

Isolation and comparative study of various polymorphic forms of inulin

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Abstract. Various forms of inulin, differing in structure and solubility in water, have been isolated and identified. A comparative reaction of periodate oxidation of α -, β - and γ -inulin was carried out. It was determined that the highest oxidation level and the rate of formation of aldehyde groups among the polymorphic forms of the polysaccharide are characteristic of α -inulin. Different forms of inulin, displaying variations in structure and solubility in water, have been successfully isolated and identified. To gain insights into their chemical properties, a comparative study involving periodate oxidation was conducted on α -, β -, and γ -inulin. The results of the study revealed that α -inulin exhibited the highest level of oxidation and the fastest rate of aldehyde group formation among the various polymorphic forms of the polysaccharide. This finding suggests that α -inulin possesses unique chemical reactivity and characteristics compared to the other forms studied. The investigation into the periodate oxidation of inulin polymorphic forms provides valuable information on their respective chemical profiles, aiding in the understanding of their diverse behaviors and potential applications in various fields. This research contributes to the broader knowledge of inulin's properties and may pave the way for its targeted use in specific applications based on its distinctive characteristics.

1. Introduction

At present time, polysaccharides are considered one of the potentially valuable macromolecular substances widely used as carriers of drugs [1-3]. First of all, it should be noted their availability, biocompatibility, non-toxicity, biodegradability and the absence of allergic effects on the body. In addition to the listed set of unique properties, the main advantage of polysaccharides is the variability of their molecular parameters and chemical structure, which makes it possible to expand the area of their practical application.

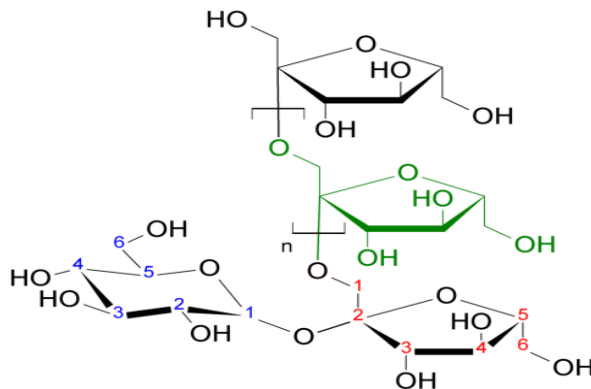
The joining of most types of physiologically active substances to polysaccharide macromolecules requires preliminary functionalization of the polymer-carrier chains. One of the most common ways of functionalization of polysaccharides is periodate oxidation [4-5]. This method is applicable for polysaccharides with vicinally located hydroxyl groups in the repeating link. The mechanism of the periodate oxidation reaction involves the formation of a cyclic complex, which then breaks down with the oxidation of the -C-C- bond and rupture of the cycle. Activated polysaccharides contain aldehyde groups in the structure, the number and rate of formation of which can be controlled by changing the conditions of the periodate oxidation process. In general, dialdehyde derivatives of polysaccharides are used as polymers-carriers of biologically active substances possessing aldehyde, phenolic, alcohol and primary amine groups in their structure. The result of chemical interaction is polymer products in which active substances are bound to aldehyde groups of polysaccharides through an azomethine bond (-C = N-). This type of chemical bond is the most acceptable because it provides a gradual isolation of a biologically active substance from the macromolecular chain of the polysaccharide [6-10].

In recent years, the interest of researchers in the field of chemistry of macromolecular compounds is directed to the development of new methods for the isolation and chemical modification of inulin. It is known that inulin is a polysaccharide found in the tubers of Asteraceae and some other plants. Inulin macromolecules have a linear structure, consist of 2-1 linked β -D-fructofuranose residues and end with a 2-D-glucopyranose residue, as in sucrose [11-15]. The molecular weight of inulin ranges from 4000-7000 Da, i.e. it occupies an intermediate region between

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oligomers and high polymers. Usually, inulin is extracted by water extraction at elevated temperatures from natural raw materials – topinambour (*Jerusalem artichoke*), yacón (*Smilax sonchifolius*) and chicory (*Cichorium intybus*) and others. Due to the fact that inulin is fermented by the microflora of the large intestine, its regular use in food products provides various therapeutic effects, mainly, for reducing sugar in the body.

The inulin formula is as follows:



However, like other polysaccharides, the structure of inulin contains exclusively hydroxyl groups, through which it is rather difficult to carry out chemical immobilization of biologically active substances [16, 17].

Based on the above-stated relevance of the tasks, the aim of the work was to isolate and carry out chemical modification of various forms of inulin using the periodate oxidation reaction.

2. Materials and methods

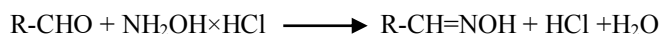
Obtaining α -inulin. 100 g of air-dried topinambour tubers, sifted through a 2 mm sieve, were extracted with water in a ratio of 1: 8, 1: 4 at 85-90°C for 1 hour. The extracts were combined, treated with CaO (4.5 g) at 80- 85°C, then centrifuged, the solution was evaporated to one third and left in the refrigerator for 10 days at -10°C. At standing, a precipitate of inulin α -form precipitated, which was separated, dried in air, and then ground into powder, the yield of α -inulin was 3% (based on air-dry raw materials). α -inulin, a white powder dissolves in water at 37 °C, $[\alpha]_D - 36,3^\circ$ (with 0.1% NaOH). IR spectrum (KBr, ν , cm^{-1}) 3367, 2930, 1606, 1418, 1033, 936, 872, 821, 601 [18].

Obtaining β -inulin. The supernatant solution was evaporated to 200 ml and precipitated with alcohol in a 1:2 ratio. The precipitate was separated, washed and dried. The β -inulin yield was 6% (based on air-dry raw materials). β -inulin is an amorphous powder, readily soluble in water at 23°C $[\alpha]_D - 35.5^\circ$ (with 0.1% NaOH). IR spectrum (KBr, ν , cm^{-1}) 3392, 2934, 1592, 1418, 1035, 935, 870, 819, 602 [19].

Obtaining γ -inulin. 2 g of β -inulin was suspended in 20 ml of water and heated at 45-50° C for 48 h, then heating was carried out at 55°C for 2 h. After cooling, γ -inulin was precipitated with alcohol in a ratio of 1:3, the precipitate was washed with acetone, ether and dried in a desiccator over P_2O_5 . The yield of the final product is 26%. Low molecular fragments of inulin remained in the alcoholic mother liquor with a yield of 52%. γ -inulin is a white powder, dissolves at 70-80 °C, $[\alpha]_D - 34.2^\circ$ (with 0.1% NaOH). IR spectrum (KBr, ν , cm^{-1}) 3395, 2935, 1588, 1418, 1035, 936, 867, 820, 603 [20].

Obtaining inulin dialdehyde. 0.5 g of α -, β - or γ -inulin was placed in a glass beaker with a capacity of 500 ml, then 100 ml of acetate buffer with pH 4.25 was added, and 0.25 N HIO_4 solution was added at a molar ratio of inulin: $\text{IO}_4^- = 1:1$. The oxidation process lasted from 0.25 to 5 hours at $t = 20^\circ\text{C}$. At the end of the periodate oxidation reaction, the reaction products were precipitated with acetone and washed with 70% ethyl alcohol until a negative reaction to the IO_4^- and IO_3^- ions (control by the reaction with a solution of silver nitrate). The target products were dried in the dark under vacuum over P_2O_5 [21].

Determination of the oxidation level (γ_{ox}) by the oxime method. 100 mg of dried dialdehydinulin weighed to the nearest 0.1 mg was placed in a conical flask with a capacity of 250 ml, containing 25 ml of a freshly prepared solution of hydroxylamine hydrochloric acid (pH 5.0). A blank control sample was prepared in parallel. The mixture was stirred for 1 h, then the liberated hydrochloric acid was titrated with 0.1 M NaOH solution (bromophenol blue indicator). The determination of the total content of aldehyde groups in the test samples is carried out by the difference in the released volume of hydrogen chloride [22].



The number of aldehyde groups is calculated using the following formula:

$$R - CHO, mole\% = \frac{0,1 \times (V_1 - V_2)}{g \times M} \times 100$$

where, V_1 is the volume in ml, 0.1 M sodium hydroxide consumed for the sample; V_2 is the volume in ml, 0.1 M sodium hydroxide consumed for the control experiment; g-sample of polysaccharide dialdehyde, $g \times M$ - molecular weight of the repeating fragment in dialdehydeinulin

Determination of the molecular weight of oxidized α -inulin and dialdehydeinulin. The molecular-weight characteristics of the synthesized derivatives were determined on an Agilent 1260 Infinity liquid chromatograph equipped with a refractometric detector using a PLAquagel OH Mixed chromatographic column 300 mm long and 7.5 mm in inner diameter (Waters, USA). The concentration and volume of the injected sample were 2 mg/ml and 100 μ l, respectively. An aqueous 0.1 N sodium nitrate solution was used as an eluent. The volumetric flow rate of the eluent was 0.8 ml/min.

3. Results and discussion

The results of the study showed that polymorphic forms of α -, β - and γ -inulin differ in appearance, instability and solubility in water; upon standing, they turn into less soluble and stable forms. The absorption bands of the IR spectra of the polymorphic forms of inulin are close and they differ only in insignificant shifts to a weak or strong field. γ -inulin is stable in contrast to the α - and β -forms [9].

Findings by the method of electron microscopic observation indicate a different supramolecular structure of polymorphic forms of inulin (Figure 1. a, b, c). α - and β -inulin have a denser structure in comparison with γ -inulin. Among the various polymorphic forms of inulin, β -inulin is distinguished by the highest crystallinity.

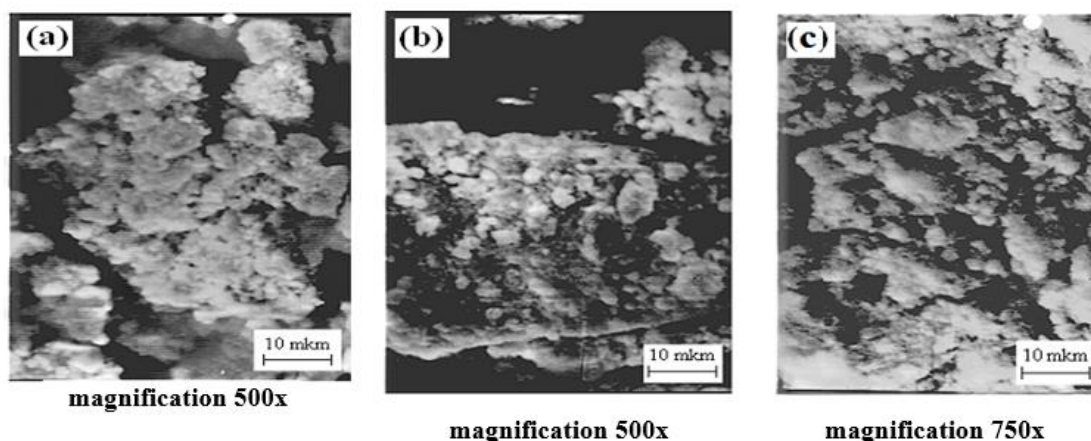
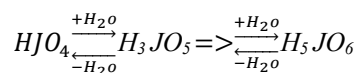


Fig. 1. Scanning electron microscopy of polymorphic forms of inulin: a) α -inulin b) β -inulin c) γ -inulin.

Under the action of iodic acid on inulin, the oxidation of the hydroxyl groups located at the C_2 and C_3 carbon atoms forming the α -glycol group occurs with the simultaneous rupture of the carbon-carbon bond between them. In this case, the oxidizing agent is H_5JO_6 , which is formed during the hydration of HJO_4 :



We studied the chemical modification of α -, β - and γ -inulin to elucidate the dependence of the rate of the periodate oxidation reaction on the structural features of various polymorphic forms of inulin.

Periodic oxidation of α -, β - and γ -inulin was carried out according to the following scheme:

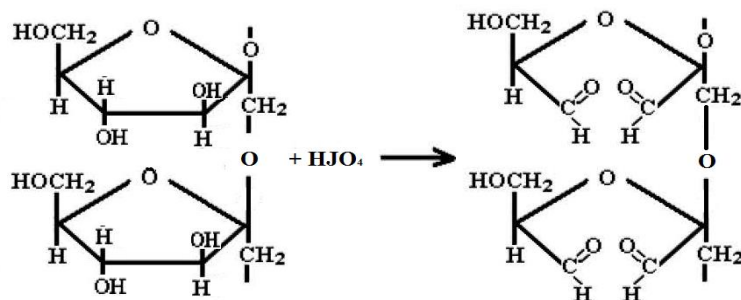


Figure 2 shows the dependences of the rate of oxidation of α -, β - and γ -inulin on the duration of the periodate oxidation reaction.

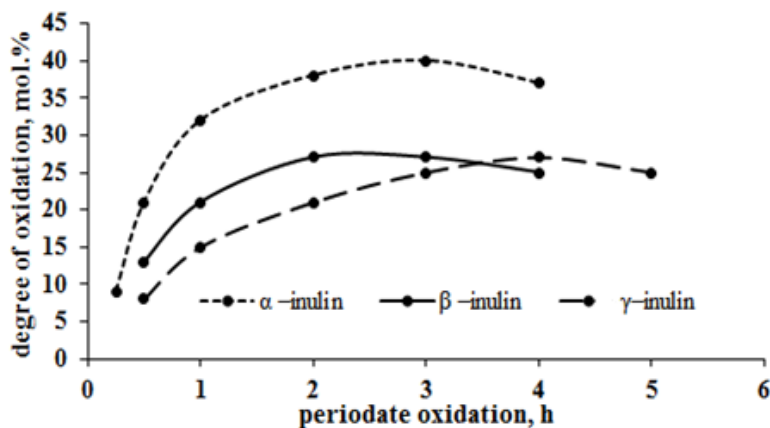


Fig. 2. Dependence of the oxidation degree of α -, β - and γ -inulin on time.

As can be seen from the data presented in Figure 2, the rate of formation of aldehyde groups differs and depends on the polymorphic forms of inulin. The highest oxidation rate is characteristic of α -inulin, since the oxidation level of the reaction products after 1-3 hours is 32-40 mole %. A further increase in the oxidation time of α -inulin leads to re-oxidation of aldehyde groups to carboxyl groups and a decrease in the value of the oxidation level. Periodic oxidation of β -inulin as compared to α -inulin occurs at a lower rate. This difference in the rate of oxidation is explained by the presence of crystalline regions in the β -inulin macromolecular chain. The lowest rate of periodate oxidation is characteristic of γ -inulin. This is due to the fact that γ -inulin does not dissolve at room temperature and the periodate oxidation reaction occurs under heterogeneous conditions.

From the data presented in Figure 3, it follows that with an increase in the oxidation level of α -inulin, a gradual decrease in the molecular weight of the initial polysaccharide is observed, which is associated with the occurrence of acid hydrolysis of the polymer chain.

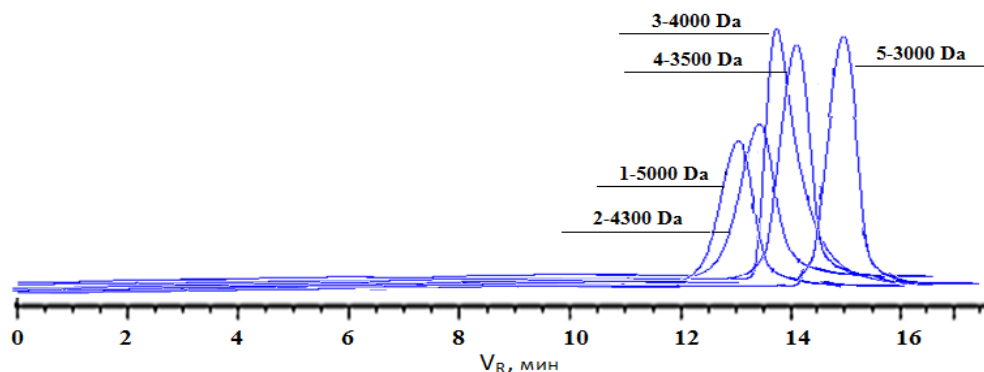


Fig. 3. Molecular characteristics of α -inulin (1) inulin dialdehyde with $\gamma_{ox} = 9$ mol% (2); $\gamma_{ox} = 21$ mol% (3); $\gamma_{ox} = 32$ mol% (4); $\gamma_{ox} = 40$ mol% (5).

4. Conclusions

Thus, on the basis of the studies carried out, the structure of various polymorphic forms of inulin has been isolated and studied. It was found that the rate of formation of aldehyde groups for α -, β - and γ -inulin is different and depends on the physicochemical properties of polysaccharides. With an increase in the oxidation level of α -inulin, a decrease in molecular weight was observed.

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