

CHROMSYM. 1807

## Effect of polystyrene coating on pore, structural and chromatographic properties of silica packings

A. KURGANOV, O. KUZMENKO and V. A. DAVANKOV

*Institute of Organo-Element Compounds, Academy of Sciences of the U.S.S.R., Moscow (U.S.S.R.)*  
and

B. ERAY, K. K. UNGER\* and U. TRÜDINGER

*Institut für Anorganische Chemie und Analytische Chemie, Johannes Gutenberg-Universität, D-6500 Mainz (F.R.G.)*

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### SUMMARY

A series of mesoporous and macroporous silicas with a mean pore diameter (PD), between 6 and 200 nm were reacted with a copolymer of styrene and vinyl-methyldiethoxysilane. The mass load of coated silicas corresponded to the monolayer capacity of the polymer calculated on the basis of the molecular cross-sectional area, except for the two mesoporous silicas of PD = 6 and 7.5 nm. Depending on the pore size, changes occurred in the specific surface area,  $a_s$ , between the native and the coated product. A comparison of the specific pore volume,  $v_p$ , of silicas before and after polymer immobilization indicated a major loss of  $v_p$  for the two mesoporous silicas whereas this effect was much less pronounced for the macroporous silicas. Surprisingly, the two macroporous silicas of PD = 50 and 100 nm exhibited a decrease in the mean pore diameter after modification, which seems to be caused by the narrowing of the pore entrances owing to polymer immobilization. The change in  $d_p$  was shown by size-exclusion separations of polystyrenes on the native and coated silica. Tests of the packings in reversed-phase chromatography with amines as solutes showed that silanophilic effects on the coated silicas were absent. It was demonstrated that the immobilization did not change the performance of the silica columns in terms of plate height and plate number.

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### INTRODUCTION

To overcome the intrinsic limited pH stability of bonded silica packings in high-performance liquid chromatography (HPLC), attempts have been made to coat the surface of silicas with polymers. The aim is to form a thin and homogeneous layer of polymer with desired functionalities that provides maximum coverage of the surface of the native silica support, maintains the column performance and permits the retention to be controlled and the selectivity to be adjusted. The various routes in the

synthesis of HPLC stationary phases with polymeric coatings have recently been reviewed by Schomburg<sup>1</sup>.

Polystyrene-coated silicas for reversed-phase HPLC were first introduced by Horváth and Lipsky<sup>2</sup> using a pellicular type of support. Caude and Rosset<sup>3</sup> prepared a polystyrene-coated silica in a two-step procedure. First, the silica was reacted with vinyltriethoxysilane and the vinyl-bonded silica was then subjected to polymerization with styrene. The unbound polystyrene was removed by extraction and the product was converted into an ion exchanger by an appropriate reaction. Quivoron<sup>4</sup> prepared coated silica supports using living polystyrene made by cationic polymerization. A drastic decrease in the specific surface area of the silica support compared with the parent material was observed when increasing amounts of polystyrene were employed. More recently, Suzuki *et al.*<sup>5</sup> polymerized styrene on the silica surface by adsorbing chloromethylstyrene and divinylbenzene on the silica from a solution in N,N-dimethylformamide. The excess of monomers was removed and the dry product was subjected to polymerization in an autoclave. The polystyrene-coated silica showed a 20% decrease in specific surface area,  $a_s$ , compared with the starting material. This decrease in  $a_s$  is the same as observed on silanized silicas with long-chain *n*-alkylsilanes<sup>6</sup>. Kurganov *et al.*<sup>7</sup> developed a procedure for coating silicas with a copolymer of styrene and vinylsilane, which was employed as a precursor to synthesize a chiral packing for the resolution of racemates by ligand-exchange chromatography. The packing exhibited improved hydrolytic stability compared with other ligand-exchange types of stationary phases, and this is assumed to be caused by a multi-point bonding of the copolymer to the silica surface.

The procedure advocated by Kurganov *et al.*<sup>7</sup> was used here to prepare reversed-phase and size-exclusion packings for HPLC. The aim was to assess the pore structure and chromatographic properties of the product relative to the parent material. Silicas of different origin and pore sizes were employed.

## EXPERIMENTAL

### *Chemicals and materials*

Zorbax PSM 60, PSM 500 and PSM 1000, all of particle diameter  $d_p$  5  $\mu\text{m}$ , were a gift from DuPont (Wilmington, DE, U.S.A.). Silasorb Si 300 and Si 600,  $d_p$  5  $\mu\text{m}$ , were obtained from Lachema (Prague, Czechoslovakia).

LiChrosorb Si 100,  $d_p$  5  $\mu\text{m}$ , and LiChrospher Si 500,  $d_p$  10  $\mu\text{m}$ , were purchased from Merck (Darmstadt, F.R.G.). A spherical silica of  $d_p$  10  $\mu\text{m}$  and a nominal pore diameter of PD = 200 nm was a gift from Merck.

Polymer standards of molecular weight 1400 (1.06), 4900 (1.04), 10 000 (1.05), 16 600 (1.02), 29 500 (1.03), 110 000 (1.20), 220 000 (1.05), 310 000 (1.04), 710 000 (1.05) and 1 250 000 (1.14) were supplied by Polymer Standards Service (Mainz, F.R.G.); the values in parantheses are the polydispersity,  $u = M_w/M_n$ , where  $M_w$  is the weight-average and  $M_n$  the number-average molecular weight.

All solvents for the syntheses were of analytical-reagent grade. HPLC-grade solvents for chromatographic measurements were obtained from Merck.

### *Preparation of coated silica supports*

A copolymer with a molecular weight of 10 000 with a silicon content of 2.0%

(w/w) was synthesized by polymerization of styrene and vinylmethyldiethoxysilane as described elsewhere<sup>7</sup>. The ratio of styrene to silane units in the copolymer was calculated to be 12:1.

All silicas were coated by the following procedure described for Zorbax PSM 60. The amount of copolymer used was adjusted to the specific surface area of native silica.

Zorbax PSM 60 (45.3 g) was weighed into a three-necked flask equipped with a stirrer and Dean-Stark separator, then 200 ml of toluene were added. The suspension was heated under reflux for 1–2 h until no water was liberated from the silica, then a solution of 4.0 g of copolymer in 50 ml of toluene was added and the suspension was agitated and heated under reflux for 4 h. The hot suspension was filtered through a porous glass frit of porosity  $< 2 \mu\text{m}$  and the remaining silica was washed twice with 100 ml of hot toluene. End-capping of the product was performed by heating the slurry of the coated silica with 6.0 ml of hexamethyldisilazane dissolved in 150 ml of toluene under reflux for 2 h. The silica was then filtered through a porous glass frit of porosity

TABLE I

## CHARACTERISTIC PARAMETERS OF UNCOATED AND COATED SILICAS

Abbreviations:  $a_s$  = specific surface area of the native silica according to BET, using  $a = 0.162 \text{ nm}^2$  per molecule;  $a_s^*$  = specific surface area of the native silica according to BET, using the weight-corrected  $a_s$  of the native silica;  $a_s^{0.162}$  = specific surface area of the native silica according to BET, using  $a_m = 0.162 \text{ nm}^2$  per molecule;  $a_s^{0.20}$  = specific surface area of the native silica according to BET, using  $a_s = 0.20 \text{ nm}^2$  per molecule; PD = nominal pore diameter of the native silica;  $\alpha_{\text{exp}}$  = surface concentration of monomer units calculated from the carbon content.

Silica	$a_s$ ( $\text{m}^2/\text{g}$ )	$a_s^*$ ( $\text{m}^2/\text{g}$ )	$a_s^{0.162}$ ( $\text{m}^2/\text{g}$ )	$a_s^{0.20}$ ( $\text{m}^2/\text{g}$ )	PD (nm)	Carbon content (% w/w)		$\alpha_{\text{exp}}$ ( $\mu\text{mol}/\text{m}^2$ )
						Before end-capping	After end-capping	
Zorbax PSM 60								
Uncoated	384	—	—	—	6	—	—	—
Coated	—	334	245	301	—	10.9	13.4	3.6
Zorbax PSM 500								
Uncoated	29	—	—	—	50	—	—	—
Coated	—	28	26	32	—	2.4	2.4	9.3
Zorbax PSM 1000								
Uncoated	17	—	—	—	100	—	—	—
Coated	—	16.8	17	21	—	1.3	1.4	8.4
LiChrosorb Si 100								
Uncoated	245	—	—	—	10	—	—	—
Coated	—	209	229	282	—	14.5	14.6	7.4
LiChrospher Si 500								
Uncoated	90	—	—	—	50	—	—	—
Coated	—	81	80	98	—	10.0	10.5	12.3
Silasorb Si 600								
Uncoated	520	—	—	—	7.5	—	—	—
Coated	—	434	400	492	—	14.0	16.5	3.5
Silasorb Si 300								
Uncoated	240	—	—	—	10	—	—	—
Coated	—	184	220	271	—	15.0	16.1	8.2
Silica, PD = 200 nm								
Uncoated	17	—	—	—	200	—	—	—
Coated	—	17	17	21	—	2.7	2.7	15.5

$< 2 \mu\text{m}$ , washed with hot toluene, ethanol and water and dried overnight at  $100^\circ\text{C}$ . The properties of the uncoated and the coated silicas are given in Table I.

### Chromatographic experiments

The coated silicas were slurry-packed into columns of  $250 \times 4.6 \text{ mm I.D.}$  or  $125 \times 4.6 \text{ mm I.D.}$  (Bischoff Analysentechnik, Leonberg, F.R.G.) using a 5% (w/w) suspension of cyclohexanol-toluene (50:50, v/v). Ethanol was employed as a displacing solvent. The native silica was slurry-packed with ethanol from a 5% (w/w) suspension.

Chromatographic experiments were performed on a system consisting of a pump (Model 2200; Bischoff), UV detector with a fixed wavelength of 254 nm (Model UV III, Latek, Heidelberg, F.R.G.) or a variable-wavelength UV detector (Model BT 3030, Biotronic, Maintal, F.R.G.) and a potentiometric recorder (Model RE 541, Metrawatt, F.R.G.; or Model 2210, Pharmacia-LKB Biotechnology, Uppsala, Sweden). The column temperature was ambient. The injection volume was  $10 \mu\text{l}$ .

### Nitrogen sorption measurements

Nitrogen adsorption-desorption isotherms at 77 K were determined gravimetrically, using a laboratory-made device, equipped with a Sartorius vacuum microbalance (Model 4433, Sartorius-Werke, Göttingen, F.R.G.). Nitrogen was of 99.99% purity (Linde, Düsseldorf, F.R.G.). The specific surface area was calculated from nitrogen adsorption using the two-parameter BET equation<sup>8</sup>. The pore-size distribution was calculated from the desorption isotherm using the Kelvin equation and the method of Pierce<sup>8</sup>.

### Mercury porosimetry

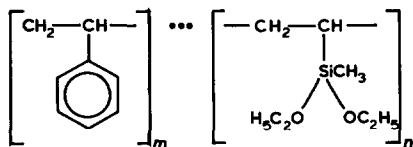
Mercury porosimetry was performed using a Carlo Erba (Milan, Italy) porosimeter with a maximum pressure of about 2500 bar. The pore-size distribution was calculated from the experimental data using the Washburn equation<sup>9</sup>.

All mathematical calculations of the experimental data and their presentation in graphical form were performed by using the program EQPLOT developed in this laboratory on an IBM-compatible computer.

## RESULTS AND DISCUSSION

### Polymer coating as a function of the mean pore diameter of the silica

The starting copolymer of  $M_w = 10\,000$  has the following structure:



In the immobilization procedure, the ethoxy groups of the copolymer react with the hydroxyl groups of the silica and ethanol is evolved. In the optimum case the copolymer unit is linked by two siloxane groups to the surface. From the mean

molecular weight of the polymer using the equation<sup>10</sup>  $r = 0.0123 M^{0.588}$ , the radius of a random coil polymer chain is calculated to be 1.4 nm. Using this value, a surface concentration equivalent to a monolayer of  $x_m = 0.07 \mu\text{mol}/\text{m}^2$  is derived.

The mass load derived from the calculated surface concentration can now be compared with the actual mass load based on the carbon content. The carbon content represents 88% of the total mass of the polymer. For the coated macroporous Zorbax PSM 1000, the mass load equivalent to the surface concentration of a monolayer is calculated to be  $(0.07 \cdot 10^{-6} \text{ mol}/\text{m}^2) \cdot (10\,000 \text{ g}/\text{mol}) \cdot (17 \text{ m}^2/\text{g}) = 11.9 \text{ mg}/\text{g}$ . The experimental value is  $(10.9 \text{ mg}/\text{g}) \cdot 100/88 = 12.3 \text{ mg}/\text{g}$ . Hence the calculated and experimental values are in fairly good agreement assuming a dense monolayer. The same is valid for the coated Zorbax PSM 500 (calculated value 20.3 mg/g, found 27.0 mg/g).

The surface concentration of the monolayer can also be related to the bound monomer unit of copolymer and then becomes  $7 \mu\text{mol}/\text{m}^2$ . The experimental values for Zorbax PSM 1000 and 500 are of the same order, namely 8.4 and 9.3  $\mu\text{mol}/\text{m}^2$ , respectively. The values calculated for LiChrospher Si 500 and the spherical silica of PD = 200 nm are higher than  $7 \mu\text{mol}/\text{m}^2$  (12.3 and 15.5  $\mu\text{mol}/\text{m}^2$ , respectively).

When the mean pore diameter of the starting silica falls into the mesopore range ( $2 < \text{PD} < 50 \text{ nm}$ ), the polymer will have limited access to the pores in the immobilization procedure and therefore the experimental mass load should be lower than the calculated value based on a freely accessible surface. This is clearly seen with Zorbax PSM 60 and Silasorb Si 600 with mean pore diameters of 6 and 7.5 nm, respectively, where the values are calculated 238 and experimental 109 mg/g for Zorbax PSM 60 and calculated 364 and experimental 140 mg/g for Silasorb Si 600. In accord with this observation, the surface concentration per copolymer unit is lowest for these two types of all the silicas studied: Zorbax PSM 60 3.6  $\mu\text{mol}/\text{m}^2$  and Silasorb Si 600 3.5  $\mu\text{mol}/\text{m}^2$ .

Further evidence that the mesopores of 6–7 nm do not remain fully accessible to the polymer is that the end-capping of the coated Zorbax PSM 60 and Silasorb Si 600 increases the carbon content by about 2–3% (w/w), whereas end-capping of the other coated silicas scarcely changes the carbon content. It is conceivable that the small hexamethyldisilazane is capable of reacting with the hydroxyl groups that were left unreacted in the polymer immobilization procedure.

#### *Effect of coating on the specific surface area ( $a_s$ )*

Changes in  $a_s$  between the native and the coated silicas can be detected by comparing the corresponding  $a_s$  values before and after coating. There are two ways to determine the specific surface area of the coated silicas. First, the actual value of  $a_s$  is measured by nitrogen adsorption applying the BET method<sup>8</sup>. The monolayer capacity of adsorbed nitrogen in mol/g is then obtained, and is multiplied by Avogadro's number and the molecular cross-sectional area of adsorbed nitrogen,  $a_m$ . Usually  $a_m$  is taken to be 0.162 nm<sup>2</sup> per molecule. Amat and Kováts<sup>11</sup> have pointed out, however, that silanized surfaces behave as low-energy surface systems with a lower packing density of adsorbed nitrogen. Hence the molecular cross-sectional area of nitrogen becomes 0.20 nm<sup>2</sup> per molecule compared with 0.162 nm<sup>2</sup> per molecule for hydroxylated surfaces. Treating the polymer-coated silicas as low-energy systems, their  $a_s$  values have to be multiplied by a factor of  $0.20/0.162 = 1.23$ . Second, neglecting the changes in the chemical composition of the surface, the  $a_s$  of a given

coated silica can be evaluated from value for the native silica by applying a correction for the weight increase,  $\Delta G$ , caused by the coating<sup>6</sup>. The corrected specific surface area,  $a_s^*$ , of the native silica is then

$$a_s^* = a_s (1 - \Delta G)$$

The different values of  $a_s$ , viz.,  $a_s$  of the native silica, using  $a_m = 0.162 \text{ nm}^2$ ,  $a_s^*$  of the coated silica, using the weight-corrected  $a_s$  of the native silica,  $a_s^{0.162}$  of the coated silica, using  $a_m = 0.162 \text{ nm}^2$  and  $a_s^{0.20}$  of the coated silica, using  $a_m = 0.20 \text{ nm}^2$ , are given in Table I. Several observations can be made. A decrease in the specific surface area of the coated silica takes place compared with that of the native material for Zorbax PSM 60 and Silasorb Si 600, taking  $a_s^*$ ,  $a_s^{0.162}$  and  $a_s^{0.20}$  into consideration, and in these instances pore blockage seems highly probable. The simple weight correction of the native silica provides no conclusive means of judging the effect of coating on  $a_s$ : the  $a_s$  values are lower than, equal to or higher than the  $a_s$  values of the coated silicas either using  $a_m = 0.162 \text{ nm}^2$  or  $a_m = 0.20 \text{ nm}^2$ ; the  $a_s$  values with  $a_m = 0.20 \text{ nm}^2$  of the coated silicas are higher than those of the native silica, using  $a_m = 0.162 \text{ nm}^2$  for PSM 500, PSM 1000, LiChrosorb Si 100, LiChrospher Si 500, Silasorb Si 300 and silica, PD = 200 nm. This increase is difficult to explain, however; it might be that the polymer coating itself develops some porosity with micropores which additionally contribute to the internal surface area.

#### *Effect of coating on the specific pore volume (SPV) and the pore-size distribution (PSD)*

In order to assess the effect of polymer coating on the SPV and PSD of the silicas, the corresponding values have to be compared before and after the polymer immobilization. Significant changes are expected for the mesoporous silicas whereas the macroporous silicas should maintain their native pore structure. For the

TABLE II

#### SPECIFIC PORE VOLUME (SPV) OF NATIVE AND POLYSTYRENE-COATED SILICAS

SPV(G) = specific pore volume according to the Gurvitch rule estimated from the saturation uptake of adsorbed nitrogen<sup>8</sup>. SPV(Hg) = specific pore volume from mercury porosimetry measurements estimated from the volume of intruded mercury at maximum pressure<sup>8</sup>.

Silica	SPV (ml/g)
Zorbax PSM 60	
Uncoated	0.70 (G)
Coated	0.41 (G)
Zorbax PSM 500	
Uncoated	0.31 (Hg)
Coated	0.26 (Hg)
Zorbax PSM 1000	
Uncoated	0.24 (Hg)
Coated	0.23 (Hg)
Silica, PD = 200 nm	
Uncoated	0.36 (Hg)
Coated	0.36 (Hg)

mesoporous Zorbax PSM 60 the SPV was calculated from the saturation uptake of adsorbed nitrogen,  $n_s$ , according to the Gurvitch rule assuming a liquid adsorbate<sup>8</sup>. The SPV of the macroporous silicas was calculated from mercury porosimetry measurements as the corrected volume of intruded mercury at maximum pressure. The results are given in Table II. As expected, the SPV of Zorbax PSM 60 as an example is reduced by about 40% after immobilization. The SPV of Zorbax PSM 500 and PSM 1000 is diminished, whereas that of the silica, PD = 200 nm, remains unaffected.

The average thickness of the polymer coating calculated from the density of the polymer, the mass load and the specific surface area of the silica is about 2 nm. Hence it is conceivable that the mean pore diameter of the Zorbax PSM 60 is drastically reduced by the coating and correspondingly the SPV changes.

The PSDs of the macroporous silicas were determined by two independent methods: (i) mercury porosimetry using the Washburn equation<sup>9</sup> and (ii) size-exclusion chromatography measuring the elution volumes of standard polystyrenes and using the calculation procedure advocated by Knox and co-workers<sup>10,12</sup>.

Fig. 1 shows as an example the cumulative pore size distribution curve assessed by mercury porosimetry (MP) and by size-exclusion chromatography (SEC) of uncoated and coated Zorbax PSM 1000. The coating slightly diminishes the PSD derived from MP measurements. The pore-size reduction is seen to be more dramatic on comparing the curves from SEC measurements: the mean pore diameter at 50% of the cumulative pore volume is about 80 nm before and to 40 nm after the immobilization of the polymer. The discrepancy in the PSD between the two methods is not surprising because they are based on different assumptions and the samples are measured under widely different conditions. In MP, mercury is forced by pressure into the pores of the dry powder. In SEC, the particles are filled with benzene as solvent and the polymer coating is solvated and might swell. This extension in volume might lead to pore blockage and limit the access of polystyrene molecules in SEC experiments.

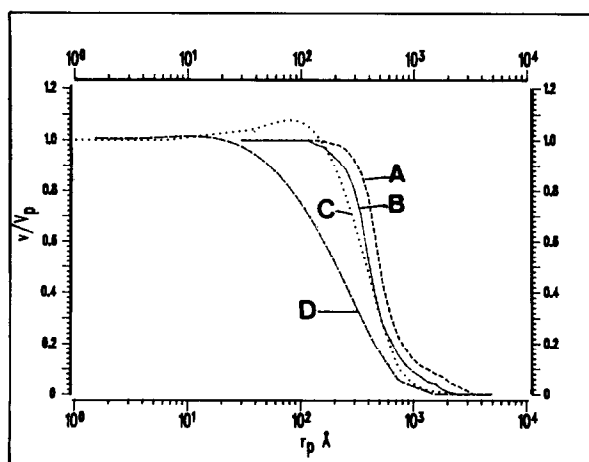


Fig. 1. Cumulative pore-size distribution of the uncoated and coated Zorbax PSM 1000. Mercury porosimetry data using a contact angle of mercury of  $140^\circ$  and a surface tension of  $480 \text{ mN/m}^2$ : A = before; B = after polymer immobilization. Data from SEC using the method of Knox and co-workers; C = before; D = after polymer immobilization.

Provided that the PSD curves from SEC measurements reflect the real chromatographic situation, the fractionation range of the coated Zorbax PSM 1000 should be shifted to the low-molecular-weight range compared with the native packing. This was indeed observed (Fig. 2). The SEC results for the polystyrene standard mixture on uncoated and coated Zorbax PSM 1000 indicate that a higher resolution in the low-molecular-weight range takes place for the coated packing. A similar effect resulted for uncoated and coated Zorbax PSM 500.

#### *Effect of coating on chromatographic properties*

The performance of the polymer-coated silicas was tested by measuring the plate height of retained and unretained solutes as a function of the linear flow-rate of the eluent in the reversed-phase (RP) mode. By plotting the data as the reduced plate height  $h$  against the reduced linear velocity  $v$ , the constants  $A$ ,  $B$  and  $C$  of the Knox equation were calculated by curve fitting using the equation<sup>13</sup>

$$h = Av^{1/3} + B/v + Cv$$

The values derived for the dimensionless constants were  $A \approx 1$ ,  $B \approx 2$  and  $C \leq 0.1$ . This indicates that the columns were well packed ( $A \approx 1$ ) and the mass transfer

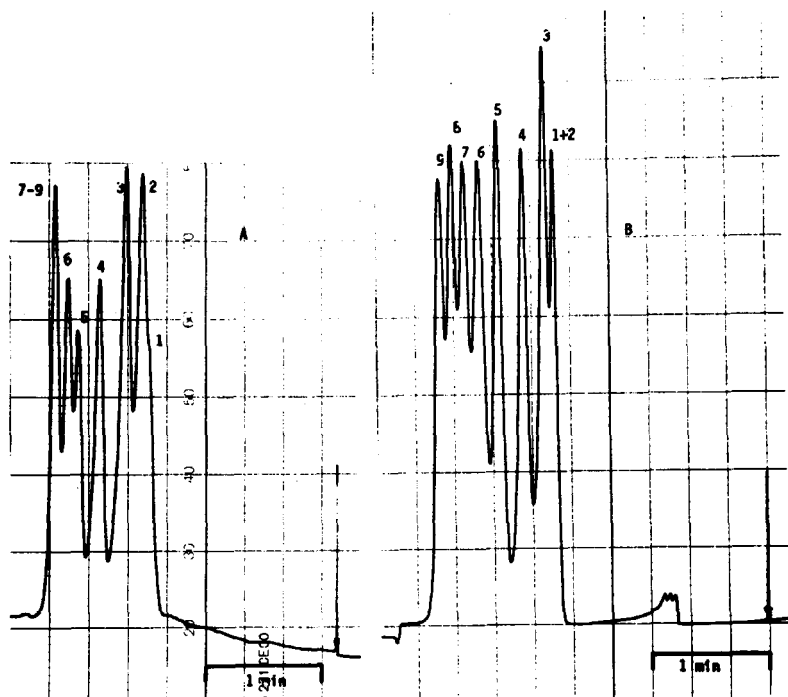


Fig. 2. Separation of a mixture of polystyrene (PS) standards on a column packed with (A) uncoated Zorbax PSM 1000 and (B) coated Zorbax PSM 1000. Column, 250 × 4.6 mm I.D.; mobile phase, tetrahydrofuran; flow-rate, 0.5 ml/min; UV detection (280 nm). Solutes: 1 = PS,  $M_w$  1250 kDa; 2 = PS, 700 kDa; 3 = PS, 310 kDa; 4 = PS, 110 kDa; 5 = PS, 29.9 kDa; 6 = PS, 10 kDa; 7 = PS, 4050 kDa; 8 = PS, 1400 kDa; 9 = benzene.



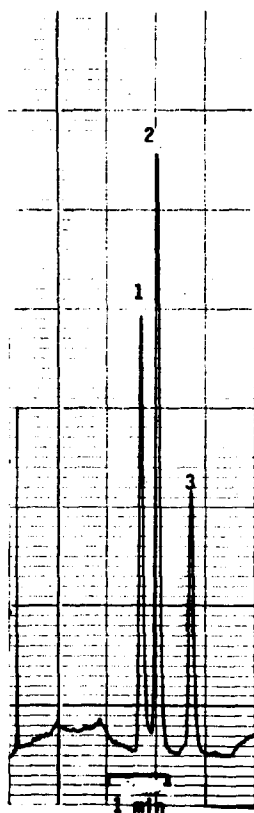


Fig. 3. Separation of a mixture of amines on a coated Zorbax PSM 500 column. Column, 250 × 4 mm I.D.; mobile phase, water-acetonitrile (40:60, v/v); flow-rate, 0.5 ml/min; UV detection (280 nm). Solutes: 1 = 2,6-dimethylpyridine; 2 = 1-naphthylamine; 3 = benzylamine.

between the mobile and the stationary phases is of the same order as in conventional RP columns ( $C \leq 0.1$ ), *i.e.*, the polymer coating did not impair the kinetic performance of the silicas in terms of plate height and plate number<sup>13</sup>.

The completeness of coverage of the silica by a polymer can be checked by monitoring the retention and peak width of basic compounds under reversed-phase conditions. The chromatograms for 2,6-dimethylpyridine, 1-naphthylamine and benzylamine on the column packed with coated Zorbax PSM 500 (Fig. 3) show totally symmetrical peaks, indicating no effect of residual silanols on retention. The excellent pH stability of the coated packings under acidic conditions was demonstrated by separating peptides and proteins during several months of continuous operation of the same column under gradient elution conditions at pH 1–2.

The highly dense coverage of the surface of silica together with the multi-point attachment of the polymer also promises a high temperature stability. This favourable property can be utilized for the rapid separation of peptides at elevated temperatures. Such studies are in progress.

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