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Chromatography of Carbohydrates on Ion-exchange Resins

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The literature data dealing with an effective method for the analysis of a mixture of carbohydrates using ion-exchange resins are reviewed. The separation of carbohydrates by this method is based on three main principles: (1) ion exchange; (2) adsorption and gel filtration; (3) partition chromatography. The use of ion-exchange resins yields clear cut qualitative and quantitative results and the analytical process may be automated. The method is distinguished by higher reliability and sensitivity and satisfactory reproducibility.
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I. INTRODUCTION

One of the main problems in carbohydrate chemistry is the separation of complex mixtures of mono- and oligosaccharides and the identification and quantitative determination of their components. A wide variety of chromatographic methods are employed for the solution of this problem. Here mention should be made primarily of paper chromatography[1,2], partition column chromatography on cellulose[3], adsorption column chromatography using a mixture of charcoal and zeolites[4], and gas–liquid chromatography[5–8]. Thin-layer chromatography of carbohydrates[9,10] is less widely used, although in many cases it yields good results[10]. It is also interesting to note that considerable advances have been achieved at the present time in the separation and quantitative determination of carbohydrates by chromatography on ion-exchange resins, although this method has not yet attracted the attention that it deserves. The separation of carbohydrates on ion-exchange resins depends on three different processes: (1) ion exchange; (2) adsorption on ion-exchange resins; (3) partition between the aqueous phase of the resin and the organic stationary phase. Nevertheless in many cases only one of these plays a decisive role and one can therefore adopt a conventional classification of such separation into ion-exchange adsorption, and partition chromatography on ion-exchange resins and treat each separately.

II. ION-EXCHANGE CHROMATOGRAPHY

1. Chromatography on Neutral Carbohydrates

The method of ion-exchange column chromatography for the separation of neutral monosaccharides was first proposed at the beginning of the 1950's.[11,12] It is well known that borate ions react with carbohydrates containing vicinal hydroxy-groups with formation of negatively charged carbohydrate-borate complexes. It has been established that a quite satisfactory separation of the mixture of carbohydrates may be achieved on a highly basic anion-exchange resin, such as Dowex-1, when the chromatography is performed in borate buffers[11]. Mannose and fructose, arabinose and xylose, and sucrose and maltose have been separated by this method. However, it should be noted that the method suffers from the disadvantage of unduly large volumes of eluates (up to 1.5 litres), despite the fact that relatively small columns (0.5 × 11 cm) are employed. Subsequently various ion exchangers and modifications of the method were used for the chromatographic separation of the borate complexes of carbohydrates[12–20].

In 1959 Nakamura and Mori[13] described the separation of carbohydrates in 50% ethanol by ion-exchange chromatography using the above principles. In order to overcome the basic disadvantage of the method (large volumes of eluates), Hallén[14] eluted the resin with solutions at a higher ionic strength (0.01–0.03 M borax solution), the fractions being analysed with the aid of anilinium acetate[15]. The method is distinguished by high sensitivity (it is suitable for the separation and determination of microgramme quantities of the carbohydrate) and can be used directly for the quantitative analysis of the hydrolysates. However, unfortunately when this method is employed, satisfactory separation cannot be achieved in many cases. In particular mannose is eluted with lyxose, ribose with fucose, arabinose with galactose, and xylose with glucose. Attempts were made to improve Hallén's system by separating the borate complexes of neutral sugars at an elevated temperature (50°C), pH 6.8,[16] and using Dowex 2 × 8 in the borate-form. The fractions were analysed with the aid of anilinium acetate by a specially developed method[17]. The authors succeeded in achieving a satisfactory separation of a mixture of rhamnose, mannose, fucose, galactose, and glucose. This modification of the method is distinguished by satisfactory reproducibility, permits a quantitative analysis of hydrolysates, and has been successfully employed in the analysis of the monosaccharide composition of many glycoproteins[17]. Automatic analysis of eluates[18–20] and the use of a special anion exchanger[20] made it possible to overcome many disadvantages of the method and to achieve a separation of 12–15 mono- and oligo-saccharides. Ion-exchange resins have also been used to separate polyols, for which paper chromatography gives unsatisfactory results. The method of separation of polyols in the form of borate complexes on highly basic anion exchangers in the borate-form[21,22], which permits the analysis of fairly complex mixtures of such compounds, has been most widely used.

2. Chromatography of Acid Carbohydrates

Chromatography of acids derived from sugars on ion-exchange resins has been described in a number of publications [14,23-34]. Highly basic ion-exchange resins have been mainly used for this purpose. In view of the instability of uronic acids in an alkaline medium (particularly at elevated temperatures), high pH values must be avoided during chromatography. Dziewiatkowski [29] employed Amberlite IRA 401 × 2 (in the acetate-form) with a particle size of 200–400 mesh in a 0.9 × 36 cm column for the separation of uronic acids. The columns were eluted with an acetate buffer (0.1 M sodium acetate, pH 5.9) at a constant flow rate through the column (1 ml/5 min). Satisfactory separation of a number of uronic acids and the corresponding lactones was achieved. Even better results were obtained by Samuelson and coworkers [30,33] who employed Dowex 1 × 8 anion-exchange resin with very small and carefully fractionated particles (down to 13–17 μm) and eluted with acetic acid or sodium acetate solutions.

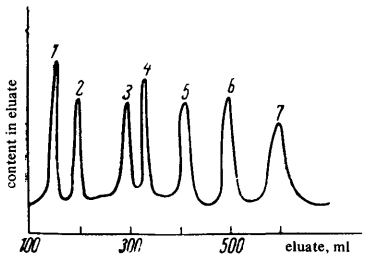


Figure 1. Chromatography of a mixture of uronic acids on Dowex-1 × 8 resin in the acetate-form (particle size 15–17 μm) in a 6 × 880 mm column; elution with 1 M acetic acid at 30°C at a rate of 0.97 ml min^{-1}; mixture components (1.5–2 μg each): 1) D-glucoheponic acid; 2) 6-β-D-glucopyranuronosyl-D-galactose; 3) D-galacturonic acid; 4) D-guluronic acid; 5) L-iduronic acid; 6) D-mannuronic acid; 7) D-glucuronic acid.

Best results were achieved with simultaneous elution by sodium acetate and acetic acid [30,31]. Satisfactory separation of a fairly complex mixture of uronic acids was then observed (Fig. 1). The use of very small particles of resin and their careful fractionation increases the column efficiency and leads to better separation. The time required to separate seven uronic acids is 10–11 h. To reduce this period, larger resin particles were employed. However this greatly impaired the results. Nevertheless, this procedure may be useful in many special cases, for example in the separation of a mixture of galacturonic and glucuronic acids [31]. In these studies the eluates were analysed by carbazole or dichromate methods. The carbazole reaction is known to give different colour intensities with various uronic acids, which permits a rapid identification of the peaks. The nature of the uronic acid may be inferred also from the ratio of the peak areas obtained in the carbazole and dichromate determinations. This quantity is constant and characteristic of different uronic acids.

In ion-exchange chromatography a fully defined elution sequence of the various components of the carbohydrate mixture is observed. Neutral monosaccharides, which are hardly separated under these conditions [34], are eluted initially, are followed by aldobiuronic acids, and finally by simple uronic acids, the elution sequence of which depends on many factors and cannot be predicted beforehand.

In order to separate aldobiuronic acids, we employed dilute sodium acetate solutions [30]. Then it was shown that the use of borax solutions as eluents greatly improves the separation of aldobiuronic acids [33]. The partition coefficients of all the uronic and aldobiuronic acids decrease very rapidly with the concentration of borax solutions.

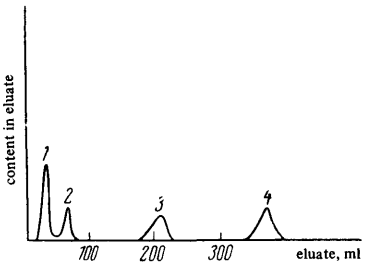


Figure 2. Separation of a mixture of certain aldobiuronic acids on Dowex-1 × 8 resin in the borate-form (particle size 23–25 μm) in a 3 × 870 mm column; elution with 0.12 M borax solution at a rate of 8.45 ml cm^{-2} min^{-1}; mixture components: 1) 4-*O*-methyl-α-D-glucopyranuronosyl-1→2-D-xylose; 2) α-D-galactopyranuronosyl-1→4-D-xylose; 3) β-D-glucopyranuronosyl-1→6-D-galactose; 4) D-glucuronic acid.

This is typical of hydroxyacids forming borate complexes [35], Therefore, in order to achieve rapid elution of the components, one may increase the borax concentration in the eluent solution. Fig. 2 presents as an example the separation of a mixture of certain aldobiuronic acids and D-glucuronic acid. Although carbohydrates are eluted in fairly diffuse bands, their separation is quite satisfactory. Knowledge about the borate complexes of hydroxy acids is very limited, but certain correlations between their positions on chromatograms and structures may be made. One of the rules states that the partition coefficients of hydroxyacids should increase with the number of free hydroxy-groups. Thus one must expect the earlier appearance of 4-*O*-methyl-D-glucuronic acid compared with D-glucuronic acid, which in fact does happen. Similarly, aldobiuronic acids are eluted more slowly than their methylated derivatives.

The study of the effect of the flow rate of the eluent solution on the separation of aldobiuronic acids showed that a sharp decrease of the flow rate leads to only a slight narrowing of the peak and therefore in the ion-exchange chromatography of aldobiuronic acids fairly rapid elution can be successfully employed.

When the temperature is increased, the separation is improved, but the degradation of aldobiuronic acids is at the same time accelerated appreciably. The use of an

automatic fraction analyser greatly accelerates and simplifies the process. In addition, automatic fraction analysis permits a more accurate determination of the peak positions on the chromatogram. It is then found that the deviation of the peak positions of uronic acids on chromatograms does not exceed 2%. The peak position characteristic of each acid can be successfully used for identification.

The separation of aldonic and aldobionic acids on anion exchangers has been described by Samuelson[36-39]. The method has been successfully applied to the analysis of sulphide liquors[41,42] and to the determination of terminal aldonic acid in cellulose[12] and hemicellulose[43].

In the elution by acetate solution a general tendency is observed in the series of hydroxyacids that for the same number of carboxy-groups acids containing more hydroxy-groups are eluted faster than those with fewer hydroxyls[44]. With few exceptions, this rule has been confirmed also in the series of aldonic and aldobionic acids[38,39]. The relation indicates that in this case the molecular size is the main factor determining the partition coefficient. An exception is mannonic acid, which is eluted with pentonic acids and D-glycero-L-mannoheptonic acid, which has a higher partition coefficient than many hexonic acids. These exceptions to the rules may be accounted for by the lower volumes of these hydrated anions, due to intramolecular hydrogen bonding, than for the other diastereoisomers.

From the practical point of view, it is interesting to note that the same elution sequence is reproduced also after prolonged operation of the columns. Anions differing in partition coefficients by 10% and more can be successfully separated. On elution by acetic acid and sodium acetate solution, the following relation between configuration and elution sequence is observed in the series of pentonic and hexonic acids: ribo-, arabo-, xylo-, lyxo-, the acids with the configurations at the beginning of the sequence being eluted earlier. Thus the position on the chromatogram can provide information about the structure of many aldonic acids and is of interest for their identification even when an authentic specimen is not available. As already pointed out, aldbiuronic acids are retained by the resin much less strongly than aldonic acids. On elution by sodium acetate, appreciable differences were observed between the partition coefficients of melibionic, lactobionic, and cellobionic acids, while the differences between cellobionic and maltobionic acids are insignificant[38]. In a borate medium aldonic acids are eluted in the following sequence: xylonic, arabonic, mannonic, gluconic, and galactonic[36,38]. Comparison of the results of chromatography in borate and acetate media shows that borate solutions are preferable for a complex mixture of aldonic acids; aldobionic acids are also satisfactorily separated. However, in a borate medium the latter are eluted almost simultaneously with aldonic acids. Therefore, when it is necessary to separate mixtures of aldonic and aldobionic acids, a preliminary separation of the acids into groups in an acetate medium is required, after which the borate method may be used to analyse the components in the individual groups. A promising method is chromatography on ion-exchange paper, which makes it possible to combine the useful aspects of ion-exchange and paper chromatography. The method has been successfully used by Oertel[25], who employed anion-exchange paper for the separation of uronic acids, lactones, and glucuronides and obtained a completely satisfactory result, despite the fact that the equilibrium between the acid and the corresponding lactone complicates the chromatographic pattern. One may also note the application of cation-exchange papers in the H^+-form for the separation of sugar acids[24]. However, in this case the ion exchanger plays the role of an acid, which is used in the developing systems in paper chromatography in order to avoid "tails". Thus the employment of ion exchange permits the analysis of mixtures of virtually any classes of mono- and oligosaccharides.

III. ADSORPTION CHROMATOGRAPHY

The results obtained by many investigators for the adsorption of polyhydric alcohols by ion-exchange resins[45-48] indicate that the latter may be used to separate carbohydrates by column chromatography. With increase in their size, the affinity of molecular for ion-exchange resins is reduced and thus large molecules are eluted first from the resin. This sequence breaks down, however, for methylated derivatives of carbohydrates, the most methylated derivatives being the last to be eluted by water from the columns[49,50]. The "molecular sieve" effect will also have an undoubted influence and therefore the percentage cross-linking in the resin and the salt form in which it is employed for chromatography are of definite importance. Both cation exchangers[49-55] and anion exchangers[56-58] have been used to separate mixtures of carbohydrates. Water was the eluent in all cases. Best results with cation exchangers were obtained on Dowex-50W (2–8% of divinylbenzene) in various salt forms. The barium-form[50] was used to separate monosaccharides. In one of our papers[51] the successful separation of D-xylose and L-arabinose on Dowex-50W (Ca^{2+}-form) was described. Mixtures of oligosaccharides or methylated monosaccharides have been separated with the aid of this resin in the lithium-form[52]. This form of the resin was also employed in the chromatography of a mixture of raffinose, sucrose, and glucose[49-52]. Satisfactory separation was then observed only for large differences in molecular weight and mixtures of carbohydrates with similar molecular weights could not be separated. More satisfactory results were obtained recently with the same ion-exchange resin in the potassium-form[53-55]. This method permits the fractionation of oligosaccharides in accordance with their molecular weights and the preparative separation of many mixtures with known monosaccharide compositions. Monosaccharides and their derivatives, particularly glucose and its mono- and di-isopropylidene derivatives, have been particularly satisfactorily separated. The use of anion exchangers did not show any advantages compared with cation exchangers. Reducing and non-reducing sugars have been separated on Amberlite IRA-400 (OH^--form) anion-exchange resin[56]. A mixture of raffinose, sucrose, and glucose have been fractionated on the basic forms of De-Acidite FF and preliminary separation of glycosides has been achieved on Dowex-1 resin[58].

The above method of fractionating mono- and oligosaccharides with the aid of ion-exchange resins is in many ways analogous to gel filtration on different types of Sephadex and biogels. The method is distinguished by rapidity and economy and constitutes a useful addition to other procedures for the separation of carbohydrate mixtures[55].

IV. PARTITION CHROMATOGRAPHY

A fundamental study by Samuelson and coworkers dealt with the partition chromatography of carbohydrates on ion-exchange resins. Preliminary studies of the sorption of monosaccharides and polyols from aqueous alcoholic solutions by ion-exchange resins[59-61] showed that the latter may be successfully employed for the partition chromatography of carbohydrates. The early attempts[62,63] gave promising results, after which the method was developed further during recent years.

Since the diffusion of carbohydrates within the resin particles is slow, very finely ground resins are employed for chromatography. The rate of sorption decreases rapidly with increasing concentration of ethanol, and therefore unduly high concentrations of the latter can lead to unsatisfactory separation. A decrease of the flow rate of the eluent solution leads to sharper peaks and the separation is appreciably improved. A higher rate of elution is permissible in the separation of small amounts of carbohydrates[64,65]. In partition chromatography on ion-exchange resins, monosaccharides are eluted before disaccharides and these in turn appear before higher oligosaccharides. Hitherto, in many studies on the partition chromatography of carbohydrates, highly basic anion exchangers with a styrene–divinylbenzene matrix (Dowex-1, Dowex-21, T4, T5B, etc.) have been employed in the sulphate-form with a wide variety of particle sizes. Thus, using a 10 × 840 mm column with Dowex-1 × 8 in the sulphate-form with a particle size of 45–75 μm and eluting with 74% ethanol, Samuelson and Swenson[64,65] successfully separated glucose and sucrose. In the separation of more complex mixtures, they employed stepwise gradient elution. Fig. 3 illustrates the separation of carbohydrates on molecular sieves by this method.

The use of a resin in the chloride-form[66] usually sharply impairs the results. Nevertheless mannose and xylose are satisfactorily separated in this case too. The chromatography of monosaccharides on highly basic resins in the bisulphite-form has been described in a number of papers[66,67]. The method ensures a satisfactory separation of ketoses from aldoses. The use of highly basic anion exchangers with more polar matrices has been described—in particular dextran containing quaternary ammonium ion-exchange groups has been employed[68]. In many cases this led to an improvement of separation but no fundamental differences were observed. Cation exchangers have also been successfully used in the partition chromatography of carbohydrates[69,70].

The fractions were initially analysed[64,65] by the phenol-sulphuric acid method and then an automatic fraction analyser began to be used[65,69,71,72]; part of the eluate and the reactants were then introduced into the analysing system, by a proportionating pump. Automatic analysis yields satisfactorily reproducible results.

In order to elucidate the possibility of separating the various carbohydrates, the positions of the peaks on the chromatograms are determined and the partition coefficients D_V are calculated[66]. Small variations in peak positions may be observed as a function of the rate of elution, the concentration of the eluent, and elution temperature. However, the relative partition coefficients of various carbohydrates depend to a lesser degree on the experimental conditions. Therefore in the identification of different carbohydrates, one may employ the ratios of their partition coefficients and the partition coefficient of any one monosaccharide chosen as a standard. The sorption of sugars by anion exchangers depends on many factors. One of these is partition between the water associated with the resin and the less polar eluent[59]. The effect of this factor should lead to an increase of the partition coefficient with increase in the number of hydroxy-groups and with decrease in the number of non-polar groups in the carbohydrate. One may then expect an increase of the partition coefficient in the series tetroses < pentoses < hexoses, and deoxy- and methylated derivatives should have smaller partition coefficients than the corresponding normal sugars. However, in addition to this factor, the interaction of the carbohydrate with the matrix and the ion-exchange groups of the resin, which may alter the above elution sequence[66], also plays an important role.

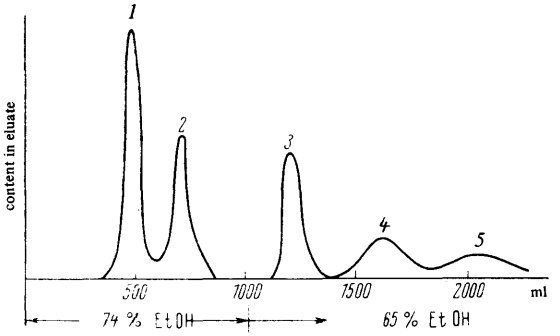


Figure 3. Separation of a mixture of carbohydrates in accordance with their molecular weights on Dowex-1 × 8 resin in the sulphate-form (particle size 45–75 μm) in a 10 × 840 mm column; gradient elution with alcoholic solutions at a rate of 0.8 ml min^{-1} cm^{-2} at 28°C; mixture components: 1) glucose; 2) sucrose; 3) raffinose; 4) stachyose; 5) verbascose.

Thus at the present time the elution sequence within each group of carbohydrates cannot be established *a priori* and is determined only empirically. It is noteworthy that in the majority of cases partition chromatography of carbohydrates and the ion-exchange resins gives a fairly satisfactory separation, which allows quantitative determinations. The sole factor which restricts the application of this method is the extremely low rate of diffusion within the resin particles. Therefore it is necessary to employ very small and as far as possible more porous particles. Thus, when a resin with a particle diameter of 45–75 μm is used, only partial separation of glucose and sucrose is observed, whereas particles with dimensions of 15–40 μm give a complete separation[73]. The employment of small particles permits the analysis also of monosaccharides[73] and it is possible to achieve a quantitative separation of mono- and oligo-saccharides[73,74]. The quality of the analysis depends to a considerable degree on the thoroughness of the fractionation of the resin. An increase of ethanol concentration also improves the results, provided that the consequent decrease of the rate of diffusion is compensated by a decrease of the resin particle size. In order to avoid under these conditions a sharp increase of column resistance to flow and to reduce the duration of analysis, the use of a mixture of an ion-exchange resin and Celite has been suggested[75]. It has been shown in a number of studies[66,69,70,72] that the partition coefficients of

the majority of carbohydrates in aqueous alcoholic mixtures differ appreciably. Fig. 4 presents the separation pattern for a mixture of twelve monosaccharides on an anion-exchange resin in the sulphate-form. The quality of the separation permits a quantitative analysis. The slight overlapping of the rhamnose and fucose peaks and of the arabinose and lyxose peaks does not affect the results of such analysis and, moreover, may be eliminated by increasing the concentration of ethanol or by increasing the column size. It has been established[76] that, in a quantitative determination of the monosaccharides present in wood hydrolysates, partition chromatography on anion-exchangers with automatic determination of the sugar concentrations in the eluates is a more convenient and accurate method than paper chromatography. The use of the orcinol method and careful fractionation of T5B anion exchanger (particle size 10–15 μm) in the sulphate-form permitted the development of an automatic micromethod for the analysis of a mixture of monosaccharides containing 6–30 μg of each component[77]. Partition chromatography on anion-exchange resins has been employed also for the separation of methylglycosides and methylated monosaccharide derivatives[78]. It was found that 94 wt. % ethanol, which ensures the complete separation of a number of the above derivatives, is optimum for elution[78].

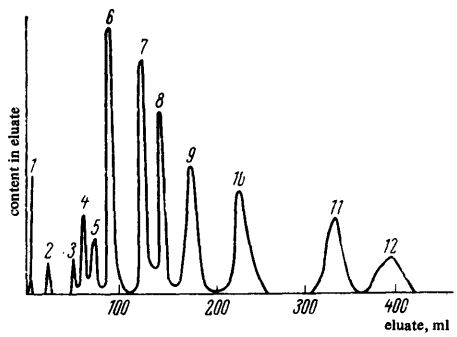


Figure 4. Analysis of a mixture of monosaccharides by partition chromatography on Dowex-21K anion-exchange resins in the sulphate-form (particle size 1–16 μm) in a 6 × 760 mm column; elution with 86% ethanol at a rate of 0.9 ml min^{-1} cm^{-2} at 75°C; mixture components (0.05–0.3 mg): 1) digitoxose; 2) 2-deoxyribose; 3) 2-deoxygalactose; 4) rhamnose; 5) fucose; 6) ribose; 7) lyxose; 8) arabinose; 9) xylose; 10) mannose; 11) galactose; 12) glucose.

Satisfactory separation of monosaccharides has been achieved on Dowex-50W × 8 cation-exchange resin with a very small particle size (14–17 μm) and elution by ethanol–water mixtures[69,70]. The ionic form of the cation exchanger has a marked influence on the partition coefficients. For monosaccharides, the latter increase in the sequence Li < Na < K. For all the sugars and all ionic forms of the cation-exchange resins, the partition coefficients decrease with increasing temperature. This finding accords with the results obtained on anion-exchange resins[71]. With small exceptions, the partition coefficients increase with the number of hydroxyls in the monosaccharide. A typical chromatogram is presented in Fig. 5, which shows that very satisfactory separation of seven different monosaccharides, including rhamnose and 2-deoxyglucose, which are not separated on anion exchangers in the sulphate-form, is achieved on a cation exchanger. Moreover, in contrast to anion exchangers, satisfactory results have been obtained for ketoses on cation exchangers. The lithium-form of the resin gives a better separation of deoxy-sugars than the sodium- and potassium-forms, but in work with the lithium-form a much higher pressure is required to maintain a constant rate of elution.

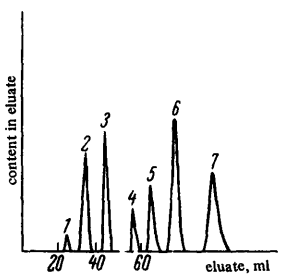


Figure 5. Separation of monosaccharides on Amberlite IR-120 cation-exchange resin in the Li^+-form; elution with 92.4% ethanol at a rate of 1.2 ml min^{-1} cm^{-2} at 75°C; mixture components: 1) 0.2 mg of rhamnose; 2) 0.4 mg of 2-deoxyglucose; 3) 0.6 mg of xylose; 4) 0.4 mg of arabinose; 5) 0.4 mg of tagatose; 6) 0.8 mg of glucose; 7) 1.5 mg of galactose.

One of the significant disadvantages of cation-exchange resins compared with anion exchangers is the poor separation of glucose and mannose. Partition chromatography has been successfully used also for the analysis of mixtures of polyols. Samuelson and Strömberg[79] suggested that in a quantitative determination of the components of such a mixture, they should be subjected to periodate oxidation with subsequent determination of the resulting formaldehyde with pentane-2,4-dione. The method permits an automatic fraction analysis, is distinguished by high accuracy and satisfactory reproducibility, and is suitable for microanalytical work. The subsequent application of the orcinol method, which does not give a colour reaction with polyols, and periodate oxidation permits the analysis of mixtures containing both aldoses and polyols. Various polyols are separated under the conditions already described for carbohydrates. A typical chromatogram obtained for polyols and aldoses on an 8 × 852 mm column with the highly basic T5B anion-exchange resin in the sulphate-form with a particle size of 3-17 μm is presented in Fig. 6. In elution with 86% ethanol at 75.5°C the duration of analysis is 11 h. Very satisfactory separation of the majority of polyols and aldoses is then observed, and only the peaks of dulcitol and mannitol overlap, which precludes an accurate quantitative determination of these polyols. The separation of mannose and dulcitol is also incomplete. To improve the results, elution with higher ethanol concentrations and longer columns are employed.

This leads to a complete separation of dulcitol and mannose, but the duration of analysis increases. To reduce the required period, very careful resin fractionation is necessary. As in the case of aldoses, the partition coefficients of polyols depend on the concentration of the alcohols and increase as it is raised, but the elution sequence is independent of alcohol concentration. A higher resin capacity leads to an increase of the partition coefficients and an improvement of separation. As for aldoses, the partition coefficients decrease with the number of hydroxyls in the polyols. The majority of polyols are eluted before the corresponding aldoses. Only ribitol and mannitol are exceptions.

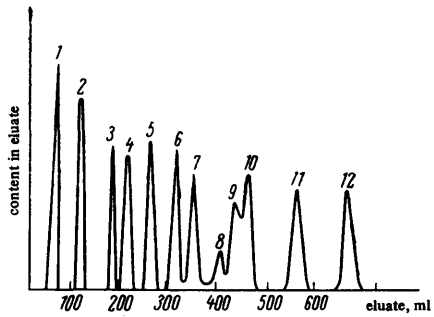


Figure 6. Analysis of a mixture of polyols and aldoses on TB resin in the sulphate-form (particle size 3–17 μm) in a 6 × 852 mm column; elution with 86% ethanol at a rate of 2.51 ml min^{-1} cm^{-2} at 75.5°C; mixture components: 1) glycerol; 2) erythritol; 3) xylitol; 4) arabitol; 5) arabinose; 6) xylose; 7) sorbitol; 8) mannose; 9) dulcitol; 10) mannitol; 11) galactose; 12) glucose.

Cation exchangers have also been used to separate polyols [70]. Resins in the lithium-form give the best results. Satisfactory separation of a complex mixture of polyols is also achieved in this case.

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Thus chromatography on ion-exchange resins permits a quantitative separation of very complex mixtures of monosaccharides and oligosaccharides. There is no doubt that the method will be very useful in the quantitative determination of the monosaccharide composition of a wide variety of carbohydrate-containing biopolymers, although the scope of the method as applied to the analysis of natural polysaccharides has so far been inadequately investigated [80, 81].

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