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FORMATION OF ANTIBACTERIAL COATINGS ON CHITOSAN MATRICES BY MAGNETRON SPUTTERING

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Chitosan as a biodegradable natural polymer is currently among the most attractive alternatives for the replacement of plastics, metals and other materials in various industries. Biodegradable chitosan matrices find their use as materials for biomedical devices, implants, scaffolds. Copper nanoparticles in their turn impart bacteriostatic and antibacterial properties to medical products, as well as copper coatings and nanopowders. We obtain thin copper coatings on the biodegradable chitosan matrices using magnetron sputtering. The optimal sputtering regimes were selected: high frequency discharge power of ~200 W at the frequency of 13.5 MHz in argon medium. The argon pressure was maintained within 1 Pa. As a target, a disk of 80 mm in diameter was made of copper grade M1. The resulting copper content in the samples does not exceed the toxicity threshold for humans 200–250 mg/day. The presence of an ultrathin copper film does not change the morphology and crystal structure of chitosan films. In the course of sample degradation in model media, copper is released from the film with the higher copper content in all variants of media (the highest release in the alkaline medium); and from all films in alkaline environment. The greatest yield is observed in the case of the smallest sputtering. To investigate the antimicrobial properties of copper-coated films, multiresistant strains of gram-positive and gram-negative microorganisms (S. aureus, E. coli and P. aeruginosa) were used. Summarizing the results, we can conclude that copper sputtering does not significantly affect the complex of the physicochemical properties of chitosan films, but imparts their antibacterial properties significantly; in particular, the films suppress the growth of S. aureus.

Keywords: *chitosan, copper, magnetron sputtering, antibacterial effect*

INTRODUCTION

Biodegradable natural and synthetic polymers are currently the most attractive alternative for the replacement of plastics, metals and other materials in various industries. These polymers are harmless to humans, and when decomposed they form elements that are not toxic, possess the property of biocompatibility, which is very important for the medical industry. Biodegradable chitosan matrices are used as a material for biomedical devices, implants, scaffolds [1]. Copper nanoparticles have recently been used to impart antibacterial properties to medical products. Copper coatings and nanopowders have bacteriostatic and bactericidal properties [2]. One of the methods is magnetron spraying of scaffolds. In particular, this method was used to modify scaffolds from polylactic acid [3]. Sputtering of thin copper films on the surface of the material can give additional antibacterial properties to chitosan materials, and also expand the scope of their application. The purpose of our work was to obtain copper coatings on chitosan matrices by magnetron sputtering, to select the optimum sputtering regime, to study the physico-chemical and antibacterial properties of the material.

MATERIALS AND METHODS

As chitosan matrices, chitosan films were used. The films were obtained by casting the 2 %

solution of chitosan in 1 % acetic acid on a Teflon substrate (resulting film thickness 0.03–0.30 mm).

For deposition of thin copper films, a magnetron sputtering method has been chosen which differs in such advantages as the possibility of films deposition on a cold substrate, high adhesion of the film to the substrate, uniformity of the films along the thickness when grown on large substrates [4].

Copper films were deposited by the method of high-frequency magnetron sputtering on chitosan substrates. Experiments on the deposition of films were carried out at the high frequency discharge power of ~200 W at the frequency of 13.5 MHz in argon medium. The argon pressure was maintained within 1 Pa. As a target, a disk of 80 mm in diameter was made of copper grade M1. The deposited biomaterial was located on a rotating substrate holder. The sputtering time and the target-substrate distance were selected in a way to minimize the effect of heating the substrate during copper sputtering.

Antibacterial properties. To investigate the antimicrobial properties of copper-coated films, multiresistant strains of gram-positive and gram-negative microorganisms (*S. aureus*, *E. coli* and *P. aeruginosa*) were used. From daily cultures of the studied microorganisms prepared, a microbial suspension to match the optical density of 0.5 units by McFarland scale (1.5×10^8). In a Petri dish with meat infusion agar 0.5×0.5 cm films were placed, then 0.1 cm poured by meat infusion agar, then bacteria were inoculated by lawn method and incubated at 37 °C for 24 and 48 h.

Optical properties were measured using a spectrophotometer KFK 3-01. Contents of copper was determined by atomic absorption spectrometry with electrothermal atomization (S115-M1, “Selmi”, Ukraine).

Fig. 1. Installation of high-frequency magnetron sputtering

RESULTS

Modified chitosan matrices were obtained.

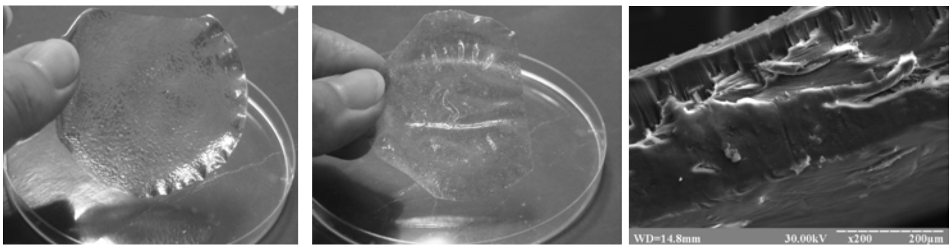

Fig. 2. Chitosan films with different types of copper coatings and electron microscopy of the film cross section

The spectra of the sputtered films exhibit maximum of 390 nm, which is due to the presence of copper nanoparticles.

Films with a minimum sputtering time of 10 s and a thickness of 0.03 mm are virtually indistinguishable in their capability to transmit light from similar films without sputtering. At the same time, the transparence of the films decreases with an increase in the sputtering time of the films of 0.3 mm.

The copper content does not exceed the toxicity threshold for humans 200–250 mg/day. The presence of an ultrathin copper film does not change the morphology and crystal structure of chitosan films, which is confirmed by SEM (see Fig. 2) and XRD.

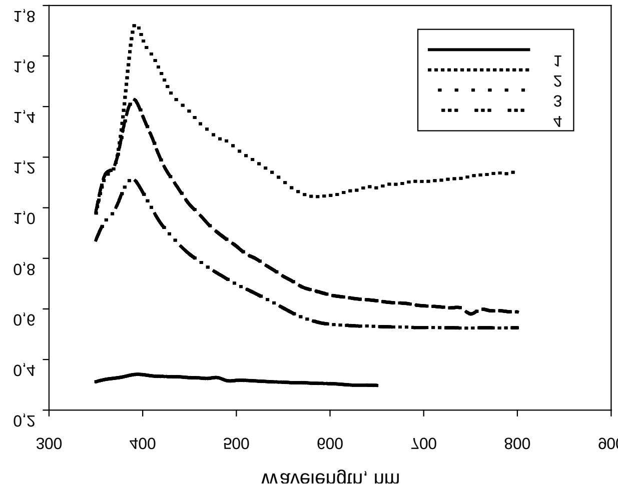

Fig. 3. Optical spectra of the chitosan-Cu films. *1* – Ch film; *2* – ChCu 20 s film; *3* – ChCu 15 s film; *4* - ChCu 10 s film; where 20, 15 and 10 s is the Cu deposition time

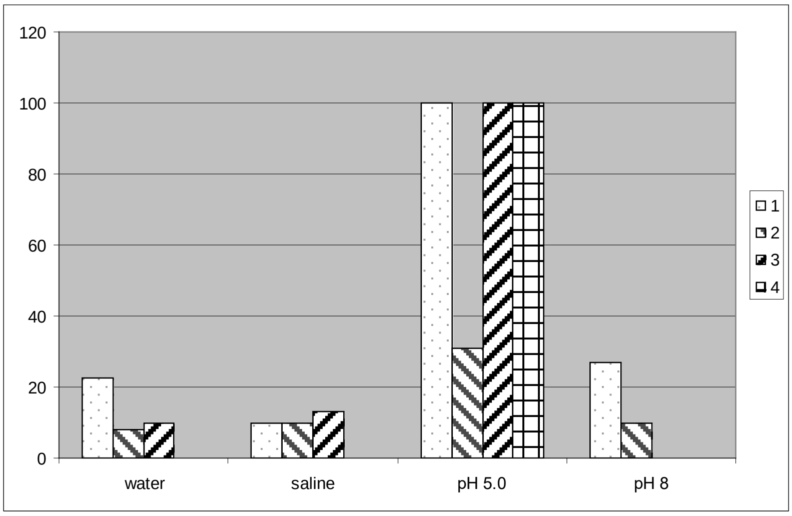

Fig. 4. Degradation of films (%) in different media (incubation for a month): *1* – Ch film; *2* – ChCu 20 s film; *3* – ChCu 15 s film; *4* – ChCu 10 s film

Table 1. Transparence (transmittance, %) of the samples, 660 nm

Sample	Transmittance, %	Thickness, mm
Ch film	45–49	0.03
ChCu 20 s film	7–8	0.30
ChCu 15 s film	24–29	0.30
ChCu 10 s film	47–48	0.03

Table 2. Copper content in the polymer films

Sample	Copper content, mg/kg	Copper content, mg/cm^2
Ch film	trace	trace
ChCu 20 s film	92.94776 RMSE 3.7892%	0.0040897 ± 0.0001549
ChCu 15 s film	50. 66775 RMSE 7.31677%	0.0020267 ± 0.0001482
ChCu 10 s film	118. 20432 RMSE 7.34273%	0.0014180 ± 0.0001041

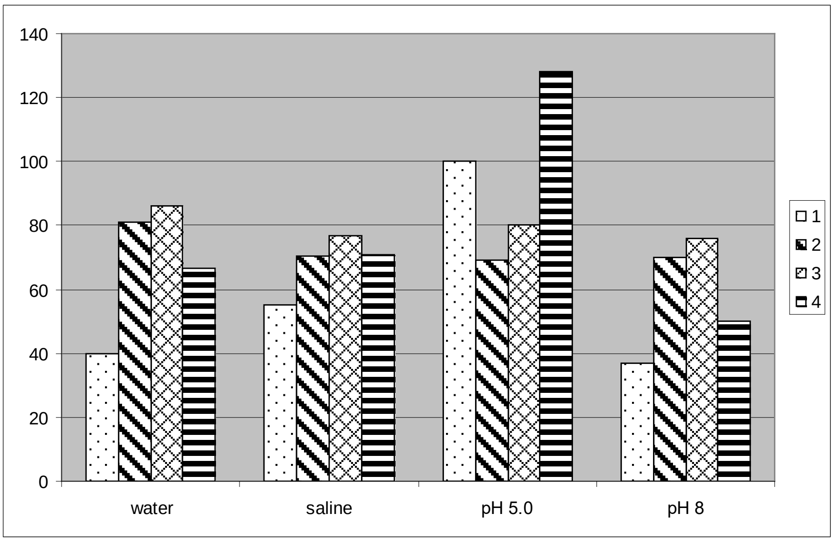

Fig. 5. Swelling of films in various media (%) for 60 min. *1* – Ch film, *2* – ChCu 20 s film, *3* – ChCu 15 s film, *4* – ChCu 10 s film

Table 3. The output of copper in the medium

Sample and medium	Mass of film, mg	Amount of medium added, ml	Copper content after 3 months, mg/ml
ChCu 20 s film in distilled water	12	1	0.00702 0.00503. RMSE 17.4%
ChCu 20 s in physiological saline (0.9% NaCl)	14	1	0.00243 RMSE 19.2%
ChCu 20 s film in physiological saline acidified with HCl (pH 5.5-6.0)	12	1	0.05618 RMSE 7.1%
ChCu 20 s film in physiological saline alkalized with NaOH (pH 8.0)	12	1	0.06450 RMSE 10.1%
ChCu 15 s film in distilled water	12	1	0
ChCu 15 s film in physiological saline (0.9% NaCl)	13	1	0
ChCu 15 s film in physiological saline acidified with HCl (pH 5.5-6.0)	12	1	0
ChCu 15 s film in physiological saline alkalized with NaOH (pH 8.0)	8	1	0.03675 RMSE 8.0%
ChCu 10 s film in distilled water	2	1	0
ChCu 10 s film in physiological saline (0.9% NaCl)	2	1	0
ChCu 10 s film in physiological saline acidified with HCl (pH 5.5-6.0)	3	1	0
ChCu 10 s film in physiological saline alkalized with NaOH (pH 8.0)	2	1	0.13673 RMSE 7.3%

Table 4. Antibacterial properties

Sample	***Pseudomonas aeruginosa***		***Staphylococcus aureus***		***Escherichia coli***	
	24 h	48 h*	24 h	48 h	24 h	48 h
ChCu 20 s film	±		±	+	+	
ChCu 15 s film	±		–		+	
ChCu 10 s film	±		–		+	

\+ – the growth of bacteria around the sample (partly on it) is identical to the growth on nutrient medium;
± – the growth of bacteria around the sample was significantly less than that on nutrient medium;
– no growth of bacteria under the sample and within about 1-2 mm around is observed;
* – data shown in case of changes in the growth of bacteria

Deposition of sputtered copper reduces the degradation of films in media with the neutral pH and pH shifted to the alkaline range. The swelling ability in sputtered films is higher.

According to the results, copper is released from the film with the higher copper content in all variants of media (the highest release in the alkaline medium); and from all films in alkaline environment. The greatest yield is observed in the case of the smallest sputtering.

Antibacterial activity summary:

- films coated during 10 and 15 s exhibit the antibacterial effect on *S. aureus*,
- films coated in 20 s exhibit bacteriostatic effect on *S. aureus*,
- all samples exhibit bacteriostatic effect on *P. aeruginosa*,
- all samples exhibit antibacterial and/or bacteriostatic effect on *E. coli*.

CONCLUSION

Copper sputtering does not significantly affect the complex of the physicochemical properties of chitosan films, but imparts their antibacterial properties significantly; in particular the films suppress the growth of *S. aureus*.

Формування антибактеріальних покриттів на хітозанових матрицях методом магнетронного напилення

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Методом магнетронного розпилення отримано тонкі мідні покриття на біодеградуючих хітозанових матрицях. Вивчено фізико-хімічні характеристики отриманих покриттів, а також їхні антибактеріальні властивості. Вміст міді не перевищує порогу токсичності для людини 200–250 мг/доба. Наявність ультратонкої мідної плівки не змінює морфологію і кристалічну структуру плівок хітозану, але значно підвищує їхні антибактеріальні властивості; зокрема, плівки пригнічують ріст S. aureus.

Ключові слова: *хітозан, мідь, магнетронне напилення, антибактеріальний эфект*

Формирование антибактериальных покрытий на хитозановых матрицах методом магнетронного напыления

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Методом магнетронного распыления получены тонкие медные покрытия на биодеградируемых хитозановых матрицах. Изучены физико-химические характеристики полученных покрытий, а также их антибактериальные свойства. Содержание меди не превышает порога токсичности для человека 200–250 мг/сут. Наличие ультратонкой медной пленки не изменяет морфологию и кристаллическую структуру пленок хитозана, но значительно повышает их антибактериальные свойства; в частности, пленки подавляют рост S. aureus.

Ключевые слова: *хитозан, медь, магнетронное напыление, антибактериальный эффект*

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