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STATIONARY PHASES FOR REVERSED-PHASE LIQUID CHROMATO-GRAPHY

COATING OF SILICA BY POLYMERS OF VARIOUS POLARITIES*

H. FIGGE, A. DEEGE, J. KÖHLER and G. SCHOMBURG*

Max-Planck Institut für Kohlenforschung, 4330 Mülheim-Ruhr (F.R.G.)

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SUMMARY

     The possibilities of syntheses and the chromatographic properties of reversed-phase materials achieved by polymer coating have been investigated. Special alkyl-polysiloxanes, which were to be immobilized on different types of silica, have been synthesized by equilibration of different mixtures of methylhydropolysiloxanes with octamethyltetrasiloxane and subsequent hydrosilylation of 1-alkenes of different chain length. The polymers obtained can easily be immobilized on silica by the usual methods, such as thermal treatment with dicumyl peroxide, γ-radiation, etc. The immobilization is even more effective if the polymer contains a low concentration of Si–H bonds. The chromatographic behaviour of such phases in regard to efficiency, selectivity, adsorptivity, and stability has extensively been tested also in comparison with reversed-phase materials obtained by silanization of SiOH groups.

INTRODUCTION

     Silica, the classic packing material for liquid chromatography (LC), is an inorganic polymer. It can be converted into a hydrophobic (lipophilic) stationary phase for selective chromatographic separations via chemical bonding or adsorptive modification of its surface by organic molecules. With such coated non-polar or weakly polar materials, phase systems can be made in which polar, especially aqueous, mobile phases are to be applied particularly for the separation of polar solutes. Different intermolecular interactions of both the polar functional group and the lipophilic carbon skeleton of the solute molecule are then effective.

     Silica of adequate average particle diameter, pore size and geometry, as well as with a sufficiently high surface concentration of silanol groups (SiOH), is usually hydrophobilized by chemical bonding via reaction with the silanol groups, particularly by formation of Si–O–Si–C bonds which are reasonably stable under the conditions of reversed-phase liquid chromatography (RPLC). Coating of silica by special

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   * Dedicated to Prof. J. F. K. Huber on the occasion of his 60th birthday.

oligomers can also be achieved without making use of silanol groups for "chemical" anchoring. Either cross-linking and/or chemical bonding of special oligomers on the unpretreated or premodified silica surface, are suitable methods for the necessary immobilization. The amount of the coating polymer to be deposited on the surfaces can be deliberately varied with the aim of synthesizing stationary phases of different phase ratios. With the silanization reaction a similar effect can be attained only when silanization reagents with different alkyl chain lengths are used.

Like any reversed phase, immobilized (*i.e.* "polymer coated") stationary reversed phases must be sufficiently stable against dissolution of the deposited material in the various mobile phase solvents and shear forces caused by the flowing mobile phase. They must also be resistant to chemically reactive mobile phases.

The advantages and disadvantages as well as the special possibilities of the two different approaches of silanization and of "polymer coating" used for the synthesis of reversed-phase materials of variable chemical structure regarding the surface layer, can be summarized as follows, in the light of our previous experience[1-3] and recent work.

Silanization

All of our own experimental work and the derived conclusions on silanization deal with the usage of monofunctional reagents only, in order to avoid complications with polymer formation, etc. The reaction involved in the achievement of chemical bonding is well defined, but is strongly influenced by the reactivity of the silanization reagent used, which depends on the reactivity of the Si–X bond and further on the steric properties of the group R to be anchored to the surface by Si–O–Si–C bonds. Because of the steric restriction, not all of the silanol groups located on the porous, fully hydrated surface can be derivatized. Different types of silanol groups (isolated, vicinal, or geminal) also exhibit different reactivities during silanization. The completeness of the silanization reaction may also be influenced by the pore geometry.

The silica to be modified must supply a sufficiently high concentration of silanol groups, which should be homogeneously distributed over the surface (*cf.* Köhler *et al.*[4]). The inhomogeneity of the distribution of the substituent alkyl groups on the surface of silica, modified by silanization, and the related "solvent clustering" effect, has been extensively discussed by Gilpin[5] in his review paper concerning the structure and dynamics of bonded phases.

A large variety of silanization reagents containing different chemical moieties which are suitable for the intended purpose to achieve a coating of special chromatographic selectivity, is available. The necessary syntheses of the required miscellaneous reagents containing more polar moieties including, for example, chiral species, may not be without complications in certain cases.

The obtainable phase ratios of the reversed-phase materials depend strongly on that steric effect that influences the reaction and on the type of the silanization reagent.

Polymer coatings

Polymer coatings are formed by cross-linking of the deposited polymers and also by chemical bonding, in particular, to presilanized (trimethylsilylated or "precapped") silica materials via radicals formed from the Si–CH$_3$ groups which are fixed

to the silica surface. Silanol groups are not needed for the immobilization, and may either be partly removed by pre-capping (only up to 75% of the silanols can react) or may remain unchanged on the surface underneath the coating layer. The shielding of residual silanols against undesirable strong (reversible or irreversible) interaction, e.g. with basic compounds, seems to be more effective than with silanized reversed-phase materials. Moreover, it can be predicted that the above-mentioned inhomogeneity of the distribution of the modifying groups across the surface, which occurs on silanization of a less suitable silica, can be avoided.

Certain oligomers of suitable chemical structure and molecular weight (of the alkylpolysiloxane or the polybutadiene type) are commercially available. Oligomers of special chemical structure, as well as with certain steric properties regarding the type of chromatographic interaction intended, could be synthesized in our previous and current work for subsequent immobilization. Such oligomers can be immobilized both on silica and on alumina. The latter material cannot, of course, be modified by silanization because of the instability of the Al–O–Si bond.

It may, however, be a disadvantage that cross-linked polymeric layers are obtained which exhibit special less favourable properties, such as swelling caused by the mobile phase solvents. Unsuitably low diffusion coefficients, which prevent fast mass transfer during the chromatographic process, may also result.

It may also be possible to contribute to the discussion about the special mechanisms of conformational changes in the polymer layers effected by the mobile phase. The residual silanols may also play an important role in this connection. The surface-modified silicas obtained with both methods may be further derivatized by *in situ* reactions, if the group R of the silanization reagent or the immobilized oligomer contains functional groups that can undergo further reactions. Low reaction rates on the solid state surface have to be considered therewith.

The aim of this paper is to report on recent experiments on the immobilization of specially synthesized and also of well characterized, commercially available oligomers, on bare or precapped silica of pore sizes between 100 and 300 Å. Chromatographic testing for efficiency and selectivity, elemental analysis for the carbon content, and measurements for surface analysis such as BET were applied as means of characterization. For comparison, silanized silicas, which were obtained by application of our "enolate" method[1] have been investigated. The polysiloxanes were characterized by gel permeation chromatography (GPC) as well as by IR and ^{29}Si NMR spectroscopy.

EXPERIMENTAL

The detailed procedures of silanization of silica by the "enolate" reaction and the different procedures of polymer coating by immobilization of various types of polysiloxane and polybutadiene have been described previously[1-3]. Polysiloxanes with a suitable molecular weight and reactivity for the necessary cross-linking reactions were synthesized by equilibration of mixtures of oligomeric methylhydropolysiloxanes (PMHS) with octamethyltetrasiloxane (D_4) and subsequent hydrosilylation of 1-alkenes or their derivatives (Fig. 1). The following methods were used to characterize the polymers: elemental analysis for carbon content; volumetric hydrogen determination after hydrolysis for SiH content; IR for SiH, ^{29}Si NMR for SiH; mo-
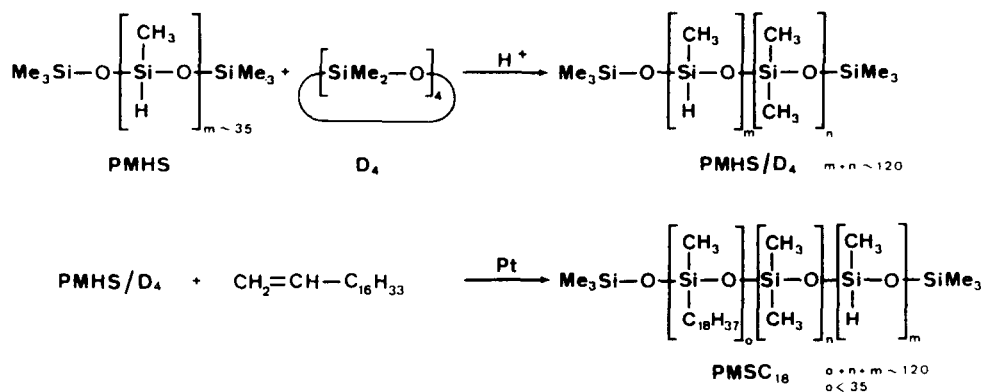
Fig. 1. Synthesis of alkyl-substituted polymethylsiloxanes: equilibration and hydrosilylation.

lecular weight determination; and, for alkyl substitution, GPC for relative characterization of the molecular weight distribution. Inverse size-exclusion chromatography[6] was also used in order to investigate the available pore volume after the coating had been applied. The silicas used were Nucleosils, with a particle diameter of either 5 or 7 μm and a pore diameter of 100 or 300 Å.

Preparation of a special SiH containing methylpolysiloxane
        A mixture of 10 g of PMHS (4 mmol) and 23.7 g of D_4 (80 mmol) containing 0.668 g of China clay and 0.5 μl of sulphuric acid was heated and stirred for 16 h at 70°C. When the reaction was complete the catalyst was removed via centrifugation and filtration. The volatile constituents of the filtrate were removed by evaporation at 120°C under a vacuum of $2 \cdot 10^{-7}$ mbar and 23.61 g of the desired polymer were obtained. The product was characterized by elemental analysis (%C, 28.11; %H, 6.74), GPC, and ^{29}Si NMR. The SiH content of the product was determined volumetrically by alkaline hydrolysis: 5.2 mmol H_2 per gram of polymer were found.

Hydrosilylation of 1-octadecene by the SiH containing polymer
        A mixture of 21 g of polymer (PMHS/D_4 1:20) and 15.5 g (62.6 mmol) of 1-octadecene were dissolved in toluene, and 0.5 mg of H_2PtCl_6 was added as catalyst. The solution was stirred for 17 h at the boiling point of toluene under reflux. The volatile constituents of the reaction mixture were removed under a vacuum of $2 \cdot 10^{-7}$ mbar at 120°C, and 34 g of the $PMSC_{18}$ polymer were obtained. Elemental analysis: %C, 50.9; %H, 10.3; alkaline hydrolysis, 0.8 mmol of H_2 per gram of polymer.
        Apparatus and technical details can be found in our previous publications[1-3].

RESULTS AND DISCUSSION

Syntheses and immobilization of $PMSC_{18}$
        We have previously cross-linked commercially available C_{18} substituted methylpolysiloxanes on native silica and also on C_1-precapped silica[1-3]. Cross-linking was achieved by applying different techniques, but thermal treatment with 2–4% dicumyl

peroxide (DCP) proved to be an easily performable and reliable method. With the precapped silica, the formation of covalent chemical bonds to the intermediate trimethylsilyl layer can also be assumed. The process of immobilization can then be considered successful when, after a rinsing procedure with several solvents such as pentane, dichloromethane, and tetrahydrofuran not more than 10–20% of the deposited oligomeric material is removable from the reaction product.

Unfortunately, different batches of PMSC$_{18}$ (C$_{18}$ substituted polymethylsiloxane), which is commercially available, exhibited completely different cross-linking properties. Even though our initial experiments proved to be successful, we were unable to cross-link batches of this particular compound that we ordered later. GPC analyses of the unsuitable oligomers revealed that the molecular weight distribution was completely different from those products we could easily immobilize. Accordingly, our next step was to synthesize alkyl-substituted methylpolysiloxanes starting from oligomeric PMHS by equilibration with D$_4$ (Fig. 1). Oligomers with suitable molecular weight and with a defined ratio of Si–H and Si–CH$_3$ bonds were obtained and qualitatively characterized by GPC. By hydrosilylation, alkyl groups of the desired carbon chain-lengths can be attached to the siloxane chain to yield PMSC$_{18}$ products that could be immobilized on different types of silica surface without difficulty (see Table I). The equilibration reaction can be executed in different modes and by varying the PMHS:D$_4$ ratio, in order to obtain oligomers of sufficiently high molecular weight and with a defined number of OSi(CH$_3$)$_2$ groups between the hydrogen- or alkyl-substituted silicon atoms. It could be proved by ^{29}Si NMR that the SiH groups are randomly distributed along the polysiloxane chain and that the SiH concentrations within the polymers correspond to the data obtained by chemical analysis via alkaline hydrolysis. Further investigations of the chemical structure and the molecular weight of such polymers are being performed. Hydrosilylation of alkenes of suitable chain-length with the SiH-containing siloxane oligomers finally

TABLE I

COATING OF PRESILANIZED SILICA WITH PMSC$_{18}$

Influence of carbon and SiH content of polymer. Silica, trimethylsilylated Nucleosil 5-100, %C = 5.8; PMSC$_{18}$, synthesized by equilibration and subsequent hydrosilylation (40% (w/w) with respect to weight of silica). SiH = mmol/g after hydrosilylation. %I = % immobilized polymer. d_f = film thickness in nanometres, as determined by the ratio of amount of immobilized polymer and surface area (assumption: no density change during cross-linking). k' = capacity ratio of n-hexylbenzene, methanol–water 5:1 (v/v), 297 K. $\alpha = k'$ (2-n-octylpyridine): k' (ethylbenzene).

Polymer				Bulk phase			
PMHS:D$_4$	%C	SiH	%I	%C	d_f	k'	α
1:50	43.3	1.10	92	16.7	1.26	7.15	0.81
1:28	49.9	0.82	95	19.2	1.43	10.09	0.67
1:20	50.9	0.80	94	19.1	1.29	9.39	0.76
1:10	58.4	0.70	87	19.1	1.19	6.34	1.00
1:5	59.0	1.34	83	19.8	1.13	5.95	1.00
1:28	62.5	0.14	52	13.8	0.72	4.90	1.00
1:20	64.9	0.30	65	17.1	0.84	5.22	0.91

TABLE II

COATING OF PRESILANIZED SILICA WITH PMSC$_{18}$

Variation of film thickness. Silica, Nucleosil 7-100-C$_1$, %C = 5.2; polymer, PMSC$_{18}$, %C = 49.9, SiH = 0.82 mmol/g. %PMSC$_{18}$ = % (w/w), with respect to weight of silica. %I = % immobilized polymer. d_f = film thickness in nanometres. k' = capacity ratio of n-hexylbenzene, methanol–water 5:1 (v/v), 296 K. α = k' (2-n-octylpyridine): k' (ethylbenzene).

%PMSC$_{18}$	%I	%C	d_f	k'	α
10	83	8.4	0.27	2.26	1.31
30	92	15.2	0.95	5.80	0.79
40	95	18.3	1.23	8.71	0.69
50	94	19.9	1.52	10.93	0.63

yields the intended oligomers. The immobilization on silica of such oligomers leads to reversed phases of the desired lipophilicity (Table I). With the execution of the hydrosilylation reaction, a certain small percentage of SiH groups remains unchanged within the oligomer because of the steric hindrance caused by the larger alkyl groups of the reacting olefin. A small proportion of residual SiH groups (mmol/g, see Table I) proved to be an advantage during the subsequent cross-linking by thermal treatment with DCP. SiH-containing oligomers can be cross-linked more easily because of the lower energy required for radical formation from SiH bonds.

The thickness of the coating layer, *i.e.* the carbon content of the bulk phase, can be changed by cross-linking different amounts of PMSC$_{18}$ on the silica, as can be seen from Table II. The cross-linked layers obtained with such oligomers proved to be very suitable from the chromatographic point of view, especially with respect to the separation efficiency of the packed columns. In Fig. 2 the HETP (u) curves of a coated polymer and a silanized reversed phase are given for comparison. The curve for the PMSC$_{18}$ reaches its minimum of h = 2.6 at a mobile phase flow-rate of *ca.* 0.06 cm/s, whereas the silanized C$_{18}$-E curve reaches its minimum of h = 2.5 at *ca.* 0.09 cm/s. Furthermore, the slope of the upper branch of the PMSC$_{18}$ curve is higher
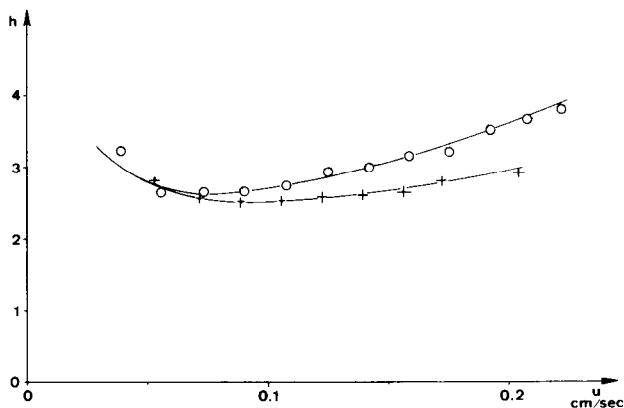

Fig. 2. Chromatographic test of polymer-coated and silanized silicas: HETP (u)-curves. k' = Capacity ratio of n-hexylbenzene 3.2; (○) Nucleosil 5-100-C$_1$-PMSC$_{18}$; (+) Nucleosil 5-100-C$_{18}$-E; mobile phase, methanol–water (90:10, v/v); temperature, 297 K.
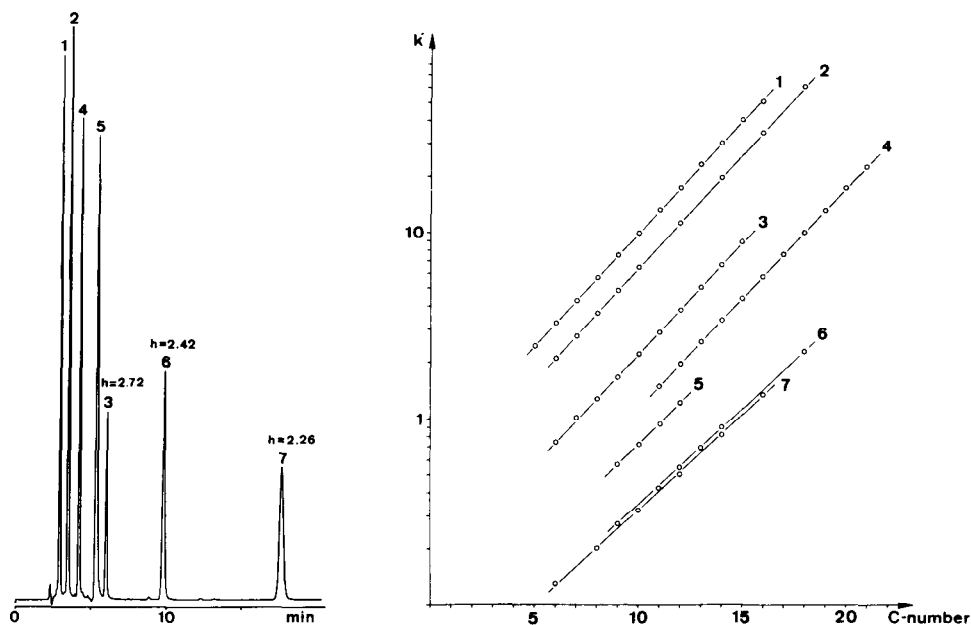
Fig. 3. Chromatographic test for selectivity and efficiency: $PMSC_{18}$-coated presilanized silica. Test solutes: 1 = acetophenone; 2 = benzophenone; 3 = ethylbenzene; 4 = benzylbenzoate; 5 = 2-n-octylpyridine; 6 = n-butylbenzene; 7 = n-hexylbenzene. Column, 250 × 4.5 mm I.D. Nucleosil 5-100-C_1-$PMSC_{18}$; temperature, 300 K; mobile phase, methanol–water (5:1, v/v); flow-rate, 1.0 ml/min; pressure, 16 MPa; detection, RI; h = reduced plate height.

Fig. 4. Retention behaviour of homologous series of various classes of compounds on $PMSC_{18}$-coated Nucleosil 5-100-C_1. Compounds: 1 = n-alkanes; 2 = n-alkenes; 3 = n-alkylbenzenes; 4 = fatty acid methyl esters; 5 = 3-alkanones; 6 = 2-n-alkylpyridines; 7 = 1-n-alcohols. Column, 250 × 4.5 mm I.D. Nucleosil 5-100-C_1-$PMSC_{18}$; temperature, 314 K; mobile phase, methanol–water (90:10, v/v); flow-rate, 1.0 ml/min; pressure, 10.4 MPa; detection, RI.

than for the C_{18}-E. These facts indicate that the C_s term of the $PMSC_{18}$ column is greater than that of the C_{18}-E, which is probably caused by a lower diffusion coefficient of the solute within the cross-linked polymer. Fig. 3 shows a chromatogram that was obtained with the test mixture, which had also been used in prior work[1–3]. It illustrates the efficiency and the selectivity of a typical polymer coated ($PMSC_{18}$) presilanized silica.

The polarities and/or the selectivities of various types of polysiloxane-coated silica were investigated by retention measurements with seven homologous series of different classes of compounds (cf. Fig. 4). In this particular Figure, typical homologous plots of log k' values *versus* carbon number[7] are given, which were obtained with the stationary phase Nucleosil-5-100-C_1-$PMSC_{18}$. This code indicates that Nucleosil-5-100 has been primarily C_1-silanized, whereby *ca.* 75% of the silanol groups were removed from the surface. Subsequently a methylpolysiloxane of a suitable molecular weight was deposited and immobilized by thermal DCP treatment. For all seven homologous series linear plots were obtained under the given conditions, *i.e.* with the composition of the mobile phase, as specified. With the phase system described the alcohols and the pyridines exhibit the same selectivity. Homologous spe-
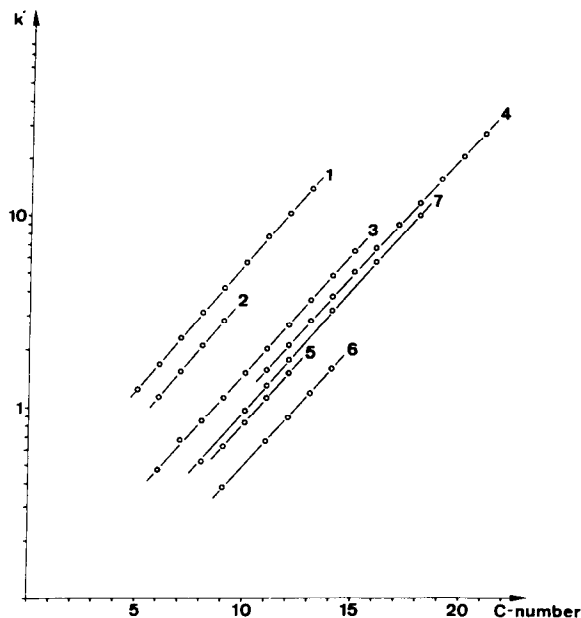
Fig. 5. Retention behaviour of homologous series of various classes of compounds on Nucleosil-5-100-C_{18}-E. Compounds: 1 = n-alkanes; 2 = n-alkenes; 3 = n-alkylbenzenes; 4 = fatty acid methyl esters; 5 = 3-alkanones; 6 = 2-n-alkylpyridines; 7 = 1-n-alcohols. Column, 250 × 4.5 mm I.D. Nucleosil-5-100-C_{18}-E; temperature, 314 K; mobile phase, methanol–water (90:10, v/v); flow-rate, 1.0 ml/min; pressure, 10.4 MPa; detection, RI.

cies of these classes of compounds with a negative difference in carbon number of *ca.* 13 (1300 index units) in comparison with the non-polar standard alkanes are eluted at the same value of log k'. The slopes of the curves of these compound classes are somewhat lower than those of the others.

Fig. 5 shows the separation of the same seven homologous series of compounds on a C_{18} silanized silica. From this diagram it can be concluded that the alkylpyridines are retarded by silanophilic interaction, whereas the 1-alkanols exhibit a similar retention behaviour as on the $PMSC_{18}$ material. In Table III the log k' differences obtained by subtraction of the log k' value of the polar compounds from that of the alkanes were converted into retention index units. (The difference in retentions between two homologues is defined to be 100.) The retention indexes obtained with four different polymer-coated columns are listed, together with those obtained with a silanized C_{18}-E column. The following statements can be made about the selectivities of the different phases:

(1) The basic pyridine compounds and the alcohols have the lowest retentions with all five of the columns at the given water content of the mobile phase.
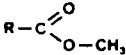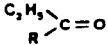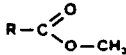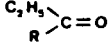
(2) The shielding of the residual silanol groups and the lower concentration of the silanol groups after the presilanization explain the much shorter retentions of the alcohols and the pyridines on the polymer-coated phases. Differences in the type and composition of the solvent–stationary phase layer may also contribute to differences in retention between individual packing materials.

TABLE III

SELECTIVITY OF HOMOLOGOUS COMPOUNDS ON POLYMER-COATED PRESILANIZED SILICA AND POLYMER-COATED ALUMINA

Comparison with C_{18} silica prepared by silanization.

Homologous series	Retention index differences				
	C_{18}-E	$PMSC_{18}$	PBD	XE 60	A_5Y-PBD*
Mobile phase, methanol–water (90:10, v/v)					
$CH_2=CH-R$	120	150	110	—	—
phenyl–R	430	540	400	—	—
$R-C(=O)-O-CH_3$	510	790	600	—	—
$\mathrm{C_2H_5} \atop R$ $>C=O$	630	990	730	—	—
R—OH	530	1360	940	—	—
pyridyl–R	810	1340	950	—	—
Mobile phase, methanol–water (80:20, v/v)					
$CH_2=CH-R$	110	130	110	100	—
phenyl–R	425	500	380	360	350
$R-C(=O)-O-CH_3$	480	700	550	520	710
$\mathrm{C_2H_5} \atop R$ $>C=O$	590	840	680	600	—
R—OH	520	1060	800	800	650
pyridyl–R	780	1110	900	950	1000

* A_5Y-PBD = polymer-coated alumina.

(3) The cyano-substituted methylpolysiloxane XE 60 does not exhibit a strong interaction in such aqueous reversed-phase systems. Alcohols and pyridines are (relatively) less retarded with this phase. This may be explained by the interaction between the cyano and the silanol groups, which weakens the interaction of the latter with the functional groups of the test solutes. It should be remembered, however, that the absolute retentions with the polar cyano phase are generally lower than with the more lipophilic phases.

(4) With the silanized C_{18}-E phase the alcohols exhibit considerably higher retentions (i.e. lower retention index differences) in comparison with the pyridines. This can be explained by strong interaction with silanol groups that are not well shielded by the coating.

Regarding the selectivity of such phases, further conclusions can be drawn only after more extensive measurements.

Another result from this part of our work turned out to be that we are now capable of synthesizing alkylpolysiloxanes of a suitable chain-length and functional-group substitution for immobilization by cross-linking at a percentage of *ca.* 80–90% without problems. For this kind of systematic investigation on immobilization, the use of commercial products with an unknown "history" of production was no longer necessary.

Immobilization of polybutadiene

Different cross-linking methods, *i.e.* γ-radiation and thermal treatment using reagents such as azo-*tert.*-octane (ATO) or DCP were primarily applied without variation of the amount (40%) of polybutadiene (PBD) on the silica surface (see Table IV). The carbon content in the reaction products was highest when DCP was used. This material also showed the highest phase ratios for elution of lipophilic test solutes (phenylalkanes). The shielding of the silanol groups against interaction with the basic test solute *n*-octylpyridine (OPY) turned out to be the best with this material.

In further experiments we tried to vary the film thickness, *i.e.* the phase ratios, by adding different amounts (5–55%) of PBD to the silica prior to the cross-linking. The corresponding increase in the measured carbon content was not quite linear. As expected, the silanol-group shielding proved to be the best with the material of the thickest polymer film. The reduced plate heights h were slightly greater with the PBD-coated phases than with the $PMSC_{18}$-coated materials.

The loading capacity of columns packed with the 55% material was as high as *ca.* 150 μg of *n*-butylbenzene. The sample capacity of the 5% PBD-coated silica was about the same at a somewhat higher reduced plate height (4–5). From the similar ascent of the upper branches of the HETP (u) curves, it can be deduced that the C_s terms of both materials must be similar in spite of the difference in the film

TABLE IV

COATING OF PRESILANIZED SILICA WITH POLYBUTADIENE

Variation of film thickness with different methods of immobilization. Base material, Nucleosil-7-100-C_1. AMA = allylmethacrylate. d_f = film thickness in nanometres. k' = capacity ratio of *n*-nonylbenzene, methanol–water (5:1, v/v), 297 K. α = k' (2-*n*-octylpyridine): k' (ethylbenzene).

%PBD	Method of immobilization	%C	d_f	k'	α
40	4%* AMA, 10 Mrad ^{60}Co	19.34	0.703	11.95	1.05
40	2%* ATO, 4 h, 180°C	21.35	0.776	17.46	0.81
40	2%* DCP, 4 h, 180°C	24.60	0.894	22.94	0.77
5	2%* DCP, 4 h, 180°C	8.18	0.297	3.37	1.68
10	2%* DCP, 4 h, 180°C	11.30	0.411	6.64	1.39
25	2%* DCP, 4 h, 180°C	19.10	0.694	14.88	0.94
40	2%* DCP, 4 h, 180°C	24.60	0.894	22.94	0.77
55	2%* DCP, 4 h, 180°C	29.93	1.088	34.16	0.68

* % (w/w) relative to polymer.

TABLE V

COATING OF SILICA WITH POLYMETHYLSILOXANE (PMSC$_1$)

Different methods of immobilization. Base materials, Nucleosil, 300 Å, 7 μm (A) and Nucleosil, 300 Å, 7 μm, TMS-presilanized (B). Amount of PMSC$_1$, 20% (w/w) with respect to silica. k' = capacity ratio of nonylbenzene, methanol–water (3:1, v/v), 297 K. α = k' (2-n-octylpyridine): k' (ethylbenzene).

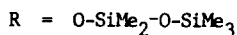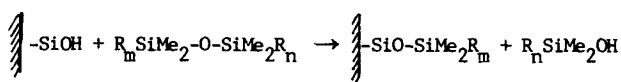| Silica	Method of immobilization	%C	k'	α
A	Thermal, 20 h, 180°C	6.02	13.95	1.69
B	Thermal, 2%* ATO, 4 h, 180°C	3.04	4.43	1.97
B	5 Mrad ^{60}Co	4.47	9.38	1.07
B	Thermal, 2%* DCP, 4 h, 180°C	5.73	11.67	0.96

* % (w/w) relative to polymer.

thickness. We assume that only the upper part of the layer of the thick film product is effective for the chromatographic process.

Immobilization of polymethylsiloxane (PMSC$_1$)

Different methods of immobilization were applied to methylpolysiloxanes on Nucleosil-300-7 and the corresponding C$_1$-precapped material. The applicability of the new methods of surface modification to wide-pore silicas was studied with 300-Å material. The products obtained were to be used for the separation of large molecules.

The results of these experiments are given in Table V. On unpretreated Nucleosil, PMSC$_1$ can be immobilized without addition of radical-generating reagents merely by heating at 180°C for 20 h. A carbon content of 6% can be achieved. The immobilization can only be explained by chemical bonding of methylpolysiloxane chains of various lengths effected by SiOH attack on Si–O–Si bonds of the PMSC$_1$:

$$\text{-SiOH} + \text{R}_m\text{SiMe}_2\text{-O-SiMe}_2\text{R}_n \rightarrow \text{-SiO-SiMe}_2\text{R}_m + \text{R}_n\text{SiMe}_2\text{OH}$$

$$R = \text{O-SiMe}_2\text{-O-SiMe}_3$$
$$Me = \text{Methyl}$$

With precapped silica this reaction does *not* lead to a noticeable immobilization of PMSC$_1$. The highest carbon contents were obtained by thermal treatment in the presence of DCP for a short time. The shielding of the residual silanol groups turned out to be the best with this product (see the α-value for OPY on the bottom line of Table IV).

Immobilization of cyanomethylpolysiloxane XE 60

Polysiloxanes of this composition can also be immobilized by the cross-linking methods applied to the unsubstituted alkylpolysiloxanes (see Table V). Such phases may be of interest because of their increased but still weak general polarity in reversed-phase systems at a similar selectivity to that obtained with other polymer-

TABLE VI

COATING OF SILICA WITH POLYMETHYLCYANOETHYLSILOXANE (XE-60)

Different methods of immobilization. Base materials, Nucleosil, 300 Å, 7 μm (A) and Nucleosil, 300 Å, 7 μm, TMS-presilanized (B). Amount of XE-60, 20% (w/w) with respect to silica. k' = capacity ratio of nonylbenzene, methanol–water (3:1, v/v), 297 K. α = k' (2-*n*-octylpyridine): k' (ethylbenzene).

Silica	Method of immobilization	%C	k'	α
A	Thermal, 2%* ATO, 4 h, 180°C	5.53	3.92	0.77
A	Thermal, 2%* DCP, 4 h, 180°C	5.98	4.05	0.75
B	Thermal, 2%* ATO, 4 h, 180°C	2.57	2.01	1.83
B	10 Mrad °°Co	6.80	6.46	0.80
B	Thermal, 2%* DCP, 4 h, 180°C	6.90	6.75	0.82

* % (w/w) relative to polymer.

coated phases. Table VI lists data concerning the immobilization and the retention behaviour of the XE 60 phase. The excellent shielding of the silanol groups has already been discussed above.

Investigation of changes in pore volume of silanized and polymer-coated silicas

The results of BET measurements (nitrogen adsorption) as shown in Fig. 6 indicate that the shape of the pore-size distribution is generally unchanged when the polymer coating is either PBD or PMSC$_{18}$. The silanized product Nucleosil-5-100-C$_1$-E was also measured for comparison. The maxima of the pore distribution curves
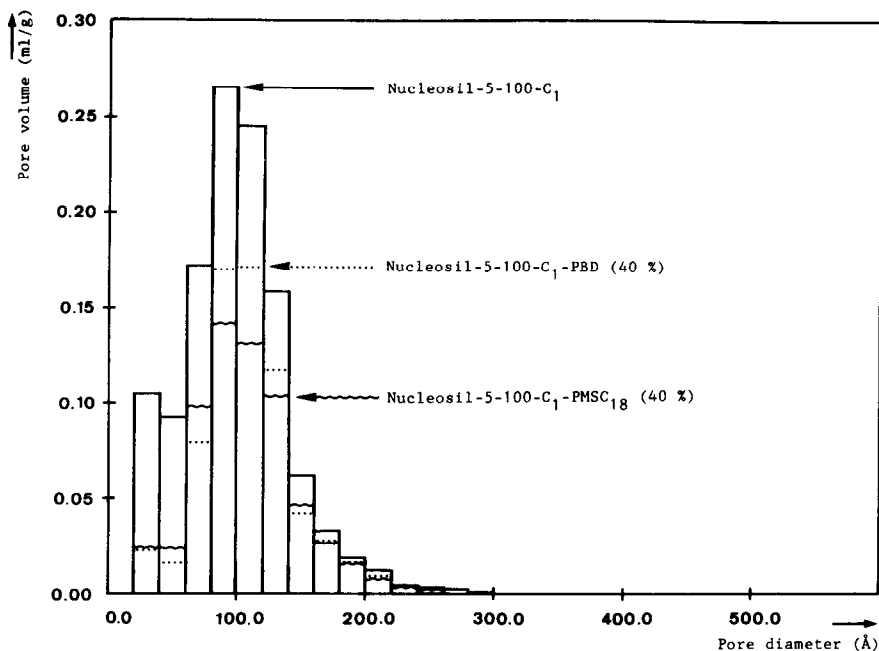

Fig. 6. Pore volume distribution by BET.

TABLE VII

PORE VOLUMES DETERMINED BY INVERSE SIZE-EXCLUSION CHROMATOGRAPHY

%P = % polymer (w/w) relative to silica. Pore porosity = pore volume/column volume.

Silica	%P	Pore volume (μl/g)	Pore porosity
Nucleosil-7-100		1074	0.46
Nucleosil-7-100-C_1		997	0.45
Nucleosil-7-100-C_1-PBD	5	870	0.43
Nucleosil-7-100-C_1-PBD	10	766	0.40
Nucleosil-7-100-C_1-PBD	25	588	0.33
Nucleosil-7-100-C_1-PBD	40	451	0.28
Nucleosil-7-100-C_1-PBD	55	327	0.22
Nucleosil-7-100-C_1-$PMSC_{18}$	10	832	0.43
Nucleosil-7-100-C_1-$PMSC_{18}$	30	493	0.31
Nucleosil-7-100-C_1-$PMSC_{18}$	50	292	0.21
Nucleosil-5-100-$PMSC_{18}$	40	674	0.37
Nucleosil-5-100-C_1-$PMSC_{18}$	40	607	0.36
Nucleosil-5-100		1097	0.50
Nucleosil-5-100-C_1		975	0.48
Nucleosil-5-100-C_8		801	0.41
Nucleosil-5-100-C_{18}		560	0.34

are still close to 100 Å with all three of the samples. However, small pores seem to be selectively removed. Inverse size-exclusion measurements using polystyrene standards, as proposed by Halasz and Martin[6], showed the expected decrease of the available pore volume. This corresponds to the space requirements of the group R of the silanization reagent or the film thickness of the polymer coating. Even with the largest group (C_{18}), 50% of the original pore volume is still available (see Table VII). With $PMSC_{18}$ materials a similar decrease of the available pore volume by *ca.* 40% was measured. Reasonable pore-size distributions could be measured for silanized silicas but not for polymer-coated silicas.

Chemical stability of polymer-coated silicas

The long-term stability against attack of mobile phase solvents, such as water or tetrahydrofuran (THF), proved to be excellent. After rinsing with 750 column volumes of THF, and 2000 column volumes of water at 50°C, no appreciable change of k' values was observed for test compounds such as *n*-hexylbenzene and OPY.

Chromatographic selectivities of polymer-coated reversed-phase materials

Five different reversed-phase materials were tested via the selectivity coefficients of certain more polar compounds in particular OPY, aminopyridine, and nitrobenzene. OPY and aminopyridine are compounds of medium or strong basicity and were partially contained in the test mixtures used in our previous work on similar subject matters. The α-values of such test solutes were standardized to ethylbenzene or other homologous phenylalkanes. A selection of the data obtained is given in Table VIII.

TABLE VIII

SELECTIVITIES OF POLYMER-COATED PHASES

Normal silicas *versus* TMS-presilanized materials. $\alpha_1 = k'$ (2-*n*-octylpyridine): k' (ethylbenzene). $\alpha_2 = k'$ (2-aminopyridine): k' (ethylbenzene). $\alpha_3 = k'$ (nitrobenzene): k' (ethylbenzene). Measured in methanol–water (3:1).

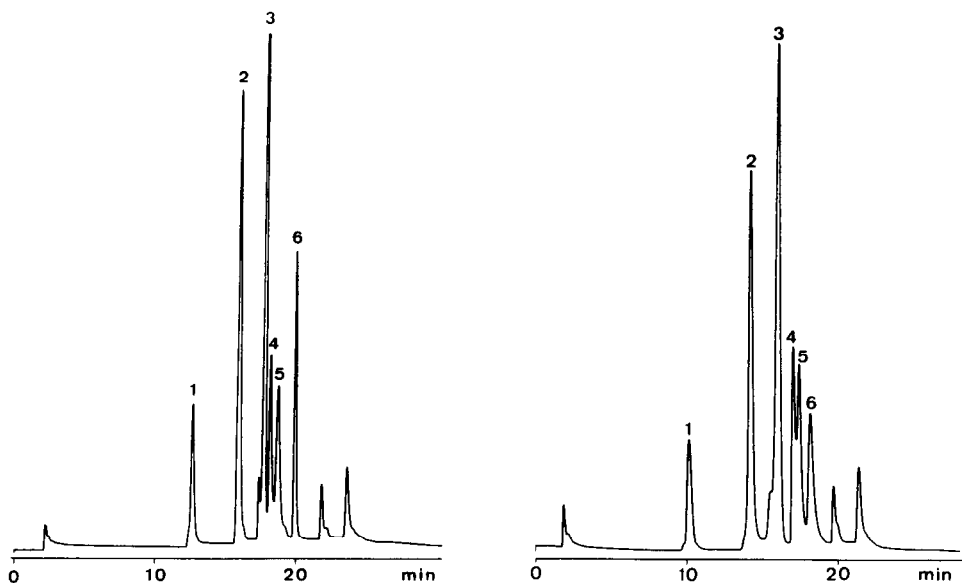| Silica	α_1	α_2	α_3
Nucleosil-5-100-C_{18}-E	2.96	0.17	0.18
Nucleosil-5-100-$PMSC_{18}$	2.02	1.23	0.22
Nucleosil-5-100-C_1-$PMSC_{18}$	1.46	0.09	0.19
Nucleosil-5-100-$PMSC_1$	1.87	0.73	0.19
Nucleosil-5-100-C_1-$PMSC_1$	1.19	0.04	0.18
Nucleosil-5-100-XE-60	0.93	2.00	0.70
Nucleosil-5-100-C_1-XE-60	1.00	0.01	0.49
Nucleosil-5-100-PBD	1.38	2.01	0.36
Nucleosil-5-100-C_1-PBD	1.28	0.04	0.21

Fig. 7. Separation of standard proteins with modified silica on TMS-silanized Nucleosil 7-300. Test solutes: 1 = ribonuclease; 2 = cytochrome *c*; 3 = lysozyme; 4 = transferrin; 5 = bovine serum albumin; 6 = haemoglobin. Column, 150 × 4.4 mm I.D. Nucleosil 7-300-C_1; temperature, 298 K; mobile phase, gradient elution, 80% A–50% A in 20 min [A = 0.1% (v/v) TFA in water, B = 0.1% (v/v) TFA in acetonitrile]; flow-rate, 0.8 ml/min; pressure, 4.8–3 MPa; detection, UV, 280 nm.

Fig. 8. Separation of standard proteins with modified silica on $PMSC_1$-coated Nucleosil 7-300. Test solutes: 1 = ribonuclease; 2 = cytochrome *c*; 3 = lysozyme; 4 = transferrin; 5 = bovine serum albumin; 6 = haemoglobin. Column, 150 × 4.4 mm I.D. Nucleosil 7-300-$PMSC_1$; temperature, 298 K; mobile phase, gradient elution, 80% A–50% A in 20 min [A = 0.1% (v/v) TFA in water, B = 0.1% (v/v) TFA in acetonitrile]; flow-rate, 0.8 ml/min; pressure, 4.8–3 MPa; detection, UV, 280 nm.
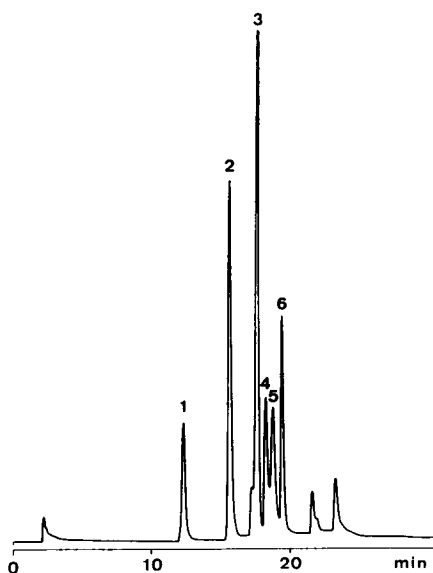
Fig. 9. Separation of standard proteins with modified silica on $PMSC_1$-coated TMS-presilanized Nucleosil 7-300. Test solutes: 1 = ribonuclease; 2 = cytochrome c; 3 = lysozyme; 4 = transferrin; 5 = bovine serum albumin; 6 = haemoglobin. Column, 150 mm × 4.4 mm I.D. Nucleosil 7-300-C_1-$PMSC_1$; temperature, 298 K; mobile phase, gradient elution, 80% A–50% A in 20 min [A = 0.1% (v/v) TFA in water, B = 0.1% (v/v) TFA in acetonitrile]; flow-rate, 0.8 ml/min; pressure, 4.8–3 MPa; detection, UV, 280 nm.

The test solute OPY had an α-value of 2.96 with a well C_{18}-silanized Nucleosil. The polymer-coated $PMSC_{18}$ and $PMSC_1$ materials show α-values of ca. 2.02 and 1.87, respectively, however, indicating that the shielding of the silanol groups is considerably better with the polymeric phases. Prior C_1-silanization reduces the silanol group concentration on the surface by ca. 70%. Therefore the α-values are considerably decreased to 1.46 and 1.19 with the polymer phases.

An interesting retention behaviour is observed with a coating layer of a CH_2CH_2CN-substituted methylpolysiloxane. For the test solute OPY, no appreciable change of the α-value is observed by precapping. Our explanation of this observation is the deactivation of SiOH groups by interaction with the polar cyano groups, which act as chemically bonded modifiers.

Surprisingly enough, no dramatic change of the OPY α-value is found when C_1-silanization is carried out before coating with PBD. A very good homogeneity of the polymeric film and the related good shielding of the silanol groups can be proposed by way of explanation. With the strongly polar aminopyridine the self-deactivation effect of the cyano group is no longer strong enough, as can be deduced from the α-data of Table VIII.

Applications

In order to demonstrate the abilities of polymer-coated columns to separate biomolecules, a mixture of standard proteins was measured using different types of column with "medium" polarity. Figs. 7–9 illustrate these separations on TMS-silanized Nucleosil with a pore diameter of 300 Å, the same silica coated with poly-

methylsiloxane ($PMSC_1$), and a presilanized and $PMSC_1$-coated stationary phase. When the same gradients were used for all three separations, no significant changes in selectivity for the different stationary phases can be observed. In terms of efficiency, the TMS-silanized materials seem to be somewhat superior, however, it should be pointed out that the peak areas for a certain protein are identical on the three different packing materials. This means that it is mainly the gradient conditions and the nature of the silica itself, and not so much the surface ligands, that influence the resolution of proteins.

CONCLUSION

Polymer coating of various types of silica by cross-linking or chemical bonding of oligomers, such as unsubstituted and substituted methylpolysiloxanes, leads to stationary phases with excellent properties for reversed-phase systems. Our results can be summarized as follows:

(1) The efficiency and sample capacity of the columns obtained with such stationary phases are comparable with efficiency and sample capacity obtained with silanized silicas.

(2) The film thickness of the coating, and therefore the phase ratio of the stationary phase, can deliberately be varied. Thick films (ca. 15 Å) give better shielding without loss of efficiency. Loadability is not dependent on film thickness.

(3) Oligomers substituted by different polar groups, such as cyano, can also be immobilized in order to produce particular polarities.

(4) The synthesized materials exhibit superior long-term stability with respect to loss of stationary phase at operation with highly polar or extremely non-polar mobile phases. At very high pH values, polymer-coated alumina phases can be applied.

The scope of our future work in this field will include the synthesis of chiral, highly polar, and ion-exchange stationary phases by the described methods. The promising applicability of inexpensive stationary phases for the separation of large biomolecules will be further explored.

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