

## Partition Chromatography of Mixtures Containing Polyols and Carbonyl Compounds (Including Sugars) on Ion Exchange Resins

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The separation of polyols, carbonyl compounds, and sugars by partition chromatography on anion- and cation-exchange resins in ethanol-water mixtures is described. All polyols investigated were well separated on a cation exchanger in the lithium form, whereas certain mixtures with other solutes were better resolved on an anion exchanger in its sulfate form.

With some complex mixtures overlapping occurred with both types of resin. Complete resolution of such mixtures can be achieved if fractions containing overlapping compounds are rechromatographed on another resin form.

In a previous paper it was shown that mixtures of alditols and sugars can be separated by partition chromatography on anion-exchange resins in their sulfate form using aqueous ethanol as eluent.<sup>1</sup> With a few solutes, however, serious overlapping occurred, and the investigations have for this reason been extended to comprise separations on cation exchange resins in their lithium, sodium, and potassium forms. A potential application of this technique is in studies of the structure of polymeric carbohydrates such as modified cellulose and starch. Hydrolyzates from such products will contain, besides sugars and alditols, simple aliphatic carbonyl compounds, and such compounds were therefore included in the present work.

### EXPERIMENTAL

Chromatographic runs were made on two resins, one of which was the cation exchanger Dowex 50 W-X8 with particle size 14-17  $\mu$  (90 % ethanol,  $K^+$ ) and exchange capacity 5.1 mequiv./g of dry resin ( $H^+$ ). The resin was used in its potassium, sodium and lithium forms. The other resin, T5C, was a strongly basic anion exchanger which was used in the sulfate form. This resin had a particle size of 8-14  $\mu$  (90 % ethanol,  $Cl^-$ ) and exchange capacity 4.2 mequiv./g ( $Cl^-$ ). The resin was kindly supplied by Dr H. W. Holy, Technicon Instrument Co., Chertsey, Great Britain.

Table 1. Volume distribution coefficients on Dowex 50W-X8 in lithium, sodium and potassium forms at 75° and various ethanol concentrations.

	Volume distribution coefficients ( $D_V$ )														
	80 %			85 %			90 %			95 %			97 %		
	Li	Na	K	Li	Na	K	Li	Na	K	Li	Na	K	Li	Na	K
Formaldehyde	0.19	0.21	0.18	0.17	0.16		0.16	0.13	0.16	0.17	0.10	0.25	0.16	0.10	0.25
Glycolaldehyde	0.37	0.45	0.45	0.37	0.41	0.48	0.37	0.40	0.48	0.39	0.48	0.74	0.40	0.67	0.95
Ethylene glycol	0.81	0.71	0.61	0.84	0.68	0.68	0.95	0.72	0.66	1.1	0.93	1.1	1.2	1.2	1.3
Glyceraldehyde	0.52	0.71	0.82	0.59	0.70	0.92	0.67	0.75	1.1	0.80	1.3	2.1	0.87	1.8	2.9
Dihydroxyacetone							1.1			1.3	4.3		1.4	6.3	
Glycerol	1.2	1.0	1.0	1.4	1.2	1.1	1.7	1.4	1.5	2.2	2.5	3.0	2.7	3.6	4.3
Erythrose	1.0	1.4	1.6	1.1	1.7		1.4	2.3		1.8	4.2		2.1	6.3	
Erythritol	1.6	1.4	1.6	2.1	1.8	1.8	2.9	2.7	2.7	4.3	5.4	6.5	5.5	9.0	10.5
Ribitol	2.1			3.0	2.6		4.4	4.1	4.4						
Arabinitol	2.5	2.5		3.7	3.4	3.1	5.7	5.7	5.4						
Xylitol	2.8	3.2		4.1	4.5	4.1	6.4	7.9	7.6						
Mannitol	3.5	3.5		5.5	5.1	4.7	9.5	9.6	9.1						
Galactitol	4.1			6.7	6.7		11.8	13.1	11.6						
Glucitol	3.8	4.5		6.1	6.9	6.0	10.4	13.4	12.2						
Xylose		2.2		1.7	2.9		2.1	4.6							
Arabinose	1.7	3.0		2.2	4.2		2.8	6.9							
Glucose	2.0	3.6		2.8	5.3		3.8	9.7							
Mannose		3.6		2.8	5.3		3.8	9.7							
Galactose		4.5		3.4	6.7		4.7	12.5							
Fructose		3.6		2.7	5.4		3.8	10.2							

The chromatographic runs were made in water jacketed glass columns under conditions similar to those applied earlier.<sup>2</sup> The ethanol concentration was varied over a wide range which resulted in appreciable changes in swelling.<sup>3</sup> The height of the resin bed given in the legends to the figures refers to experiments in 90 % ethanol. At higher concentration the height was somewhat less and at a lower concentration greater. The volume distribution coefficients ( $D_v$ ) were calculated from the peak elution volumes as usual<sup>4</sup> and no corrections were applied for changes in swelling. The swelling of the resin in ethanol is different with different ionic forms, and with the cation exchanger the swelling increases in the same order as in water<sup>3</sup> ( $K < Na < Li$ ), which means that the exchange capacity of the resin beds decreases in that order.

The eluate was fed into a Technicon Auto Analyzer for automatic colorimetric analysis. Furfural and 5-hydroxymethylfurfural were determined with the orcinol method,<sup>5</sup> while the other compounds were determined by periodate oxidation at pH 7.<sup>1</sup>

## RESULTS AND DISCUSSION

The volume distribution coefficients determined from runs on cation exchange columns in their lithium, sodium, and potassium forms over a wide range of ethanol concentration are given in Table 1. It is seen that with all solutes containing more than one hydroxyl group the  $D_v$ -values increase markedly with increased ethanol concentration. Within the concentration range of practical interest the order of elution is, with one exception, independent of the ethanol concentration. A similar behavior was observed in previous work with monosaccharides<sup>5</sup> and alditols<sup>1</sup> on anion exchange resins. With formaldehyde and glycolaldehyde, which as expected have lower distribution coefficients than the polyhydroxy compounds, the effect of the ethanol concentration is small and possibly within the limits of experimental error.

It is seen that the ionic form of the resin has a great influence upon the distribution coefficients. With the sugars the uptake increases in the order  $Li < Na < K$ , in agreement with earlier observations.<sup>6</sup> Interestingly this rule holds true also with the lower hydroxy-aldehydes. As pointed out previously<sup>6</sup> the observation that the distribution coefficients of monosaccharides are markedly lower with the lithium form than with the other ionic forms can be explained by the fact that, compared at constant ethanol concentration in the external solution, the ethanol concentration inside the resin is much higher with the lithium than with the sodium and potassium forms.

With the polyols the influence of the ionic form differs from one solute to another, and the distribution coefficients vary with the ethanol concentration in a complex manner which depends upon the ionic form of the resin. For example, at an ethanol concentration of 80 % the distribution coefficients of erythritol increase in the order of  $Na < K = Li$ , whereas at high ethanol concentration the order is  $Li < Na < K$ .

It is noteworthy that despite the unfavourable solvent composition inside the lithium resin, several polyols are held more strongly by this resin form than by the sodium and potassium forms. This fact, which is most striking at comparatively low ethanol concentration, shows that specific interaction forces between the polyols and the counter-ions in the resin phase are of great importance and that, as expected from the degree of solvation of the cations, these forces are stronger with lithium ions than with sodium and potassium ions.

The existence of such forces was demonstrated in previous work<sup>7</sup> with almost water-free ethanol where solvation forces explain the fact that polyols are strongly held by ion exchange resins. A comparison between the behavior of polyols and the corresponding aldehydes confirms the conclusion that forces of this type are of importance within the range of ethanol concentration of interest in chromatographic work. With an anion exchange resin in the sulfate form, most alditols are held less strongly than the corresponding aldoses,<sup>1</sup> whereas with a cation exchanger in the lithium form, all polyols are held much more strongly than the corresponding aldehydes. As expected, the sodium resin occupies an intermediate position.

Another interesting feature of the cation exchange resins is that with some solutes, *e.g.* ethylene glycol and glyceraldehyde, a change in the counter ion may result in a reversed order of elution. This gives additional support to the conclusion that specific forces between the solutes and the counter-ions have a great influence upon the distribution coefficients.

Among the resin forms studied, the most favorable separations of the polyols were obtained with the lithium form. A chromatogram which shows the separation of the first seven solutes listed in the table is reproduced in Fig. 1. This separation, which was made in 95% ethanol, required about 3 h. At this ethanol concentration the elution of the solutes with higher distribution coefficients is lengthy, but excellent separations of the other polyols can be achieved at lower ethanol concentration. A chromatogram in which all polyols studied in this work are included is given in Fig. 2. This run was made in 85% ethanol and took about 5 h.

In a few separations of less complex mixtures the sodium or potassium resins offer advantages over the lithium form. Examples are the separations

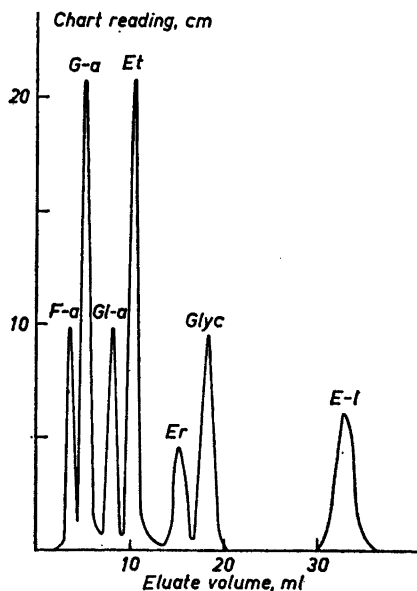


Fig. 1. Partition chromatography in 95% (w/w) ethanol at 75°. Resin bed: 2.6 × 1310 mm, Dowex 50 W-X 8, Li<sup>+</sup>, 14–17 μ.

Flow rate: 3.8 ml cm<sup>-2</sup> min<sup>-1</sup>.

F-a = Formaldehyde, 5 μg; G-a = Glycolaldehyde, 40 μg; Gl-a = Glyceraldehyde, 80 μg; Et = Ethylene glycol, 30 μg; Er = Erythrose, 40 μg; Glyc = Glycerol, 20 μg; E-l = Erythritol, 20 μg.

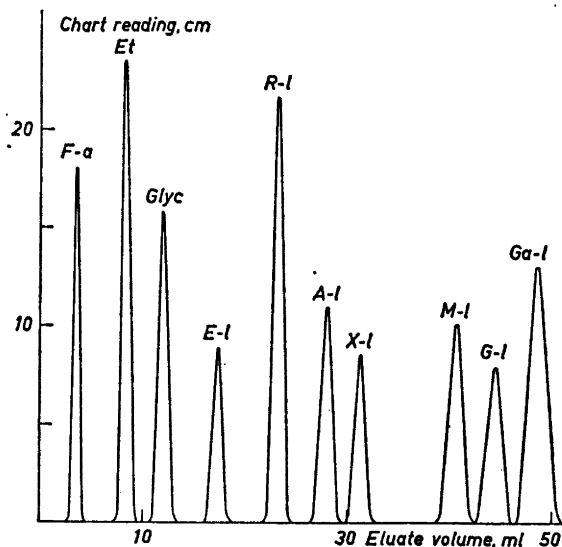


Fig. 2. Partition chromatography in 85 % (w/w) ethanol at 75°. Resin bed: 2.6 × 1310 mm, Dowex 50 W-X 8, Li<sup>+</sup>, 14–17 μ. Flow rate: 3.0 ml cm<sup>-2</sup> min<sup>-1</sup>. F-a = Formaldehyde, 20 μg; Et = Ethylene glycol, 50 μg; Glyc = Glycerol, 50 μg; E-l = Erythritol, 20 μg; R-l = Ribitol, 100 μg; A-l = Arabinitol, 50 μg; X-l = Xylitol, 50 μg; M-l = Mannitol, 60 μg; G-l = Glucitol, 60 μg; Ga-l = Galactitol, 100 μg.

of formaldehyde, glycolaldehyde, and glyceraldehyde, for which the potassium form is to be preferred.

The results obtained with monosaccharides (Table 1) confirm the earlier observation that cation exchange resins cannot be used in some separations of common monosaccharides<sup>8</sup> which can be carried out satisfactorily by means of anion exchange resins in their sulfate form.<sup>5</sup> Since it was of interest to examine the behavior of the lower carbonyl compounds under conditions suitable for the separation of the monosaccharides their distribution coefficients were determined on a resin in the sulfate form. The results are given in Table 2 together with those obtained with erythrose, which has not been studied previously. To permit a comparison with previously published  $D_v$ -values, some determinations with erythritol and xylitol are included in the table.

A chromatogram from a run on the sulfate column is reproduced in Fig. 3. In this experiment the elution was made at 40°, since with some solutes some decomposition was detected at 75°. Six of the seven compounds included in this run were well separated. Glycerol and dihydroxyacetone overlapped seriously but these compounds can be well separated on cation exchangers in the lithium or sodium forms. Erythrose is easily separated from glycerol and erythritol without any overlapping, whereas their separation on a cation exchange resin in the lithium form is less favorable (Fig. 1). From the distribution coefficients given in Tables 1 and 2 it is seen that it is not possible to

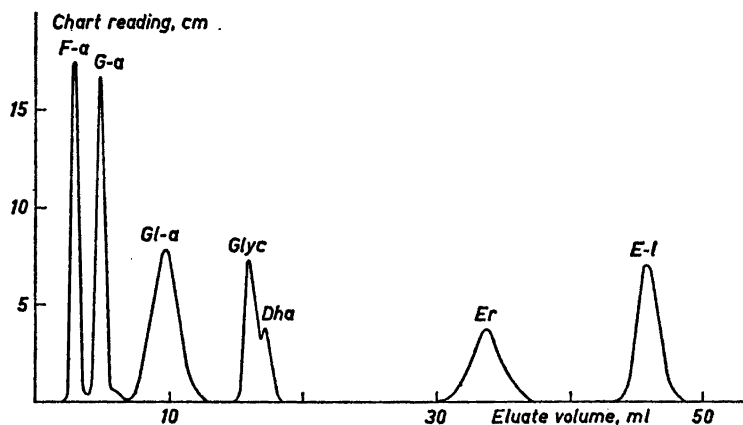


Fig. 3. Partition chromatography in 92% (w/w) ethanol at 40°. Resin bed: 2 × 1130 mm, T5C, SO<sub>4</sub><sup>2-</sup>, 8–14 μ. Flow rate: 1.9 ml cm<sup>-2</sup> min<sup>-1</sup>. F-a = Formaldehyde, 20 μg; G-a = Glycolaldehyde, 50 μg; Gl-a = Glyceraldehyde, 150 μg; Glyc = Glycerol, 30 μg; Dha = Dihydroxyacetone, 50 μg; Er = Erythrose, 150 μg; E-1 = Erythritol, 70 μg.

separate all solutes in a single run on one column. With complex mixtures of aldehydes, polyols, and sugars it is therefore necessary to make a preliminary separation on one column and a final separation of fractions containing overlapping compounds on a second column. Guidance in the choice of working conditions is obtained from the  $D_v$ -values given in the tables. In most systems

Table 2. Volume distribution coefficients on resin T5C in the sulfate form at 75° and 40° and various ethanol concentrations.

	Volume distribution coefficients ( $D_v$ )						
	75°					40°	
	80 %	84 %	88 %	92 %	96 %	92 %	96 %
Furfural	0.46	0.52	0.53	0.48		0.47	0.53
Formaldehyde	0.58	0.54	0.49	0.51	0.50	0.76	0.61
5-Hydroxymethyl-furfural	0.73	0.79	0.80	0.96		1.1	1.6
Glycolaldehyde	1.0	1.0	0.92	1.0	1.3	1.0	1.4
Ethylene glycol	1.1	1.1	1.1	1.2	1.4	1.2	1.5
Glyceraldehyde	1.4	1.5	1.8			2.4	4.3
Dihydroxyacetone	1.8	2.0	2.5	3.4		4.5	8.2
Glycerol	1.9	2.1	2.6	3.6	6.2	4.2	8.0
Erythrose	2.6	2.9	4.5	7.0		9.2	22.0
Erythritol	3.3	4.0	5.8	9.6	22.2	12.5	32.0
Xylitol	4.7	6.5	9.8	18.8			

containing the common monosaccharides together with alditols and lower carbonyl compounds a combination of the sulfate form of an anion exchange resin and the lithium form of a cation exchange resin will give the best results. With the lower carbonyl compounds, which are less well resolved than the polyhydroxy compounds, it can be advantageous to combine the method with ion exchange chromatography on an anion exchanger in its hydrogen sulfite form.<sup>9</sup> Another interesting combination is separation on an ion exchange column with a separation and identification of the individual compounds by gas chromatography-mass spectrometry.

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