PREPARATION AND CHARACTERIZATION OF POLYSACCHARIDE FILMS BASED ON CHITOSAN

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Abstract

The present work is focused on preparation of chitosan films, which were doped with silver nanoparticles (AgNPs). AgNPs were prepared and stabilized by reduction of silver nitrate by chitosan without addition of other reducing agents. It was tested whether the use of UV light could affect the AgNPs in the process. The UV light irradiation took 5 min: during the preparation of chitosan solution and at the end of the process when the chitosan films were done. The presence of AgNPs was studied by X-ray photoelectron spectroscopy. Wettability and water absorption of the films were evaluated. Solid films were dissolved and then the solution was observed by the ultraviolet-visible spectroscopy and transmission electron microscopy. Concentration of the AgNPs released into the solution during dissolution was studied by atomic absorption spectroscopy. The presence of AgNPs was confirmed both in the solid films and in the solutions by the above mentioned methods. Our 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, chitosan, film preparation and characterization

1. INTRODUCTION

Chitin is one of the most abundant polysaccharides on Earth. It is very important for production of chitosan, because chitosan occurs in nature very rarely. Chitosan is produced by chemical or enzymatically deacetylation of chitin which is found in invertebrates as crustacean shells or insect coticules. Other sources of chitin are mushrooms envelopes, green algae cell walls and yeasts [1]. Chemically, chitosan is *N*-deacetylate derivative of chitin. Nowadays, chitosan is well known polycationic biopolymer with a wide spectrum of biological activities including antibacterial and antifungal effects and is used in both industry and pharmacy [2-4]. For better antibacterial activity of chitosan are added silver nanoparticles (AgNPs). Lately, a lot of studies were published about preparation, properties and utilization of chitosan films with AgNPs [3,5-7]. AgNPs can be prepared by different kinds of reactions. The first who have used chitosan in synthesis of gold and silver nanoparticles were Huang et al. [8,9]. Very cheap and easy was how to prepare and stabilize AgNPs is reduction of silver nitrate by chitosan without adding reducing agents [3,4]. This mean of preparation belongs to the field called green chemistry. Shao and Yao also used silver nitrate for the preparation of AgNPs but on the top of that they used UV light irradiation to initiate direct decomposition of silver nitrate [10].

This work is focused on the preparation of polysaccharide films based on chitosan. The prepared films were doped with silver nitrate yielding AgNPs embedded in the polysaccharide matrix. It was tested whether the use of UV light could affect the AgNPs. UV light was used for 5 min: during the preparation of chitosan solution and at the end of the process when the chitosan films were done. Concentration of elements in the surface layer of the prepared films was studied by X-ray photoelectron spectroscopy. Wettability and water absorption of the films were evaluated and the ultraviolet and visible spectroscopy of the dissolved films was measured. The concentration of AgNPs of the dissolved films was determined by atomic absorption spectroscopy. Size and shape of the AgNPs was studied by transmission electron microscopy.

2. EXPERIMENTAL

2.1. Material

High molecular weight chitosan (Chit) and silver nitrate ($AgNO_3$) were obtained from Sigma-Aldrich, acetic acid was purchased from Lach-Ner.

2.2. Preparation of polysaccharide films

Polysaccharide films were prepared via process shown in **Figure 1**. Chronologically: chitosan (1 g) was dissolved in 200 mL of 0.2 % (v/v) solution of acetic acid. The solution was constantly stirred and heated at 40 °C. After 2 h, 10 mL of $AgNO_3$ was added (0.34 g of $AgNO_3$ was dissolved in 10 mL of water, to achieve final concentration of 0.01 mol L^{-1}). Then the solution was heated to 90 °C under constant stirring. The colour of the solution changed from colourless to yellow or ochre during 1 h. This colour change indicated reduction of silver ions to AgNPs [12]. Then the solution was cooled down to the laboratory temperature and aliquots of 15 mL were poured into round silicone moulds, 5 cm in diameter. The samples were dried for 16 h at 60 °C. Finally, we got round homogenous yellowish, brown and violet-brown polysaccharide films (**Figure 2**). The other films were prepared by the same method under the same conditions with one extra step of 5 min UV light irradiation. Second set of films was irradiated after reduction of $AgNO_3$ to AgNPs, the solution changed colour to violet and then the solutions were poured into silicone moulds and dried for 16 h at 60 °C. The third set of polysaccharide films was irradiated at the end of the process when the chitosan films were done and dried.

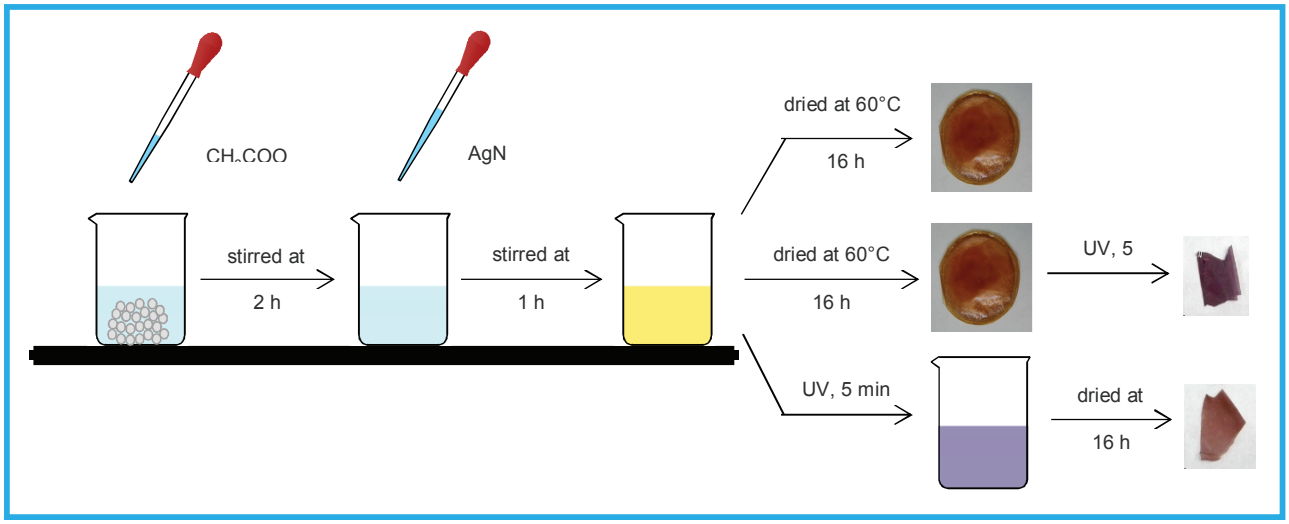

Figure 1 Schema of the preparation of polysaccharide films with AgNPs

2.3. Characterization methods

The presence and concentration of AgNPs and other elements on the surface of the prepared films were studied by X-ray photoelectron spectroscopy (XPS) (ESCAProbeP spectrometer, Omicron Nanotechnology Ltd., Germany). Wettability of the prepared films was evaluated by Surface Energy Evalution System Advex Instruments, Brno). Water absorption of the films was evaluated gravimetrically (RADWAG MAX 60, Poland). Solid films were dissolved in acetate buffer and the solutions were measured by Ultraviolet-visible spectroscopy (UV-Vis) (LAMBDA 25, PerkinElmer, USA) and transmission electron microscopy (JEOL JEM-1010, Japan). Transmission electron microscopy (TEM) was used to study the AgNPs size and shape. Concentration of AgNPs of dissolved films was determined by atomic absorption spectroscopy (AAS).

3. RESULTS AND DISCUSSION

3.1. Characterization of solid films

Three types of polysaccharide films based on chitosan were prepared (**Figure 2**). All of these films contained AgNPs. From **Figure 2** it is apparent that UV light irradiation affected the colour of polysaccharide films.

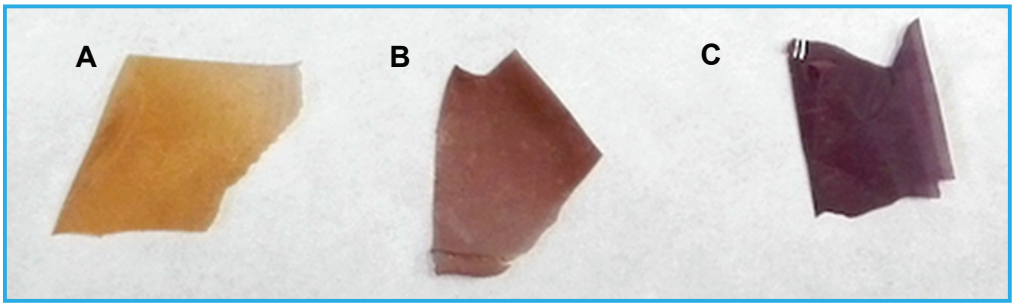

Figure 2 Prepared films: A - Chit-Ag, B - Chit-Ag-UV light (solution), C - Chit-Ag-UV light (film)

3.1.1. X-ray photoelectron spectroscopy

Concentration of elements on the surface of the prepared chitosan films was studied by X-ray photoelectron spectroscopy (XPS). The XPS showed the elemental composition of the superficial layer where we expected silver, results are summarised in the **Table 1**. As expected, the surface of samples contained elements typical for chitosan (carbon, oxygen and nitrogen) and silver. Silver was detected on the surface of all samples. **Table 1** shows that the highest concentration of silver was detected on the surface of the Chit-Ag samples, where UV light was used during preparation (irradiation of the solution). The silver content on the surface of these chitosan films was ranging from 2.7 to 4.4 at.%. The highest percentage of silver on the surface was found for the Chit-Ag films, where UV light was used during preparation. A slightly lower concetration of silver was observed for pristine films Chit-Ag and the lowest amount of silver was found for Chit-PEG 400-Ag films.

Table 1 Concentration of elements studied by XPS

Sample	**Concentration of elements** (at. %)				
	C (1s)	**O** (1s)	**N** (1s)	**Ag** (3d)	**impurities**
Chit-Ag	54.93	30.13	6.08	4.08	4.78
Chit-Ag-UV light (solution)	56.45	28.22	5.83	4.42	5.08
Chit-Ag-UV light (film)	56.86	33.09	6.31	2.69	1.04

3.1.2 Wettability and water absorption

Wettability and water absorption are very important properties of films which could be used as a wound cover. The values of the contact angle and water absorption determined wettability of the films. It can subsequently be used as an indicator of the ability to absorb exudate from injury. This testing is necessary to design wound healing material. **Figure 3** shows results of the wettability and water absorption measurement. From **Figure 3** it is apparent that samples where UV light irradiation was used have lower value of the contact angle than the pristine film Chit-Ag. The highest wettability has pristine film Chit-Ag. The water absorption of films where UV light irradiation was used have lower value of the water absorption than the pristine film Chit-Ag. We found out that the addition of silver significantly reduced the wettability and water absorption [7]. From these experiments we found out that UV light irradiation reduced the wettability and water absorption too.

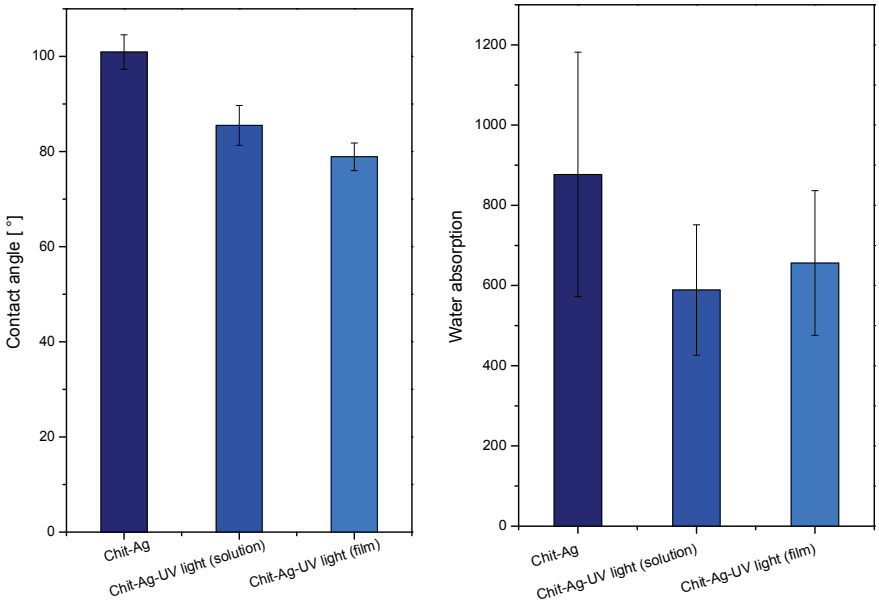

Figure 3 Wettability and water absorption of the prepared films

3.2. Characteristics of dissolved films

Due to the natural properties of chitosan, the prepared films were soluble only at acidic pH. We dissolved a quarter of the prepared film in acetate buffer (pH = 4.65) and then transmission electron microscopy (TEM) and ultraviolet-visible spectroscopy (UV-Vis) were measured. The concentration of AgNPs of the dissolved films was also determined by atomic absorption spectroscopy (AAS).

3.2.1 Ultraviolet-visible spectroscopy (UV-Vis)

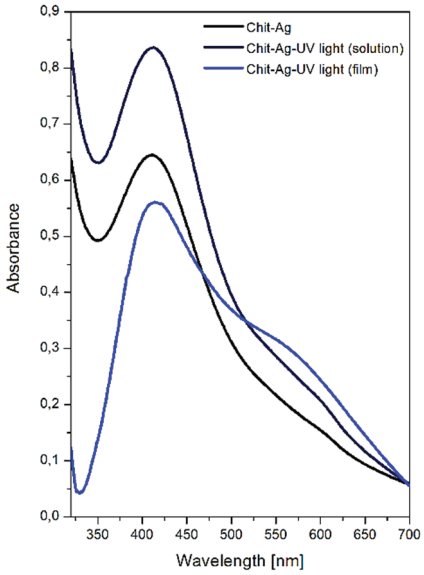

Figure 4 UV-Vis spectra of dissolved chitosan films in acetate buffer: A - Chit-Ag, B - Chit-Ag-UV light (solution), C - Chit-Ag-UV light (film)

AgNPs were confirmed by UV-Vis spectroscopy. AgNPs have characteristic peak at wavelength around 400 nm [7]. **Figure 4** shows that the highest absorbance had solution of dissolved Chit-Ag-UV light (solution)

film. Very similar peak with lower absorbance was observewd for the solution of dissolved Chit-Ag film. The lowest absorbance had solution of the dissolved Chit-Ag-UV light (film) film.

3.2.2 Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) was used to get information about particle size and shape of prepared AgNPs. **Figure 5** shows that AgNP had various shapes. Samples which were prepared with UV light irradiation had more AgNPs in the form of clusters than nonirradiated ones.

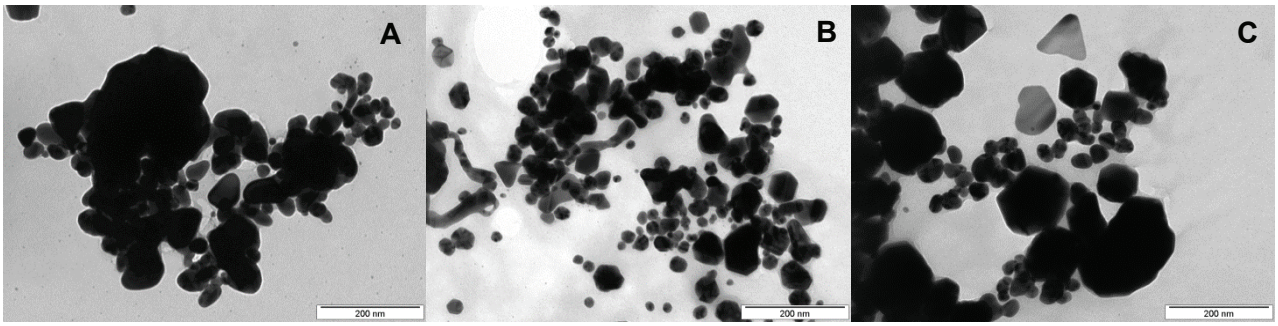

Figure 5 TEM images of dissolved chitosan films in acetate buffer: A - Chit-Ag, B - Chit-Ag-UV light (solution), C - Chit-Ag-UV light (film)

3.2.3 Atomic absorption spectroscopy (AAS)

Atomic absorption spectroscopy was used to examine the rate of released AgNPs from the prepared chitosan films. **Figure 6** shows that after 1 h the highest amount of AgNPs was released from the pristine Chit-Ag film. Lower amount of AgNPs was released from those chitosan films which were prepared with UV light irradiation. In this case, the UV light irradiation acted as a crosslinker so the release of AgNPs from the crosslinked film was difficult and therefore slower. After 18 h the amount of released AgNPs from the UV light irradiated films was approximately the same as for the other UV treated samples.

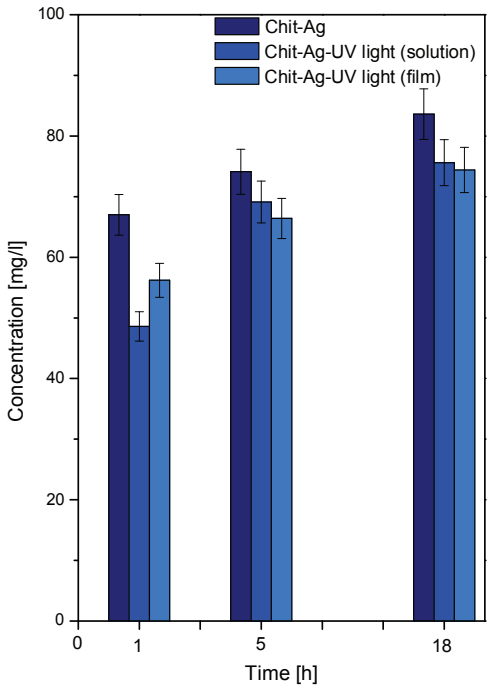

Figure 6 Concentration of released AgNPs from the films

CONCLUSION

Three polysaccharide films were prepared. The silver content on the surface of these chitosan films was ranging from 2.7 to 4.4 at. % of silver. The highest percentage of silver on the surface was found for the Chit-Ag-UV light (solution) films and the lowest silver percentage had the Chit-Ag-UV light (film) films. The wettability and water absorption of films which were UV light irradiated had lower value than pristine film Chit-Ag. Transmission electron microscopy showed that AgNPs in these polysaccharide films have an average size of tens of nanometers and various shape of AgNPs.

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