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## Note

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### Determination of the interstitial volume of ion-exchange columns

KENNETH LARSSON and OLOF SAMUELSON

*Department of Engineering Chemistry, Chalmers University of Technology, Fack, S-402 20 Göteborg (Sweden)*

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For calculations of chromatographic retention data by the usual methods<sup>1</sup>, it is necessary to know the interstitial volume ( $V_I$ ) of the column. An error in the interstitial volume has little effect on the distribution coefficients calculated per unit weight of the resin ( $D_g$ ) or unit volume of the resin bed ( $D_v$ ) provided that the peak elution volume is much larger than the interstitial volume. In reporting data on exclusion chromatography and ion-exchange chromatography of compounds that exhibit low distribution coefficients, it is, however, highly desirable to have determinations of the interstitial volume that are as accurate as possible.

We report here on a comparison between different methods, including a technique which, to our knowledge, has not been described previously.

#### CATION-EXCHANGE RESINS OF THE GEL TYPE

For ion-exchange columns, the interstitial volume is equal to the total bed volume minus the volume of the swollen resin. Accurate determinations of the swelling can be made by studies of the penetration of electrolytes into the resin (ion exclusion). The amount of “non-solvent” water or the “non-solvent” (“salt-free”) volume is determined at different electrolyte concentrations and the values are extrapolated to zero concentration<sup>2,3</sup>. This technique is conveniently applied to sulphonic acid resins where Donnan hydrolysis can be neglected. An alternative method for the determination of  $V_I$  is to determine the peak elution volume,  $\bar{V}$ , for a compound which, owing to its large molar volume or high charge, cannot penetrate into the swollen resin<sup>4,5</sup>. To obtain an unambiguous comparison between these methods, experiments were made with the sodium form of a sulphonic acid resin (Aminex A4; 16–24  $\mu\text{m}$ ).

The resin bed (22.3  $\text{cm}^3$ ), which was supported by wire netting, was conditioned with sodium chloride solution of known molarity ( $c$ ). The solution above and beneath the bed was removed and the sodium chloride was then displaced by washing with water and determined in the effluent ( $b$  mmole) by conductimetric titration with silver nitrate solution. The ratio  $b/c$  was plotted against  $c$  and the interstitial volume obtained by extrapolation to zero concentration. When  $c$  was decreased from 0.025 to 0.005, the ratio  $b/c$  decreased from 6.47 to 6.27, which indicates that  $V_I$  was very close to 6.25.

Interestingly, this value was significantly higher than the peak elution volume obtained when Dextran T 2000 was eluted with water from the same column. The average value calculated from eight experiments was 5.58 ml (standard deviation 0.05 ml).

These results indicate that high-molecular-weight solutes are partially or completely excluded from regions in the resin bed in which the concentration of low-molecular-weight compounds is virtually the same as in the bulk of the mobile phase. This would mean that the application of high-molecular-weight compounds in determinations of  $V_I$  is impaired by uncertainty and may contribute to a significant error in the distribution coefficients unless the difference between  $\bar{V}$  and  $V_I$  is great.

As the technique based on Donnan exclusion is not generally applicable, a new approach was tested, based on the observation that the concentration of oligomeric sugars is lower inside the resin phase than in the external solution. The logarithm of the distribution coefficient is linearly related to the degree of polymerization (DP) of the sugar<sup>6</sup>, so that a plot of the peak elution volume ( $\bar{V}_{n+1}$ ) for an oligomer of DP =  $(n+1)$  against that of its next lower oligomer ( $\bar{V}_n$ ) will result in a straight line. When an oligomer of DP =  $n$  is completely excluded,  $\bar{V}_n = \bar{V}_{n+1}$ . Hence, an extrapolation of the observed values (Fig. 1) to the diagonal will give the interstitial volume of the column. The determinations were made by recording the peak elution volumes for oligomeric sugars of the  $\beta$ -(1 $\rightarrow$ 4)-linked xylose series (xylose to xyloheptaose) as described elsewhere<sup>7</sup>. The value obtained by this technique,  $V_I = 6.25$ , was in excellent agreement with that obtained by the Donnan exclusion method. Good agreement was obtained in a duplicate run ( $V_I = 6.23$ ). This corresponds to an interstitial fraction in the column of  $\epsilon = 0.280$ .

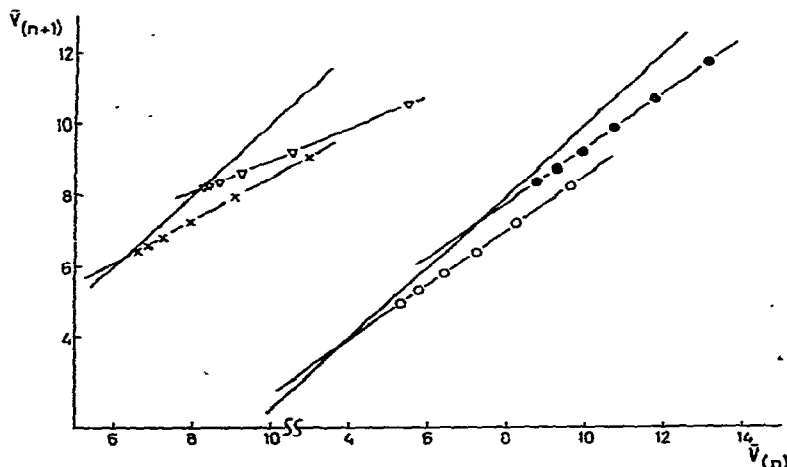


Fig. 1. Plots for determination of the interstitial volume by the oligomer method. Experiments at 30°.  $\times$ , Aminex A-4, 16–24  $\mu\text{m}$ , 1435  $\times$  4.4 mm (sodium form in water);  $\nabla$ , Dowex 1-X8, 13–20  $\mu\text{m}$ , 1405  $\times$  4.5 mm (acetate form in 0.08  $M$  sodium acetate solution). Dowex 1-X2, 23–40  $\mu\text{m}$  (acetate form in 0.02  $M$  sodium acetate solution):  $\bullet$ , before compression (1200  $\times$  4.5 mm);  $\circ$ , after compression (968  $\times$  4.5 mm).

## ANION-EXCHANGE RESINS OF THE GEL TYPE

Owing to Donnan hydrolysis, the exclusion technique used for the cation exchanger is not generally applicable, and cannot be used for anion exchangers in the acetate form.

Experiments with Dowex 1-X8, 13–20  $\mu\text{m}$ , showed that the oligomer method gave promising results. Determinations of the peak elution volume in 0.08  $M$  sodium acetate solution of the same sugars as above gave the results included in Fig. 1. Extrapolation gave  $V_I = 8.18$  ml, corresponding to an interstitial fraction of  $\epsilon = 0.368$ . Experiments in 0.02  $M$  sodium acetate solution gave an identical interstitial fraction. Similarly, the peak elution volume of Dextran T 2000 was unaffected by the change in eluent concentration. In agreement with the results obtained with the cation exchanger, the calculated interstitial fraction was significantly lower ( $\epsilon = 0.330$ ) than that determined by the oligomer method.

The above experiments were carried out on columns that had been used in experiments at high flow-rates. Hence, no change in column volume occurred due to compression during the experiments. To study the effect of bed compression, experiments were carried out with the acetate form of a lightly cross-linked resin (Dowex 1-X2, 23–40  $\mu\text{m}$ ). The experiments were carried out in 0.02  $M$  sodium acetate solution with oligomers of DP 2–8 belonging to the same oligomeric series. The results are given on the right in Fig. 1.

For a freshly packed column, the oligomer method gave the value  $V_I = 7.09$  ml, which corresponds to  $\epsilon = 0.374$ . Dextran T 2000 was eluted at  $\bar{V} = 6.12$  ml, which corresponds to  $\epsilon = 0.323$ . To avoid compression, these runs were carried out at a very low flow-rate.

The resin bed was then compressed from a total bed volume of 18.9 ml to 15.3 ml by pumping sodium acetate solution through the column at a high flow-rate. The same oligomers were then eluted at a very low flow-rate. The oligomer method gave  $V_I = 3.67$  ml, corresponding to  $\epsilon = 0.240$ , while in subsequent experiments with Dextran T 2000 this compound was eluted at  $\bar{V} = 2.89$  ml, corresponding to  $\epsilon = 0.189$ .

The compression of the bed resulted in a decrease in the total volume of 3.6 ml. The corresponding decrease in the interstitial volume determined by the oligomer method was only slightly lower (3.4 ml), indicating that the compression of the resin beads was very small.

In summary, these results show that the Donnan exclusion technique, when applicable, seems to give reliable determinations of the interstitial volume. Good agreement was obtained between this method and the oligomer method, while for all systems studied the polymer exclusion method gave significantly lower values. It should be stressed that all of the results refer to gel-type resins. With macroporous resins it may even be questioned if it is fruitful to distinguish between the interstitial volume and a resin phase<sup>8</sup>.

## ACKNOWLEDGEMENT

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