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Simultaneous separation of oligomeric sugars and oligomers with a terminal aldonic acid group on anion-exchange resins

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In separations of oligomers with a terminal aldonic acid group by anion-exchange chromatography in aqueous solution, the oligomers are eluted in order of decreasing degree of polymerization[1] (DP, defined as the total number of monomeric moieties, including the aldonic acid end group). Ion-exchange resins can also be employed in the gel permeation chromatography of strongly polar non-electrolytes such as polyols and sugars[2]. We now report on the simultaneous separation of acid oligomers and oligomeric sugars, and on a correlation between their distribution coefficients.

RESULTS AND DISCUSSION

In a previous paper[1], it was shown that oligomers with an aldonic acid end group and which contained more than seven sugar moieties (DP > 8) were effectively excluded from anion-exchange resins that have a styrene–divinylbenzene matrix with a nominal degree of cross-linking of 8% (Dowex 1-X8). Most experiments were therefore carried out with a resin with 2% nominal divinylbenzene (Dowex 1-X2). The chromatogram presented in Fig. 1 shows that an excellent separation of sugars of DP 1–5, belonging to the β-(1→4)-linked xylose series (xylose: DP = 1), was achieved on the acetate form of this resin under conditions suitable for the complete separation of the acid oligomers of DP 2–16 belonging to the same oligomeric series (xylobionic acid: DP = 2) in the same run.

A prerequisite for an efficient separation of the oligomeric sugars is that only small amounts are applied to the column and that, in any event, with the system employed for automatic colorimetric analysis, the flow-rate is very low during the first part of the elution. In addition, a low flow-rate was necessary in order to prevent severe broadening of the peaks corresponding to the aldonic acids of very high DP. Evidently the height equivalent to a theoretical plate for these compounds was determined mainly by the rate of diffusion inside the resin particles. A decreased plate height can, as previously reported[1], be obtained at high temperature. Relevant data required for the theoretical discussion refer to room temperature and no results obtained at high temperature will therefore be given. Under the working conditions applied, the separation of the five sugars required 10 h. The elution of the aldonic acids
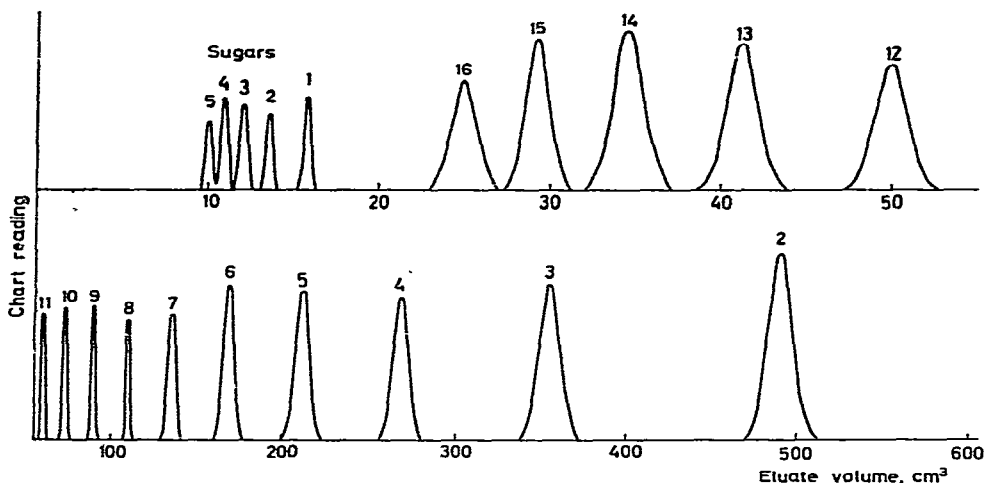
Fig. 1. Separation of xylose, O-β-D-xylopyranosyl-(1→4)-[O-β-D-xylopyranosyl-(1→4)]$_{DP-2}$-D-xylose and O-β-D-xylopyranosyl-(1→4)-[O-β-D-xylopyranosyl-(1→4)]$_{DP-2}$-D-xylonic acid.·Numbers on peaks indicate DP. Resin bed: Dowex 1-X8, 1273 × 4.5 mm, 23–40 μm, acetate form. Eluent: 0.02 M sodium acetate solution, 30°.

with DP 8–16 was completed after 38 h while the last compound (xylobionic acid) was eluted after 66 h. Separate experiments showed that the time required could be shortened by less than two thirds by increasing the eluent concentration during the run. An increased flow-rate can also be used[3] after the elution of the sugars and the acids of highest DP.

From experiments at constant eluent concentration, the distribution coefficients, D_v, were calculated from the peak elution volume ($\overline{V}$) by the equation $D_v = \overline{V}/X — \varepsilon$, where X is the bed volume and ε the interstitial fraction. This value was determined from the peak positions of the sugars by the extrapolation method described elsewhere[4].

For a resin with a normal degree of cross-linking (Dowex 1-X8), a linear relationship between ln D_v and DP was obtained for acid oligomers of DP 2–6 (Fig. 2). The values for the acids of DP 7–9 were slightly higher than predicted from the linear relationship. The results are in agreement with those obtained in a previous investigation[1] in which ε was determined by a less reliable method.

The D_v values of the individual acids in 0.08 M sodium acetate solution were very close to those calculated from experiments in 0.02 M sodium acetate solution by dividing by four. As very small amounts of aldonic acids were chromatographed, it can be concluded that the ratio between the activity coefficients of the eluted aldonate ions and the acetate ions in the external solution was almost constant. The slope of the straight line calculated by the least-squares method, for oligomers of DP 2–6, was —0.78 for the experiments in 0.02 M sodium acetate solution and —0.77 for those in 0.08 M sodium acetate solution.

The D_v values of the sugars were very low on the resin with a high degree of cross-linking (Fig. 2). The determinations are therefore subject to considerable errors. An estimate of the slope from the values recorded for sugars of DP 2–4 gave the value —0.79 in 0.02 M solution and —0.76 in 0.08 M solution.
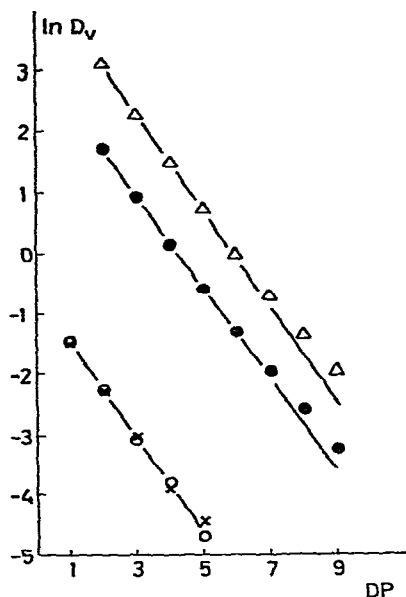
Fig. 2. Relationship between ln D_v and the number of monomeric units (DP). Dowex 1-X8, 13–20 μm, acetate form, 30°. O-β-D-Xylopyranosyl-(1→4)-[O-β-D-xylopyranosyl-(1→4)]$_{DP-2}$-D-xylonic acid: △, 0.02 M sodium acetate solution; ●, 0.08 M sodium acetate solution. Xylose and O-β-D-xylopyranosyl-(1→4)-[O-β-D-xylopyranosyl-(1→4)]$_{DP-2}$-D-xylose: ×, 0.02 M sodium acetate solution; ○, 0.08 M sodium acetate solution.
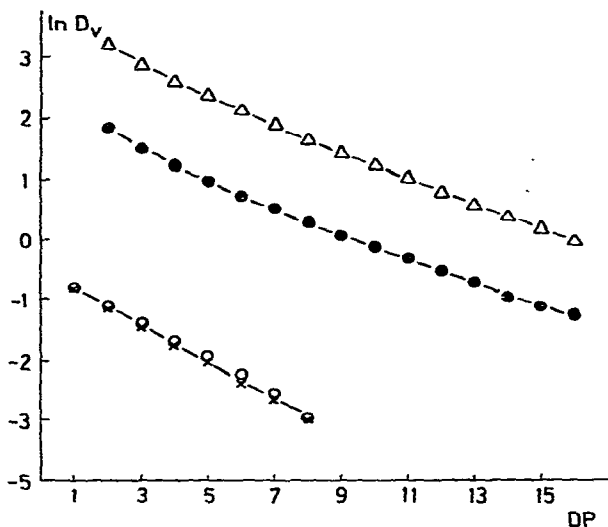
Fig. 3. Relationship between ln D_v and the number of monomeric units (DP). Dowex 1-X2, 23–40 μm, acetate form, 30°. O-β-D-Xylopyranosyl-(1→4)-[O-β-D-xylopyranosyl-(1→4)]$_{DP-2}$-D-xylonic acid: △, 0.02 M sodium acetate solution; ●, 0.08 M sodium acetate solution. Xylose and O-β-D-xylopyranosyl-(1→4)-[O-β-D-xylopyranosyl-(1→4)]$_{DP-2}$-D-xylose. ×, 0.02 M sodium acetate solution; ○, 0.08 M sodium acetate solution.

In a previous study[3] of acid oligomers it was shown that for a lightly cross-linked resin, the plot of ln D_v against DP in 0.02 M sodium acetate solution exhibited slight deviations from linearity. Fig. 3 shows that this is also true for Dowex 1-X2 at a higher eluent concentration and when the improved method for the determination of ε is applied.

Shrinkage of the resin bed by 2% occurred at the higher eluent concentration. A comparison between the distribution coefficients at the concentrations applied was therefore made per unit weight of the dry resin. For the acids of DP 2–8 the distribution coefficients in 0.08 M sodium acetate solution differed by less than 2% from those calculated from the determinations in 0.02 M solution. The difference increased with increasing DP and amounted to 7% for the acid of DP 16. A possible explanation is a decrease in swelling pressure with increasing eluent concentration, which has the greatest influence for the oligomers of the highest molar volume.

Again, the D_v values for the lower acid oligomers must be more reliable than those of the highest oligomers. The slope calculated for the DP range 2–5 was —0.29 for chromatography in 0.02 M sodium acetate solution and —0.28 in 0.08 M sodium acetate solution. For the sugars, the determinations of the D_v values for oligomers of

DP 6 and higher are impaired by great uncertainty. The slope for a straight-line relationship between ln D_v and DP for the same range as used for the acid oligomers was —0.28 at the lower eluent concentration and —0.27 at the higher concentration.

The results show that for both degrees of resin cross-linking the slope was virtually the same for the acid oligomers that participate in an ion-exchange process and for the oligomeric sugars that are partially excluded from the resin. Within the range studied, the influence of the eluent concentration was small and hardly significant while, in agreement with the Gibbs–Donnan theory[5], an increased degree of cross-linking led to a marked increase in the slope. As D_v is an equilibrium distribution coefficient, the results show that each new sugar moiety adds a constant increment $[-\Delta(\Delta G°)]$ to the change in free energy for the transfer of an oligomer from the external solution to the resin phase and that this increment is the same for charged and uncharged oligomers.

The results strongly indicate that the ratio between the activity coefficients of two oligomers belonging to the same series approaches unity both in the external solution and in the resin phase. According to the Gibbs–Donnan equation, the slope should therefore be $-\pi\Delta\bar{v}/RT$, where π is the swelling pressure, $\Delta\bar{v}$ the incremental change in partial molar volume for each additional sugar moiety, R the gas constant and T the thermodynamic temperature.

For the β-(1→4)-linked xylose oligomers, $\Delta\bar{v}$ is equal to 0.085 cm³/mole (ref. 6). The swelling pressure calculated from the slopes of the straight lines is 23 MPa for the acetate form of Dowex 1-X8, which is in good agreement with values obtained by isopiestic measurements[7]. For Dowex 1-X2, the calculated pressure is 8.5 MPa in 0.02 M sodium acetate solution and 8.1 MPa in 0.08 M sodium acetate solution.

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