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Journal of Chromatography A, 697 (1995) 159–164

JOURNAL OF CHROMATOGRAPHY A

Stability of high-performance liquid chromatography columns packed with C_1 and C_8 polysiloxanes sorbed into porous silica particles

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Abstract

Columns, packed with 10 μm diameter porous silica particles (15 nm diameter pores) containing polydimethylsiloxane (20%, w/w) or polymethyloctylsiloxane (40%, w/w) sorbed into the pores, were tested for chromatographic stability as a function of elution volume. No significant changes in efficiency, retention factor or separation factor were observed following extended washing (up to 5000 column volumes) of either stationary phase with methanol–water mobile phases.

1. Introduction

Liquid–liquid chromatography (LLC) underwent considerable development from its introduction in 1941 [1] to the 1970s [2]. During this period liquid stationary phases became widely used for both normal- and reversed-phase chromatography. Packing materials having chemically bonded stationary phases have since displaced LLC: only a few specialized applications make use of LLC today. This is true despite the unique and potentially important characteristics of liquid-based stationary phases. To put this into perspective the advantages and disadvantages of these stationary phases should be considered.

Bonded phases have one great advantage over conventional liquid stationary phases: the structural groups (C_8, C_{18}, phenyl, etc.) are anchored to the silica support surface by covalent bonds which give a relatively high stability to these phases when used with conventional mobile phases. In contrast, most of the liquid stationary phases used in LLC since 1970 have consisted of small molecules ($M_r \leq 200$) which are retained in the pores of the support particles by much weaker dipolar (Van der Waals) attractive forces. Hence, these molecules are much more susceptible to loss (wash-out) in typical mobile phases. Indeed, to maintain the stationary liquid phase normally involves replacement of the molecules which are lost. This requires the presence of replacement stationary phase molecules in the mobile phase and very careful control of column temperature [2,3].

Two advantages of LLC over bonded-phase chromatography are equally impressive. Without the burden of chemical synthesis of a bonded phase, many different liquid materials, covering an enormous range of properties, can be considered. This can, in principle, open up HPLC to separations with a vast array of specific-interaction stationary phases. Another advantage is

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0021-9673/95/$09.50
SSDI 0021-9673(94)00810-8

the high reproducibility which can be achieved in routine separations with LLC columns [4], the reason being that the silica–stationary phase interface in LLC does not affect the chromatographic separation as much as in the case of bonded-phase HPLC. In addition, LLC stationary phases, but not bonded phases, can be repaired —or even replaced— to restore the initial separation properties of a column.

To decrease the stability (wash-off) problem in LLC, polymeric liquids can be considered. In this way the molecules which constitute the stationary phase can have very many weak (physical) bonds to the support surfaces and to

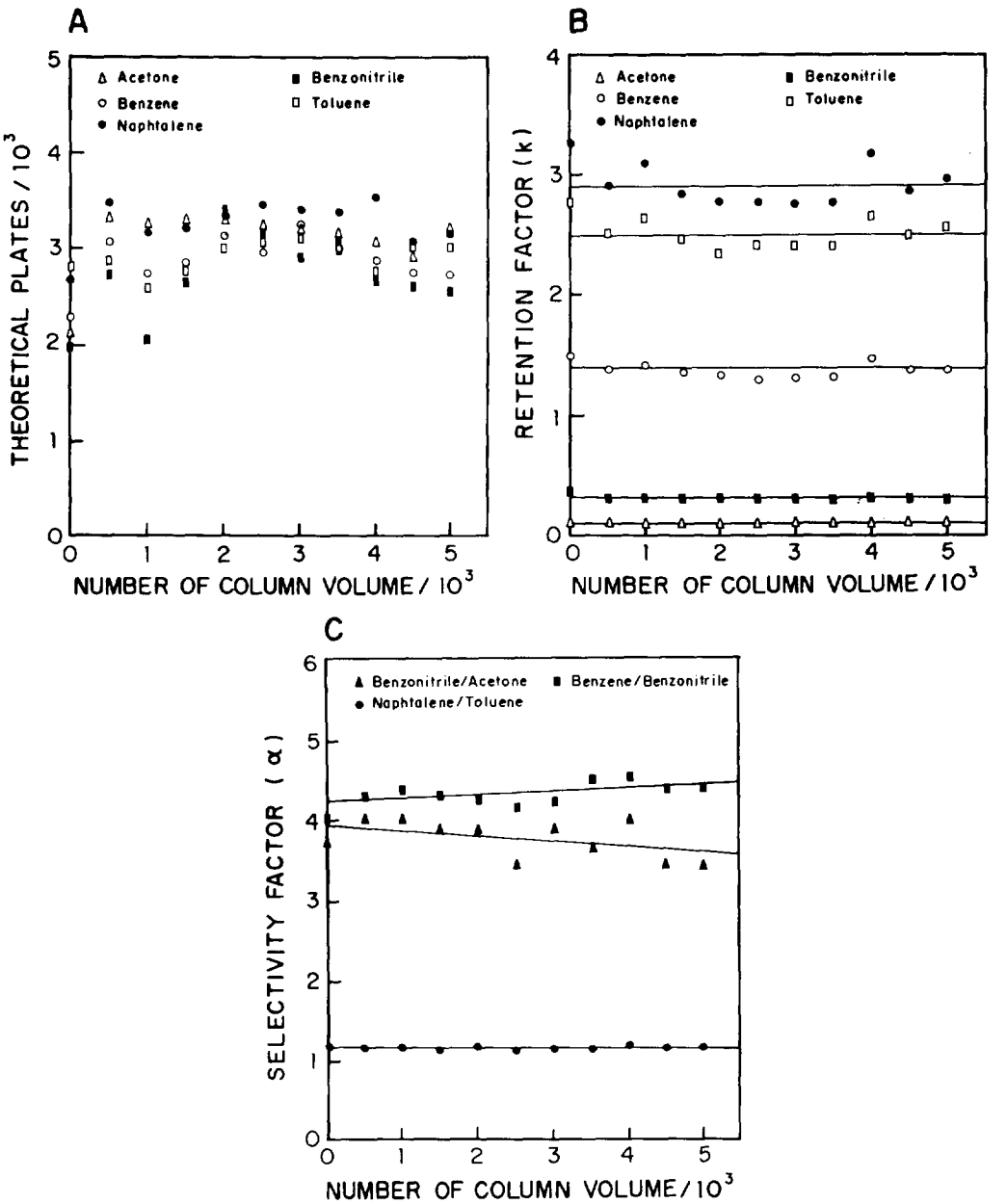

Fig. 1. (A) Column efficiency (theoretical plates, N); (B) retention factor (k) and (C) selectivity factor (α) as functions of volume of mobile phase passing a column packed with 20% (w/w) PDMS-loaded silica support.

each other, resulting in a degree of general stability of the phase even in the presence of moderately strong mobile phases. A number of polymeric materials were tested as possible LLC stationary phases prior to 1970 [5]. This work, largely with polyethylene glycols (Carbowaxes) with molecular masses in the range 200–20 000, led to the conclusion that column efficiency decreases with increasing molecular mass [5]. An explanation was that the viscosity of the polymer, which increases with its molecular mass, affects the diffusive mobility of solutes in the pores of porous supports and thus leads to increased band dispersion in the column.

However, we have recently shown [6] that separations carried out with a polymethyloctylsiloxane (PMOS) liquid phase on silica has a chromatographic behaviour remarkably similar to that obtained with conventional C_8 bonded phases.

In the present paper we show that high-molecular-mass polydimethylsiloxane (PDMS) and PMOS stationary phases are quite stable to extended washing with methanol–water mobile phases and that good chromatographic efficiency is achieved and maintained with these viscous liquid phases.

2. Experimental

2.1. Materials

Methanol (LiChrosolv), dichloromethane (LiChrosolv) and carbon tetrachloride (analytical-reagent grade) were obtained from Merck (Rio de Janeiro, Brazil).

The chromatographic test substances (acetone, benzonitrile, benzene, toluene and naphthalene) were analytical-reagent grade and not further purified.

Davisil silica having a mean particle diameter of 10 μm, a 15 nm mean pore diameter and a 240 $m^2\ g^{-1}$ specific surface area was obtained from Alltech (USA).

PDMS (product PS-048; M_r 139 000) and PMOS (product PS-140; M_r 6200) were obtained from Hüls America (USA).

2.2. Preparation of packing materials

Following drying at 150°C for 24 h, weighed quantities of the silica were added to solutions containing weighed quantities of polysiloxane (PDMS or PMOS) in 60 ml of dichloromethane. The mixtures were gently stirred for 3 h at room temperature and then the solvent was allowed to evaporate, without stirring, at room temperature.

2.3. Preparation of columns

Columns of 125 × 2.9 mm (PDMS) or 125 × 3.4 mm (PMOS) were made from type 316L stainless-steel tubing with highly polished interior surfaces. The columns were slurry packed at 38 MPa using a 10% (w/v) slurry of the prepared packing material in carbon tetrachloride.

2.4. Instrumentation

Two medium dispersion chromatographic systems were used. System 1 (PDMS column) used an Altex Model 110A pump, a Rheodyne Model 7125 injector with a 10-μl loop, and a Schoeffel Model SF 770 spectrophotometric detector (254 nm; 8 μl cell volume), coupled to an ECB Model RB 102 recorder. System 2 (PMOS column) consisted of a Waters Model 510 pump, SSI Model 3XL pneumatic injector with a 10-μl loop and a Waters Model 481 spectrophotometric

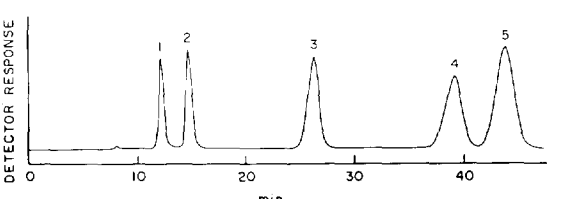

Fig. 2. Chromatogram obtained with a column packed with 20% (w/w) PDMS-loaded silica support at the end of the washing test. Peaks: 1 = acetone; 2 = benzonitrile; 3 = benzene; 4 = toluene; 5 = naphthalene. Column: 125 × 2.9 mm, mobile phase: methanol–water (50:50, v/v), flow-rate: 0.1 ml min^{-1}, detection: UV at 254 nm.

detector (254 nm; 14 μl cell volume), coupled to a Waters Model 740 integrator.

2.5. Stability testing

Methanol–water mobile phases (50:50 or 70:30, v/v) were passed through the columns at 2 ml min^{-1} to a total of 5000 column volumes. Chromatograms of a mixture of test compounds were obtained periodically with the same mobile phases as used for washing, at flow-rates of 0.1 and 0.2 ml min^{-1}, with the columns containing PDMS and PMOS, respectively. Efficiency (N), retention factor (k) and selectivity factor (α)

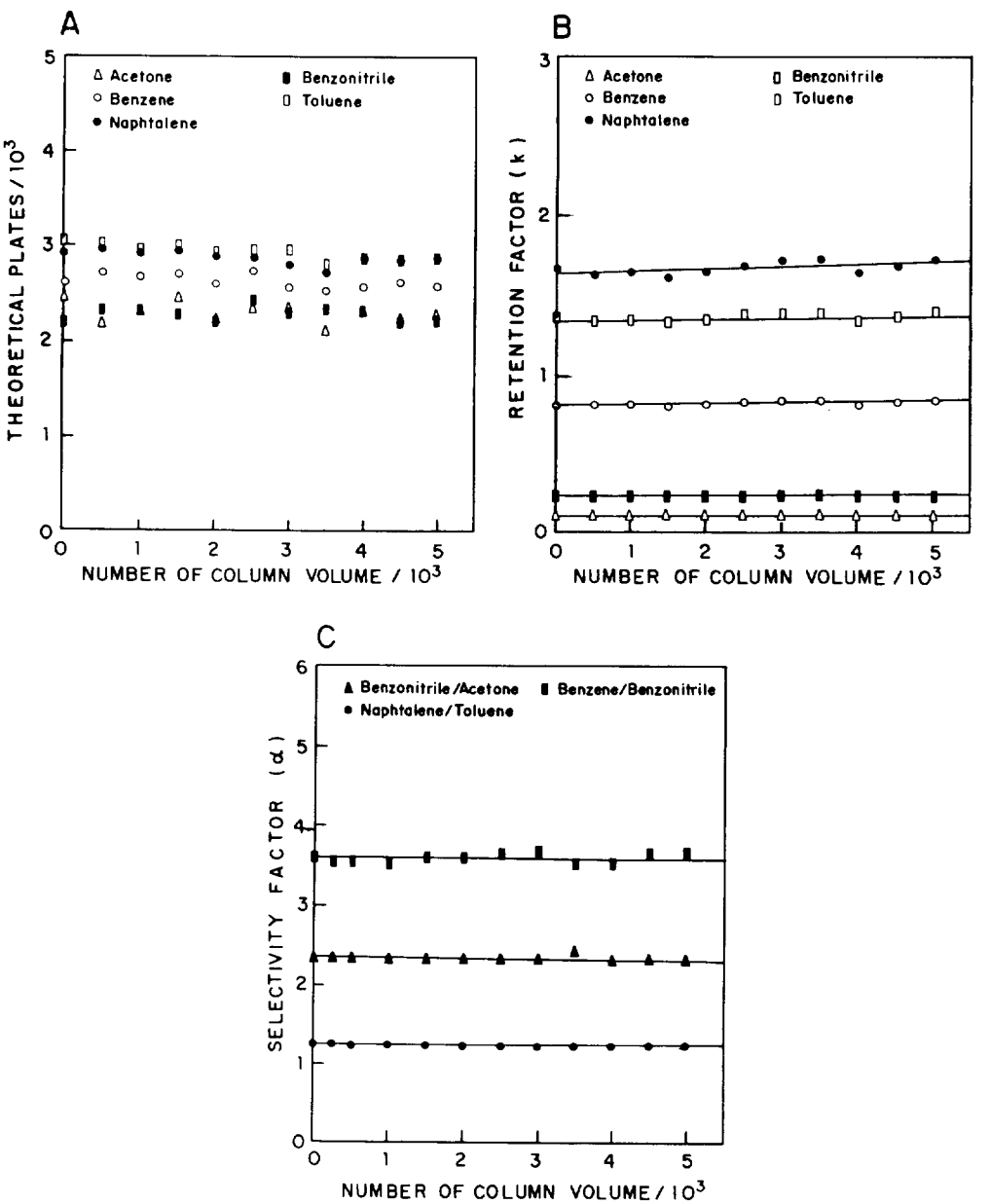

Fig. 3. (A) Column efficiency (theoretical plates, N); (B) retention factor (k) and (C) selectivity factor (α) as functions of volume of mobile phase passing a column packed with 40% (w/w) PMOS-loaded silica support.

values were determined from the chromatograms. The high flow-rate (2 ml min^{-1}) was used for the column washing so that the total time for the 5000 column volumes would not be excessive and to have somewhat stronger "wash-out" conditions. The lower flow-rates (0.1 and 0.2 ml min^{-1}) were near optimal for separations made with the two columns.

3. Results and discussion

Fig. 1 shows measurements of N, k and α for a column packed with 20% (w/w) PDMS-loaded packing material during column washing with a methanol–water (50:50) mobile phase. The column efficiency values fluctuate, reflecting the fact that the column temperature (and perhaps other experimental parameters) were not constant during the different chromatographic tests. Nevertheless, no general trend is apparent as evidence of column deterioration. The retention and selectivity factors, being relative parameters, show much less fluctuation and serve as indicators that no significant changes in column structure or function have taken place. The chromatogram obtained at the end of the washing test for this column is shown in Fig. 2.

Fig. 3 shows the corresponding chromatographic results for a column packed with 40% PMOS-loaded packing material. With no apparent changes in retention or selectivity factors resulting from the extended washing treatment, there is no indication of significant changes in this column. Chromatograms taken at the beginning and the end of the washing test (Fig. 4) likewise show that no significant changes have occurred.

The efficiencies obtained for the columns packed with PDMS and PMOS were ca. 26 000 and ca. 23 000 plates/m, respectively, for the naphthalene solute. Thus, the efficiencies of the two packings are similar. A comparison of the separations obtained with the five test solutes on the two packings can be made from ratios of corresponding retention factors (Figs. 1B and 3B). These k_{PDMS}/k_{PMOS} ratios are 0.91, 0.69, 0.55, 0.52 and 0.55 for acetone, benzonitrile, benzene, toluene and naphthalene, respectively.

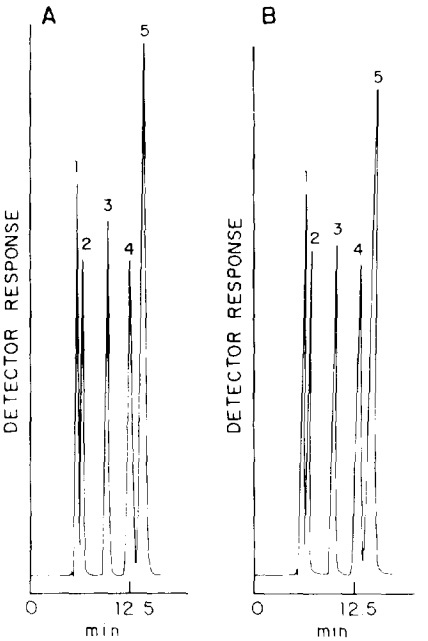

Fig. 4. Chromatograms obtained with a column packed with 40% (w/w) PMOS-loaded silica support at the beginning (A) and the end (B) of the washing test. Peaks as in Fig. 2. Column: 125 × 3.4 mm, mobile phase: methanol–water (70:30, v/v), flow-rate: 0.2 ml min^{-1}, detection: UV at 254 nm.

Thus, the more polar solutes (acetone and benzonitrile) behave differently than the less polar solutes on the two packings, with the residence time of the more polar solutes longer in the PDMS stationary phase than in the PMOS one. We can only speculate on the mechanistic basis for these chromatographic differences but, presumably, geometric differences in the "packing" of the long-chain polysiloxane molecules in the pores, as well as the viscosity differences, are controlling factors.

4. Conclusions

Moderately high viscosity (ca. 10^3–10^5 cSt) polysiloxane liquids, and presumably many other large-molecule liquids, can be used as stationary phases for reversed-phase HPLC. The polysiloxane phases are remarkably stable to wash-off by typical mobile phases such as methanol–water solutions. The efficiencies of columns prepared

with polysiloxane-loaded packing materials are similar to those obtained with conventional bonded-phase packing materials of the same pore diameter.

Thus, LLC with large-molecule stationary phases should be considered for chromatographic applications in which exotic functional group properties, long-term reproducibility or packing material economy are especially important.

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