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Carbohydrate separation by ligand-exchange liquid chromatography

Correlation between the formation of sugar–cation complexes and the elution order

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ABSTRACT

Carbohydrate separation (hexoses, pentoses and corresponding polyols) was studied by liquid chromatography using ligand exchange on cation-exchange resin column with water as eluent. Seven cations (Ca^{2+}, Sr^{2+}, Ba^{2+}, Pb^{2+}, Y^{3+}, La^{3+} and Pr^{3+}) were tested. The carbohydrate elution order is considered in connection with the complexing sites likely to be involved for each sugar or polyol molecule and with the exclusion processes.

INTRODUCTION

Extraction of sugars from plants by diffusion or pressing and by acid or enzymatic hydrolysis yields sugar mixtures. Catalytic or enzymatic hydrogenation gives polyol mixtures which may contain some residual sugars, the separation of which is required. Liquid chromatography with ligand exchange on ion-exchange resins and water as eluent seems to be the most efficient method. Techniques for the analytical separation of sugars developed over the last 15 years [1–3] are now being used for industrial carbohydrate separations, especially glucose–fructose separation [4–6]. This type of separation was studied by Angyal *et al.* [7] and Goulding [6]. The formation of a donor–acceptor complex between the cations immobilized on the ion-exchange resin and the carbohydrate hydroxyl groups is the mechanism effecting the separation [7–9]. Some water molecules of cation hydration are thus displaced and replaced by the carbohydrate [8]. The movement of the latter through the column is caused by the water molecules of the eluent, which in turn replace sugar or polyol molecules. Their separation is directly proportional to the stability of the complex formed with the cation: the more stable the complex, the more immobilized the molecule will remain [8,10]. The influence of the cation fixed on the resin constitutes one of the major parameters of the separation process.

This study was aimed at selecting the most appropriate resin counter ion to

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achieve the separation of carbohydrate compounds produced by treatment of plants. The components of the mixtures studied were D-glucose, D-xylose, D-galactose, D-mannose, L-arabinose, mannitol, arabinitol, galactitol, xylitol and sorbitol. Seven cations (Ca^{2+}, Sr^{2+}, Pb^{2+}, Y^{3+}, La^{3+} and Pr^{3+}) were selected according to their characteristics and the literature [7,8,11–16]. In order to form a bidentate complex (less stable, $K_{stab} = 0.1\ mol^{-1}$) or a tridentate complex (more stable, $K_{stab} = 1–5\ mol^{-1}$), with two or three sequences of hydroxyl groups supported by adjacent carbons, the cation must have an octahedrial structure—in solution the hydration of non-octahedrial cations of Group 1A in the Periodic Table causes a structural change allowing complex formation [8]; the highest possible electronic deficit [11]; and an optimum size with respect to the complexing site, *i.e.*, a 1 Å ionic radius [17] for the tridentate complex produced on adjacent hydroxyl sequences, in axial–equatorial–axial (a–e–a) on a pyranose ring, in *cis–cis* on a furanose ring and on a so-called M–P sequence on an open carbonated chain where the first and second carbon are *gauche* clockwise and the second and third carbons are *gauche* anticlockwise or *vice versa* [18]. This sequence is analogous to the a–e–a pyranose sequence on an open chain. Below 1 Å, the small cation will tend to produce axial–equatorial (a–e) bidentate complexes. Above 1 Å, the large cations will tend to produce 1–3 diaxial (1–3 a–a) or equatorial–equatorial (e–e) bidentate complexes [8].

Concerning complex formation, one may observe a difference between sugars and polyols. Sugars will yield complexes with cations function as their own immobilized sites whereas polyols can all achieve the M–P configuration by rotation around carbon–carbon bonds. The formation of more or less stable complexes will largely depend on the energy supplied to obtain this M–P configuration. When the energy consumption averages 1–2 kcal/mol, complexing occurs but weakly. With a greater energy no complexing occurs [17]. The complex stability is linked to the polyol structure. It decreases in the following structural order: *threo–threo* (t–t) a pair of *threo* adjacent to a primary hydroxyl (w–t) *erythro–threo* (e–t) a pair of *erythro* adjacent to a primary hydroxyl (w–e) [18].

EXPERIMENTAL

A 200 cm × 1.67 cm I.D. glass column, thermostated at 50°C by fluid circulation in the jacket, was packed with Duolite C204/2078 resin (6.4% divinylbenzene, 0.15–0.3 mm diameter) in the appropriate form by the sedimentation method. The counter ions used for resin permutation were supplied by Prolabo (calcium, strontium and barium chloride), Degussa (lead nitrate) and Rhone-Poulenc Rare Earth Unit (yttrium, lanthanum, praseodymium nitrates).

The products used to reconstitute the carbohydrate mixtures were supplied by Fluka (glucose, xylitol, mannitol), Prolabo (xylose, galactose, galactitol) Jansen (mannose, arabinose), Aldrich (sorbitol) and Extrasynthese (arabinitol).

The mixtures were reconstituted in deionized water with a resistivity higher than 10 MΩ/cm and from 45 to 75 g/l of each component.

The injection of 1.5 cm^3 of separating solution was performed by direct deposition on the resin. In order to prevent dilution of the injected solution, elution water with was initiated only after complete penetration of the solution into the resin. A Gilson Minipuls 2 peristaltic pump fed the system with water as eluent at 50°C. An

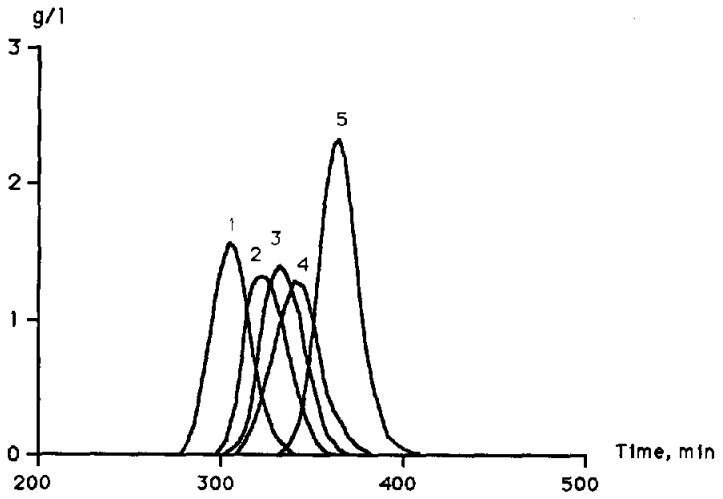

Fig. 1. Chromatogram of sugars on Ca^{2+} ion-exchange resin. Flow-rate, 0.83 ml/min. In Figs. 1–14 the numbers on the peaks correspond to the carbohydrates listed in Table I.

MTDC Gilson automated fraction collector recovered the effluent at the column output. When the separation procedure was completed, the collected fractions were analysed by high-performance liquid chromatography (HPLC) on an LDC Milton Roy III apparatus with refractometric detection.

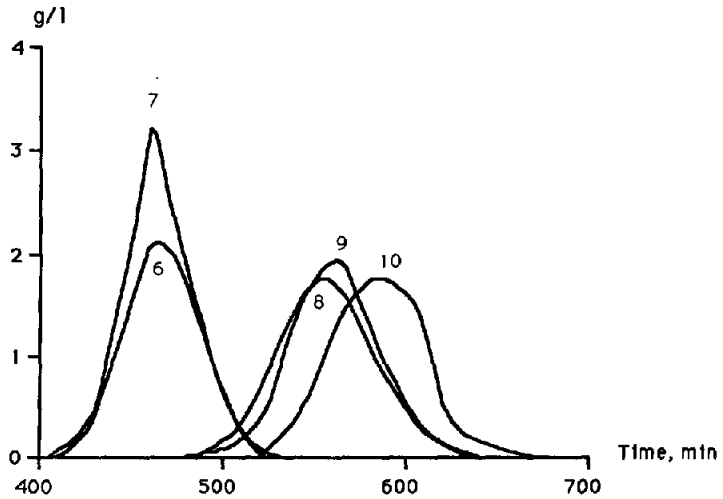

Fig. 2. Chromatogram of polyols on Ca^{2+} ion-exchange resin. Flow-rate, 0.83 ml/min.

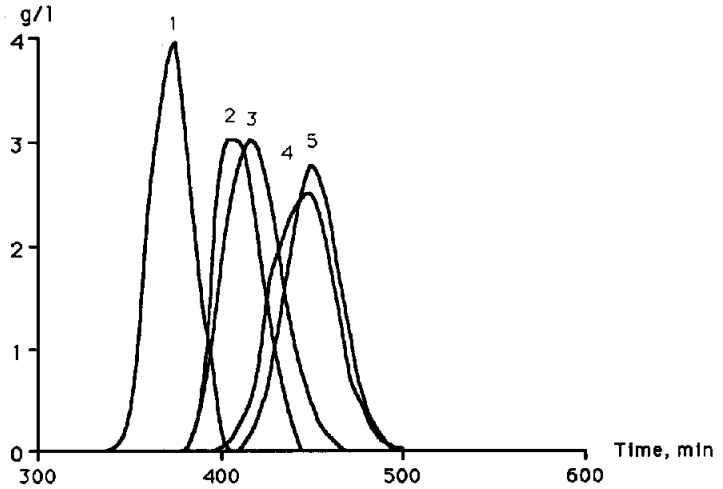

Fig. 3. Chromatogram of sugars on Sr^{2+} ion-exchange resin. Flow-rate, 0.69 ml/min.

RESULTS

Fig. 1–14 illustrate the chromatograms for each of the seven cations. The capacity factor (k') was calculated (Table I) for each monosaccharide, polyol and cation. The k' order corresponds to the elution order of the monosaccharides and polyols. It should be noted that the values of k' are similar to those determined by Goulding [8].

One can observe that sugars always elute before polyols whatever the cation used. The separation of the two groups is complete with lanthanum, praseodymium,

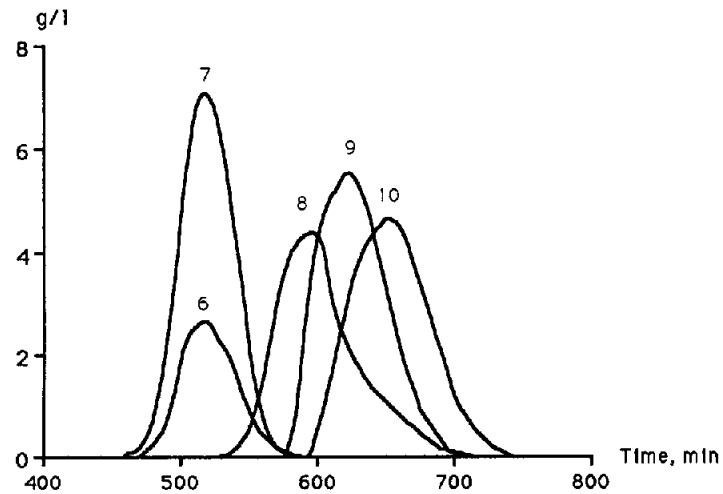

Fig. 4. Chromatogram of polyols on Sr^{2+} ion-exchange resin. Flow-rate, 0.69 ml/min.

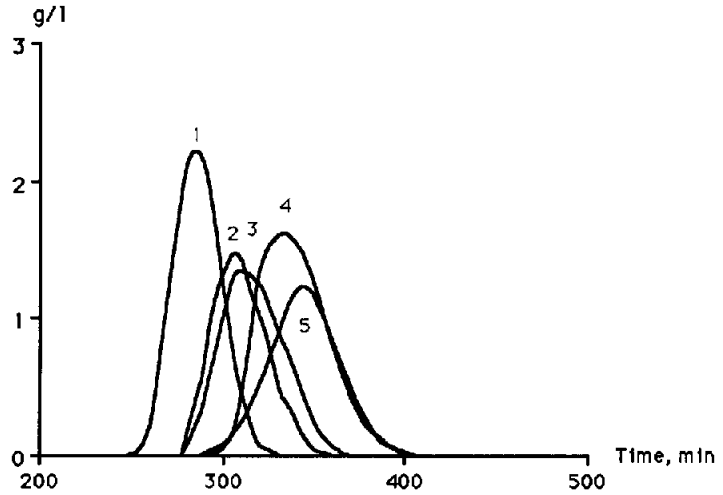

Fig. 5. Chromatogram of sugars on Ba^{2+} ion-exchange resin. Flow-rate, 0.90 ml/min.

calcium and lead cations and almost complete with the yttrium cation. This is not the case for strontium (Figs. 3 and 4) and barium (Figs. 5 and 6), although the overlapping remains minor and restricted to the mannose–arabinose and mannitol–arabinitol pairs. These results are in agreement with the theoretical data available on the stability of the sugar–cation complexes [3,8,17]. All the polyols are likely to form tridentate complexes. This is not the case for the monosaccharides studied.

One can also say that in general the elution order is (1) glucose, (2) xylose, (3) galactose, (4) mannose, (5) arabinose, (6) mannitol, (7) arabinitol, (8) galactitol, (9)

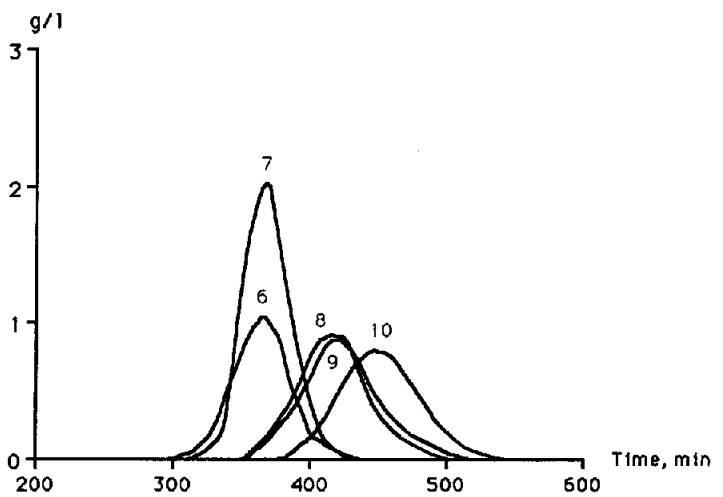

Fig. 6. Chromatogram of polyols on Ba^{2+} ion-exchange resin. Flow-rate, 0.90 ml/min.

TABLE I

CAPACITY FACTORS (k') FOR THE DIFFERENT SEPARATED COMPONENTS ON DUOLITE C204/2078 RESIN WITH WATER AS ELUENT

$$k' = \frac{t_{ri} - t_0}{t_0} = t_{ri} \cdot \frac{D}{LS\varepsilon} - 1$$

where t_{ri} = retention time of component i measured at the maximum of the chromatographic peaks (min), t_0 = residence time of the mobile phase in the column, $t_0 = L/\mu$ (min), L = length of the column (cm), μ = linear flow-rate of the eluent, $\mu = D/S\varepsilon$ (cm/min), D = eluent flow-rate (ml/min), S = column cross-section (cm^2) and ε = void volume = volume of the eluting phase contained in the column/volume of the column ($\varepsilon = 0.4$).

Counter ion	(1) Glucose	(2) Xylose	(3) Galactose	(4) Mannose	(5) Arabinose	(6) Mannitol	(7) Arabinitol	(8) Galactitol	(9) Xylitol	(10) Sorbitol
Ca^{2+}	0.46	0.54	0.57	0.61	0.73	1.24	1.202	1.65	1.67	1.78
Sr^{2+}	0.48	0.61	0.65	0.76	0.78	1.04	1.06	1.36	1.46	1.60
Ba^{2+}	0.49	0.57	0.63	0.73	0.79	0.89	0.89	1.15	1.17	1.32
Pb^{2+}	0.59	0.66	0.83	1.09	0.95	2.10	2.09	3.14	3.02	3.69
Y^{3+}	0.29	0.36	0.36	0.39	0.50	0.84	1.03	1.23	1.50	1.52
La^{3+}	0.34	0.43	0.43	0.53	0.54	1.52	1.81	2.69	3.36	3.69
Pr^{3+}	0.41	0.48	0.50	0.56	0.61	2.66	3.31	6.16	6.03	7.96

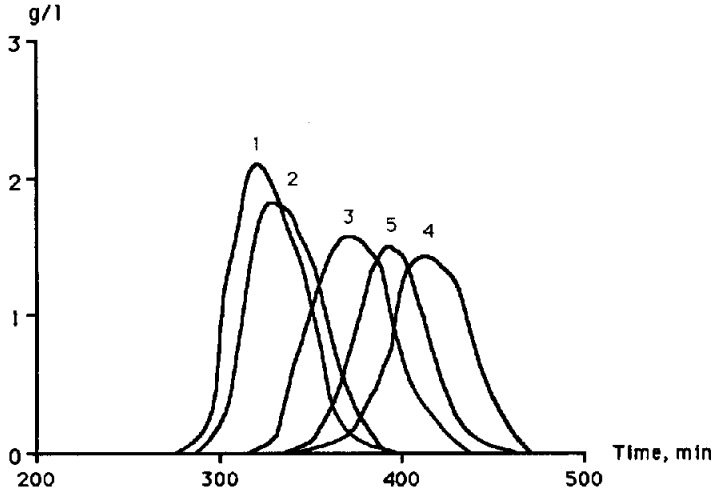

Fig. 7. Chromatogram of sugars on Pb^{2+} ion-exchange resin. Flow-rate, 0.88 ml/min.

xylitol and (10) sorbitol. There are several exceptions with Pb^{2+}. For mannose–arabinose, mannitol–arabinitol and xylitol–galactitol (Figs. 7 and 8), three inversions were observed. These results, already reported by Baker and Himmel [13] for mannose and arabinose, were corroborated by HPLC on Pb^{2+} and Ca^{2+} analytical columns (Table II).

Ca^{2+} seems to be the most suitable cation for obtaining an efficient separation of the components of sugar–polyol, glucose–galactose, –mannose or –arabinose, ara-

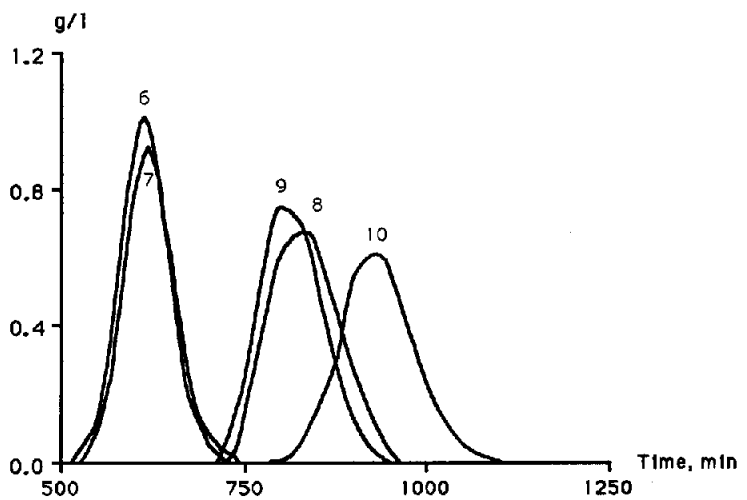

Fig. 8. Chromatogram of polyols on Pb^{2+} ion-exchange resin. Flow-rate, 0.88 ml/min.

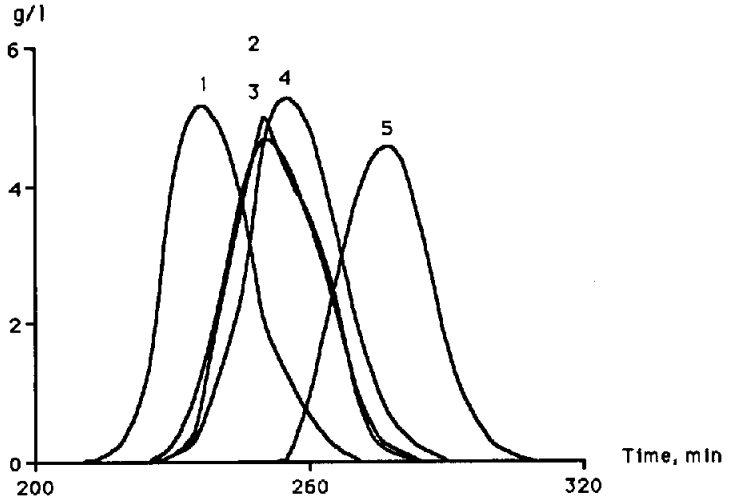

Fig. 9. Chromatogram of sugars on Y^{3+} ion-exchange resin. Flow-rate, 0.95 ml/min.

binose–xylose, –galactose or –mannose, mannitol–galactitol, –xylitol –sorbitol and arabinitol–galactitol, –xylitol –sorbitol mixtures.

In some instances, separation can be improved by using other cations (Table III). For example, the glucose–xylose separation is more efficient with Sr^{2+} (Figs. 1 and 3) and the arabinitol–galactitol, –xylitol or –sorbitol separation is better with La^{3+} (Figs. 1 and 12). Pr^{3+} leads to capacity factors that are sharply higher for polyols. For the separation of alditols, a Pr^{3+} column considerably smaller than one

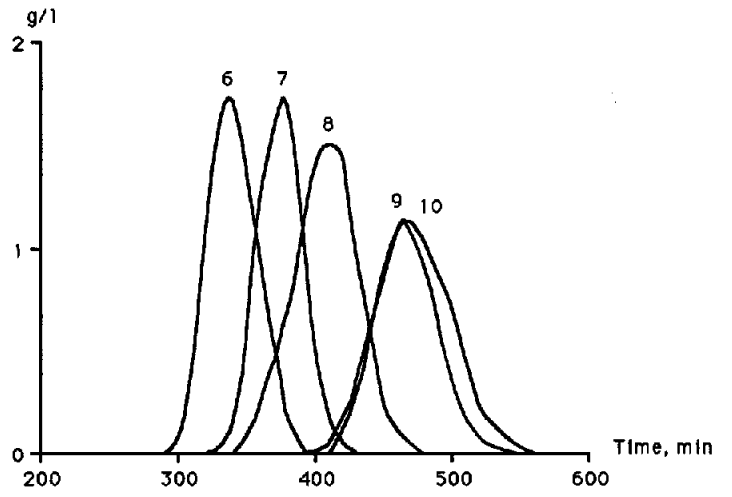

Fig. 10. Chromatogram of polyols on Y^{3+} ion-exchange resin. Flow-rate, 0.95 ml/min.

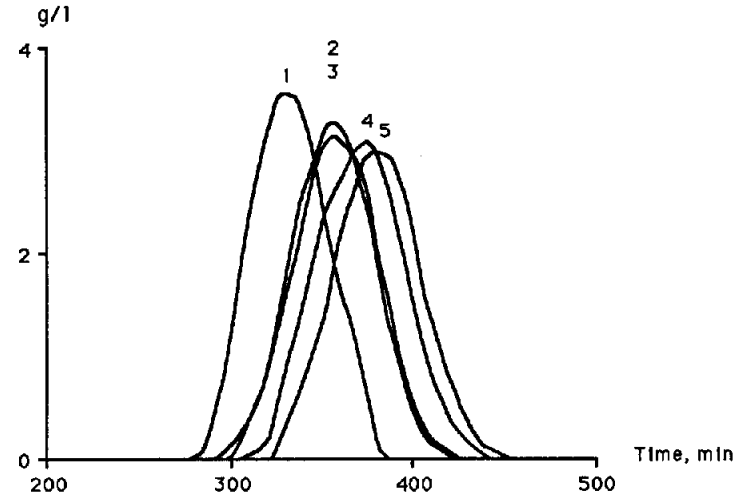

Fig. 11. Chromatogram of sugars on La^{3+} ion-exchange resin. Flow-rate, 0.70 ml/min.

made with La^{3+} could be used. Lastly, separation on Ca^{2+} resin is almost or completely impossible in certain situations. Table IV reports unsolved separations with Ca^{2+} and suggests the use of other cations. Although Ba^{2+} allows a satisfactory separation of D-tagatose and D-talose [19], in our study it did not yield better results than the other cations tested.

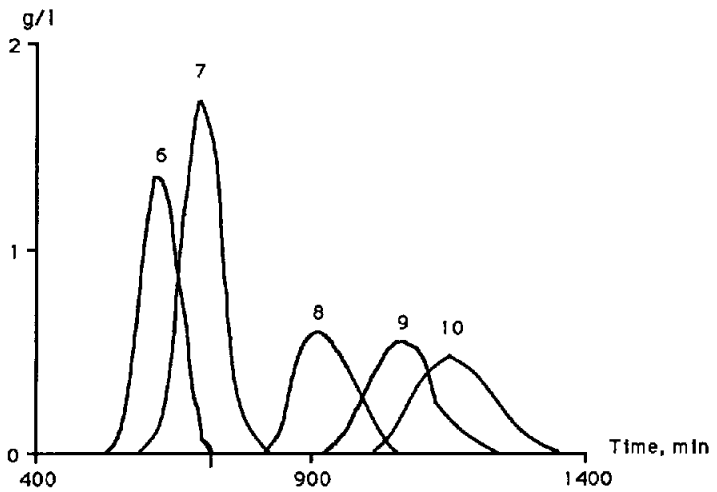

Fig. 12. Chromatogram of polyols on La^{3+} ion-exchange resin. Flow-rate, 0.70 ml/min.

TABLE II

RETENTION TIMES (min) ON ANALYTICAL COLUMNS FOR THE THREE PAIRS WITH INVERTED ELUTION ORDER BETWEEN CALCIUM AND LEAD

Carbohydrate	Brownlee Labs. Cartridge, 20 cm, 0.3 ml/min, 85°C (Ca^{2+})	Bio-Rad Labs. HPX 87P 30 cm, 0.6 ml/min, 85°C (Pb^{2+})	Bio-Rad Labs. HPX 87C, 30 cm, 0.6 ml/min, 85°C (Ca^{2+})	Interaction CHO-682, 30 cm, 0.4 ml/min, 90°C (Pb^{2+})
Mannose	6.97	16.44	12.47	25.44
Arabinose	7.59	15.48	13.75	24,27
Mannitol	9.57	–	–	40.72
Arabinitol	9.74	–	–	40.42
Galactitol	10.94	–	–	53.07
Xylitol	11.24	–	–	51.32

TABLE III

COMPARATIVE QUALITATIVE RESULTS FOR POSSIBLE SEPARATIONS FOR CALCIUM AND OTHER CATIONS

The separation obtained with the cation is equal to (=) greater than (>) or much better than (≫) that obtained with Ca^{2+}.

Mixture	Ca^{2+}	Sr^{2+}	Pb^{2+}	Y^{3+}	La^{3+}	Pr^{3+}
Sugar–polyol	+		>		>	>
Glucose–galactose	+	>				
Glucose–mannose	+	>				
Glucose–arabinose	+	>				
Xylose–arabinose	+		=			
Galactose–arabinose	+				>	≫
Mannose–arabinose	+			>	≫	≫
Mannitol–galactitol	+				>	≫
Mannitol–xylitol	+			>		>
Mannitol–sorbitol	+	>	>			>
Arabinitol–galactitol	+		>		>	
Arabinitol–xylitol	+				>	
Arabinitol–sorbitol	+	>			>	

TABLE IV

CATION SELECTION WHEN SEPARATION IS IMPOSSIBLE WITH CALCIUM

Mixture	Sr^{2+}	Pb^{2+}	Y^{3+}	La^{3+}	Pr^{3+}
Glucose–xylose	Fig. 3				
Xylose–galactose		Fig. 7			
Xylose–mannose		Fig. 7			
Galactose–mannose		Fig. 7			
Mannitol-arabinitol			Fig. 10	Fig. 12	Fig. 14
Galactitol–xylitol			Fig. 10	Fig. 12	
Galactitol–sorbitol				Fig. 12	
Xylitol–sorbitol		Fig. 8			Fig. 14

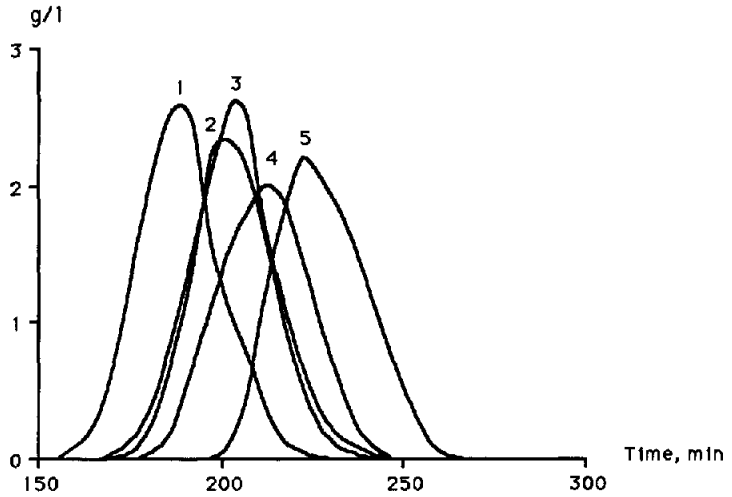

Fig. 13. Chromatogram of sugars on Pr^{3+} ion-exchange resin. Flow rate, 1.30 ml/min.

DISCUSSION

The results obtained illustrate the different behaviours of monosaccharides and polyols. Both will be considered separately in order to check whether the complex formation theory is consistent with the elution order in both groups. The contribution of the exclusion phenomena related to the use of ion-exchange resins as chromatographic supports will be discussed according to the separation efficiency.

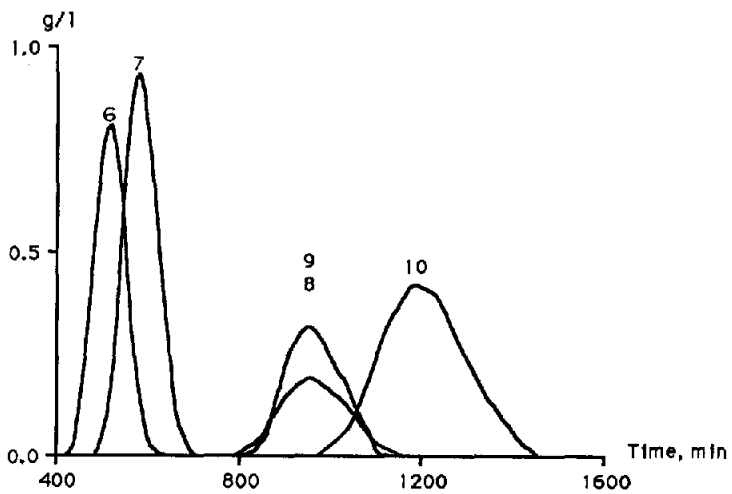

Fig. 14. Chromatogram of polyols on Pr^{3+} ion-exchange resin. Flow-rate, 1.30 ml/min.

Monosaccharides

Contribution of complex formation. According to theory, the sugar and related configuration sites (a–e–a, a–e, e–e, 1–3 a–a) allow the formation of more or less strong complexes. Although there are no available current data, to our knowledge, on the formation speed of sugar–cation complexes, the kinetics of mutarotation seem to be a restrictive factor compared with those of sugar–cation complexes formation. In order to substantiate this observation, we may add that the mutarotation equilibrium is achieved slowly [20,21]; the separation of some sugar anomers by HPLC on cation-exchange resins with calcium or lead columns has already been completed [8,13]; and sugar analysis by NMR spectroscopy identifies the five anomeric forms (α- and β-pyranose, α- and β-furanose and open-chain) whenever they exist in sufficient amounts. The kinetics of exchanges between the different forms are slow compared with the NMR time scale. However, when there is a cation, only a average spectrum corresponding to the free sugar and to the sugar-cation complex will be observed [17]. The kinetics of the complex formation are very fast compared with the NMR time scale.

The contributions of the sites likely to form a complex with a cation will be proportional to each tautomeric form of dissolved sugars. All the monosaccharides are present in water solution in several forms at equilibrium. For the five sugars studied, the α- and β-pyranose forms prevail [22]. According to Angyal *et al.* [23], apart from β-L-arabinose, which has the two confirmation, $^1C_4$ and $^4C_1$, the prevailing conformation of the studied aldopyranoses is $^4C_1$. The energy to change conformation is always greater than 2 kcal/mol.

Although there are no direct data on the energies generated by the formation of a bi- or tridentate sugar–cation complex, the stabilization energy brought about by the formation of a tridentate complex does not seem to be sufficient to reach the level required to induce a conformation change. According to Angyal [17], with polyols and when the conformation change requires very little energy (1–2 kcal/mol), the tridentate complex will occur but its stability will be weak. Beyond this level, it will no longer occur. This is even more true for bidentate complexes. Symons *et al.* [24] related some sugar NMR spectroscopic changes with variation in calcium chloride concentration to the shift of conformation equilibrium, $^1C_4 \rightleftharpoons {}^4C_1$. However, and in compliance with Goulding's observation [8], only the complexes likely to occur with the $^4C_1$ prevailing conformations will be studied, except for β-L-arabinose. The part played by the a–e–a complex of the β-D-mannopyranose $^1C_4$ conformation in the separation will be discounted. In the same way, no 1–3 a–a complex will be taken into account as they could only occur with D-glucose and D-xylose in the $^1C_4$ forms. The contribution of the open-chain forms will also be discounted because they only occur as trace components [25]. As far as the furanose forms of sugars are concerned, their proportion is small [26,27]. The contribution of the bidentate complex which they may generate remains negligible. This is also the case for the tridentate complex of β-D-mannofuranose [8].

The a–e complexes will form preferentially the e–e forms proportionally to the equilibrium obtained at 50°C. Table V summarizes the contribution of each complex for each sugar.

The chromatographic elution order of the monosaccharides is linked to the proportion of a–e bidentate complexes likely to form. With calcium the contribution of the weaker e–e bidentate complexes seems to be minor with this small-sized cation

(*ca.* 1 Å). In contrast, this contribution can be higher [1] with strontium and barium, whose ionic radii are larger (1.12 and 1.34 Å, respectively). This might account for the poor xylose–galactose and mannose–arabinose separations observed with these cations (Figs. 3 and 5). However, the elution order of sugars remains identical with that obtained with Ca^{2+}. The same applies to the yttrium, lanthanum and praseodymium cations. However, the total lack of xylose–galactose separation demonstrated by identical capacity factors (0.36 for Y^{3+}, 0.43 for La^{3+}) or close capacity factors (0.48–0.5 for Pr^{3+}) cannot only be accounted for by the ionic radius of the cations (0.89, 1.02 and 1.1 Å, respectively). In the same way, although the contributions of the a–e and e–e complexing sites are identical for glucose and xylose, these sugars are always clearly separated by all the cations.

Contribution of exclusion phenomena. Another factor limiting the contribution of sugar–cation complexes in the separation process will have to be taken into account. The comparison of cation structures or of their electronic deficit is not sufficient to explain the separation. However, the contribution of exclusion phenomena linked to the differences in steric crowding between hexoses and pentoses might clarify the interpretation of the observed phenomena.

For all the cations described, xylose and glucose on the one hand and galactose and arabinose on the other are always clearly separated. These hexose–pentose pairs do not differ from a structural viewpoint except for the presence of the hydroxymethyl group on the pyranose ring. As far as xylose and glucose are concerned, the complexing site contributions are equivalent but the exclusion phenomenon remains prevalent.

TABLE V

CONTRIBUTION TO THE SEPARATION OF EACH SUGAR COMPLEX

Sugar	Form[a]	Proportion at 50°C (%)	Complexation sites	
			a–e	e–e
D-Glucose	α-P	37	1	1
[26,27]	β-P	63	0	2
	Total	100	0.37	1.63
D-Xylose	α-P	40	1	1
[3]	β-P	60	0	2
	Total	100	0.4	1.6
D-Galactose	α-P	33	2	0
[26,27]	β-P	57	1	1
	Total	90[b]	1.23	0.56
D-Mannose	α-P	62	1	0
[3]	β-P	38	2	0
	Total	100	1.38	0
L-Arabinose	α-P	63	2	0
[31]	β-P	34	1	1
	Total	97[c]	1.60	0.34

[a] P = pyranose.
[b] 10% of furanose forms.
[c] 3% of furanose forms.

The permutation of the exchanger by trivalent ions is shown by the moderate swelling of the resin, which will increase the exclusion penomena. The capacity factors observed with Y^{3+}, La^{3+} and Pr^{3+} are weaker for the sugars than for those observed with Ca^{2+}, Sr^{2+} and Ba^{2+}. Nevertheless the glucose–xylose and galactose–arabinose separations, for which the exclusion contribution to the separation is important, remain effective. However, the penetration of galactose and mannose into the cation-exchange resin in their Y^{3+}, La^{3+} or Pr^{3+} forms is less extensive. This restricts the contribution of complex formation and accounts for the weaker retention and poor separation with xylose.

Lead

The situation is even more complex for lead. Glucose, galactose and mannose are separated as with calcium. Xylose is no longer separated from glucose, and arabinose is eluted between galactose and mannose. It seems as if hexoses were more immobilized than pentoses. This phenomenon might be linked to the increase in the contribution of complex formation compared with the exclusion phenomena. This assumption is substantiated by the observation of the capacity factors: they are much higher for most of the sugars with Pb^{2+} than those obtained with other cations. Pb^{2+} is located at the hard and soft acid limit owing to its partially vacant $5d$ orbital ($4f^{14}$, $5d^{8}$, $6s^{2}6p^{2}$) [28,29]. The association with oxygen free pairs of hydroxyl groups which are soft bases are therefore stronger (soft acid–soft base association) than those obtained with alkaline earth metal cations (hard acid). The prevailing contribution of complexing phenomena levels off the different xylose and glucose behaviours which have the same contributions on complexing sites, and in smaller proportions than those for arabinose and galactose. Also, the larger ionic radius of the Pb^{2+} cation (1.2 Å) could lead to a modification in relation to the contribution on the a—e and e e sites. This cation accelerates the speed of mutarotation of sugars [13], which could alter the proportions of α- and β-forms.

To conclude, without totally leaving aside the complexing tridentate site contribution, the elution order of the investigated monosaccharides can thus be correlated with a–e or e–e bidentate complex formation. Depending on the type of cation, the phenomena will be more or less affected by exclusion factors linked to the size of resin pores.

Polyols

In contrast to monosaccharides, polyols in aqueous solution have an acyclic form, the prevailing conformation being [18,19] either planar or "zig-zag" when there is no 1–3 diaxial interaction between the oxygen atoms, or bent or sickle form in the opposite case. By rotating round carbon–carbon bonds, all the alditols studied can form tridentate M-P-type complexes with a cation. These complexes are strong. It would appear reasonable to assume that the contribution of the exclusion phenomena to separation will be minor compared with that of complex formation. This assumption is confirmed by the following observation: for the same ionic radius (Ca^{2+} and La^{3+}, for instance), passing from two to three load units does not entail any decrease in capacity factors as occurs with monosaccharides (Table II). In the same way, the sharp increase in capacity factors observed with Pr^{3+} corroborated the predominant role of the complex formation. The formation of strong complexes with lanthanide ions is widely used in NMR spectroscopy [12,18,32].

The elution order observed on rare earths, *i.e.*, mannitol, arabinitol, galactitol, xylitol, sorbitol, appears to be consistent with the work of Angyal *et al.* [18] on ^1H NMR chemical shifts induced by the addition of lanthanum ions (Table VI). The best separation pattern is obtained with La^{3+} (Fig. 12), whose ionic radius averages 1 Å. The sorbitol–xylitol separation could be explained by the fact that in addition to the sequence t–t formed on O_2–O_3–O_4, sorbitol can also lead to the formation of two other M–P complexes from the sequences w–t formed on O_1–O_2–O_3 and w–e formed on O_4–O_5–O_6. Such an alternative does not occur with xylitol, which can only yield one M–P complex from either of the w–t sequences formed O_2–O_3–O_4 or on O_3–O_4–O_5.

Moreover, in addition to the O_1–O_2–O_3 and O_2–O_3–O_4 complexing sites similar to those of arabinitol, galactitol can form another M–P complex from the w–t sequence of O_4–O_5–O_6 oxygen atoms; this second complex is independent of the first. With the Pr^{3+} cation whose ionic radius averages 1 Å, this alternative induces a higher galactitol retention with no xylitol separation (Fig. 14).

A decrease in the ionic radius of the cation (Y^{3+}, 0.89 Å) results in a lack of xylitol–sorbitol separation (Fig. 10) together with a 50% decrease in the capacity factors of the polyol group (Table I). This result is in agreement with the hypothesis of a 1 Å cation for the formation of M–P complexes [8,17]. On the other hand, this prerequisite does not appear to be applicable for alkaline earth metal cations. Despite the optimum 1 Å ionic radius, the calcium ion does not lead to an efficient separation of the mannitol–arabinitol and galactitol–xylitol pairs (Fig. 2). The situation is identical for Sr^{2+}, Ba^{2+} and Pb^{2+} for the mannitol–arabinitol pair (Figs. 4, 6 and 8).

The results tend indicate that 2+ cations do not carry enough energy to compensate for the energy needed to modify the conformation by rotation around a C–C axis facilitating the formation of sequences yielding complexes formation. As is the case for monosaccharides, the phenomena ruling the polyol separation involve the capacity to form complexes. The strength of these complexes stimulated by a 1 Å ionic radius cation and a three unit loading deficit allows for a larger retention of aditols and a efficient separation of each component. Exclusion phenomena appear to play a minor role.

TABLE VI

COMPLEXATION IDENTIFIED BY NMR ANALYSIS

^1H chemical shifts induced by the addition of lanthanum [18].

Polyol	Complexation sites		
	t–t	w–t	e–t
Mannitol			O_2–O_3–O_4
Arabinitol		O_1–O_2–O_3	O_2–O_3–O_4
Galactitol		O_1–O_2–O_3	O_2–O_3–O_4
Xylitol	O_2–O_3–O_4		
Sorbitol	O_2–O_3–O_4		

CONCLUSIONS

The contribution of carbohydrate–cation complex formation appears to be directly correlated with the elution order of sugars and polyols in ligand-exchange chromatography. In the former instance, the complexes are of the weak bidentate type and the exclusion phenomena leading to steric crowding of the different sugars can also result in chromatographic separation. In the latter, the complexes are of the tridentate type and the separation occurs as a function of the energy needed for the polyols to have the appropriate configuration. In both instances, a suitable choice of the cation improves the separation process, the quality of which also depends on the characteristic of the support (granulometry, cross-linking, swelling, etc.). The study of the influence of these different factors is in progress.

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REFERENCES

1 A. Meunier, M. Caude and R. Rosset, *Analusis*, 14 (1986) 363.
2 P. Jandera and J. Churacek, *J. Chromatogr.*, 98 (1974) 55.
3 T. Carillon, *Thèse de Docteur Ingénieur*, Institut National Polytechnique, Toulouse, 1987.
4 Colonial Sugar Refining, *Br. Pat.*, 1 083 500 (1967).
5 V. S. H. Liu, N. E. Lloyd and K. Khaleeluding, *US Pat.*, 4 096 036 (1978).
6 H. Ishikawa, H. Tanabe and K. Usui, *US Pat.*, 4 182 633 (1980).
7 S. J. Angyal, G. S. Bethell and R. J. Beveridge, *Carbohydro. Res.*, 73 (1979) 9.
8 R. W. Goulding, *J. Chromatogr.*, 103 (1975) 229.
9 F. Helfferich, *Ion Exchange*, McGraw-Hill, New York, 1962.
10 B. Porch, *J. Chromatogr.*, 253 (1982) 49.
11 S. J. Angyal, *Aust. J. Chem.*, 25 (1972) 1957.
12 S. J. Angyal and J. A. Mills, *Aust. J. Chem.*, 38 (1985) 1279.
13 J. O. Baker and M. E. Himmel, *J. Chromatogr.*, 357 (1986) 161.
14 S. J. Angyal and R. J. Hickamn, *Aust. J. Chem.*, 28 (1975) 1279.
15 S. J. Angyal and D. Greeves, *Aust. J. Chem.*, 29 (1976) 1223.
16 R. M. Saunders, *Carbohydr. Res.*, 7 (1968) 76.
17 S. J. Angyal, *Tetrahedron*, 30 (1974) 195.
18 S. J. Angyal, D. Greeves and J. A. Mills, *Aust. J. Chem.*, 37 (1974) 1447.
19 J. K. N. Jones and R. A. Wall, *Can. J. Chem.*, 38 (1960) 2290.
20 A. de Grandchamp-Chaudun, *C. R. Acad. Sci.*, 258 (1964) 6564.
21 A. de Grandchamp-Chaudun, *C. R. Acad. Sci. Ser. C*, 262 (1966) 1441.
22 S. J. Angyal, *Angew. Chem. Int. Ed. Engl.*, 8 (1969) 157.
23 S. J. Angyal, *Aust. J. Chem.*, 21 (1968) 2737.
24 M. C. R. Symons, J. A. Benbow and H. Pelmore, *J.Chem. Soc., Faraday Trans. 1*, 80 (1984) 1999.
25 L. D. Hayward and S. J. Angyal, *Carbohydr. Res.*, 53 (1977) 13.
26 S. J. Angyal and V. A. Pickles, *Aust. J. Chem.*, 25 (1972) 1695.
27 L. Rigal, *Thèse de Doctorat d'Etat*, Institut National Polytechnique, Toulouse, 1987.
28 R. G. Pearson, *J. Am. Chem. Soc.*, 85 (1963) 3533.
29 J. Seyden-Penne, *Bull. Soc. Chim. Fr.*, 9 (1968) 3871.
30 J. A. Mills, *Aust. J. Chem.*, 27 (1974) 1433.
31 S. J. Angual and V. A. Pickles, *Carbohydr. Res.*, 4 (1967) 269.
32 P. Girard, H. Kagan and S. David, *Tetrahedron*, 27 (1971) 5911.