

Novel synthesis of PEGylated chitosan. Part II: Research

Nowa synteza PEGylovanego chitozanu. Część II: Badania

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Abstract

This article is a continuation of the article: Synthesis and evaluation of the possibility of using some chitosan derivatives in the leather industry. Part I: Literature Review [1]. Part II describes the synthesis of methoxy poly (ethylene glycol) chitosan (mPEGylated chitosan). Methoxy poly(ethylene glycol) with two different molecular weights was used. The effective grafting of poly(ethylene glycol) (PEG) with chitosan was preceded by the activation of the hydroxyl group in the PEG molecule. Benzenesulfonyl chloride was used to activate the hydroxyl group. The course of the reaction for the preparation of PEG esters and PEG-chitosan was confirmed by chromatography.

Abstrakt

Praca jest kontynuacją artykułu: Synteza i ocena możliwości wykorzystania niektórych pochodnych chitozanu w przemyśle skórzanym. Część I: Przegląd literatury [1]. W części II opisano syntezę metoksy poli(glikolu etylenowego) chitozanu (mPEGylovanego chitozanu). Stosowano metoksy poli(glikolu etylenowy) o dwóch różnych masach cząsteczkowych. Efektywne szczepienie poli(glikolu etylenowego) (PEG) chitozaniem poprzedzone było aktywacją grupy hydroksylowej w cząsteczce PEG. Chlorek benzenosulfonylu zastosowano do aktywacji grupy hydroksylowej. Przebieg reakcji otrzymywania estrów PEG i PEG-chitozanu potwierdzono chromatograficznie.

Keywords: synthesis, chitosan, poly(ethylene glycol), PEGylated chitosan

Słowa kluczowe: synteza, chitozan, poli(glikol etylenowy), PEGylowany chitozan

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1. Introduction

The process of PEGylation, grafting (bonding) of covalent PEG with the chitosan molecule increases its solubility in water and also increases its biocompatibility, which is especially useful in drug development [2]. Methoxylated PEG (mPEG) is commonly used in the process of chitosan functionalization to avoid cross-linking. In this case, only one mPEG-activated hydroxyl group reacts with the amino group of chitosan, while the second OH group blocked with a methoxy group cannot react with another amino group of another glucosamine. Activation of the mPEG hydroxyl group can be done with following compounds: aldehyde [3], carboxyl [4], tosylate [3], p-nitrophenyl carbonate [5, 6], iodide [7] and sulfonate [8]. The aim of our research was the synthesis of PEGylated chitosan as a result of the reaction of a mPEG ester with chitosan. The effective grafting of poly(ethylene glycol) (PEG) with chitosan was preceded by the activation of the hydroxyl group in the mPEG molecule. The benzenesulfonyl chloride was used to activate the hydroxide group. The course of the reaction for the preparation of PEG esters and PEG-chitosan was confirmed by chromatography.

2. Experimental

2.1. Materials

The following raw materials were used in the research: chitosan, methoxy polyethylene glycols: 750 (mPEG 750), 2000 (mPEG 2000), benzenesulfonyl chloride diethylene glycol monobutyl ether, were purchased by Sigma Aldrich (USA). The following reagents: sodium hydroxide (NaOH), taurine, urea were purchased by Sigma Aldrich (USA), methylene chloride, bismuth(III)nitrate(V), hydrochloric acid (HCl), potassium iodide (KI) were purchased by Pol-Aura (Poland), acetone, methanol, n-propanol, ammonia of analytical purity were used as solvents (from Chempur-Poland).

2.2. Synthesis of poly(ethylene glycol) ester

Activation of the hydroxyl group with benzenesulfonyl chloride was performed for the following polyglycols:

- methoxy poly(ethylene glycol) 750 (mPEG 750),
- methoxy poly(ethylene glycol) 2000 (mPEG 2000),

0.063 mol of mPEG, 18 cm³ (0,1406 mol) of benzenesulfonyl chloride and 26 cm³ of a 26% NaOH solution were introduced into a 500 cm³ sulfonation flask equipped with a stirrer, a thermometer and two dropping funnels. The mixture was stirred for 1.5 hours at a temperature of 25-30°C (in the case of PEG 2000 the temperature was 55-65°C). Then 4 g of taurine were added to the reaction mass to remove unreacted benzenesulfonyl chloride. The mixture was stirred for 1.5 h and then 60 cm³ of water and 180 cm³ of methylene chloride were added. After stirring for 1 h, the layers were separated. The methylene layer containing the product, after drying with magnesium sulfate, was subjected to vacuum distillation.

2.3. TLC chromatographic method to evaluate the degree of conversion and separation of products

TLC conditions :

- stationary phase (fluorescent plates silica gel TLC 60 F₂₅₄ (Merck))
- mobile phases (developing system): 5% aqueous urea solution/acetone/methanol (3:1:1 v/v/v), n-propanol : ammonia solution 25% - 2:1 v/v),
- Dragendorff's reagent (color developing reagent): 1 g of bismuth(III) nitrate(V) was dissolved in 40 cm³ of hot distilled water at about 80°C, and then 3 cm³ of concentrated hydrochloric acid was added to the obtained solution. 6 g of potassium iodide were separately dissolved in 50 cm³ of distilled water. The two solutions were then mixed, and made up to 100 cm³ with water. The reagent thus obtained was in the form of a red-gold, opalescent solution. The completion of the reaction

synthesis methoxypoly(ethylene glycol) ester and PEGylated chitosan was monitored by thin layer chromatography (TLC). The samples were taken from the reaction mass. They were then dissolved in acetone:water (1:1 v/v), then basified with sodium bicarbonate to pH 6, and applied to a silica gel 60 F₂₅₄ chromatography plate. After drying it, it was placed in a chromatographic chamber containing:

- 5% aqueous urea solution: acetone: methanol 3:1:1 v/v/v for synthesis of mPEG ester. Retardation factor of product - mPEG ester $R_f = 0.45$, substrate - polyethylene glycol $R_f = 0.58$, the absence of a mPEG spot at $R_f = 0.58$ indicates the end of the reaction. After drying the chromatogram, the color was developed with Dragendorff's reagent.
- n-propanol: ammonia solution 25% 2:1 v/v for synthesis of mPEGylated chitosan. Retardation factor of substrate – mPEG ester $R_f = 0.72$. The absence of a fluorescent PEG ester spot at $R_f = 0.72$ under UV 254 light indicates the end of the reaction

2.4. PEGylated chitosan synthesis

mPEG 750 – chitosan

220 cm³ of 2% acetic acid and 2.25 g of chitosan ($M = 161\text{g/mol}$) were added into the 500 cm³ flask. The ingredients were mixed until the chitosan was dissolved. Then, 2.4 g of the PEG-750 ester ($M = 890\text{g/mol}$) was added to the colorless, clear, viscous solution and stirred at room temperature for 2 h. The reaction was deemed complete by chromatographic evaluation in the system: n-propanol : ammonia solution 25% - 2:1 v/v, showing conversion of the ester. After the reaction, 300 cm³ of chloroform was added to the viscous, clear mass, stirred for 0.5 h and placed in the freezer. The solidified product layer was easily separated from the solvent layer, and then dried to obtain 3.9 g of highly water-soluble product in the form of flakes.

mPEG 2000 – chitosan

220 cm³ of 2% acetic acid and 2.25 g of chitosan were added into the 500 cm³ flask. After the ingredients had dissolved, 2,9 g of the PEG 2000 ester (M=2140 g/mol) dissolved in 30 cm³ of water was added. The mixture was stirred at room temperature for 2 h. Chromatographic evaluation in the system: n-propanol : ammonia solution 25% - 2:1 v/v showed no chitosan and a trace of unreacted ester. After the reaction, 360 cm³ of chloroform was added to the clear, viscous mass, then the mixture was stirred for 1 h, after which the layers were separated. 4.8 g of product were obtained in the form of a foil disc. The product dissolves well in water.

2.5. Optimization of mPEGylated chitosan

Following the description of the mPEG 750-chitosan and mPEG 2000-chitosan syntheses, several attempts were made to optimize PEGylated chitosan by changing the molar ratio of mPEG 750 ester and mPEG 2000 ester to chitosan. The reaction was carried out in two temperature intervals: 20-25°C and 33-35°C. The results of the weight percentages of PEG content in the PEGylated chitosan copolymer are illustrated in Tab. 1. The weight percent (%_{PEG}) of PEG in PEG-chitosan was calculated from equation (1):

$$\% \text{ PEG} = (\text{Wt} - \text{Wc})/\text{Wt} \times 100 \quad (1)$$

where:

Wt - mass of PEG- chitosan [g]

Wc- mass chitosan, which was added for PEG- chitosan synthesis [g]

Tab. 1. Samples prepared in various molar proportions of reagents.

PEGylated chitosan	T [°C]	Molar ratio ester:chitosan	%PEG in PEG-chitosan
PEG 750	20-25	1:1	62.72
	33-35	1:1	81.32
	20-25	0.6:1	46.40
	33-35	0.6:1	60.50
	20-25	0.2:1	42.65
	33-35	0.2:1	44.38
PEG 2000	20-25	1:1	53.40
	33-35	1:1	80.22
	20-25	0.6:1	46.70
	33-35	0.6:1	77.30
	20-25	0.2:1	36.71
	33-35	0.2:1	38.75

3. Results and discussion

The work presents the synthesis of polyethylene methoxy glycol esters with molecular weights of 750 and 2000. The end product was PEGylated chitosan based on the above-mentioned esters. Effective grafting of methoxypolyethylene glycol (mPEG) with chitosan is possible after the activation of the hydroxyl group. According to the literature data, activation can be performed with such groups as: aldehyde, carboxyl, tosylate, p-nitrophenyl carbonate, iodide, sulphate, maleimide and succinimidyl ester. The reactions take place in organic solvents such as: dimethylsulfoxide, methylene chloride, acetonitrile and dimethylformamide. The methods of obtaining polyglycol esters (activation of the hydroxyl group) described in the literature take place in organic solvents, so they are dangerous methods that may have limited application on a larger scale. Moreover, despite the fivefold excess of the alkylating agent, it is not possible to complete the reaction completely. The new glycol activation method developed by us takes place in an aqueous

medium, in a shorter time and with the use of a smaller excess of the alkylating agent - benzenesulfonyl chloride. The activation of the free hydroxyl group of PEG was carried out in an aqueous medium using a 2-2.2-fold excess of benzenesulfonyl chloride at a temperature allowing, depending on the PEG used, to maintain the PEG in a dissolved form for a time allowing maximum conversion of polyethylene glycol (Fig. 1).

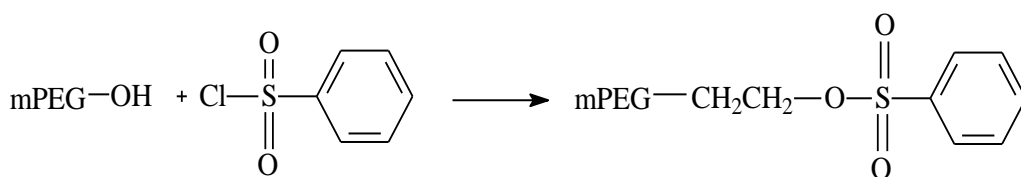


Fig. 1. Mechanism of obtaining PEG esters.

The reaction was monitored by TLC using silica gel 60 F₂₅₄ plates and eluent: 5%: aqueous urea solution : acetone: methanol 3:1:1 v/v/v. The absence of a PEG spot at $R_f = 0.58$ after induction with Dragendorff's reagent indicates the end of the reaction. The product was observed at $R_f = 0.45$. The efficiency of esterification was about 80%. The resulting copolymers dissolve well in water. The PEG 2000 and PEG 750 were selected for detailed research on PEGylated chitosan. By changing the molar ratio of PEG 750 ester and PEG 2000 ester to chitosan at two temperatures: 20-25°C and 33-35°C, PEG-g-chitosan samples with different weight percentages of PEG in PEGylated chitosan were obtained. The data in Tab. 1 shows that for both the mPEG 750 ester and the mPEG 2000 ester, the amount of mPEG attached to chitosan increased with increasing temperature at 1: 1 molar ratios of the reactants. The synthesis of PEGylated chitosan is shown in Fig. 2.

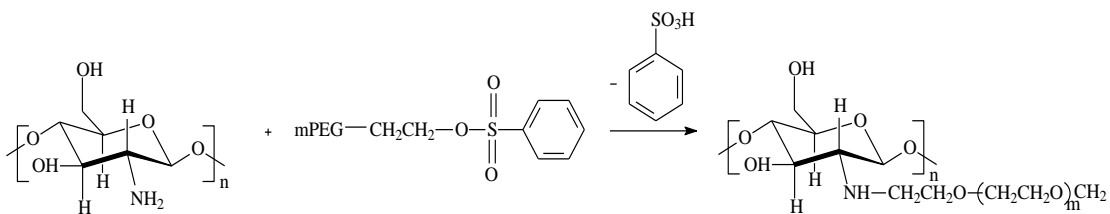


Fig. 2. Synthesis of PEGylated chitosan.

The reaction was monitored by TLC using silica gel 60 F254 plates and eluent: n-propanol: ammonia 2: 1 v/v. The absence of a fluorescent mPEG ester spot at $R_f = 0.72$ under UV 254 light indicates the completion of the reaction PEGylation chitosan. It should be mentioned that, according to the literature [9], PEGylated chitosan was secreted by dialysis and lyophilization. In our case, using the ester obtained as a result of the reaction of polyglycols with benzenesulfonyl chloride, copolymers of satisfactory chromatographic purity were obtained in powder form by drying the reaction mass, thus omitting the mentioned literature methods.

4. Conclusions

As part of the research work, a new method of obtaining polyglycol esters was developed. The developed method of activating polyglycols takes place in an aqueous environment in a short time and with the use of a new alkylating agent - benzenesulfonyl chloride. The methods of chromatographic evaluation of polyglycol esterification allow to monitor the progress of the reaction and thus allow for obtaining high-quality products. Their purity was confirmed by chromatographic analysis. Based on the newly developed PEG esters, PEGylated chitosan derivatives with high quality parameters were synthesized without cumbersome and costly dialysis and lyophilization processes.

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