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ANION-EXCHANGE CHROMATOGRAPHY OF OLIGOMERIC ALDONIC ACIDS

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SUMMARY

Oligomeric aldonic acids were separated on strongly basic resins with a styrene–divinylbenzene matrix and sodium acetate as eluent. With a resin with 8% divinylbenzene, species containing more than eight monomeric units were excluded, while with 2% divinylbenzene, species containing up to sixteen monomeric units were obtained as distinct peaks provided that the column loading was low. With both matrices, the practical specific-exchange capacity decreased drastically with increased size of the oligomer.

INTRODUCTION

Oligomeric aldonic acids are present in waste waters from industries using polymeric carbohydrates. Their separation is of interest also in studies of the structure of polysaccharides. As previously shown[1], lower oligomers can be separated completely by anion-exchange chromatography in sodium acetate on a resin of the styrene–divinylbenzene type with a nominal cross-linking of 8% (Dowex 1-X8). Later investigations showed that the method failed with oligomers containing more than eight monomeric units. Even with lower oligomers losses occurred when the method was used for preparative purposes. This was not unexpected since it had been observed with other anions that the practical specific exchange capacity of anion-exchange resins decreases with increasing molecular size and that above a certain limit the exclusion is virtually complete[2–4]. In contrast to the ions studied in these reports, the aldonate ions exhibit very low ion-exchange affinities and no interfering non-polar interactions. Additional studies of oligomeric aldonic acids would therefore be of interest also for elucidating the ability of anion-exchange resins to accommodate large hydrophilic anions.

EXPERIMENTAL

The aldonic acids of the O-β-D-xylopyranosyl-(1,4)-[O-β-D-xylopyranosyl-

(1,4)]$_n$-D-xylonic acid series containing 1–6 monomeric units (DP = 1–6) were the same as those used previously[1]. A sample that contained in addition higher oligomeric acids of the same series was prepared by hypoiodite oxidation[5] of neutral saccharides prepared from birch xylan by hydrolysis in distilled water at 140° for 8 h. The hydrolyzate was freed from high-molecular-weight material by ultrafiltration (Diaflo PM-10) and from acidic products by treatment with Dowex 2-X8 (HCO_3^-) and subsequently Dowex 1-X2 in large excess. The average molecular weight[6] of the saccharide mixture was about 760.

Chromatographic separation and determination of the total exchange capacity were made as described previously[1]. The capacity per unit weight refers to the chloride form. The weight was determined by drying the chloride form at 105°. In the batch experiments, the aldonic acids were determined by chromic acid oxidation. The excess of chromic acid was determined iodimetrically[7], except in the kinetic studies where the external solution was analyzed automatically with a Technicon Auto-Analyzer[8].

RESULTS AND DISCUSSION

The chromatogram in Fig. 1 shows that the resin with 2% nominal divinylbenzene (Dowex 1-X2) is well suited for chromatographic analysis of higher oligomeric aldonic acids. A fairly high sodium acetate concentration (0.02 M) was chosen to permit the elution of xylonic acid within a reasonable elution volume. Xylonic acid (1) was recorded in the chromic acid and periodate–formaldehyde channels, while xylobionic acid (2) and the higher oligomers were recorded in the chromic acid and carbazole channels. The highest oligomer that gave a distinct peak contained sixteen monomeric units. The first peaks that appeared on the chromatogram exhibited severe overlapping. As shown in separate experiments, improved separation of these compounds was achieved in 0.005 M and 0.01 M solution, but at these con-

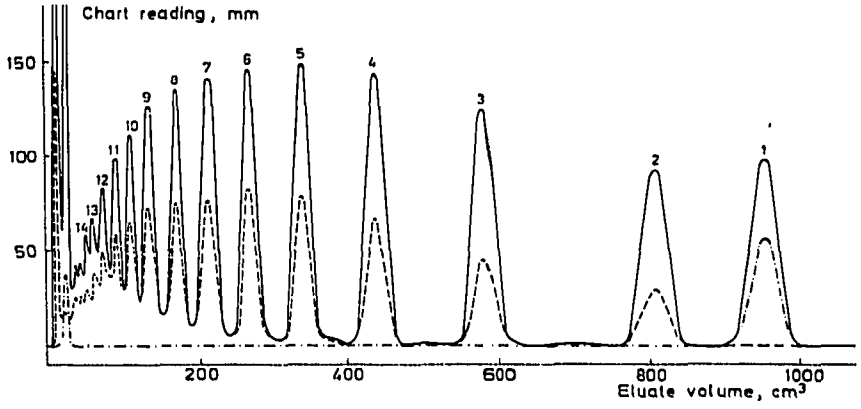

Fig. 1. Separation of a mixture (100 mg) of aldonic acids of the xylonic acid series which was prepared from a hydrolyzate of birch xylan as described in Experimental. Column (1320 mm × 6 mm I.D.) packed with Dowex 1-X2, 20–40 μm. Eluent, 0.02 M sodium acetate; nominal linear flow-rate, 2.5 cm/min. Channels: ———, chromic acid; –·–·–, periodate–formaldehyde; — — —, carbazole. Peak numbers refer to the numbers of monomeric units.

centrations xylonic and xylobionic acids appeared very late as broad peaks. Improved separation within a reasonable time can be obtained by gradient elution with increasing acetate concentration from 0.005 to 0.1 *M*. This is recommended in routine analysis of solutions that contain previously identified oligomers. The identification is, however, simplified when the eluent concentration is kept at a constant value.

Authentic samples were available for xylonic acid and the oligomers with *DP* = 2–6. Separate runs showed that their positions on the chromatogram were in close agreement with those of peaks 1–6 in Fig. 1. A graph of the logarithm of the distribution coefficient, D_V (calculated from the peak position), *versus DP* in Fig. 2 shows that slight but significant deviations from linearity were obtained for the lower oligomers (*DP* = 2–4), while the positions of the higher oligomers were in close agreement with a linear relation. This permits the identification of the higher oligomers for which no authentic samples are available.

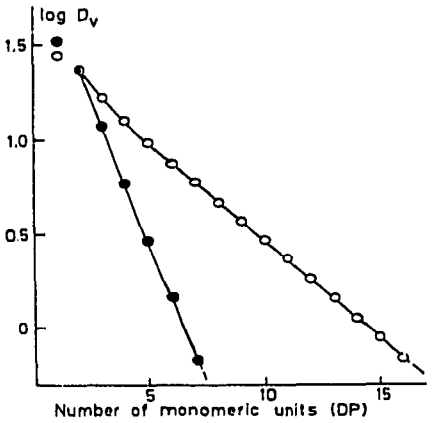

Fig. 2. Relation between log D_V and *DP* for oligomeric aldonic acids. ○, Dowex 1-X2, 20–40 μm; ●, Dowex 1-X8, 14–17 μm. Eluent, 0.02 *M* sodium acetate (pH 5.9).

A parallel experiment with the resin with 8% nominal divinylbenzene (Dowex 1-X8) showed that distinct peaks were obtained for species with *DP* = 1–8 and that the higher oligomers appeared as an overlapping band close to the interstitial volume of the column. In agreement with the results reported previously, a linear relation between log D_V and *DP* was obtained starting with xylobionic acid (Fig. 2). Xylonic acid was retained more strongly by this resin than by Dowex 1-X2. The distribution coefficient, D_V, of xylobionic acid, calculated per unit volume of the resin bed, was virtually the same on both resins, while the higher oligomers were retained much more effectively by the lightly cross-linked resin, Dowex 1-X2, although the number of exchangeable acetate ions per unit volume was much lower with this resin. Qualitatively, the results can be explained by the lower swelling pressure of Dowex 1-X2 resin. The results clearly show that the resin with the tighter network structure, Dowex 1-X8, is only suitable for analysis of solutions containing lower oligomers, while resins with a lower number of cross-linkages can be used to advantage for analysis of solutions which also contain higher oligomeric acids.

An example of the application of Dowex 1-X2 resin for the isolation of higher oligomeric acids on a preparative scale is given in Fig. 3. First, the mixture of aldonic acids with a molecular weight of 760 was fractionated on a column (950 mm × 14 mm I.D.) packed with Dowex 1-X2 (particle size, 20–40 μm) under conditions similar to those in Fig. 1. Acids that appeared between the peak containing xylodecaonic acid (peak 10 in Fig. 1) and the large peak containing virtually non-retarded compounds (oligomers with $DP > 18$ and saccharides) were isolated as a group. The sodium ions were exchanged for hydrogen ions and the solution evaporated to dryness at 30°. The residue was then re-chromatographed on the same column in 0.005 *M* sodium acetate solution. The results show that, although a shorter and wider column was used in this run than in that referred to in Fig. 1, the solutes with $DP = 11$–17 were separated much more efficiently than at the higher eluent concentration. Fractions were cut so that the yield of the oligomers with $DP = 11$–15 was about 80–90%. Re-chromatography of the isolated acids on an analytical column under otherwise unchanged conditions indicated that only one oligomer was present in each fraction.

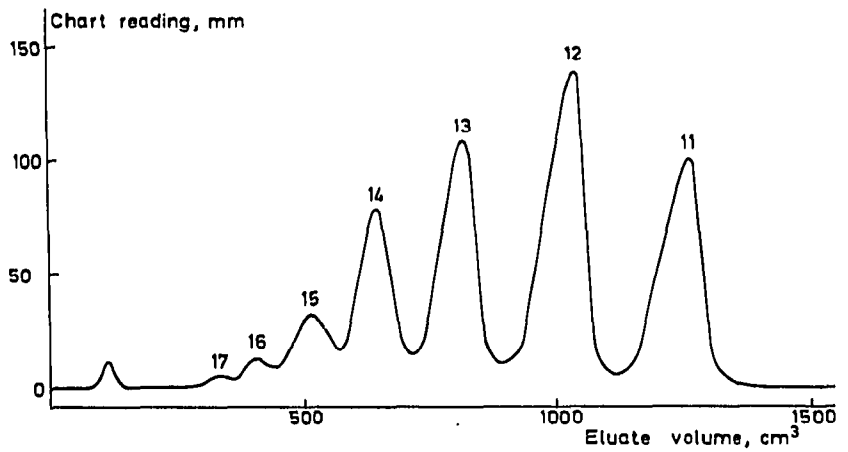

Fig. 3. Separation of a mixture of oligomeric aldonic acids of $DP = 11$–17 on a preparative scale. Column (950 mm × 14 mm I.D.) packed with Dowex 1-X2, 20–40 μm. Eluent, 0.005 *M* sodium acetate (pH 5.9); nominal linear flow-rate, 0.74 cm/min. Detector: Waters R4 differential refractometer. Peak numbers refer to the numbers of monomeric units.

All results reported above refer to elution analysis of sample solutions containing small amounts of exchangeable ions compared to the total number of exchange sites in the columns. In analyses of complex mixtures that contain large amounts of other solutes, *e.g.*, other carboxylic acids and sugars, it is desirable to perform a group separation of the monocarboxylic acids before separating them chromatographically. With solutions containing only acids of low molecular weight, this separation is conveniently carried out by stirring the solution containing the free acids with an anion exchanger in the bicarbonate form. This method can also be applied to advantage for the sorption of acids present as lactones[9].

In the batch experiments with the individual acids in Fig. 4, sorption by Dowex 1-X8 (HCO_3^-) resin was studied as a function of time. The amount of acid added was

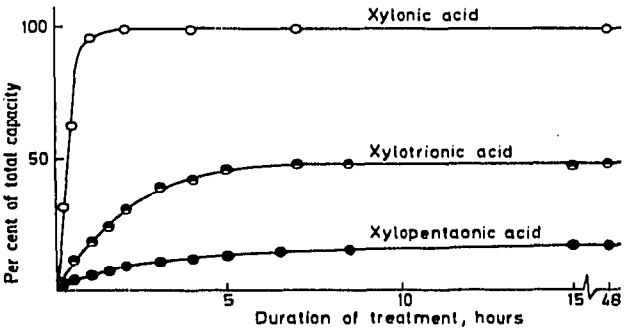

Fig. 4. Uptake of xylonic (○), xylotrionic (◓) and xylopentaonic (●) acids on Dowex 1-X8 (HCO_3^-), 50–100 mesh, at room temperature. Batch experiments were made with 0.2–0.3 g of resin per 10 ml of solution.

1.5 times the total exchange capacity of the resin (determined for chloride). Under the applied conditions, the uptake of xylonic acid reached an asymptotic value after about 2 h. This value corresponded to 98 % of the total exchange capacity. With xylotrionic acid an asymptotic value corresponding to 47 % of the total exchange capacity was reached after about 7 h, demonstrating that less than half of the bicarbonate ions in the resin could be replaced by aldonate ions although an excess of free acid was present in the external solution. The sorption of xylopentaonic acid was even slower, the asymptotic value being reached first after about 15 h. The kinetic studies clearly show that a very long contact time between the solution and the resin is required before the sorption of the oligomeric species corresponds to the practical specific capacity, Q_A, and that this capacity decreases drastically with increased *DP* of the oligomers.

Separate experiments were carried out to determine the effect of the molecular size on Q_A. The resin (0.2–0.3 g) was stirred with the sample solution for 24 h, drained in a column and washed with 10 ml of water. The aldonic acids were determined both in the combined effluent and washings and in the eluate obtained after elution with 100 ml of 0.2 *M* sodium acetate solution. Finally, the resin was converted into its chloride form and weighed. The values calculated for the amount of aldonic acids adsorbed and eluted were in close agreement. This shows that, in contrast to results observed with large ions which were retained very strongly by the resin[10], no trapping effect was obtained. No detectable amounts of acids were found in the effluent when, after an initial washing with 10 ml of water, the column was rinsed with an additional 50 ml of water. This shows that, despite the low exchange affinity of the oligomeric acids, Donnan hydrolysis had no significant effect on the determination of Q_A.

The practical specific exchange capacity for xylonic acid was very close to the total exchange capacity determined for chloride ions (3.42 mmoles/g). A slight but significant decrease was observed for xylobionic acid (Fig. 5). With the higher oligomers, Q_A decreased drastically. The highest oligomer that was retained in detectable amounts was xylooctaonic acid. For this oligomer, $Q_A = 0.05$ mmoles/g, *i.e.*, only 1.5 % of the total exchange capacity. For xylononaonic acid the sorption was negligible. For comparison, an experiment was made with xylopentaonic acid and Dowex 1-X2 resin. The practical specific capacity was much higher for this resin than for Dowex 1-X8, but the value was still only about one third of the total capacity of the

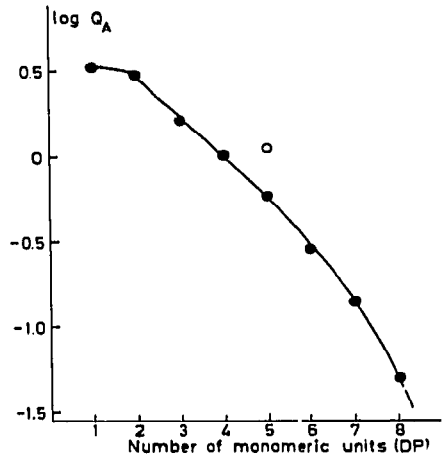

Fig. 5. Relation between the practical specific capacity (Q_A, mmoles/g) and the number of monomeric units (*DP*) of oligomeric aldonic acids. ●, Dowex 1-X8, 50–100 mesh; ○, Dowex 1-X2, 50–100 mesh.

resin as determined for chloride (3.58 mmoles/g). The results show that the ability of anion-exchange resins to accommodate oligomeric acids is restricted and that the fraction of the exchange sites that can be utilized decreases drastically with increased *DP* of the oligomers and with increasing degree of cross-linking of the resin.

When mixtures of oligomeric acids have to be analyzed, it is often necessary to isolate sufficient amounts of the individual acids for identification. The results given above show that the practical specific capacity is very low, even for oligomers that can be well separated when applied in small amounts on a column packed with Dowex 1-X8 resin (*e.g.*, pentamers and hexamers[1]). For such analyses and for preparative purposes, a resin with a low number of cross-linkages is recommended, even when higher oligomers are absent. In all instances, a large excess of anion exchanger has to be applied to obtain complete sorption of the oligomeric acids.

ACKNOWLEDGEMENT

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