Journal of Chromatography, 588 (1991) 340–343
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 715

Short Communication

Synthesis and characterization of stationary phases on the basis of silicas modified with epoxidized polybutadienes

IV☆. Chromatographic experiments on a new amino-functionalized stationary phase for high-performance liquid chromatography

U. Erler* and G. Heublein☆☆

Department of Chemistry, Friedrich-Schiller-University of Jena, Humboldt-Strasse 10, D-6900 Jena (Germany)

(First received July 12th, 1991; revised manuscript received August 29th, 1991)

ABSTRACT

Amino-functionalized stationary phases derived from polybutadiene epoxide-coated silica possess a good stability in aqueous buffer solutions at different pH. Pressure stability of the highly polymer-loaded support, separation of carbohydrates, purines and pyrimidines, and the influence of the pH of the eluent mixture on the retention behaviour of ionisable samples, such as substituted benzoic acids, are demonstrated.

INTRODUCTION

In our last paper [1] we described different ways of synthesizing amino-functionalized stationary phases within our concept of heterogeneous polymer-analogous reactions of epoxidized polybutadienes and silicas [2–4]. The aim of the present paper is to prove some characteristic properties of those amino-functionalized stationary phases under conditions of high-performance liquid chromatography.

EXPERIMENTAL

The following chromatographic equipment was used: an LC-6A pump, an SCL-6B system controller, an SPD-6AV UV spectrophotometric detector, an RID-6A differential refractometer and a C-R4A Chromatopac multifunctional data processor, all from Shimadzu. The chromatographic experiments were carried out at ambient temperature. The solvent acetonitrile was of UV grade. The test compounds were obtained from various chemical suppliers and were used as received.

Silica used was LiChrosorb Si 100 (Merck 9340, 7 μm, LC 100). The modification of the surface of LC 100 with epoxidized Polyöl 110 (Chemische Werke

☆ For Part III, see ref. 1.
☆☆ Author deceased.

0021-9673/91/$03.50

Hüls) was carried out according to an optimized variant for the synthesis of polymer-modified supports [4]. This procedure gave the following product: LC 100 P(EPO): 16.90% C, $O_{Spec.}$(BET) = 65 $m^2 g^{-1}$. The reaction of epoxide groups on the surface of the composite LC 100 P(EPO) with ethylene diamine was carried out at 80°C as described in ref. 1.

The resulting material was LC 100 P(NH): 19.54% C, 3.07% N, $O_{Spec.}$(BET) = 58 $m^2 g^{-1}$. LC 100 P(NH) was used to fill a 250 × 4 mm I.D. column using a high-pressure, balanced-density slurry-packing technique [5,6]. The column was rinsed with 200 ml of methanol after packing, followed by rinsing with the elution mixture until equilibration was attained. The ion-exchange capacities (IEC) of amino-functionalized stationary phases were determined by means of potentiometric titration in 1 *M* sodium chloride with 0.1 *M* hydrochloric acid after washing with acetic acid (10%), sodium hydrogencarbonate (10%), water, methanol and diethyl ether and drying at room temperature.

RESULTS AND DISCUSSION

One of the advantages of polymer-modified stationary phases in high-performance liquid chromatography (HPLC) is the combination of the properties of the organic polymer (high pH stability, functionality) with those of the inorganic support (porosity, pressure stability). However, highly polymer-loaded materials could show some disadvantages of polymer packing materials, such as swelling in certain organic solvents and poor pressure stability. In our first attempt we tested the pressure stability of LC 100 P(NH), measuring the dependence of the column pressure on the chosen flow-rate. We found a linear relationship between column pressure and flow-rate in the investigated range (0.1–3 ml/min, p = 0.2–26 MPa). On increasing the flow-rate several times, the measured pressures remained on the same straight line, demonstrating the pressure stability and incompressibility of the highly polymer-loaded and amino-functionalized support.

The separation, identification and quantitative determination of carbohydrates by liquid chromatography have received increasing attention in recent years. Verzele *et al.* [7] published a critical review of some liquid chromatographic (LC) systems for the separation of sugars. Other reviews were published by, for example, Heyrand and Rinando [8], Robards and Whitelaw [9], Hicks [10] and Hanai [11]. Silica gels, derivatized with aminopropyl- or aminoethylaminopropyl-trialkoxysilanes are the most widely used stationary phases for carbohydrate and sugar LC. These stationary phases separate mono-, di- and trisaccharides with eluent compositions such as acetonitrile–water in the ratio 80:20 or 75:25 [7]. We tried to separate some carbohydrates on our amino-functionalized stationary phase LC 100 P(NH) using acetonitrile–water in the ratio 75:25 as an eluent. Fig. 1 demonstrates the ability of the material to separate some mono- and disaccharides as well as other sugar derivatives. As expected, splitting of the peaks because of separation of the anomers was not a problem with the amino-functionalized stationary phase. The comparison of the k' values in Fig. 2 demonstrates the possibility of separating groups of compounds (mono- from disaccharides, desoxy sugars from monosaccharides, ketohexose fructose from aldohexoses, aldopentoses from aldohexoses) as well as compounds within groups (for example separation of individual mono- or disaccharides).

One of the drawbacks of aminopropyl-derivatized silica gel phases is the rather short life expectancy due to the self-hydrolysis of the basic material. Some reports on the hydrolytic stability of ami-

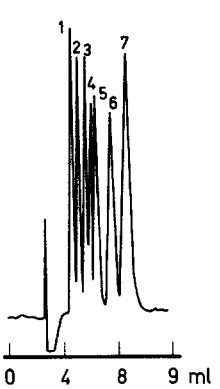

Fig. 1. Separation of rhamnose (1), arabinose (2), fructose (3), sorbitol (4), glucose (5), saccharose (6) and maltose (7). Column, LC 100 P(NH), 7 μm, 250 × 4 mm I.D.; eluent, acetonitrile–water (75:25); flow-rate, 1 ml/min; refractive index detector; 5-mg sample in 2 ml of eluent; dosier volume, 20 μl.

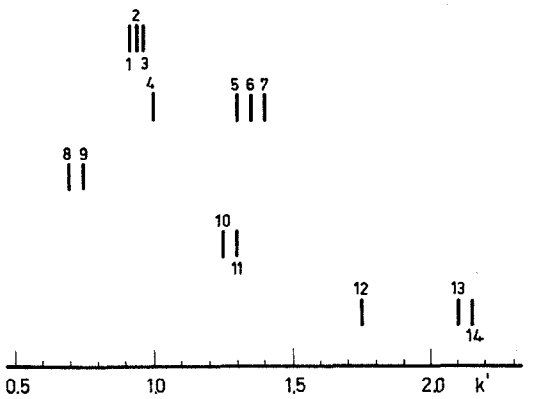

Fig. 2. Comparison of k' values of lyxose (1), xylose (2), arabinose (3), fructose (4), mannose (5), glucose (6), galactose (7), rhamnose (8), fucose (9), sorbitol (10), mannitol (11), saccharose (12), lactose (13) and maltose (14). Conditions as in Fig. 1.

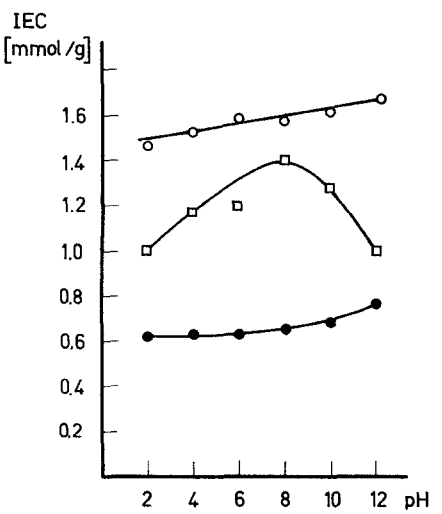

Fig. 3. Comparison of the stability of amino-functionalized stationary phases in aqueous buffer solutions at room temperature (10 days). □ = Kieselgel 60 (Merck, d = 0.04–0.063 mm), modified with aminoethylaminopropyl-triethoxysilane (Wacker, GF 94). Starting material: 7.1% C, 2.31% N, IEC = 1.65 mmol/g; after 10 days at pH 2: 4.5% C, 1.38% N; after 10 days at pH 12: 4.8% C, 1.35% N. ○ = Kieselgel 60, modified with epoxidized polybutadiene and functionalized with ethylene diamine [1]. Starting material: 18.21% C, 2.38% N, IEC = 1.68 mmol/g; after 10 days at pH 2: 16.10% C, 2.10% N; after 10 days at pH 12: 18.02% C, 2.35% N. ● = Kieselgel 60, modified with epoxidized polybutadiene and functionalized with ethylene diamine [1]. Starting material: 13.26% C, 1.05% N, IEC = 0.75 mmol/g; after 10 days at pH 2: 12.12% C, 0.88% N; after 10 days at pH 12: 13.10% C, 1.03% N.

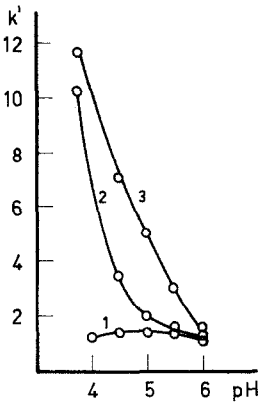

Fig. 4. Dependence of the retention behavior of phenol (1), 4-hydroxybenzoic acid (2) and 2,4-dihydroxybenzoic acid (3) on the pH of the eluent mixture. Column, LC 100 P(NH), 7 μm, 250 × 4 mm I.D.; eluent, phosphate buffer pH 4–6, (I = 0.1)–acetonitrile (80:20); flow-rate, 1 ml/min; UV detection at 280 nm.

no-functionalized stationary phases and reversed-phase materials are given in refs. 12 and 13. In contrast to aminosilane-modified silicas amino-functionalized stationary phase based on silicas modified with expoxidized polybutadienes possess good stability in aqueous buffer solutions at different pH values (Fig. 3).

As is well known, the pH of the buffer solution used as a mobile phase in ion-exchange chromatography influences the retention behavior of ionizable solutes. Fig. 4 demonstrates the increasing resolution of three ionizable samples with decreasing pH value of the mobile phase in the pH range 6–4. The order phenol, 4-hydroxybenzoic acid, 2,4-dihydroxybenzoic acid shows that the higher the possibility of ionization of the test solute, the more changes in the pH value of the eluent mixture influence the retention behavior. These results are in good agreement with those obtained by Heinemann *et al.* [14] on similar stationary phases.

Previously we demonstrated the possibility of separating some xanthine derivatives on columns filled with a new polyhydroxy-functionalized stationary phase derived from poly(butadiene)epoxide-modified silica [15]. We obtained a good separation of the test solutes caffeine, theophylline and 1,3-dimethyl-4-amino-5-formylaminouracil. Because of the strong tendency for xanthine or hypoxanthine to form hydrogen bonds, the system was not suitable for separating these samples in an ac-

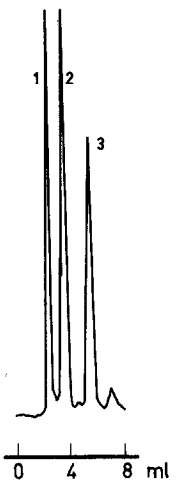

Fig. 5. Separation of caffeine (1), xanthine (2) and hypoxanthine (3). Column, LC 100 P(NH), 7 μm, 250 × 4 mm I.D.; eluent, phosphate buffer pH 5 (I = 0.1)–acetonitrile (80:20); flow-rate, 1 ml/min; UV detection at 280 nm.

ceptable time. As shown in Fig. 5, separation of caffeine, xanthine and hypoxanthine was achieved on LC 100 P(NH) at pH 5 with good efficiency. An important property of purines and pyrimidines is their ability to undergo tautomerization, affecting the chromatographic behavior of those compounds. In addition, the acid–base character of those molecules plays an important role in their chromatographic behavior.

ACKNOWLEDGEMENT

We greatly appreciate the support of Professor Dr. S. Reissmann (Friedrich-Schiller-University of Jena, Department of Biochemistry) for placing the chromatographic equipment at our disposal.

REFERENCES

1 U. Erler and G. Heublein, *Angew. Makromol. Chem.*, 182 (1990) 43.
2 G. Heublein, B. Heublein, P. Hortschansky, H. Meissner and H. Schütz, *J. Macromol. Sci.*, A25 (1988) 183.
3 G. Heublein, B. Heublein, P. Hortschansky, H. Schütz and H.-J. Flammersheim, *Makromol. Chem.*, 190 (1989) 9.
4 G. Heublein, U. Erler, A. Ulbricht and H. Winkelmann, *Angew. Makromol. Chem.*, 182 (1990) 29.
5 R. E. Majors, *Anal. Chem.*, 44 (1972) 1722.
6 J. J. Kirkland, *Chromatographia*, 8 (1975) 661.
7 M. Verzele, G. Simoens and F. Van Damme, *Chromatographia*, 23 (1987) 292.
8 A. Heyrand and M. Rinando, *J. Liq. Chromatogr.*, 4 (Suppl. 2) (1981) 175.
9 K. Robards and M. Whitelaw, *J. Chromatogr.*, 373 (1986) 81.
10 K. B. Hicks, *Adv. Carbohydrate Chem. Biochem.*, 46 (1987) 17.
11 T. Hanai, *Adv. Chromatogr.*, 25 (1986) 280.
12 D. M. Wonnacott and E. V. Patton, *J. Chromatogr.*, 389 (1987) 103.
13 J. L. Glajch, J. J. Kirkland and J. Köhler, *J. Chromatogr.*, 384 (1987) 81.
14 G. Heinemann, J. Köhler and G. Schomburg, *Chromatographia*, 23 (1987) 435.
15 U. Erler, S. Spange and G. Heublein, *Acta Polym.*, 42 (1991) 389.