Journal of Chromatography, 325 (1985) 287–293
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17 694

Note

Retention of carbohydrates on silica and amine-bonded silica stationary phases: application of the hydration model

ZIVKO L. NIKOLOV and PETER J. REILLY*
Department of Chemical Engineering, Iowa State University, Ames, IA 50011 (U.S.A.)
(First received January 24th, 1985; revised manuscript received February 28th, 1985)

The introduction of chemically bonded silica packings has opened a new dimension in liquid–solid chromatography with respect to selectivity and convenience. Among polar-bonded phases, the aminoalkyl stationary phase has gained considerable popularity since its first application in the analysis of sugar mixtures[1,2]. Many articles have been published since then dealing with the separation of different sugars using various aqueous organic eluents, but very few have covered the separation mechanism of carbohydrates in these systems. This fact is not surprising, since the complex interactions between polar solute and solvent molecules and the polar stationary phase make elucidation of the retention mechanism very difficult.

There have been several reports[3–6] suggesting that the principal interaction in carbohydrate separation is hydrogen bonding between amine-bonded packing and sugar hydroxyls, the total number and distribution of which play an important role.

On the other hand, Verhaar and Kuster[7] and Orth and Engelhardt[8] determined experimentally that the concentration of water in the pores of the packing was higher than that in the acetonitrile–water eluent. On the basis of the observed relationship between water uptake by the stationary phase, amine group surface concentration, and capacity factor values of a number of sugars, they explained the separation process as a partition of the solute between a stagnant water-enriched phase and a moving acetonitrile–water mixture.

In this work we have made a further contribution to the understanding of the two mechanisms by (1) measuring water concentrations in mobile and stationary liquid phases associated with amine-bonded and free silica surfaces, and by (2) relating retention of carbohydrates to two parameters, the number of hydroxyl groups per molecule that could hydrogen bond to amine groups on the surface and the calculated hydration number of the molecule. Eight carbohydrates, most of them non-reducing to avoid difficulty with slow mutarotation in free silica columns, were used.

EXPERIMENTAL

The chromatographic apparatus, separation conditions (23 ± 1°C, 1 ml/min aqueous acetonitrile eluent flow), and solvents were described earlier[9].

Deuterium oxide came from Fisher (Pittsburgh, PA, U.S.A.). Sigma (St. Louis,

0021-9673/85/$03.30

MO, U.S.A.) supplied 2-deoxy-D-glucose, digitoxose (2,6-dideoxy-D-*ribo*-hexose), D-glucose, myo-inositol (1,2,3,5/4,6-hexahydroxycyclohexane), and α,α-trehalose (O-α-D-glucopyranosyl-(1 → 1)-α-D-glucopyranoside). Sucrose O-α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside) was purchased from Baker (Phillipsburg, NJ, U.S.A.). Dextran (mol. wt. $40 \cdot 10^6$ daltons) was donated by Professor John F. Robyt of Iowa State University, α,β-trehalose (O-α-D-glucopyranosyl-(1 → 1)-β-D-glucopyranoside) by Dr. Frederick W. Parrish of the Southern Regional Research Center, and β,β-trehalose (O-β-D-glucopyranosyl-(1 → 1)-β-D-glucopyranoside) by Dr. Riaz Khan of Tate & Lyle.

The columns employed in this work were a DuPont (Wilmington, DE, U.S.A.) Zorbax-NH_2 (column T2535), a Supelco (Bellefonte, PA, U.S.A.) Supelcosil LC-NH_2 (column 91,164), and a Supelcosil LC-Si (column 15,571), all 250 mm × 4.6 mm I.D. No precolumn was used. The properties of the stationary phases are summarized in Table I.

The total column porosity ($\varepsilon_t = V_t/V_c$), defined as the column fraction filled with intra- and interparticle eluent, was determined from the total column volume (V_c) and the elution volume of unretained ^2H_2O (V_t). The interstitial volume (V_i) necessary to calculate the interstitial porosity ($\varepsilon_i = V_i/V_c$) was obtained from the retention time of a dextran that was totally excluded from the pores. Both volumes V_t and V_i were corrected for the dead volume between the injector and the detector. Elution volumes of unretained and excluded solutes were measured using water as a mobile phase.

The pore volume fraction was calculated from the difference of the total and interstitial porosities ($\varepsilon_p = \varepsilon_t - \varepsilon_i$). This value, determined from our experimental results, agreed well with the supplier's value, in the case of the Zorbax-NH_2 packing.

The amount of water retained by the columns was determined by the modified

TABLE I

CHARACTERISTIC COLUMN PACKING PROPERTIES

Property	*Column*		
	Zorbax-NH_2	*Supelcosil LC-NH_2*	*Supelcosil LC-Si*
Particle shape*	Spherical	Spherical	Spherical
Average particle size (μm)*	7	5	5
Specific surface area (m^2/g)*	320	170	169
Average pore diameter of parent silica (nm)*	6.5	11.5	11.5
Aminopropyl surface concentration (μmol/m^2)*	3.6	2.8	–
Average area available per bonded group (nm^2)**	0.46	0.59	–
Average distance between bonded groups (nm)**	0.76	0.87	–
Packing density (mg/ml)*	0.67	0.62	0.62
Intraparticle porosity (ε_p)	0.40	0.38	0.42
Interstitial porosity (ε_i)	0.39	0.38	0.34
Total porosity (ε_t)	0.79	0.76	0.76

* Information obtained from the suppliers.

** Calculated assuming close-packed hexagonal packing in a plane.

equilibrium method[10] described previously[7]. The experimental setup and procedure for measuring the water holdup in the columns was that of Verhaar and Kuster[7], with an eluent flow-rate of 0.6 ml/min.

Capacity factor values [$k' = (t_R - t_0)/t_0$] were calculated from the solute retention times (t_R) and the ^2H_2O retention times (t_0).

RESULTS AND DISCUSSION

The water uptake by the three stationary phases as a function of the water concentration in the eluent mixture is plotted in Fig. 1. For all three column packings the water concentration in the pores was higher than that in the mobile phase, a result of preferential adsorption of water by the stationary phase. The greater the polarity of the organic component of the aqueous organic eluent mixture, the smaller the water uptake is, because of the competition between both polar components for the polar adsorbent active sites. This is well illustrated in Fig. 1 by the reduced water uptake when a methanol–water mobile phase was used instead of acetonitrile–water in the Supelcosil LC-NH_2 column.

Comparison of the water uptake curves of the two Supelcosil packings indicates that bonded amine groups enhance water uptake. The considerably higher water uptake by the Zorbax-NH_2 stationary phase over either Supelcosil column is a result of its higher specific area and amine loading. These results are in agreement with the observation of Orth and Engelhardt[8] that the amount of water adsorbed by the stationary phase increases with increasing amine group surface concentration.

In order to measure the retention of carbohydrates with varying structures and numbers of hydroxyl groups on stationary phases of free or amine-bonded silica, the eight compounds previously described were chromatographed on Supelcosil LC-Si and Supelcosil LC-NH_2 columns with different acetonitrile–water eluent mixtures.

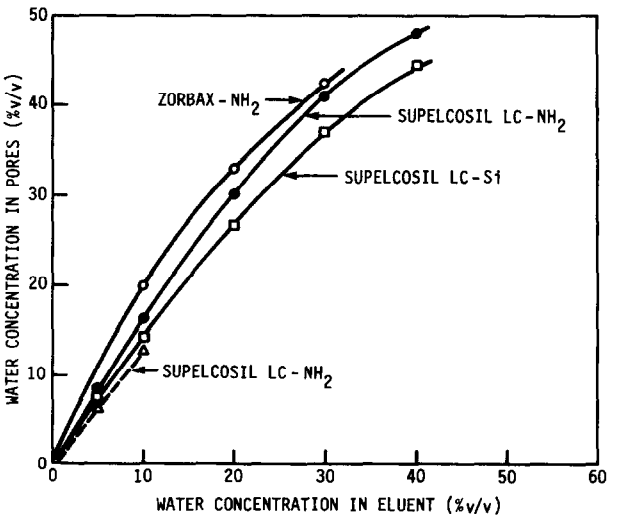

Fig. 1. Water concentrations in mobile and stationary liquid phases in Zorbax-NH_2, Supelcosil LC-NH_2, and Supelcosil LC-Si columns: (——) acetonitrile–water mobile phase, (------) methanol–water mobile phase.

TABLE II

RETENTION OF CARBOHYDRATES CHROMATOGRAPHED ON SILICA COLUMNS WITH AQUEOUS ACETONITRILE

Carbohydrate	*No. of free hydroxyl groups*	*Max. No. of hydrogen bonds per molecule*	*Capacity factors (k')*							
			Supelcosil LC-Si				*Supelcosil LC-NH_2*			
			Acetonitrile (%, v/v)				*Acetonitrile (%, v/v)*			
			60	*70*	*80*	*90*	*60*	*70*	*80*	*90*
Digitoxose	3	2–3	0.18	0.18	0.12	0.10	0.22	0.22	0.17	0.17
2-Deoxy-D-glucose	4	3	0.20	0.26	0.28	0.46	0.31	0.41	0.54	1.11
D-Glucose	5	3	0.22	0.32	0.42	0.90	0.40	0.62	1.03	2.82
Sucrose	8	4	0.24	0.39	0.65	1.56	0.46	0.83	1.75	7.89
α,β-Trehalose	8	4	0.26	0.45	0.80	2.26	0.50	0.98	2.31	>80
α,α-Trehalose	8	4	0.26	0.45	0.82	2.52	0.50	1.02	2.57	>80
β,β-Trehalose	8	4	0.26	0.45	0.85	2.56	0.50	1.05	2.60	>80
myo-Inositol	6	3	0.30	0.48	0.87	2.02	0.56	1.05	2.28	>80

The range of the eluent composition, from 60 to 90% aqueous acetonitrile, is that usually applied in sugar separations.

Elution order was the same on both columns and, except for myo-inositol, corresponded to the number of free hydroxyl groups per molecule (Table II). Separation of the carbohydrates on the Supelcosil LC-Si column could be made comparable to that on the Supelcosil LC-NH_2 column simply by increasing the acetonitrile concentration in the eluent. For example, approximately the same separation resulted when the eight compounds were chromatographed on the Supelcosil LC-Si column with 90% acetonitrile, versus 80% acetonitrile on Supelcosil LC-NH_2. Throughout the entire range of eluent concentrations tested, similar separations in the two columns occurred at similar ratios of pore to eluent water concentrations.

In a test of the assumption that specific interactions between carbohydrate hydroxyls and the stationary water-rich phase play an important role in the separation process, we attempted to link carbohydrate-water hydrogen bonding, as expressed by the specific carbohydrate hydration model[11], to the overall retention. We did this because one would expect that those carbohydrates that are more fully hydrated in aqueous solution would be more strongly attracted by the water-rich layer at the silica and amine-bonded silica surfaces.

In their mutarotation study of sugars, Kabayama and Patterson[11], on the basis of the conformation of carbohydrates in solution and tetrahedrally bonded water molecules, were able to predict that an equatorial hydroxyl group would be more strongly hydrated than an axial one. Since it is equatorial secondary hydroxyl groups on the pyranosyl ring which possess spacing compatible with the oxygen atoms in the water structure, the primary hydroxyl on the C-6 atom —and to an even greater extent any axial groups— would not be expected to contribute as much to specific hydration. Furthermore, less specific hydration of carbohydrate furanosyl forms would be anticipated due to the geometric incompatibility of the furanosyl ring with the tetrahedrally bonded water oxygen atoms. Thus, the specific hydration model

indicates that not only the number of hydroxyl groups and their orientation, but also their position on the ring and the conformation of the whole molecule, might be factors determining the hydration effect.

There are a number of methods to estimate hydration numbers of carbohydrates, but that using ultrasonic velocities to measure solution compressibilities, which can be extrapolated to zero solute concentration, appears best suited to our needs. Therefore, the hydration number data of Shiio[12], Franks *et al.*[13] and Juszkiewicz[14], together with those estimated from the compressibility data of Høiland and Holvik[15] by eqn. 1 of Shiio[12], are shown in Table III. The apparent molal compressibility of myo-inositol has been reported only once[13], at 5°C, but by use of the variations in compressibilities between 5°C and 25°C for nine other organics, it is possible to estimate its value at 25°C, and from it a hydration number at that temperature. The very high value of the latter is expected, as myo-inositol, with five equatorial secondary hydroxyl groups, should be more strongly hydrated than any of the monosaccharides, which have up to four equatorial secondary hydroxyls in the case of β-anomers and up to three for α-anomers.

These hydration numbers can be compared with relative retention times, assembled from the composite data of Verhaar and Kuster[16] and some of ours[9,17,18] (Table III). With a few exceptions correlation between the two parameters is good. The unexpectedly high retention time of myo-inositol is in line with its high hydration number.

The differences in hydration between the carbohydrates in Table III, which result from differences in the number of hydroxyl groups, their orientation and position on the ring, and the conformation of the molecule, reveal the very specific nature of the sugar–water interactions as suggested by the hydration model. The observed correlation between the retention times and hydration numbers implies the existence of similar specific interactions between carbohydrate hydroxyls and stagnant water molecules.

Therefore, based on these observations, the same elution order of the eight carbohydrates chromatographed on Supelcosil LC-NH_2 and Supelcosil LC-Si columns (Table III) would be expected. In general, the same elution order of carbohydrates is to be observed in any chromatographic system where the partitioning of carbohydrates occurs between a water-enriched stagnant phase and a moving aqueous organic eluent. This occurs with amine-modified silica and acetone–water[6], hydroxylated silica and acetone–water[6], amine-bonded silica and methanol–water[17], sulfate-treated amine-bonded silica and acetonitrile–water[19], and to a substantial extent with anion-exchange resin and ethanol–water[20].

The possibility of hydrogen bonding between mono- and disaccharides and amine groups was investigated with space-filling molecular models, taking into consideration the orientation flexibility of the aminopropyl group on the silica surface and the average distance between bonded groups, as given in Table I. In all cases the axial hydroxyls above the pyranosyl ring plane are the only ones not accessible for hydrogen bonding. We concluded that monosaccharides cannot be engaged in hydrogen bonding with more than three and disaccharides with more than four aminopropyl groups (Table II), except for the (1 → 6)-linked disaccharides isomaltose and gentiobiose, which can form up to five hydrogen bonds.

In general, retention of the eight carbohydrates increased with increasing po-

TABLE III

RELATION BETWEEN RELATIVE RETENTION TIME AND HYDRATION NUMBER FOR A SERIES OF CARBOHYDRATES*

Carbohydrate	*Relative retention time***		*Hydration number at 25°C*			
	Nikolov et al.	*Verhaar and Kuster*[16]	*Shiio*[12]	*Franks et al.*[13]	*Høiland and Holvik*[15]	*Juszkiewicz*[14]
D-Ribose	–	0.40	–	2.7	2.6	–
D-Xylose	0.30***	0.47	2.3	–	2.8	4.1
D-Arabinose	–	0.53	3.5	–	4.1	4.0
D-Fructose	0.46***	0.53	3.8	–	–	–
D-Mannose	–	0.60	–	–	3.3	5.1
D-Glucose	0.56***, 0.59§	0.67	3.5	3.4	3.8	5.2
D-Galactose	0.61***	0.73	–	–	4.3	–
Sucrose	1.00	1.00	3.8	–	3.8	7.0
Cellobiose	1.25§§	1.20	4.8	–	–	–
Maltose	1.26§§	1.20	4.2	–	4.9	–
myo-Inositol	1.30§	–	–	8.0	–	–
Lactose	1.49§§	1.33	–	–	6.2	8.2
Melezitose	2.03§§§	–	–	–	5.2	–
Raffinose	2.56§§§	2.33	6.2	–	6.4	–

* Amine-bonded silica stationary phase, 80% aqueous acetonitrile eluent.
** Relative to sucrose.
*** Ref. 17.
§ This paper.
§§ Ref. 9.
§§§ Ref. 18.

tential hydrogen bonding to amine groups, with the exception of myo-inositol. At eluent concentrations below 80% acetonitrile, myo-inositol was retained on the amine-bonded column as long or longer than any of the other eight carbohydrates, though it could engage in only three hydrogen bonds. In fact, using the relative retention time of sucrose as a base, it eluted later than 10 of 21 disaccharides previously chromatographed on Supelcosil LC-NH_2 with 80% aqueous acetonitrile eluent[9]. On the basis of hydrogen bonding, myo-inositol would be expected to have a lower retention time than any disaccharide and one approximately the same as glucose, which it resembles in structure, size, and number of hydroxyl groups positioned for bonding.

In conclusion, it may be stated that hydration number is a good parameter for correlating retention times of carbohydrates on silica and amine-bonded silica stationary phases. In the case of myo-inositol, it is distinctly preferable to total number of hydroxyl groups or to number of potential hydrogen bonds. It should be noted that myo-inositol may be retained in a different manner than the other carbohydrates tested, as its order of retention in comparison to theirs varies with acetonitrile concentration in both silica and amine-bonded silica. Finally, it has been found that capacity factors of individual carbohydrates were similar in the two stationary phases when the ratios of pore to eluent water concentrations in the two phases were similar.

ACKNOWLEDGEMENTS

This project was supported by National Science Foundation Grant CPE-8311521 and by the Engineering Research Institute, Iowa State University. We thank Dr. Daryl J. Gisch of Supelco and Mr. Alan Goldberg of DuPont for information on the stationary phases and Drs. J. F. Robyt, F. W. Parrish, and R. Khan for their gifts of carbohydrates.

REFERENCES

1 J. C. Linden and C. L. Lawhead, *J. Chromatogr.*, 105 (1975) 125.
2 J. K. Palmer, *Anal. Lett.*, 8 (1975) 215.
3 R. E. Majors, *J. Chromatogr. Sci.*, 18 (1980) 488.
4 H. Binder, *J. Chromatogr.*, 189 (1980) 414.
5 M. D'Ambiose, D. Noël and T. Hanai, *Carbohydr. Res.*, 79 (1980) 1.
6 M. Boumahraz, V. Ya. Davidov and A. V. Kiselev, *Chromatographia*, 15 (1982) 751.
7 L. A. Th. Verhaar and B. F. M. Kuster, *J. Chromatogr.*, 234 (1982) 57.
8 P. Orth and H. Engelhardt, *Chromatographia*, 15 (1982) 91.
9 Z. L. Nikolov, M. M. Meagher and P. J. Reilly, *J. Chromatogr.*, 319 (1985) 51.
10 J. F. K. Huber and R. G. Gerritse, *J. Chromatogr.*, 58 (1971) 137.
11 M. A. Kabayama and D. Patterson, *Can. J. Chem.*, 36 (1958) 563.
12 H. Shiio, *J. Amer. Chem. Soc.*, 80 (1958) 70.
13 F. Franks, J. R. Ravenhil, and D. S. Reid, *J. Sol. Chem.*, 1 (1972) 3.
14 A. Juszkiewicz, *Arch. Acoust.*, 6 (1981) 307.
15 H. Høiland and H. Holvik, *J. Sol. Chem.*, 7 (1978) 587.
16 L. A. Th. Verhaar and B. F. M. Kuster, *J. Chromatogr.*, 220 (1981) 313.
17 Z. L. Nikolov, unpublished results.
18 Z. L. Nikolov, M. M. Meagher and P. J. Reilly, *J. Chromatogr.*, 321 (1985) 393.
19 V. Kahle and K. Tesařík, *J. Chromatogr.*, 191 (1980) 121.
20 O. R. Ramnäs and O. Samuelson, *Acta Chem. Scand. B*, 28 (1974) 955.