ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES REDUCED ON POLYSACCHARIDE FILMS

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Abstract

The present work is focused on preparation of polysaccharide films which were doped with silver nanoparticles for enhancement of antibacterial properties. Silver nanoparticles (AgNPs) were prepared and stabilized by reduction of silver nitrate with chitosan without addition of reducing agents. The presence of AgNPs was studied by X-ray photoelectron spectroscopy. Wettability and water absorption of the films were evaluated. Antibacterial activity of solid films with AgNPs was tested by disc diffusion test on two bacterial strains, Gram-positive (*Staphylococcus epidermidis*) and Gram-negative (*Escherichia coli*). Solid films were dissolved and then the solution was observed by the Transmission electron microscopy. Aging of the solutions which were stored in the day light and dark were tested. The presence of AgNPs was confirmed both in the solid films and in the solutions by the above mentioned methods and the films exhibited antibacterial activity against both bacterial strains. The research was aiming on use of these films in medicine as a new type of wound dressing with antibacterial properties. These films could be used as a wound dressing, antimicrobial packaging material or as a long-term storage of AgNPs for various applications.

Keywords: Polysaccharides films, silver nanoparticles, preparation and characterization of films

1. INTRODUCTION

Natural polymers have been used as biomaterials for thousands of years. Their main advantages are good mechanical properties, easy fabrication and low cost. Biological properties can be changed by means of chemical or physical modification of their surface. Surface properties of biomaterials are inevitably bonded with tissue engineering, especially for polymers when improvement of the cell adhesion to the surface, the cell growth and uniformity are studied [1].

Cellulose and chitosan belong among a wide group of natural polysaccharides. These natural polymers are the most abundant polysaccharides on the Earth. Natural polysaccharides have excellent biological properties, such as non-toxicity, biocompatibility and biodegradability [2 - 4]. Their composition is often advantageous, very similar and in some cases may even coincide with the tissue of human body. Cellulose wound dressings are commonly used in health care. Nowadays, chitosan is well known polycationic biopolymer with a wide spectrum of biological activities including antibacterial and antifungal effects. Antibacterial activity can be increased by addition of silver. Silver nanoparticles (AgNPs) can be prepared and stabilized by reduction of silver nitrate ($AgNO_3$) with chitosan without addition of reducing agents [5, 6]. The first, who have used chitosan in synthesis of gold and silver nanoparticles were Huang et al. [7, 8]. It is known, that chitosan is not soluble in water and in common organic solvents, but is soluble in aqueous solutions of organic or mineral acids. In acidic solutions amino groups of chitosan are readily protonated and chitosan becomes soluble [9 - 11]. The most common solvent is 1% (w/v) solution of acetic acid [12].

This work is focused on the preparation of polysaccharide films based on chitosan or composite films based on chitosan and cellulose. The prepared films were doped with $AgNO_3$ yielding AgNPs embedded in the polysaccharide matrix. The influence of addition of polyethylene glycol on the material properties and formation of AgNPs was studied. Wettability and water absorption of the films were evaluated and the transmission

electron microscopy of the dissolved films was measured. Antibacterial activity of the prepared films was also tested.

2. EXPERIMENTAL

2.1. Material

Chitosan (Chit) was obtained from Sigma-Aldrich, microcrystalline cellulose (MCC) was purchased from Modernist Pantry. Polyethylene glycol 400 (PEG 400) was obtained from Sigma-Aldrich and it was used as plasticizer. Silver nitrate ($AgNO_3$) was obtained from Sigma-Aldrich, acetic acid was purchased from Lach-Ner and distilled water.

2.2. Preparation polysaccharide films

Polysaccharide films were prepared by process shown at **Figure 1**. Chitosan (1 g) was dissolved in 200 mL of 0.2 % (v/v) solution of acetic acid. The solution was constantly stirred and heated to 60 °C. After 1 h, 10 mL of $AgNO_3$ (0.34 g of $AgNO_3$ was dissolved in 10 mL of water to achieve final concentration of 0.01 mol L^{-1}) was added. Then the solution was heated to 95 °C at constant stirring. During 3 h, colour of the solution changed from colourless to yellow or ochre. This colour change indicated reduction of silver ions to AgNPs [13]. Then MCC (1 g) was added and heated to 60 °C for 1 h at constant stirring. Then it was cooled down to the room temperature and aliquots of 10 mL were poured into circular silicone moulds, diameter 5 cm. The samples were dried for 16 h at 60 °C. The final products were homogenous polysaccharide films of yellowish colour (**Figure 2**). The next set of films was prepared by the same method without the addition of PEG 400 or MCC.

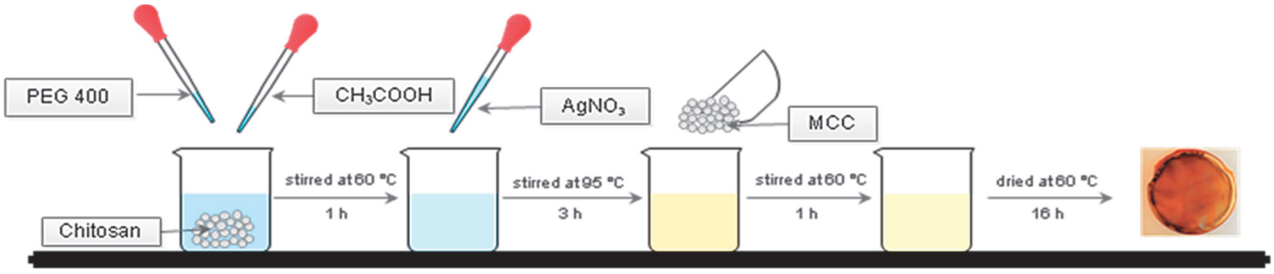

Figure 1 Schematic depiction of polysaccharide film preparation

2.3. Characterization methods

The presence of AgNPs was studied by X-ray photoelectron spectroscopy (ESCAProbeP spectrometer, Omicron Nanotechnology Ltd., Germany). Antibacterial activity of solid films was tested by disc test on two bacterial strains (Gram-positive *Staphylococcus epidermidis* and Gram-negative *Escherichia coli*). Solid films were dissolved in acetate buffer and the solution was measured by transmission electron microscopy (JEOL JEM-1010, Japan). Wettability was determined by contact angle measurement (Surface Energy Evalution System, Advex Instruments, Brno) and water absorption of the films was evaluated gravimetrically (UMX 2, Mettler Toledo, USA).

3. RESULTS AND DISCUSSION

3.1. Characteristics of solid films

Six types of polysaccharide films were prepared by the process described above (**Figure 2**). Chit-MCC and Chit-MCC-PEG 400 films were white and the films containing AgNPs were yellow to brown. PEG 400 was used for to improve material properties of the films.

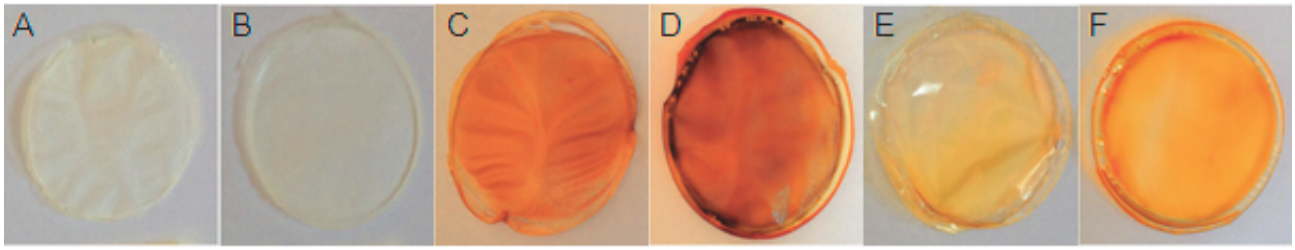

Figure 2 Prepared films:
A - Chit-MCC, B - Chit-MCC-PEG 400, C - Chit-MCC-Ag,
D - Chit-MCC-PEG 400-Ag, E - Chit-Ag, F - Chit-PEG 400-Ag

3.1.1. X-ray photoelectron spectroscopy

Concentration of elements on in the superficial layer of prepared films was studied by X-ray photoelectron spectroscopy (XPS). The analysis showed expected elements and the results are summarised in **Table 1**. The surface of samples contained elements typical for chitosan, cellulose and polyethylene glycol (carbon, oxygen and nitrogen). All samples with the addition of $AgNO_3$ had silver detected on the surface. **Table 1** shows that the highest concentration of silver was detected in the film of Chit-Ag and the lowest has Chit-PEG 400-Ag film [14]. The silver content in the surface layer of AgNPs modified chitosan films was ranging from 2.0 to 4.4 at. %.The highest amount of oxygen was present in the samples containing PEGs, which was expected result for the use of PEGs.

Table 1 Concentration of elements analysed by XPS

Sample	**Concentration of elements** (at. %)			
	C (1s)	**O** (1s)	**N** (1s)	**Ag** (3d)
Chit-MCC	57.07	28.47	14.47	-
Chit-MCC-PEG 400	60.28	33.90	5.83	-
Chit-MCC-Ag	57.34	32.61	6.19	3.86
Chit-MCC-PEG 400-Ag	58.82	34.15	4.04	3.00
Chit-Ag	57.92	31.16	6.47	4.44
Chit-PEG 400-Ag	63.12	30.23	4.56	2.09

3.1.2. Wettability and water absorption

Wettability and water absorption are very important properties of biomaterials that are in direct contact with living tissues. Values of the water contact angle determine surface wettability and the ability of material adhesion to the wound and the moist absorption. PEGs are known as stabilizing agents of AgNPs and we found out that PEG significantly altered the material properties of the prepared films [14]. **Figure 3** shows results of the wettability (water contact angel) and water absorption. The prepared samples with PEG have lower wettability and lower water absorption as well. Samples without addition of PEG have higher wettability and water absorption. We found out that the addition of silver significantly reduced the wettability and water absorption. The highest wettability has film Chit-MCC in another words sample without any additives (PEG 400 or silver). On the other hand the highest water absorption has film which contains only chitosan and silver.

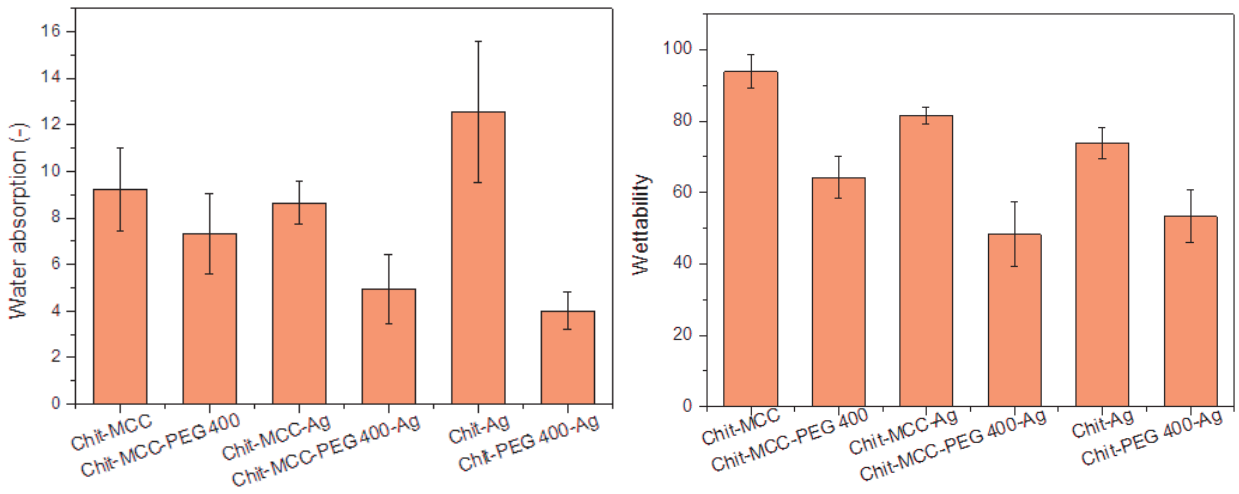

Figure 3 Wettability and water absorption of the prepared films

3.1.2. Antibacterial disc tests

Antibacterial activity of solid films was tested by the disc test on two bacterial strains, Gram-positive *S. epidermidis* and Gram-negative *E. coli.* **Figure 4** shows that samples with addition of silver have higher antibacterial activity than samples without silver. The antibacterial activity of samples without silver was not observed, even though chitosan is considered as an antibacterial biopolymer. The explanation is this observation is that the antibacterial activity of chitosan depends on pH, chitosan is active against bacteria only at pH<6 [15, 16] and this antibacterial tests were performed at pH equal to 7.

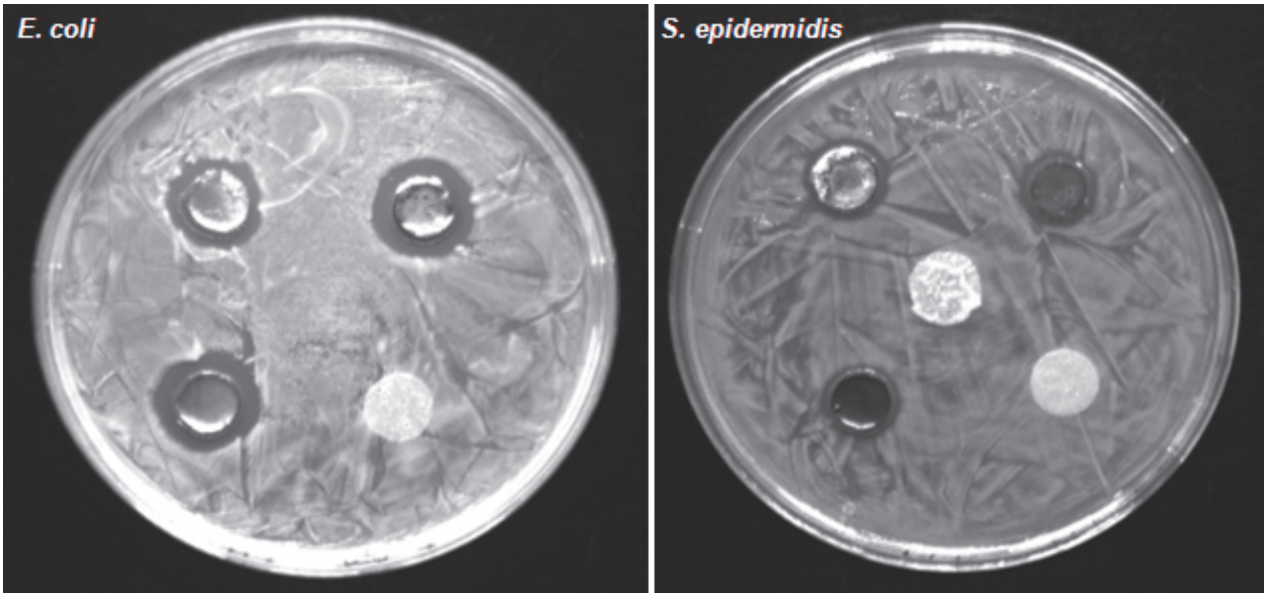

Figure 4 Antibacterial disc tests: Gram-negative *E. coli* and Gram-positive *S. epidermidis*

3.2. Characteristics of dissolved films

The prepared films are soluble only in the acidic region of pH. We dissolved a quarter of the prepared film at acetate buffer (pH = 4.65) and then TEM images were recorded.

3.2.2. Transmission electron microscopy (TEM)

Analysis of TEM images was used to obtain information about the size and shape of prepared AgNPs. **Figure 5** shows that AgNPs have different shape and the spherical shape prevails. Chit-MCC-Ag films contents AgNPs in the range of 10 to 80 nm, Chit-MCC-PEG 400-Ag 9 to 50 nm, Chit-Ag 7 to 50 nm and Chit-PEG 400-Ag 15 to 40 nm. TEM analysis confirmed that the PEG affect particle size as well a wettability and water absorption. Films with the addition of PEG have smaller silver nanoparticles than films without the use of PEG.

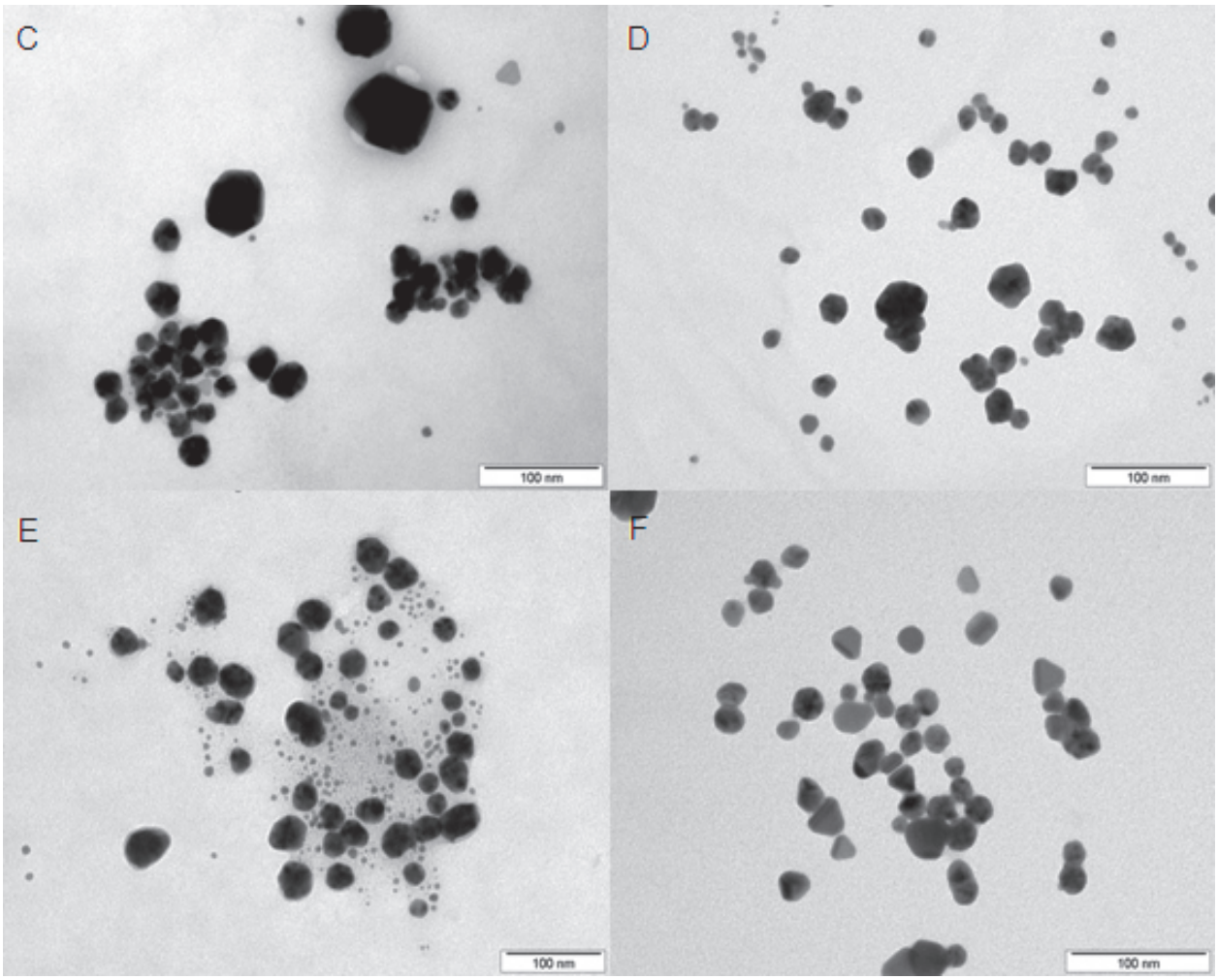

Figure 5 TEM images of dissolved chitosan films in acetate buffer:
C - Chit-MCC-Ag, D - Chit-MCC-PEG 400-Ag,
E - Chit-Ag, F - Chit-PEG 400-Ag

4. CONCLUSION

Six polysaccharide films with antibacterial activity were prepared. The silver content in the surface layer of the chitosan films was ranging from 2.0 to 4.4 at. %. The highest content of silver on the surface was found for Chitosan-Ag film and the lowest content was observed for Chit-PEG 400-Ag film. It was found out that polysaccharides films containing PEG 400 had reduced wettability and water absorption and the addition of PEG decreased the AgNPs size. TEM showed that AgNPs in the polysaccharide films have an average size of tens of nanometers with prevailing spherical shape.

ACKNOWLEDGEMENTS

This work was supported by the Grant Agency of CR (project No. 108/12/1168), by the Ministry of Health of CR (15-33018A) and by the specific university research (MSMT No 20/2016).

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