Chromatographic separation of alditols on a cation-exchange resin in the lanthanum form

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Chromatography on a cation-exchange resin in its La form using elution with water was found to be an effective separation method for mixtures of alditols, as demonstrated on example of fourteen different alditols. The results obtained by this method were correlated with the separation of alditols on the same resin in the Ca, Ba, Mg, Li, Na, K, and H form. The highest retention of acyclic polyols was observed on the resin in the La form. Aldoses were retained most on the resin in the Ba form.

Хроматографией на катионите в La-форме при использовании воды в качестве элюэнта достигается эффективное разделение смесей альдитолов, которое демонстрируется на примере 14 разных альдитолов. Метод разделения сравнивается с разделением альдитолов на катионите в Ca, Ba, Mg, Li, Na, K и H-форме. Наиболее высокое удержание ациклических полиолов достигается на катионите в La-форме, а удержание альдоз самое высокое в случае катионита в Ba-форме.

Separation of acyclic saccharides still represents a serious problem of preparative and analytical chemistry. Almost all of the described procedures for separation of alditols are based on chromatographic methods. Borate ions are often used as a component of the elution systems in chromatography on paper [1, 2], columns of anion-exchange resins in the borate form [3], or charcoal-celite columns [4]. A good resolution of some alditol mixtures can be achieved on columns of Fuller's earth clay and cellulose [5], anion-exchange resins in the sulfate form [6] or cation-exchange resins in the Ba [7], Li [8] or Ca form [9]. Acyclic polyols having the clockwise-anticlockwise *gauche-gauche* arrangement of hydroxyl groups are capable to form complexes with alkaline earth and rare earth metal ions, which were proved by electrophoresis and ^{1}H-n.m.r. spectroscopy [10—13]. The complexes with rare earth metal ions are more stable [14, 15]. In attempts to elaborate an effective method for separation of alditols (also isotopically labelled alditols) we have examined their chromatography on a cation-exchange resin in its La form.

An effective separation of alditols was achieved on a column of Dowex 50 W (X-8, 200/400 mesh) in the La form when developed with water (Table 1, Fig. 1).

Table 1

Ion-exchange chromatography of polyols on columns of the cation-exchange resin in the La, Ca, and Ba form
(Relative elution volumes are referred to the elution volume of pentaerythritol)

Polyol	Form of the resin		
	La	Ca	Ba
Glycerol	0.99	—	—
Erythritol	0.97	—	—
D-Threitol	1.48	—	—
Ribitol	0.89	0.97	0.98
L-Arabinitol	1.39	1.30	1.20
Xylitol	2.52	1.78	1.60
Allitol	0.91	—	—
D-Mannitol	1.20	1.37	1.15
D-Altritol	1.35	—	—
Galactitol	1.98	1.77	1.40
D-Glucitol	2.50	1.86	1.61
L-Iditol	3.15	2.35	—
D-*glycero*-D-Taloheptitol	1.17	—	—
D-*glycero*-D-Galactoheptitol	1.70	—	—
Pentaerythritol	1.00	1.00	1.00
D-Galactose	0.65	0.80	0.94
D-Talose	1.61	2.08	2.60

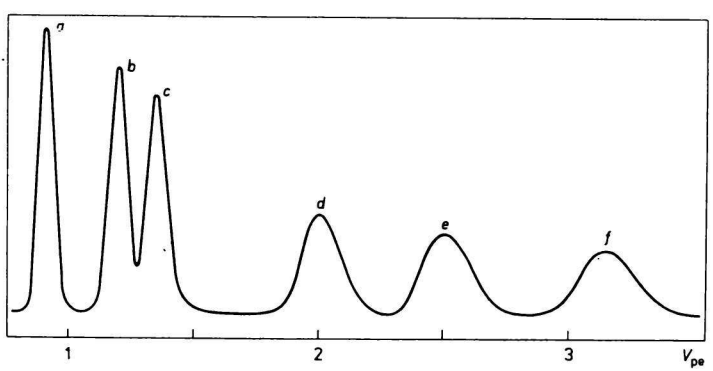

Fig. 1. Chromatographic separation of hexitols on a column of the cation-exchange resin in the La form using elution with water.
a) Allitol; *b*) D-mannitol; *c*) D-altritol; *d*) galactitol; *e*) D-glucitol; *f*) L-iditol.
(Relative elution volumes are referred to the elution volume of pentaerythritol.)

All stereoisomers of tetritols, pentitols, hexitols as well as a mixture of two heptitols (D-*glycero*-D-taloheptitol and D-*glycero*-D-galactoheptitol) could be resolved in this way. Mixtures of ^{2}H-labelled alditols obtained on reduction of D-arabinohexulose, D-erythropentulose, L-erythropentulose, D-threopentulose, and *erythro*-3-pentulose with sodium borodeuteride were resolved on a preparative scale. The La form of the resin gave better separation of alditols than its Ca or Ba forms (Table 1). The same resin in the Mg, Li, Na or K form exhibited only slight differences from the H form in ability to separate alditols (Table 2), and galactose from talose (Table 3). About the same is the effectiveness of separation of galactose from talose on the cation-exchange resin in the La, Ca and Ba form (Table 3), however, the elution volume of both aldoses increases in the given order of cations (Table 1).

Table 2

Relative elution volumes of xylitol and D-glucitol referred to the elution volume of pentaerythritol during chromatography on the cation-exchange resin in the given form

Alditol	Form of the resin				
	Mg	Li	Na	K	H
Xylitol	0.87	0.92	1.0	1.0	0.87
D-Glucitol	0.81	0.85	1.0	1.0	0.83

Table 3

Relative elution volumes of D-talose referred to the elution volume of D-galactose during chromatography on the cation-exchange resin in the given form

	Form of the resin							
	La	Ca	Ba	Mg	Li	Na	K	H
D-Talose	2.48	2.60	2.76	1.11	1.15	1.32	1.25	1.09

The results of the separation of alditols on the cation-exchanger in the La, Ca, and Ba form pointed out that the La form is the most effective in this regard (Table 1). Somewhat better separation of alditols is achieved on the Ca form than on the Ba form of the resin, however, none of these two forms separated satisfactorily galactitol from glucitol. The effectiveness of the separation of alditols apparently depends on their ability to form complexes with the cations of the used

resins. This is relevant to the known fact that the stability of the cation-polyol complexes decreases in the order La, Ca, and Ba [14, 15]. The ability of the cation-exchange resin to separate both cyclic and acyclic polyols is rather low in its Mg, Li, Na, K, and H form when developed with water (the separation here involves in small extent effects resulting from differences in molecular weight and size of the polyol molecules). This means that chemisorption of alditols plays only a minor role during their chromatography on the resin in the above-quoted cationic forms. However, when a cation-exchange resin in the Li form was eluted with 85% ethanol at 75°C, stereoisomers of pentitols and some hexitols (mannitol, glucitol, galactitol) were well separated [8]. It is interesting to note that under these conditions (Li form, elution with 85% ethanol) galactitol and glucitol were eluted in reverse order as is the order of their elution with water from the resin in the La, Ca or Ba form.

Of all cationic forms of the resin the largest differences in the retention of stereoisomeric alditols were observed on the resin in the La form. Therefore the column chromatography on the cation-exchange resin in the La form using elution with water can be suggested as the most effective method for separation of alditols both on the analytical (Fig. 1) and preparative scale. The separation method carried out on a larger scale was employed for isolation of various ^{2}H-labelled alditols (used in a study of effects of the presence of deuterium in alditol molecules on their biochemical dehydrogenation [16]). Optimum separation of alditol mixtures can be achieved when the weight of the sample applied was 5—15% of the column packing weight.

Separation of cyclic polyols on Dowex 50 W in the La, Ca, and Ba form was demonstrated on example of galactose and talose (Table 1). Retention of both aldoses on the resin decreases in the order Ba, Ca, and La form, however, in the view of their separation, all three cationic forms exhibit about the same effect (Table 3). In our previous work [17] separation of epimeric aldoses of the homomorphous series of galactose and talose was accomplished on Dowex 50 W (X-8, 100/200 mesh) in its Ba form. According to *Angyal* [9], however, for certain technical reasons it is more convenient to use for such separations the cation-exchange resin in the Ca form.

Significant differences in the adsorptive strength of alditols and cyclic aldoses on the cation-exchange resin in the La, Ca, and Ba form, when eluted with water, are illustrated on the example of separation of glucitol and talose in the presence of an internal standard, pentaerythritol, which, due to arrangement of its isolated hydroxyl groups, does not meet the conditions for complexing with alkaline and rare earth metal ions [11]. As it is apparent from Table 1 and Fig. 2, on the resin in the La form, the chemisorption of acyclic structures having the clockwise-anti-clockwise *gauche-gauche* arrangement of hydroxyl groups is substantially stronger than that of cyclic structures having the *ax-eq-ax* arrangement of hydroxyl groups.

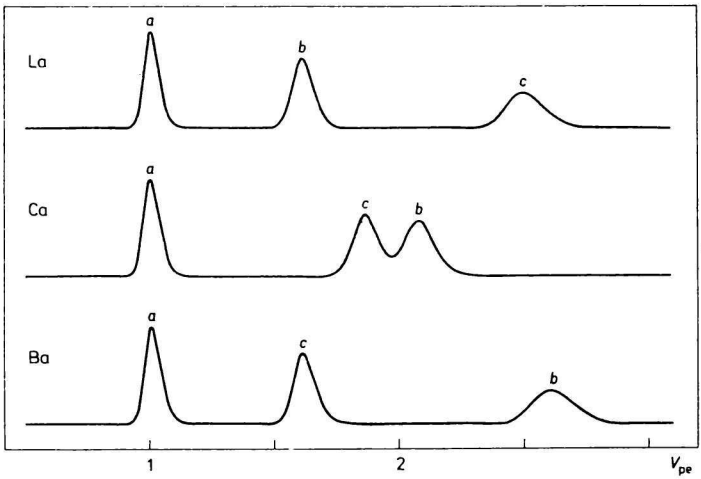

Fig. 2. Differences in the separation of pentaerythritol (*a*), D-talose (*b*), and D-glucitol (*c*) during chromatography on the cation-exchange resin in the La, Ca, and Ba form.

Just opposite is true for the resin in the Ba form. On the resin in the Ca form, the chemisorption of the two groups of polyols is involved in about the same extent. This means that in the choice of suitable separation conditions on cation-exchange resins, cyclic or acyclic character of polyol molecules should be taken into consideration in addition to stability constants of their complexes and arrangement of their hydroxyl groups.

Experimental

Chromatographic separation of alditols, pentaerythritol, galactose, and talose was performed on columns (90 × 0.9 cm) of Dowex 50 W (X-8, 200/400 mesh) in the La, Ca, Ba, Mg, Li, Na, K, and H form using elution with water deprived of gases, at a constant flow rate. A closed system, consisting of a peristaltic pump (VCM 150, Czechoslovak Academy of Sciences, Prague), a packed column, and a detector (differential refractometer 5100, Knauer, Berlin), was used. Tested samples were applied on the column without interruption of the flow by means of a six-way cock [18] placed between the pump and the column.

Testing of column packings for separation of polyols

Mixtures containing various combinations of alditols (glycerol, erythritol, D-threitol, ribitol, L-arabinitol, xylitol, allitol, D-mannitol, D-altritol, galactitol, D-glucitol, L-iditol, D-*glycero*-D-taloheptitol, D-*glycero*-D-galactoheptitol), D-galactose, D-talose, and

pentaerythritol as an internal standard, usually three up to six components (20 mg of each), were dissolved in water (0.2 ml) and applied on a column of the cation-exchange resin in the La, Ca, Ba, Mg, Li, Na, K or H form and chromatographed in water at a flow rate of 10 ml h^{-1}. Relative elution volumes of polyols, V_{pe}, are related to the elution volume of pentaerythritol ($V = 54$ ml) which showed only little changes (± 3 ml) depending on the cationic form of the used resin.

Preparative separation of ^{2}H-labelled alditols on the cation-exchange resin in the La form

a) A mixture (4 g) of D-[2-^{2}H]mannitol and D-[2-^{2}H]glucitol (obtained by reduction of D-arabinohexulose with sodium borodeuteride followed by deionization on a cation-exchanger and three successive evaporations of the sirupy residue with methanol) was dissolved in water (3 ml) and chromatographed on the resin eluted with water at a flow rate 18 ml h^{-1} to separate D-[2-^{2}H]mannitol (1.9 g; elution volume 54—88 ml) from D-[2-^{2}H]glucitol (1.9 g; 100—165 ml).

b) A mixture (0.8 g) of ^{2}H-labelled alditols obtained on reduction of D-erythropentulose with sodium borodeuteride (as described above) was dissolved in water (1 ml) and chromatographed using elution with water at a flow rate 10 ml h^{-1} to separate D-[2-^{2}H]ribitol (0.38 g; 40—52 ml) from D-[2-^{2}H]arabinitol (0.36 g; 60—75 ml). The same procedure was successfully employed for separation of L-[2-^{2}H]arabinitol and D-[4-^{2}H]ribitol present in a mixture obtained by reduction of L-erythropentulose.

c) A mixture (0.8 g) of ^{2}H-labelled pentitols obtained on reduction of D-threopentulose with sodium borodeuteride was chromatographed on the resin eluted with water at a flow rate 30 ml h^{-1}. D-[4-^{2}H]Arabinitol (0.37 g; 60—82 ml) and D-[2-^{2}H]xylitol (0.37 g; 110—150 ml) were isolated. In a similar run performed at a flow rate 40 ml h^{-1}, a mixture (0.8 g) of alditols prepared by reduction of *erythro*-3-pentulose with sodium borodeuteride was separated to give [3-^{2}H]ribitol (0.38 g; 40—60 ml) and [3-^{2}H]xylitol (0.35 g; 110—155 ml).

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