Journal of Chromatography, 253 (1982) 49–54
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,262

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF SUGAR MIXTURES CONTAINING XYLOSE AND ARABINOSE ON PRIMARY AMINO-BONDED PHASES

B. PORSCH
Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, CS-16206 Prague 6 (Czechoslovakia)
(Received July 27th, 1982)

SUMMARY

The extent of the chemical reaction between aldehydic sugars and primary amino groups chemically bonded to the surface of a silica-based sorbent is even larger when silica modified *in situ* by sorbed diethylenetriamine is employed as the stationary phase. However, the effect of this reaction can be eliminated by employing a suitably buffered mobile phase.

INTRODUCTION

Recently, silica with chemically bonded primary amine functions has become the more or less standard stationary phase recommended[1,2] for the analysis of mixtures of mono- and oligosaccharides by high-performance liquid chromatography (HPLC). However, its low stability[3–5] is a distinct drawback: the amino group can apparently react with a number of impurities both in the sample and in the mobile phase. Consequently, the character of the sorbent changes gradually and irreversibly and with it the retention characteristics of the column.

Aitzetmüller[6] recommends an alternative to the chemically bonded amino phase for the analysis of sugars: bare silica as the stationary phase with acetonitrile–water and a small amount of a polyfunctional amine[7], *e.g.*, tetraethylenepentamine[8], as eluent. The amine is sorbed onto the surface on the silica resulting in a stationary phase having similar characteristics to those of the chemically bonded amino phase. Among the advantages of this method it is claimed that, in contrast to the chemically bonded amino phase where reaction with impurities leads to irreversible deterioration of the sorbent, a dynamic equilibrium can be assumed to exist within the column and the sorbed polyamine can react with an impurity, be released from the sorbent and replaced by a fresh amine molecule. As a result the column should be more tolerant[7] of impurities that are capable of reacting with the amine group.

An additional objection has been raised[1] against the use of chemically bonded amino phases in the analysis of sugars, *viz.*, that reducing sugars, in particular aldopentoses and aldohexoses, can also react[9] with the primary amine group; this problem

0021-9673/82/0000–0000/$02.75 © 1982 Elsevier Scientific Publishing Company

seems generally to be underestimated. Attention to this effect was drawn by Abbot[10], who showed that this reaction can result in a loss of as much as 60% of ribose originally present in a sample.

It is the aim of the present study to illustrate the chromatographic consequences of the reaction between aldopentoses and amino phases, to test the behaviour of unmodified silica used together with a polyamine-containing mobile phase under comparable conditions and, finally, to propose a procedure for eliminating the undesirable consequences of the reaction between aldehydic sugars and the amino phase.

EXPERIMENTAL

Materials

Two types of silica (mean particle diameter, d_p = 10 μm) with chemically bonded γ-aminopropyl groups were used: Separon SI NH_2 (Laboratory Instruments Works, Prague, Czechoslovakia) and LiChrosorb NH_2 (E. Merck, Darmstadt, G.F.R.). Glucose, fructose, arabinose, xylose (analytical grade) and diethylenetriamine (pure) were products of Fluka (Buchs, Switzerland). Acetonitrile (reagent grade) was purchased either from Croft Labs. (Brackley, Great Britain) or from Fluka. Potassium hydrogen phosphate and sodium dihydrogen phosphate (both analytical grade) were obtained from Lachema (Brno, Czechoslovakia).

Instrument and columns

The liquid chromatograph consisted of a positive displacement pump and a stop-flow sample injector[11,12]; alternatively, a reciprocating membrane pump VCM 300 (Development Workshop, Czechoslovak Academy of Sciences, Prague) was used. A differential refractometer R 401 (Waters Assoc., Milford, MA, U.S.A.) was employed as the detector together with a Servogor 2S potentiometric recorder (Goerz Electro, Vienna, Austria). The stainless-steel columns[13] (100 × 6 mm I.D.) and the pressure-resistant glass columns CGC[14] (150 × 3.2 mm I.D.) (Laboratory Instruments Works) were slurry-packed with the corresponding amino phase in methanol–dioxane (1:1) by means of a high-pressure membrane pump at 40 MPa. CGC glass columns with bare silica Separon SI VSK (d_p = 5 μm) were packed by the manufacturer. In chromatography on bonded amino phases a saturation glass column (150 × 5 mm) packed with Separon SI NH_2 was always connected between the pump and the sample injector.

RESULTS AND DISCUSSION

Reducing sugars with primary amines form glycosylamines[9] (*e.g.*, L-arabinosylamine), which compounds are stable to hydrolysis at pH >9. They are hydrolysed at pH >1.5 and <9, however, the reaction being most rapid at around pH 5. Considering that the pH of a suspension of the bonded amino phase is about 9.5, it is expected that, once formed, the product of the reaction between aldose and the amine groups on the silica surface will be quite stable.

The chromatograms in Fig. 1 differ in the flow-rate of the mobile phase (14.2%, w/w water in acetonitrile), *i.e.*, in the time of contact between the column packing

and the sample (a mixture of xylose, arabinose, fructose and glucose, 1.71, 2.47, 3.71 and 3.65%, w/w, respectively in water). It is clear that a state is soon reached where one of the sugars —arabinose— reacts almost quantitatively and its peak disappears completely, at a flow-rate that is near to the optimum from the point of view of column efficiency. In quantitative analyses it may be possible, under appropriate conditions (particularly the flow-rate), by following a reproducible routine and a suitable calibration procedure, largely to eliminate the effect of this reaction. However, the situation is far worse in the analysis of natural mixtures of sugars, where a number of other compounds are always present and where it is often imperative to operate the instrument near the optimum flow-rate in order to enhance the peak resolution and to suppress the interference of non-sugar impurities with sugars. Under such conditions, compounds such as arabinose, present as a major component, can easily be completely missed.

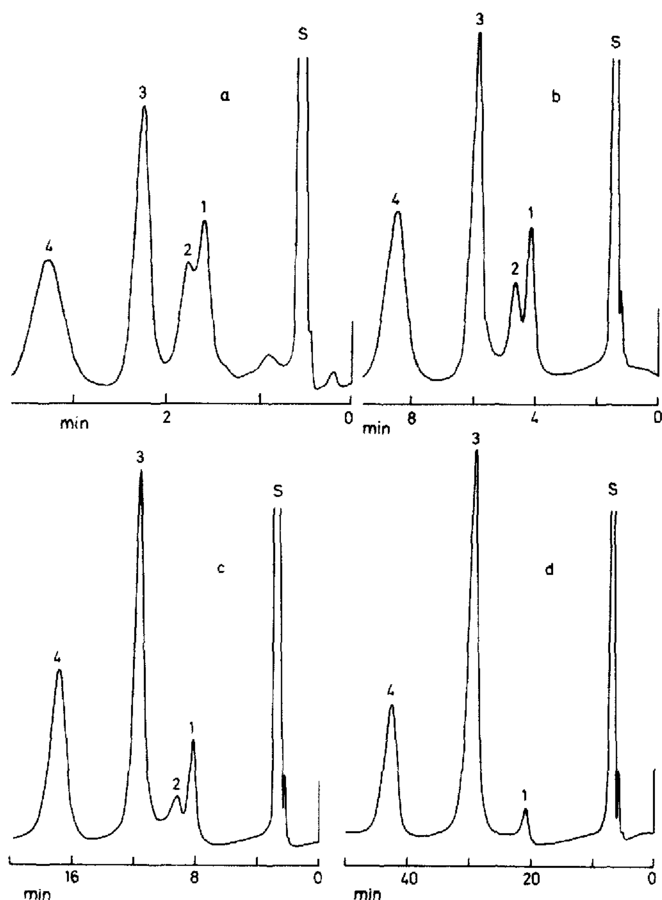

Fig. 1. Separation of xylose, arabinose, fructose and glucose on LiChrosorb NH_2 (d_p = 10 μm) at mobile phase flow-rates of 5.5 ml/min (a), 2.16 ml/min (b), 1.05 ml/min (c) and 0.43 ml/min (d). Sample: 7 μl of a solution containing 1.71% xylose (1), 2.47% arabinose (2), 3.71% fructose (3) and 3.65% glucose (4) in water (S). Mobile phase: 14.2% (w/w) water in acetonitrile. Refractive index detector R 401, attenuation 4×. Stainless-steel column: 100 × 6 mm.

Replacement of the bonded amino phase by silica and a mobile phase containing polyamine[6–8], although more tolerant of the presence of impurities in the mobile phase, is not necessarily more adyantageous from the point of view of undesirable formation of glycosylamines. In comparison with the bonded amino phase, a high excess of polyamine is present which is also able to react with aldoses; the pH of the mobile phase is also usually close to 9. The chromatogram in Fig. 2a of the same sugar mixture as before shows that, under conditions comparable to those in Fig. 1c (from the point of view of the sample residence time in the column), all three aldoses disappear. Fig. 2b, 2c and 2d illustrate the apparent loss of glucose from a sample (which in this case contained 33 % glucose and 3.8 % fructose) at different flow-rates; at a residence time of about 60 min (Fig. 2c) glucose disappears completely even at this high content of the sugar. The negative peak just before peak 2 can probably be ascribed to consumption of polyamine in the eluent; be as it may, it is clear that the

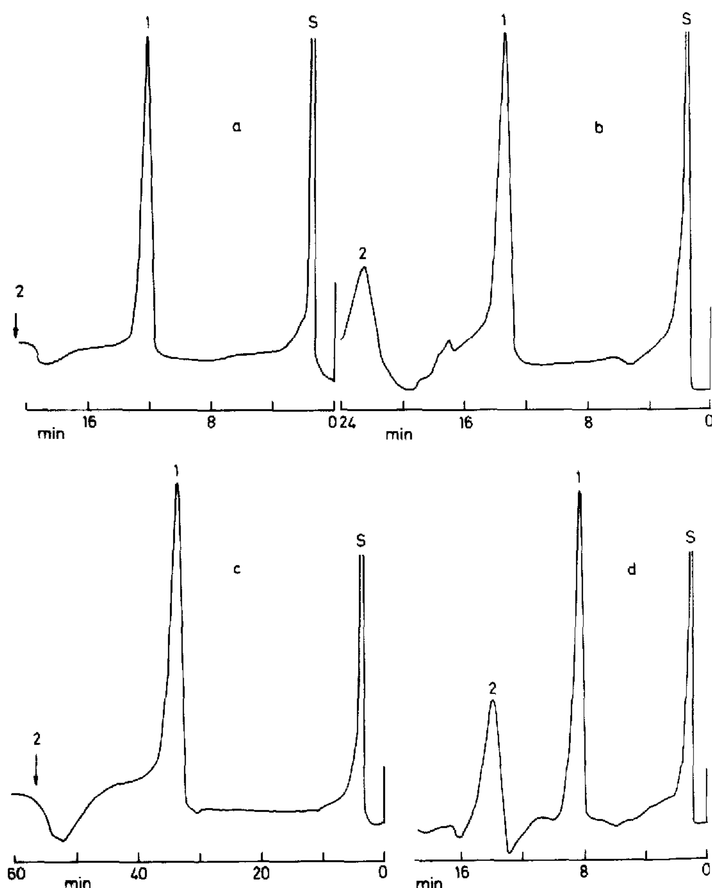

Fig. 2. Separation of sugars on silica (Separon SI VSK d_p = 5 μm) modified by sorbed diethylenetriamine. a, The same sample as in Fig. 1, 10 μl injected, flow-rate 1.58 ml/min; b–d, 3.8 % (w/w) fructose (1) and 33 % glucose (2) in water (S), 10 μl injected. Flow-rates: 1.58 ml/min (b), 0.63 ml/min (c) and 2.34 ml/min (d). Mobile phase: 14.2 % (w/w) water in acetonitrile with 0.01 % diethylenetriamine. Refractive index detection, attenuation 4 ×. Glass column CGC: 150 × 3.2 mm.

situation here is even worse than with the chemically bonded amino phase, and one can hardly expect it to change much if diethylenetriamine were to be replaced by some other polyamine.

As the hydrolytic stability of glycosylamines depends on pH, it is reasonable to expect that these troublesome effects can be suppressed by adjusting the pH of the mobile phase, as in the analysis of D-glucosone[15]. This expectation was fully confirmed by the use of a mobile phase containing 20.3 % (w/w) of 0.011 *M* phosphate buffer, pH 5.9.

Fig. 3 shows two chromatograms of the standard sugar mixture (identical with that used in Figs. 1 and 2a): one was recorded at the beginning and the other at the end of pumping about 1000 ml of the above buffered eluent through a CGC twin (two pressure-resistant glass columns, each 150 mm long, connected directly in series) packed with Separon SI NH_2. (At the end, 980 ml of the eluent had passed through the column, corresponding to some 3 m*M* of buffer, and the surface is expected to be saturated.) The area of the glucose peak is markedly larger in Fig. 3b. The progress of this "buffering" of the surface is shown in Fig. 4, where the ratio of (i) xylose and fructose peak heights and (ii) arabinose and fructose peak heights is plotted against the volume of the eluent pumped through the system (fructose serves as a sufficiently inert standard).

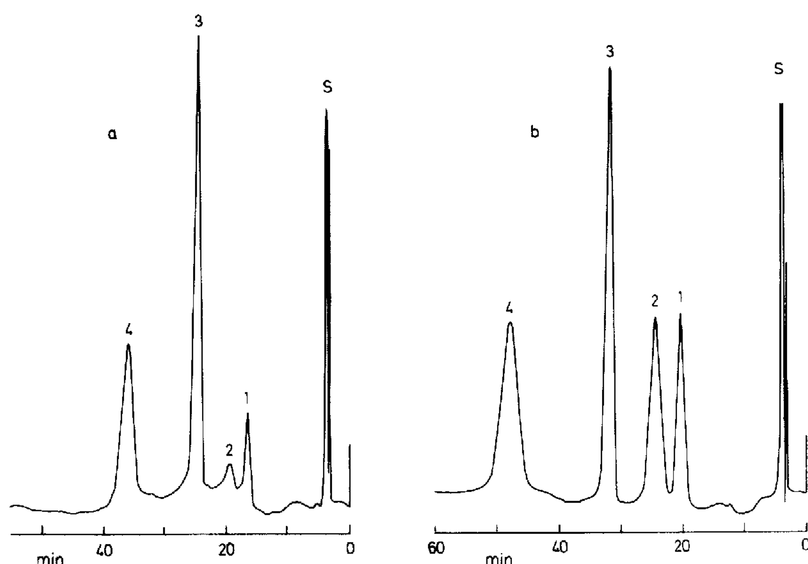

Fig. 3. Separation of a sugar mixture (see Fig. 1) on Separon SI NH_2 (d_p = 10 μm) at the beginning and at the end of equilibration in a buffered mobile phase (acetonitrile + 20.3 %, w/w of 0.011 *M* phosphate buffer, pH 5.9). a, After 270 ml of the eluent had passed; b, after about 1000 ml of the eluent. Flow-rate: 0.7 ml/min. 8 μl injected. Refractive index detection, attenuation 4 ×. Two CGC glass columns in series.

The capacity factors, k', of the four sugars increased almost simultaneously and in parallel during the equilibration: at the end, k' had increased by 41 % for xylose, 43 % for arabinose, 52 % for fructose and 53 % for glucose. It is apparent from Fig. 4 that, after sufficient equilibration, the peak heights of both aldoses no longer increase; at the same time, no changes in peak heights with the flow-rate can be

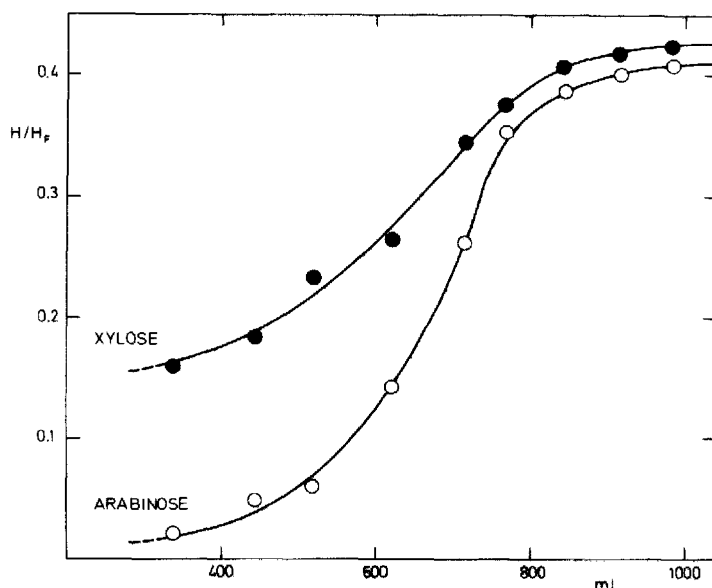

Fig. 4. Ratio of peak heights of xylose to fructose and of arabinose to fructose during equilibration of the CGC twin column packed with Separon SI NH_2 (d_p = 10 μm) and with buffered (pH 5.9) mobile phase; chromatographic conditions as in Fig. 3.

detected (under conditions corresponding to those in Fig. 1), so that apparently the hydrolysis of glycosylamines (if they are indeed formed) is sufficiently rapid under these conditions. Thus, one can be reasonably certain that by changing the pH of the eluent it is possible to eliminate the effect of the reaction between the amino phase and aldoses.

ACKNOWLEDGEMENT

The author wishes to thank Dr. S. Vozka for the prepacked CGC columns and for samples of Separon SI VSK and Separon SI NH_2.

REFERENCES

1 R. E. Majors, *J. Chromatogr. Sci.*, 18 (1980) 488.
2 M. Ugrinovits, *Chromatographia*, 13 (1980) 386.
3 F. M. Rabel, *J. Chromatogr. Sci.*, 18 (1980) 394.
4 I. Molnar, *Chromatographia*, 12 (1979) 371.
5 N. W. H. Cheetham and P. Sirimanne, *J. Chromatogr.*, 196 (1980) 171.
6 K. Aitzetmüller, *J. Chromatogr.*, 156 (1978) 354.
7 B. B. Wheals and P. C. White, *J. Chromatogr.*, 176 (1979) 421.
8 D. L. Hendrix, R. E. Lee, J. G. Baust and H. James, *J. Chromatogr.*, 210 (1981) 45.
9 G. Ellis and J. Honeyman, *Advan. Carbohydr. Chem.*, 10 (1955) 95.
10 S. R. Abbot, *J. Chromatogr. Sci.*, 18 (1980) 540.
11 M. Kubín, B. Porsch, P. Špaček, J. Švantner, J. Voslář and S. Vozka, *Chem. Prum.*, 29/54 (1979) 190.
12 S. Vozka, B. Porsch, J. Voslář, P. Špaček and M. Kubín, *Czech. Pat.*, 187,774 (1978).
13 S. Vozka, J. Voslář, B. Porsch, L. Řeřicha, M. Kubín and P. Špaček, *Czech. Pat.*, 200,755 (1980).
14 P. Špaček, S. Vozka, J. Čoupek, M. Kubín, J. Voslář and B. Porsch, *Czech. Pat. Appl.*, 4635-81.
15 J. Geigert, D. S. Hirano and S. L. Neidleman, *J. Chromatogr.*, 202 (1980) 319.