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STUDIES OF IMMOBILIZATION OF SOME DIAMINES TO DIALDEHYDINULIN MACROMOLECULES

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Abstract

Modified derivatives of polysaccharides containing aldehyde groups in their structure are unique intermediate materials for the production of new polymeric compounds. In the present study, periodate oxidation of inulin was carried out. The physicochemical properties of the resulting dialdehyde inulin with varying degrees of oxidation were investigated. Due to the presence of aldehyde groups in the structure of oxidized inulin, immobilization of hydrazine, ethylenediamine, and propylenediamine was performed via covalent azomethine bonds. The presence of diamines in the dialdehyde inulin structure was confirmed by elemental analysis (by nitrogen content) and FTIR-spectroscopy.

Keywords: *polymers, inulin, periodate oxidation, dialdehyde inulin, hydrazine, ethylenediamine, propylenediamine*

Introduction

The application area of macromolecular compounds covers a wide range of polymer materials intended for use in various fields of practical activity. The results achieved to date demonstrate the possibility of producing polymer products with specified physicochemical, mechanical, operational, and medical-biological properties (Bhatt Sh., Pathak A., Grover P., Kaurav M., 1985; Andrews G. P., Laverty T. P., Jones D. S., 2009; Peponi L., Lopez D., 2018). Among the different classes of polymers, special importance is given to macromolecular compounds that contain specific functional groups in their structure, which later determine a number of key properties of the synthesized polymers. One such class of polymer compounds is macromolecular systems containing amine groups in their structure (Xuan M., Gu X., Li J., Huang D., Xue Ch., He Yu., 2023; Kuksa V., 2000; Pegg A. E., McCann P. P., 1982; Abdulhussein A. A., Wallace H. M., 2014). It is well known that many polymeric amines exhibit antimicrobial, hemostatic, wound-healing, and antitumor properties.

Inulin is a heteropolysaccharide that has been increasingly used in medicine in recent years as a hypoglycemic agent, sorbent, and biologically active supplement. Unlike many polysaccharides, inulin has its own biological activity and a low molecular weight, which eliminates the need for hydrolysis to impart new physicochemical and pharmacological properties to the macromolecular chain. To date, only a limited number of chemical transformations of inulin have been explored, mainly involving the immobilization of low-molecular fragments into the structural units of inulin. Among these studies, the synthesis of amino-containing derivatives of inulin is a particularly interesting area of research. The aim of our study was to investigate the chemical immobilization of amino-containing compounds into the monomeric units of inulin to obtain new derivatives.

Materials and method

The following reagents were used for the synthesis of amino-containing derivatives of inulin: inulin, extracted from Jerusalem artichoke (variety «Mujiza») with an average molecular weight of 5600 Da; hydrazine sulfate – CAS 10034–93–2; ethylenediamine – CAS 107–15–3; propylenediamine – CAS 109–76–2.

Synthesis of dialdehydeinulin. 1–2 g of thoroughly dried inulin were placed in a heat-resistant dark glass flask with a volume of 300 mL, and 100 mL of acetate buffer (pH = 5) and 0.2 N freshly prepared sodium periodate solution were added, maintaining a molar ratio of inulin: $NaIO_4$ = 1 : 1. The reaction mixture was left to stir in the dark for 1–5 hours at 20–25 °C. To complete the reaction, 30 mL of ethylene glycol was added. After the reaction, the mixture was dialyzed against distilled water to remove any residual IO_4^- and IO_3^- ions (monitored using silver nitrate solution). The final products were isolated by sublimation drying. The degree of oxidation of inulin was determined by iodometric titration (Rhee, K. C., 2001).

Modification of Hydrazine, Ethylenediamine, and Propylenediamine with Dialdehyde Inulin Samples. 0.5–1.0 g of dialdehyde inulin with varying degrees of oxidation was dissolved in 50 mL of water at room temperature to form a clear solution. Then, hydrazine, ethylenediamine, or propylenediamine was added with stirring at a molar ratio of DAI: $H_2N\text{-}R$ = 1 : 2.5. The resulting solution was stirred for 1 hour at 20–25 °C. After the reaction, the clear solution was placed in dialysis membranes and purified against distilled water for 24 hours. The final reaction products were isolated by sublimation drying.

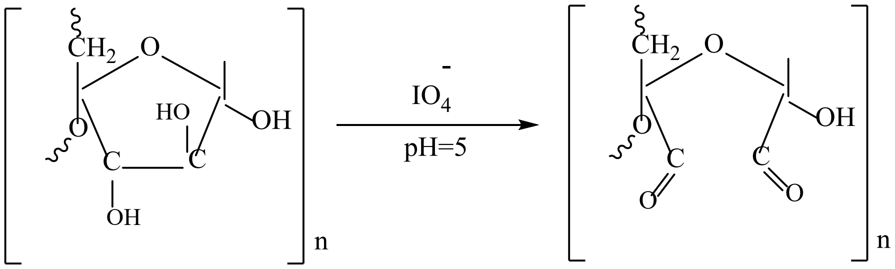
FTIR-spectra of the synthesized compounds were recorded on a Vector-22 IR spectrometer in the wavelength range of 400–4000 cm^{-1} using KBr pellets (3–5 mg of sample/300 mg of KBr). The nitrogen content by weight in the synthesized compounds was determined using the Kjeldahl method (Tunik T. V., Nemchenko U. M., Ganenko T. V., Yurinova G. V., Dzhioev Yu.P., Sukhov B. G., Zlobin V. I., Trofimov B. A., 2019). ζ-potential of the samples was measured on a Zetasizer Nano ZS instrument (Malvern, UK) in plastic cuvettes using the immersion cell ZEN1002.

The molecular weight of the synthesized samples was determined by size-exclusion liquid chromatography on Yedrogel 100, 500, and 1000 columns from Waters (USA), at a temperature of 20±1 °C, equipped with a refractive index detector. The eluent used was a 0.1 N NaCl solution, with a flow rate of 0.1 mL/min. The calibration curve was standardized using dextran with a narrow molecular weight distribution.

Determination of pKa values of amino-containing derivatives of inulin. 100–150 mg of the dried sample was dissolved in water, and then 0.1 N sodium hydroxide solution was added to the solution. Titration was started while stirring continuously, adding aliquots of 0.1 N hydrochloric acid solution. The pKa values of the samples were calculated based on the pH value at which the midpoint of the plateau appeared on the resulting titration curve (Rawlinson L. B., Ryan S. M., Mantovani G., Syrett J.A., Haddleton D. M., Brayden D. J., 2010).

Result and discussions

It is known that oxidized polysaccharides containing electrophilic aldehyde groups in their structure exhibit high reactivity and can interact with molecules containing primary amine groups. These characteristics are typical for dialdehyde derivatives of polysaccharides, which are widely used for the chemical immobilization of low-molecular-weight biologically active substances. When aldehyde groups react with primary amines, Schiff bases (–C = N-) are formed, which can decompose in acidic or alkaline environments. Based on this, we have developed methods for the fixation of amine groups into the structure of dialdehyde inulin with varying degrees of oxidation. The periodate oxidation of inulin was carried out according to the following scheme:



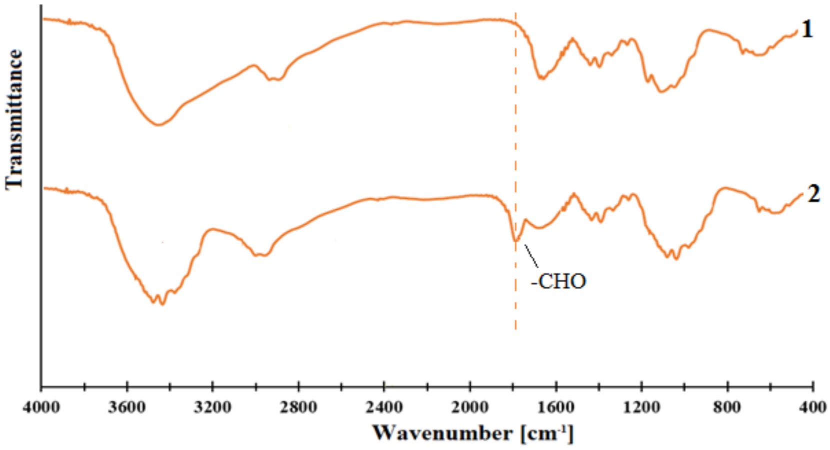
The table 1 presents the physicochemical characteristics of dialdehyde inulin with varying degrees of oxidation, which were obtained by altering the reaction time.

Table 1. *Physicochemical characteristics of dialdehyde inulin with varying degrees of oxidation*

№	Degree of oxidation of DAI, mol%	[η], dL/g	M×10⁻⁴, Da	ζ- potential, mV	Color of the sample
1.	13	0.64	5.4	–12.0±1.5	White
2.	20	0.58	5.1	–19.0±1.6	White
3.	28	0.52	4.8	–21.5±1.0	White
4.	35	0.45	3.7	–23.7±2.1	Cream
5.	37	0.41	3.0	–25.4±1.0	Cream

Experimental data presented in Table 1 show that the periodate oxidation of inulin proceeds with partial destruction of the original macromolecular chain, resulting in a decrease in molecular weight and the yield of reaction products. The data also reveal that the intensity of the molecular weight decrease of inulin occurs in two stages: the first stage is characterized by a slight reduction in the average molecular weight of inulin (from 5600 to 4800 Da) over the first 2.5 hours, while the second stage involves a more significant drop in the molecular weight of dialdehyde inulin (DAI) to 3000 Da. This indicates that as the number of aldehyde groups increases, the stability of DAI in acidic media decreases. Furthermore, with an increase in the degree of oxidation of inulin, there is a consistent shift in the ζ-potential of the investigated samples into the negative region.

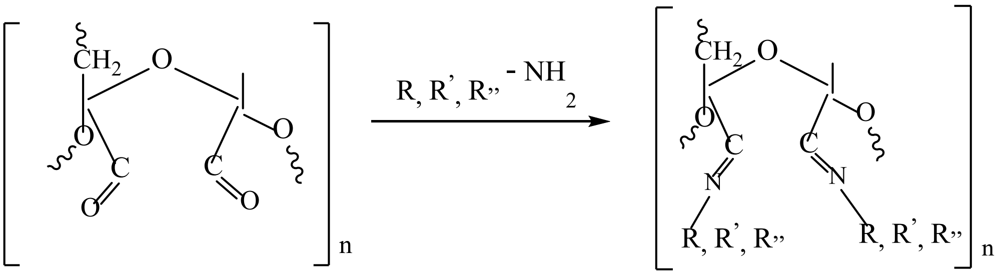
Figure 1. *FTIR-spectra of inulin (1) and dialdehyde inulin (2) with an oxidation degree of 37 mol%*



The reason for the decrease in the ζ-potential with increasing degree of oxidation of DAI is explained by the fact that aldehyde groups carry a partial negative charge due to the polarization of the π-bond, imparting polymeric acid properties to the main macromolecular chain of oxidized inulin.

In the FTIR-spectrum of inulin (Fig. 1), absorption bands are observed at 3429 cm^{-1} (–OH), associated with the formation of hydrogen bonds, 2924 cm^{-1} (–CH_2^-), 1133 cm^{-1} (C-O-C bonds), and 1032 cm^{-1}, corresponding to the vibration of primary -OH groups. In the FTIR-spectra of DAI, a new absorption band appears at 1730 cm^{-1}, corresponding to the vibration of the aldehyde group, the intensity of which increases with the degree of oxidation of inulin.

Since the oxidized derivatives of inulin contain electrophilic aldehyde groups, we carried out the immobilization of diamines (hydrazine, ethylenediamine, and propylenediamine) based on a nucleophilic addition reaction according to the following scheme:



R = $-NH_2$
R' = $-CH_2-CH_2-NH_2$
R'' = $-CH_2-CH_2-CH_2-NH_2$

The research results illustrating the effect of the degree of oxidation of inulin on the composition and substitution degree of the reaction products are presented in tables 2–4.

Table 2. *Effect of the degree of oxidation of inulin on the composition of the reaction products (t = 20 °C; DAI: hydrazine=1:2.5; τ=1 hour)*

№	Degree of oxidation of DAI, mol%	$\gamma_{ок}$ DAI	Nitrogen content, %	Degree of substitution, mol%	pKa value
1.	13	26	4.2	25	7.2
2.	20	40	6.5	38	7.4
3.	28	56	8.8	54	7.6
4.	35	70	10.9	70	7.8
5.	37	74	11.5	72	7.8

Note: $\gamma_{ок}$ DAI – The content of aldehyde groups in 100 elementary units of inulin

Table 3. *Effect of the degree of oxidation of inulin on the composition of the reaction products (t = 20 °C; DAI: ethylenediamine=1:2.5; τ=1 hour)*

№	Degree of oxidation of DAI, mol%	$\gamma_{ок}$ DAI	Nitrogen content, %	Degree of substitution, mol%	pKa value
1.	13	26	4.5	26	7.8
2.	20	40	6.8	36	8.0
3.	28	56	9.1	55	8.2
4.	35	70	11.6	68	8.4
5.	37	74	12.0	74	8.5

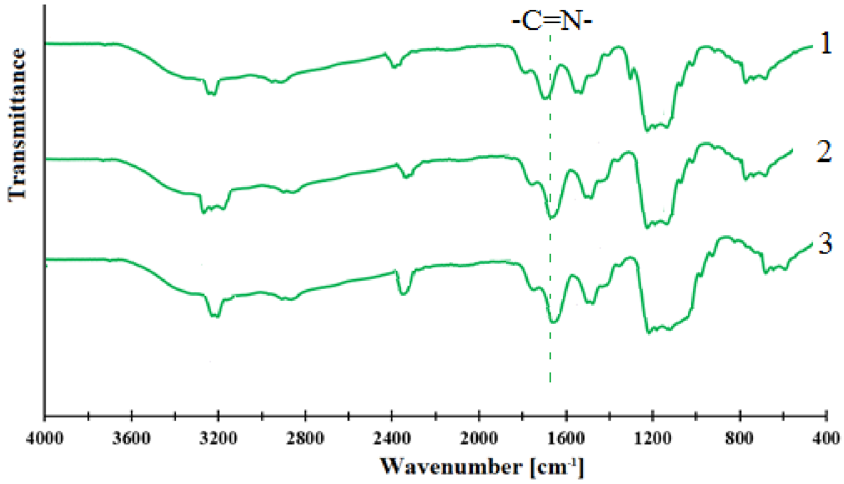
Table 4. *Effect of the degree of oxidation of inulin on the composition of the reaction products (t = 20 °C; DAI: propylenediamine =1:2.5; τ=1 hour)*

№	Degree of oxidation of DAI, mol%	$\gamma_{\text{ок}}$ DAI	Nitrogen content, %	Degree of substitution, mol%	pKα value
1.	13	26	4.7	23	7.5
2.	20	40	7.0	36	7.9
3.	28	56	9.5	54	8.1
4.	35	70	11.8	68	8.3
5.	37	74	12.3	72	8.3

The results presented in Tables 2–4 show that with an increase in the degree of oxidation of inulin, the nitrogen content and the degree of substitution of the reaction products increase. By comparing the degree of oxidation with the degrees of substitution of the synthesized compounds, it can be concluded that each aldehyde group corresponds to one molecule of hydrazine, ethylenediamine or propylenediamine. After the immobilization of hydrazine, ethylenediamine, and propylene diamine onto the macromolecules of dialdehyde inulin, the resulting samples begin to exhibit properties characteristic of polymeric bases. The data presented in Tables 2–4 show that the pKa value ranges from 7.2 to 8.5. As the degree of substitution of the samples increases, the pKa value also increases.

The presence of Schiff bases (–C=N-) in the synthesized azomethine derivatives of inulin, which contain fragments of hydrazine, ethylenediamine, and propylenediamine in their structure, was confirmed by FTIR-spectroscopy (Fig. 2). A common absorption band in the range of 1634–1652 cm^{-1} indicates the presence of covalent -C=N- Schiff base linkages.

Figure 2. *FTIR-spectra of the azomethine derivatives of inulin containing hydrazine (1), ethylenediamine (2), and propylenediamine (3) fragments in their structure*



Conclusion

Thus, in the present research work, dialdehyde inulins with varying degrees of oxidation were obtained. The presence of aldehyde groups in oxidized inulin was confirmed by iodometric titration and IR spectroscopy. The molecular weight characteristics of dialdehyde inulin were studied. In the next stage, hydrazine, ethylenediamine, and propylenediamine were immobilized onto the monomeric units of oxidized inulin. It was established that as the content of aldehyde groups in oxidized inulin increases, the degree of substitution of the reaction products also increases. IR spectroscopy confirmed that the chemical binding of hydrazine, ethylenediamine, and propylenediamine to the aldehyde groups of dialdehyde inulin occurs through covalent azomethine (–C=N-) linkages.

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