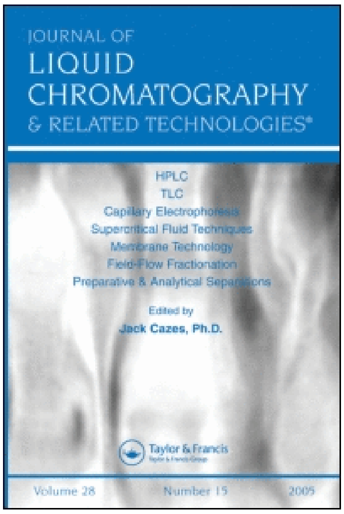

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THE CHROMATOGRAPHIC BEHAVIOR OF COATED STATIONARY PHASES WITH DIFFERENT SILICAS

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ABSTRACT

Different silicas coated with poly(methyloctylsiloxane) are compared in terms of the effect of particle shape, particle diameter, mean pore diameter and specific surface area. For irregular particles, the carbon contents (% C) coated are essentially identical even though there are considerable variations in surface area for the different silica supports. Sample retentions (k) increase with increasing surface area while efficiences (N/m, naphthalene) increase as the particle diameter decreases. Excellent peak symmetries (As) are obtained with most packings. Spherical particles, with significantly lower surface areas, have lower carbon loadings and higher surface coverage but produce columns with significantly higher efficiences than the irregular particles having similar particle diameter.

INTRODUCTION

Porous silica is the most widely used support material in liquid chromatography (LC) today. It is not only employed as a polar stationary phase *per se* , but also serves as support for bonded phases. It follows that both the chemical and the physical properties of the silica can have a strong influence on the chromatographic performance of the resulting columns.

Commercially available silicas vary in their characteristics, not only from manufacturer to manufacturer but also from batch to batch of one manufacturer.[1-3] Most silicas also contain significant levels of impurities, mostly metals and alumina.[4]

We have recently prepared a stationary phase consisting of a C_8 polysiloxane coated on porous silica particles, without cross-linking, that showed good chromatographic behavior and stability.[5,6] In the present paper we extend our work using several other silicas to determine the influence of the different physical properties on the chromatographic behavior of the coated stationary phases.

EXPERIMENTAL

Chemicals and Material

Methanol, dichloromethane (Merck, HPLC-grade) and carbon tetrachloride (Merck, AR) were used without further purification.

The chromatographic test substances (acetone, aniline, benzene, benzonitrile, N,N-dimethylaniline, naphthalene, toluene and o-, m- and p-toluidine) were AR grade and not further purified.

Poly(methyloctylsiloxane) (PMOS) polymer (average molecular mass of 6 200) was obtained from Hüls America (USA).

The silicas tested as chromatographic supports were Davisil, 10 μm (Alltech Associates, USA), Sigma, 10 μm (Sigma, USA), Lichrosorb Si-100, 10 μm, and Lichrosorb Si-60, 10, 7, and 5 μm (Merck, Germany) and Spherisorb, 8 and 5 μm (Phase Separations, UK).

Preparation of the Packing Materials

The silicas were dried in air at 150°C for 24 hours prior to preparation of the packing material. Determined quantities of the silicas were added to solutions of PMOS dissolved in 60 mL of dichloromethane.

The mixtures were gently stirred for 3 hours at room temperature and then the solvent was allowed to evaporate, without stirring, at room temperature in the fume hood.

Preparation of Columns

Columns (125 mm x 3.4 mm i.d) were made from type 316 L stainless-steel tubing. The internal surface was polished using a technique developed in our laboratories.[7]

The columns were slurry packed using 10% (irregular) or 20% (spherical) slurries (w/v) of the coated stationary phase in carbon tetrachloride. A packing pressure of 38 MPa (Haskel Packing Pump) was used, with methanol as propulsion solvent.

Columns were conditioned for four hours with mobile phase (methanol:water, 70:30, v/v) at 0.2 mL min^{-1} prior to testing.

Instrumentation

The chromatography was performed with a modular HPLC system equipped with a SSI Model 3XL pneumatic injector with a 10 μL loop, a Waters 510 pump, a Waters Model 481 spectrophotometric detector (254 nm; 14 μL cell volume), and a Waters 740 integrator.

Elemental determinations on the packing materials were made using a Model 2400 Perkin Elmer CHN analyzer.

The specific surface areas of the silicas and of the prepared packings were determined by the conventional BET method[8] using a Model 2300 Micromeritics Flow Sorb II instrument. Atomic absorption determination of metals in some silicas were made using a Varian Model AA6 atomic absorption spectrometer.

Table 1

Characterization of the Silicas: Manufacturer of Supplier, Particle Shape, Particle Diameter (dp), Mean Pore Diameter (pd) and Specific Surface Area

Silica	Manufacturer or Supplier	Particle Shape	dp[a] (μm)	pd[a] (nm)	S[b] (m^2g^{-1})
Davisil	Alltech	Irregular	10	15	237
Sigma	Sigma	Irregular	10	6	393
Lichrosorb Si-100	Merck	Irregular	10	10	290
Lichrosorb Si-60	Merck	Irregular	10	6	267
Lichrosorb Si-60	Merck	Irregular	7	6	305
Lichrosorb Si-60	Merck	Irregular	5	6	402
Spherisorb	Phase Sep.	Spherical	8	8	149
Spherisorb	Phase Sep.	Spherical	5	8	186

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[a] As furnished by manufacturer/supplier.
[b] Determined in our laboratories by the BET method.

Calculation of Surface Coverage

The surface coverage, S.C., was calculated according to Berendsen and de Galan[9]:

$$\text{S.C. } (\mu\text{mol m}^{-2}) = \frac{10^6\,C}{1200n_c - C(M-1)}\,\frac{1}{S}$$

where C is the percent carbon loading of the prepared phase after packing, n_c is the number of carbon atoms in the silane reagent (8 in our case), S is the surface area of the non modified silica, and M is the molecular weight of the silane ligand ($OSi(CH_3)(C_8H_{17})$).

Evaluation of Columns

All columns were operated at room temperature. The mobile phase flow rate of 0.2 mL min^{-1} was near optimal for separations made with these columns. The column dead time, t_M, was determined using methanol as an unretained compound. Column efficiency values (N/m) were determined from

Table 2

Comparation of the Different Coated Stationary Phases Using Test Mixture 1

Stationary Phase	dp	$S(m^2g^{-1})$		$C(\%)^d$		S.C.	Chrom. Parameters				
(% Initial Loading)	(μm)		SP^b	SP		$(\mu mol\ m^{-2})$	N/m^e	As^e	k^e	R_s^f	α^f
		Silica[a]	a.p.[c]	Initial	a.p.[c]		(m^{-1})				
40%PMOS-Davisil	10	237	161	22.8	11.5	6.36	24 000	1.4	1.8	1.8	1.2
50%PMOS-Sigma	10	393	87	30.2	17.7	6.85	34 400	1.1	3.6	2.7	1.2
50%PMOS-Si-100	10	290	99	29.7	16.9	8.68	33 600	1.0	3.2	2.4	1.2
50% PMOS-Si-60	10	267	183	30.4	8.5	3.91	35 200	1.2	1.5	2.0	1.2
50%PMOS-Si-60	7	305	---	30.5	18.0	9.05	41 200	1.3	3.1	2.7	1.2
50%PMOS-Si-60	5	402	62	30.0	18.8	7.32	50 400	1.2	4.8	3.2	1.2
40%PMOS-Spher.	8	149	2	24.9	14.9	14.8	58 400	1.0	4.1	3.4	1.2
40%PMOS-Spher.	5	186	99	23.8	13.5	9.95	74 400	1.3	2.1	3.3	1.2

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[a] Pure silica, [b] SP = stationary phase, [c] a.p. = after packing, [d] obtained by elemental analysis, [e] calculated for the naphthalene peak, [f] calculated for the toluene-naphthalene pair.

peak widths at half height. The asymmetry factors (As) were calculated at 10% of the peak height from the ratio of the widths of the rear and front sides of the peak. Retention factors (k), resolutions (R_S) and separation factors (α) were also determined.

Two test mixtures were used during this study: (I) acetone, benzonitrile, benzene, toluene, and naphthalene and (II) aniline, o-, m-, p-toluidine, and N,N-dimethylaniline. Injections of 10 μL of appropriate concentrations of these mixtures produced satisfactory chromatographic peaks with a x128 attenuation at 254 nm.

RESULTS AND DISCUSSION

Table 1 shows the characteristics of the silicas utilized as supports in the preparation of the coated stationary phases. For both irregular and spherical silicas having the same mean pore diameter (from the same manufacturer) the specific surface area increases as the particle diameter decreases.

As can be seen in Table 2, the initial carbon contents (% C) of the packing materials prepared from silicas with irregular shape (with exception of the Davisil) are essentially identical even though there are considerable variations in the surface area of the initial silica supports. After packing, the % C decreases due to the loss of some PMOS during the process of column packing as a result of the solubility of the PMOS in CCl_4. Note, however, that the specific surface area of the stationary phases, after packing, is smaller than that of the initial silica, indicating that a part of PMOS is strongly sorbed into the porous silica particles. The remaining loading of PMOS on the different silicas remains practically constant during the chromatographic testing.

Previous work[6] has shown that the PMOS phase is remarkably stable to extended washing with methanol-water mobile phase. This was also confirmed in the present work by the constancy of the chromatographic parameters even after extensive testing.

The surface coverage values are, in general, higher than those for chemically bonded phases.[10] Probably this results from using a polymeric phase which coats the support, giving rise to a higher %C than for chemically bonded phases. However, when the %C are similar for our phases, prepared from either irregular or spherical silicas, the surface coverage increases as the surface area decreases.

The value of k depends on the thickness and uniformity of the coating. For thick coatings, k reflects interactions with the polymer film, which are not perturbed by silica surface.

In general, for irregular particles of the same particle diameter, 10 μm, the %C increases with increasing surface area of the initial silica. This leads to a decrease in the velocity of mass transfer and, consequently, an increase in the retention factor.

Chromatograms of some stationary phases obtained with test mixture I are ilustrated in Figure 1. Excellent peak symmetries (As) are obtained with most of the stationary phases. In general, higher efficiences are obtained with smaller particle diameters. The stationary phases prepared from spherical silica have significantly lower surface areas and lower carbon loadings, but produce chromatographic columns of significantly higher efficiences than the irregular particles having similar particle diameters; this is as expected, as spherical particles result in a more homogeneous bed in the column.

Among the different irregular supports with 10 μm particle diameters, the Davisil silica gave the poorest values of the chromatographic parameters, showing that the mean pore diameter influences the results. The two 6 nm pore size silicas (Sigma and Lichrosorb Si-60), although quite different, provide the best efficiencies.

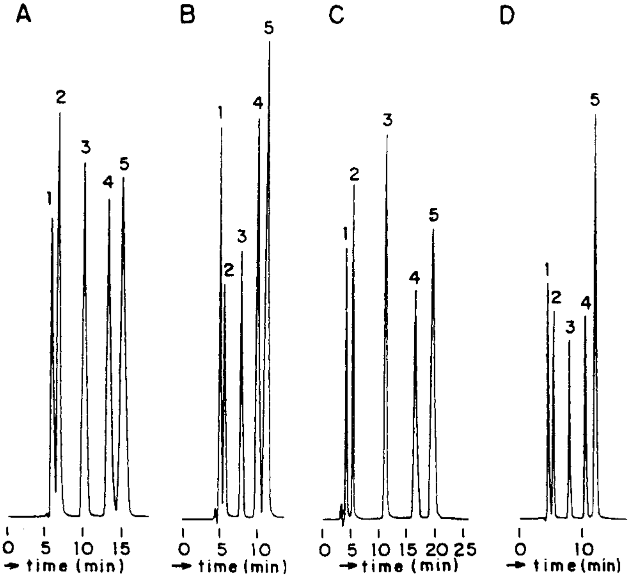

Figure 1. Chromatograms of test mixture I: 1= acetone, 2= benzonitrile, 3= benzene, 4= toluene and 5= naphthalene, obtained with columns packed with (% initial loading): (A) 40% PMOS on Davisil, 10 μm, (B) 50% PMOS on Lichrosorb Si-60, 10 μm, (C) 50% PMOS on Lichrosorb Si-60, 5μm and (D) 40% PMOS on Spherisorb, 8 μm. Chromatographic conditions: mobile phase: methanol: water (70:30, v/v), flow-rate: 0.2 mL min^{-1}, volume of injected sample: 10 μL, detection: UV, 254 nm and paper speed: 0.2 cm min^{-1}.

Comparing the packings prepared from 6 nm irregular silica (50% (initial) PMOS on Lichrosorb Si-60, 10, 7 and 5 μm), the efficiency increases as the particle diameter decreases. A similar result is seen for the two spherical silicas that have the same mean pore diameters.

Table 3 shows the results obtained using mixture II, part of the mixture proposed by Engelhardt and co-workers[11,12] as basic probes, with aniline as a weak base, N,N-dimethylaniline (N,N-DMA) as a strong base, and the isomeric o-, m- and p-toluidines as probes for silanophilic interactions. Methanol:water (55:45, v/v), without addition of buffer or salt solution, was used as the mobile phase.

Table 3

Chromatographic Parameters Obtained Using Test Mixture II

Stationary Phase (% Initial Loading)	dp (μm)	Chromatogr. Parameter	Compound Aniline	N,N-DMA	p-/m-t	m-/o-t
40% PMOS-Davisil	10	As	2.5	3.3		
		α			1.3	1.0
50% PMOS-Sigma	10	As	1.3	1.2		
		α			1.2	1.0
50% PMOS-Lich. Si-100	10	As	1.8	1.9		
		α			1.2	1.0
50% PMOS-Lich Si-60	10	As	1.5	1.3		
		α			1.0	1.0
50% PMOS-Lich. Si-60	7	As	1.6	1.4		
		α			1.0	1.5
50% PMOS-Lich Si-60	5	As	2.2	2.1		
		α			1.0	1.4
40% PMOS-Spherisorb	8	As	1.6	1.8		
		α			1.2	1.2
40% PMOS-Spherisorb	5	As	problems of absorption of the compounds			
		α				

p-/m-t = p-/m-toluene
m-/o-t = m-/o-toluene

A column can be considered "good" for the analysis of basic compounds, according to the criteria of Engelhardt and co-workers,[11,12] if the isomeric toluidines coelute or have a ratio of k values below 1.3. Some chromatograms of these test compounds are shown in Figure 2. In general, we have found that aniline, despite having a lower pKa value than N,N-DMA, usually gives peaks with somewhat more asymmetry than N,N-DMA.

All the columns, except those based on 5 μm Spherisorb, where the basic solutes were irreversibly adsorbed, show ratios of k values smaller than 1.3, indicating their good quality. However, the asymmetry values for several of the columns are higher than the values usually accepted in the literature.[13]

The poor performance for basic compounds of the column packed with 40% PMOS on Spherisorb, 5 μm, might be due to the presence of strongly acidic silanol groups on the surface of the silica or to significant levels of some metal impurities that could enhance the acidity of silanol groups in their immediate vicinity. It is interesting to note that these problems bring undesirable behavior, principally for particles of 5 μm, both for irregular and spherical silicas. In the irregular supports the distribution of silanol groups on

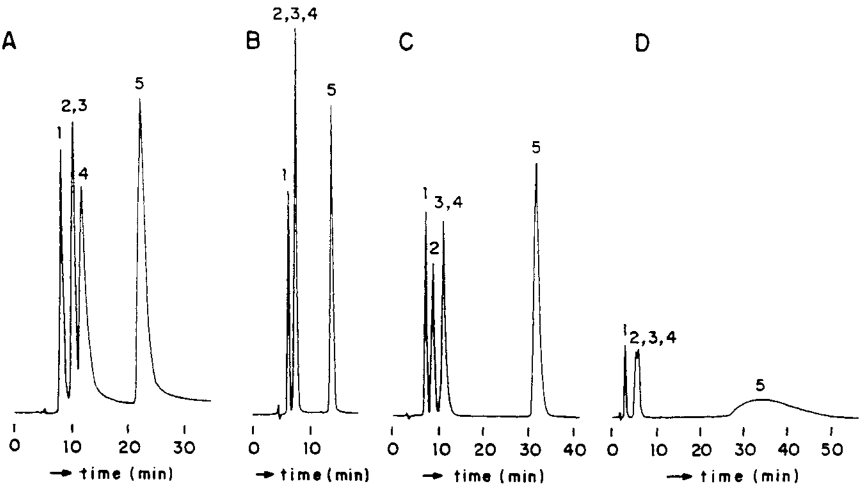

Figure 2. Chromatograms of test mixture II: 1= aniline, 2, 3 and 4= o-, m- and p-toluidine and 5= N,N-DMA, obtained with columns packed with (% initial loading): (A) 40% PMOS on Davisil, 10 μm, (B) 50% PMOS on Lichrosorb Si-60, 10 μm, (C) 50% PMOS on Lichrosorb Si-60, 5μm and (D) 40% PMOS on Spherisorb, 8 μm. Chromatographic conditions: mobile phase: methanol: water (55:45, v/v), other conditions as in Figure 1.

the surface can be more heterogeneous so that the basic solutes interact less due to problems of steric hindrance, while in the spherical supports the distribution is more homogeneous and thus results in a higher interaction with the basic solutes, creating irreversible adsorption.

Atomic absorption spectrometry found that the spherical silicas have higher sodium and iron contents than the irregular silicas. For example, for the Sigma silica the sodium content is 1 070 μg g^{-1} while for 8 and 5 μm Spherisorb it is, respectively, 4 315 and 2 070 μg g^{-1} and for iron it is 83, 263, and 123 μg g^{-1} for Sigma and 8 and 5 μm Spherisorb, respectively. As the content of these metals in the spherical silicas is higher than in the irregular ones, the basic compounds are more retained, up to complete adsorption, as is the case of the 5 μm Spherisorb.

It is important to point out that excellent results are obtained with the 50% (initial) PMOS on Lichrosorb Si-60, 10μm packing material, that the analysis time is shorter than for the other columns, and furthermore, the three toluidines elute together (see Figure 2), indicating a column of good quality.[11,12]

This represents an advantage of this phase in relation to bonded phases which need an endcapping reaction to reduce the effect of residual silanols. However, this packing material has, after packing, a low (8.5%) C content, which accounts for the short analysis time and low retention factor (Figure 1 and Table 2, respectively).

CONCLUSIONS

The chromatographic behavior of coated stationary phases depends on the properties of the silica base material. These properties may differ, despite having the same particle shape and diameter. In general, the specific surface area of the silica support has a significant impact on the solute retention of the prepared phases. It is important to note that coated stationary phases show excellent chromatographic properties, even for basic compounds.

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