Polyoxyethylene containing chitosan derivatives for the development of hydrogels

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Abstract – ***Polyoxyethylene containing derivatives of succinic acid (2SA-PEG) have been synthesized via the acylation of polyethylene glycols with succinic anhydride. Water-soluble chitosan derivatives and films based on them have been obtained through the interaction between 2SA-PEG and chitosan (CS). Water-insoluble films have been developed via crosslinking during thermal treatment of the products of the interaction between 2SA-PEG and chitosan. The films form hydrogels after swelling in water. The hydrogel films, filled with rhodamine G and malachite green dyes, are capable of prolonged releasing them into an aqueous medium.***

Key words – chitosan, polyoxyethylene containing chitosan derivatives, reagentless stitching of chitosan, pH-sensitive hydrogels.

I. Introduction

The development of new polymeric materials via the modification of natural polymers, chitosan in particular, has been of great interest recently. These materials are nontoxic, have high biocompatibility with living tissues, antibacterial properties and are hydrophilic. They adsorb heavy metal ions and other toxic substances. They can swell in aqueous solutions depending on pH [1]. These properties allow to use goods from chitosan derivatives, films in particular, for micro- and nanocapsules, hydrogels, coatings for wounds, fibers and bandages in surgery in the treatment of wounds, as materials for implants, for matrices for tissue bioengineering, in pharmacy as carriers of enzymes, drugs and other biologically active compounds [2], cosmetic preparations for making both in care and treatment of affected skin and burns.

Aldehydes, such as dialdehydes, methanal, glyoxal as well as henipin, diepoxides, nucleosides, nucleotides, [2,3], diisocyanates, monocarbonates derivatives, cyclic anhydrides of dicarboxylic acids, epoxy compounds, crown ethers, oxyaldehides [2] have been used as crosslinkers for the development of chitosan-based hydrogels.

Chitosan is soluble in water and forms three-dimensional structures in the presence of crosslinking agents. To dissolve chitosan, the presence of acids is required for the protonation (e.i., ionization) of primary amino groups in the glucosamine fragments.

The aim of this work was to synthesize polyethylene glycol (PEG) succinates with the different PEG chain length and polyoxyethylene containing water soluble chitosan derivatives for the development of pH-sensitive hydrogels by thermostatting.

II. Experimental part

Synthesis of polyoxyethylene disuccinates (2SA-PEG) – polyoxyethylene containing derivatives of succinic acid were synthesized via the acylation of polyethylene glycol of a different chain length with succinic anhydride (SA). The reaction was carried out in a 1,4-dioxane solution at 80 ˚ C and a molar ratio of PEG to SA as 1:2 (Fig. 1).

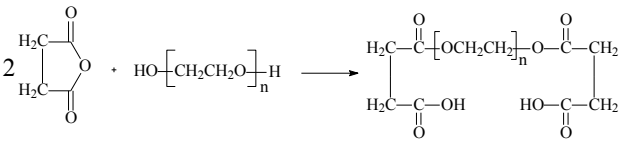

Fig. 1. The scheme of formation of 2SA-PEG, n = 1÷34

Water-soluble chitosan polyoxyethylene derivatives (CS-2SA-PEG) were obtained via the interaction of CS and 2SA-PEG in distilled water at room temperature and stirring for 1-2 hours. Chitosan concentrations in aqueous solutions were equal to 0.5-3.0%wt. 2SA-PEG concentration was calculated from the ratio of NH_2-groups in chitosan and carboxylic -C(O)OH groups in 2SA-PEG, which were varried in the range from 1,0:0,5 to 1,0:1,0.

Chitosan based films (CS-2SA-PEG) were prepared from their aqueous solutions or the aqueous solutions of mixtures CS-2SA-PEG and polyvinyl alcohol (PVA). The ratio of CS-2SA-PEG to PVA in dry films was from 1:0 to 1:2 (by weight). To form films, the aqueous solutions were cast on polyethylene or polytetrafluoroethylene substrates and dried at room temperature.

Crosslinking the films and the formation of three-dimensional structures from the film-forming polymers have been carried out by thermostatting of dry films at 100 ÷ 120 ˚ C for 15 ÷ 210 minutes.

Determination of total content of the amino- and carboxylic groups in the crosslinked films (overall functionality) during their thermostatting was performed by potentiometric titration with 0.1 N aqueous NaOH in the presence of HCl (back titration).

Determination of the amino groups in the crosslinked films was performed by potentiometric titration with perchloric acid in acetic acid as a non-aqueous medium.

The swelling degree of the obtained films was determined from the equation [4]:

$$\alpha = \left(W_S - W_D\right) \Big/ W_D \times 100, \% \qquad (1)$$

where α – film absorption of water, thickness 0,15 ÷ 0,2 mm; W_D and W_S – weight of a dry and swollen film, respectively, g.

The equilibrium swelling degree was determined from the equation [4]:

$$\alpha_p = \left(W_E - W_D\right) \Big/ W_D \times 100, \% \qquad (2)$$

where α_p – the maximum film absorption of water (equilibrium); W_D and W_E – weight of a dry and swollen film, respectively, g.

Absorption of Rhodamine G (RG) and Malachite Green (MG) in the hydrogel films. An RG solution was prepared in a mixture of benzene and ethanol (volume ratio as 11.0:0.1). An MG solution was prepared in water.

Hydrogel films were placed in the prepared dye solutions. The concentration of RG in the benzene-ethanol solution and the concentration of MG in the aqueous solution were determined using UV-Vis spectroscopy and calibration curves. The RG or MG absorption (mg/g of a polymer film) was calculated as the difference between the initial current concentration of RG in benzene-alcohol solution during the experiment, referred to the mass of the film. Films with the adsorbed dyes were dried.

Dye release from the films. Dried films with the absorbed dyes were placed in an aqueous solution at room temperature and the weight ratio of the film to the solution 1:50. The concentration of the released dyes in the aqueous solution was determined from the calibration curves.

FTIR spectroscopy

The FTIR spectra of synthesized CS-2SA-PEG were recorded in a thin layer deposited from a benzene solution on a potassium bromide tablet, using a Thermo Scientific Nicolet Fourier Transform Infrared Spectrometer, in the range of 400-4000 cm^{-1} with compensation of atmospheric CO_2 and H_2O.

NMR spectroscopy

NMR spectra of CS-2SA-PEG were recorded in acetone-d_6 and chloroform-d solutions of CS-2SA-PEG using a 500 MHz Varian Inova spectrometer.

Antibacterial activity of hydrogel films CS-2SA-PEG was determined on Escherichia coli and Staphylococcus aureus, as an example. A thin layer of hydrogels of CS-2SA-PEG_{1000} were applied to aluminum discs for this. The impact of hydrogel films on growth was analyzed and cell viability of microorganisms was compared to the blank experiments.

III.Results and discussion

The structure of synthesized 2SA-PEG has been confirmed using FTIR- (Fig. 2) and NMR (Fig. 3) spectroscopy.

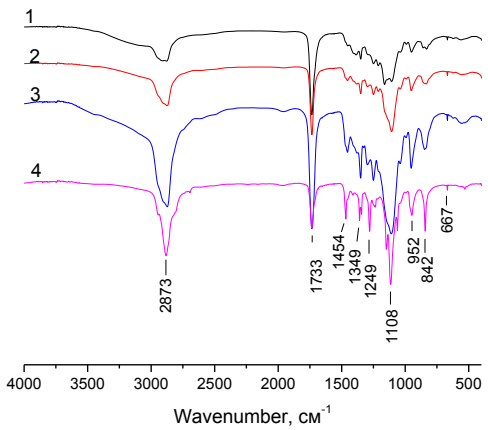

Fig. 2. FTIR-spectra 2SA-PEG with the different length of chains of PEG: 1 – PEG-300, 2 – PEG-600, 3 – PEG-1000, 4 – PEG-2000.

^{1}H NMR spectra has also confirmed the structure of the synthesized 2SA-PEG (Fig. 3 and Table 1).

The presence of the absorption bands in the spectra of 2SA-PEG (Fig. 2) at 2873 (ν_s, CH_2), 1454 (δ_s, CH_2), 1349 cm^{-1} (δ_s, CH_2) corresponds to the aliphatic fragments of succinic acid and the methylene fragments of PEG. The absorption bands at 1733 cm^{-1} (ν, C=O) and 952 (δ, C–O–H) indicates the presence of carboxylic groups. The absorption band at 1733 cm^{-1} (ν, C=O) has been attributed to the carbonyl group in the ester group whereas the absorption band at 1249 cm^{-1} (ν, O=C–O–) has been classified as the ester fragment. The presence of polyethylene glycol fragments in 2SA-PEG has been confirmed by the absorption band at 1108 cm^{-1} (ν, C–O–C), whose intensity increases with the increasing PEG chain length.

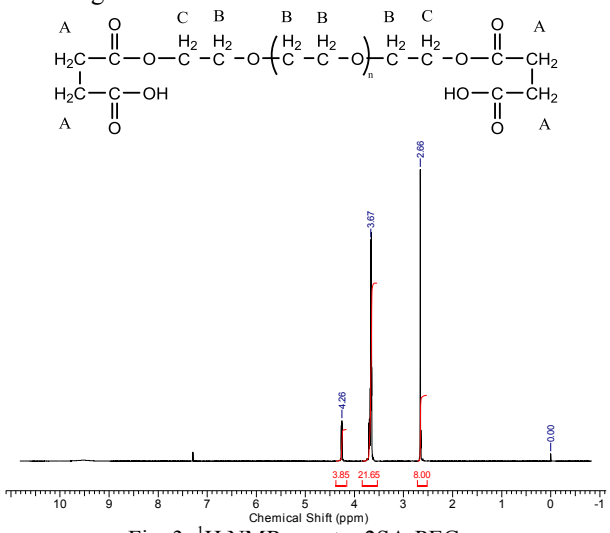

Fig. 3. ^{1}H NMR-spectra 2SA-PEG_{300}

The integral values of the protons in the spectra of samples 2SA-PEG with the different PEG chain length indicate the coincidence with the theoretical composition (Table 1).

TABLE 1

CHARACTERIZATION OF 2SA-PEG USING ^{1}H NMR-SPECTROSCOPY*

Mw PEG	Chemical shift	Integral	Proton	H amount	Location of the protons
300	2.50-2.66	8.00	A	8	SA
	3.67	21.6	B	22	PEG
	4.26	3.8	C	4	α -CH_2 PEG
600	2.49-2.65	8.00	A	8	SA
	3.65	49.8	B	50	PEG
	4.26	3.8	C	4	α -CH_2 PEG
1000	2.49-2.65	8.00	A	8	SA
	3.65	74.8	B	75	PEG
	4.26	3.8	C	4	α -CH_2 PEG
2000	2.49-2.65	8.0	A	8	SA
	3.65	163.7	B	164	PEG
	4.26	3.9	C	4	α -CH_2 PEG

* Protons indicated on Fig. 3

Synthesized 2SA-PEG are water soluble. The interaction of the samples CS-2SA-PEG in water is the protonation of the primary amino groups in the glucosamine fragments of chitosan, their ionization and the formation of salt bonds in the water-soluble adducts CS-2SA-PEG (Fig. 4).

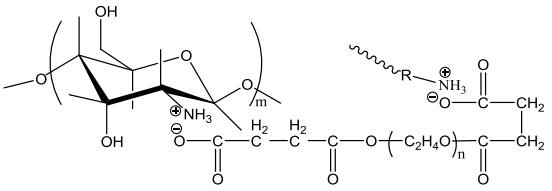

Fig. 4. The scheme of the fragment of water-soluble chitosan-polyethylene glycol succinate adducts (CS-2SA-PEG), where R is chitosan macromolecule fragment.

Films have been formed on polymer substrates from aqueous solutions of CS-2SA-PEG and they have been dried at room temperature. The resulting dry films are soluble in water.

Crosslinking polymer molecules and the formation of three-dimensional structures of the CS-2SA-PEG films has been carried out via thermostatting at 100 ÷ 120 ˚ C. It has been found that, at these temperatures, the covalent crosslinking of the fragments occurs with the formation of ester bonds.

Potentiometric titration has showed the decrease of the total content of the carboxyl and amino groups in the films during thermostatting at 100 ÷ 120 ˚ C. Using selective potentiometric titration of amino group, it has been determined that the number NH_2-groups in the films do not decrease during the crosslinking. It has been shown that the reduce of the polymer film functionality is a result of the depletion of the carboxyl groups. It has been also shown that reduce only a limited portion of the carboxyl groups, which is about half of their original number.

The analysis of the dependences of the swelling degree of the CS-2SA-PEG films and the content of gel and sol fractions during crosslinking at 120 ºC allows to draw some conclusions about the chemistry of the crosslinked films (Fig. 5). It has been shown that the swelling degree of the films decreases during to the thermostatting. However, the content of a gel fraction increases during certain thermostatting time, which begins to rise above the sol fraction (Fig. 5). This suggests that, besides to the esterification reaction through the interaction between –C(O)OH groups of 2SA-PEG and –OH groups of chitosan, the peresterification reaction occurs after 40-60 min of thermostatting at 120 ºC.

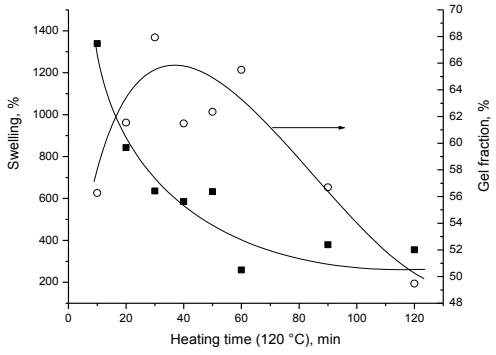

Fig. 5. Dependence of the swelling degree and gel-sol fraction of films CS-2SA-PEG_{600} from the thermostatting time at 120 ºC (chitosan Mw = 33100)

The peresterification reactions of covalently bound chitosan and PEG disuccinates occur during their interaction with the OH groups of chitosan. It leads to the formation of a dense polymer network and a sol fraction containing monosuccinates and PEG. This conclusion is confirmed by the ^{1}H NMR spectrum of the sol fraction film obtained via thermostatting for 60 minutes at 120 ° C (Fig. 6).

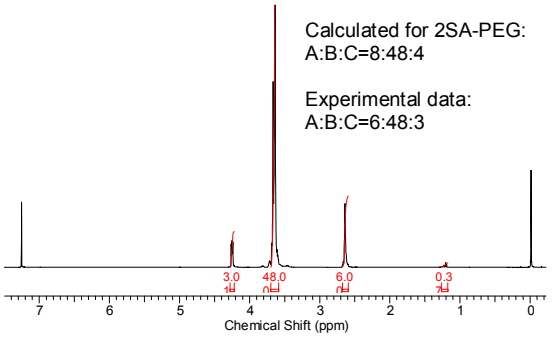

Fig. 6. ^{1}H NMR-spectra of sol fraction of CS-2SA-PEG_{600} film, thermostatting during 60 min at 120 ºC.

It has been shown that ^{1}H NMR-spectra of the sol fraction contained signals with chemical shifts typical for ^{1}H NMR-spectra of 2SA-PEG_{600}. However, the found ratio of the proton integral values (A:B:C=6:48:3) is different from the value in the initial 2SA-PEG_{600} (A:B:C=8:48:4). It indicates a deviation from the composition of 2SA-PEG_{600} aside more PEG fragments. Considering the content of a gel fraction (Fig. 5), it should be noted that the contribution of the peretherification process is quite significant at the deep stages of crosslinking.

However, it has been experimentally confirmed that the interaction of monoglucosamine (with a protected aldehyde group) with 2SA-PEG occurs mainly due to the amino group. This difference in the interaction between 2SA–PEG and monoglucosamine and the interaction between 2SA–PEG and the polymer molecules of chitosan can be explained by steric hindrace in the case of chitosan.

CS-2SA-PEG film, crosslinked through heat treatment without the addition of a crosslinking agent, is not soluble in water, but it swells in an aqueous medium and forms hydrogels. The equilibrium swelling degree is inversely proportional to the film incubation time, due to the formation of a dense polymer network with a longer cross-linking time (Fig. 7).

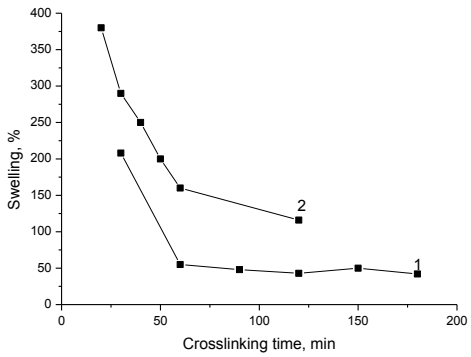

Fig. 7. The dependence of the swelling degree (for 30 minutes in water with a pH of 6.5) of 2SA-PEG_{Mm}-CS films with different molecular weight of the PEG fragment (Mm) after incubation at 120 ° C: 1 – Mm – 100, 2 – Mm – 600.

It has been found that the increasing length of the PEG fragment in 2SA-PEG-CS leads to the increase in the swelling degree (Fig. 7), and the rate and the equilibrium swelling degree depends on the pH and increases in acidic aqueous solutions.

To increase the mechanical strength of the obtained hydrogel films, 0.1÷0.5 %wt. of PVA has been added. It has been shown that films formed from the 2SA-PEG-CS-PVA solutions swell in water well, and their swelling degree and rate increase at lower pH (Fig. 8).

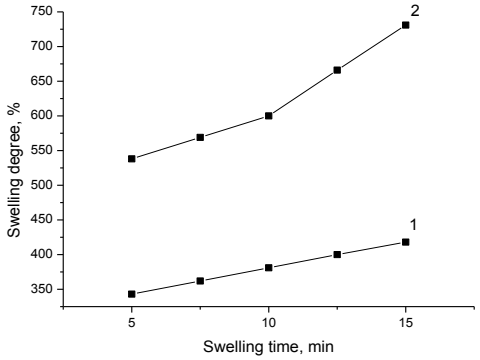

Fig. 8. Kinetics of swelling of 2SA – PEG_{Mm} -CS – PVA films at different pH: 1 – pH 6,5; 2 –pH 3,2. (Mm- 100, ratio CS : PVA = 1:1, crosslinking at 120 ° C for 30 minutes).

The resulting hydrogel films 2SA-PEG-CS-PVA, pre-swollen in water, can absorb dyes, RG and MG in particular, and release them in an aqueous solutions. It has been found that the absorption of RG can be controlled via the time of film thermostatting. If time of thermostatting is more than 120 minutes, it leads to an increase in absorption, which can be explained by the formation of a sol-fraction and its leaching from the polymer network during film swelling in water (Table 2).

TABLE 2

RG FILM ABSORPTION, MG/G OF POLYMER FILM*

№	Absorption time of RG, min	Film crosslinking time, min		
		120	150	180
1	10	0,48	1,49.0	1,45.9
2	20	0,72	1,64.0	1,76.8
3	30	1,22	1,71.9	1,79.3

*structure of 2SA-PEG-CS-PVA film, CS:PVA = 1:1 (wt.)

TABLE 3

KINETICS OF MG RELEASE INTO AN AQUEOUS MEDIUM FROM THE 2SA-PEG_{100}-CS-PVA HYDROGEL FILM

Release time, min.	30	60	90	120	180	1440
Amount of released MG, mg/g of polymer	0.37	0.60	0.62	0.74	1.20	0.74

It has been found, that the MG absorption by the 2SA-PEG-CS-PVA film (Mm PEG 100, CS:PVA = 1:1, crosslinking at 120 ºC for 60 minutes) in an aqueous solution at pH 3.1 is 1.44·mg/g polymer.

Xerogel films, filled with the dyes, are capable of prolonged releasing them in an aqueous medium. Within 2-3 hours, near 50% of the dye has been released in the aqueous solution from the film filled with dyes both.

It has been shown that 2SA-PEG-CS films have antibacterial activity against Escherichia coli and Staphylococcus aureus (Fig. 9).

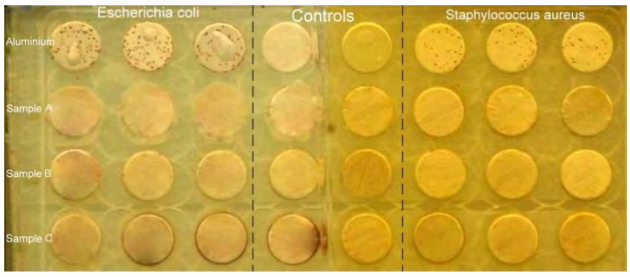

Fig. 9. Aluminum wheels, placed in culture medium with Escherichia coli and Staphylococcus aureus: top line – wheels without 2SA-PEG-CS, two vertical lines averages – in the absence of bacterial cultures, samples A, B, C – wheels, coated 2SA-PEG_{1000}-CS with different crosslinking time at 120˚C: A – no crosslinking, B – 45 minutes, C – 120 minutes.

It can be seen that there is no growth of the bacterial cultures in the aluminum wheels with the films.

Conclusions

Polyoxyethylene containing derivatives of succinic acid (2SA – PEG) have been synthesized via the acylation of polyethylene glycols with succinic anhydride. The interaction between 2SA – PEG and chitosan (CS) leads to the formation of water-soluble chitosan derivatives, containing polyoxyethylene, and films based on them. Water-insoluble films, able to swell in water and to form hydrogels, have been prepared through thermal treatment resulting in crosslinking. The swelling degree increases in an acidic solution. The hydrogel films based on chitosan derivatives, filled with rhodamine G and malachite green dyes, are capable of prolonged releasing them into the water solution and they exhibit antibacterial properties.

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