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MODIFICATION OF POLYSACCHARIDES BY *N*-DERIVATIVES OF GLUTAMIC ACID USING STEGLICH REACTION

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Abstract. The work describes dextrin modifications with the purpose to obtain a functional product with an extensive structure of macromolecules, capable to form self-stabilized dispersions in an aqueous environment. To ensure the branching, the modification was performed using the functional agents – *N*-derivatives of glutamic acid. The substitute in the *N*-position provides the introduction of the required function. Modification was performed by using the Steglich reaction that provides the progress of the process in soft, controlled conditions.

Keywords: Steglich reaction, dextrin, modified polysaccharides, glutamic acid.

1. Introduction

It is considered that the polysaccharides were the first polymer materials. Moreover, the use of polysaccharides penetrates, practically, all spheres of life. First of all polysaccharides are a food product. But this is not a limit. For a human polysaccharides become a source of energy and a construction material. That was facilitated by distribution of polysaccharides and a number of their unique properties. The role of polysaccharides has not diminished in modern life and in the future their role may increase due to the fact that the polysaccharides are renewable materials. But there is another very important property of the polysaccharides that makes them so attractive for humans. They are tolerant to the human organism. This can explain the desire of a large number of researchers to formulate the polymer materials for medical and biomedical purposes based on the polysaccharides.

Researches on formulating biomaterials based on polysaccharides are carried out mainly in three directions: the creation of hydrogels with special properties [1, 2], micro-hydrogels [3] and the delivery of therapeutic agents [4, 5].

A number of natural properties of the polysaccharides do not allow their direct use for these purposes. To create biomaterials based on them they need to be modified. Very often the modification is made by controlled cross-linking of the polysaccharides macromolecules. However, one should not forget the advantages of this natural polymer. The materials obtained by the modification should stay polysaccharides and preserve the property of being tolerant to the human body.

We have previously reported [6] the synthesis of pseudo-polyamino acids of polyester type based on *N*-derivatives of glutamic acid and polyols of polyoxyethylene polyoxypropylene series by the Steglich reaction. The reaction takes place in mild conditions, and the obtained polyester, as shown by the relevant studies, is non-toxic, depyrogenized and biodegradable material. It is also an important fact that the products of decomposition of the polymer in the body are also non-toxic.

In view of the above, the modification of polysaccharides by *N*-derived of dicarboxylic acid using Steglich reaction to produce polymer materials for medical and biomedical purposes represents a considerable interest.

2. Experimental

2.1. Materials

As a model of polysaccharides was used dextrin with a molecular weight of 40,000 Da purchased from Sigma-Aldrich (cas number: 9004-53-9) without further purification. In this work we studied its modification by the 2-(stearoylamino) pentane dicarboxylic acid (Glu(St)), which was received by the interaction of glutamic acid with chlorides of stearic acid by the method described in [7]. *N,N*-Dicyclohexylcarbodiimide, (DCC) and 4-dimethylaminopyridine (DMAP), purchased from Alfa

Aesar (cas num: A10973, H63520 in accordance), were used without further purification. Dextrin modification was performed in dimethylformamide (DMF) purified by the method [8].

2.2. Research Methods

2.2.1. Modification of the dextrin by the Steglich reaction

The reaction between dextrin and Glu(St) was carried out in a thermostatted reactor of 50 cm³ volume, equipped with a reflux condenser and a dropping funnel. Mixing of the reaction mixture was done by a magnetic stirrer. Glu(St) was dissolved in DMF in the reactor at 278–280 K and solutions of dextrin and DMAP in the same solvent were sequentially added while stirring to the obtained solution. A solution of DCC, charged into the dropping funnel, was added by drops to the reaction mixture during 10 min. After reagents were loaded into the reactor, the reaction mixture was kept for 15 min at the same temperature and then heated to 287 ± 1 K. Depending on the purpose of the experiment and composition of the reaction mixture it was kept at this temperature from 4 to 20 h.

After the reaction was completed dicyclohexyl urea (DCU) was removed from the mixture by filtration and DMF was partially evaporated in a vacuum of a waterjet pump. Then tenfold volume of methanol was administered into the reaction mixture. One of the dextrin modification products and unreacted dextrin were precipitated from the reaction mass and washed with water. The residue after washing was dried in a vacuum-desiccator over the phosphoric anhydride. The second product of dextrin modification was extracted from methanol solution by evaporation under vacuum of the waterjet pump.

2.2.2. NMR spectroscopy of dextrin esterification products

The spectrums of dextrin modified samples were obtained in deuterium-dimethylsulfoxide using the JEOL's ECA Series Nuclear Magnetic Resonance (NMR) Spectrometer at 400 MHz in an automatic mode scan.

Analysis of NMR-spectrums was carried out using the tables of chemical shifts characteristic described in [9] and the program Shem Bio Draw Ultra 11.0.1.

2.2.3. Rheological and turbodimetric investigation

The change of viscosity was determined on a rotary viscometer Rheotest 2 using cylinder N 373 from a standard set of device. The study was performed at the thermostatic control of the cylinder at the temperature of 288 ± 0.5 K.

Turbodimetric studies were conducted by precipitation of the reaction product from the DMF solution using the methanol. Uniform supply of the methanol was ensured by pump HPP 4001 laboratorni pristroje. Turbidity was recorded using the turbidimeter LMF-72 M

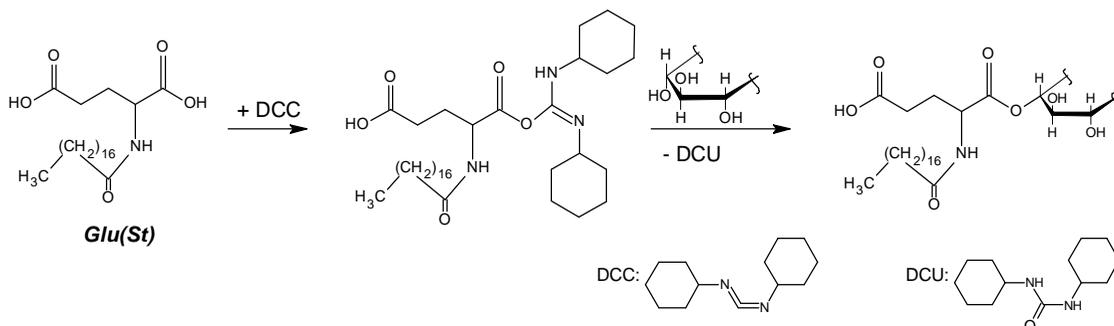
3. Results and Discussion

In work [6] we reported the regularities of polycondensation of the *N*-derivatives of glutamic acid with polyols of polyoxyethylene and polyoxypropylene series by the Steglich reaction. Results of the research which were described in this work gave reason to believe that the reaction can be an effective tool for polymer-analogous transformation in polysaccharides. The first results of the interaction of these derivatives on the model disaccharides were published in the work [10]. In particular it was shown that by the Steglich reaction, with quite significant conversion, up to 3–4 acid residues to one molecule of sucrose through the formation of the ester bonds can be entered. Based on these studies in this article the specifics of Steglich reaction between dextrin hydroxyl groups and carbonyl groups of Glu(St) will be discussed. In general, the interactions between the hydroxyl group of dextrin and carbonyl group of Glu(St) can be represented by Scheme 1.

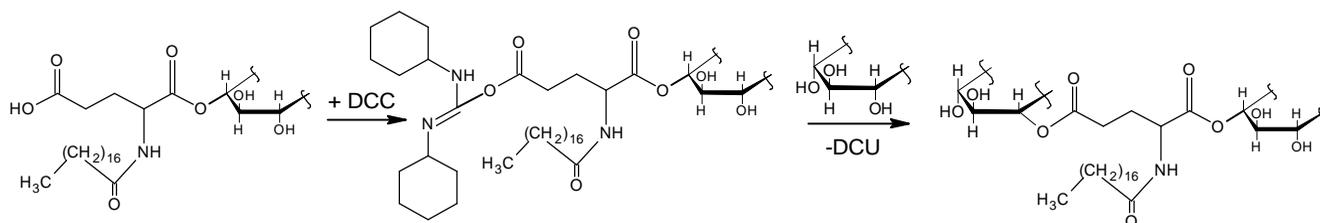
The degree of substitution of hydroxyl groups was determined by the efficiency (selectivity) of the reaction and by the ratio between the reagents. Because the Glu(St) is a dicarboxylic acid, the cross-linking reaction between dextrin macromolecules is possible under the conditions of sufficient process efficiency, at any ratio of the reagents. This process can be reflected by Scheme 2.

With moderate increase of quantities of cross-links, as a result of the reaction, modified dextrin with significantly increase of molecular weight and a dendrite structure of macromolecules will be formed. The modified product, with the dendrite structure of macromolecules, should not lose solubility in water and, by introducing the residues of Glu(St) and the formation of ester group must obtain the ability to dissolve in some organic solvents, particularly in methanol.

On the other hand, the formation of a significant amount of cross-links between the macromolecules of dextrin should lead to the formation of spatial net. The modified dextrin, whose macromolecules formed cross-linked spatial nets, loses its ability to dissolve in solvents, including water, and is can only limitedly swell. Furthermore, in case of involving a critical amount of macromolecules of dextrin to the process of creating a three-dimensional net it will lead to gel formation, which, as it is known, is observed with a rapid increase of the viscosity of the environment.



Scheme 1. Schematic image of the interaction between Glu(St) and dextrin by the Steglich reaction



Scheme 2. Cross-linking of the dextrin macromolecules

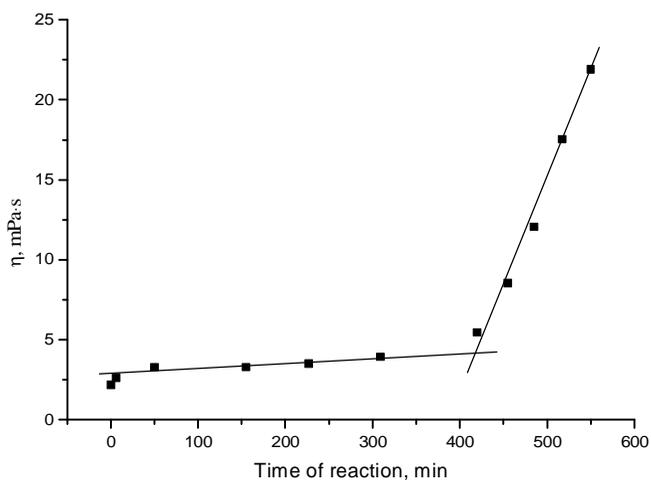


Fig. 1. Viscosity vs. time

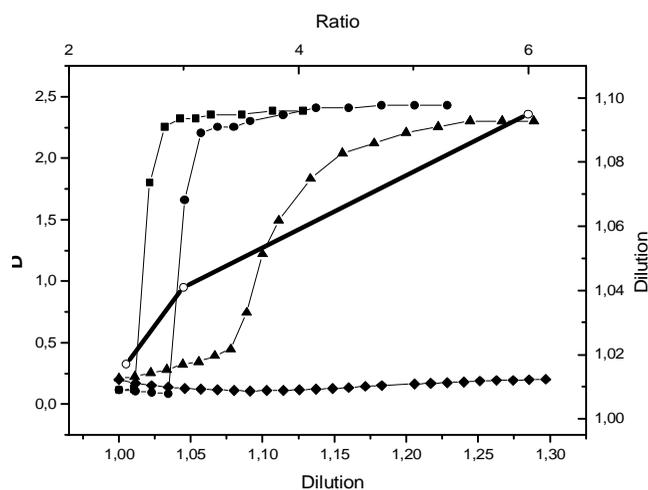


Fig. 2. Turbidimetric titration

Confirmation of the fact that in the investigated system the progress of described above processes observed can be seen in Fig. 1, which shows the change of viscosity of the reaction mixture during the process at 1:1 ratio of carboxyl groups of Glu(St) to the hydroxyl groups of dextrin. As it can be seen from the curve, the viscosity of the mixture increases during the whole process of the reaction and when the process reaches a certain conversion its rapid magnification occurs and the reaction environment is transformed into the gel. Therefore, it can be stated that processes of structuring are passing during all the reaction flow, and when a certain molecular weight is reached, a mass interaction starts between the cross-linked macromolecules at the point of

percolation, leading to the formation of the gel. Chemical processes of structuring also occur and after percolation point.

Time of the percolation point achievement depends on the ratio of the reagents and conversion. In the above case, the percolation point is observed after 6.5–7.5 h from the beginning of the process, which corresponds to approximately 80–90 % conversion.

The conducted research showed that the ratio, under which 92–95 % conversion is achieved, is the determining factor for obtaining modified products of the different molecular weight and composition.

The investigation of the structure and composition of significantly structured product that was obtained after

percolation point is complicated due to the loss of its solubility. Therefore, within this work, the study of modified dextrin that formed up to percolation point, but under the condition of high conversion was conducted. To avoid massive structuring, syntheses were carried out at the ratio of carboxyl to hydroxyl groups in the range of 1:(2–6). At these ratios the transformation of the reaction mixture into the gel-like structure was not observed. At the same time, structuring processes were observed at the entire range of investigated ratios. This suggests that the share of dextrin modified by Scheme 1 was low at investigated ratios of reagents. This is confirmed by turbidimetric titration that is shown in Fig. 2. For the curves reception, was used the fact that in the result of the modification of dextrin, products insoluble in water were formed. From these curves we can see that the dilution at which a massive precipitation of dextrin modification is observed increased almost linearly with increasing ratio of carboxyl groups to hydroxyl. This encourages the belief that in all ratios a significant increase in molecular weight of reaction products is observed, which is determined by the ratio between the reagents in the initial mixture.

However, even with ratios that significantly differ from the equimolar by the surplus of hydroxyl groups, a heterogeneous product by the substitution degree and the number of transverse cross-links is formed during the reaction. According to the methodology of the research, the resulting product was separated by extraction of samples with methanol and the product soluble in methanol and the one insoluble in it were distinguished. Several additional observations such as swelling of methanol separated products in a variety of solvents showed that the fraction soluble in methanol endlessly swells in DMF and the product insoluble in methanol has a limited swelling in some solvents. From this we can conclude that the one dissolved by methanol is modified and heavily branched product, whereas insoluble in methanol fraction is a cross-linked product that forms the spatial net during modifications.

Fig. 3 shows the NMR spectrum of methanol soluble fraction of the product obtained with 1:2 ratio of hydroxyl to carboxyl groups with referring to the basic signals. This spectrum confirms the reaction of grafting Glu(St) to dextrin macromolecules. In addition, NMR spectroscopy allows assessing the degree of substitution by the ratio of signal strength of hydrogen at the first carbon atom of glucopyranose residue *A* (5.7 ppm) to the signal intensity of the three hydrogens of the methyl group (*E*) of alkyl fragment Glu(St). In particular, for the above given spectrum this ratio is 0.39. This ratio means that on every glucopyranose residue of dextrin one molecule Glu(St) is instilled. With the mentioned ratio it is possible

to replace 3 hydroxyl groups from every two glucopyranose residues. So at the ratio of 1:2 the grafting efficiency is 56 % of the possible. In the work [9] it was shown that the efficiency of the disaccharide modification was 70–80 % for relevant ratios of the reagents. From this fact it is possible to conclude that the substantial increase of molecular weight of polysaccharide has no material affect on the efficiency of the reaction. The absence of the substantial dependence of weight from the efficiency of grafting molecular weight of polysaccharide can explain weightless reduce of the cooperation effectiveness of the carboxyl group of already grafted fragment with another macromolecule of dextrin (by Scheme 2).

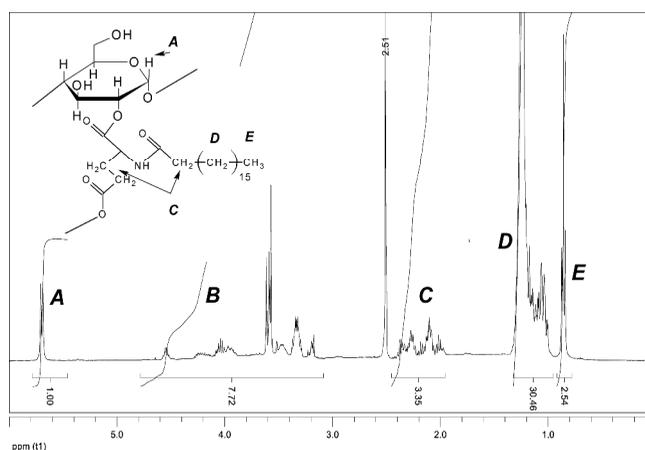


Fig. 3. NMR spectrum of the dextrin esterification product

Table shows some results of research of dextrin interaction with Glu(St) with different ratio of reactants.

From the data given in Table we can conclude that in all investigated ratios both dextrin modification with dendrite structure of macromolecules and dextrin modification which formed a spatial nets were formed. It is logical that reducing the number of carboxyl groups leads to decrease of the particle of modified dextrin fraction of the network structure. However, the average degree of substitution also decreases, leading to formation of less substituted product or a product with a lower degree of branching. The highest grafting efficiency, that is the ratio of obtained degree of substitution to the theoretically possible, was observed at the ratio of 1:3.

The resulting products were tested for the ability to form polymer dispersions in water environments. These studies have shown that modified dextrin with a dendrite structure of macromolecule in an aqueous environment forms a self-stabilized dispersion with particles of nanometric size.

Results of studies of dextrin interaction with Glu (St)

Carboxyl:hydroxyl groups ratio	Exit of reaction products			Degree of substitution*	Effectiveness of substitution, %
	Conversion, %	Factions, %			
		Soluble	Insoluble		
1:1	–		~100		
1:2	93.4	21	79	0.84	56
1:3	91.7	25	75	0.74	74
1:6	96.2	60	40	0.26	52

Note: *averaged quantity of ester group on one glucopyranose residue

4. Conclusions

As a result of conducted studies it was found that modification of dextrin by *N*-derivatives of the glutamic acid by the Steglich reaction proceeds with high efficiency and provides substitution of 50 to 75 % out of the possible hydroxyl groups, at a given proportion of reagents. As a result of the modification the products with branched and cross-linked structure of macromolecules are formed. These products can be separated and the ratio between them can be determined by the ratio of reagents at the stage of synthesis. Of practical interest is the product with branched structure of macromolecules, as it is able to form stable in time self-stabilized aqueous dispersion with nano- and micro-metric size of the particles.

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МОДИФІКАЦІЯ ПОЛІСАХАРИДІВ N-ПОХІДНИМИ ГЛУТАМІНОВОЇ КИСЛОТИ ЗА РЕАКЦІЄЮ СТЕГЛІХА

Анотація. В роботі викладено ряд досліджень стосовно модифікації декстрину з метою одержання функціонального продукту з розгалуженою структурою макромолекул, здатного до формування в водних середовищах самостабілізованих дисперсій. Для забезпечення розгалуження макромолекул декстрину модифікацію здійснювали біфункціональними агентами – *N*-похідними глутамінової кислоти. Замісник в *N*-положенні забезпечує введення необхідної функції. Модифікацію здійснювали за реакцією Стегліха, що забезпечує перебіг процесу в м'яких, контрольованих умовах.

Ключові слова: реакція Стегліха, декстрин, модифікація полісахаридів, глутамінова кислота.