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Polytrifluorostyrene-coated silica as a packing for column liquid chromatography

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ABSTRACT

A reversed-phase hydrolytically stable packing material prepared by chemisorption of a trifluorostyrene-containing copolymer on to the surface of porous silica was tested by high-performance liquid chromatography of various aromatic compounds. It was found that the polytrifluorostyrene (PTFS) phase has an intermediate adsorption activity between those of polytetrafluoroethylene and polystyrene phases. A high selectivity of PTFS-coated silica in the separation of haloaryl compounds was observed. Wide-pore silica coated with PTFS was found to give an efficient resolution of peptides and proteins.

INTRODUCTION

The great expansion of reversed-phase high-performance liquid chromatography (RP-HPLC) has been largely the result of the development of new stationary phases and recently some aspects of support materials and their bonded stationary phases have been reviewed in a special issue of this journal [1]. At present there are three general approaches to the synthesis of adsorbents for reversed-phase chromatography: polymerization of monomers (such as styrene and tetrafluoroethylene) and the use of polymer particles with appropriate pore size and diameter as adsorbents; surface modification of silica by low-molecular-mass substances (such as alkyl- and fluoroalkylsilanes); and modification of packing materials by coating with thin layers of polymers. Polymeric sorbents are compressible under pressure and frequently change their bead volumes on replacement of eluents, so that their application in HPLC is restricted. Silica-based stationary phases are still the most widely used sorbents in HPLC owing to their mechanical stability and availability in a wide range of pore

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sizes and particle diameters. Polymer-coated silicas are promising materials having improved pH stability over bonded silica packings [2–4]. This is assumed to be caused by multi-point attachment of the polymer to the silica surface [2].

Adsorbents for RP-HPLC based on fluorine-containing substances are attractive owing to their high chemical stability and their useful chromatographic properties, and they have been used for the separation of low-molecular-mass solvents and biopolymers [5]. Recently, it was shown that silicas coated with PTFE are very useful for the separation of biomolecules such as proteins, peptides and nucleic acids [6,7]. These sorbents, however, do not allow the attachment of some specific ligands and groups that could be useful for certain separation tasks. To overcome these disadvantages of PTFE-silica sorbents, efforts have been made to modify the silica by incorporating trifluorostyrene chains [8] using the approach of chemical binding of copolymers with active functional groups on to the silica surface [2].

This paper describes the preparation of polytrifluorostyrene-coated silica by binding a copolymer of α,β,β-trifluorostyrene and vinyl-

methyldiethoxysilane on to the silica surface, some aspects of this procedure and the chromatographic properties of the column packing obtained in comparison with PTFE- and polystyrene (PST)-coated silicas.

EXPERIMENTAL

Chemicals and materials

Controlled-pore glass (CPG-2000) with a pore diameter of 200 nm ($d_p = 0.25$–0.5 mm) (GOZ VNII NP, N. Novgorod, Russian Federation), Silasorb 300 ($d_p = 5$ μm) (Lachema, Brno, Czech Republic) and Nucleosil 1000 ($d_p = 7$ μm) (Machery–Nagel, Düren Germany) were used as packing materials.

α,β,β-Trifluorostyrene (TFS) was of laboratory grade and vinylmethyldiethoxysilane (VMDES) was of research grade and both were purified by distillation under vacuum and stored at low temperature in sealed glass tubes.

All solvents used for the syntheses were of research grade. Mobile phases were prepared from HPLC-grade methanol and acetonitrile (Merck, Darmstadt, Germany) and deionized water purified with a Milli-Q system (Millipore).

A copolymer of VMDES and TFS (TFS–VMDES) was synthesized by the radical polymerization method [8] with dicyclohexyl peroxydicarbonate as initiator. The copolymer was precipitated by hexane from a solution in benzene. The ratio of TFS to VMDES units in the copolymer was calculated using results of elemental analysis.

The molecular mass of the copolymers was determined by size-exclusion chromatography on a column of Zorbax PSM 60S (DuPont) using tetrahydrofuran (Fluka, Buchs, Switzerland) as the mobile phase.

Kinetic data for the polymer binding were obtained by incubation of 2% (w/w) copolymer solution in dry toluene with CPG or silica at 110°C. The amount of chemisorbed copolymer was determined by elemental analysis of the coated packings and UV spectrometry of non-bonded copolymer in solution. The extraction of coated packings with toluene in a Soxhlet apparatus was performed for testing for the presence of physically sorbed copolymer.

Preparation of coated silica supports

Silasorb 300 (3 g) was weighed into a three-necked flask equipped with a stirrer and a Dean–Stark separator, then 30 ml of dried toluene were added. The suspension was heated under reflux for 1 h until no further water was liberated from the silica, then a solution of 0.5 g of copolymer in 10 ml of toluene was added and the suspension was agitated and heated under reflux for 3 h. The hot suspension was filtered through a porous PTFE membrane and remaining silica was washed twice with 30 ml of hot, dried toluene. End-capping of the product was performed by heating the coated silica with 1 ml of hexamethydisilazane dissolved in 15 ml of dried toluene under reflux for 2 h. The silica was then filtered through a porous PTFE membrane, washed with hot toluene, ethanol and water and dried overnight at 100°C.

All other silicas and porous glass were coated using the procedure described for Silasorb 300. The amount of copolymer used depended on the specific surface area of the silica or glass packings.

Chromatographic experiments

The coated silicas were slurry packed into columns (250 × 4.6 mm I.D.) using a 5% (w/w) suspension of cyclohexanol–toluene (50:50, v/v). Ethanol was employed as a displacing solvent. The column packing pump (Shandon) was used in this step. The pressure used to pack the columns was 310 bar. Chromatographic experiments were performed on a system consisting of a Model 114M pump, a Model 160 UV detector with a fixed wavelength of 214 or 254 nm and a Model 421A controller (all from Beckman). The column temperature was ambient and the injection volumes were 1–5 μl.

RESULTS AND DISCUSSION

Polymer coating

The synthesized TFS–VMDES copolymer was analysed by size-exclusion chromatography and it was found that the profile of elution contained two peaks with molecular masses (M_r) of 12 700 ($M_w/M_n = 2.9$) and 400 ($M_w/M_n = 1.1$). The substance with $M_r = 400$ was the dimer of TFS.

This mixture was fractionated by precipitation of the copolymer from the solution in benzene by adding n-hexane. Four fractions of the polymer had $M_r = 11\,700$, 6000, 6000 and 4000 with M_w / M_n from 1.12 to 1.3.

The ratio of TFS to VMDES units in the polymer found from element analysis was 10:1, so the copolymer had the following structure:
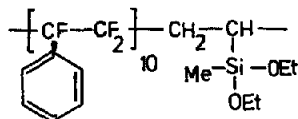
CPG-2000 was used for the investigation of the kinetics of the chemisorption of copolymers of TFS or styrene (ST) with VMDES ($M_r = 11\,700$ and 10 000, respectively) on a glass surface to avoid size-exclusion effects. Two conclusions could be drawn by comparing the data found for PST and PTFS copolymers. First, a reaction time of only 10–15 min was sufficient for completion of these procedures owing to the large amount of reacting ethoxysilyl groups in each of the macromolecules. Second, the rates of chemisorption were very similar for ST- and TFS-containing copolymers, so the type of polymer backbone had no influence on the reaction ability of the polymer.

No polymer was liberated from the coated samples after extraction with hot toluene, which is evidence that all the copolymer was chemisorbed rather then physisorbed on the glass surface.

The ligand density values were calculated from the data on carbon content and the specific surface area of the native silica. For Silasorb 300–PTFS it was found to be ca. 7.5 μmol/m^2 (carbon content 9%, w/w; specific surface area 240 m^2/g [2]).

Chromatographic properties

The completeness of coverage of the silica by the copolymer can be checked by monitoring the retention and peak width of basic compounds under the reversed-phase chromatographic conditions at neutral pH. The separation profiles for pyridine, 4,4'-diaminodibenzyl and 3,4-dimethylpyridine on the column with Silasorb 300
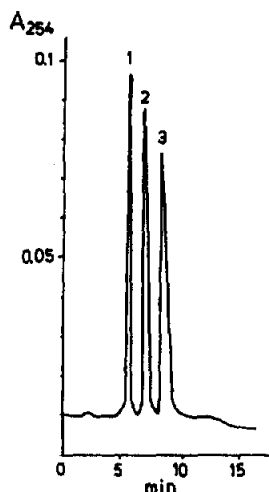

Fig. 1. Separation of a mixture of basic compounds on a column of Silasorb 300–PTFS (250 × 4.6 mm I.D.). Eluent, water–acetonitrile (50:50, v/v); flow-rate, 1 ml/min. Peaks: 1 = pyridine; 2 = 4,4'-diaminodibenzyl; 3 = 3,4-dimethylpyridine.
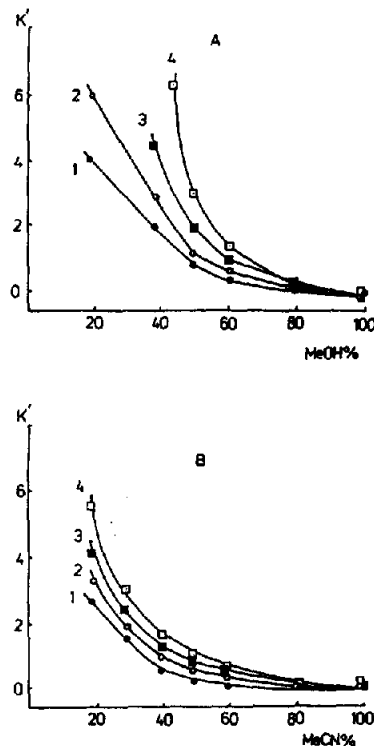

Fig. 2. Dependence of capacity factors (k') of halobenzenes on the contents of (A) methanol and (B) acetonitrile in the eluent using a column of Silasorb 300–PTFS. 1 = Benzene; 2 = fluorobenzene; 3 = bromobenzene; 4 = iodobenzene.

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coated with copolymer (Fig. 1) showed symmetrical peaks, indicating the absence of a significant effect of the residual silanols on retention.

Further characterization of the chromatographic properties of PTFS-coated silica was carried out with a variety of functionalized benzenes. The dependences of the retention of haloaryl compounds on the type and composition of the mobile phase are shown in Fig. 2. The rapid decrease of k' with increase in acetonitrile content in the mobile phase can be attributed to the greater enrichment of the stationary phase by acetonitrile compared with methanol. The same situation was pointed out by Siergiej and Danielson [9] for the chromatographic properties of polychlorotrifluoroethylene powder (KEL-F) modified with phenyllithium. The shapes of the dependences of k' on organic content in the mobile phase were the same as for the usual n-alkyl-bonded reversed phases, hence the re-

tention mechanism on polytrifluorostyrene-coated silica was the same.

Capacity factors (k') and hydrophobic selectivies $(\alpha^* = k'_{\text{substituted benzene}}/k'_{\text{benzene}})$ for functionalized benzenes are given in Table I. Previously, it has been reported [10,11] that fluorocarbon-bonded phases exhibited specific selectivity toward halogen-containing compounds. Confirmation of this effect could be made by comparing the retentions of the compounds in Table I. The retention times of halogen-containing benzenes are higher than those for their analogues without halogen groups.

The k' and α^* values for Silasorb 300–PTFS were compared with those for the PTFE- and PST-coated silicas. These sorbents were synthesized as described [2,4,6] using Silasorb 300 as the support. The results indicated that the retentions of all the compounds tested increase in the order PTFE < PTFS < PST phases. This is evidence that the polymer backbone and the

TABLE I

CAPACITY FACTORS (k') AND HYDROPHOBIC SELECTIVIES (α^*) FOR VARIOUS FUNCTIONALIZED BENZENES

Mobile phase: methanol–water (45:55, v/v) (pH 4.5).

Functional group	PTFE		PTFS		PST	
	k'	α^*	k'	α^*	k'	α^*
H	2.17	1.0	2.5	1.0	5.6	1.0
NO$_2$	2.32	1.07	2.5	1.0	5.5	0.98
F	2.7	1.3	2.9	1.16	6.1	1.09
Cl	3.13	1.44	3.17	1.3	10.07	1.9
Br	3.1	1.43	5.1	2.0	14.0	2.5
I	3.16	1.46	6.7	2.68	19.0	3.4
CH$_3$	2.53	1.17	3.8	1.52	8.3	1.48
o-F; CH$_3$	3.27	1.51	5.13	2.12	12.8	2.29
CH$_2$CH$_3$	3.37	1.56	5.0	2.0	>9.0	>1.61
OH	1.54	0.7	1.2	0.48	2.0	0.36
p-Cl; OH	1.56	0.72	1.6	0.64	4.1	0.73
CH$_2$NH$_2$	1.97	0.92	2.0	0.8	3.0	0.54
COH	2.09	0.96	2.13	0.8	3.6	0.64
o-Cl; COH	2.05	0.94	2.15	1.16	4.0	0.71
COOH	1.5	0.7	0.34	0.14	2.6	0.46
p-F; COOH	1.6	0.71	0.57	0.23	4.6	0.82
o-F; COOH	1.97	0.92	0.13	0.05	5.13	0.90
o-Br; COOH	1.00	0.5	0.43	0.17	2.2	0.39

phenyl ligand contributed to the adsorption activity of the sorbents and to the retention of the adsorbates.

The intermediate adsorption activity of the PTFS phase helped to provide a good resolution for the four halobenzenes, as shown in Fig. 3. With PTFE-coated silica the differences in retention times were too small to separate these molecules. However, the PST phase turned out to be much more active under the same conditions and provided acceptable retention times.

A Nucleosil 1000-7–PTFS HPLC column was tested for the reversed-phase chromatography of peptides and proteins. The dependences of k' on the content of acetonitrile in 0.1% TFA for several peptides and proteins are shown in Fig. 4. There was a sharp decrease in the retention of all peptides tested with increase in acetonitrile concentration in the eluent. The retention curves on PTFS-coated silica have essentially the same shape as on the usual C_8–C_{18} reversed-phase packings. This column was able to separate both relatively small peptides and proteins. Some enhancement of the retention of proteins due to silanophilic interactions could be observed on
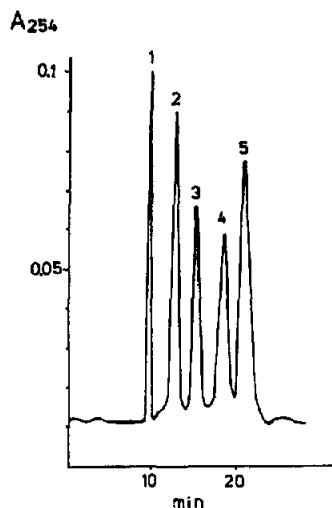

Fig. 4. Dependence of capacity factors (k') of proteins and peptides on the content of acetonitrile in the eluent using a column of Nucleosil 1000–PTFS (250 × 4.6 mm I.D.). Eluent, acetonitrile–water containing 0.1% TFA. 1 = Met-Cys-bradikinin; 2 = cytochrome c; 3 = bovine serum albumin.

the PTFS-coated packing at concentrations of acetonitrile higher than 70%.

The PTFS-coated sorbents easily separated peptide and protein mixtures under gradient elution conditions. The separation of bee venom components (natural sample) using a gradient of acetonitrile in 0.1% TFA is shown in Fig. 5.

Fig. 3. Separation of a mixture of halobenzenes on a column of Silasorb 300–PTFS (250 × 4.6 mm I.D.). Eluent, acetonitrile–water (50:50, v/v); flow-rate, 1 ml/min. Peaks: 1 = benzene; 2 = fluorobenzene; 3 = chlorobenzene; 4 = bromobenzene; 5 = iodobenzene.
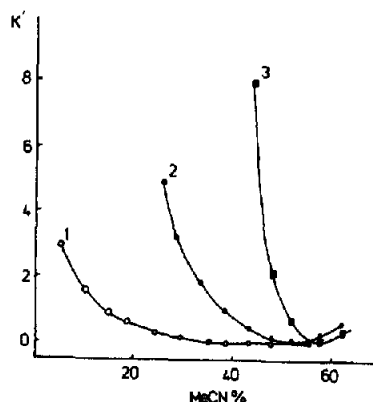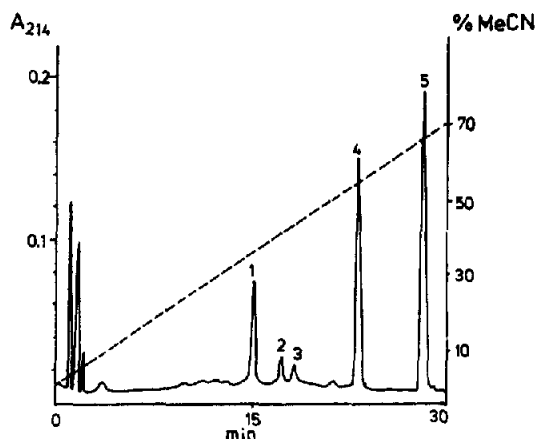

Fig. 5. Separtion of bee venom components on a column of Silasorb 300–PTFS (250 × 4.6 mm I.D.). Eluent, gradient from 5 to 70% acetonitrile in 0.1% TFA in 30 min; flow-rate, 1 ml/min. Peaks: 1 = apamin; 2 = MCD peptide; 3 = secapin; 4 = phospholipase A^2; 5 = melittin.

The higher hydrolytic stability of silicas with a covalently bonded PTFS phase was typical for materials obtained by such a procedure [2,6]. An HPLC column containing Silasorb 300–PTFS was used for 1 year at pH 2–8.5 without considerable changes in peak resolution. The unusual adsorption properties and the possibility of introducing several functional groups make PTFS-coated silicas attractive for future studies.

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