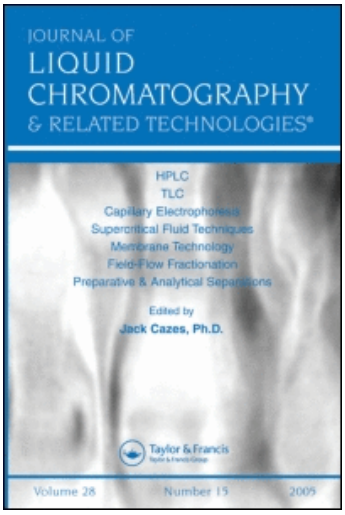

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G. R. Noll[a]; D. J. Mitchell[a]; J. O. Baker[a]; K. Grohmann[a]; M. E. Himmel[a]; N. J. Nagle[b]
[a] Applied Biological Sciences Section Biotechnology Research Branch Solar Fuels Research Division Solar Energy Research Institute, Colorado [b] Algal and Applied Plant Sciences Section Biotechnology Research Branch Solar Fuels Research Division Solar Energy Research Institute, Colorado

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EVALUATION OF ALKALINE EARTH AND TRANSITION METALS FOR USE IN THE ION MODERATED PARTITION CHROMATOGRAPHY OF SUGARS

G. R. NOLL[1], N. J. NAGLE[2],
D. J. MITCHELL[1], J. O. BAKER[1],
K. GROHMANN[1], AND M. E. HIMMEL[1]*
[1]*Applied Biological Sciences Section*
[2]*Algal and Applied Plant Sciences Section*
Biotechnology Research Branch
Solar Fuels Research Division
Solar Energy Research Institute
1617 Cole Boulevard
Golden, Colorado 80401

ABSTRACT

The elution behavior of 28 sugars and related compounds from high performance chromatography columns packed with alkaline earth and transition metals was investigated. As expected from the well-known chromatography of the commercially available calcium, silver, and lead form columns, elution behavior is highly metal-ion form specific. A significant data base of sugar elution versus column cationic-form was generated to permit the selection of new columns to enhance the separation of specific eluting species. Sugars resulting from the hydrolysis of wood were closely examined. Arabinose and galactose, known to be difficult to separate on commercially available lead and silver-form columns, were well separated on custom-packed rubidium and cesium-form columns.

INTRODUCTION

The demonstration by Jonsson and Samuelson in 1967 (1) that neutral sugars form complexes of varying stability with the metal ions coordinated to cation exchange resins initiated investigation of an analytical method that now is recognized as an invaluable chromatographic tool (2,3). In 1981, Jupille and coworkers (4) described

this separation method as ion-moderated partition (IMP) chromatography. This complex interaction of solute and immobilized cation leads to a mixed partition chromatography, which has been used successfully to resolve many unlikely compounds, even dissolved gases (5). Although widely used today as an analytical tool for the separation and measurement of monomeric sugars, oligosaccharides, alcohols, amino acids, and other neutral hydroxylated compounds, the range of applicability of ion-moderated partition chromatography has remained limited by the availability of commercial columns packed in only the calcium, lead, silver, sodium, and potassium metal-ion forms. The cation specific selectivity shown by Goulding (3) for sugar separations by the low performance (i.e., open column) IMP chromatography of 12 cation resin forms clearly showed the potential of this method for fine tuning improvements in problem separations by the use of non-commercial resin forms.

We present here the results of studies designed to evaluate a larger data base of IMP separations than those previously reported, in that we have examined 13 resin cation forms and their effect on the elution of 28 compounds.

MATERIALS AND METHODS

Metal Salts, Elution Standards, and Chromatography Resin

The chromatographic resin used in this study was the Aminex A-7 divinylbenzene-crosslinked sulfonated polystyrene cation-exchange resin (8% crosslinking and 7-11 microns diameter, BioRad, Richmond, CA). This resin is normally supplied in the sodium form. Chloride and hydroxide salts of lithium, potassium, calcium, and barium were obtained from J.T. Baker Chemicals (Phillipsberg, NJ) as reagent grade (99+% or higher purity). Chlorides, hydroxides, or oxides of magnesium, strontium, rubidium, cobalt, cadmium, and cesium were obtained from Aldrich Chemicals (Milwaukee, WI) as reagent grade (99+%). Silver nitrate (99+%) was obtained from Aldrich and cadmium oxide (99.9%) was from Johnson-Matthey (Seabrook, NH). Commercial columns in the lead and calcium-forms (HPX-87P and HPX-87C) were obtained from BioRad.

Elution standards were obtained from Sigma Chemicals (St. Louis, MO). These included DL-glyceraldehyde, erythritol, D(-)ribose, xylitol, D(+)arabitol, D(-) arabinose, α-L(-)fucose, xylulose, D-lyxose, D-sorbitol, D-mannitol, D(+)xylose, β-D(-)fructose, D(+)mannose, D(+)galactose, α-L-rhamnose, D-tagatose, L(-)sorbose, α-D(+)glucose, D-mannoheptulose, D-3-O-methyl glucose, lactulose, maltose,

D(+)cellobiose, β-gentibiose, D(+)melezitose, and deuterium oxide. D-turanose was from Aldrich.

Instrumentation and Chromatography

Column chromatography was performed with a Hewlett-Packard model 1081 B liquid chromatography system equipped with autoinjection and a column temperature control oven. Elutions were monitored with a Waters model 401 refractive index detector. Eluant flow rate and column temperature were maintained at 0.3 mL/min and 60°C, respectively. Output from the detector was stored on disk using the Baseline Chromatography software (Dynamic Solutions, Ventura, CA). Deuterium oxide elution was used to perform theoretical plate analysis (6) and to establish the total column volume, V_t (taken as equal to flow rate x D_2O retention time, t_{D_2O}), of the custom-packed columns. Most standards were chromatographed twice to insure reproducibility.

Interconversion of Resin Forms

The pH and salt concentration ranges required to maximize metal cation availability were calculated by the use of stability constants critically selected by Smith and Martell (7). The equilibria between each "free" cation and its hydroxide, nitrate, and chloride complexes were plotted versus pH in order to estimate both the acceptable pH range and the limits of total cation solubility. These pH values and salt concentrations were then used to assure both total cationic molarities well in excess of equivalent resin sites, and concentrations of "free" cation sufficient to make the resin-conversion processes kinetically efficient. In solutions of pH equal to or less than pH 11, chloride salts of lithium, potassium, strontium, rubidium, and cesium yield 80% to 100% free cation per mole of salt. Under these conditions barium chloride is notably less dissociated, with only 52% free cation per mole of salt available. In solutions ranging from pH 7 to 8, chloride salts of cadmium, cobalt, and magnesium yield 2%, 50%, and 85% of free cation per mole of salt, respectively. Calcium chloride solutions maintained below pH 8 yield 100% available cation/mole of salt.

Following chromatographic evaluation of each metal-ion form of the resin, the packing (~ 30 g) was removed from the column, slurried for one hour in 300 mL of gently boiling 6 N HCl, then recovered by vacuum filtration on a glass-fiber filter and washed with 7 to 10 successive 75-mL portions of boiling 6 N HCl. After a final wash with deionized water, the resin, now hydrogen form, was slurried for 24 to 72 hours

at 25°C in 250 mL of a 0.5 to 1.0 M solution of the chloride salt (or, in the case of silver, the nitrate salt) of the "new" metal counter-ion. Using this procedure, the resin was effectively titrated until the pH of the slurries indicated that no further significant displacement of hydrogen ions from the resin was taking place. When available, the hydroxide or oxide forms of the cations were used to adjust slurry pH rapidly. Prior to column packing, the resins were washed extensively with distilled water.

Column Packing

HPLC columns were packed using a 40-mL commercial packing reservoir (Alltech, Deerfield, Il) attached to a standard 7.8 x 30 mm stainless steel column. After introducing a 50% dilution of the resin slurry to the column/column reservoir system, the system was pumped at a flow rate of 0.3 mL/min for approximately 5 min. The flow rate was then increased to 2 mL/min, which corresponded to approximately 2,000 psi. Over the course of 4 to 5 h, the flow rate was slowly decreased manually to maintain the column back pressure in the 1200 to 1500 psi range. Finally, a flow rate of 0.3 mL/min resulted in a stable back pressure of 500-700 psi.

Cation Analysis

Three mLs of settled column resin were pipetted into a centrifuge tube, to which 10 mL of 6 N HCl was also added. Samples were shaken for 1 h, centrifuged and filtered through a 0.22 micron filter. Cations were analyzed on a Plasma 100 (Allied Analytical Services) Inductively Coupled Plasma (ICP) Spectrophotometer. Samples were aspirated though a peristaltic pump at 1.0 mL/min. Plasma gas was argon, regulated at 15 mL/min. Nebulizer pressure was 40 psi. Three readings were performed for each sample, with mean and standard deviations reported.

RESULTS AND DISCUSSION

Eleven columns were packed in the lithium, potassium, rubidium, cesium, magnesium, calcium, strontium, barium, cobalt, silver, and cadmium forms using the standard slurry method (8). These custom columns demonstrated theoretical plate counts (calculated from the injection of deuterium oxide), which range from 4000 to 7900 (see Table 1). These values compare favorably with the two commercially packed columns tested (i.e., calcium, 7200 and lead, 8100). Table 1 also shows that the methodology used for stripping the column packing material with acid and changing the cation form was efficient and effective, provided the equilibria between

TABLE 1

Characteristics of Custom-Packed IMP Columns

Cation form	Number of Theoretical Plates*	Efficiency of Conversion**
Lithium	6900	99.5%
Sodium	7800	commercial resin
Potassium	6500	NT
Rubidium	6300	NT
Cesium	5900	NT
Magnesium	6800	99.9%
Calcium	6600	99.99%
Calcium	7200	commercial-column
Strontium	6800	99.99%
Barium	4000	95.5%
Cobalt	6900	99.99%
Silver	7900	99.99%
Lead	8100	commercial-column
Cadmium	7900	99.99%

*Calculated from the injection of D_2O. NT is not tested. **Percentage new cationic form relative to cationic form previous to acidic stripping procedure.

metal ion and anions present (hydroxide and the salt anion) are taken into account as described under Materials and Methods.

The sugars and related compounds chosen as elution standards for these columns were chromatographed individually and, with the exceptions noted in Tables 2 and 3, were eluted as single, symmetrical peaks. Elution data is shown in Tables 2 and 3 as elution time ratios, $t_e/t_{D_2O} \times 100$, which provides a normalization of the data from columns packed with various cationic-forms of the Aminex resin because the differences in elution of deuterium oxide should reflect only variance in purely physical, non-interactive properties, such as different total available volumes, which can result from differences in packing efficiencies. Examination of Tables 2 and 3 shows some limited conservation of elution behavior between selected column cation-forms. For example, the elution of the disaccharides, the ketohexoses, the aldofuranoses, and the alditols is quite similar for the lithium and sodium-form columns. Also, a strong correlation of elution performance for nearly all standards was observed between the cobalt and the cadmium columns, even though these metal ions are from the transition metal groups VIII and II, respectively. Considering the

TABLE 2
Elution of Sugars and Sugar Derivatives from Aminex A-7 Columns in Various Cationic Forms
Values given are elution time ratios, t_e/t_{D_2O} x 100

	mel	gen	cell	mal	tur	glu	mgl	gal	fuc	man	rha	sor	tag	fru	D_2O (min)
Li	39.4	41.6	42.4	43.1	43.7	53.1	50.3	56.9	60.6	60.6	56.3	54.4	55.4	57.4	34.8
Na	42.1	41.7	42.1	43.1	NoPk	53.8	47.5	58.0	64.3	57.0	55.4	54.4	55.0	57.4	33.1
K	42.3	52.9	51.0	56.1	51.4	69.9	57.0	76.2	82.6	78.8	72.1	69.1	69.1	79.9	31.4
Rb	43.2	55.3	52.3	54.5	52.3	71.4	57.9	78.6	85.1	81.5	73.2	70.5	70.5	77.9	30.7
Cs	45.6	58.5	54.8	56.8	54.6	74.8	60.9	80.7	85.4	83.6	75.6	73.1	73.4	79.5	30.5
Mg	46.6	48.5	49.0	49.8	50.1	57.2	56.3	60.2	65.8	60.6	61.5	58.0	59.3	60.2	35.8
Ca	46.7	49.8	50.8	52.4	57.5	64.1	58.4	72.0	80.8	72.8	72.6	70.6	103	85.2	32.7
Sr	50.5	54.8	56.2	58.5	58.9	67.5	61.2	74.7	81.7	78.0	75.4	75.4	110	87.3	34.3
Ba	67.7	68.8	70.4	73.7	74.1	80.5	72.4	87.8	93.4	95.5	87.3	87.8	110	102	33.6
Co	47.7	49.4	50.3	51.0	51.6	58.7	57.3	62.4	68.1	62.8	63.2	59.5	61.9	62.6	36.3
Ag	68.2	80.3	81.9	81.5	81.5	92.3	74.1	108	102	108	97.9	91.5	91.3	106	32.6
Pb	60.6	---	64.5	66.9	---	77.2	---	98.9	94.8	113	88.5	93.0	197	119	33.3
Cd	47.0	48.7	49.5	50.7	51.3	58.9	57.3	62.8	69.8	63.5	64.1	59.9	61.8	63.7	34.2

mel = melezitose, gen = beta-gentibiose, cell = cellobiose, mal = maltose, tur = turanose, glu = glucose, mgl = 3-O-methyl glucose, gal = galactose, fuc = fructose, man = mannose, rha = rhamnose, sor = sorbose, tag = tagatose, and fru = fructose.

TABLE 3
Elution of Sugars and Sugar Derivatives from Aminex A-7 Columns in Various Cationic Forms
Values given are elution time ratios, t_e/t_{D_2O} x 100

	xyl	lyx	arab	rib	matol	sotol	artol	xytol	ertol	glyol	glde	xyul	laul	maul	D_2O (min)
Li	57.7	60.2	61.8	66.1	58.3	59.5	62.8	64.0	66.5	73.8	66.6	60.3	45.4	51.7	34.8
Na	58.4	59.8	64.5	70.1	58.0	61.4	62.9	66.5	65.3	72.5	MPk	MPk	45.1	51.6	33.1
K	76.3	80.1	65.7	92.3	62.3	64.6	65.5	69.5	68.0	77.8	85.2	79.1	58.3	62.3	31.4
Rb	78.4	82.1	88.4	92.4	61.2	64.5	65.3	68.8	67.9	74.2	86.2	80.5	59.9	63.6	30.7
Cs	81.0	84.8	90.0	90.0	63.1	65.5	66.8	69.4	69.7	74.9	86.9	81.8	61.8	66.4	30.5
Mg	61.1	63.2	64.9	66.7	61.5	62.4	65.1	66.2	68.8	75.9	67.5	63.4	51.4	55.9	35.8
Ca	69.6	88.0	83.2	157	99.1	149	116	143	96.6	102	91.5	84.9	61.0	73.4	32.7
Sr	74.0	89.2	84.2	158	104	---	106	---	91.4	94.5	90.6	87.9	65.7	77.2	34.3
Ba	86.6	96.2	95.7	---	103	126	106	125	95.8	98.6	100	99.3	81.6	88.0	33.6
Co	62.6	65.4	67.2	71.7	68.5	71.1	72.7	74.7	75.5	82.6	70.6	64.8	53.1	57.8	36.3
Ag	93.6	99.0	111	116	96.6	105	98.3	108	94.8	96.6	98.5	91.1	107	91.1	32.6
Pb	84.8	---	102	183	189	122	---	266	---	124	---	96.0	---	146	33.3
Cd	63.0	62.8	68.4	72.3	67.1	69.4	71.6	73.9	74.3	82.3	71.4	62.8	53.2	58.9	34.2

xyl = xylose, lyx = lyxose, arab = arabinose, rib = ribose, matol = mannitol, sotol = sorbitol, artol = arabitol, xytol = xylitol, ertol = erythritol, glyol = glycerol, glde = glyceraldehyde, xyul = xylulose, laul = lactulose, maul = mannoheptulose, and MPk = multiple peaks.

TABLE 4

Variance in Chromatographic Data (elution ratio, t_e/t_{D_2O})x100

Sugar	Mean of all cations	Std. dev.
oligosaccharides:		
melezitose	50	9.5
gentibiose	54	11
cellobiose	55	11
maltose	57	11
turanose	57	11
aldohexoses:		
glucose	68	12
3-O-methyl glucose	59	7.6
galactose	75	16
fucose	80	13
mannose	78	18
rhamnose	73	13
ketohexoses:		
sorbose	71	14
tagatose	86	39
fructose	80	20
aldofuranoses:		
xylose	73	12
lyxose	78	15
arabinose	81	16
ribose	103	41
alditols:		
mannitol	84	37
sorbitol	85	32
arabitol	80	20
xylitol	100	59
erythritol	78	13
glycerol	87	15
others:		
glyceraldehyde	83	12
xylulose	79	14
lactulose	62	17
mannoheptulose	73	26

significant data base of elution times resulting from this 13 x 28 matrix of elution standards and cation resin forms, no attempt was made to perform chromatography of mixed standards and make a careful determination of resolution factors. In general, however, a separation in elution ratio of 4.0 corresponds approximately to an elution-time separation of 2 min, which provides nearly baseline separation under the conditions of this experiment. The results from Tables 2 and 3 were also examined for trends from the perspective of the standard deviation of elution times as a function of all sugars for each cation-form column and for all columns for each sugar. These results are shown in Tables 4 and 5. From these tables the general sensitivity of the sugars to cation form can be assessed. Table 4 shows that the elution of oligosaccharides vary over a narrow range of elution times whereas ribose, tagatose, and xylitol appear to be very sensitive to column cation-form. Table 5 shows that chromatography on most Group IA columns produces small deviations in elution times for all sugars; however, the transition metal column lead, and the Group IIA columns calcium and strontium, produce much higher variations in sugar elution.

TABLE 5

Variance in Chromatographic Data (elution ratio, t_e/t_{D_2O})x100

Cation form	Mean of all sugars	Std. dev.
Group IA:		
Lithium	56	8.7
Sodium	56	8.9
Potassium	68	12
Rubidium	70	12
Cesium	72	12
Group IIA:		
Magnesium	60	7
Calcium	84	29
Strontium	81	23
Barium	92	15
Transition metals:		
Cobalt	63	8.8
Silver	95	12
Lead	119	53
Cadmium	63	8.8

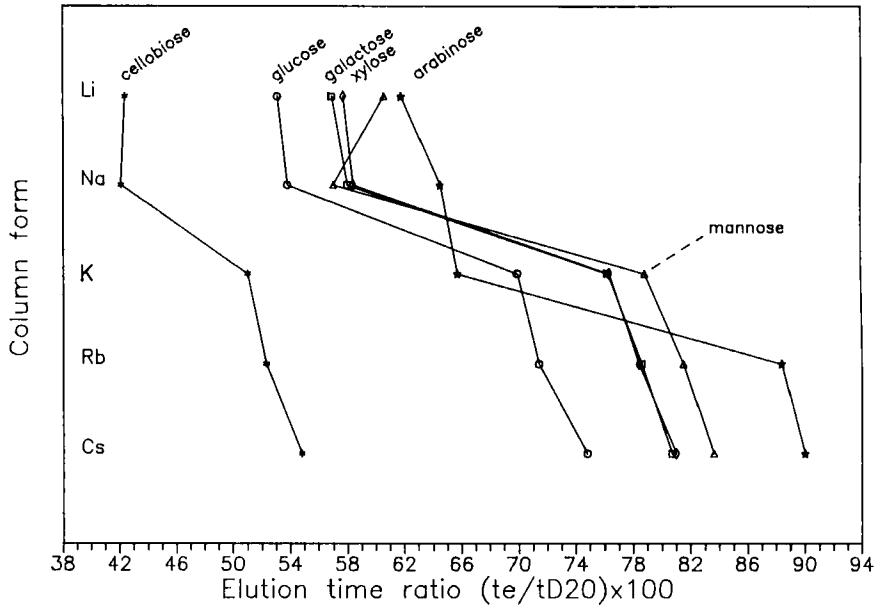

FIGURE 1. Elution of wood hydrolysate sugars from custom packed HPLC columns. Columns were slurry packed with BioRad Aminex A-7 chromatographic resin coordinated with Group IA cations. Data are shown as the elution time ratio, $t_e/t_{D_2O}x100$, which normalizes all chromatographic experiments to the elution of a non-interactive solute.

The separation of sugars produced by the hydrolysis of wood is an example of a traditional analytical separations problem. Figures 1 through 3 show the relationships between elution ratio and column cation-form for cellobiose, glucose, galactose, xylose, arabinose, and mannose. The analytical separation of mannose and galactose on calcium-form columns has long posed a problem for researchers examining wood hydrolysates. The other commercially available columns, silver and lead-form, resolve poorly the pairs, glucose/xylose and galactose/mannose, and galactose/arabinose, respectively. A further consequence of the use of lead-form columns is that the chromatography of certain sugars, such as ketoses, has been shown to result in degradation (9). Of the tested column forms that are not available commercially, the strontium-form column is the best overall performer, separating all but the xylose and galactose pair quite well. Also, arabinose and galactose, which are difficult to

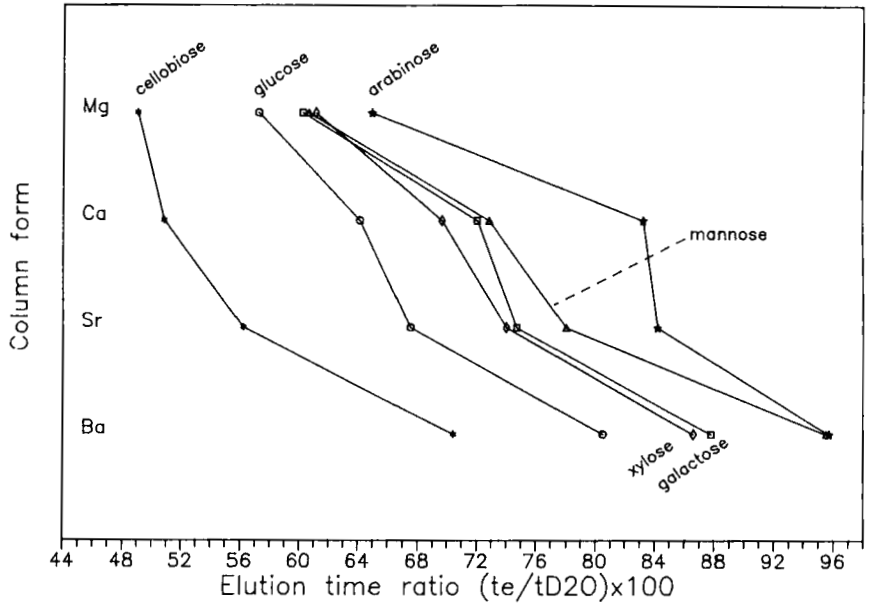

FIGURE 2. Elution of wood hydrolysate sugars from custom packed HPLC columns. Columns were slurry packed with BioRad Aminex A-7 chromatographic resin coordinated with Group IIA cations.

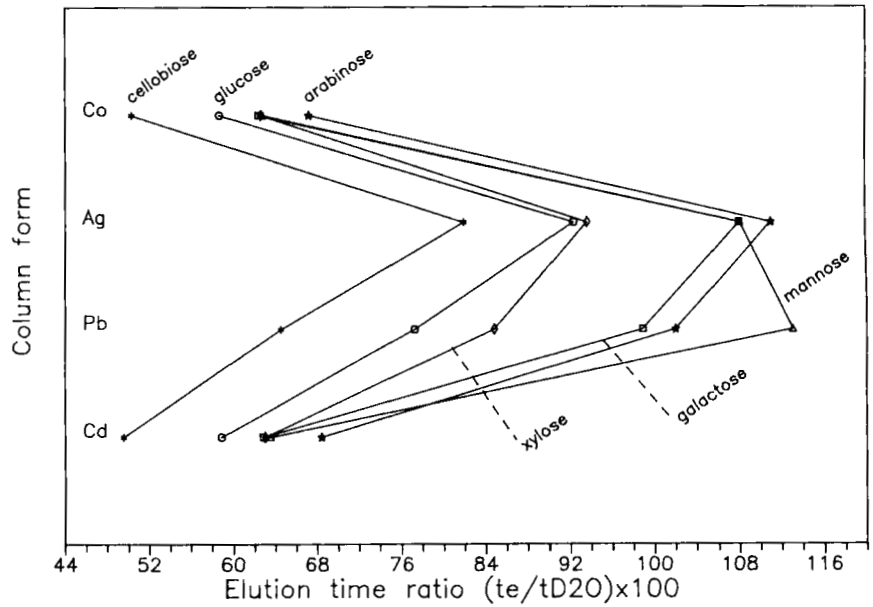

FIGURE 3. Elution of wood hydrolysate sugars from custom packed HPLC columns. Columns were slurry packed with BioRad Aminex A-7 chromatographic resin coordinated with selected transition metals.

separate adequately on either of the widely used commercial lead- and silver-form columns, are readily resolved on rubidium- or cesium-form columns.

In general, the interactions between the sugars and the various metal-ion forms of the resins are highly individual -- no two sugars show exactly the same pattern of retention times with the array of metal counter-ions tested, and no two metals show the same elution pattern for all of the sugars. By proper selection of metal counter-ion, therefore, any pair of sugars drawn from those present in wood hydrolysates may be resolved sufficiently to allow quantitation of both sugars.

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