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Polyalkylenes used as stationary phases for preparative reversed-phase liquid chromatography

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ABSTRACT

Polyethylene and polypropylene were employed as stationary phases for preparative liquid chromatography and compared with LiChroprep RP-18. Polyalkylene phases are chemically stable and have homogeneous hydrophobic surfaces, but they are physically less resistant than silica-based phases. The influence of organic modifiers and salt ions on the capacity factors of aromatic hydrocarbons and peptides was determined. Peptides prepared by solid-phase peptide synthesis were purified on polypropylene. The results demonstrate the usefulness of polyalkylenes in preparative reversed-phase liquid chromatography, particularly for basic peptides.

INTRODUCTION

Reversed-phase liquid chromatography has become an efficient method for the analysis and purification of biologically active substances such as peptides and proteins. Alkyl-bonded silica provides effective stationary phases with uniform particle size, which are reproducible and versatile with respect to many separation problems. However, the presence of residual silanols causes interactions with basic solutes, for example amines, leading to peak tailing and reduced recoveries. A further limitation of alkyl-bonded silica phases is the chemical instability at low and high pH. This prevents the effective regeneration of the stationary phases, especially for peptides and proteins in preparative work.

Porous poly(styrene–divinylbenzene) stationary phases have been shown to be excellent alternatives to alkyl-bonded silica for the chromatography of peptides and proteins [1,2]. The polymer phases are stable with eluents from pH 1 to 14. The homogeneous non-polar adsorbent surface with aromatic groups leads to a retention behaviour different to that of the alkyl phases [3,4]. Octadecyl-bonded vinyl alcohol copolymer gels [5] and a macroporous polyacrylamide-based packing having an octadecyl chemically bonded phase [4] have been developed for reversed-phase liquid chromatography. Such stationary phases operate with capacity factors for aromatic hydrocarbon solutes that are similar to those with alkyl-bonded silica and are particularly advantageous for basic compounds.

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Polyethylene and polypropylene are chemically stable polymers and have hydrophobic surfaces without polar groups. Therefore, from the chemical point of view they are ideal phases without polar solid–solute interactions. In this work, polyalkylenes were examined for their use as stationary phases for reversed-phase liquid chromatography. The results were compared with those with alkyl-bonded silica.

EXPERIMENTAL

Polyethylene was obtained from Leuna (G.D.R.) and modified by additional precipitation procedures to 5–85 μm irregular particles. Surface areas were measured by the conventional BET method and were found to be 15–20 m^2/g. The density was 0.922/g cm^3. Polypropylene was supplied by Enka (F.R.G.) as porous Accurel polypropylene with a 75% void volume. Its particle size was 100–200 μm.

The dry polymer particles were dispersed in methanol and the slurry was packed into a glass column (150 × 9 mm I.D.) at a pressure of up to 1 MPa. LiChroprep RP-18 (15–25 μm) (Merck, F.R.G.) was packed in the same way.

Analytical high-performance liquid chromatographic (HPLC) separations of the peptides were carried out on a column (150 × 3.3 mm I.D.) containing Separon SIX, C_{18} (5 μm), purchased from Laboratorní Přístroje (Czechoslovakia).

The instrumentation consisted of an HP 10.1 pump (Chromatron, G.D.R.), a Rheodyne Model 7125 injection valve (20-, 100- and 200-μl loops) and an LCD 2040 variable-wavelength UV detector (Laboratorní Přístroje) operated at 254 nm for aromatic hydrocarbons and at 215 nm for peptides. Solute concentrations were 200 μg/ml of pyridine and phenol, 1 mg/ml of aromatic hydrocarbons and 1–10 mg/ml of peptides. Separations were performed at ambient temperature and at an eluent flow-rate of 0.25–0.5 ml/min.

Capacity factors (k') were calculated from the retention time, t_R, of each solute: $k' = (t_R - t_0)/t_0$. Data for t_0 were obtained by injecting a liquid mixture with a volume composition different from that of the eluent.

RESULTS AND DISCUSSION

Retention of aromatic hydrocarbons

Capacity factors of benzene, toluene, nitrobenzene and phenol were determined on polypropylene, polyethylene and LiChroprep RP-18. The content of water in the water–methanol eluent varied from 10% (v/v) ($X = 0.1$) to 50% (v/v) ($X = 0.5$). The variations of log k' with X are shown in Fig. 1.

The results indicate a similar behaviour of the polyalkylene stationary phases and the alkyl-bonded silica. The retention orders of the aromatic hydrocarbons on the polymer phases and RP-18 were identical, indicating that the dominant retention mechanism was hydrophobic in nature. However, the capacity factors of the polymer phases were lower than those of RP-18.

The hydrophobic selectivities (α) of the polyalkylene stationary phases for benzene–toluene and benzene–nitrobenzene were compared with data for octadecyl-bonded silica and PRP-1 (Table I). The data for α, as shown in Table I, confirm the higher selectivity of the polyalkylene stationary phases in comparison with LiChroprep RP-18. The selectivity of PRP-1, a macroporous copolymer of styrene and divinylbenzene, is also lower than that of the polyalkylenes.

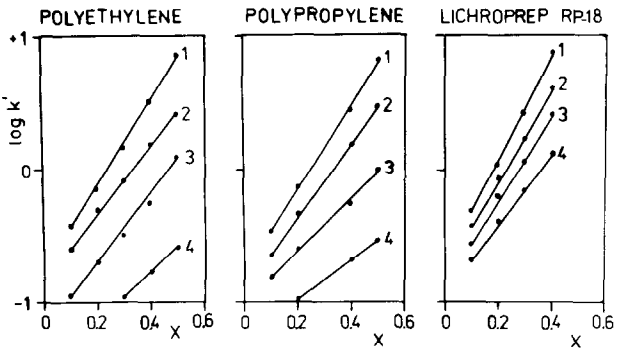

Fig. 1. Change in the capacity factors with increasing volume content of water (X) for (1) toluene, (2) benzene, (3) nitrobenzene and (4) phenol.

On alkyl-bonded silica phases pyridine was eluted later than phenol, with greater peak tailing owing to the interaction with residual silanol groups. The capacity factors for the elution of pyridine and phenol are given in Table II.

On polypropylene the more hydrophilic pyridine was eluted earlier than phenol without peak tailing. This emphasizes the importance of a homogeneous hydrophobic surface for the elution to be in order of the hydrophobicities of solutes in reversed-phase liquid chromatography and makes the polyalkylene stationary phases very suitable for the separation of basic substances.

TABLE I

SELECTIVITY FOR BENZENE–TOLUENE AND BENZENE–NITROBENZENE

Stationary phase	α	
	Benzene–toluene	Benzene–nitrobenzene
Polyethylene	2.20	2.41
Polypropylene	2.17	2.98
LiChroprep	2.07	1.21
PRP-1 [6]	–	1.19

TABLE II

CAPACITY FACTORS FOR PYRIDINE AND PHENOL

Mobile phase: polypropylene, methanol–water (40:60); LiChroprep RP-18, methanol–water (80:20).

Solute	k'	
	Polypropylene	LiChroprep RP-18
Pyridine	0.12	19.8
Phenol	0.39	0.2

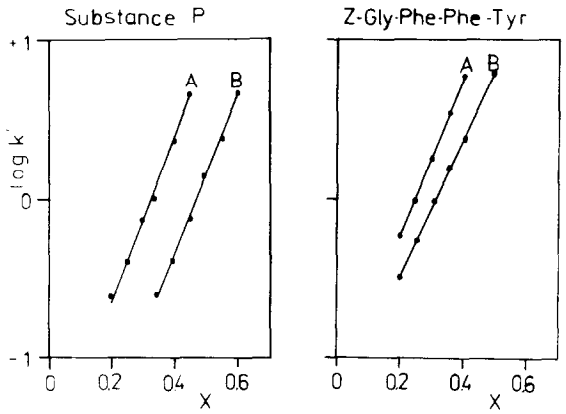

Fig. 2. Change in the capacity factors with increasing volume content of water in methanol (X). (A) LiChroprep RP-18; (B) polypropylene. Eluent: methanol–water containing 0.1% trifluoroacetic acid.

Retention behaviour of peptides

The main aim of this study was to demonstrate the use of polyalkylenes as stationary phases for the preparative chromatography of peptides. The basic peptide substance P and the acidic *Z*-Gly–Phe–Phe–Tyr were chromatographed with aqueous methanol as eluent and the capacity factors were plotted as a function of the methanol concentration in the eluent, as shown in Fig. 2.

The dependence of log k' on methanol concentration was very close to linear and the capacity factors of polypropylene were lower than those of LiChroprep RP-18, particularly for the basic substance P. It is obvious that the polyalkylenes have retentive properties similar to those of alkyl-bonded silica phases and can be used as an alternative stationary phase in reversed-phase chromatography. The increase in the capacity factor of substance P on LiChroprep RP-18 may arise from solute interactions with acidic silanol groups.

Studies on the influence of salt ions illustrate the difference in retention properties between polyalkylenes and alkyl-bonded silica, phases, as shown in Fig. 3.

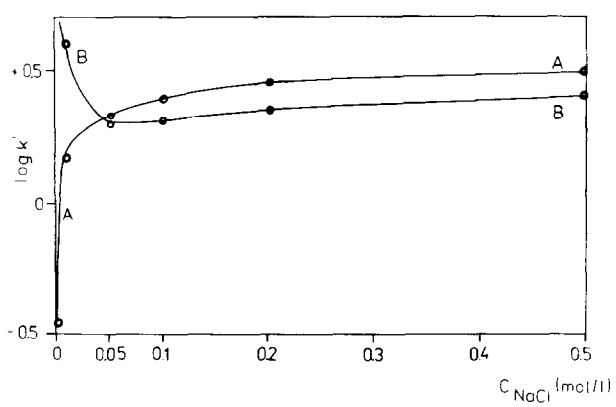

Fig. 3. Effect of salt ions on retention of substance P. (A) Polypropylene, methanol–water (50:50). (B) LiChroprep RP-18, methanol–water (60:40).

Whereas the capacity factors on RP-18 are considerably decreased by addition of 0.5 *M* sodium chloride, the reverse effect is observed on polypropylene. This salt effect on RP-18 results from the elimination of polar solid–solute interactions, which increase the retention of the basic peptide.

Purification of peptides

In preparative liquid chromatography the yield of pure substance per run is an important parameter. Therefore, polyalkylenes used as stationary phases were evaluated with respect to sample capacity and peptide recovery. To compare the efficiencies of stationary phases having different diameters, the influence of the amount of sample injected on the reduced plate height, *h*, was determined (Fig. 4).

Comparing the polymer and RP-18, the reduced plate heights with polypropylene is lower than those with LiChroprep RP-18, particularly for substance P. In contrast to the alkyl-bonded silica, polypropylene shows no tailing of the substance P peak with sample loads up to 1 mg of peptide per ml of stationary phase. The recoveries of substance P, *Z*-Gly–Phe–Phe–Tyr and human insulin chromatographed under acidic conditions (0.1% trifluoroacetic acid) were 98.5, 94.2 and 92.1%, respectively.

Preliminary investigations were made to examine the potential of polyalkylenes for the purification of synthetic peptides. The preparative chromatograms of crude substance P and GnRH (decapeptide), prepared by solid-phase peptide synthesis, are shown in Figs. 5 and 6. The purity of the peptides was determined by reversed-phase HPLC on Separon SIX C_{18} to be >96%.

Non-polar compounds adsorbed on the stationary phase were removed after each preparative separation by washing with organic solvent. Irreversibly adsorbed peptides or proteins can be removed with 1 *M* sodium hydroxide solution. The polyalkylenes were stable under these conditions. There was no change in selectivity and efficiency after exposing the column to 1 *M* hydrochloric acid, 1 *M* sodium hydroxide solution and pure organic solvents.

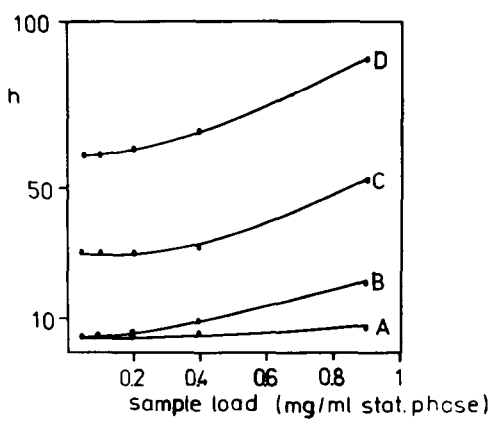

Fig. 4. Reduced plate height, *h*, *versus* sample load for (A, B) polypropylene and (C, D) LiChroprep RP-18. Eluent: polypropylene, methanol–water (50:50)–0.1% NaCl; LiChroprep RP-18, methanol–water (65:35)–0.1% NaCl. Sample: (B, D) substance P; (A, C) *Z*-Gly–Phe–Phe–Tyr.

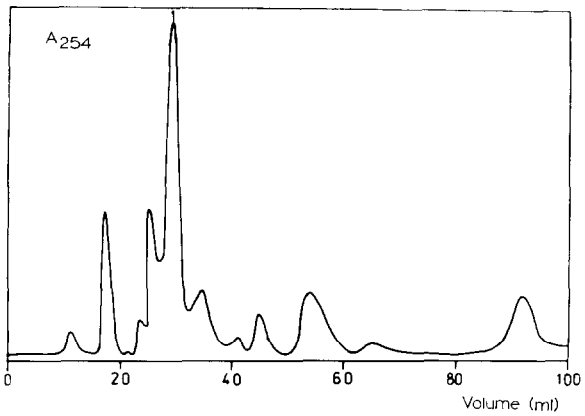

Fig. 5. Elution of crude substance P (peak 1) on a 120 × 9 mm I.D. polypropylene column. Flow-rate: 0.5 ml/min. Linear gradient from 0 to 50% isopropanol in water. Sample size: 4 mg.

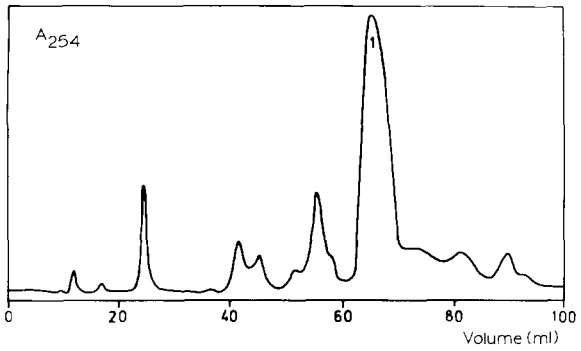

Fig. 6. Elution of crude GnRH (peak 1) on a 120 × 9 mm I.D. polypropylene column. Flow-rate: 0.5 ml/min. Linear gradient from 20 to 50% methanol in water containing 0.1% trifluoroacetic acid. Sample size: 4 mg.

The mechanical stabilities of the polyalkylenes are much lower than those of silica phases. The pressure of 1 MPa should not be exceeded because distortion of particles and a drastic pressure drop may occur, resulting in a reduced efficiency of the stationary phases.

CONCLUSION

These preliminary results show that polyalkylenes can be useful stationary phases in reversed-phase liquid chromatography. Polyethylene and polypropylene have homogeneous hydrophobic surfaces and operate with retention and selectivity for peptide solutes similar to those of LiChroprep RP-18. The polymer phases are particularly advantageous for basic solutes owing to the absence of residual polar groups on the adsorbent surface.

The excellent chemical stability over the wide pH range of 1–14, the compatibility with organic solvents and the high recoveries make the polyalkylene stationary phases highly appropriate for preparative separations.

The aim of our current work is to improve the column efficiency by optimizing the particle size distribution, surface area and mechanical stability.

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