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PRELIMINARY ANALYSIS OF THE COMPOSITION OF POLY-SACCHARIDE FRACTIONS

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Work on the analysis and separation of mixtures of polysaccharides has been carried out for a long time [1], but advances in this field have been recorded only in recent years using quaternary ammonium salts [2], DEAE-cellulose [3–6], Sephadex [7–9], and Bio-Gels [10–13].

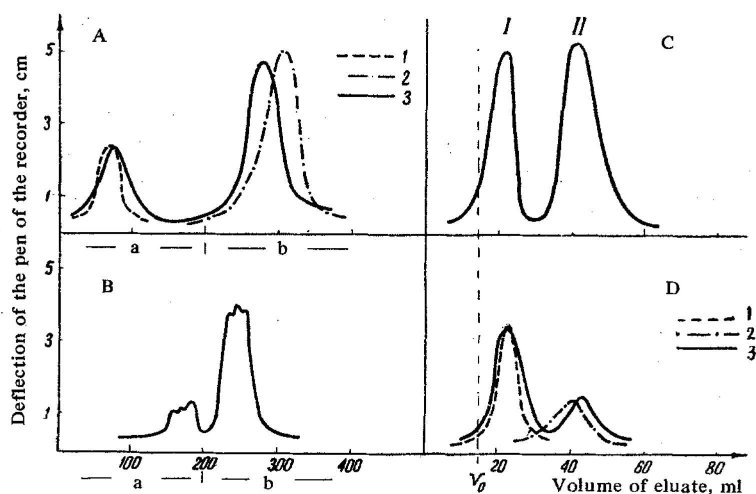
In this paper we give the results of the use of a more rapid and convenient method for the preliminary analysis of mixtures of polysaccharides which requires little time and material.

The method is based on gel filtration and ion-exchange chromatography using small columns with automatic monitoring of the separation of the polysaccharides.

We have found that polysaccharides, especially the polysaccharide fractions isolated from natural sources, absorb appreciably in UV light at wavelengths below 250 mμ. This has enabled us to carry out the continuous densitometric measurement of the eluates obtained from columns and to perform the automatic monitoring of the separation of the polysaccharides and the automatic recording of the elution curves. An analysis of the fractions by the phenol–sulfuric acid method [14] carried out in parallel led to identical elution curves in the case of polysaccharide fractions purified from phenolic impurities and unbound protein.

The sensitivity of the method has made it possible successfully to analyze a mixture of polysaccharides containing 5–10 mg of each of the individual components. The use of a photomultiplier greatly increased the sensitivity of the method and also reduced the amount of material and the time for analysis.

The separation of the polysaccharide mixtures into acid and neutral components was carried out by chromatography on DEAE-cellulose in the phosphate form [3]. In all cases we observed a good separation of the mixture of polysaccharides into acidic and neutral components; indeed, in a number of cases, separation within the individual groups as well (figure, A and B).



Gel filtration on Bio-Gels and chromatography on DEAE-cellulose of polysaccharide mixtures. A) Chromatography of inulin and zosterol on DEAE-cellulose: 1) inulin; 2) zosterol; 3) mixture of inulin and zosterol; a) elution with 0.05 M sodium dihydrogen phosphate; b) elution with 0.2 M caustic potash. B) Chromatography of gum arabic on DEAE-cellulose: a) elution with 0.05 M sodium dihydrogen phosphate; b) elution with 0.2 M caustic potash. C) Gel filtration of zosterol (I) (v_e = 20 ml) and of alginic acid (II) (v_e = 38 ml) on Bio-Gel P = 60 (v_0 = 14 ml). D) Gel filtration of zosterol (I) (v_e = 20 ml) and dextran (2) (v_e = 45 ml) on Bio-Gel P = 100 (v_0 = 15 ml); mixture of zosterol and dextran (3).

Elution Volume of Some Polysaccharides on Gel Filtration Through Bio-Gels

Bio-Gel		V_0, ml	Dextran mol. wt. 15-20 000	Alginic acid	Gum arabic	Inulin	Zosterol
type	weight						
P-20	2.5	13	25*, 29	15*, 30*	15*, 23*, 30	13, 25*, 30*	13*
P-30	2.5	13	28, 35*	—	17, 30*, 40	25, 50*, 70	13*
P-60	2.5	14	—	19, 38*	—	—	20*
P-100	2.5	15	45	—	70, 100*	—	20*

*Strongest peaks.

To separate the mixture of polysaccharides and analyze the polysaccharide fractions by partition-adsorption chromatography an attempt was made to use as carriers the salt forms of cation-exchange resins, especially Amberlite IR-20 in the sodium form, the adsorption of the polysaccharides by this resin being negligible and the diffusion of the polysaccharides within the particles of resin being highly hindered. This led to the finding that it was impossible to separate mixtures of polysaccharides, even when the dimensions of the column were increased and various solvents were used as eluants (water, solutions of urea, 1–10% aqueous solutions of alcohols, etc.).

When the carrier was used in the form of a mixture of carbon and Celite (1:2), the polysaccharides were strongly adsorbed and could not be eluted but when Celite alone was used there was no absorption and all the polysaccharides were eluted immediately from the free volume of the column, which did not lead to their separation.

Valuable results were obtained in the separation of the polysaccharides according to their molecular weights by gel filtration on Bio-Gels of various types. Above all, the Bio-Gels permitted a rough determination of the molecular weights of the components of the mixture of polysaccharides, which presented considerable interest [11].

In addition, in all cases a separation both of artificial mixtures of polysaccharides and of natural polysaccharide fractions was found. This made it possible to analyze the latter (figure, C, D). The free volume of the columns (V_0) was determined with the aid of dextran sulfate having mol. wt. 500 000.

The elution volumes of the individual polysaccharides (V_e) were found from the number of milliliters corresponding to the maximum peak. The table gives the elution volumes of the individual components of a number of polysaccharides. It can be seen from the table that the majority of the polysaccharides studied are not homogeneous with respect to their molecular weights.

Analysis required 1–2 hr, and in all cases good separation took place with satisfactory reproducibility of the results. The method has been used successfully in the analysis of the polysaccharide fractions isolated from the Araliaceae and Potamogetonaceae families, from brown algae, etc.

Experimental

Chromatography was carried out on DEAE-cellulose (H_2PO_4 form, capacity 0.61 meq/g), Celite-545 (greater than 400 mesh), and Amberlite IR-120 (100/200 mesh, Na^+ form); gel filtration was carried out on Bio-Gels of various types of the firm of "Bio Rad Laboratories" (Richmond, Calif., USA).

The analyses were run on standard columns 27 mm in diameter treated with a 1% solution of dichlorodimethylsilane in benzene.

The work was performed with commercial samples of dextran (mol wt 15–20 000), dextran sulfate (mol wt 500 000), inulin, gum arabic, and alginic acid. The zosterol was prepared from marine plants of the family Zosteraceae [15]. The course of the separation of the polysaccharide was monitored with a DPU-2M flow-through densitometer with an EPP-0.9MZ recorded. The fractions were analyzed parallel by the phenol-sulfuric acid method [14].

Chromatography of the polysaccharides on DEAE-cellulose. Five grams of DEAE-cellulose was washed three times with 0.5 N HCl and with water and was converted into the OH form by treatment with a 0.5 N solution of alkali with subsequent washing with distilled water to neutrality.

The carrier obtained was charged into a standard column and was washed with a 0.05 M solution of dihydrogen phosphate to convert the DEAE-cellulose into the dihydrogen phosphate form. Solutions of the polysaccharide fraction in 1 ml of 0.05 M sodium dihydrogen phosphate were deposited on the top of the column. The neutral polysaccharides were eluted with the same solution and the acidic polysaccharides with 0.2 M caustic potash solution (see figure, A, B).

Gel filtration of polysaccharides on Bio-Gels. 2.5 g of a Bio-Gel of a particular type (from P-10 to P-100) was treated with a 0.05 M solution of sodium dihydrogen phosphate at room temperature for 48 hr. The resulting carrier was

charged into a standard column and washed with water to eliminate the sodium phsophate. A solution of a polysaccharide fraction in 1 ml of 0.05 M sodium dihydrogen phosphate was deposited on the top of the column. Elution was carried out with distilled water (see figure, C, D).

Conclusions

A simple and convenient method for analyzing polysaccharide fractions isolated from natural sources has been proposed.

The method is based on ion-exchange chromatography on DEAE-cellulose and gel filtration on Bio-Gels using small columns and automatic monitoring of the separation with the aid of a flow-through ultraviolet densitometer. The method is characterized by rapidity and good reproducibility.

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