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High-performance anion-exchange chromatography of sugars and sugar alcohols on quaternary ammonium resins under alkaline conditions

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ABSTRACT

Ninety-three sugars and sugar alcohols of one to four monosaccharide units were subjected to high-performance anion-exchange chromatography on three quaternary ammonium columns of the same size and virtually the same composition under identical eluent flow rates (1 $mL \cdot min^{-1}$) and compositions (aqueous 0.1N NaOH). Although the columns had very different histories of use, their different capacity factors for individual carbohydrates can be linearly correlated. Those for monosaccharides are lowest for sugar alcohols, and are higher and roughly similar for analogous aldoses and ketoses. There is a general trend, strongest with sugar alcohols, for capacity factors to increase with increasing numbers of carbon atoms. Increasing numbers of hydroxyl groups on residues of similar structure lead to increasing capacity factors. For members of homologous oligosaccharide series, capacity factors increase in a regular and predictable manner with chain length. Peak areas for different sugars generated by a differential refractometer are roughly correlated with mass concentration; with a pulsed amperometric detector, there are equally rough correlations with either molar or mass concentration.

INTRODUCTION

Chromatographic separation of carbohydrates of the same molecular weight, especially those containing more than one monosaccharide unit, is often a difficult undertaking. As chain length increases, resolution between isomers progressively decreases. Even analysis of monosaccharides of similar structural and chemical properties can pose severe problems.

There are two ways to enhance the separation of similar materials. One is to choose a chromatographic technique that yields very narrow peaks, so that even small differences in retention times yield acceptable resolutions. The other is to find a technique that magnifies subtle variations in structure and chemistry into large differences in retention times.

Two chromatographic methods appear to be most effective in attaining both goals. The first is capillary gas chromatography of derivatized carbohydrates, which can yield widely dispersed retention times for even similar di- and tri-saccharides[1,2]. This

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method becomes convenient if the derivatization method is not overly tedious. The second method, much newer, is h.p.l.c. with strongly basic resins using high-pH eluents to take advantage of the slight acidity of carbohydrates[3] and is most effective when used with a pulsed amperometric detector (p.a.d.)[4]. A number of research groups have used this separation technique, not only with simple mono-, di-, and oligo-saccharides[3,5–9], but also with series of homologous oligosaccharides[10,11] and with oligosaccharides containing several different monomeric residues[12–15].

This article is the account of a joint effort between two laboratories to investigate the use of strong-base h.p.l.c. with quaternary ammonium resins to separate sugars and sugar alcohols of one to four residues under the same operating conditions. No effort has been made to optimize the separation of any particular pair of components. Instead work has been directed to correlate the following: (*i*) retention times of the same carbohydrates measured by the two laboratories; (*ii*) retention times with carbohydrate structural and chemical properties; (*iii*) retention times with chain lengths of members of homologous oligosaccharide series; (*iv*) responses to the same carbohydrates by differential refractive index (r.i.) and p.a. detectors. An article from the same two laboratories that concentrated on the separation of sugars found in sucrose processing has already appeared[16].

EXPERIMENTAL

Materials. — Except as indicated, sugars and sugar alcohols used in this project were purchased from laboratory suppliers and were used without further purification. Of the materials used at the Zuckerinstitut at Technische Universität Braunschweig (ZIB), D-fructose, D-glucose, and sucrose were recrystallized from aqueous ethanol, while 6-*O*-(α-D-glucopyranosyl)-D-mannitol, isomaltitol, isomaltose, palatinose, and trehalulose were donated by Südzucker AG, Mannheim/Ochsenfurt, F.R.G. Leucrose came from Pfeifer & Langen, Köln, F.R.G. Galactinol was separated from beet molasses by gel chromatography on Fractogel TSK HW-40S and purified by repeated preparative chromatography on amino-bonded silica gel. Among the sugars used at Iowa State University (ISU), maltulose and 3-*O*-β-cellobiosyl-D-glucose were gifts of Dr. H. D. Scobell of A. E. Staley Manufacturing Co., Decatur, IL, U.S.A., and Dr. Bent Stig Enevoldsen of the Carlsberg Research Laboratory, Copenhagen, Denmark, respectively. Xylo- and isomalto-oligosaccharides were prepared as previously described[19,20], as were α,β- and β,β-trehalose[21]. Sodium hydroxide solution was made either from a 10N carbonate-free NaOH solution added to deionized water and stored in a high-density polyethylene container under nitrogen bubbling (ISU), or from NaOH pellets dissolved at high concentration in double-distilled water, followed by filtration of the carbonate precipitate and dilution with double-distilled water (ZIB). Storage of the latter preparation was in polyethylene containers under helium bubbling.

Chromatography. — Three different experimental systems were used for this work. The first (ZIB) had a Knauer 64.00 h.p.l.c. pump, Dionex HPIC-AG6 (25 mm × 3 mm i.d.) precolumn, Dionex HPIC-AS6 (250 mm × 4 mm i.d.) column, Dionex type

II p.a.d. with a gold working electrode, and Shimadzu C-R1B integrator. The second (also ZIB) had a Dionex GMP pump, Dionex CarboPac PA (25 mm × 3 mm i.d.) precolumn, Dionex CarboPac PA1 (250 mm × 4.0 mm i.d.) column, Dionex type II p.a.d. with a gold working electrode, and Hitachi D-2000 detector. In both systems the working electrode was maintained at 0.1 V for 60 ms, 0.6 V for 60 ms, and −0.8 V for 240 ms, the voltages measured against a standard calomel electrode[4]. The third system (ISU) had an ISCO 2350 h.p.l.c. pump, Erma ERC-3510 degasser, Dionex HPIC-AG6A precolumn, Dionex HPIC-AS6 column, Knauer r.i. detector, and Hewlett–Packard 3392A integrator.

Eluent was 0.1N NaOH, eluent flow rate was 1 mL·min^{-1}, and temperature was close to 25° for all experiments with the three columns so that the factors that influence column performance and detector response could be identified and explained more completely. Better separation of individual pairs, especially monosaccharides, could have been attained with lower NaOH concentrations and with added anions such as acetate[5,6]. Carbohydrates at ISU were dissolved in 0.1N NaOH and quickly chromatographed, as use of samples dissolved in water caused instability of the baseline detected by the r.i. detector. Samples at ZIB were dissolved in water. All three columns had seen extensive use before this project, and none had been cleaned or regenerated before use. All data sets were obtained within an elapsed time of two months, and standards gave consistent retention times throughout.

Calculation of capacity factors and substituent parameters. — The retention time of component i (t_{Ri}) was converted into a capacity factor k'_i by the relationship

$$k'_i = (t_{Ri} - t_0)/t_0 \qquad (1)$$

where t_0 is the dead time, measured from the retention time at the beginning of the initial peak. In turn, capacity factors of components of different compositions and linkages were used to obtain substituent parameters τ_{ij} by the equation[22,23]

$$\ln(k'_i/k'_p) = (\Delta G^0_p - \Delta G^0_i)/RT = \sum_{j=1}^{m} \tau_{ji} \qquad (2)$$

where each component arises from the addition of a substituent at the nonreducing end of a parent compound p, and m is the total number of substituents[22]. If values of τ_{ji} for a given substituent in a given position do not vary with different parent compounds, but are additive with different substituents in the same or different positions, τ_{ji} simplifies[22] to τ_j. All substituents of the materials studied here were carbohydrate residues, and therefore a plot of ln k'_i vs. chain length should give straight lines for homologous oligosaccharide series, each having a characteristic slope τ_j, and should give common slopes for the same substituent added with the same linkage to different parent compounds if the assumption holds.

Measurement of detector response. — For measurement of detector linearity and detection limit, D-glucose was dissolved in either water (ZIB) or 0.1N NaOH (ISU). The

TABLE I

Capacity factors (k') of carbohydrates measured with three strong-base columns eluted with 0.1N NaOH

Carbohydrate	*Capacity factor*			*Carbohydrate*	*Capacity factor*		
	ZIB[a]		*ISU*[a]		*ZIB*[a]		*ISU*[a]
	AS6[b]	*PA1*	*AS6*[b]		*AS6*[b]	*PA1*	*AS6*[b]
D-Allose			2.01	D-*glycero*-Tetrulose[c,d]			--
D-Altrose			2.78	D-Gulose			1.94
D-Arabinose	1.57	2.21	1.13	L-Iditol			0.40
L-Arabinose			1.13	D-Idose			3.85
D-Arabinitol	0.43			*myo*-Inositol	0.21	0.26	0.18
L-Arabinitol			0.35	Isomaltitol	0.86		
Cellobiose	7.00	11.3	4.15	Isomaltose	3.93	6.43	2.94
3-*O*-β-Cellobiosyl-D-glucose			4.82	Isomaltriose			5.96
Cellotetraose			30.7	3-Ketosucrose[c]			
Cellotriose			12.3	Kojibiose[c]			3.43
Chloralose			7.13	Lactose	4.21	6.67	2.69
2-Deoxy-D-*arabino*-hexose[c]			1.15	Lactulose	4.14	6.59	2.92
2-Deoxy-D-*erythro*-pentose[c,e]	1.00	1.49	0.75	Laminaribiose[c]			4.63
2-Deoxy-D-*lyxo*-hexose[c]			0.97	Leucrose	3.36		
D-Digitoxose			0.68	D-Lyxose			1.38
1,3-Dihydroxy-2-propanone[c,f]			--	Maltitol	2.00		
DL-Dithiothreitol			1.61	Maltose	11.4	19.2	6.87
Erythritol	0.21	0.33	0.21	Maltotritol	5.79		
D-*erythro*-Pentulose[c,f]			--	Maltotriose			25.5
D-Erythrose			1.99	Maltulose			2.87
D-Fructose[c]	2.50	3.51	1.73	D-Mannitol	0.71	1.08	0.56
D-Fucose	0.86	1.19	0.65	D-Mannose			1.44
L-Fucose			0.65	L-Mannose			1.43
DL-1-*O*-α-D-Galactopyranosyl-*myo*-inositol	0.43			Melezitose	7.80	13.1	4.51
D-Galactitol[c]	0.57	0.80	0.44	Melibiitol			0.62
D-Galactose	2.07	3.04	1.50	Melibiose			2.01
L-Galactose			1.52	α-Methyl-D-mannoside			0.21
Gentiobiose			3.92	3-*O*-Methyl-D-glucose			0.92
D-Glucitol	0.57	0.84	0.45	Nigerose[c]			6.32
D-Glucoheptose			3.03	Palatinose	6.79	11.4	4.82
D-Glucoheptulose			3.03	Panose			6.26
6-*O*-α-D-glucopyranosyl)-D-mannitol	1.21			D-Psicose			1.82
D-Glucose	2.14	3.05	1.51	Raffinose	8.07	15.1	5.54
L-Glucose			1.50	L-Rhamnose	1.07	1.61	0.90
DL-Glyceraldehyde[c]	0.93	1.27	0.84	Ribitol			0.44
Glycerol			0.17	D-Ribose	2.79	3.97	1.83
				Sedoheptulose			0.23
				Sophorose			8.86
				D-Sorbose	2.36		
				L-Sorbose			1.80
				Stachyose			6.29
				Sucrose	4.43	7.52	3.30
				D-Tagatose[c]			1.50
				D-Talose			3.02

TABLE I, continued

Capacity factors (k_i') of carbohydrates measured with three strong-base columns eluted with 0.1N NaOH

Carbohydrate	*Capacity factor*			*Carbohydrate*	*Capacity factor*		
	ZIB[a]		*ISU*[a]		*ZIB*[a]		*ISU*[a]
	AS6[b]	*PA1*[c]	*AS6*[d]		*AS6*[b]	*PA1*[c]	*AS6*[d]
DL-Threitol			0.20	Turanose[e]	6.29	10.8	4.29
D-*threo*-Pentulose			3.26	Xylitol			0.30
D-Threose			1.82	Xylobiose			3.23
α,α-Trehalose	0.86	1.34	0.63	D-Xylose	2.21	3.19	1.59
α,β-Trehalose			3.13	Xylotetraose			11.5
β,β-Trehalose			1.15	Xylotriose			5.70
Trehalulose	4.57						

[a] ZIB indicates work carried out at the Zuckerinstitut, Technische Universität Braunschweig; ISU indicates work carried out at the Iowa State University. [b] Dead time: 1.40 min; column: Dionex HPIC-AS6; eluent flow rate: 1 mL·min^{-1}; isocratic NaOH concentration: 0.1N; temperature: 25°; sample concentration: 0.1 g·L^{-1}; sample volume: 20 μL; detector: Dionex p.a.d. with gold electrode. [c] Dead time: 1.25 min; column: Dionex CarboPac PA1; eluent flow rate: 1 mL·min^{-1}; isocratic NaOH concentration: 0.1N; temperature: 25°; sample concentration: 22–192 mg·L^{-1};sample volume: 20 μL; detector: Dionex p.a.d. with gold electrode. [d] Dead time: 1.42 min; column: Dionex HPIC-AS6; eluent flow rate: 1 mL·min^{-1}; isocratic NaOH concentration: 0.1N; temperature: 25°; sample concentration: 2.8mM; sample size: 20 μL; detector: Knauer differential refractometer. [e] Degradation in alkaline solution can cause multiple peaks. [f] Trivial names (in parentheses) include the following: 2-Deoxy-D-*arabino*-hexose, (2-deoxy-D-glucose); 2-deoxy-D-*erythro*-pentose, (2-deoxy-D-ribose); 2-deoxy-D-*lyxo*-hexose, (2-deoxy-D-galactose); 1,3-dihydroxy-2-pentanone, (dihydroxyacetone); D-*erythro*-pentulose, (D-ribulose); D-galactitol, (dulcitol); 1L-1-*O*-α-D-galactopyranosyl-*myo*-inositol, (galactinol); D-*glycero*-tetrulose, (D-erythrulose); D-glucitol, (sorbitol); D-*threo*-pentulose, (D-xylulose).

resulting samples were chromatographed under standard conditions with the second (ZIB) or third (ISU) chromatographic systems to compare the responses of p.a. and r.i. detectors.

RESULTS AND DISCUSSION

A total of 93 carbohydrates, 37 at ZIB and 82 at ISU, including 26 measured at both places, were chromatographed on strong-base anion exchange columns. Their capacity factors are presented in alphabetical order in Table I. Three ketoses, 1,3-dihydroxy-2-propanone (dihydroxyacetone), D-*glycero*-tetrulose (D-erythulose), and D-*erythro*-pentulose (D-ribulose), along with 3-ketosucrose, were so badly degraded by the alkaline solution in the column that no dominant peak survived. Two other ketoses, D-fructose and D-tagatose, and in addition 3-deoxy-D-*erythro*-pentose (3-deoxy-D-ribose), glyceraldehyde, and four disaccharides, kojibiose, laminaribiose, nigerose, and turanose, gave several peaks when dissolved in NaOH solution and then chromatographed, although the peak representing the original material remained easily identifiable. Of the disaccharides, all but kojibiose are linked through the 3-OH, and,

therefore, would be especially susceptible to β-elimination reactions. Solution in water before chromatography, as is possible if a p.a.d. is used, helps to prevent degradation of carbohydrates that are especially sensitive to alkaline conditions.

Capacity factors of both enantiomeric forms of carbohydrate measured with the same column, as they were with arabinose, fucose, galactose, glucose, and mannose, are essentially equal, as would be expected (Table I). Therefore it is possible to use values of k'_i in correlations without regard to enantiomeric form.

Effect of column on capacity factor. — Values of k'_i of carbohydrates measured in both laboratories under the same conditions are quite different, those from ISU with an HPIC-AS6 column being lowest, followed by those from ZIB with the same type of column and finally by those from ZIB with a CarboPac PA1 column (Table I). Several reasons for these differences are possible, the most likely reason being the different histories of use of the columns, the different packing diameters employed in the precolumns used in the first two systems (5 μm in the HPIC-AG6A *vs.* 10 μm in the HPIC-AG6 precolumn), and the slightly different composition of the CarboPac PA1 column compared to the HPIC-AS6 columns. However, k'_i values follow an almost linear correlation that passes near the origin (Fig. 1), giving confidence that results from one column can be extrapolated to another operated under the same conditions if k'_i values of several benchmark standards are measured with each.

Effect of molecular structure on capacity factor. — All fifteen aldoses containing three to six carbon atoms, along with one heptose, were subjected to chromatography with the same column at ISU; their capacity factors are listed by structural type in Table II. The order of capacity factors is the same as that previously reported[6,7] except that

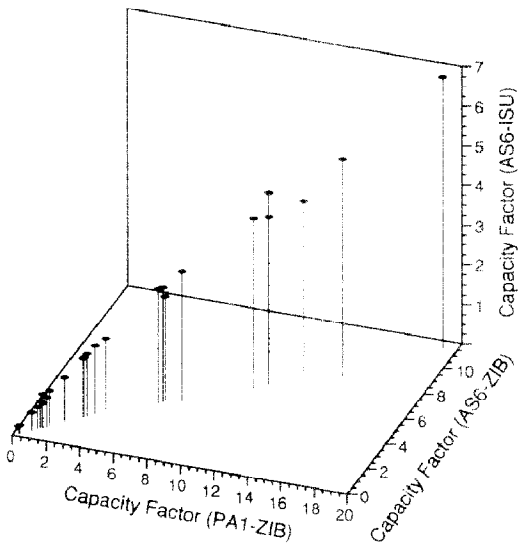

Fig. 1. Comparison of capacity factors of identical carbohydrates with Dionex HPIC-AS6 columns at the Zuckerinstitut, Braunschweig and at Iowa State University, and a Dionex CarboPac PA1 column at the Zuckerinstitut Braunschweig.

TABLE II

Capacity factors (k_i') determined for various sugars at Iowa State University[a]

Compound	k_i'	*Compound*	k_i'
Triose		D-*threo*-Pentulose[b]	3.26
D-Glyceraldehyde	0.84	Ketohexoses	
Aldotetroses		D-Psicose	1.82
D-Erythrose	1.99	D-Fructose	1.73
D-Threose	1.82	D-Sorbose	1.80
Aldopentoses		D-Tagatose	1.50
D-Ribose	1.85	Ketoheptose	
D-Arabinose	1.13	D-Glucoheptulose	3.03
D-Xylose	1.59	Triol	
D-Lyxose	1.38	Glycerol	0.17
Aldohexoses		Tetritols	
D-Allose	2.01	Erythritol	0.21
D-Altrose	2.78	D-threitol	0.20
D-Glucose	1.51	Pentitols	
D-Mannose	1.44	Ribitol	0.44
D-Gulose	1.94	D-Arabinitol	0.35
D-Idose	3.85	Xylitol	0.30
D-Galactose	1.50	D-Lyxitol	—[d]
D-Talose	3.02	Hexitols	
Aldoheptose		Allitol	—[d]
D-*glycero*-D-*gulo*-Heptose[b]	3.03	D-Altritol (D-Talitol)	—[d]
Ketotrioses		D-Glucitol[b]	0.45
1,3-Dihydroxy-2-propanone[b]	—[c]	D-Mannitol	0.56
Ketotetrose		D-Gulitol	—[d]
D-*glycero*-Tetrulose[b]	—[c]	D-Iditol	0.40
Ketopentose		D-Galactitol[b]	0.44
D-*erythro*-Pentulose[b]	—[c]		

[a] Column: HPIC-AS6. For details, see Experimental section. [b] Trivial names (in parentheses) include the following: D-*glycero*-D-*gulo*-heptose, (D-glucoheptose); 1,3-dihydroxy-2-pentanone, (dihydroxyacetone); D-*glycero*-tetrulose, (D-erythrulose); D-*erythro*-pentulose, (D-ribulose); D-*threo*-pentulose, (D-xylulose); D-glucitol, (sorbitol); D-galactitol, (dulcitol). [c] Decomposes under conditions of chromatography. [d] Not determined.

D-mannose precedes D-galactose, D-glucose, and D-xylose, instead of following them, perhaps because of the higher NaOH concentration used in this work. Although there is a overall increase of k_i' with increasing numbers of carbon atoms, variations are so great that no trustworthy correlation is possible. Those ketoses that are sufficiently stable in 0.1N NaOH to be successfully chromatographed gave values of k_i' roughly similar to those of the equivalent aldoses (Table II). Sugar alcohols have k_i' values that steadily increase with increasing numbers of carbon atoms (Table II). Their capacity factors generally are less than one-third of aldoses of equivalent structure. The same behavior occurs with sugar–sugar alcohol pairs of greater than one residue, such as isomaltose–isomaltitol, maltose–maltitol, melibiose–melibiitol, and maltotriose–maltotriitol. Decreases of k_i' were also noted when 2- and 6-OH groups are replaced by hydrogen atoms, such as with the pairs D-galactose–2-deoxy-D-*lyxo*-hexose, D-glucose–2-deoxy-D-*arabi*-

no-hexose, and D-ribose 2-deoxy-D-*erythro*-pentose, on one hand, and D-galactose–D-fucose and L-mannose–L-rhamnose, on the other. D-Digitoxose, a monosaccharide with both 2- and 6-OH groups replaced by hydrogen atoms, has a k'_2 value similar to that of D-fucose and lower than that of any other carbohydrate possessing an anomeric carbon atom except D-sedoheptulose.

Effect of acidic dissociation constant on capacity factor Capacity factors of carbohydrates measured in this work may be correlated with carbohydrate acidic dissociation constants (Fig. 2). A number of values of pK_a, mainly of monosaccharides, have been measured near 25° and at low ionic strength and carbohydrate concentration. Although the chromatographic eluent used here was of high ionic strength, if we assume that ionic strength affects all pK_a values relatively equally, the correlation should be valid.

It is known that the high lability of the hydrogen atom of the anomeric hydroxyl group, caused by the inductive effect of the ring oxygen, is the chief source of reducing sugar acidity[24]. Stabilization of anionic oxygen occurs by intramolecular hydrogen bonding with adjacent hydroxyl groups[24,25], and the lack of 2-OH decreases the acidity of the resulting sugar compared to the corresponding sugar with 2-OH. When no anomeric hydroxyl group exists, as in sugar alcohols and glycosides, hydroxyl acidity decreases[24] in the order 2-OH >> 6-OH > 3-OH > 4-OH. Fig. 2 shows that among monosaccharides, sugar alcohols[26,27] have the highest pK_a values, followed by 2-deoxy-D-*arabino*-hexose[28] and 2-deoxy-D-*erythro*-pentose[29], and then by aldoses and ketoses[25,27–31], and that decreasing values of pK_a lead to increasing capacity factors. Di- and tri-saccharides such as maltose, lactose, sucrose, and raffinose have values[29] of pK_a (11.74, 12.22, 12.62, and 12.74, respectively, at 16.5–19°) and k'_2 (6.87, 2.69, 3.30, and 5.54, respectively) that do not correspond with this plot. Therefore it is clear that

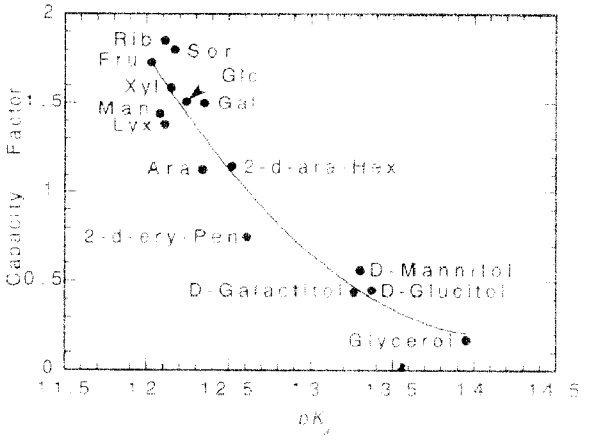

Fig. 2. Effect of pK_a on capacity factors of monosaccharides measured at Iowa State University. Values of the latter for D-galactitol, D-glucitol, glycerol, and D-mannitol were adjusted to 25° from their actual measured values at lower temperatures. Abbreviations are the following: Ara, D-arabinose; 2-d-ara-Hex, 2-deoxy-D-*arabino*-hexose; 2-D-ery-Pen; 2-deoxy-D-*erythro*-pentose; Fru, D-fructose; Gal, D-galactose; Glc, D-glucose; Lyx, D-lyxose; Man, D-mannose; Rib, D-ribose; Sor, D-sorbose; Xyl, D-xylose.

separation of oligosaccharides by anion-exchange chromatography is strongly affected by factors other than oligosaccharide acidity, such as by the accessibility of oxyanions to functional groups attached to the stationary phase, as suggested by Hardy and Townsend[12]. This conclusion is strongly buttressed by consideration of the three trehaloses, each of which is composed of two D-glucosyl residues lacking the anomeric hydroxyl groups that provide relatively high acidity. These three disaccharides, similar in structure and probably in pK_a value and different only in the configuration of their glucosidic bonds, have radically different k_i' values, which can be caused only by their different orientations when adsorbed by the stationary phase.

Effect of chain length and substituent on capacity factor. — To test the assumptions underlying Eq. *2*, values of ln k_i' of members of the homologous cello-, isomalto-, maltitol-, malto-, and xylo-oligosaccharide series, three of the series having three members and the other two four members, are plotted *vs.* chain length (Fig. 3). In all five cases straight lines that follow Eq. *2* are obtained. Values of τ_j, the slopes of these lines, based on the ISU column and obtained by linear regression, are listed on Table III, and conform with the results of Koizumi *et al.*[11] in the three cases where the same series have been tested. Members of the malto-oligosaccharide series have higher capacity factors than those of the cello-, xylo, and isomalto-oligosaccharide series because the value of τ_j for the malto-oligosaccharide series is higher than those of the other three series. This again is almost surely because the conformation of the malto-oligosaccharide chain is such that negatively charged portions of each residue are more easily brought into contact with positively charged sites on the adsorbent than are the analogous areas of other chains[12]. Capacity factors of the lower members of the maltitol-oligosaccharide series are lower than those of other series because the value of k_p' of the parent

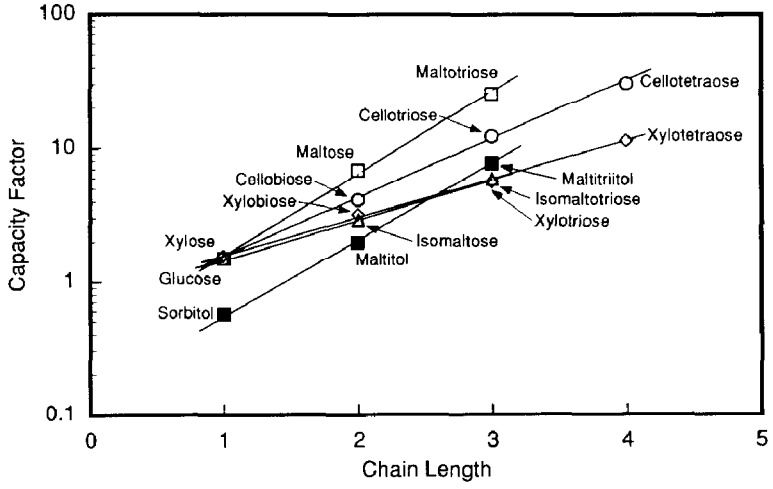

Fig. 3. Effect of chain length on capacity factors of members of homologous series of oligosaccharides. Closed symbols: measured at the Zuckerinstitut Braunschweig; open symbols: measured at Iowa State University.

TABLE III

Values of substituent parameter (τ_i) and capacity factor of parent compound (k'_p) for homologous oligosaccharide series chromatographed with Dionex HPIC-AS6 columns eluted with 0.1N NaOH

Oligosaccharide series	*Substituent*	*Parent compound*	τ_i	k'_p *(calc.)*[a]	k'_p *(measured)*[b]
Cello-[c]	4-*O*-β-D-glucopyranosyl	D-Glucose	1.01	1.52	1.51
Isomalto-[c]	6-*O*-α-D-glucopyranosyl	D-Glucose	0.69	1.49	1.51
Maltitol-[d]	4-*O*-α-D-glucopyranosyl	D-Glucitol	1.26	0.44	0.45
Malto-[c]	4-*O*-α-D-glucopyranosyl	D-Glucose	1.42	1.55	1.51
Xylo-[c]	4-*O*-β-D-xylopyranosyl	D-Xylose	0.65	1.61	1.59

[a] Intercept of linear regression at chain length = 1. [b] Experimental value for parent compound. [c] Chromatographed at ISU with a Dionex HPIC-AS6 column. Eluent flow rate: 1 mL·min^{-1}; isocratic NaOH concentration: 0.1N; temperature: 25 °. [d] Chromatographed at ZIB with a Dionex HPIC-AS6 column under the above conditions. Values of k'_p converted to ISU basis by use of Fig. 1.

compound, D-glucitol, is lower than those of D-glucose and D-xylose. The values of τ_i for malto- and maltitol-oligosaccharide series are similar, since in both cases 4-*O*-α-D-glucopyranosyl residues are added sequentially to an already existing parent compound. Furthermore, values of τ_i for isomalto- and xylo-oligosaccharide series are almost the same because in both cases chain extension causes an increase in the number of residues lacking the relatively acidic and spatially flexible 6-OH group.

A further test of the assumptions was attempted by calculating the values of τ_i for different pairs of carbohydrates where the same substituent is added to different parent compounds through the same linkage. For instance, nigerose–D-glucose and turanose–D-fructose pairs can be used to test the effect of the 3-*O*-α-D-glucopyranosyl substituent, the maltulose–D-fructose pair for the 4-*O*-α-D-glucopyranosyl substituent, lactose–D-glucose and lactulose–D-fructose pairs for the 4-*O*-α-D-galactopyranosyl substituent, the 3-*O*-β-cellobiosyl-D-glucose laminaribiose pair for the 4-*O*-β-D-glucopyranosyl substituent, and 6-*O*-(α-D-glucopyranosyl)-D-mannitol–D-mannitol, isomaltitol–D-glucitol, palatinose–D-fructose, and panose–maltose pairs for the 6-*O*-(α-D-glucopyranosyl) substituent. Not only do pairs representing the same substituent often give very different results from each other, but when applicable they are very different than τ_i values calculated from the five homologous series just tested. In contrast to this behavior, addition of the same residues to the nonreducing ends of different parent compounds through the same linkages led to similar τ_i values in h.p.l.c. with amino-bonded silica carriers[32]. Furthermore, Koizumi *et al.*[33] found that β-(1→2)- and α-(1→3)-linked oligosaccharide series had radically different k'_p values for D-glucose than those of other D-glucosyl-oligosaccharide series subjected to anion-exchange h.p.l.c., a phenomenon not seen when similar series were subjected to h.p.l.c. on amino-bonded silica columns[31,32]. This suggests that, unlike other chromatographic systems, anion-exchange chromatography of carbohydrates at high pHs does not always follow the assumption[22] that addition of the same residue through the same linkage to different

parent compounds should give the same value of τ_j, and the further assumption that addition of the same or different residues through the same or different linkages to the same parent compound should give the same value of k'_p.

Response of pulsed amperometric and differential refractive index detectors. — The r.i. detector at ISU yielded a nearly linear response on glucose, a plot of ln (area) *vs.* ln (concentration) giving a slope of 0.945 (Fig. 4). The detection limit was 40 ng. The slope of the corresponding plot with the p.a.d. at ZIB was 0.894, with an even less linear response at higher glucose concentrations. The detection limit of this system was 20 ng.

Responses of the two detectors to 26 sugars at ZIB and 24 of the same sugars at ISU, including monosaccharides of three, five, and six carbons, sugar alcohols, deoxy sugars, and both reducing and nonreducing di- and trisaccharides, are shown in Table

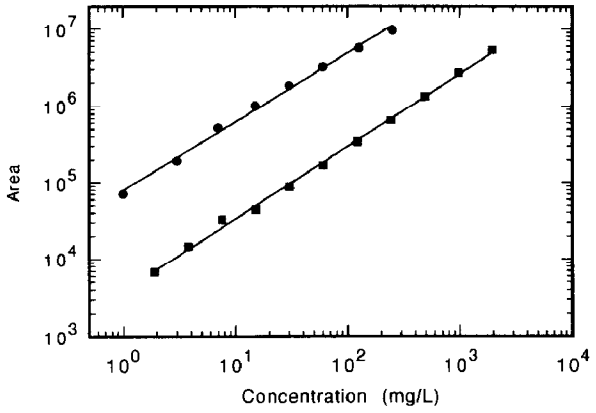

Fig. 4. Effect of D-glucose mass concentration on integrator areas generated by an r.i. detector at Iowa State University (■) and a p.a. detector at the Zuckerinstitut Braunschweig (●).

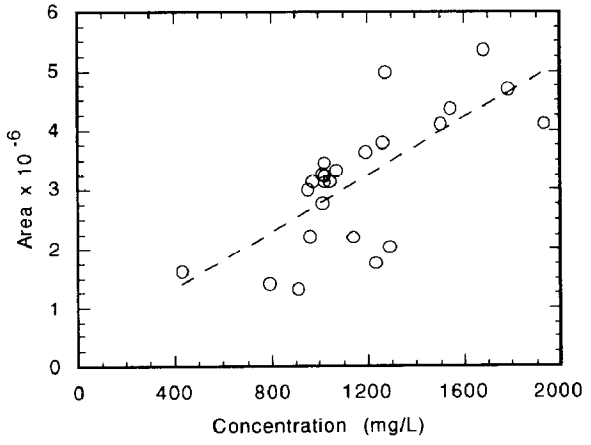

Fig. 5. Effect of carbohydrate mass concentration on integrator areas generated by an r.i. detector at Iowa State University.

TABLE IV

Response of pulsed amperometric and differential refractive index detectors to carbohydrates in 0.1 N NaOH solution

Carbohydrate	*Molecular weight*	*ZIB*			*ISU*		
		Conc. (mg L⁻¹)	*Area × 10⁻⁶*	*Relative area/mol*	*Conc. (mg L⁻¹)*	*Area × 10⁻⁶*	*Relative area/mol*
D-Arabinose	150.13	26.5	2.265	0.67	1020	3.233	0.93
Cellobiose	342.30	101.3	7.177	1.27	1020	3.129	2.06
2-Deoxy-D-*erythro*-pentose	134.13	23.5	0.915	0.27			
meso-Erythritol	122.12	23.7	3.634	0.98	430	1.627	0.91
D-Fructose	180.16	51.4	4.054	0.74	1930	4.109	0.75
D-Fucose	164.16	22.2	2.152	0.83	1010	3.263	1.04
D-Galactitol	182.17	29.9	4.132	1.32	1260	3.796	1.07
D-Galactose	180.16	53.0	6.138	1.09	1010	2.765	0.97
D-Glucitol	182.17	30.1	3.474	1.10	1040	3.145	1.08
D-Glucose	180.16	61.7	6.549	1.00	1540	4.351	1.00
DL-Glyceraldehyde	90.08	23.2	0.376	0.08	790	1.409	0.31
myo-Inositol	180.16	25.3	4.198	1.56	1270	4.984	1.39
Isomaltose	342.30	90.4	6.025	1.19	960	2.203	1.54
Lactose	342.30	95.1	6.728	1.27	1500	4.098	1.84
Lactulose	342.30	93.6	4.115	0.79			
Maltose	342.30	189.5	9.227	0.87	1200	2.034	1.06
D-Mannitol	182.17	24.6	2.619	1.01	950	3.006	1.13
Melezitose	504.44	96.6	3.448	0.94	1190	3.626	3.02
Palatinose	342.30	192.1	7.330	0.68	910	1.820	0.97
Raffinose	504.44	174.6	5.150	0.78	1020	3.458	3.35
L-Rhamnose	164.16	30.7	2.498	0.70	1070	3.333	1.00
D-Ribose	150.13	49.9	3.894	0.61	1140	2.196	0.57
Sucrose	342.30	119.9	3.058	0.46	1680	5.353	2.14
α,α-Trehalose	342.30	29.2	1.651	1.01	970	3.143	2.17
Turanose	342.30	104.2	3.952	0.68	1230	1.749	0.98
D-Xylose	150.13	23.6	2.634	0.88	1280	4.706	0.78

[a] For some trivial name equivalents, see Tables I and II, footnotes *f* and *b*, respectively. [b] ZIB indicates work carried out at the Zuckerinstitut at the Technische Universität Braunschweig, ISU indicates work carried out at Iowa State University. [c] Column: Dionex CarboPac PA1, eluent flow rate: 1 mL·min⁻¹; isocratic NaOH concentration: 0.1 N; temperature: 25 °; sample volume: 20 μL; detector: Dionex type II p.a.d. with gold electrode; integrator: Hitachi D-2000. [d] Column: Dionex HPIC-AS6, eluent flow rate: 1 mL·min⁻¹, isocratic NaOH concentration: 0.1 N; temperature: 25 °; sample size: 20 μL; detector: Knauer RI detector; integrator: Hewlett Packard 3392A.

IV. Integrator areas generated by the r.i. detector are related more to mass concentrations (Fig. 5) than to molar concentrations. Those of the p.a.d. are only roughly correlated with either molar or mass concentration (Figs. 6 and 7); the former correlation may be slightly favored because a linear regression of the data passes closer to the origin of Fig. 6 than to the origin of Fig. 7. In any case it is clear that responses of both detectors are affected by a number of factors that make simple correlations covering many different sugars of different chemical and physical properties unreliable.

It might be expected that pK_a would affect p.a.d. response in a straightforward

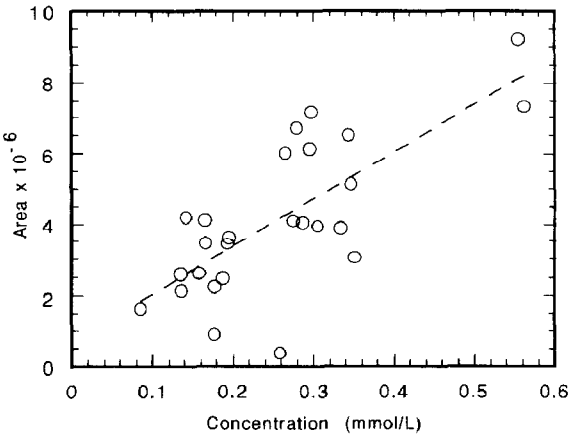

Fig. 6. Effect of carbohydrate molar concentration on integrator areas generated by a p.a. detector at the Zuckerinstitut, Braunschweig.

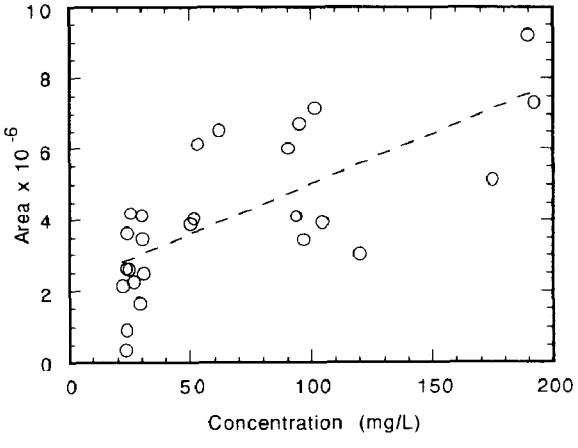

Fig. 7. Effect of carbohydrate mass concentration on integrator areas generated by a p.a. detector at the Zuckerinstitut, Braunschweig.

manner, but such is not the case. Three sugar alcohols, D-galactitol, D-mannitol, and D-glucitol, have high pK_a values but yield high molar response factors, opposite to their expected behavior. Molar response factors of other sugars show only a slight inverse correlation with pK_a. As with capacity factor, it seems clear that p.a.d. response is a complicated function of pK_a and structural factors.

Strong-base anion-exchange h.p.l.c. is a powerful and largely predictable tool for the separation of carbohydrates. With proper treatment of samples, nearly all sugars can be analyzed, despite the extremely high pHs employed. It is hoped that this project, combining the results of two laboratories on a very large number of carbohydrates, will make the technique more accessible to the prospective user.

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