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ION-EXCHANGE CHROMATOGRAPHY OF ALDEHYDES, KETONES, ETHERS, ALCOHOLS, POLYOLS AND SACCHARIDES

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CONTENTS

1. INTRODUCTION

This is the fourth and last review in the series surveying ion-exchange chromatography of the main classes of organic compounds and it is divided into the following sections: aldehydes and ketones; ethers; alcohols and polyols; and saccharides. The survey deals mainly with the literature for the period 1962–1970 and, as in the previous reviews, biochemical aspects have not been dealt with but mainly the chemical aspects of the separation process are discussed.

2. ALDEHYDES AND KETONES

Aldehydes and ketones are not ionized in aqueous solutions, but ion exchangers, however, can be used to effect some useful separations of these compounds. For this purpose, complex formation with the hydrogen sulphite form of anion-exchange resins is of possible use. Salting-out chromatography or differences in solubilities of individual compounds in aqueous–organic solutions are more advantageous separation mechanisms.

Carbonyl compounds are sorbed on the hydrogen sulphite form of strongly basic anion-exchange resins with the formation of the corresponding complex α-hydroxysulphonic acids. These complexes are retained by the resin, in contrast to the non-complexed compounds. Thus carbonyl compounds were separated quantitatively from alcohols by sorption on a 550 × 9.8 mm column of Amberlite IRA-400

(HSO_3^-) (0.12–0.30 mm)[1,2]. The alcohols passed into the effluent, while ketones were retained and could be recovered by elution with hot water or a solution of carbonate and hydrogen carbonate.

Glyceraldehyde 3-phosphate was separated from inorganic phosphates on the hydrogen sulphite form of anion-exchange resins[3]. A similar approach has been also applied to the retention of higher aldehydes in wines[4].

There are great differences in the stabilities of the hydrogen sulphite complexes of individual carbonyl compounds. Aromatic ketones, such as acetophenone and benzophenone, do not form stable hydrogen sulphite complexes and are only slightly retained on anion-exchange columns in the hydrogen sulphite form when water is used as the mobile phase. On the other hand, cyclic ketones such as cyclohexanone and diketones such as diacetyl and acetylacetone are sorbed relatively strongly and the use of elevated temperatures is recommended for splitting the complexes and facilitating the elution of these ketones with water. α,β-Unsaturated ketones such as mesityl oxide form strong complexes that are sorbed even from alcoholic solutions and cannot be split with hot water. These compounds, however, could be completely recovered by elution with salt solutions[2].

Likewise, differences exist in the sorption of aldehydes on the hydrogen sulphite form of anion exchangers. As a rule, the complexes of aldehydes with the hydrogen sulphite form of anion-exchange resins are more stable than those of ketones and their elution with hot water is usually not possible. Formaldehyde, acetaldehyde, furfural, benzaldehyde, salicylaldehyde, vanillin, glyoxal and other aldehydes are strongly retained by the resin.

It is possible to separate some aldehydes from ketones by selective elution of the ketones with hot water, followed by the recovery of aldehydes using solutions of salts (1 *N* sodium chloride, sodium carbonate–hydrogen carbonate). In this way, it was possible to separate acetaldehyde and furfural from acetone and methyl ethyl ketone on Amberlite IRA-400 (refs. 5 and 6).

The complete separation of mixtures containing acetic acid, ethanol, furfural or acetaldehyde and acetone could be accomplished. Acetic acid was sorbed on a column of Amberlite IRA-400 (HCO_3^-) (0.12–0.30 mm) while the other compounds passed into the effluent. The acid was then recovered by elution with 0.1 *M* sodium carbonate solution. The effluent from the hydrogen carbonate column was passed through a column of the same resin in the hydrogen sulphite form. The alcohol was not retained and could be determined pycnometrically in the effluent. The carbonyl compounds were sorbed on the column; acetone was eluted quantitatively with water at 75° and, finally, the elution of furfural or acetaldehyde was effected with 1 *M* sodium chloride solution at ambient temperature[2].

The differences in the sorption behaviour of the individual compounds have been utilized for a number of separations of ketones and aldehydes. Thus, diacetone alcohol, acetylacetone and mesityl oxide were separated from each other on a 450 × 9 mm column of Amberlite IRA-400 (HSO_3^-), 0.12–0.30 mm, by stepwise elution with water at 40° and 70° and, finally, with 1 *M* sodium chloride solution at 20° (ref. 2). The quantitative separation of a mixture of acetone and isopropyl *tert.*-butyl ketone was effected on the same Amberlite column by elution of acetone with water at 60° and subsequent elution of the other ketone with 1 *N* sodium chloride solution[7].

Stepwise or gradient elution with sodium or potassium hydrogen sulphite

solution of increasing concentration has brought about a marked improvement in the separation of carbonyl compounds on the hydrogen sulphite form of anion-exchange resins. The presence of hydrogen sulphite stabilizes the volatile compounds, and those which tend to polymerize or oxidize readily, as the hydrogen sulphite complexes. Micromolar amounts of 1-hydroxy-2-propanone, lactaldehyde, pyruvic acid and pyruvaldehyde have been completely separated from each other by this method, using Dowex 1-X10 (200–400 mesh) in 9.5 × 600 mm columns. The elution was carried out at 24° with 0.1, 0.2, 0.4, 0.8 and 1.6 *M* hydrogen sulphite solutions applied successively. The peaks were symmetrical, reproducible and sharp, in contrast to the asymmetric, irregular and broad elution curves obtained when sodium carbonate–hydrogen carbonate solutions were used for elution. It has also been possible to separate acetaldehyde and formaldehyde on a similar column[8].

Christofferson[9,10] achieved efficient separations of mixtures containing acetaldehyde, formaldehyde, furfural, 5-hydroxymethylfurfural, vanillin, pyruvic acid and pyruvaldehyde on Dowex 1-X8 (HSO_3^-) resin. A 150–300 mesh resin bead size gave a better resolution than coarser particles. Using a 410 × 11 mm column packed with this resin, the quantitative separation of the first five compounds was achieved by elution of acetaldehyde and formaldehyde with 0.2 *M* sodium hydrogen sulphite, then 0.4 *M* sodium hydrogen sulphite eluted 5-hydroxymethylfurfural and furfural. Finally, the most strongly sorbed compound, vanillin, was recovered with 0.8 *M* sodium hydrogen sulphite in 10–20% ethanol[9]. This method has been successfully applied to the determination of carbonyl compounds in sulphite spent liquor. Fig. 1 shows the elution positions of seven aldehydes in an industrial sample in chromatography on an 81 × 11 mm column of Dowex 1-X8 (HSO_3^-), 200–300 mesh. In this run, the above method was modified in that the elution with 0.2 *M* sodium hydrogen

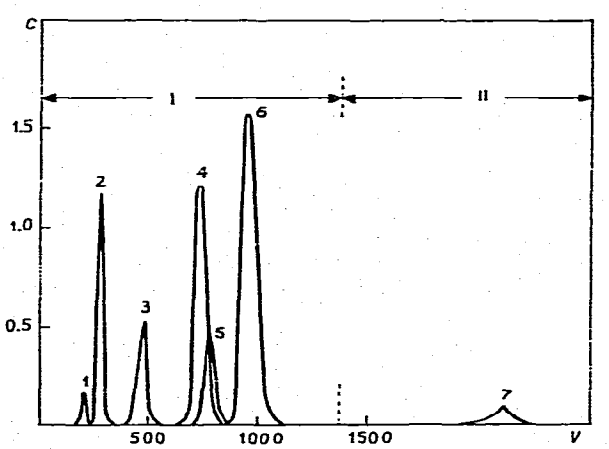


Fig. 1. Chromatography of carbonyl compounds in sulphite spent liquor on an anion-exchange resin in the hydrogen sulphite form. (1) Acetaldehyde; (2) formaldehyde; (3) pyruvic acid; (4) pyruvaldehyde; (5) 5-hydroxymethylfurfural; (6) furfural; (7) vanillin. Ion exchanger: Dowex 1-X8 (HSO_3^-), 200–300 mesh. Column dimensions: 81 × 11 mm. Mobile phase: I, 0.4 *M* $NaHSO_3$; II, 0.8 *M* $NaHSO_3$ in 20% ethanol. Flow-rate: 0.66–0.67 ml/cm²·min. Temperature: ambient. Detection: UV photometry and colorimetry in the effluent fractions. Fraction size: 19 ml. c = concentration (μmoles/l); V = volume of eluate (ml).

sulphite solution was omitted and a 0.4 *M* solution was used as the starting eluent[10]. The concentration of aldehydes in the eluate was measured by UV photometry and colorimetry using the reaction with chromotropic acid, after the excess of hydrogen sulphite had been removed by reaction with iodine[11].

Anion-exchange resins in the cyanide form sorb aldehydes and ketones with the formation of complexes. However, the chromatography of carbonyl compounds on columns packed with the cyanide form of anion exchangers (Dowex 2) was not successful, because of the excessively strong retention of most compounds and the possible polymerization of some of the aldehydes on the column[12].

Ketones that are able to form enol forms with a slightly acidic character, *e.g.* acetylacetone, can be sorbed on strongly basic anion-exchange resins in the free hydroxyl form, while they are not retained by the carbonate form of anion-exchange resins. This sorption can be used for the separation of the enolizable compounds from other ketones and aldehydes[2].

Breyer and Rieman[13] applied salting-out chromatography to separations of aldehydes and ketones, and found ammonium sulphate solutions to be satisfactory eluting agents. The utility of strongly acidic sulphonated cation-exchange resins (Dowex 50 and Amberlite CG-120) and strongly basic quaternary ammonium anion-exchange resins (Dowex 1 and Amberlite CG-400) of different degrees of cross-linking was tested. The capacity ratios of a number of compounds on three cation-exchange and three anion-exchange resins are given in Table I for various concentrations of ammonium sulphate solution.

Both the selectivity and broadening of the elution curves increased with increasing degree of cross-linking of the anion-exchange resin. With cation-exchange resins, the distribution ratios decreased as the degree of cross-linking increased from 4 to 12% but increased slightly with a further increase in the degree of cross-linking[14].

The separation efficiency was better on 200–400 mesh resins than that on exchangers with coarser particles, as expected. The anion exchangers Dowex 1-X8 and Amberlite CG-400 yielded the greatest differences in the distribution ratios of individual compounds. As the elution curves on Dowex 1-X8 were narrower than those on Amberlite CG-400, the former resin gave more satisfactory separations.

A number of valuable separations could be achieved using Dowex 1-X8. A nearly quantitative separation of formaldehyde, acetaldehyde, propionaldehyde and butyraldehyde could be effected on a 30 cm × 3.88 cm² column of this resin, 200–400 mesh, in 3 h using stepwise elution with 4.0 and 0.5 *M* ammonium sulphate solution. Acetoin, 2,5-hexanedione, 2,4-pentanedione, and diethyl ketone were separated in 10 h on a 57.8 cm × 2.28 cm² column using stepwise elution with 2.0 and 1.5 *M* ammonium sulphate solution and water. The separation of another, more complex, mixture of five ketones (acetone, diacetyl, diacetone alcohol, 2,3-pentanedione and cyclohexanone) required 35 h when a 108 cm × 2.28 cm² column was used with the same sequence of the eluents as in the former example. The separation of a seven-component mixture of aldehydes and ketones on a column of dimensions 110 cm × 2.28 cm² took about 65 h when the elution was carried out with 4.0, 2.0 and 1.0 *M* ammonium sulphate solution. This separation is shown in Fig. 2 (ref. 13).

2,3-Butylene glycol, acetoin and diacetyl were separated on a 3.5 × 54 cm column of Dowex 1-X8, 200–400 mesh, with 0.5 *M* sodium sulphate solution as the eluent. The compounds emerged in order of decreasing polarity[15].

TABLE 1

CAPACITY RATIOS OF CARBONYL COMPOUNDS IN SALTING-OUT CHROMATOGRAPHY

Resin	*Carbonyl compound*	*Concentration of ammonium sulphate solution (M)*						
		0.00	*0.50*	*1.0*	*1.5*	*2.0*	*3.0*	*4.0*
Cation-exchange resins								
Dowex 50-X4	Acetone	1.53	—	2.89	3.84	5.17	10.0	—
	Methyl ethyl ketone	1.89	—	4.43	6.50	9.71	22.8	—
	Methyl propyl ketone	2.55	—	6.89	11.11	18.9	—	—
Dowex 50-X8	Acetone	1.01	—	2.15	3.16	4.47	8.60	—
	Methyl ethyl ketone	1.41	—	3.60	5.64	8.73	—	—
	Methyl propyl ketone	1.96	—	5.98	10.3	17.5	—	—
Amberlite CG-120	Acetone	1.05	1.47	2.14	2.80	3.94	8.28	—
	Methyl ethyl ketone	1.42	2.18	3.28	4.74	7.24	19.4	—
	Methyl propyl ketone	1.95	3.10	4.93	8.62	14.5	—	—
Anion-exchange resins								
Dowex 1-X4, 200–400 mesh	Acetone	0.57	1.02	1.32	—	2.58	4.56	8.07
	Acetoin	0.55	1.06	1.39	—	2.58	4.24	7.33
	Diacetyl	0.85	1.46	1.89	—	3.45	5.69	12.3
	2,5-Hexanedione	0.73	1.41	2.17	—	5.15	13.3	30.8
	Diacetone alcohol	0.61	1.30	2.04	—	5.85	12.7	—
	Methyl ethyl ketone	0.83	1.60	2.41	—	5.10	12.3	24.0
	Cyclopentanone	1.09	1.96	2.95	—	6.24	13.9	30.8
	2,3-Pentanedione	1.26	2.49	3.28	—	7.28	14.3	—
	2,4-Pentanedione	1.24	2.20	3.30	—	7.18	14.8	—
	Methyl isopropyl ketone	1.24	2.42	3.88	—	10.1	27.6	—
	Methyl propyl ketone	1.39	2.77	4.32	—	11.5	32.2	—
	Diethyl ketone	1.27	2.70	4.32	—	11.3	33.0	—
	Cyclohexanone	1.67	3.09	4.89	—	12.7	—	—
Dowex 1-X8, 200–400 mesh	Formaldehyde	1.05	0.93	1.00	—	0.98	0.97	0.88
	Acetaldehyde	0.80	0.98	1.22	—	1.52	2.18	2.92
	Acetone	0.70	0.93	1.53	—	3.00	6.12	10.9
	Acetoin	0.76	0.96	1.38	—	2.91	6.13	10.8
	Diacetyl	1.25	2.06	2.20	—	4.60	8.50	13.9
	2,5-Hexanedione	1.06	1.47	2.56	—	6.87	22.4	—
	Diacetone alcohol	0.78	1.36	2.31	—	7.31	22.5	—
	Propionaldehyde	1.37	1.86	2.50	—	4.58	7.70	—
	Methyl ethyl ketone	1.28	2.00	3.02	—	8.21	21.1	—
	Cyclopentanone	1.70	2.59	4.01	—	8.83	—	—
	2,3-Pentanedione	1.92	2.59	4.49	—	12.8	—	—
	2,4-Pentanedione	1.90	2.73	4.34	—	12.9	21.8	—
	Methyl isopropyl ketone	2.12	3.59	5.76	—	16.5	—	—
	Butyraldehyde	2.82	4.42	5.81	—	—	—	—
	Methyl propyl ketone	2.51	4.56	7.52	—	19.6	—	—
	Diethyl ketone	2.52	4.42	7.16	—	19.6	—	—
	Cyclohexanone	3.09	5.17	8.61	—	—	—	—
Amberlite CG-400	Acetone	0.34	0.49	0.75	—	1.43	3.67	—
	Methyl ethyl ketone	0.73	1.05	1.97	—	4.52	—	—
	Methyl propyl ketone	1.49	2.55	4.16	—	—	—	—

TABLE 2

CAPACITY RATIOS OF KETONES ON DOWEX 50-X8 (H^+) 200-400 MESH

Ketone	*Eluent*																		
	Methanol					*Ethanol*					*Acetic acid*					*Propanol-2*			
	0.0 M	*2.0 M*	*4.0 M*	*6.0 M*	*8.0 M*	*0.0 M*	*1.0 M*	*2.0 M*	*4.0 M*	*6.0 M*	*0.0 M*	*1.0 M*	*2.0 M*	*4.0 M*	*6.0 M*	*0.0 M*	*1.0 M*	*2.0 M*	*4.0 M*
Methyl *n*-butyl ketone	5.55	4.14	3.04	2.50	2.01	5.55	4.33	3.58	2.21	1.51	5.55	4.08	3.32	1.98	1.26	5.55	4.17	3.14	1.42
Methyl *n*-amyl ketone	8.26	5.95	4.00	3.23	2.40	8.26	6.13	4.96	2.66	1.47	8.26	6.00	4.41	2.10	1.12	8.26	5.03	3.84	1.47
Methyl *n*-hexyl ketone	16.4	10.1	6.94	4.93	3.30	16.4	11.3	8.16	3.36	1.45	16.4	10.4	6.46	2.77	1.33	16.4	9.17	6.00	1.60
Acetophenone	15.3	12.1	9.56	7.25	4.94														
Methyl isobutyl ketone	3.76	3.21	2.76	2.32	1.95														
Methyl *n*-heptyl ketone	24.7	17.1	11.6	8.24	4.60														
Methyl *n*-octyl ketone	52.5	33.6	21.3	12.7	6.93														
Methyl *n*-nonyl ketone	104	63.1	39.6	22.9	11.3														

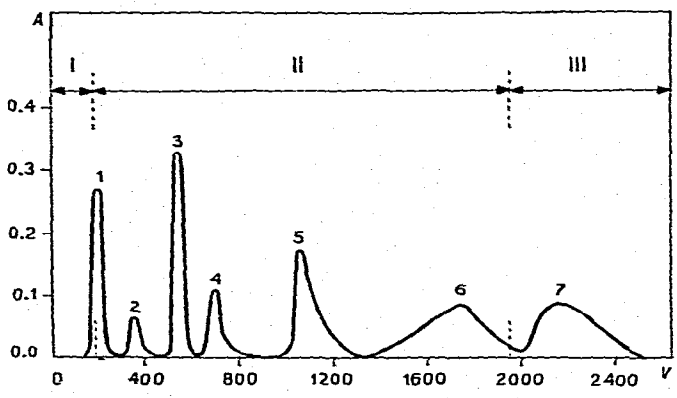


Fig. 2. Separation of a seven-component mixture of carbonyl compounds by salting-out chromatography. (1) Formaldehyde; (2) acetaldehyde; (3) acetone; (4) propionaldehyde; (5) methyl ethyl ketone; (6) butyraldehyde; (7) methyl propyl ketone. Ion exchanger: Dowex 1-X8 (SO_4^{2-}), 200–400 mesh. Column dimensions: 110 cm × 2.28 cm². Mobile phase: ammonium sulphate solution: (I) 4.0 *M*; (II) 2.0 *M*; (III) 1.0 *M*. Flow-rate: 0.35 cm/min. Temperature: ambient. Detection: colorimetry in fractions after reaction with bichromate. *A* = absorbance of Cr(III); *V* = volume of eluate (ml).

The two stereoisomers of 2-acetyl-5-ethyl-3,5-dihydroxy-4-propionylcyclopenta-2-en-1-one were separated on a 50 × 2 cm column of Dowex AG 1-X2 (CH_3COO^-), 200–400 mesh, using a gradient elution system with 0.1 and 0.05 *M* aqueous sodium acetate solutions[16].

Specially prepared resins, analogous to Dowex 50-X8 and Dowex 1-X8 except for a lower capacity, were tested[17]. This decreased capacity is supposed to diminish the relative importance of the attraction of the functional groups in the resin for dipoles and to increase the effect of dispersion forces. Consequently, the sorption of organic non-electrolytes from aqueous solutions should increase. On the other hand, if the capacity is too low, the swelling of the resin is restricted to such an extent that diffusion in the resin is considerably limited. In agreement with these considerations, the sorption rose to a maximum and then decreased with further decrease in the capacity of the resins. Furthermore, the differences in the distribution ratios of any two organic compounds were greater for cation exchangers of low capacities than for the fully sulphonated resins. Capacities lower than 2 mequiv./g are to be avoided, otherwise severe tailing can occur owing to the limited diffusion rate.

Higher carbonyl compounds, such as hexanone-2, heptanone-2 and octanone-2, have only limited solubility in water. An attempt was made to apply salting-out chromatography to the separation of these compounds[18]. Methanolic–aqueous solutions of salts were tested as possible salting-out eluents, and the less soluble salts (magnesium sulphate and sodium acetate) were found to be the most powerful salting-out agents. The salting-out process in partially non-aqueous solvents is much more complicated than the salting-out chromatography of water-soluble non-electrolytes in water. There is a strong indication that the solubilizing effect of the organic constituent of the eluent is more powerful than the salting-out effect of the salt. In some instances, even salting-in was noted.

Chromatography with mixed organic–aqueous solvents as the mobile phase is superior to salting-out chromatography for the separation of water-insoluble carbonyl

TABLE 3

VOLUME DISTRIBUTION COEFFICIENTS ON TECHNICON T_5C (SO_4^{2-}) RESIN, 8–14 μm, AT 75° AND 40° AND ON DOWEX 50-X8 (Li^+, Na^+ AND K^+), 14–17 μm, AT 75° IN SOLUTIONS WITH VARIOUS ETHANOL CONCENTRATIONS

Resin	*Carbonyl compound*	*Ethanol concentration (%)*						
		80	*84*	*88*	*92*	*96*	*92*	*96*
		75°					*40°*	
Technicon T_5C (SO_4^{2-})	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-Hydroxymethylfurfural	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
	Glyceraldehyde	1.4	1.5	1.8			2.4	4.3
	Dihydroxyacetone	1.8	2.0	2.5	3.4		4.5	8.2

Resin	*Carbonyl compound*	*Ethanol concentration (%)*														
		80			*85*			*90*			*95*			*97*		
		Form of resin														
		Li^+	*Na^+*	*K^+*	*Li^+*	*Na^+*	*K^+*	*Li^+*	*Na^+*	*K^+*	*Li^+*	*Na^+*	*K^+*	*Li^+*	*Na^+*	*K^+*
Dowex 50-X8	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
	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	

compounds on columns of ion-exchange resins. Methanol, ethanol, acetic acid and propanol-2 of various concentrations were tested as the eluting agents for methyl *n*-butyl, methyl *n*-amyl and methyl *n*-hexyl ketones[19]. As expected, the eluting strength of the organic solvents studied increased with increasing hydrocarbon chain-length, *i.e.*, from methanol to propanol-2. The differences in the elution volumes of the three ketones were maximal in methanol and decreased through ethanol and acetic acid to propanol-2. Thus, methanol proved to be the best differentiating elution agent. The results of these experiments are summarized in Table 2, together with the data for some other ketones in methanol[19].

Based on these data, conditions could be chosen which allowed the quantitative separation of seven of the ketones studied. A 54.5 cm × 2.28 cm² column packed with Dowex 50-X8 (H^+), 200–400 mesh, was used for the separation and stepwise elution was performed with 1, 5, 8 and 16 *M* methanol (Fig. 3). Acetophenone, which was not included in the mixture, would have been eluted between the peaks of methyl *n*-hexyl and methyl *n*-heptyl ketones, partially overlapping the former[19].

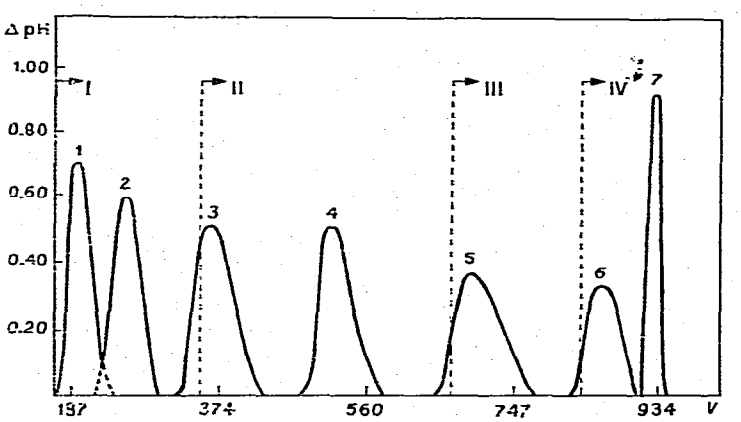


Fig. 3. Separation of a seven-component ketone mixture in mixed aqueous-organic medium. (1) Methyl isobutyl ketone; (2) methyl *n*-butyl ketone; (3) methyl *n*-amyl ketone; (4) methyl *n*-hexyl ketone; (5) methyl *n*-heptyl ketone; (6) methyl *n*-octyl ketone; (7) methyl *n*-nonyl ketone (0.2 mmole of each). Ion exchanger: Dowex 50-X8 (H^+), 200–400 mesh. Column dimensions: 54.5 cm × 2.28 cm². Mobile phase: methanol: I, 1 *M*; II, 5 *M*; III, 8 *M*; IV, 16 *M*; eluent changed at 350, 670 and 835 ml. Flow-rate: 0.28 cm/min. Temperature: ambient. Detection: concentration of compounds in fractions was determined from ΔpH, the difference between the pH of the fraction containing ketones after the addition of hydroxylamine hydrochloride and the blank experiment. *V* = volume of eluate (ml).

Ethanolic solutions have also been used for separations of some carbonyl compounds, on both cation- and anion-exchange resins. Good results were achieved with resins with very fine particle diameters. Table 3 lists the distribution coefficients of some aldehydes and ketones on the strongly acidic cation-exchange resin Dowex 50-X8 (Li^+, Na^+ and K^+), 14–17 μm. Distribution coefficients on the anion-exchange resin Technicon T_5C (SO_4^{2-}), 8–14 μm, are also given. An elevated temperature has a beneficial effect on the sharpness of the elution curves, but the use of excessive temperatures is to be avoided because of the poorer selectivity and possible thermal decomposition of certain compounds[20].

The potassium form of the resin gave a better separation of formaldehyde, glycolaldehyde and glyceraldehyde than the lithium form. The lithium form of the resin, on the other hand, is preferable for the more complex separations of mixtures containing polyols and sugars in addition to the lower carbonyl compounds.

When the sulphate form of the T_5C resin was used, a complete separation of formaldehyde, glycolaldehyde, glyceraldehyde, dihydroxyacetone and some other polyols and sugars could be achieved on a 2 × 1130 mm column by elution with 92% ethanol at 40°. This indicates the usefulness of chromatography on both cation- and anion-exchange resins in aqueous–ethanolic media[20].

A good separation of Δ^4-3-keto steroids was obtained on columns of partially esterified Amberlite IRC-50 by using ethanol–methanol–water (15:5:1) as the mobile phase. The components were eluted, as in the above experiment with ketones, in order of decreasing polarity[21].

Thin layers, 200 μm thick on 8 × 8 in. glass plates, were prepared from the strongly basic quaternary ammonium anion-exchange resin Amberlite CG-400 (Cl^-), 200–400 mesh[22]. Starch binder was used to prepare consistent layers. The utility of these layers for the separation of various ketones was tested using aqueous–organic developing solutions. Other ionic forms (bromide, iodide and sulphate) brought about no improvement in the separation, as compared with the chloride form. R_F values for eight ketones are compared in Table 4 for methanol and acetone solutions of different concentration as the mobile phase. Based on the data in Table 4, separations were possible of binary mixtures involving almost all combinations of these eight ketones. Table 5 surveys the separations achieved together with the composition of the mobile phase which was used in each case to achieve these separations. The

TABLE 4

R_F VALUES OF VARIOUS KETONES ON AMBERLITE CG-400 ANION-EXCHANGE THIN LAYERS

(a) R_F of front; (b) R_F of rear.

No.	Ketone		*Methanol concentration (M)*					*Acetone concentration (M)*	
			12.0	*14.0*	*17.0*	*19.0*	*22.0*	*6.0*	*8.0*
1	Phenyl-2-propanone	(a)	0.42	0.53	0.68	0.72	0.76	0.66	0.75
		(b)	0.32	0.45	0.59	0.62	0.68	0.48	0.61
2	4'-Methylacetophenone	(a)	0.37	0.48	0.70	0.74	0.81	0.58	0.73
		(b)	0.27	0.40	0.60	0.64	0.75	0.42	0.63
3	*trans*-4-Phenyl-3-buten-2-one	(a)	0.25	0.35	0.59	0.63	0.69	0.57	0.57
		(b)	0.15	0.26	0.47	0.52	0.61	0.37	0.04
4	1-Phenyl-1,3-butanedione	(a)	0.13	0.21	0.38	0.42	0.60	0.37	0.55
		(b)	0.06	0.16	0.31	0.33	0.52	0.33	0.39
5	Hexanophenone	(a)	0.15	0.27	0.58	0.64	0.83	0.18	0.57
		(b)	0.09	0.20	0.50	0.54	0.76	0.00	0.30
6	Phenyl 2-thienyl ketone	(a)	0.11	0.17	0.35	0.38	0.58	0.26	0.56
		(b)	0.04	0.11	0.30	0.31	0.50	0.14	0.36
7	4'-Phenylacetophenone	(a)	0.05	0.15	0.34	0.38	0.59	0.21	0.46
		(b)	0.00	0.06	0.25	0.29	0.51	0.13	0.33
8	2-Tridecanone	(a)	0.07	0.20	0.70	0.85	1.0	0.16	0.77
		(b)	0.01	0.12	0.57	0.73	0.90	0.00	0.08

TABLE 5

MOBILE PHASES GIVING RELIABLE SEPARATIONS OF PAIRS OF KETONES IN CHROMATOGRAPHY ON AMBERLITE CG-400 ANION-EXCHANGE THIN LAYERS

*Ketone mixture**	*Methanol concentration (M)*	*Acetone concentration (M)*
1–2	—	—
1–3	14	
1–4	14 or 19	
1–5	14	
1–6	14 or 19	
1–7	14 or 19	
1–8	14	
2–3	22	
2–4	14 or 19	
2–5	14	
2–6	14 or 19	
2–7	14 or 19	
2–8	14	
3–4	14 or 19	
3–5	22	
3–6	14 or 19	
3–7	14 or 19	
3–8	19 or 22	
4–5	19	
4–6	—	6
4–7	—	6
4–8	19	
5–6	14 or 19	
5–7	14 or 19	
5–8	22	
6–7	—	—
6–8	19	
7–8	19	

* See Table 4 for identification of ketones.

numbers of the ketones in Table 5 are as in Table 4. Some ternary mixtures were also resolved, *e.g.*, that of 4′-methylacetophenone, *trans*-4-phenyl-3-buten-2-one and 4′-phenylacetophenone by development with 14 *M* methanol. Similarly, 19 *M* methanol could be used for the separation of hexanophenone, phenyl 2-thienyl ketone and 2-tridecanone[22].

Ion-exchange papers containing the same anion-exchange resin have also been used successfully for separations of ketones involved in experiments with anion-exchange thin layers[23]. The major difference is the presence of the cellulose matrix in the paper instead of a relatively small percentage of starch binder in layers. In both instances, the separation is based on a partition process. The occurrence of interactions between cellulose and the solutes results in R_F values on paper that differ from those on thin layers when developing agents of the same composition are used in both techniques.

Table 6 lists R_F values for eight ketones when developed with methanol of different molarity on Amberlite SB-2 (Cl^-) paper. Ketones with molecular weights

TABLE 6

R_F VALUES OF KETONES ON AMBERLITE SB-2 ANION-EXCHANGE PAPER (CONTAINING AMBERLITE IRA-400) IN METHANOL SOLUTIONS OF VARIOUS CONCENTRATIONS

(a) R_F of front; (b) R_F of rear.

Ketone		*Methanol concentration (M)*								
		0.0	*6.0*	*8.0*	*10.0*	*12.0*	*14.0*	*16.0*	*18.0*	*20.0*
Phenyl-2-propanone	(a)	0.33	0.45	0.52	0.61	0.64	0.80	0.86	0.88	0.97
	(b)	0.24	0.32	0.41	0.48	0.54	0.69	0.73	0.80	0.83
4'-Methylacetophenone	(a)	0.17	0.31	0.39	0.40	0.60	0.69	0.74	0.83	0.93
	(b)	0.10	0.18	0.30	0.33	0.48	0.62	0.67	0.76	0.83
trans-4-Phenyl-3-buten-2-one	(a)	0.12	0.21	0.28	0.36	0.49	0.63	0.76	0.81	0.89
	(b)	0.04	0.09	0.17	0.21	0.34	0.51	0.64	0.69	0.78
1-Phenyl-1,3-butanedione	(a)	0.07	0.09	0.10	0.19	0.28	0.53	0.58	0.67	0.77
	(b)	0.00	0.00	0.04	0.08	0.16	0.35	0.46	0.54	0.65
Hexanophenone	(a)	0.00	0.04	0.05	0.09	0.40	0.50	0.61	0.74	0.92
	(b)	0.00	0.00	0.00	0.00	0.08	0.37	0.44	0.64	0.80
Phenyl 2-thienyl ketone	(a)	0.03	0.04	0.06	0.11	0.25	0.38	0.46	0.55	0.65
	(b)	0.00	0.00	0.00	0.00	0.14	0.28	0.38	0.47	0.58
4'-Phenylacetophenone	(a)	0.00	0.04	0.05	0.06	0.12	0.26	0.38	0.52	0.68
	(b)	0.00	0.00	0.00	0.00	0.00	0.00	0.28	0.40	0.60
2-Tridecanone	(a)	0.00	0.01	0.01	0.01	0.05	0.19	0.36	0.64	0.86
	(b)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.23	0.59

TABLE 7

R_F VALUES OF AROMATIC ALDEHYDES AND KETONES ON AMBERLITE SB-2 ANION-EXCHANGE PAPER

Solvent A: cyclohexane–ethyl acetate–acetic acid (5:1:1). Solvent B: *n*-butanol–water–acetic acid (6:2:1).

Compound	R_f *value*	
	Solvent A	*Solvent B*
p-Hydroxybenzaldehyde	0.07	0.71
2,4-Dihydroxybenzaldehyde	0.08	0.61
2,5-Dihydroxybenzaldehyde	0.07	0.57
3,4-Dihydroxybenzaldehyde	0.02	0.42
2-Hydroxy-3-methoxybenzaldehyde (*o*-vanillin)	0.64	0.84
Vanillin	0.20	0.75
Veratraldehyde	0.45	0.87
Syringaldehyde	0.10	0.74
Coniferyl aldehyde	0.08	0.71
Cinnamaldehyde	—	0.92
Hydroxymethylfurfural	0.08	0.68
5-Formylvanillin	0.16	0.40
5-Carboxylvanillin	0.03	0.26
2,4-Dihydroxyacetophenone	0.18	0.68
Acetovanillone	0.20	0.78
Acetosyringone	0.34	0.78
2,4-Dihydroxypropiophenone	0.30	0.73
3,4-Dihydroxypropiophenone	0.06	0.55
Maltol	0.42	0.81

less than 120 were too volatile to be suitable for paper chromatography. Table 6 indicates that each ketone can be separated from all others, except for 4'-phenylacetophenone and tridecanone, which cannot be separated from one another at any methanol concentration[23].

Table 7 lists the R_F values of various aromatic aldehydes and ketones on Amberlite SB-2 paper containing Amberlite IRA-400 (Cl^-) anion-exchange resin in the chloride form[24]. Two solvents were tested as the mobile phase: (A) cyclohexane–ethyl acetate–acetic acid (5:1:1) and (B) *n*-butanol–water–acetic acid (6:2:1). An R_F difference of about 0.05 was required for both solvents in order to permit the separation of individual compounds. With this criterion, separations can be found from Table 7 which can be achieved by means of this technique.

3. ETHERS

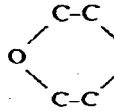
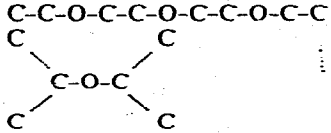
Ethers, like esters, hydrocarbons, alcohols and carbonyl compounds, are not ionized in aqueous solutions. As no useful complex formation is known with this class of compounds, two separation mechanisms can be used for their chromatography on ion exchangers: salting-out effects or differences in solubilities in aqueous–organic solutions.

Sargent and Rieman[25] applied salting-out chromatography to the separation of aliphatic and polyglycol ethers. The cation-exchange resin Dowex 50-X4, 200–400 mesh, gave more efficient separations than anion exchangers or cation-exchange resins with a higher degree of cross-linking. Ammonium sulphate solution was found to be a suitable eluent for ethers, as for carbonyl compounds. The capacity ratios of 15 ethers and ethylene glycol on this resin are shown in Table 8 for various ammonium sulphate concentrations. Based on the data in Table 8, conditions could be chosen for the separation of several mixtures containing 0.05 mmole or less of each ether dissolved in 1 ml of water. For example, the separation of ethylene glycol and four ethers (Nos. 2, 5, 9 and 12 in Table 8) was carried out on a 12.0 cm × 3.9 cm^2 column by stepwise elution with 3.0, 2.0 and 0.5 *M* ammonium sulphate solution. Fig. 4 shows as an example the separation of a seven-component mixture of ethers on a 14.0 cm × 3.9 cm^2 column of this resin with 3.0, 2.0, 1.0 and 0.01 *M* ammonium sulphate solutions used gradually as the eluents[25]. The low solubility of the aliphatic ethers in aqueous solutions causes difficulties in the chromatographic procedure. This technique, however, would be useful for separations of high-boiling hydroxy ethers, for which gas–liquid partition chromatography is not as effective as with volatile ethers.

In experiments on the chromatography of ethers in aqueous–organic solutions, acetic acid was chosen as the organic solvent because it does not interfere in the oxidation with bichromate used for the convenient determination of small amounts of ethers in effluent fractions. Dowex 50-X4 cation exchanger, 200–400 mesh, with a low degree of cross-linking, has proved to be a convenient resin for these separations, as in the salting-out chromatography of ethers. The capacity ratios of eight ethers in solutions containing various amounts of acetic acid are presented in Table 9 (ref. 26). The results indicate that the branched-chain ethers are eluted before the corresponding straight-chain isomers, owing to their greater solubility in aqueous solvents. Ethers with longer chains, such as diisoamyl, di-*n*-amyl and di-*n*-hexyl ethers, gave elution curves of a form which indicated that most of these large molecules were excluded

TABLE 8

CAPACITY RATIOS OF ETHERS IN SALTING-OUT CHROMATOGRAPHY ON THE CATION-EXCHANGE RESIN DOWEX 50-X4 (NH_4^+), 200–400 MESH

No.	*Structural formula of ether*	*Capacity ratios for ammonium sulphate eluents*					
		0.01 M	*1.0 M*	*2.0 M*	*3.0 M*	*4.0 M*	*0.0 M*
1	HO-C-C-OH	1.32	1.60	1.96	2.37	2.98	1.32
2	HO-C-C-O-C-C-OH	1.37	2.11	3.40	5.30	8.84	1.33
3	C-O-C-C-OH	1.34	2.41	3.80	6.25	10.0	1.46
4		1.69	3.52	6.63	12.4	24.7	1.90
5	C-C-O-C-C-OH OH	1.41	2.86	5.83	11.5	23.7	1.44
6	C-O-C-C-C- OH OH	1.37	2.81	5.72	11.3	24.5	1.38
7	C-C-C-O-C-C-C	1.32	3.10	6.81	15.8	37.7	1.33
8	C-O-C-C-O-C	1.36	3.36	7.10	16.1	38.9	1.41
9	C-C-O-C-C-O-C-C-OH	1.37	3.88	9.20	23.9	67.7	1.41
10	C-C-O-C-C	1.68	4.52	11.4	–	–	1.70
11	C-C-C-C-O-C-C-OH	2.50	7.28	20.9	–	–	2.50
12	C-C-O-C-C-O-C-C-O-C-C	1.54	6.24	23.0	–	–	1.70
13		2.04	6.96	–	–	–	2.04
14	C-C-C-O-C-C-C	3.14	12.1	–	–	–	3.14
15	C-C-C-C-O-C-C-O-C-C-C-C	5.33	–	–	–	–	5.33
16	C-C-C-C-O-C-C-C-C	6.90	–	–	–	–	6.90
–	C-C-OH	1.36	2.25	3.40	5.08	7.75	1.48

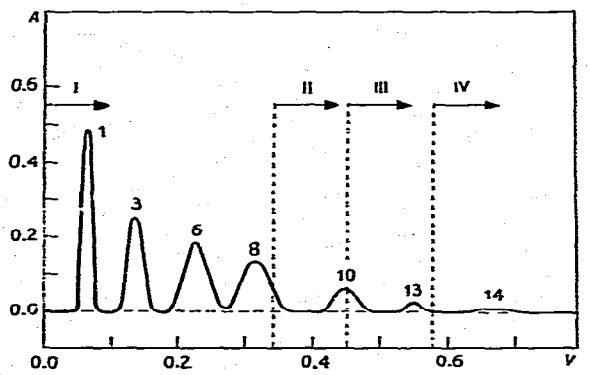


Fig. 4. Separation of a seven-component mixture of ethers by salting-out chromatography. The numbers of the compounds are as in Table 8. Ion exchanger: Dowex 50-X4 (NH_4^+), 200–400 mesh. Column dimensions: 14.0 cm × 3.90 cm^2. Mobile phase: ammonium sulphate: I, 3.0 *M*; II, 2.0 *M*; III, 1.0 *M*; IV, 0.01 *M*. Flow-rate: 0.7 cm/min. Temperature: ambient. Detection: colorimetry in fractions after oxidation with bichromate. *A* = absorbance; *V* = volume of eluate (l).

TABLE 9

CAPACITY RATIOS OF ETHERS ON DOWEX 50-X4 (H^+), 200–400 MESH

Ether	*Concentration of acetic acid (M)*				
	0.0	*1.0*	*2.0*	*4.0*	*6.0*
Diisopropyl ether	3.51	3.11	2.88	2.13	1.30
Di-*n*-propyl ether	4.94	4.16	3.37	2.08	1.65
Ethyl *n*-butyl ether	5.25	4.19	3.60	2.48	1.69
Di-*n*-butyl ether	12.5	9.36	6.81	3.90	1.83
Anisole	15.2	11.6	9.09	5.36	3.08
Diphenyl ether	–	–	54.6	19.2	6.65
Diisoamyl ether	–	–	–	–	2.28
Di-*n*-amyl ether	–	–	–	–	3.03

from the resin and appeared almost immediately after the effluent volume equalled the interstitial volume. Tailing for a large volume of effluent then occurred, probably owing to the slow diffusion of the molecules which diffused inside the resin and were trapped there. This behaviour was observed for concentrations of acetic acid less than 6 *M* with the amyl ethers, while di-*n*-hexyl ether behaved in this manner even in 6 *M* acetic acid. Attempts to separate these three ethers also failed when cation exchangers were used with a smaller degree of cross-linking, such as Dowex 50-X2, as well as with a phenolic matrix-type resin (Duolite S-30) and a "snake cage" resin (Retardion 11-A8). However, the separation of the six remaining lower ethers was possible, with the exception of di-*n*-propyl ether and ethyl *n*-butyl ether, which showed very similar solubility characteristics. A clear-cut separation of a five-component mixture of ethers achieved on a 102 cm × 2.28 cm^2 column of Dowex 50-X4, 200–400 mesh, is shown in Fig. 5. The concentration of acetic acid was gradually increased from 2.0 *M*

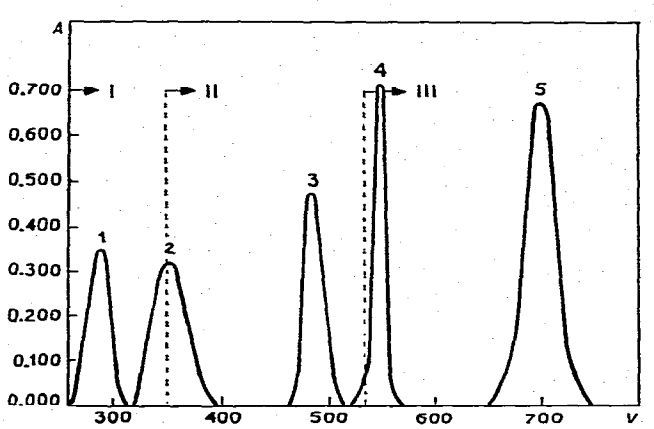


Fig. 5. Separation of a five-component mixture of ethers in acetic acid medium. (1) Diisopropyl ether; (2) ethyl *n*-butyl ether; (3) di-*n*-butyl ether; (4) anisole; (5) diphenyl ether. Ion exchanger: Dowex 50-X4 (H^+), 200–400 mesh. Column dimensions: 102 cm × 2.28 cm^2. Mobile phase: acetic acid: I, 2.0 *M*; II, 6.0 *M*; III, 8.0 *M*. Flow-rate: 0.28 cm/min. Detection: colorimetry in fractions after oxidation with bichromate. *A* = absorbance; *V* = volume of eluate (ml).

through 6.0 *M* to 8.0 *M* during the elution. Di-*n*-propyl ether could have been included in the separation experiment instead of ethyl *n*-butyl ether, but it would have overlapped the peak of diisopropyl ether[26].

4. ALCOHOLS AND POLYOLS

Gas chromatography (GC) is the method of choice for the separation of volatile lower alcohols. GC of the trimethylsilyl ethers or acetates of polyols offers distinct advantages in terms of speed and sensitivity over chromatography on ion-exchange resins. However, ion-exchange methods are useful for the analysis of unknown mixtures as solute peaks can be collected for identification by other methods. The other advantages over GC methods are that many other substances which are present in hydrolyzates from material of biological origin and in various technical liquors do not interfere, and that direct separations can be performed without the need to prepare any derivatives.

The sorption of aliphatic alcohols on ion-exchange resins increases with increasing molecular weight, as the matrix attraction forces become more important than the salting-out of the organic molecules from the inner solution in the resin particles. This sorption increase stops and a decrease is observed when the molecular size exceeds a certain limit, where diffusion in the interior of the particles becomes sterically hindered by the cross-linkage. A decrease in the degree of cross-linking of a resin results in an increase in the sorption[27–30].

It has been found, on the other hand, that the sorption of polyols from aqueous solutions decreases with increasing number of hydroxyl groups (with increasing polarity), *e.g.* in the order glycol > glycerol > sorbitol[31].

The uptake of an alcohol depends on the ionic form of the ion-exchange resin, as can be seen in Figs. 6 and 7 (ref. 28). This sorption behaviour is essentially the same as that of other classes of non-polar compounds.

The differences in sorption behaviour in aqueous solutions have been utilized

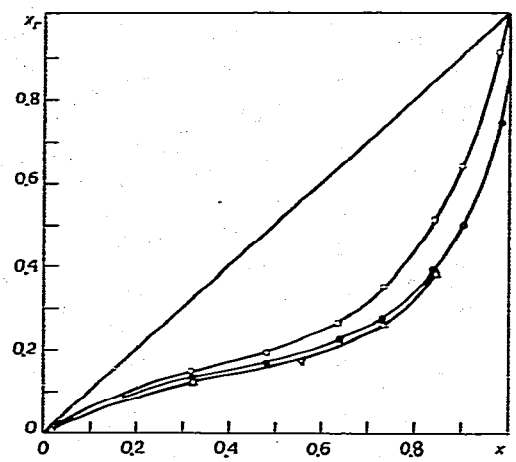


Fig. 6. Distribution curves of ethanol for Dowex 50 cation-exchange resin in various ionic forms. The molar ratio of ethanol in the "inner" solution, x_r, is plotted as a function of the molar ratio of ethanol in the "outer" solution, x. Ionic form of resin: ○, Li^+; △, Na^+; ●, K^+.

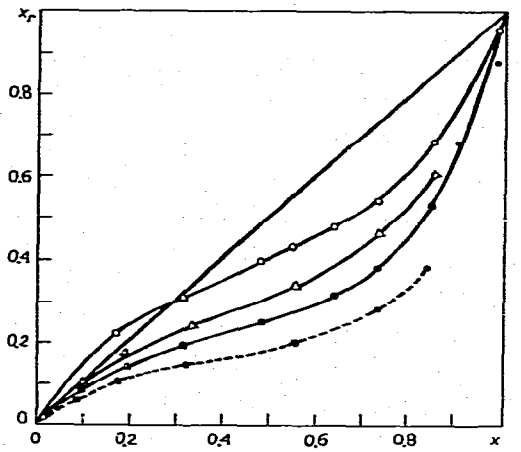


Fig. 7. Distribution curves of ethanol for Dowex 2 (solid lines) and Dowex 2-X1 (broken line) anion-exchange resins in various ionic forms. The molar ratio of ethanol in the "inner" solution, x_r, is plotted as a function of the molar ratio of ethanol in the "outer" solution, x. Ionic form of resin: ○, ClO_4^-; △, Cl^-; ●, SO_4^{2-}.

by several workers to effect separations of alcohols on ion-exchange resins with water as the eluent. This approach was introduced by Wheaton and Bauman[32], who separated sucrose, glycerol, triethylene glycol and phenol reasonably well, in addition to a number of two-component mixtures.

Glycerol was separated from sugar alcohols on a 240 × 2.5 cm column packed with Dowex 50-X12 (H^+), 100–200 mesh, by elution with water at 60° (ref. 33). A pure glycerol fraction was obtained, while erythritol and sorbitol were only incompletely resolved from each other and their fractions also contained trace amounts of xylitol. Another fraction containing ethylene glycol and propylene glycol was also obtained. A resin with a lower degree of cross-linking, Dowex 50-X8, gave a resolution similar to that with Dowex 50-X12.

Columns of Dowex 50-X12 and KU-2 (a Russian sulphonated polystyrene cation exchanger) with 12% cross-linking were used for the separation of sorbitol, xylitol, erythritol, glycerol, ethylene glycol and 1,2-propanediol in mixtures in about 34–36 h using the same elution technique (water at 60°)[34]. The hydrogen form of the resins gave the best resolution. Using the calcium form of the resin, the order of elution was changed; xylitol is sorbed selectively on the calcium form and is eluted as the last compound. The desorption of polyols from the calcium form with water as the eluent is slow and the efficiency of separation is low. However, two analytical methods have been developed that are useful for the control of industrial hydrolytic processes with sugars and polyols. First, small admixtures of xylitol can be separated from glycol on the hydrogen form of a cation exchanger, while on the other hand small amounts of glycol in xylitol can be resolved on the calcium form of a cation-exchange resin[35].

Anion-exchange resins have also been used for separations of alcohols and polyols with water as the eluent. Good separations of a mixture containing glycol, glycerol and sorbitol were obtained on a column of Dowex 2 (ClO_4^-) anion-exchange resin[31].

The use of a column of the strongly basic anion-exchange resin Dowex 1-X4 (OH^-) with water as the eluent effected the separation of xylitol, arabinitol, galactitol, ribitol, glucitol, mannitol and maltitol. The elution of the maltitol could be accelerated by using 0.25 *M* ammonium carbonate solution for elution instead of water[36].

Small and Bremer[37] studied the use of anion-exchange resins in the form of amphophilic anions of higher saturated carboxylic acids as a means of increasing the sorption affinity of less polar organic compounds for the resin. On this ionic form of the resin, additional interaction forces occur between the organic compounds and the hydrocarbon chains of the counter ions, in addition to the interactions of compounds with the resin matrix and the water-structure promoting effects. The increased sorption affinity of the less polar compounds can bring about an improvement in the separation of these compounds. As with the ion-exchange resins in the conventional ionic forms, the separation efficiency can be significantly influenced by an appropriate choice of temperature.

It was possible to effect an almost complete separation of a mixture of propylene glycol and *tert.*-butanol and satisfactory separations of ethanol from *n*-propanol and ethylene glycol from *n*-propanol by elution with water at 70° on Dowex 1-X1 columns in the amphophilic anionic form. These compounds showed no resolution when the chloride form of the resin was used under these conditions[37].

Salting-out chromatography gives a significant improvement in the efficiency of many separations of alcohols and glycols in mixtures compared with elution with water. For example, elution with water resulted in no separation of a mixture of diethylene glycol and dipropylene glycol on a 70-cm column of Dowex 1-X8 (SO_4^{2-}), 200–300 mesh, whereas elution with 3.0 *M* ammonium sulphate resulted in a quantitative separation using a column only 10 cm long; diethylene glycol emerged from the column before dipropylene glycol[38]. Similarly, complete separation of methanol, ethanol and propanol-1 was possible by elution with 3.0 *M* ammonium sulphate solution using a 25.7 cm × 2.28 cm^2 column packed with Dowex 1-X4 (SO_4^{2-}) anion-exchange resin, 200–300 mesh, while this separation failed when water was used as the mobile phase. In order to accelerate the elution of propanol-1, this alcohol was eluted with water instead of the salt solution, after ethanol had been eluted[38].

The capacity ratios for various alcohols on the anion-exchange (Dowex 1-X8) and cation-exchange (Dowex 50-X8) resins in solutions containing various amounts of ammonium sulphate are given in Table 10.

A 32 cm × 2.28 cm^2 column packed with Dowex 1-X8 (SO_4^{2-}), 200–400 mesh, allowed the complete separation of nine alcohols: glycerol, methanol, propylene glycol, ethanol, isopropanol, *tert.*-butanol, *sec.*-butanol, *n*-butanol and *n*-amyl alcohol. The separation was carried out in 12 h by stepwise elution with ammonium sulphate solutions of sequentially decreasing concentration (4.0, 2.5, 2.0 and 0.1 *M*). Water could be used for the elution of the most strongly retained compound, *n*-amyl alcohol, but the elution is accelerated considerably by using 1.0 *M* acetic acid as the eluent. This separation is shown in Fig. 8. Straight-chain primary alcohols higher than *n*-amyl alcohol are sorbed strongly on the resin and would require excessively long elution times. *n*-Propanol and isobutanol, which were not included in the separation experiment, would be eluted with their elution maxima very close to those of *tert.*-butanol and *n*-butanol, respectively, under these experimental conditions[39].

The use of mixed aqueous–organic solutions instead of water as the mobile

TABLE 10

CAPACITY RATIOS FOR VARIOUS ALCOHOLS AND RESINS IN SOLUTIONS OF AMMONIUM SULPHATE

Alcohol	*Dowex 1-X8*						*Dowex 50-X8*				
	Ammonium sulphate concentration (M)						*Ammonium sulphate concentration (M)*				
	0.0	*0.5*	*1.0*	*2.0*	*3.0*	*4.0*	*0.0*	*0.5*	*1.0*	*2.0*	*3.0*
Glycerol	0.427	0.453	0.473	0.622	0.843	1.16	0.586	0.635	0.688	0.901	1.20
Propylene glycol	0.477	0.647	0.740	1.29	2.21	3.83	0.644	0.856	1.08	1.77	3.07
Methanol	0.507	0.670	0.804	1.15	1.68	2.33	0.839	0.950	1.13	1.59	2.29
Ethanol	0.673	0.843	1.12	2.00	3.72	–	0.839	1.08	1.46	2.46	4.57
Isopropanol	0.753	1.02	1.53	3.48	7.76	–	0.744	1.21	1.61	3.58	8.34
tert.-Butanol	0.883	1.19	1.96	5.12	13.4	–	0.662	1.20	1.90	4.78	12.9
n-Propanol	1.28	1.71	2.57	5.45	12.3	–	1.03	1.62	2.35	5.01	–
sec.-Butanol	1.56	2.26	3.79	9.30	26.5	–	1.07	1.73	2.88	7.48	–
Isobutanol	2.47	3.34	5.17	12.3	–	–	1.33	2.20	3.38	8.74	–
n-Butanol	2.37	3.52	5.67	13.8	–	–	1.28	2.17	3.53	8.44	–
n-Amyl alcohol	6.63	11.0	–	–	–	–	2.69	5.03	8.33	–	–

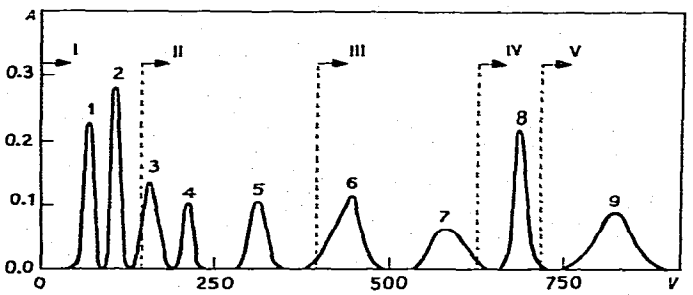


Fig. 8. Separation of a nine-component alcohol mixture by salting-out chromatography. (1) Glycerol; (2) methanol; (3) propylene glycol; (4) ethanol; (5) isopropanol; (6) *tert.*-butanol; (7) *sec.*-butanol; (8) *n*-butanol; (9) *n*-amyl alcohol. Ion exchanger: Dowex 1-X8 (SO_4^{2-}), 200–400 mesh. Column dimensions: 32.0 cm × 2.28 cm². Mobile phase: I, 4.0 *M*; II, 2.5 *M*; III, 2.0 *M*; IV, 0.1 *M* ammonium sulphate; V, 1.0 *M* acetic acid. Flow-rate: 0.6 cm/min. Detection: colorimetry in fractions after oxidation with bichromate. *A* = absorbance; *V* = volume of eluate (ml).

phase is another means of improving separations. This method gives better results than salting-out chromatography, especially if the less polar higher aliphatic alcohols are to be separated. The separation is based essentially on the differences in solubilities in the outer solution and the solution in the resin phase, which has a different composition. Mattisson and Samuelson[40] studied the sorption of glycol, glycerol, sorbitol and mannitol between cation-exchange resins (Dowex 50-X8 and Dowex 50-X2) and aqueous ethanolic solutions. It was found that the ethanol concentration has a predominating influence upon the rate of uptake. The equilibrium uptake increases considerably with increase in the ethanol concentration, but also the nature of the counter ion and the degree of cross-linking have an effect which is explained by the differences in swelling. At higher water concentrations, the lithium form of the resin

is a more effective sorbent than the potassium form, but the opposite holds true at low water concentrations (to 0.6%).

The order of sorption affinities of the polyols studied (sorbitol > mannitol > glycerol > glycol) is the reverse of that with water. Moreover, the separation factors differ much more in aqueous–ethanolic solutions than in pure water and an increase in the ethanol concentration causes an increase in the separation factor so that it is advantageous to use high ethanol concentrations. The rate of diffusion (and the attainment of equilibrium) is very slow in an almost water-free medium, which is a considerable disadvantage and for practical chromatographic work the ethanol concentration must not be too high.

Glycol, glycerol and sorbitol could be separated easily on a column containing Dowex 50 (Na^+) by elution with 95% ethanol. Glycol and glycerol appear as sharp peaks and sorbitol, which is bound much more strongly, appears as a broad band of low concentration. After the elution of glycol and glycerol, sorbitol can be displaced quickly using water[41].

Elution with aqueous ethanol has been used for the chromatographic separation of complex mixtures of polyols on ion-exchange resins. A number of polyols were separated on the fine-mesh cation-exchange resin Dowex 50W-X8 (Li^+), 14–17 μm. Some mixtures containing polyols together with other compounds (sugars) were better resolved on an anion-exchange resin in the sulphate form[31].

The distribution coefficients on the sulphate form of anion-exchange resins increase with increase in the number of hydroxyl groups in the solutes, as with the potassium form of sulphonated cation-exchange resins. The elution behaviour of individual isomeric sugar alcohols, however, may differ to a considerable extent on both ion-exchange resins. For example, the order of elution of mannitol and glucitol is reversed on the sulphate form of an anion exchanger compared with the potassium form of a cation exchanger. No simple rule seems to apply to the order of elution of different isomers. Most of the alditols have lower distribution coefficients than the corresponding aldoses.

For illustration, the values of the volume distribution coefficients of alditols on Dowex 50W-X8 (Li^+, Na^+ and K^+), 14–17 μm, and on a strongly basic anion-exchange resin Technicon T_5B (SO_4^{2-}), 13–17 μm, are listed in Table 11 for various ethanol concentrations at 75° (ref. 31).

A number of valuable separations were achieved on Technicon T_5B (SO_4^{2-}) resin. Thus, a good separation of glycerol, erythritol, xylitol, arabinitol, glucitol, galactitol and mannitol from each other and from arabinose, xylose, mannose, galactose and glucose was achieved on a 6 × 852 mm column of this resin by elution with 86% ethanol at 75.5°. Most of the solutes were separated quantitatively from each other under the conditions used, and only the elution curve of galactitol overlapped with those of mannose and mannitol. The analysis required about 11 h. The separation of the three overlapping compounds could be improved by using a longer column and a more concentrated ethanol solution, on account of the separation time[42].

More rapid separations of some sugar alcohols were possible by means of this technique, and Fig. 9 shows an example of such a separation of five alditols. The total time of elution was less than 2 h, using 86% ethanol for elution at 90° and a column of dimensions 650 × 2 mm. The flow-rate used was about seven times higher than that in the former experiment with a more complex mixture. It can be seen that the

TABLE 11

VOLUME DISTRIBUTION COEFFICIENTS ON DOWEX 50W-X8 (Li^+, Na^+ AND K^+) AND ON TECHNICON T_5B (SO_4^{2-}) IN ETHANOL OF VARIOUS CONCENTRATIONS AT 75°

Resin	*Alditol*	*Ethanol concentration (%)*								
		80			*85*			*90*		
		Form of resin								
		Li^+	Na^+	K^+	Li^+	Na^+	K^+	Li^+	Na^+	K^+
Dowex 50-X8	Ethylene glycol	0.81	0.71	0.61	0.84	0.68	0.68	0.95	0.72	0.66
Dowex 50-X8	Glycerol	1.2	1.0	1.0	1.4	1.2	1.1	1.7	1.4	1.5
Dowex 50-X8	Erythritol	1.6	1.4	1.6	2.1	1.8	1.8	2.9	2.7	2.7
Dowex 50-X8	Ribitol	2.1	–	–	3.0	2.6	–	4.4	4.1	4.4
Dowex 50-X8	Arabinitol	2.5	2.5	–	3.7	3.4	3.1	5.7	5.7	5.4
Dowex 50-X8	Xylitol	2.8	3.2	–	4.1	4.5	4.1	6.4	7.9	7.6
Dowex 50-X8	Mannitol	3.5	3.5	–	5.5	5.1	4.7	9.5	9.6	9.1
Dowex 50-X8	Galactitol	4.1	–	–	6.7	6.7	–	11.8	13.1	11.6
Dowex 50-X8	Glucitol	3.8	4.5	–	6.1	6.9	6.0	10.4	13.4	12.2

Resin	*Alditol*	*Ethanol concentration (%)*			
		84	*86*	*88*	*90*
Technicon T_5B (SO_4^{2-})	Glycerol	1.72	2.07	2.34	2.68
Technicon T_5B (SO_4^{2-})	Erythritol	3.39	4.15	5.19	6.16
Technicon T_5B (SO_4^{2-})	Xylitol	5.49	7.09	9.17	12.14
Technicon T_5B (SO_4^{2-})	Ribitol	6.12	7.91	10.38	14.00
Technicon T_5B (SO_4^{2-})	Arabinitol	6.31	8.25	10.90	14.70
Technicon T_5B (SO_4^{2-})	Glucitol	10.64	14.28	20.15	28.60
Technicon T_5B (SO_4^{2-})	Galactitol	13.18	17.20	–	–
Technicon T_5B (SO_4^{2-})	Mannitol	13.83	19.00	27.50	40.20

curves corresponding to xylitol and arabinitol showed some overlapping, which had no serious influence on the evaluation of the chromatogram for quantitative purposes.

The distribution coefficients of polyols decrease with increase in temperature, and the elution curves are sharper and the column pressure drop decreases. The separation factors however are less favourable at high temperatures and this disadvantage may have great practical importance in planning the separations. For example, the elution bands of ribitol and arabinitol overlapped seriously at 93°, whereas an excellent separation was achieved at 40° (ref. 42).

The method described has proved to be useful for the determination of glucitol, mannitol and other alditols in the presence of saccharides in various commercial food products[43]. Alditols and sugars are extracted with water, the extract is dissolved in ethanol and a chromatographic run is carried out on a strongly basic anion-exchange resin (Technicon T_5B or T_5C) in the sulphate form. Alditols and monosaccharides in the eluate are determined automatically in a Technicon AutoAnalyzer using the periodate and orcinol method.

Sherma and Rieman[44] separated higher aliphatic alcohols by stepwise elution with acetic acid solutions. The elution data of the higher alcohols in acetic acid solutions of different concentrations are listed in Table 12 for two cation- and two anion-exchange resins. Dowex 50 was chosen for the separation because it takes up the higher

TABLE 12

CAPACITY RATIOS OF ALCOHOLS ON ION-EXCHANGE RESINS IN ACETIC ACID MEDIA

Alcohol	*Dowex 50-X4*					*Dowex 50-X8*					*Dowex 1-X4*					*Dowex 1-X8*				
	Acetic acid concentration (M)					*Acetic acid concentration (M)*					*Acetic acid concentration (M)*					*Acetic acid concentration (M)*				
	0.0	*1.0*	*2.0*	*4.0*	*6.0*	*0.0*	*1.0*	*2.0*	*4.0*	*6.0*	*0.0*	*1.0*	*2.0*	*4.0*	*6.0*	*0.0*	*1.0*	*2.0*	*4.0*	*6.0*
tert-Amyl alcohol						2.19	1.86				1.18	1.18	1.12	0.998	0.754	—				
n-Amyl alcohol	4.97	4.22	3.51	2.51	1.77	4.61	3.85	3.30	2.21	1.43	2.97	2.58	2.36	1.93	1.41	3.64	2.91	2.29	1.50	1.02
n-Hexanol	8.60	6.44	5.34	3.32	1.85	7.97	6.12	4.88	2.88	1.46	5.39	4.78	4.04	2.83	1.67	8.38	6.17	4.40	2.38	1.36
n-Heptanol	16.4	11.0	8.44	4.45	1.85	14.9	10.8	8.28	3.81	1.65	10.7	9.37	7.70	5.59	2.17	20.3	14.1	9.79	4.12	2.12
n-Octanol	—	—	—	—	—	27.5	20.7	13.8	5.39	1.85	25.4	20.4	16.1	9.99	3.10	—	—	—	—	—
n-Nonanol	—	—	—	—	—	55.1	36.7	21.6	7.47	1.98	63.6	48.2	37.7	22.7	4.19	—	—	—	—	—
n-Decanol	—	—	—	—	—	—	—	—	9.60	2.69	—	—	—	—	5.31	—	—	—	—	—
n-Undecanol	—	—	—	—	—	—	—	—	—	3.55	—	—	—	—	—	—	—	—	—	—
n-Dodecanol	—	—	—	—	—	—	—	—	—	4.77	—	—	—	—	—	—	—	—	—	—
Benzyl alcohol	—	—	—	—	—	5.71	4.30	3.76	2.53	1.55	—	—	—	—	—	—	—	—	—	—
Cyclohexanol	—	—	—	—	—	4.80	4.04	3.58	2.55	1.67	—	—	—	—	—	—	—	—	—	—

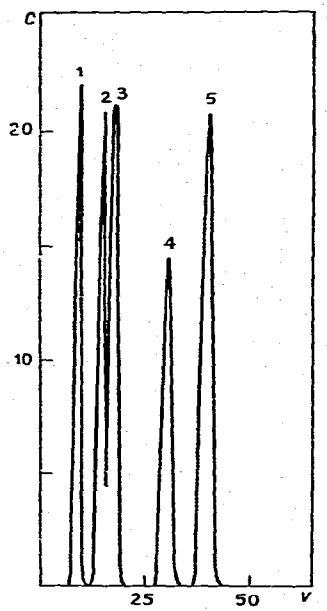


Fig. 9. Separation of a mixture of five alditols on an anion-exchange resin in aqueous–ethanolic medium. (1) Erythritol; (2) xylitol; (3) arabinitol; (4) glucitol; (5) mannitol (0.05 mg of each). Ion exchanger: strongly basic anion-exchange resin Technicon T_5B (SO_4^{2-}), 13–17 μm. Column dimensions: 650 × 2 mm. Mobile phase: 86% ethanol. Flow-rate: 16.2 ml/cm²·min. Temperature: 90°. Detection: Technicon AutoAnalyzer, periodate oxidation. C = chart reading (cm); V = volume of eluate (ml).

alcohols less strongly than does Dowex 1. The X8 degree of cross-linking was chosen because of its greater dimensional stability, as there is little difference between Dowex 50-X4 and 50-X8 in terms of selectivity and shape of the elution peaks.

A mixture of six higher aliphatic alcohols (*tert.*-amyl alcohol, *n*-amyl alcohol, *n*-hexanol, *n*-heptanol, *n*-octanol and *n*-nonanol) was completely separated on a column packed with Dowex 50-X8 (H^+), 200–400 mesh. The elution was started with 1.0 *M* acetic acid, then the acid concentration was increased to 2.0 *M* and the separation was completed with 3.0 *M* acetic acid in order to obtain both separation and rapid elution of the remaining components (Fig. 10). The separation of the alcohols higher than nonanol failed on Dowex 50-X8, 50-X4 and 50-X2 because of the severe spreading of the longer-chain alcohols[44].

The separation of homologous aliphatic alcohols by partition chromatography on the macroporous cation-exchange resin Amberlyst 15 has not been as successful as on conventional gel-type resins[45].

Polyhydroxy compounds containing vicinal hydroxy groups are known to form negatively charged borate complexes. This led Zill *et al.*[46] to extend the anion-exchange chromatography of the borate complexes of sugars to the analysis of sugar alcohols. They separated a mixture of mannitol, dulcitol and sorbitol on an 11 cm × 0.85 cm² column of the strongly basic Dowex 1, *ca.* 300 mesh, by elution of the first two components with 0.015 *M* potassium tetraborate solution, followed by the elution of mannitol with 0.030 *M* potassium tetraborate solution.

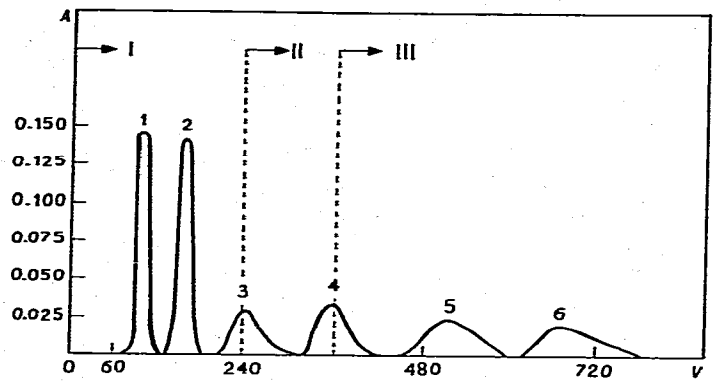


Fig. 10. Separation of a six-component mixture of higher aliphatic alcohols on a cation-exchange resin in acetic acid medium. (1) *tert.*-Amyl alcohol; (2) *n*-amyl alcohol; (3) *n*-hexanol; (4) *n*-heptanol; (5) *n*-octanol; (6) *n*-nonanol. Ion exchanger: Dowex 50-X8 (H^+), 200–400 mesh. Column dimensions: 39.0 cm × 2.28 cm^2. Mobile phase: I, 1 *M*; II, 2 *M*; III, 3 *M* acetic acid. Flow-rate: 0.45 cm/min. Detection: colorimetry in fractions (bichromate method). *A* = absorbance; *V* = volume of eluate (ml).

The borate method has been applied successfully to the analyses of glycols. For example, a mixture containing diethylene glycol, ethylene glycol, 1,2-propylene glycol, the *meso*- and the *d,l*-isomers of 2,3-butylene glycol, and glycerol was separated on a 20 cm × 2.28 cm^2 column packed with Dowex 1-X8 (BO_3^-), 200–300 mesh, using 0.02 *M* borax solution as the mobile phase. Under these conditions, a satisfactory separation of the glycols was achieved, except for propylene glycol and *meso*-2,3-butylene glycol, which could be resolved, however, in another run on a 76.5 cm × 2.28 cm^2 column of the same resin with 0.925 *M* sodium borate solution as the eluent. In this run, a clear-cut separation into four peaks was obtained. Diethylene glycol was eluted first followed by ethylene glycol, then a mixture of propylene glycol and glycerol was eluted as one common band and, finally, *meso*-2,3-butylene glycol and *d,l*-2,3-butylene glycol were eluted. It can be seen that both runs are complementary. The time required for the analysis, when run simultaneously, was 10 h[47,48].

The separation of a nine-component mixture of polyols has been reported by Spencer[49] using a 60 × 0.8 cm column of De Acidite FF anion-exchange resin (3–5% cross-linked; size <200 mesh) by elution with two borate buffers (0.18 and 0.36 *M* boric acid adjusted to pH 9 with triethylamine) at 35°. This separation is shown in Fig. 11. The effect of temperature on the separation was particularly marked; at 25°, the affinity of the polyol–borate complexes for the resin is decreased so that many of the polyol peaks overlap, while at 45° the polyols are so strongly bound to the resin that the time required for elution is impractical. At 35°, only mannitol and galactitol overlap. When the less commonly occurring polyols rhamnitol and fucitol are included in the mixture, they overlap with arabinitol and glucitol, respectively. The method has been applied to the analysis of human urine[49].

The cation-exchange paper Amberlite SA-2 (Na^+) has been used for the separation of higher normal aliphatic alcohols from *n*-heptanol to *n*-dodecanol by development with aqueous organic solvents. Acetic acid (8.0 *M*) could be used as the developing solution, but methanol was preferred because it did not produce the very

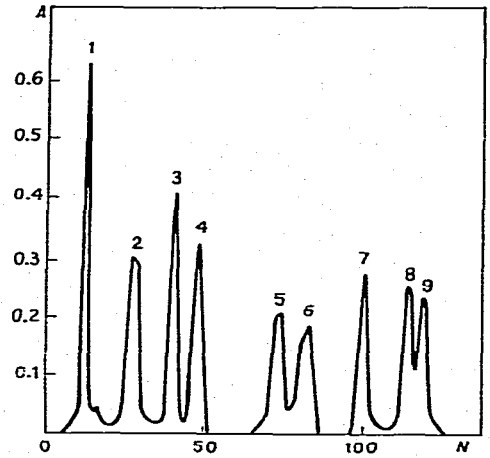


Fig. 11. Separation of a nine-component mixture of polyols by anion-exchange chromatography of their borate complexes. (1) Glycerol; (2) threitol; (3) erythritol; (4) xylitol; (5) arabinitol; (6) ribitol; (7) glucitol; (8) galactitol; (9) mannitol. Ion exchanger: De-Acidite FF (BO_3^-), 3–5% cross-linked, -200 mesh. Column dimensions: 60 × 0.8 cm. Mobile phase: I, 0.18 *M*; II, 0.36 *M* boric acid adjusted to pH 9 with triethylamine; II was changed for I after the elution of ribitol. Flow-rate: 25 ml/h. Temperature: 35°. Detection: periodate oxidation followed by the colorimetric determination of formaldehyde in fractions. Fraction size: 5 ml. *A* = absorbance; *N* = fraction number.

faint trailing that was observed with acetic acid. The R_F values decreased with increasing chain length of the alcohol and increased with increasing concentration of the organic solvent in the developing solution. Development of a mixture of all six alcohols with 75.0% methanol yielded a separation of all except the C_7 and C_8 alcohols. The spots were detected with vanadium oxinate or with potassium permanganate in 0.1 *M* sulphuric acid. This separation is a rare example of the paper chromatographic separation of uncombined monohydric alcohols without the preparation of derivatives[50].

5. SACCHARIDES

The ion-exchange behaviour and separation of carboxylic sugar acids is discussed in the review dealing with carboxylic acids. The separation of neutral saccharides only will be described here.

A. Separation of saccharides on ion-exchange resins using elution with water or aqueous solutions of non-complexing agents

Sugars contain a number of very weakly acidic alcoholic groups and this very slightly acidic character may account, at least partly, for the sorption of sugars on the free (hydroxyl) form of strongly basic anion-exchange resins[51]. This sorption, however, is often accompanied by conversion reactions of the individual sugars, which in many instances prevent the use of the hydroxyl form of anion-exchange resins for the chromatography of sugars.

The differences in non-ionic sorption of individual sugars on ion-exchange resins enable some useful separations to be carried out. An increase in the number of

hydroxyl groups brings about a marked decrease in affinity for the resin and the higher the number of hydroxyl groups in the sugar, the more the aqueous phase is preferred. This means that monosaccharides have higher distribution coefficients than the disaccharides. Disaccharides are more strongly sorbed than trisaccharides, which are, on the other hand, retained more than tetrasaccharides, etc.

The substitution of a hydroxyl group by an alkyl group causes a decrease in polarity and alkyl sugars have higher distribution coefficients than the corresponding non-alkylated sugars. As would be expected, the distribution coefficients increase with an increase in the number of alkyl groups present and, if the number of alkyl groups remains constant, with an increase in the length of the alkyl chains[52,53]. This behaviour occurs with various ionic forms of both cation and anion exchangers. The degree of cross-linking of the resin used is of great importance, as steric exclusion may control the sorption to a certain extent, mainly with saccharides of higher molecular weights. If strongly polar substituents, such as halogen atoms, are incorporated in the structure of a saccharide, interactions with the functional groups of the resin (*e.g.*, quaternary ammonium groups in anion-exchange resins) may alter the sorption order[51].

A few simple separations of saccharides could be effected using the hydroxyl form of anion-exchange resins, but the possibility of undesired conversions of the sugars has to be kept in mind when planning such a separation. The distribution coefficients are higher on this form than on other ionic forms of anion-exchange resins, which confirms that both non-ionic and ion-exchange interactions take place on the hydroxyl form. The elution sequence of the individual saccharides from anion-exchange columns in the hydroxyl form is thus controlled not only by the differences in the non-polar attractive forces with the resin matrix, but also by the differences in acidity of the various hydroxyl groups of carbohydrates[51].

Several reducing sugars were separated from non-reducing sugars by chromatography on a column of Amberlite IRA-400 (OH^-) or Dowex 1 (OH^-). The non-reducing sugars passed into the effluent, while the reducing sugars were retained. The elution of the retained carbohydrates was accelerated by using solutions of acetic acid, sodium phosphate or sodium chloride. By this method, glucose was resolved from sorbitol and methyl-α-D-glucopyranoside in mixtures containing different concentrations of glucose[54].

Separations of various glycosides were achieved on Dowex 1-X2 (OH^-), Dowex 2-X8 (OH^-) and De Acidite FF (OH^-) anion-exchange resins. Furanosides are sorbed more strongly than pyranosides, and β-anomers than α-anomers. Rapid separations of mixtures containing anomeric α-D- and β-D-galactopyranosides together with α-D- and β-D-galactofuranosides, mixtures containing α-D- and β-D-glucopyranosides and α-D- and β-D-glucofuranosides, and mixtures containing glycerol, 2-O-α-D- and 2-O-β-D-glucosylglycerols were achieved with water as the eluent. Mixtures of methyl-α-D-glucopyranoside and α-D-galactopyranoside were partially separated[55], and so were mixtures containing anomeric methyl-6-deoxy-, methyl-6-chloro-6-deoxy- and methyl-α- and -β-glucopyranosides[51]. Dowex 1 and De Acidite yielded better results than Dowex 2 (ref. 55).

The α- or β-anomeric mixtures of the above glucopyranosides were also separated on Dowex 1-X2 (Cl^-), but the elution order was different from that observed on Dowex 1-X2 (OH^-)[51].

Some sugars are known to form complexes with carbon dioxide, but no signifi-

cant differences were noted in the sorption behaviour of sugars on anion-exchange columns (De Acidite, 3.5% cross-linked, <200 mesh) in the chloride, carbonate and hydrogen carbonate forms. This indicates that no carbonate–sugar complexing occurs on the column, apparently owing to the instability of these complexes in solutions that are not strongly alkaline. The carbonate form of this resin packed in a 100 × 2 cm column was used for the separation of raffinose, sucrose and D-glucose[56]. By elution with water, separation was achieved within 36 h. Although L-arabinose could be separated from D-xylose, D-ribose and D-lyxose, the last three pentoses could not be separated.

The use of cation-exchange resins (Dowex 50W) together with water as the mobile phase resulted in a better separation of saccharides than when anion exchangers were used. A column packed with Dowex 50W-X4 (Ag^+), 50–100 mesh, was used for the separation of glucose from fructose, with a recovery of about 90% (ref. 57). Xylose and arabinose were separated on Dowex 50W-X8 (Ca^{2+}), 50–100 mesh[58].

The successful separation has been reported of a mixture containing sucrose, raffinose and glucose on a 2 × 100 cm column of Dowex 50W-X2 (Li^+), 200–400 mesh, with water as the eluent[52]. Clean separations of mixtures containing raffinose, mellibiose and glucose and those containing maltose, lactose and galactose were achieved by this method. A number of O-alkylated sugars and hydrolyzates of polysaccharides have also been fractionated[53-59].

Saunders[60] introduced a method for the separation of sugars on the cation-exchange resin Dowex 50W-X4 (K^+), 200–400 mesh, using water as the eluent. A large number of saccharides were separated by this method, including oligosaccharides, hexoses, pentoses, acetals, methyl-α-D-glycosides and other sugar derivatives, with recoveries of greater than 95%. The resin column was used continuously in the analyses for 12 months with no loss of resolution. Aminex A-20 cation-exchange resin with fine particles is recommended for sharp resolution. The method has been applied to analyses of the sugars of white beans, green coffee beans and other typical plant sugars[61].

The use of non-complexing salt solutions as the eluting agents has been reported only rarely. In this case, salting-out effects control the distribution of saccharides between the resin and the external solution.

The chloride and sulphate forms of Dowex 1 anion-exchange resin have been used for the chromatographic fractionation of some polysaccharide mixtures[62]. Glycogen is only weakly sorbed on these resins and can be eluted with 0.1 *N* sodium sulphate solution, while the more acidic pectin is sorbed quantitatively and a more concentrated eluent (1 *N* sodium sulphate solution) has to be used for its recovery.

Xylan-rich hemicelluloses were subjected to chromatographic fractionation on Dowex 1-X2 anion-exchange resin[63]. While elution with water and aqueous salt solutions did not always give a sharp separation, the elution pattern changed significantly when the elution was carried out in the presence of 7 *M* urea. The addition of urea should eliminate the effects of secondary binding forces to the resin.

An oligosaccharide mixture, containing 1-kestose and nystose, was separated by chromatography on an ion-exchange column of Dowex 50W-X4 using 0.2% benzoate (pH 7.3) as the eluting agent[64].

Pure 6-O-sulphate esters of D-glucose, D-galactose, N-acetyl-D-glucosamine

and N-acetyl-D-galactosamine were obtained by the fractionation of the products of the direct sulphonation of the corresponding monosaccharides, using Dowex 1 anion-exchange resin at 10° with a continuous gradient of sulphuric acid[65].

Oligosaccharides from chitin hydrolyzates were separated into fractions by chromatography on a 54 × 3.6 cm column of Dowex 50-X4 (H^+), using continuous non-linear gradient elution with an increasing concentration of hydrochloric acid[66].

B. Separation of saccharides by elution with aqueous ethanol solutions

Very good results were obtained by partition chromatography of saccharides in mixed aqueous–organic solutions. Sugars that are strongly polar solutes have a greater affinity for the solution inside the resin where the water concentration is higher compared with that in the external solution. Interaction forces between the resin and the polar solutes are also important, however, and even the interactions between sugars and counter ions have some effect[67-68]. The sorption behaviour and possibilities for the separation of sugars have been studied in aqueous ethanol solutions. It is likely that other systems, such as aqueous acetone solutions, can be also used.

The chromatography of sugars on ion-exchange resins in aqueous ethanol solutions has been studied by Samuelson and co-workers with remarkable results. They found that the distribution coefficients of sugars on ion exchangers increase with increase in the concentration of ethanol[69], but the order of elution from individual resins is unaffected by the ethanol concentration. On the other hand, an increase in the ethanol concentration results in a broadening of the elution bands. At low ethanol concentrations, diffusion inside the resin particles is facilitated while the limited diffusion at high ethanol concentrations often causes broad overlapping curves. For this reason, it is important to avoid excessive ethanol concentrations. A small resin particle size and an adequate flow-rate are decisive for accelerating the diffusion and the rate of sorption so that satisfactory separations can be achieved[70].

The elution order of sugars in aqueous ethanol solutions is opposite to that in pure water and the distribution coefficients would therefore be expected to increase with an increase in the number of hydroxyl groups. Thus, monosaccharides are eluted ahead of the disaccharides, and higher oligosaccharides then follow.

Pentoses have lower distribution coefficients than hexoses, but the sorption of saccharides would decrease when non-polar groups such as methyl are present. The deoxy sugars derived from hexoses (2-deoxygalactose, 2-deoxyglucose, rhamnose and fucose) have distribution coefficients that are lower than those of the pentoses. As expected, digitoxose (2,6-dideoxyribohexose) has a lower distribution coefficient than the monodeoxyhexoses.

Fructose and other ketohexoses are more soluble in ethanol and should be eluted ahead of aldohexoses. The order or elution of the sugars within each group (*e.g.*, for the pentoses) is very difficult to predict and should be determined experimentally[71].

It was possible to separate satisfactorily a mixture of the monosaccharide glucose, the disaccharide sucrose, the trisaccharide raffinose, the tetrasaccharide stachyose and the pentasaccharide verbascose using a 10 × 840 mm column of the anion exchanger Dowex 1-X8 (SO_4^{2-}), 45–75 μm. A suitable choice of the ethanol concentration is decisive for the separation. In 65% ethanol, it was possible to separate glucose, raffinose and stachyose, but sucrose could not be resolved from glucose and

raffinose. The separation of these three saccharides could be achieved in 74% ethanol, but an excessive broadening of the elution curves of stachyose and verbascose occurred. For this reason, stepwise elution should be used for the separation of mixtures containing all five saccharides. Glucose and sucrose were separated in 74% ethanol, then the concentration was decreased to 65% for the elution of the three remaining saccharides. The elution curves of the last two oligosaccharides were considerably broadened even with this lower ethanol concentration, however[69-72]. It was possible to separate quantitatively other saccharides, such as glucose from cellobiose, lactose or maltose, using 74% ethanol as the eluent[69].

Individual differences exist between the sorption of various mono-, di- and trisaccharides and can be used for the separation of these sugars. Using Dowex 1-X8 (45–75 μm), it was possible to separate some monosaccharides from each other. Xylose, arabinose and mannose are held less strongly than glucose and can be separated easily as a group by elution with 74% ethanol[72]. Similarly, the trisaccharide melezitose could be separated from raffinose[72].

The use of fine particles (8–13 and 10–15 μm) of Dowex 1-X8 allowed the separation of sugars from wood hydrolyzates in about 150 min. Automated analysis of the column effluent was performed using a three-channel flow-through photometer after reaction with anthrone (625 nm) and aniline (385 nm)[73].

The separation of saccharides was markedly improved when the macroporous anion exchanger Dowex 21K (SO_4^{2-}) with fine particles (15–40 μm) was used. 2-Deoxy-D-glucose, arabinose, glucose, sucrose, melezitose and raffinose could be separated quantitatively by elution with 74% ethanol. With the small resin particles, the lower rate of diffusion inside the resin beads does not offset the advantages of higher separation factors at higher ethanol concentrations. A great improvement in the separation of monosaccharides is achieved when the concentration of ethanol is increased to 88%. A complete separation of arabinose, xylose, mannose, galactose and glucose could be achieved under these conditions[74,75]. Further, ribose, arabinose, xylose, mannose, galactose and glucose were separated and determined in hydrolyzates from wood and wood pulp[76].

As the use of finer particles is connected with an increased pressure drop across the column, the operation technique has to be adapted to higher pressure requirements. The pressure drop was increased by the irregular particle shape of the crushed resin used in the experiments described. A further improvement in resolution and speed of analysis could be expected with even smaller particles. To avoid an excessive pressure drop across the column, the possibilities were studied of using columns packed with a mixture of equal volumes of very finely crushed particles of Dowex 21K (1–16 μm) and the inert Celite 545. The improvement resulting from this column packing was, however, not very significant. It seems probable that Celite has a negative effect on the separation owing to the decreased column capacity and, consequently, the lower resolution power of the column[77].

A more attractive method for the improvement of separation is to use increased temperatures, which results in less broadening of the elution curves owing to an increased rate of diffusion inside the resin particles. Using elution with 88% ethanol at 47°, a marked improvement resulted in the separation of the monosaccharides arabinose, xylose, mannose, galactose and glucose. A further improvement was obtained at 60° (ref. 77).

When columns packed with irregular crushed particles of Dowex 21K (1–16 μm) without the addition of Celite were tested, a high pressure drop occurred in the column even at very low flow-rates. When this column was started, very broad elution curves were observed but after several months very sharp elution curves resulted. On a 6 × 440 mm column of this resin, it was possible to separate quantitatively a mixture containing twelve monosaccharides (digitoxose, 2-deoxyribose, 2-deoxygalactose, rhamnose, fucose, ribose, lyxose, arabinose, xylose, mannose, galactose and glucose) by elution with 86% ethanol at 75° (ref. 71).

Better column packings could be obtained by using a spherical macroporous resin. Sharp separations of various monosaccharides present in hydrolyzates from wood and wood pulp were obtained using Technicon T_4 (SO_4^{2-}), particle size 10–35 μm, with 89% or 92% ethanol as the eluent.[71]

The macroporous spherical anion-exchange resin Technicon T_5B with a smaller particle diameter (3–17 μm) packed in a 6 × 950 mm column was used for the automated chromatography of saccharides. The eluate from the column was analyzed automatically by the orcinol method with a Technicon AutoAnalyzer. By elution with 94% ethanol at 75°, it was possible to separate very complicated mixtures. Thus, a mixture of ten monosaccharides was separated in about 34 h, and, under the same conditions, twelve sugar derivatives, three pentoses and one deoxy sugar were separated from each other (Fig. 12). Both the separation of sugar derivatives and the separation of the monosaccharides from each other was much more effective in this run than in the experiment with the Technicon T_4 resin, which has a lower capacity and coarser particles. Consequently, less favorable separation factors and broader elution bands resulted with the Technicon T_4 resin[78]. Table 13 gives separation factors

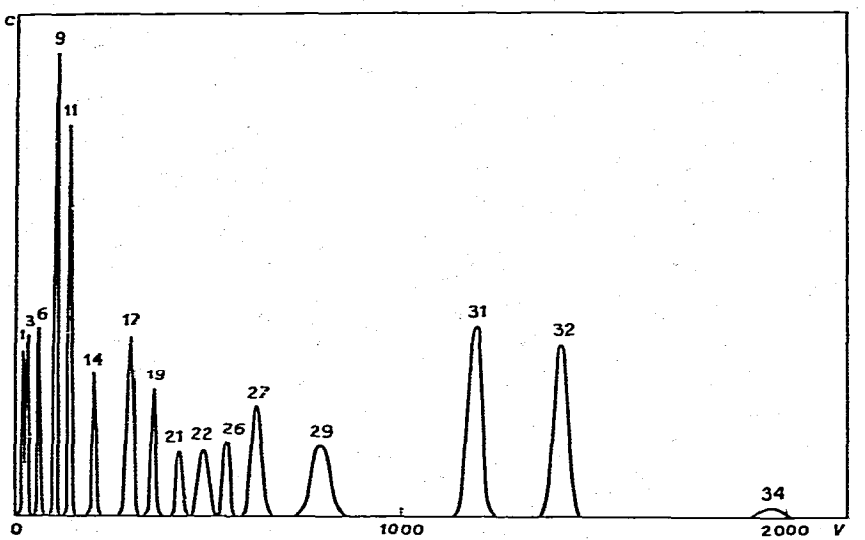


Fig. 12. Separation of monosaccharides and sugar derivatives (6–70 μg) in aqueous ethanol medium on an anion-exchange resin. The numbers of the compounds separated are as in Table 13. Ion-exchange resin: Technicon T_5B (SO_4^{2-}), 3–17 μm. Column dimensions: 6 × 950 mm. Mobile phase: 94% ethanol. Flow-rate: 3.5 ml/cm^2·min. Temperature: 75°. Detection: Technicon AutoAnalyzer, orcinol method. c = concentration (arbitrary units); V = volume of eluate (ml).

TABLE 13

SEPARATION FACTORS RELATIVE TO FUCOSE IN 94% ETHANOL (% BY WEIGHT)

No.	*Compound*	*Technicon T_4*	*Technicon T_5B*
1	2,3,4,6-Tetra-O-methyl-D-glucose	0.02	0.01
2	2,3,6-Tri-O-methyl-D-glucose	0.03	—
3	Methyl 2,3-di-O-methyl-β-D-glucopyranoside	0.04	0.02
4	2,3-Di-O-ethyl-D-glucose	0.05	—
5	2,3-Di-O-methyl-D-glucose	0.07	—
6	3,6-Di-O-methyl-D-glucose	0.08	0.07
7	Methyl-4-O-methyl-β-D-glucopyranoside	0.08	—
8	D-Digitoxose	0.11	0.10
9	Methyl β-L-arabinopyranoside	0.17	0.14
10	Methyl β-D-arabinopyranoside	0.17	—
11	Methyl α-D-xylopyranoside	0.23	0.24
12	2-Deoxy-D-ribose	0.23	0.22
13	Methyl β-D-xylopyranoside	0.30	—
14	2-O-Ethyl-D-glucose	0.33	0.31
15	Ethyl β-D-glucopyranoside	0.40	—
16	Methyl α-D-galactopyranoside	0.46	—
17	3-O-Benzyl-D-glucose	0.50	0.46
18	Methyl-α-D-mannopyranoside	0.57	—
19	Methyl α-D-glucopyranoside	0.58	0.57
20	2-Deoxy-D-galactose	0.63	0.63
21	2-O-Methyl-D-glucose	0.70	0.68
22	3-O-Methyl-D-glucose	0.77	0.77
23	Methyl β-D-glucopyranoside	0.77	—
24	4-O-Methyl-D-glucose	0.78	—
25	L-Rhamnose	0.82	0.82
26	2-Deoxy-D-glucose	0.86	0.87
27	L-Fucose	1.00	1.00
28	D-Ribose	1.22	—
29	6-O-Methyl-D-glucose	1.26	1.28
30	3-O-Hydroxyethyl-D-glucose	1.33	—
31	D-Lyxose	1.78	1.93
32	D-Arabinose	2.09	2.30
33	D-Xylose	2.74	3.04
34	6-O-Hydroxyethyl-D-glucose	2.87	3.19

of various monosaccharides and sugar derivatives on both resins under the experimental conditions used for the chromatographic run[78].

The distribution coefficients of sugar derivatives are controlled by rules similar to those for sugars. As might be expected, all of the derivatives studied are eluted ahead of the sugar from which they are derived. Not only etherification of sugars, but also conversion into glycosides, results in a decrease in the distribution coefficients. As a rule, the distribution coefficient increases with a decrease in the number of substituents. As expected, the methyl ethers exhibit higher distribution coefficients than the less polar ethyl ethers. Hydroxyethyl derivatives are retained more strongly than the methylated sugars because no hydroxyl groups disappear during this substitution. An ethylglycoside appears ahead of the corresponding methylglycoside, and the glyco-

sides derived from pentoses exhibit lower distribution coefficients than those from hexoses[78].

The experimental conditions used made it possible to obtain good separations at high flow-rates even with solutes whose distribution factors differ by only 10%, which means that the separation of a large number of sugars and sugar derivatives can be achieved. In general, the conditions for a quantitative separation are better with less substituted derivatives than with those which contain several substituents[78].

With finer mesh size (10–15 μm) Technicon T_5B (SO_4^{2-}) resin, it was possible to separate quantitatively microgram amounts of various monosaccharides in columns with a small inner diameter, using the orcinol method for the determination of sugars in the eluate. The complete separation of rhamnose, ribose, arabinose, xylose, mannose, galactose and glucose was accomplished in 150 min on a 650 × 2 mm I.D. column by elution with 86% ethanol at a flow-rate of 0.44 ml/min with a pressure drop of 60 atm[79].

Using Technicon T_5B resin (10–15 μm), separations were possible of mixtures containing various saccharides, deoxy sugars and alditols. Thus an almost complete separation of a 16-component mixture could be achieved on a 915 × 6 mm I.D. column by elution with 89% ethanol at 78.5° in about 16 h (ref. 42).

With the anion exchanger Technicon T_5C (SO_4^{2-}), 10–17 μm (capacity 4.2 mequiv./g), separations of mixtures containing various mono-, di- and trisaccharides have been reported. The distribution coefficients of various mono-, di- and trisaccharides on this resin are given in Table 14 at various temperatures and ethanol concentra-

TABLE 14

VOLUME DISTRIBUTION COEFFICIENTS OF MONO-, DI- AND TRISACCHARIDES ON ION-EXCHANGE RESINS AT VARIOUS TEMPERATURES AND ETHANOL CONCENTRATIONS

EtOH = Ethanol.

Saccharide	*Dowex 50W-X8, 14–17 μm*							*Technicon T_5C (SO_4^{2-}), 10–17 μm*			
	82% EtOH, 90°		*82% EtOH, 75°*		*85% EtOH, 75°*		*88% EtOH, 75°*	*70% EtOH, 90°*	*76% EtOH, 90°*	*80% EtOH, 75°*	*83% EtOH, 75°*
	Li^+	K^+	Li^+	Na^+	Li^+	Na^+	Li^+				
Arabinose	1.9	4.4	2.1	3.6	2.3	4.9	2.6	1.8	3.0	4.5	6.0
Galactose	–	5.4	3.1	5.5	3.6	7.4	4.4	2.9	5.3	8.4	12.7
Maltose	3.3	10.4	3.5	8.6	4.7	13.6	6.5	4.6	10.9	20.4	37.4
Cellobiose	3.4	11.5		9.5	5.0	15.3		4.7	11.6	21.3	39.8
Turanose	3.6	8.0	3.9	7.5	5.5	–	–	3.3	7.6	–	–
Palatinose	3.5	10.3	4.0	9.4	5.5	–	–	4.4	–	–	–
Saccharose	d*	5.9	4.2	6.2	5.9	9.7	8.8	4.4	10.6	19.4	35.1
Gentibiose	–	–	4.8	13.7	6.7	–	–	7.7	20.3	–	–
Lactose	4.7	15.4	5.2	12.6	7.0	20.1	9.6	4.4	10.3	19.3	35.1
Trehalose	–	–	5.7	9.6	8.2	–	11.8	6.3	15.6	–	–
Melizitose	–	–	6.4	11.5	10.2	–	–	6.6	19.3	–	–
Melibiose	6.6	27.1	7.5	22.0	10.7	37.1	16.2	5.8	14.9	29.2	48.7
Raffinose	d*	–	11.8	26.4	–	–	–	8.4	–	–	–

* d = decomposition.

tions. A mixture containing eight monosaccharides (threose, 6-deoxyglucose, talose, xylose, allose, gulose, galactose and glucose) was completely separated on a 4 × 550 mm column of the Technicon T_5C (SO_4^{2-}) resin, 14–17 μm, using 88% ethanol as the eluent at 75° (ref. 80).

Using longer columns of this resin and lower ethanol concentrations, a mixture containing eleven mono- and trisaccharides (rhamnose, ribose, arabinose, xylose, mannose, galactose, turanose, palatinose, melibiose, melizitose and raffinose) was completely separated in about 10 h. A 4 × 1500 mm column was used and the elution was carried out with 70% ethanol at 90°, the pressure drop across the column being 60 atm. The low ethanol concentration made possible the elution of the oligosaccharides in a reasonable time, but some resolution was sacrificed. Thus, glucose could not be separated from turanose under the experimental conditions used[81].

An aqueous ethanol concentration of 70% is too low for separations of complicated mixtures in which mono- and disaccharides are involved. This ethanol concentration was, however, suitable for the complete separation of the oligosaccharides in the glucose–cellohexaose series. Another example of the separation of mixtures containing mono-, di-, tri- and higher oligosaccharides is the separation of mycinose, xylose, cellobiose, maltotriose, planteose, nystose and stachyose on a 4 × 550 mm column packed with Technicon T_5C (SO_4^{2-}) resin (14–17 μm) by elution with 70% ethanol[80].

Chromatography on a 4.7 × 605 mm column packed with Technicon T_5C (SO_4^{2-}) resin (10–17 μm) has been used for the automatic determination of the carbohydrate composition in rayon pulp, alkali cellulose and rayon. After hydrolysis and neutralization, elution with 88% ethanol resulted in the separation of laevoglucosan (pyranose form), laevoglucosan (furanose form), arabinose, xylose, fructose, mannose and glucose[82].

Jonsson and Samuelson[83] studied the possibilities of using strongly basic anion exchangers with a more polar resin matrix for the separation of sugars in aqueous ethanol media. Cross-linked dextran, containing quaternary ammonium groups, holds the sugars more strongly than do styrene–divinylbenzene resins because of the higher ionic concentration inside the styrene–divinylbenzene resins. The order of elution, together with the influence of the ethanol concentration, on the dextran anion exchanger are the same as with the polystyrene resin, and can be seen the from results in Table 15.

As with styrene–divinylbenzene anion-exchange resins, the sulphate form of the dextran anion exchanger yielded much greater separation factors than the chloride form. An interesting exception, which can be used to advantage in certain practical analyses, is the separation of xylose from mannose, which is more favorable with the chloride form of the anion exchanger. As a rule, the distribution coefficients of sugars are much lower on the chloride form than on the sulphate form, but the chloride form of the dextran anion exchanger, however, can be used for certain separations. It was possible to separate completely a mixture of monosaccharides (rhamnose, ribose, xylose, mannose and glucose) on an 890 × 6.0 mm column in less than 3 h by elution with 95% ethanol at 90° (ref. 83).

The sulphate form of the exchanger is to be preferred in most other instances. Four pentoses (ribose, lyxose, arabinose and xylose) were separated on an 840 × 6.0 mm column of the dextran anion exchanger (5–30 μm) in the sulphate form in less than

TABLE 15

VOLUME DISTRIBUTION COEFFICIENTS ON QAE-SEPHADEX ANION-EXCHANGER IN 95% ETHANOL AT DIFFERENT TEMPERATURES

Values in parentheses refer to experiments in 92.4% ethanol. A = low cross-linking, 10–40 μm; B = higher cross-linking, 5–30 μm.

Saccharide	*A(SO_4^{2-})*		*B(SO_4^{2-})*				*B(Cl^-)*
	75°		*75°*	*90°*	*97°*	*110°*	*90°*
Digitoxose	—	(0.7)	0.8	0.7	—	—	0.4
2-Deoxyribose	1.4	(1.3)	1.8	1.4	—	—	0.7
2-Deoxygalactose	—	—	3.7	3.1	—	—	1.4
Rhamnose	—	—	4.8	4.0	3.6	3.2	1.4
2-Deoxyglucose	—	—	—	4.1	—	—	1.6
Fucose	5.4	—	4.4	3.9	—	—	
Ribose	5.1	(4.3)	6.9	6.5	5.8	4.9	2.0
Lyxose	—		9.7	7.9	6.9	—	—
Arabinose	6.9	(6.1)	10.5	8.9	7.8	6.5	2.7
Xylose	8.2	(7.1)	14.0	11.5	9.9	8.0	2.9
Fructose	—	—	14.6	12.1	10.6	—	—
Mannose	9.3	(7.9)	15.2	12.4	10.7	8.8	3.7
Tagatose	—	—	16.7	13.1	11.1	—	—
Sorbose	—	—	16.9	13.7	11.3	—	—
Galactose	12.6	(11.0)	23.0	18.1	15.3	12.1	4.2
Glucose	14.7	(12.1)	29.7	22.0	18.3	14.3	4.4

4 h using the same eluting agent as in the experiment with the chloride form. Under the same conditions, a mixture of monosaccharides (rhamnose, ribose, arabinose, xylose, mannose, galactose and glucose) could be separated in less than 7 h. The separation of xylose from mannose was, however, less effective than that obtained by using anion-exchange resins with a styrene–divinylbenzene matrix. On the other hand, the separations of arabinose from xylose and of galactose from glucose were better on the dextran anion exchanger. At 65°, a successful separation of fructose from tagatose could be achieved on an 890 × 4.3 mm column of the sulphate form of dextran anion exchanger by elution with 95% ethanol, while the separation of these ketoses failed on styrene–divinylbenzene anion exchangers. It is obvious that chromatography on a dextran anion exchanger can be employed, in some instances, as a valuable complementary method to that on styrene–divinylbenzene anion exchangers[83].

When columns of styrene–divinylbenzene cation exchangers are used for chromatography in aqueous–organic media, saccharides are most strongly sorbed on the potassium form of the resin. Use of the sodium form results in lower distribution coefficients and the sorption is the least with the lithium form of the resin. This indicates that the interaction forces between the ions inside the resin and the sugar molecules have a marked influence upon the sorbability. In most instances, an increased number of hydroxyl groups in the sugar results in an increased distribution coefficient, as with the sulphate form of anion-exchange resins. Among the individual sugars within each group, the order of elution was reversed with some species. This reversal of the order of elution occurred in several instances even when the counter ion of the cation-exchange resin was changed. For all sugars and all ionic forms of the resin, the distribution coefficients decreased with increase in temperature and increased with in-

crease in the concentration of ethanol. An increase in temperature resulted in a considerable sharpening of the elution curves. Table 14 gives the distribution coefficients of some sugars on different ionic forms of the cation-exchange resin[84].

The separation of deoxy sugars on the lithium form of the resin is superior to that on the potassium form in terms of efficiency and speed of operation. A mixture of digitoxose, 2-deoxyribose, 2-deoxyglucose and 2-deoxygalactose was completely separated on a 460 × 6 mm column of the strongly acidic cation exchanger Amberlite IR-120 (Li^+), 3–17 μm, by elution with 92.4% ethanol at 75°. Under the same conditions, a mixture of seven monosaccharides (rhamnose, 2-deoxyglucose, xylose, arabinose, tagatose, glucose and galactose) was separated quantitatively in 5 h. Of these sugars, rhamnose and 2-deoxyglucose cannot be separated on anion-exchange resins in the sulphate form. The separation of xylose and tagatose or allose and altrose is feasible on the lithium form of the cation exchanger, while it fails on the sulphate form of the anion exchanger.

Chromatography on the lithium form of the cation exchanger has been used for the separation of complex mixtures of mono- and disaccharides. A nine-component mixture of microgram amounts of rhamnose, xylose, mannose, galactose, maltose, sucrose, lactose, trehalose and melibiose was quantitatively separated on a 2.6 × 1225 mm column of Dowex 50W-X8 (Li^+), 14–17 μm, by elution with 85% ethanol at 75°. The pressure drop was about 40 atm and the separation required about 5 h. The result is shown in Fig. 13 (ref. 81).

This system can be useful in the analysis of various types of foods containing maltose, sucrose and lactose. Separations on the sulphate form of anion-exchange

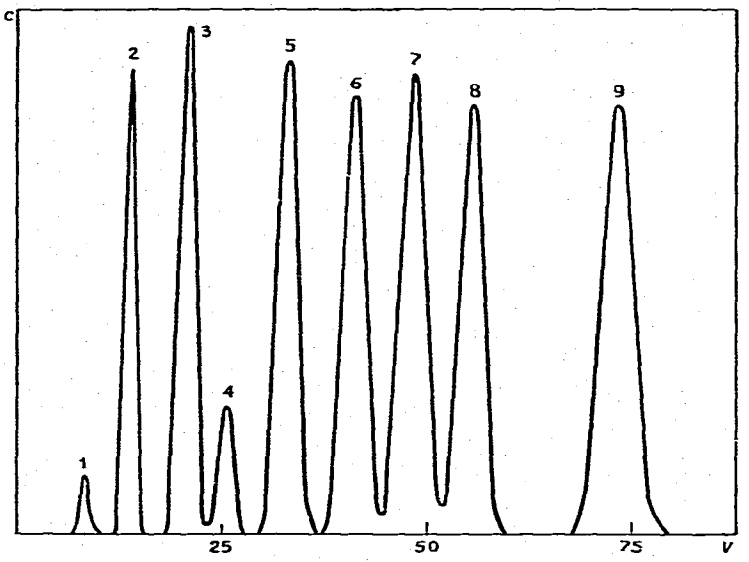


Fig. 13. Separation of mono- and disaccharides in aqueous ethanol medium on a cation-exchange resin. (1) Rhamnose, 2 μg; (2) xylose, 20 μg; (3) mannose, 70 μg; (4) galactose, 10 μg; (5) maltose, 100 μg; (6) sucrose, 100 μg; (7) lactose, 100 μg; (8) trehalose, 100 μg; (9) melibiose, 100 μg. Ion-exchange resin: Dowex 50W-X8 (Li^+), 14–17 μm. Column dimensions: 2.6 × 1225 mm. Mobile phase: 85% ethanol. Flow-rate: 5.2 ml/cm²·min. Temperature: 75°. Detection: Technicon Auto-Analyzer, orcinol method. c = concentration (arbitrary units); V = volume of eluate (ml).

resins suffer, however, from the great disadvantage that these important disaccharides cannot be separated from each other without serious overlapping[81].

The quantitative separation of glucose, cellobiose, isomaltose, gentianose, 1-kestose, planteose, nystose, cellohexaose and stachyose on a 4 × 1050 mm column packed with Dowex 50W-X8 (Li^+), 17–24 μm, with 85% ethanol as the eluent is an example of the separation of complex mixtures containing mono-, di- and higher oligosaccharides[80].

In some separations, the sodium form offers certain advantages over the lithium form of the resin. Palatinose and turanose, which cannot be distinguished from each other on the lithium form, can be well separated on the sodium form of a cation exchanger or on the sulphate form of an anion exchanger. The sodium form can resolve cellobiose and maltose, whereas serious overlapping occurs on the other ionic forms.

The potassium form of the cation exchanger yields broad elution curves and high elution volumes and thus offers no advantages over the other types of resin. At low ethanol concentrations, the potassium form could be used for some simple separations, such as that of lactose from sucrose.

The distribution coefficients of some saccharides on the sulphate and lithium forms of resins are compared in Table 16.

The separation of tagatose and fructose could not be achieved on the anion exchanger, while it was easily achieved on the cation-exchange resin. On the other hand, mannose cannot be separated from glucose on the cation exchangers, in contrast to the sulphate form of the anion exchangers.

Both methods are complementary. The main advantage of the use of cation exchangers is in analysis of the common disaccharides, deoxy sugars and ketoses[84]. The sulphate form of anion-exchange resins is recommended in group separations of monosaccharides from higher saccharides. With complex mixtures, it is often necessary to re-chromatograph some bands obtained from the lithium form of the resin either on the cation exchanger in the sodium form or on the anion exchanger in the sulphate form. With mixtures containing several monosaccharides which are to be determined, it may be preferable to start with the anion exchanger in the sulphate form and re-chromatograph appropriate fractions containing disaccharides on the lithium or sodium form of the cation exchanger[81].

Chromatography of multicomponent mixtures of saccharides in aqueous ethanol solutions usually requires about 7 h of column operation. The need to use high temperatures and ethanol concentrations leads to difficulties owing to the rapid exhaustion of the AutoAnalyzer tubing. In addition, the solubility of polysaccharides in alcohol decreases as the degree of polymerization increases, thus limiting the use of the aqueous ethanol chromatographic system to lower saccharides only.

C. Chromatography of saccharides on anion-exchange resins in the borate and hydrogen sulphite forms

Borate ions react with sugars to produce negatively charged sugar–borate complexes and the differences in the strengths of the sugar–borate complexes formed can be used for the separation of sugars on strong anion exchangers with borate solutions as the eluents, as for the separation of hydroxy and sugar acids or polyols.

As the stability of the sugar–borate complexes is greatly dependent on the pH and the concentration of the borate ions in the mobile phase, appropriate control of

TABLE 16
VOLUME DISTRIBUTION COEFFICIENTS OF SOME SACCHARIDES AT 75°
EtOH = Ethanol.

Saccharide	*Technicon T_5C (SO_3^{2-}) resin (14–17 μm)*		*Dowex 50W-X8 (Li^+) resin*		
			17–24 μm	*17–21 μm*	
	70% EtOH	*88% EtOH*	*85% EtOH*	*92.4% EtOH*	*96% EtOH*
Erythrose		3.1		1.9	2.3
Threose		3.8		1.4	1.8
Arabinose		10.1		3.5	4.2
Lyxose		8.9		2.7	3.2
Ribose		6.6			4.6
Xylose	2.3	12.5	1.9	2.7	3.2
Allose		16.6		6.0	8.3
Altrose		16.6		4.2	5.6
Galactose		23.4			7.6
Glucose	3.1	28.1	3.0	4.8	6.0
Gulose		20.4		4.9	6.3
Mannose		16.4			5.9
Talose		10.7		5.8	8.3
Fructose		13.5			6.8
3-Ribohexulose		9.8		4.7	6.6
6-Deoxy-D-glucose		7.3		0.9	1.1
Rhamnose		4.8		1.4	1.6
1,6-Anhydro-β-D-glucofuranose		6.3		1.4	1.6
1,6-Anhydro-β-D-glucopyranose		3.8		2.3	2.9
2,3-Di-O-methyl-6-deoxyallose (mycinose)		0.53		0.4	0.4
Mannobiose	2.9	41.6	7.8		
Xylobiose	3.0	35.8	2.0	5.4	7.3
Cellobiose	4.4		4.9		
Isomaltose	6.5		8.6		
Trehalose	6.8		8.2		
Cellotriose	5.8		8.7		
Gentianose	9.6		12.3		
1-Kestose	8.7		16.2		
Maltotriose	6.6		7.7		
Planteose	9.1		19.3		
Cellotetraose	7.9		15.4		
Nystose	14.9		27.8		
Stachyose	18.5		64.7		
Cellopentaose	10.9		27.4		
Cellohexaose	15.2		44.4		

these values is a convenient method for selecting conditions favourable for efficient separations. A higher pH (about the pK of the boric acid, 9.2) increases the concentration of the borate ions in the solution, thus minimizing the total volume of borate solution required. Borate solutions with pH values between 8 and 9 are suitable for the separation of various sugars.

The differences in the affinity of the various sugar–borate complexes for the anion exchanger are controlled by structural differences and other factors, such as mutarotation and pyranose–furanose interconversion. Polyhydroxy compounds,

such as the non-reducing sugars sucrose and trehalose, which possess no adjacent *cis*-hydroxyl groups, have only a slight affinity for an anion exchanger[85].

Cellobiose, maltose, lactose and melibiose are reducing sugars and can have adjacent *cis*-hydroxyl groups through mutarotation and can therefore form borate complexes thought to be highly ionised. In fact, the reducing disaccharides show a greater affinity for the exchanger than do sucrose and trehalose[86].

Compounds that contain *cis*-hydroxyl groups in a pyranose ring form complexes which exhibit only a weak affinity for a strongly basic anion exchanger. The affinity for the exchanger of the borate complexes formed by the 1,4-disaccharides maltose, lactose and cellobiose, which cannot form furanose isomers, is much less than the affinity of the monosaccharides that can form furanose structures. Raffinose also cannot form a furanoid structure and has only a slightly greater affinity than sucrose[46]. Melibiose and gentiobiose, owing to their 1,6-linkage, are capable of forming a furanose isomer and the affinity of their borate complexes for the exchanger parallels that of the monosaccharides[86]. The greater affinity of melibiose compared with that of gentiobiose may be attributed to the presence of an additional pair of *cis*-hydroxyl groups on the galactose portion of melibiose[46].

It is assumed that the accumulation of hydroxyl groups in the vicinity of the reducing carbon atom is the main factor in the formation of the borate complexes of the ketoses, such as fructose, and accounts for their great affinity for the ion exchanger[46].

The ease of mutarotation of a sugar is also a factor that could affect the rate of reaction of a borate complex with the exchanger, and it appears significant that among the monosaccharides the sugars that have the highest mutarotation constants are the ones that are eluted most easily and that these constants correspond roughly with the elution order of the pentoses and hexoses[86].

The separation technique using borate medium was developed by Khym and Zill[85,86] in 1951. They reported the separation of fructose, galactose and glucose on a 0.85 $cm^2 \times 11$ cm column packed with Dowex 1, *ca.* 300 mesh, by elution of the first two sugars with 0.015 *M* sodium borate solution and of glucose with 0.03 *M* sodium borate solution[85].

If mannose is present in this mixture, it is eluted in one band together with fructose. However, mannose and fructose can be separated on the same column with a lower pH and a lower borate concentration used for the elution of mannose (0.05 *M* boric acid + 0.004 *M* potassium tetraborate). The elution of fructose is then performed with 0.015 *M* potassium tetraborate solution[86].

Some pentoses (ribose, arabinose and xylose) could be separated under the same conditions as the hexoses (elution with 0.015 *M* potassium tetraborate solution), but a better separation was achieved by elution of ribose and arabinose at a lower pH (0.08 *M* boric acid + 0.004 *M* potassium tetraborate) followed by the elution of xylose with 0.03 *M* potassium tetraborate solution[86].

The separation of more complex mixtures containing pentoses and hexoses (ribose, fructose, arabinose, galactose, xylose and glucose) employing the same conditions as those used for the separation of fructose, galactose and glucose has been reported[86]. In this case, the elution bands of fructose and arabinose and those of galactose and xylose overlapped almost completely, but the individual sugars could be differentiated and determined quantitatively by using a combination of the orcinol and anthrone methods for the analysis of the fractions.

The disaccharides are eluted ahead of the monosaccharides. Mixtures of sucrose, fructose and glucose (after an incomplete hydrolysis of sucrose) could be easily separated using stepwise elution with 0.005, 0.015, 0.02 and 0.03 *M* potassium tetraborate solution[86]. The separation of the disaccharides sucrose and maltose could be accomplished by a single elution with 0.005 *M* potassium tetraborate solution. In this case, maltose is retained more strongly on the column.

The hydrolytic products of melezitose were separated on the same column by stepwise elution with 0.015 *M* potassium tetraborate solution (melezitose, followed by an elution band containing both turanose and fructose) and 0.03 *M* potassium tetraborate solution (glucose). Turanose could be separated from fructose by eluting the first compound with 0.1 *M* boric acid and the fructose with 0.1 *M* potassium tetraborate solution[46].

Sedoheptulosan was separated from sedoheptulose using stepwise elution with 0.015 and 0.1 *M* potassium tetraborate solution[46]. Similarly, the products formed during the acid treatment of 2,7-anhydro-β-D-altroheptulopyranose were separated on the same column as in the previous experiments; 0.01 *M* potassium tetraborate solution eluted 2,7-anhydro-β-D-altroheptulofuranose and 2,7-anhydro-β-D-altroheptulopyranose as two separated bands, and 0.1 *M* potassium tetraborate solution then eluted a mixture of D-altroheptulose and 5-(1,2-dihydroxyethyl)-2-furfuraldehyde in a single peak[87].

A distinct disadvantage of the method of Khym and Zill was that although small columns were used, the elution volumes of the monosaccharides were very large, making the separation very lengthy (requiring up to 60 h). Therefore, Hallén[88] modified this method by eluting at a higher ionic strength. As an example, a mixture of mannose, fucose, galactose and glucose was separated on a 0.6 × 150 cm column of Dowex 2-X8 (BO_3^-), 200–400 mesh, by a single elution using 0.01 *M* borax in 0.20 *M* sodium hydrogen carbonate solution. The resolution was complete and the elution volumes were considerably lower than in the method of Khym and Zill. If galacturonic and glucuronic acids were present in the mixture, they could be separated each from the other by elution with 0.03 *M* sodium tetraborate in 0.6 *M* sodium hydrogen carbonate solution after the appearance of the last neutral monosaccharide (glucose)[88].

Parr[89] separated sugars and sugar phosphates on a column of the strongly basic anion-exchange resin De Acidite FF (BO_3^-) using gradient elution with borate buffers containing sodium chloride, its concentration being gradually changed.

Similarly, Syamananda, Staples and Block[90] used elution with a solution adjusted to pH 8.0 and containing a constant borate concentration upon which was superimposed a smooth positive chloride ion concentration gradient. This procedure reduced the time required for the separation by a factor of at least three over the method of Khym and Zill, but the resolution of individual sugars remained largely incomplete.

This method has been applied to the separation and determination of sugars in acid hydrolyzates of soil[91]. A 600 × 6 mm column packed with Bio-Rad AG 1-X8 (BO_3^-) anion-exchange resin has been used in connection with gradient elution using 0.1 *M* sodium borate (pH 8.0) in the eluent with a sodium chloride gradient. The sugars were eluted in the order rhamnose, mannose, fucose, arabinose, galactose, xylose and glucose, with recoveries of about 90%.

Some phosphate esters of sugars, glucose and lactic and pyruvic acids in glycolysis intermediates were quantitatively separated, identified and recovered by anion-exchange chromatography on a 30 × 1 cm column packed with Bio-Rad AG 1-X4, 200–400 mesh[92]. Gradient elution was performed using ammonium chloride solutions, continually increasing in concentration and containing sufficient alkaline borate to ensure complexing of the sugar phosphates.

The Technicon sugar chromatography system is based on a column packed with the spherical strongly basic anion-exchange resin Chromobeads S (20 μm) and gradient elution with borate buffer–sodium chloride solution. The gradient of gradually increasing chloride concentration and pH is formed in a nine-chamber Autograd apparatus. The elution is carried out at 45° at a pressure of 300–400 p.s.i. and the amount of sugars in the eluate is determined automatically by the orcinol method using a Technicon AutoAnalyzer. Sharp separations were obtained. As an example, a 14-component mixture containing furfural, hydroxymethylfurfural, trehalose, cellobiose, maltose, rhamnose, lactose, ribose, mannose, fructose, arabinose, galactose, xylose and glucose was separated in about 5.5 h[93].

A fixed chloride ion concentration in the eluent can result in a sharper separation of sugar mixtures than elution with chloride ion concentration gradients, if the temperature is suitably adjusted. For example, a mixture of mannose, arabinose, galactose, xylose and glucose was separated on a 0.6 × 70 cm column of Dowex 1-X8 (BO_3^-), 30–40 μm, by elution with an eluent containing 5.84 g of sodium chloride per litre of the sodium borate buffer of pH 8.0. An increase in temperature from 40° to 60° resulted in an improved separation of mannose, arabinose, galactose and xylose, while the separation of glucose from xylose was impaired at higher temperatures. For this reason, after the elution of xylose, borate buffer without sodium chloride was used for elution instead of the sodium chloride solution for a period of about 15 min. This was sufficient for the complete resolution of glucose and xylose when working at 50–55°. The elution of glucose could then be accelerated by using the initial eluent. By this method, the five-component sugar mixture could be separated quantitatively in about 6 h[94].

Similarly, Green[95] found that the effect of increasing the column temperature was to shift the eluted bands to longer elution times, while simultaneously enhancing resolution. Little further advantage, however, could be gained between 55° and 70°.

It must be taken into account that transformation of certain reducing disaccharides (lactose, maltose and cellobiose) may occur during chromatography in borate medium under alkaline conditions, *e.g.*, lactulose is formed from lactose. These isomeration reactions are promoted by higher temperatures[96].

The use of chloride ions in the eluent can be dispensed with, and a solution of borate buffers of changing pH and/or concentration is a suitable eluent.

Kesler[97] used a combination of increasing pH (from 7 to 10) and increasing borate concentration (usually from 0.1–0.2 to 0.6 *M*) for the elution of sugars at an elevated temperature (53°). This system represented a major development in separation of saccharides and allowed quantitative analyses of multicomponent mixtures of mono-, di- and trisaccharides in 4–6 h. It was possible to separate almost quantitatively an 18-component mixture (containing 2 aldehydes and 15 saccharides) in about 6 h on a 3 × 700 mm column packed with Technicon strong anion exchanger, 8% cross-linked, 5–40 μm size. This separation is shown in Fig. 14.

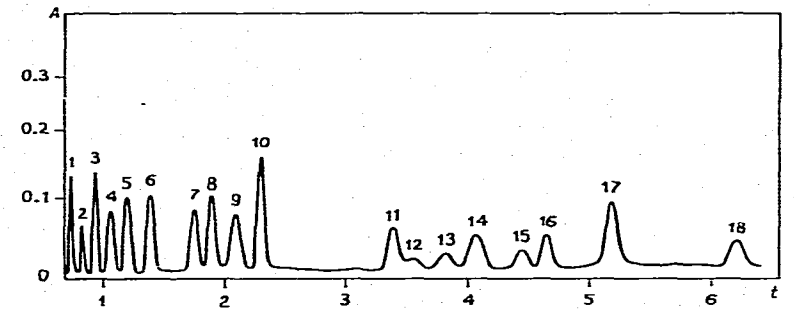


Fig. 14. Separation of an 18-component mixture of saccharides and aldehydes by anion-exchange chromatography in borate medium, using elution with gradients of pH and borate concentration. (1) Furfural, 1.0 μg; (2) hydroxymethylfurfural, 2.0 μg; (3) sucrose, 2.4 μg; (4) cellotetraose, 2.2 μg; (5) cellotriose, 2.3 μg; (6) cellobiose, 2.4 μg; (7) maltose, 2.2 μg; (8) rhamnose, 2.0 μg; (9) lactose, 2.2 μg; (10) ribose, 1.6 μg; (11) mannose, 2.0 μg; (12) unknown; (13) fructose, 2.4 μg; (14) arabinose, 1.1 μg; (15) galactose, 1.3 μg; (16) xylose, 0.8 μg; (17) glucose, 2.5 μg; (18) gentiobiose, 1.6 μg. Ion-exchange resin: Technicon 3/28 VI (BO_3^-) strongly basic styrene-divinylbenzene anion-exchange resin, 8% cross-linked, 5–40 μm. Column dimensions: 3 × 750 mm. Mobile phase: gradient elution with a solution containing 0.60 *M* boric acid, pH 10.0, being continually introduced into the mixing chamber filled with 70 ml of solution containing 0.125 *M* boric acid, pH 7.0. Flow-rate: 0.30 ml/min. Temperature: 53°. Detection: Technicon AutoAnalyzer, orcinol method. *A* = absorbance; *t* = time elapsed (h).

Under the same conditions, sorbose is eluted together with xylose, lyxose with ribose, *d*-2-deoxyribose with furfural, raffinose with cellobiose and trehalose with sucrose. The small particle size resin used has proved to be far more efficient than other resins of this type with coarser particles (Dowex 1-X8, 200–400 mesh; Bio-Rad AG 1-X8, 30–40 μm). Aminex A-15 is suitable for this operation.

A mixture of cellodextrins (celloheptaose, cellohexaose, etc.) was separated by gradient elution using four mixing chambers (Autograd) filled with 100 ml of 0.050 *M* boric acid, 0.100 *M* boric acid at pH 7.0, 0.125 *M* boric acid at pH 8.5 and 0.150 *M* boric acid at pH 9.0, respectively. The separation was accomplished at 53° in about 5 h using a 6 × 1000 mm column of the same resin as in the previous experiment, and only the elution peaks of celloheptaose and cellohexaose overlapped considerably[97].

Later, Kesler's system was slightly modified for the faster routine analysis of neutral sugars in glycoproteins (rhamnose, mannose, ribose, fucose, arabinose, galactose, xylose and glucose). Using Technicon Chromobeads S (low-pressure type) in a 750 × 3 mm column, the elution was performed at 55° with a linear gradient formed with 0.4 *M* sodium borate of pH 10.00 being continuously introduced into the mixing chamber containing 100 ml of 0.15 *M* sodium borate buffer of pH 7.00. Under these conditions, the elution of saccharides was about 30–50% faster than in the original Kesler system. Some pairs of saccharides, however, such as mannose–ribose and fucose–arabinose, were not completely separated. Good reproducibility of the analysis was achieved (standard deviations were less than 2.2% for sugar standards). The system can easily be adapted for the analysis of amino sugars or glycosidase digestion products[98].

Stepwise elution with borate buffers increasing both in borate concentration and pH is used in the JEOL system for automated sugar analysis[99].

Green[95] developed an automated carbohydrate analyzer based on a slightly different procedure. In this system, 15 sugar–borate complexes were eluted from the anion-exchange column is about 12 h. The eluate was monitored in a through-flow colorimeter after reaction with phenol in sulphuric acid.

Ohms *et al.*[100] reported the chromatographic separation of neutral sugars (sucrose, raffinose, cellobiose, maltose, lactose, ribose, rhamnose, mannose, fructose, arabinose, galactose and glucose) in a synthetic mixture in about 7.5 h on a strongly basic anion-exchange resin, Beckman 1-S, using instrumentation and methodology similar to that of Green[95].

Jolley and Freeman[101] developed an automated high-pressure carbohydrate analyzer consisting of a heated, high-pressure anion-exchange column (0.62 × 150 cm), made of stainless steel, filled with 6–12-μm Dowex 1-X8 (or Aminex A-27) resin; an eluent gradient-making device; a detection system consisting of a reaction column containing glass beads, in which the column effluent, 5% phenol and concentrated sulphuric acid are mixed; and a colorimeter to measure the absorbance of the reaction mixture at 480 and 490 nm[102,103]. In this system, a measured volume (1–2 ml) of standard or borated physiological fluid was introduced at the top of the chromatographic column via a six-port injection valve. Elution of sugars was accomplished in 20 h at an eluent flow-rate of 1.14 ml/min by increasing the buffer concentration linearly from 0.029 *M* sodium tetraborate–0.057 *M* boric acid to 0.147 *M* sodium tetraborate–0.283 *M* boric acid. The pH of the eluent was 8.5–8.6 during the elution and the column was operated at 55° and a pressure of 1000–2000 p.s.i. The column was regenerated for the next run by stripping the resin for 1 h with the most concentrated buffer and then equilibrating the resin for 3 h with the dilute buffer[101,104].

Using this system, about 40–50 peaks of carbohydrates or carbohydrate-like material appeared in a typical urine chromatogram, and a number of these could be tentatively identified by co-chromatography with standards (sucrose, raffinose, N-acetylglucosamine, maltose, lactose, ribose, fructose, arabinose, fucose, galactose, xylose, mannoheptulose, glucose and glucose-1-phosphate). Gas chromatographic identification of the trimethylsilyl derivatives of sugars has also been used[105].

Another useful identification method is the determination of the absorbance ratio of the sulphuric acid–phenol–eluate reaction mixture at 480 and 490 nm. Pentoses exhibit greater absorbance at 480 nm than at 490 nm, whereas hexoses have approximately the same absorbance at each wavelength. The lower detection limit for sugars is approximately 1 μg. Sugars, such as glucose, can be analyzed quantitatively with a satisfactory accuracy of ± 0.025 μmole over a range of sample amounts from 0.5 to 1 μmole[101,104].

Compounds other than simple sugars are also separated in the chromatographic apparatus and additional detection systems can be used for identification and quantitation. The compounds that form complexes with borate include both aliphatic and aromatic hydroxy and keto acids, as well as vitamins and polysaccharides. Sugars and some of their derivatives, however, can be measured without interference from these other compounds.

The possibility has been outlined of using modern computer techniques in which the chromatographic data from several automated analyzers are fed to a small

on-line digital computer[106]. This chromatographic data handling system can significantly increase the usefulness of the automated carbohydrate analyzer. It is possible that high-pressure anion-exchange chromatographic analysis may become a valuable tool in the clinical investigation of inherited disorders of carbohydrate metabolism by allowing the quantitation of many different sugars[107].

Using elution at a lower pH (about 7), the possible isomerization of sugars during analysis in alkaline borate solutions is eliminated. As the pH is decreased, a buffer of lower ionic strength, and consequently lower buffering capacity, is required for the elution. In order to increase the amount of ionizable borate at low pH and, at the same time, to obtain a higher capacity buffer, a system has been introduced[108] that contains an alcohol which forms ionized complex anions with borates.

A borate elution buffer containing an organic solvent (50% ethanol) was first used for sugar analysis by Nakamura and Mori[109]. Ethanol, of course, does not form borate complexes. Walborg *et al.*[108] employed an eluting buffer consisting of 0.4 *M* boric acid, 1.0 *M* glycerol, 0.050 *M* sodium chloride and 0.5 ml of toluene per litre, adjusted to pH 6.80 with sodium hydroxide. Using this eluent, good separations of sugar mixtures with high resolution were possible. Sub-micromole amounts of rhamnose, mannose, fucose, galactose and glucose were quantitatively separated in about 40 h on a 0.6 × 155 cm column of Dowex 2-X8 (200–400 mesh)[108]. The elution was performed at 50° at a flow-rate of 3.0 ml/h. The sugars in fractions of the eluate were analyzed by measuring the absorbance at 365 nm of the reaction mixture after the addition of aniline dissolved in acetic and orthophosphoric acids. This system is compatible with eluents that contain glycerol[110].

The use of a resin with a lower degree of cross-linkage and a higher porosity, Dowex 1-X4, allowed the attainment of exchange equilibrium at increased rates and greatly improved the chromatographic behaviour of the di- and trisaccharides. This made possible an extension of Walborg's method to higher molecular weight saccharides. The flow-rate could be increased three-fold in comparison with that reported previously. With a 0.6 × 150 mm column of Dowex 1-X4 (200–400 mesh), elution with a buffer composed of 0.4 *M* boric acid, 1.0 *M* glycerol, 0.035 *M* sodium chloride, 0.1% Brij-35 SP (polyoxyethylene(23) lauryl ether) and 0.5 ml of toluene per litre, adjusted to pH 6.70, at 60° resulted in a good separation of mixtures containing mono- and higher saccharides. A 10-component mixture containing sucrose, lactose, rhamnose, mannose, fucose, ribose, galactose, glucose, fructose and sorbose was separated completely in about 24 h. A higher resolution of di- and trisaccharides was possible when a buffer containing 0.2 *M* boric acid, 0.6 *M* glycerol, 0.1% Brij-35 SP and 0.5 ml of toluene per litre, adjusted to pH 6.70, was used for the elution at 40°. Using this system, the complete separation of sucrose, cellobiose, maltose, lactose and rhamnose was possible[111].

It is obvious that the separation of more complex mixtures can be improved by programming the composition of the eluting solvent during the elution. Mixtures of oligosaccharides were separated and determined using a 0.6 × 65 cm anion-exchange column of Chromobeads S (20 ± 1 μm). Boric acid–glycerol, boric acid–glycerol–sodium chloride and sodium tetraborate solutions were used in a nine-chamber Autograd apparatus to form a buffer gradient. A colorimetric determination of the saccharides was performed after reaction with cysteine–hydrochloric acid reagent in sulphuric acid. Sucrose, raffinose, maltose, stachyose, verbascose and an unidentified

saccharide were separated and determined in tissues from healthy and infected plants[112].

Glycerol in the eluting buffer may interfere with the colorimetric assay in the Walborg–Lantz method[111], as it contains primary hydroxyl groups that are subject to oxidation, and for this reason, 2,3-butanediol was substituted for glycerol in the buffer system[113].

A two-buffer stepwise elution system, utilizing a 0.6 × 85 cm column of Dowex 1-X4 (—400 mesh) operated at elevated temperatures, yielded an improved separation of complex saccharide mixtures as compared with the original Walborg–Lantz system. The elution was begun at 40 with a buffer of pH 7.0, containing 0.15 *M* boric acid, 0.5 *M* 2,3-butanediol, 0.1 % Brij-35 and 0.5 ml of toluene per litre. At an effluent volume of about 230 ml, the remaining sugars were eluted with a buffer of pH 7.0 composed of 0.8 *M* boric acid, 1.6 *M* 2,3-butanediol, 0.1 % Brij-35 and 0.5 ml of toluene per litre, changing the operating temperature to 60 (ref. 113).

This system, a two-stepwise elution with two borate–2,3-butanediol buffers at neutral pH, has been automated by employing the Hitachi Perkin-Elmer Model 034-04 liquid chromatograph. Aminex A-14, a 4 % cross-linked anion-exchange resin of 20 μm particle diameter, was used packed in 0.4 × 100 cm columns. The elution was performed under the same conditions as described above, except that higher flow-rates (20 ml/h) could be used with a column back-pressure of 15–28 atm. The analysis of the column effluent was performed by continuous monitoring at 310, 365 and 390 nm of the colour formed by reaction with aniline–acetic acid–orthophosphoric acid reagent. This reagent has the advantages of greater stability compared with the phenol sulphuric acid and orcinol reagents. The ratio of the absorbances at 365 and 390 nm has been utilized as a qualitative tool to distinguish between classes of saccharides, *e.g.*, between aldopentoses, aldo- and ketohexoses, and 6-deoxyaldohexoses[114].

Table 17 gives the retention times of various saccharides studied in this system. The separation can be achieved of any pair of saccharides whose retention times differ by as much as 30 min. The precision of analysis is about ±5 % and the resolution is comparable with those with the systems of Green[95], Ohms *et al.*[100] and Kesler[97]. As an example, the separation of an 11-component mixture containing melezitose, raffinose, maltose, rhamnose, lyxose, ribose, mannose, fucose, fructose, galactose and glucose can be achieved in about 11 h[114].

Another procedure has been reported for the automated determination of hexoses in the effluents from Dowex 1 columns in borate medium, involving the measurement of the fluorescence of the derivatives produced by reaction of 5-hydroxy-α-tetralone with hexoses in concentrated sulphuric acid. The high sensitivity of this method allowed determinations of amounts of <20 μg of hexoses[115]. Solms and Deuel[116] prepared a special resin containing covalently bound borate groups and studied the possibilities of separating sugar mixtures on this resin, utilizing specific interactions between the saccharides and the functional groups of the resin. Water, aqueous ethanol and hydrochloric acid solutions were employed as the eluents. This method gave no advantages over chromatography on the borate form of the strongly basic anion-exchange resin. It was possible to achieve a partial separation of binary mixtures containing galactose and ribose, and arabinose and ribose, on a 0.8 × 10 cm column packed with this resin[116].

The formation of complexes of saccharides with hydrogen sulphite ions has been utilized by several workers for the chromatographic separation of sugars on

TABLE 17

RETENTION TIMES OF SACCHARIDES IN AUTOMATED ANION-EXCHANGE SYSTEM

Stepwise elution with two borate–2,3-butanediol buffers on an Aminex A-14 (20 μm) column, 0.4 × 100 cm. Temperatures: 40° and 60°. The retention times were calculated on replicate samples containing saccharides present in the following amounts: 0.300 μmole of melezitose, 0.500 μmole of raffinose, 0.320 μmole of maltotriose, 0.250 μmole of stachyose, 2.0 μmoles of rhamnose, 1.5 μmoles of lyxose, 1.0 μmole of mannose, 1.5 μmoles of fucose, 2.0 μmoles of arabinose, 2.5 μmoles of xylose, 1.88 μmoles of sorbose and 2.5 μmoles of glucose. The values for sucrose, cellobiose, maltose, lactose, ribose, fructose and galactose were calculated using four different sample loads, varying between 0.2 and 5.0 μmole of each saccharide (as monosaccharide).

Saccharide	*Retention time*	
	Min	± *S.D.*
Sucrose	52	1.3
Melezitose	57	0.8
Raffinose	90	1.3
Cellobiose	96	1.7
Maltotriose	127	1.8
Maltose	151	3.6
Stachyose	243	6.1
Rhamnose	287	1.7
Lactose	306	5.5
Lyxose	457	5.1
Ribose	480	5.4
Mannose	510	1.0
Fucose	569	5.8
Arabinose	602	6.0
Fructose	604	5.4
Xylose	635	5.2
Sorbose	650	4.5
Galactose	657	4.2
Glucose	736	7.6

strongly basic anion-exchange columns in the hydrogen sulphite form. Ketoses do not form stable complexes with hydrogen sulphite ions, while aldoses yield stable α-oxysulphonic acids, and these two groups can be separated on the hydrogen sulphite form of anion-exchange resins.

Samuelson and Sjöström[117] separated a mixture of fructose, glucose and mannose and of fructose, xylose and mannose on a 150 × 9 mm column packed with strongly basic Amberlite IRA-400 (HSO_3^-), 0.12–0.30 mm. The sorption of sugars was much greater from aqueous ethanol than from water. Stepwise elution with 99.5% ethanol, 95% ethanol and water was used and the sugars were eluted in the order fructose, glucose and mannose.

Adacki and Sugiwara[118] could not achieve separations by elution with methanol or ethanol. Using stepwise elution with 75% *n*-propanol and water, they succeeded in obtaining the preparative separations of monosaccharidic and disaccharidic aldoses from ketoses on a 2 × 30 cm column of Amberlite IRA-400 (HSO_3^-), 0.2–0.4 mm. The presence of *n*-propanol influences the stabilities of the sugar–hydrogen sulphite complexes. It was possible, for example, to separate binary mixtures of fructose and glu-

cose, fructose, and maltose, lactulose and lactose, and rhamnose and xylose[118].

Chromatography on a hydrogen sulphite column has been used for the preparative separation of sugars in mixtures after epimerization of aldoses. The less common epimerization products (D-threopentulose, L-erythropentulose, D-lyxohexulose and D-glucoheptulose) could be thus separated from their parent compounds. Not only the separation of ketoses from aldoses was possible by this method, but also the preparation of chromatographically pure D-fructose, D-glucose and D-galactose from commercial products[119].

D. Chromatography of saccharides on dextran and cellulose ion exchangers

The chromatography of sugars on QAE-Sephadex (SO_4^{2-}) in mixed aqueous ethanol medium is discussed together with separations on polystyrene–divinylbenzene ion-exchange resins. The distribution coefficients of various sugars on this exchanger in 95% ethanol are given in Table 15 (ref. 83).

Chromatography on Sephadex ion exchangers is useful for the separation of various polysaccharides. As an example, Barker *et al.*[120] fractionated soil polysaccharides of bacterial origin on a 3.4 × 45 cm column of DEAE-Sephadex A-50, using a continuous linear ionic strength gradient from 0 to 2 *M* sodium chloride in a phosphate buffer at pH 6 (ref. 120).

Chromatography on a 0.9 × 58 cm column of DEAE-Sephadex A-25, using elution with a linear gradient (0–0.4 *M*) of sodium chloride, resulted in good separation of three monoglucosiduronates (estrone-3-glucosiduronate, 17β-estradiol-3-glucosiduronate and 17β-estradiol-17-glucosiduronate). This procedure has proved to be of particular value in the study of estrogen glucosiduronate metabolism[121].

DEAE-cellulose columns can be used for the efficient chromatographic fractionation of polysaccharide mixtures. At about pH 6, neutral polysaccharides are not sorbed or are only weakly sorbed by DEAE-cellulose (Cl^- or SO_4^{2-}), and can be readily eluted at the same pH by using buffers of increasing strength or dilute hydrochloric acid. Acidic polysaccharides such as pectic acid are sorbed readily at neutral and weakly acid pH, but can be eluted only by increasing the pH. The neutral polysaccharides are strongly sorbed on DEAE-cellulose (OH^-) and can be fractionated by elution with sodium hydroxide solutions, gradually increasing in concentration[122].

Wheat starch dextran was separated into three fractions by chromatography on a column of DEAE-cellulose (OH^-) using stepwise elution with water, 0.01, 0.05 and 0.1 *N* sodium hydroxide solution. A mixture of sugar beet araban and pectic acid was separated on a column of DEAE-cellulose (PO_4^{3-}). Araban was eluted gradually with 0.025, 0.05, 0.1 and 0.25 *M* sodium phosphate solution at pH 6.1, while gradient elution with sodium hydroxide concentration changing from 0.01 to 0.5 *N* was used for pectic acid. Both araban and pectic acid appeared in the effluent clearly resolved into several further peaks. Using the same elution technique, sugar beet pectic acid was further fractionated. Wheat pentosan was separated from serum albumin on DEAE-cellulose (PO_4^{3-}) by stepwise elution with 0.005 *M* sodium phosphate buffer (pH 8). The proteins remained sorbed on the column under these conditions and could be recovered at a lower pH and higher ionic strength by subsequent elution with 0.01 *M* sodium phosphate buffer (pH 6) and a solution 0.1 *M* in sodium chloride and 0.05 *M* in sodium dihydrophosphate[122].

The fractionation effect was increased when DEAE-cellulose (BO_3^-) and elution with sodium borate of increasing strength was used. Using this method, cold water-soluble wheat flour polysaccharides could be fractionated. Elution with water yielded a fraction of pentosan composed of xylose and arabinose only, and 0.01 *M* borate eluted a main pentosan fraction, containing small amounts of galactose and "spots" of protein. The fraction eluted with 0.1 *M* borate consisted of about 50% protein, 20% pentosan and 30% hexosan. Finally, a fraction of hexosan composed of only glucose was eluted with 0.5 *M* borate solution[122].

When a column of DEAE-cellulose (OH^-) was used for the chromatography of the hemicelluloses from black spruce, elution with water and 2 *N* ammonium acetate solution did not separate the xylan and mannan components. Elution with 7 *M* aqueous urea minimized hydrogen bonding between polysaccharides and cellulose and yielded a fraction free from xylose. By subsequent use of ammonium acetate in 7 *M* urea, mainly xylose polymers were eluted[93,123].

Five different polysaccharides (hyaluronic acid, chondroitinsulphuric acid A, heparin, β-heparin and a polysaccharidic by-product obtained during heparin preparation) were chromatographed on a 10 × 1 cm column of ECTEOLA-cellulose. For good separations, it was necessary to work at an acidic pH. The column was eluted stepwise with buffers of increasing chloride concentration (equimolar amounts of sodium chloride and hydrochloric acid, 0.1, 0.5, 0.7, 1.1, 1.4 and 2.5 *M*). Hyaluronic acid, chondroitinsulphuric acid and heparin had very different affinities for ECTEOLA-cellulose, which allowed their separation. Polysaccharidic by-product could be separated into four distinct fractions. Similar results were obtained when a constant concentration of 0.05 *M* hydrochloric acid was used in all steps, while the chloride concentration was regulated by increasing the amount of sodium chloride in the eluent. All separations were performed at 0–5° (ref. 124).

6. SUMMARY

A systematic survey of applications of ion-exchange chromatography in the analysis of aldehydes, ketones, ethers, alcohols, polyols and saccharides is given. The main part is concerned with the separation of saccharides on anion-exchange resins in the borate and hydrogen sulphite forms, and the separation of saccharides, aldehydes, ketones, ethers and alcohols by salting-out and solubilization chromatography on ion-exchange columns. Other ion-exchange chromatographic methods, the sorption behaviour of the compounds on ion-exchange resins and chromatography on ion-exchange papers and thin layers are also covered. The survey deals mainly with the literature for the period 1962–1970.

REFERENCES

1 G. Gabrielson and O. Samuelson, *Acta Chem. Scand.*, 6 (1952) 738.
2 O. Samuelson, *Z. Elektrochem.*, 57 (1953) 207.
3 B. Koser and P. Oesper, *Anal. Biochem.*, 17 (1966) 120.
4 V. Racotta and V. Iliescu, *Rev. Chim. (Bucarest)*, 18 (1967) 100.
5 G. Gabrielson and O. Samuelson, *Sv. Kem. Tidskr.*, 62 (1950) 214.
6 G. Gabrielson and O. Samuelson, *Sv. Kem. Tidskr.*, 64 (1952) 150.
7 E. Sjöström, *Sv. Kem. Tidskr.*, 64 (1952) 301.

8 E. Huff, *Anal. Chem.*, 31 (1959) 1626.
9 K. Christofferson, *Anal. Chim. Acta*, 33 (1965) 303.
10 K. Christofferson, *Sv. Papperstidn.*, 70 (1967) 540.
11 K. Christofferson, *Anal. Chim. Acta*, 31 (1964) 233.
12 G. Gabrielson, *J. Appl. Chem.*, 7 (1957) 533.
13 A. Breyer and W. Rieman, *Anal. Chim. Acta*, 18 (1958) 204.
14 A. Breyer and W. Rieman, *Talanta*, 4 (1960) 67.
15 R. A. Speckman and E. B. Collins, *Anal. Biochem.*, 22 (1968) 154.
16 S. J. Shaw and P. J. Smith, *J. Chem. Soc.*, (1968) 1882.
17 G. D. Manalo, A. Breyer, J. Sherma and W. Rieman, *J. Phys. Chem.*, 63 (1959) 1511.
18 J. Sherma, D. Locke and D. Bassett, *J. Chromatogr.*, 7 (1962) 273.
19 J. Sherma and W. Rieman, *Anal. Chim. Acta*, 19 (1958) 134.
20 O. Samuelson and H. Strömberg, *Acta Chem. Scand.*, 22 (1968) 1252.
21 T. Seki, *J. Chromatogr.*, 2 (1959) 667.
22 J. Sherma, D. A. Goldstein and R. Lucek, *J. Chromatogr.*, 38 (1968) 54.
23 J. Sherma and L. H. Pignolet, *Anal. Chim. Acta*, 34 (1966) 185.
24 I. T. Clark, *J. Chromatogr.*, 15 (1964) 65.
25 R. Sargent and W. Rieman, *Anal. Chim. Acta*, 18 (1958) 197.
26 J. Sherma and W. Rieman, *Anal. Chim. Acta*, 20 (1959) 357.
27 R. W. Gable and H. A. Strobel, *J. Phys. Chem.*, 60 (1956) 513.
28 H. Rückert and O. Samuelson, *Acta Chem. Scand.*, 11 (1957) 303.
29 D. Reichenberg and W. F. Wall, *J. Chem. Soc.*, (1956) 3364.
30 P. Radzitsky, *C. R. 31e Congr. Int. Chim. Ind., Liège, 1958.*
31 M. Mattisson and O. Samuelson, *Acta Chem. Scand.*, 12 (1958) 1386.
32 R. M. Wheaton and W. C. Bauman, *Ann. N.Y. Acad. Sci.*, 57 (1953) 159.
33 I. T. Clark, *Anal. Chem.*, 30 (1958) 1676.
34 N. S. Dabagov and A. A. Balandin, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1966) 1308.
35 N. S. Dabagov and A. A. Balandin, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1966) 1315.
36 F. Yaku and Y. Matsushima, *Nippon Kagaku Zasshi*, 87 (1966) 969.
37 H. Small and D. N. Bremer, *Ind. Eng. Chem., Fundam.*, 3 (1964) 361.
38 R. Sargent and W. Rieman, *J. Org. Chem.*, 21 (1956) 594.
39 R. Sargent and W. Rieman, *J. Phys. Chem.*, 61 (1957) 354.
40 M. Mattisson and O. Samuelson, *Acta Chem. Scand.*, 12 (1958) 1395.
41 O. Samuelson, *Nord. Kemikermöde, 9th, Aarhus, 1956*, Vol. II, p. 105.
42 O. Samuelson and H. Strömberg, *Carbohyd. Res.*, 3 (1966) 89.
43 O. Samuelson and H. Strömberg, *J. Food Sci.*, 33 (1968) 308.
44 J. Sherma and W. Rieman, *Anal. Chim. Acta*, 18 (1958) 214.
45 J. Sherma and J. D. Lowry, *Anal. Lett.*, 1 (1968) 707.
46 L. P. Zill, J. X. Khym and G. M. Cheniae, *J. Amer. Chem. Soc.*, 75 (1953) 1339.
47 R. Sargent and W. Rieman, *J. Phys. Chem.*, 60 (1956) 1370.
48 R. Sargent and W. Rieman, *Anal. Chim. Acta*, 16 (1957) 144.
49 N. Spencer, *J. Chromatogr.*, 30 (1967) 566.
50 J. Sherma and D. E. Thompson, *Anal. Chim. Acta*, 32 (1965) 181.
51 M. E. Evans, L. Long and F. W. Parrish, *J. Chromatogr.*, 32 (1968) 602.
52 J. K. N. Jones, R. A. Wall and A. O. Pittet, *Chem. Ind. (London)*, (1959) 1196.
53 J. K. N. Jones, R. A. Wall and A. O. Pittet, *Can. J. Chem.*, 38 (1960) 2285.
54 S. Roseman, H. Abeles and A. Dorfman, *Arch. Biochem. Biophys.*, 36 (1952) 232.
55 P. W. Austin, F. E. Hardy, J. G. Buchanan and J. Baddiley, *J. Chem. Soc.*, (1963) 5350.
56 L. Hough, J. E. Priddle and R. S. Theobald, *Chem. Ind. (London)*, (1960) 900.
57 L. J. Lefevre, *U.S. Pat.*, 3,044,906 (1962).
58 V. F. Felicetta, M. Lung and J. L. McCarthy, *Tappi*, 42 (1959) 496.
59 J. K. N. Jones and R. A. Wall, *Can. J. Chem.*, 38 (1960) 2290.
60 R. M. Saunders, *Carbohyd. Res.*, 7 (1968) 76.
61 H. G. Walker and R. M. Saunders, *Cereal Sci. Today*, 15 (1970) 140.
62 K. Steiner, H. Neukom and H. Deuel, *Chimia*, 12 (1958) 150.
63 D. A. Applegarth and G. G. S. Dutton, *Tappi*, 48 (1965) 204.
64 R. M. McCready and J. C. Goodwin, *J. Chromatogr.*, 22 (1966) 195.

65 A. G. Lloyd, *Biochem. J.*, 83 (1962) 455.
66 V. I. Maksimov and V. A. Mosin, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1969) 2579.
67 H. Rückert and O. Samuelson, *Sv. Kem. Tidskr.*, 66 (1954) 337.
68 H. Rückert and O. Samuelson, *Acta Chem. Scand.*, 11 (1957) 315.
69 O. Samuelson and B. Swenson, *Acta Chem. Scand.*, 16 (1962) 2056.
70 B. Arwidi and O. Samuelson, *Sv. Kem. Tidskr.*, 77 (1965) 84.
71 L. I. Larsson and O. Samuelson, *Acta Chem. Scand.*, 19 (1965) 1357.
72 O. Samuelson and B. Swenson, *Anal. Chim. Acta*, 28 (1963) 426.
73 P. Jonsson and O. Samuelson, *Sci. Tools*, 13 (1966) 17.
74 J. Dahlberg and O. Samuelson, *Sv. Kem. Tidskr.*, 75 (1963) 178.
75 J. Dahlberg and O. Samuelson, *Acta Chem. Scand.*, 17 (1963) 2136.
76 B. Arwidi and O. Samuelson, *Sv. Papperstidn.*, 68 (1965) 330.
77 B. Arwidi and O. Samuelson, *Anal. Chim. Acta*, 31 (1964) 462.
78 L. I. Larsson, O. Rammäs and O. Samuelson, *Anal. Chim. Acta*, 34 (1966) 394.
79 L. I. Larsson and O. Samuelson, *Mikrochim. Acta*, (1967) 329.
80 E. Martinsson and O. Samuelson, *J. Chromatogr.*, 50 (1970) 429.
81 O. Samuelson and H. Strömberg, *Z. Anal. Chem.*, 236 (1968) 506.
82 L. I. Larsson and O. Samuelson, *Sv. Papperstidn.*, 71 (1968) 432.
83 P. Jonsson and O. Samuelson, *J. Chromatogr.*, 26 (1967) 194.
84 P. Jonsson and O. Samuelson, *Anal. Chem.*, 39 (1967) 1156.
85 J. X. Khym and L. P. Zill, *J. Amer. Chem. Soc.*, 73 (1951) 2399.
86 J. X. Khym and L. P. Zill, *J. Amer. Chem. Soc.*, 74 (1952) 2090.
87 L. P. Zill and N. E. Tolbert, *J. Amer. Chem. Soc.*, 76 (1954) 2929.
88 A. Hallén, *Acta Chem. Scand.*, 14 (1960) 2249.
89 C. W. Parr, *Biochem. J.*, 56 (1954) 27.
90 R. Syamananda, R. C. Staples and R. J. Block, *Contrib. Boyce Thompson Inst.*, 21 (1962) 363.
91 C. M. Mundie, M. V. Cheshire and J. S. D. Bacon, *Biochem. J.*, 108 (1968) 51P.
92 G. Bedetti, G. D'Agnolo and F. Pocchiari, *J. Chromatogr.*, 49 (1970) 53.
93 *Technicon Sugar Chromatography System Brochure*, Technicon, Ardsley, N.Y., 1968.
94 R. B. Kesler, *Technicon Int. Symp. on Autom. Anal. Chem., 5th, New York, Oct. 1965*, London, 1966, p. 174.
95 J. G. Green, *Nat. Cancer Inst. Monogr.*, No. 21 (1966) 447.
96 R. Carubelli, *Carbohyd. Res.*, 2 (1966) 480.
97 R. B. Kesler, *Anal. Chem.*, 39 (1967) 1416.
98 Y. C. Lee, J. F. McKelvy and D. Lang, *Anal. Biochem.*, 27 (1969) 567.
99 *JLC-3BC, Liquid Chromatograph Brochure*, JEOL, Tokyo.
100 J. I. Ohms, J. Zec, J. V. Benson and J. A. Patterson, *Anal. Biochem.*, 20 (1967) 51.
101 R. L. Jolley and M. L. Freeman, *Clin. Chem.*, 14 (1968) 538.
102 C. D. Scott, R. L. Jolley, W. W. Pitt and W. F. Johnson, *Amer. J. Clin. Pathol.*, 53 (1970) 701.
103 R. L. Jolley, W. W. Pitt and C. D. Scott, *Anal. Biochem.*, 28 (1969) 300.
104 R. L. Jolley, K. S. Warren, C. D. Scott, J. L. Jainchill and M. L. Freeman, *Amer. J. Clin. Pathol.*, 53 (1970) 793.
105 W. C. Butts and R. L. Jolley, *Clin. Chem.*, 16 (1970) 722.
106 C. D. Scott, J. M. Jansen and W. W. Pitt, *Amer. J. Clin. Pathol.*, 53 (1970) 739.
107 D. S. Young, *Amer. J. Clin. Pathol.*, 53 (1970) 803.
108 E. F. Walborg, L. Christensson and S. Gardell, *Anal. Biochem.*, 13 (1965) 177.
109 M. Nakamura and K. Mori, *Biochim. Biophys. Acta*, 34 (1959) 546.
110 E. F. Walborg and L. Christensson, *Anal. Biochem.*, 13 (1965) 186.
111 E. F. Walborg and R. S. Lantz, *Anal. Biochem.*, 22 (1968) 123.
112 P. Auriol, *C. R. Acad. Sci., Ser. D*, 269 (1969) 1183.
113 E. F. Walborg, D. B. Ray and L. E. Öhrberg, *Anal. Biochem.*, 29 (1969) 433.
114 E. F. Walborg and L. E. Kondo, *Anal. Biochem.*, 37 (1970) 320.
115 P. Weber, I. Bornstein and R. J. Winzler, *Anal. Biochem.*, 14 (1966) 100.
116 J. Solms and H. Deuel, *Chimia*, 11 (1957) 311.
117 O. Samuelson and E. Sjöström, *Sv. Kem. Tidskr.*, 64 (1952) 305.
118 S. Adacki and H. Sugiwara, *Arch. Biochem. Biophys.*, 100 (1963) 468.
119 B. Lindberg and K. N. Slessor, *Carbohyd. Res.*, 5 (1967) 286.

120 S. A. Barker, M. H. B. Hayes and R. G. Simmonds, *Carbohyd. Res.*, 5 (1967) 13.
121 R. Hobkirk and M. Nilsen, *Anal. Biochem.*, 37 (1970) 337.
122 H. Neukom, H. Deuel, W. J. Heri and W. Kündig, *Helv. Chim. Acta*, 43 (1960) 64.
123 D. A. Applegarth and G. G. S. Dutton, *J. Chromatogr.*, 15 (1964) 246.
124 N. R. Ringertz and P. Reichard, *Acta Chem. Scand.*, 13 (1959) 1467.