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Chitosan scaffolds, films and microgranules for medical application — preparation and drug release studies

Summary — The paper presents various structural forms of chitosan for medical applications. These are films and microgranules for transdermal and oral application of active substances and porous scaffolds constituting support for the regenerating cells in bone tissue engineering. Tested porous systems were obtained by lyophilization or electrolytic method. Methods of sample preparation and the morphology analyses were presented. The influence of water on properties of transdermal systems and changes in release kinetics of active substances for microgranules and films under modification of their structures, were carried out.
Keywords: scaffolds, microgranules, kinetics of drug release, lyophilization, electrolysis.

SKAFOLDY, BŁONKI I MIKROGRANULE CHITOZANOWE DO ZASTOSOWAŃ MEDYCZNYCH — OTRZYMYWANIE I BADANIA KINETYKI UWALNIANIA LEKU
Streszczenie — W artykule przedstawiono różne formy strukturalne chitozanu znajdujące różnorodne zastosowania w medycynie. Omawiane formy to błonki i mikrogranule do transdermalnej oraz doustnej aplikacji substancji aktywnych, oraz porowate skafoldy stosowane w inżynierii tkankowej, stanowiące rusztowanie dla regenerującej się tkanki kostnej. Badane w pracy układy porowate otrzymano metodą liofilizacyjną lub elektrolityczną. Opisano sposób przygotowania oraz morfologię otrzymanych próbek. Zbadano wpływ wody na właściwości układów transdermalnych oraz zmiany w kinetyce uwalniania substancji aktywnych z mikrogranul oraz błonek wywołane modyfikacją ich struktury.
Słowa kluczowe: skafoldy, mikrogranule, kinetyka uwalniania leku, liofilizacja, elektroliza.

INTRODUCTION

Progress in medicine persisting since the beginning of the nineties of the twentieth century to the present day, brought the possibility of treating the lesions at an unprecedented scale. Modern methods of treatment using techniques inaccessible before, start to become a daily routine in tissue engineering, pharmacology and diagnostics. Progress has been made possible mainly by the use of modern materials, particularly natural polymers. Biopolymers have several properties that favor them as preparation materials for bone implants and matrices for controlled release of drugs.

Many studies in recent years have focused on chitosan [1—3], a natural polymer obtained from chitin deacetylation process. The interest in chitosan stems mainly from its unique properties. It has a bactericidal and fungicidal ability and moreover, it is biocompatible [4, 5] which means, which introduced into a living organism do not cause any negative immune response. The products of biodegradation are non-toxic, which allows for their metabolism and removal from the body. For years, the studies are focused on bioactive properties of chitosan, and its effect on the activation and inhibition of various processes in living organisms [6]. Numerous researches are devoted to the work associated with producing porous biopolymer systems based on chitosan scaffolds, as implants for applications in tissue engineering. Such systems can be used as templates for the reconstruction of damaged skin [7, 8], liver [9] and bone [10—13].

There are several methods to prepare scaffolds for tissue engineering. The most commonly used method is freeze-drying method based on freeze-evaporation of the solvent from previously frozen chitosan solution. The sites from which solvent crystals were evaporated have been formed in the polymer structure so far [14, 15]. Variable process conditions allow for controlled porosity and obtaining a specific pore size. Properties of structures can also be modified by adding additives to systems of other polymers such as polycaprolactone (PCL), polyvinyl alcohol (PVA) or using fillers such as calcium and phosphorus compounds which are designed to stimulate the process of osteogenesis.

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A relatively new method for obtaining scaffolds is electrospinning. Systems obtained with this method constitute porous network resulting from the imposition of successive layers of each strand of the polymer. Preparation of such structures involves spraying through the nozzle of the polymer strands having a positive charge in the direction of the grounded anode. The process requires a large potential difference between the nozzle and a grounded collecting plate, towards which polymer strands are directed. The amount of polymer solution fed to the nozzle is measured exactly by the infusion pump [16].

Due to the increasing interest in natural biopolymers for the controlled release of drugs flat films (transdermal patches) deserve on attention [17—20]. This is the most friendly form of drug administration to the patient. The transdermal patch is applied to the surface of human skin, so that the drug penetrates into the body bypassing the digestive system of the patient. This method can provide a number of substances such as lidocaine, ibuprofen, testosterone, antibiotics and vitamins. Flat matrices usually consist of a layer containing the drug substance, adjacent to the outer protective layer. Numerous advantages of investigated systems influenced the abundance of their applications, among others as slices of a sedative, analgesic activity, in heart disease, and others.

An important issue is also enteric-coated drugs of prolonged activity, based on a polymer matrix in the form of granules [21]. Modifications involving the transfer of the protective layers allow significantly extending the drug release, maintaining its therapeutic concentration at the patient's blood for a longer period of time. There is some interesting literature concerning models describing drug release from various morphological polymer structures matrix [22—27]. Most of them are found to justify a Fickian diffusion mechanism, some of them are modified by a dissolution profile, polymer erosion during drug release into the dissolution medium. The modeling of our results will be the topic of further studies.

The paper presents sample preparation of various medical structures of chitosan, used as controlled release systems for drugs (granules, films) and biodegradable bone implants (scaffolds). The ways to modify obtained systems to improve their properties are shown. The article demonstrates also several studies, including kinetics investigations as useful for preparation of medical materials, based on biopolymers. There are ways to modify such systems to improve their properties and to demonstrate the usefulness of the medical structures, based on biopolymers.

EXPERIMENTAL

Materials

In the experiment one applied chitosan (CS) of a deacetylation degree 73.3 % and viscosity 200 mPas (beads and films) produced by Sea Fisheries Institute in Gdynia and of a deacetylation degree 85 % and viscosity 120 mPas (scaffolds) of the company BioLog GmbH.

To improve the osteoconductive properties of scaffolds hydroxyapatite (HAp) $Ca_5(PO_4)_3OH$ of the company Sigma-Aldrich was applied.

The model active substance used for the study of the kinetics of drug release was salicylic acid [SA, $C_6H_4(OH)COOH$], of the company Chempur.

For crosslinking of chitosan two substances were used. They were sodium tripolyphosphate ($Na_5O_{10}P_3$, STPP) of the company Fluka or glutaraldehyde ($C_5H_8O_2$) produced by Sigma-Aldrich.

For coating of chitosan granules polylactide (PLA) (of glass transition temperature T_g = 57 °C) of the Nature Works company 4042D (USA) was used.

All reagents used in the experiment had analytical purity.

Methods of sample preparation

Production of scaffolds by lyophilization

2 % chitosan solution in 1 % water acetic acid solution was prepared, next 5—15 % of hydroxyapatite (HAp) in relation to chitosan mass was added. The solution was subjected to mixing in a magnetic stirrer for 20 min, and next it was subjected to ultrasonic mixing (ultrasonic cleaner 21.5 kHz, time 20 min) to obtain a good dispersion of HAp in the solution. The process of magnetic and ultrasound mixing was repeated twice. The solution was transferred on Petri dishes and placed on a heating plate at the temperature of 55 °C to preliminary evaporation of the solution. After 4 h the thickened solution (to 30 %) was frozen at -37 °C and kept at this temperature for 24 h. The frozen systems were subjected to the process of lyophilization, where freeze drying was carried out under the conditions of deep vacuum and at the temperature of -80 °C.

Preparation of scaffolds by the electrolytic method

To 1 % of chitosan solution in 1 % aqueous acetic acid 15 % of HAp in relation to the mass of chitosan was added and stirred first, with a magnetic stirrer for 20 min and then using ultrasounds for 20 min (the process was repeated twice). The solution was introduced into an electrolytic cell with capacity of 50 cm^3. Electrodes were made of stainless steel. The system was powered by a laboratory stabilized power supply MNGR-300. The process was conducted at a constant voltage of 20 V, a current flowing through the system depended on the instantaneous resistance of the electrolyte and the thickness of the scaffolds formed on the electrode. The process was carried out for about 20 min, until the complete disappearance of the current flowing through the system. The scaffold obtained at the cathode was removed using a scalpel and tran-

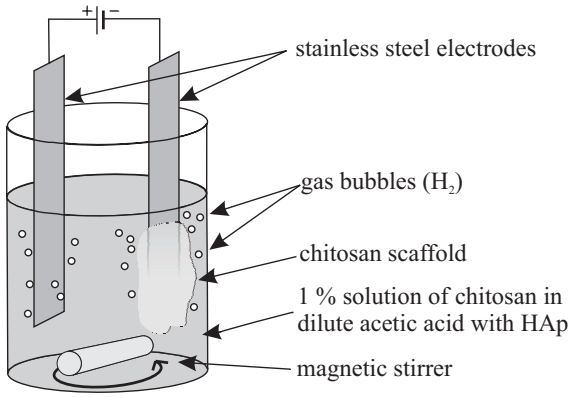
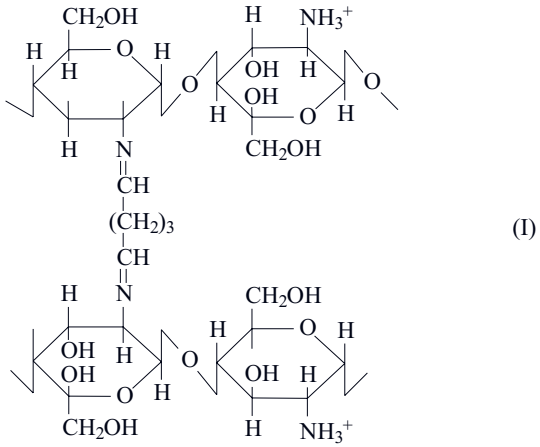


Fig. 1. The scheme of scaffolds preparation with the electrolytic method

sferred to a Petri dish. Preliminarily frozen at the temperature of -37 °C and then subjected to freeze-drying. The diagram of the manufacturing process by electrolytic method is shown in Figure 1.

Production of flat chitosan films as drug carriers

To obtain flat polymer films 1.5 % chitosan solution in dilute acetic acid was used. Next, 10 % m/m of salicylic acid in relation to chitosan mass in the solution was added. The system was subjected to mixing on a magnetic stirrer for 30 min at the stabilized temperature 27 °C. Chitosan crosslinked with glutaraldehyde was prepared to investigate the differences in the kinetics of active substance release. Formula (I) shows the structure unit of the product of crosslinking chitosan by glutaraldehyde.



(I)

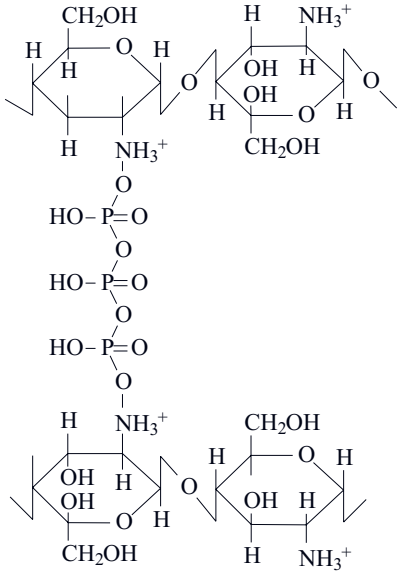
The crosslinked solution was obtained by adding 1 % glutaraldehyde in mass ratio relative to the mass of chitosan in solution. Then a certain part of the solution was transferred to a Petri dish and the solvent was evaporated on a hot plate at a stabilized temperature of 50 °C. After complete evaporation of the solvent, obtained films were transferred for 2 h to a bath in methyl alcohol to remove the acid residues. After the etching process films were redried.

The obtained crosslinked and uncrosslinked chitosan films ~100 μm thick were subjected to their coating with a layer of PLA. The process was designed to slow down drug release from chitosan matrix. The coating was made by dipping chitosan film in a solution of PLA in methylene chloride. The average thickness of the applied layer of PLA was ~30 μm. Predicted good adhesion of PLA to the chitosan layer occurs due to ionic interactions.

Preparation of chitosan beads

Chitosan hydrogel granules were prepared based on the phenomenon of coacervation, *i.e.* dehydration of hydrophilic sols evoked by a high concentration of electrolyte. 1 % m/m solution of chitosan in acetic acid was added using compressed air passing through a nozzle 0.8 mm in diameter to 1 % m/m solution of STPP at pH = 3. At low pH in the reaction of chitosan with STPP there occurs ionic crosslinking [25] which product has structural units shown by formula (II).

After coacervation the obtained hydrogel granules were separated from the solution and frozen at -27 °C.



(II)

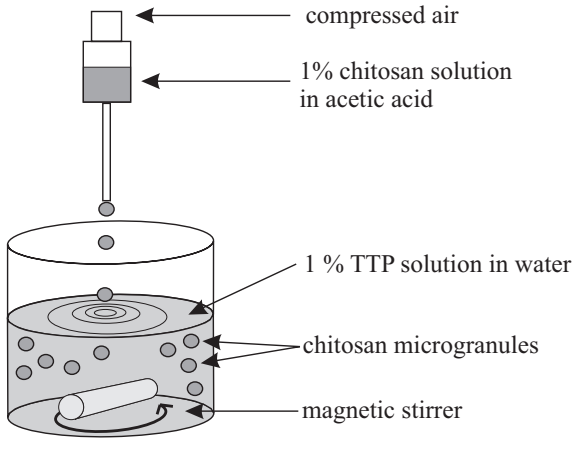


Fig. 2. Scheme of chitosan beads formation process

Then they were subjected to lyophilization. Figure 2 shows a scheme of production of chitosan granules. This process produced beads ~1.5 mm in diameter were obtained.

Filling the beads with salicylic acid

Filling the chitosan beads by SA was carried out in the vacuum chamber (of the company ArtVac). The SA solution in deionized water at a concentration $2.1 \cdot 10^{-4}$ mol/dm^3 was placed in the chamber and chitosan granules on a grid of movable arm of the chamber. In order to degasify granules and the solution, the pressure in the chamber was lowered to 0.2 bar, and then the arm of the chamber was moved, so as the mesh with the granules was immersed in the filling solution. The chamber was aerated again, and the granules were left in the solution for 5 to 30 min. Filled beads were transferred to a vacuum drier to evaporate water, the drying process was carried out for 60 min at 60 °C.

Modification of chitosan beads filled with salicylic acid

To achieve extended time of SA release from chitosan granules, some CS-SA granules after the impregnation process were coated with a layer of a second polymer, which was PLA.

CS-SA beads were coated with a solution of PLA in ethyl acetate by immersion. CS-SA beads were placed in a container with a solution of PLA in ethyl acetate (2.2 % m/m), and mixed with a magnetic stirrer. After 3 min beads were filtered with a sieve and dried for 5 min at 50 °C to evaporate ethyl acetate. This process was repeated 3 or 5 times to modify the thickness of PLA outside control layer.

Test of the scaffold porosity degree

Porosity of the obtained scaffolds was determined as follows:

$$P = \frac{V_s - V_c}{V_s} \cdot 100\ \% = \frac{V_s - (m_c / \rho_c)}{V_s} \cdot 100\ \% \qquad (1)$$

where: V_s *— scaffold volume,* V_c *— volume of chitosan in the scaffold,* m_c *— mass of scaffold,* ρ_c *— density of scaffold (~1.61 g/cm*^{3} *average density of chitosan scaffold with 15 % m/m of HAp).*

To examine the morphology and distribution of hydroxyapatite microparticles in the structure of scaffold, scanning electron microscope (SEM) images were made. SEM pictures were taken with a microscope Joel JSM-5500LV.

Examination of swelling degree of chitosan films

For crosslinked and uncrosslinked CS films swelling kinetics has been measured. Films that do not contain SA were etched in methyl alcohol for 2 h, then they were subjected to drying at 80 °C for 1 h. After complete drying, the films were put into vessels containing distilled water at the stabilized temperature of 23 °C. Films were weighed at specified intervals, and the degree of swelling was determined by the formula:

$$\alpha = \left(\frac{m_m - m_s}{m_m} \right) \cdot 100\ \% \qquad (2)$$

where: α *— degree of swelling, the amount of adsorbed water,* m_m *— mass of wet sample,* m_s *— dry sample mass.*

Examination of intrinsic viscosity of dilute chitosan solutions obtained from the films

To determine changes in the structure of chitosan the investigations of reduced viscosity ($\eta_{reduced}$) of dilute chitosan solutions (in 1 % acetic acid) were made. The study was conducted for various dilutions of CS solution in the thermostated system at the temperature of 25 °C, using an Ubbelohde viscometer. To determine the intrinsic viscosity the following dependence was used:

$$\eta_{reduced} = [\eta] + K[\eta]^2 \cdot C \qquad (3)$$

where: *[*η*] — intrinsic viscosity, K — constant, C — concentration of chitosan.*

$\eta_{reduced}$ was determined according to equation:

$$\eta_{reduced} = \frac{\eta - \eta_o}{\eta_o \cdot C} \cong \frac{t - t_o}{t_o \cdot C} \qquad (4)$$

where: η, η_o *and* t, t_o *— viscosity and time of solution and solvent, respectively.*

[η] was found by extrapolation of $\eta_{reduced}$ to $C = 0$.

Examination of the release kinetics of active substances

The kinetics of active substance (SA) release from the beads and flat films to the buffer system at pH equal to 5.6 and 7.2 was examined using the UV-Vis spectrometry. The release process was conducted in a glass vessel with a volume of 50 cm^3 at 37 °C. The solution was subjected to stirring with a magnetic stirrer in order to offset the concentration of the total volume of the system. Measurements of the spectrum in order to observe changes in the characteristic peak of SA (λ = 297.2 nm) were made using a spectrophotometer Jasco V-630.

Kinetics of SA release is described by two equations.

The first is the following equation of I-order reaction kinetics:

$$f_t = \frac{C_t}{C_{\max}} = 1 - e^{-kt} \qquad (5)$$

where: $f_t = C_t/C_{max}$ *— the fraction of SA released to the buffer at the time t(*C_t*) in relation to the maximal amount of released drug in the release process (*C_{max}*), k — the kinetic constant of the I-order reaction.*

The second is the equation derived by Peppas:

$$f_t = a \cdot t^n \qquad (6)$$

where: $f_t = C_t/C_0$ — *fraction of salicylic acid released to the buffer at the time t(C_t) in relation to the initial amount of a drug contained in the polymer (C_0), a — the kinetic constant of the Peppas equation, n — exponent defining the mechanism of the drug release.*

RESULTS AND DISCUSSION

Morphology of scaffolds

Porosity of obtained scaffolds ranged from 87 to 94 % for systems obtained with the freeze-drying method. The higher was the amount of HAp, the lower was the porosity of scaffolds. Structures obtained with the electrolytic method were characterized by porosity ranging from 75 to 84 %, however the pore size was much greater than in case of systems obtained in the process of freeze-drying. Figure 3 shows electron microscope images of a scaffold obtained with freeze-drying (a, b) and electrolytic method (c, d).

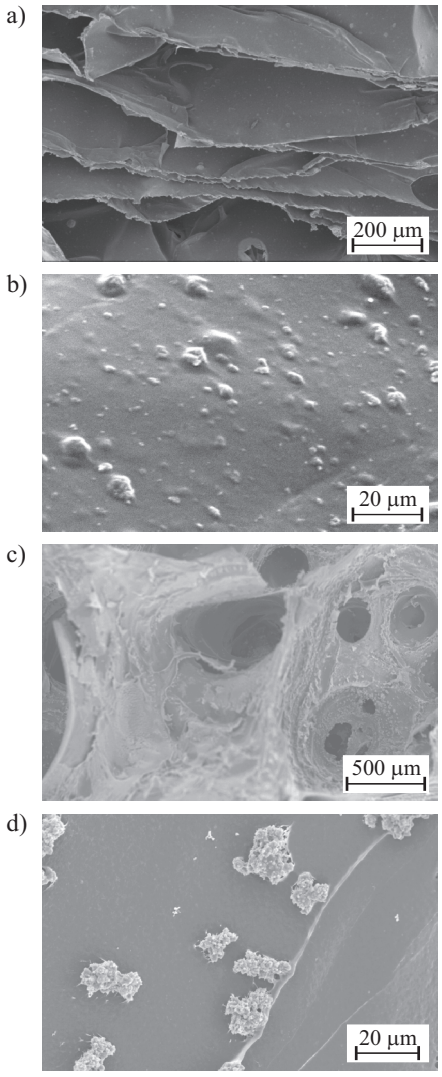


Fig. 3. SEM images of scaffolds obtained by freez-drying: cross section (a), surface with visible hydroxyapatite (b) and with electrolitic method: cross section (c), surface with visible hydroxyapatite (d)

Microscopic examination showed that HAp particles are located in the structure of the polymer surface, and their size does not exceed 15 μm when they were obtained with freeze-drying method and 30 μm for particles obtained with electrolytic method. Dispersion of HAp in the system is irregular.

The swelling degree of the chitosan films

The swelling process proceeds fast, reaching equilibrium in a few minutes. Figure 4 shows the graph of the swelling kinetics of chitosan crosslinked with glutaraldehyde and uncrosslinked one. Uncrosslinked chitosan absorbs more than two times more water than crosslinked chitosan. The process proceeds according to the kinetics of I-order reaction.

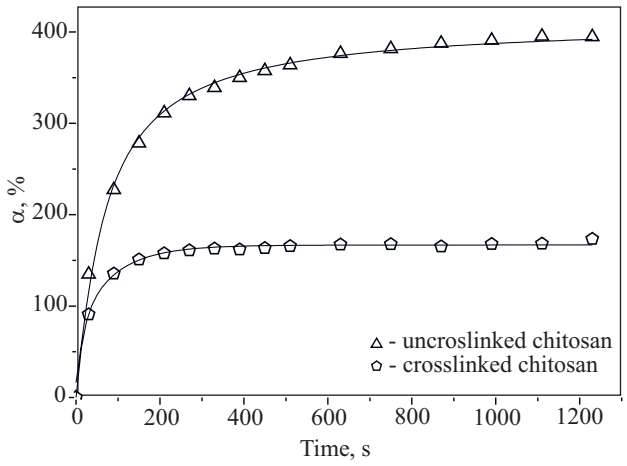


Fig. 4. The curves of the swelling kinetics for non-crosslinked chitosan and chitosan crosslinked with glutaraldehyde (points — experimental results, lines — I-order kinetics)

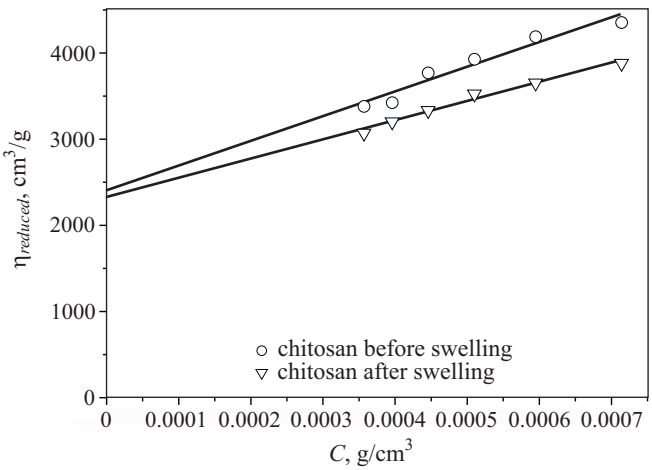


Fig. 5. Diagram of the reduced viscosity versus the concentration of chitosan solution

Reduced viscosity before and after swelling

The study of the reduced and intrinsic viscosities before and after release showed a slight decrease of their values. Figure 5 shows plots of reduced viscosity $\eta_{reduced}$ versus the concentration of the chitosan solution (chitosan samples before and after release — time equal to 200 h).

Change in the reduced and intrinsic viscosities of chitosan dilute solutions may indicate a slight decrease of molecular weight of the polymer in buffer solutions (during the release process) as a result of hydrolysis.

Kinetics of release of active substance

Release from chitosan granules

Granules at different time of filling absorbed different amounts of active substance. Figure 6 shows the kinetic release of drug [$C_t(t)$] into the buffer from 10 mg of chitosan granules. Using the measured maximum concentration of drug released into the buffer (C_{max}), average concentrations of the drug in 10 mg of chitosan granules was estimated. Amount of drug closed in the granules depending on the filling time is shown in Table 1.

T a b l e 1. Amount of a drug introduced to 10 mg of granules in the filling process

Filling time, min	Amount of a drug closed in 10 mg of granules, mg
5	2.27
15	3.46
30	5.62

A study of the kinetics of active substance (SA) release from chitosan granules subjected to different impregnation times (different filling time) was conducted.

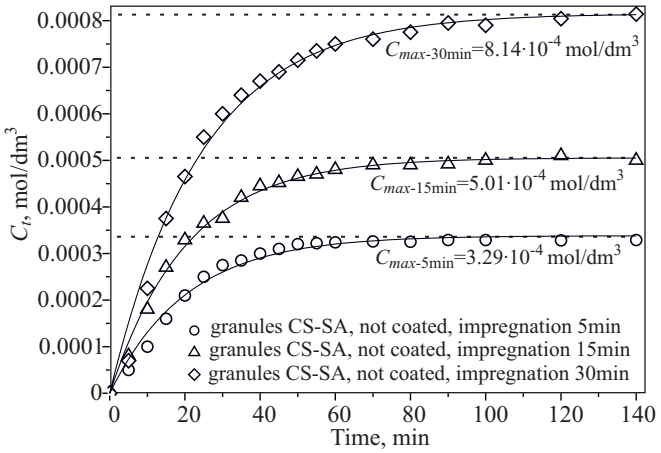


Fig. 6. Drug release kinetics [C = f(t)] to a buffer of pH = 7.2

The I-order kinetic model, given by eq. (5) matches the results.

Figure 7 shows plots of the kinetics of drug release [$f_t(t)$] for different times of impregnation (filling).

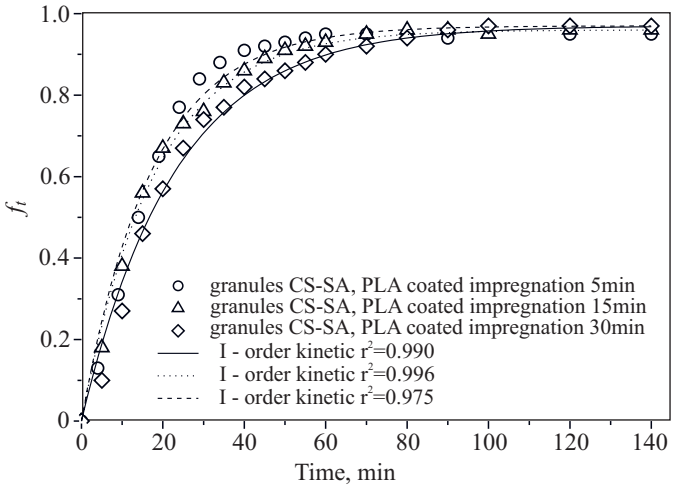


Fig. 7. Kinetics of SA release [$f_t = f(t)$] from chitosan granules for different impregnation times

The shorter the time of filling, the faster the salicylic acid releases from chitosan granules. This can be related to lower deep sedimentation of the drug in the pores of chitosan matrix. Drug release from such a sample is

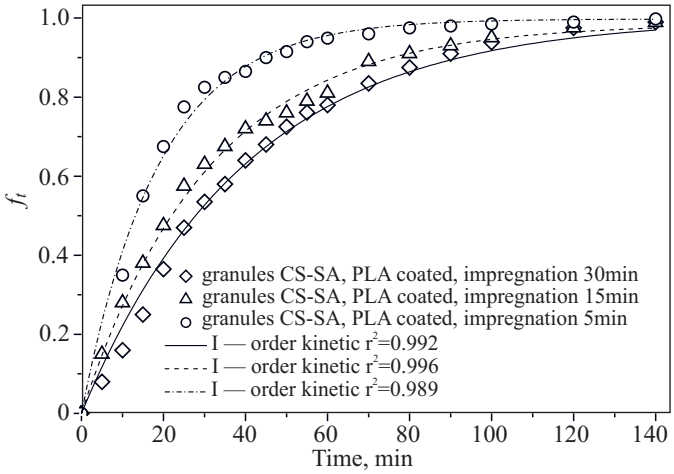


Fig. 8. The kinetics of SA release [$f_t = f(t)$] from chitosan granules coated with PLA

easier and faster. A similar trend occurred for PLA-coated granules, the release rate decreased for long filling time. Figure 8 shows the kinetics of coated PLA release [$f_t(t)$] from chitosan beads.

In Table 2 the kinetic parameters of the model for the release of granules of varying impregnation are listed.

T a b l e 2. Kinetic parameters of the release model

Impregnation time (filling), min	Parameter *k*, 1/min	
	uncoated with PLA	coated with PLA
5	0.058	0.042
15	0.055	0.032
30	0.043	0.024

Release from the chitosan films

To compare the rate of active substances release from various chitosan films the kinetics for non-modified films crosslinked with glutaraldehyde and coated with PLA were studied. Figure 9 shows the release kinetics [$f_t(t)$] from the unmodified chitosan film and crosslinked with glutaraldehyde.

The release of SA from crosslinked chitosan and uncrosslinked chitosan has very similar courses (swelling of both samples is sufficiently high). Figure 10 shows the release curves for crosslinked (with glutaraldehyde) and coated (with PLA) chitosan films.

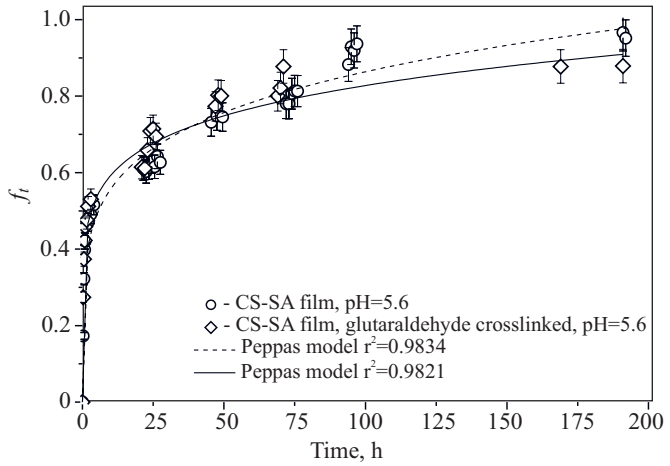


Fig. 9. Kinetics of SA release [$f_t = f(t)$] from unmodified chitosan films and films crosslinked with glutaraldehyde

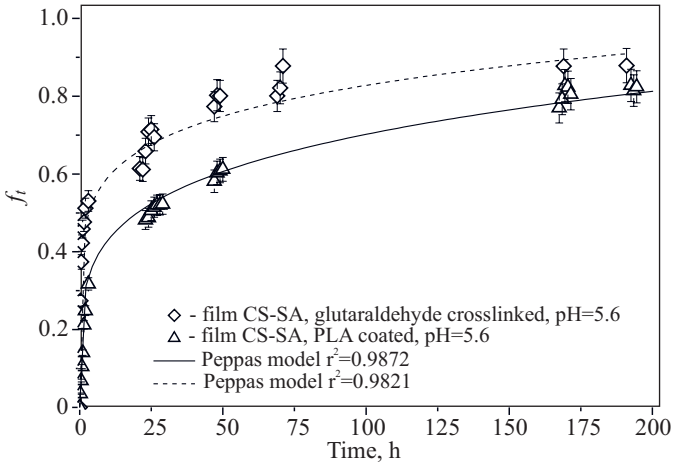


Fig. 10. Kinetics of SA release [$f_t = f(t)$] from chitosan film crosslinked with glutaraldehyde and chitosan film coated with a 30 μm layer of PLA

The outer layer covering chitosan is made of a hydrophobic polymer, which is PLA. In comparison to chitosan PLA swell is small (~5 %) thus its presence on the chitosan surface affects the rate of drug diffusion outside into the buffer.

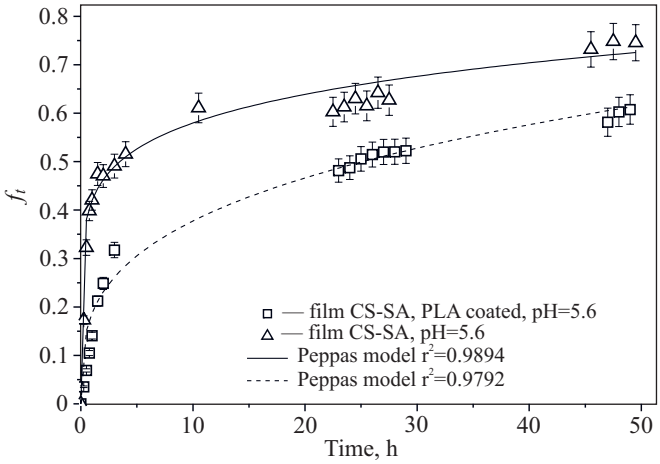


Fig. 11. Kinetics of SA release [$f_t = f(t)$] from the unmodified chitosan films and from chitosan films coated with PLA

Figure 11 shows the release curves for unmodified and coated with PLA chitosan films.

T a b l e 3. Kinetic parameters of the Peppas model

Chitosan	Parameter of the Peppas model	
	a, 1/h	*n*
Unmodified	0.36	0.19
Crosslinked by glutaraldehyde	0.42	0.14
Coated with PLA	0.28	0.11

After fifty hours of the process about 55 % of drug was released in the case of coated chitosan film. For the sample without protective layer more than 70 % of the active substance is released in the same time. The curves of the release process do not fit well to the I-order reaction kinetics. Thus the Peppas model [eq. (6)] was applied. The curve fitting allows determining the model parameters which are listed in Table 3.

CONCLUSIONS

The results presented in the work show that chitosan-based materials properties allow their medical use. Porous scaffolds with the addition of hydroxyapatite constitute a good environment for the growth of bone tissue in the process of regeneration. In order to compare,

scaffolds received in the presented paper were obtained with two methods: freeze-drying and electrolytic. The electrolytic method is new and innovative method for formation of scaffold from chitosan, insoluble in water. In other methods were obtained chitosan acetate scaffolds, which require crosslinking or deactivating of NH_3^+ group. Freeze-drying scaffolds have higher porosity (average 90 %) in comparison to scaffolds obtained by electrolytic method. At the same time in freeze-drying scaffolds the average size of HAp agglomerates is almost twice higher than in electrolytic scaffolds (equal to 30 μm).

Systems for the drug release, both transdermal patches in the form of films, and oral ones in the form of microgranules after appropriate modifications allow for considerable elongation of time for the release of the active substance. In the case of chitosan granules coated with PLA layer double extension of release time was observed. 80 % of drug located in polymer matrix was released after 60 min, whereas from unmodified granules after 30 min. No significant loss of drug from the polymer matrix during coating with PLA layer was observed.

In the case of chitosan films no influence of crosslinking with glutaraldehyde on release rate was observed. Covering of chitosan film with a PLA layer results in significant extension of the release time. For coated chitosan films 60 % of drug is released after 50 h, however for not coated film as early as after 25 h.

Following the presented results on preparation and application of different chitosan structures in medicine, the following tests are now continued:

— preparation of scaffolds of different structural composition and pore size,

— preparation of complex flat structures containing microspheres with drugs or multilayer structures of variable thickness and different drug concentration for drug release control.

Further research in this topic will allow for better understanding of the mechanisms of the drug release kinetics and for design of systems with pre-established release time.

REFERENCES

1. Lijun K., Yuan G., Guangyuan L., Yandao G.: *Eur. Polym. J.* 2006, **42**, 3171.
2. Wenshui X., Ping L., Jiali Z., Jie Ch.: *Food Hydrocolloids* 2011, **25**, 170.
3. Chunmeng S., Ying Z. *et al*.: *J. Surg. Res.* 2006, **133**, 185.
4. Muzzarelli R. A. A.: *Carbohydr. Polym.* 2009, **76**, 167.
5. Muzzarelli R. A. A.: *Carbohydr. Polym.* 2011, **83**, 1433.
6. Lie M., Changyou G., Zhengwei M., Jie Z.: *Biomaterials* 2003, **24**, 4833.
7. Wang X. H., Wang Q. L, Feng F. Z.: *Biomaterials* 2003, **24**, 3213.
8. Sundararajan V. M., Howard W. T. M.: *Biomaterials* 1999, **20**, 1133.
9. Lifeng Q., Zirong X., Xia J., Caihong H.: *Carbohydr. Res.* 2004, **339**, 2693.
10. Cheng X., Li Y., Zuo Y., Zhang L.: *Mater. Sci. Eng.* 2009, **29**, 29.
11. Chunmeng S., Ying Z., Xinze R., Meng W.: *J. Surg. Res.* 2006, **133**, 185.
12. Prashanth K. V. H., Tharanathan R. N.: *Trends Food Sci. Tech.* 2007, **18**, 117.
13. Mucha M.: „Chitosan — universal polymer from renewable sources", WNT, Warszawa 2010.
14. Xi-Guang Ch., Cheng-Sheng L., Chen-Guang L., Xiang-Hong M.: *Biochem. Eng. J.* 2006, **27**, 269.
15. Tylman M., Mucha M.: *Prog. Chem. Appl. Chitin Derivat.* 2010, **15**, 97.
16. Jayakumar R., Prabaharan M., Nair S. V., Tamura H.: *Biotechnol. Adv.* 2010, **28**, 142.
17. Prausnitz M., Langer R.: *Nat. Biotechnol.* 2008, **26**, 1261.
18. Guy R. H., Hadgraft J.: *Pharm. Sci. Technol.* 2000, **3**, 318.
19. Kalia Y. N., Guy R. H.: *Adv. Drug. Deliver. Rev.* 2001, **48**, 159.
20. Michalak I., Traczyk D., Mucha M.: *Prog. Chem. Appl. Chitin Derivat.* 2010, **15**, 107.
21. Balcerzak J., Mucha M.: *Prog. Chem. Appl. Chitin Derivat.* 2010, **15**, 117.
22. Macheras P., Dokoumetzidis A.: *Pharm. Res.* 2000, **17**, 108.
23. Siepmann J., Göpferich A.: *Adv. Drug Deliver. Rev.* 2001, **48**, 229.
24. Wada R., Hyon S., Ikada Y.: *J. Controlled Release* 2001, **37**, 151.
25. Siepmann F., Le Brun V., Siepmann J.: *J. Controlled Release* 2006, **115**, 298.
26. Shu X. Z., Zhu K. J.: *Eur. J. Pharm. Biopharm.* 2002, **54**, 235.
27. Korsmeyer R. W., Gurny R., Doelker E., Buri P.: *Int. J. Pharm.* 1983, **15**, 25.

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