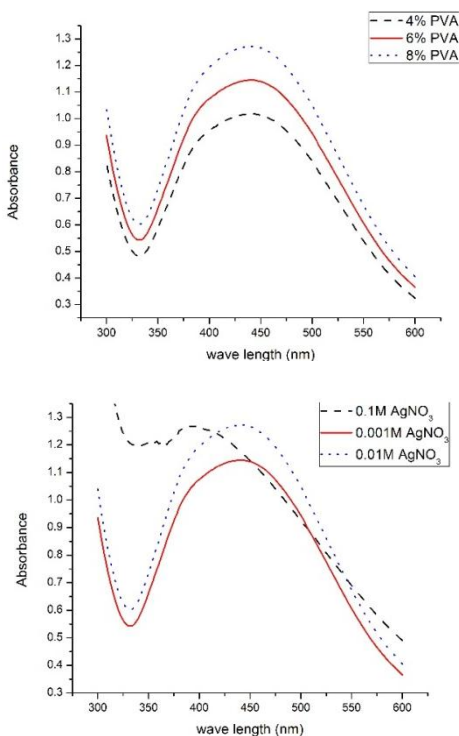


Fig. 2: Ultraviolet-Visible spectra of ANPs at different concentrations.

The formation of silver nanoparticles we achieved by UV-Vis. Absorption peaks at ~ 430 nm as shown in figure 2. . Figure 2a shows the effect of different PVA concentrations (3, 6, 8 wt. %) on silver nanoparticles formation at constant AgNO_3 concentration at 0.001M. UV-vis. spectra shows that formation of Silver stabilized by increasing of PVA wt. %. Although the maximum peak position and size didn't change, due to the coordination bond between oxygen n-electrons of PVA and silver (Ag^+ and Ag^0)(Jin *et al.*, 2007). Figure 2b illustrate the effect of

silver nitrate concentration (0.001M-0.1M) at constant PVA concentration (6wt. %) and this change monitored by UV-Vis. Spectra. The UV-Vis peak intensity and formation of spherical shaped of AgNPs increased as AgNO_3 concentration decreased(Petica *et al.*, 2008).

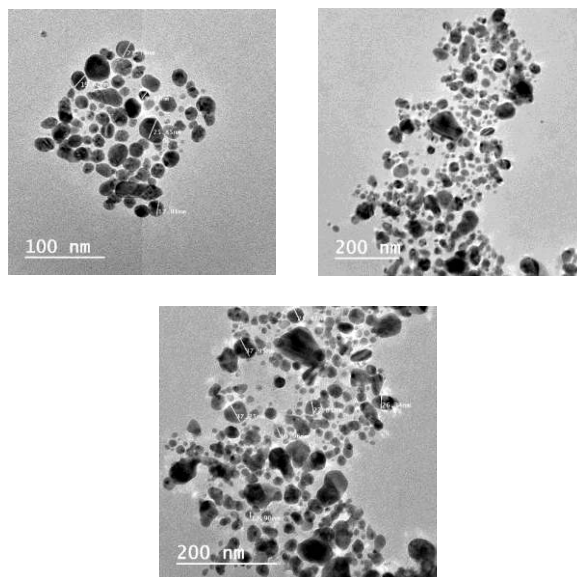


Fig. 3: TEM micrographs of AgNPs obtained in the presence of 6 g/100 ml aqueous solution (a), 0.001M AgNO_3 (b) 0.01M AgNO_3 (c).0.1M AgNO_3

Figure 3 shows that the images of AgNPs by TEM, which proof that these nanoparticles has spherical shapes, uniform scattered and its diameter ranged from 15-25 nm. In addition, aggregation of the particles associated with an increasing of AgNO_3 concentrations.

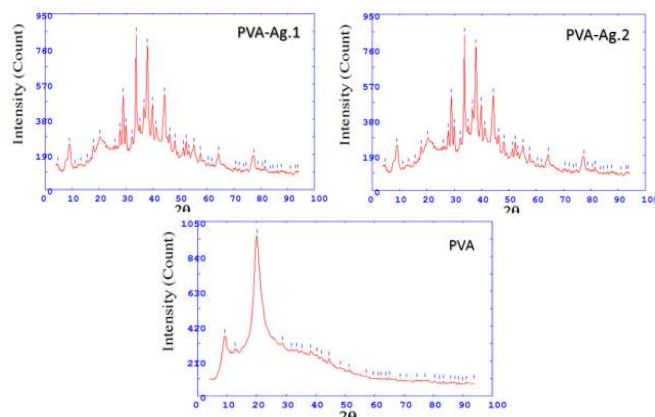


Fig. 4: XRD pattern of PVA and AgNps;

Crystalline behavior of PVA nanofibers containing AgNPs was confirmed by using XRD. XRD profiles of PVA nanofibers loaded AgNPs by electrospinning and pure PVA nanofibers using electrospinning technique are showed in Figure 4. Diffraction peak corresponds to crystallization of silver nanoparticles present at ($2\theta=38$) which can differentiate between pure PVA nanofibers from PVA nanofibers containing AgNPs (figure 4) (Ricciardi *et al.*, 2005; Nguyen *et al.*, 2010).

Electrospinning of PVA/AgNPs/CMCS aqueous solutions

Carboxymethylchitosan (CMCS) is polyelectrolyte have high viscosity so that its water solution is not be easily electrospun alone but it need to blends with another polymer as aiding fiber formation to form nanofibers. Polyvinyl alcohol (PVA) act as a “guest” polymer due to it can form hydrogen bonding with CMCS and could be electrospun from its aqueous solutions. In addition, PVA used as both reducing and capping agent for AgNPs as we discussed before. In previous study (Ibrahim *et al.*, 2015a) we concluded that 7% CMCS concentration showed more uniform nanofiber morphology. Therefore, 7% wt., CMCS concentration mixed with PVA containing AgNPs to get antibacterial nanofibers used in biomedical applications. The morphology of prepared nanofibers characterized by SEM images as we seen in Figure 5. When CMCS percentage is 100% we obtain beads with films as seen in figure 5a. As the percent of PVA containing AgNPs increased the beads formation starts disappears with appears fibers as seen in figure 5b and 5c., and as the percent increased more than 50% the beads were completely disappear and we got nanofibers appears as seen in figure 5d and 5e.(Zhou *et al.*, 2007; Ibrahim *et al.*, 2015a).

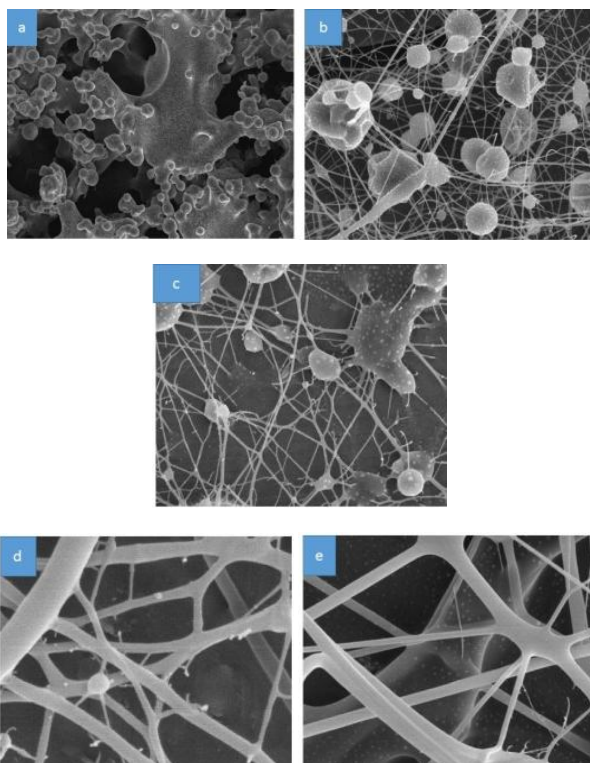


Fig. 5: SEM images of PVA/AgNPs./CMCS nanofibers. CMCS/PVA weight ratio: (a) 100/00; (b) 75/25; (c) 50/50; (d) 25/70; (e) 00/100 at 1 ml/h. extrusion rate and (30Kv/10cm) field

Figure 6 shows the FTIR of the pure PVA, pure CMCS and PVA-AgNPs-CMCS nanocomposites (Mbhele *et al.*, 2003). Disappearance of 837 and 711, 650, and 570 cm^{-1} spectral bands for out of plane CH and OH groups respectively indicate that AgNPs increased in the composite and it react with CMCS through OH groups. Also AgNPs oriented within CMCS chains illustrated

by appearing bands at 1322 cm^{-1} from coupling of OH in plane and at 1420 cm^{-1} form CH wagging vibrations.

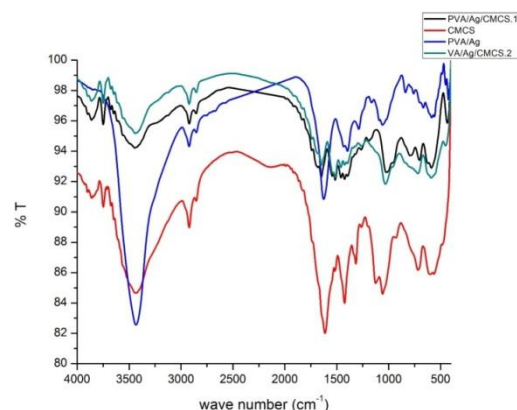


Fig. 6: FTIR spectra of CMCS, PVA-AgNPs and PVA-AgNPs-CMCS nanofibers.

Antibacterial activity of PVA (AgNPs) CMCS nanofiber composites

Figure 7 shows the antibacterial activity expressed in inhibition zone of the electrospun carboxymethylchitosan, according to the disk diffusion method, on Gram-positive and Gram-negative bacteria as mentioned in the experimental section.

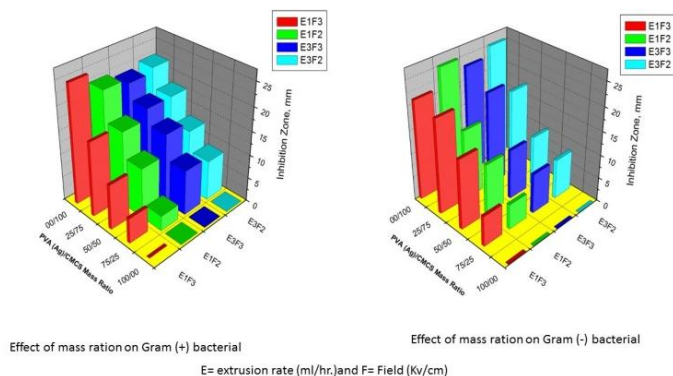


Fig. 7: Effect of PVA and CMCS Ratio and antibacterial activity. E: Extrusion rate and F: Electric Field.

Chitosan used as wound dressing because it is bactericidal material and its antibacterial mechanism explained through the reaction between chitosan NH_2 groups and bacterial cell membrane and then its growth inhibited, bacterial cell death or transfer its components outside the bacterial cell.(Helander *et al.*, 2001; Ghoujeh and Mousavi, 2002).

From figure 7, we found that the antibacterial activity of the nanofibers increased as CMCS ratio increased which indicate that the reaction with CMCS and AgNPs takes place at OH groups not NH_2 groups. In other words, the area covered by the nanofibers from CMCS higher with concentration a matter that lead to an increase of the inhabitation zone with concentration(Seyam *et al.*, 2012; Ibrahim *et al.*, 2015a).

Although chitosan had more effective towards Gram-positive bacteria than gram-negative bacteria due to cell membrane

of bacteria (Abou-Zeid *et al.*, 2011). However, previous results shows that electrospun carboxymethylchitosan nanofibers shows the similar antibacterial activity towards both Gram-positive and Gram-negative bacteria due to its transform to nano structure that made it able to penetrate the bacterial cell wall even it had potential barrier. (Seyam *et al.*, 2012; Ibrahim *et al.*, 2015a).

As shown in Figure7, the changing of extrusion rate from 1 to 3 ml/h and the changing of the needle tip-to-collector distance didn't affect the morphology and the antibacterial activity of the electrospun nanofibers because the amount of both CMCS and AgNPs in nanofiber web deposit at the collectors are the same (Seyam *et al.*, 2012; Ibrahim *et al.*, 2015a).

CONCLUSION

Antibacterial composite from carboxymethylchitosan (CMCS) nanofibers and silver nanoparticles (AgNPs) by using poly (vinyl alcohol) (PVA) were prepared. Antibacterial activities of electrospun nanofibers increased by increasing of both CMCS and Ag nanoparticles percent in the electrospun membrane and showed good antibacterial effects towards Gram (+ve) and Gram (-ve) bacteria. Also, we can used it as biomedical application. PVA act as reducing, capping and fiber aiding material. The prepared AgNPs has spherical shapes and its diameter ranged from 15 to 25 nm and distributed within the prepared nanofibers.

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