Journal of Chromatography, 316 (1984) 163–181
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 500

INFLUENCE OF SUBSTRATE PARAMETERS ON COLUMN SELECTIVITY WITH ALKYL BONDED-PHASE SORBENTS

LANE C. SANDER* and STEPHEN A. WISE
Organic Analytical Research Division, Center for Analytical Chemistry, National Bureau of Standards, Gaithersburg, MD 20899 (U.S.A.)

SUMMARY

Differences in bonded-phase properties were studied for monomeric and polymeric C_{18} phases prepared on a variety of silica substrate materials. A total of 22 silicas with pore diameters ranging from 50–1000 Å were used in syntheses. Phase loadings for the resulting bonded phases ranged from 1.3–5.4 μmol/m^2. Physical properties of the substrates including surface area, pore volume, packing density, and background carbon were measured prior to bonding. Large differences were observed in the properties of the silica substrates and in the chromatographic behavior of the resulting phases. Differences in selectivity as well as absolute retention were observed as a function of pore size, with the greatest changes in selectivity occurring for the polymeric phases. The effect of silica pretreatment on phase synthesis and column selectivity was also examined for wide- and narrow-pore substrates. Phases prepared from silica pretreated with acid had greater polymeric character than those prepared from base-pretreated silica. Variation in phase loading and column selectivity is thought to be a function of both the reactivity of the silica surface and pore size. A model for polymeric phase synthesis is proposed where the extent of reaction is limited by a size-exclusion mechanism.

INTRODUCTION

The study of bonded stationary phases for use in liquid chromatography (LC) remains an area of considerable interest both from theoretical and practical points of view. Part of this interest is a result of differences that exist among columns of the same phase type. In previous work[1,2] we have shown that selectivity differences exist between monomeric and polymeric C_{18} phases for the separation of polycyclic aromatic hydrocarbons. Others have reported differences among C_{18} columns for the separation of acidic and basic compounds[3,4], nucleotides[5], quinones[6], and a variety of other solutes[7–10]. Such differences have prompted investigations into the effect of certain bonded-phase parameters on retention. The effects of phase length[11–14], surface coverage[2,15,16], carbon loading[17], and endcapping[18,19] have been studied in detail. To a lesser extent, the effect of polymerization of the bonded phase has also been examined[1,20]. Relatively little work, however, has involved changes in column selectivity as a function of the silica substrate.

Silica substrates prepared by various manufacturers differ in a number of important ways. Surface area, pore size and pore volume are interrelated variables that directly affect retention and mechanical stability of bonded-phase sorbents. For constant pore volume, surface area increases with decreasing pore diameter. Similarly, for a given pore diameter, surface area is dependent on pore volume, and thus increases with substrate porosity. In reversed-phase systems, solute retention increases with surface area simply due to increased phase ratio —more bonded phase is contained within the column. Other parameters that can affect chromatographic performance include particle shape[21,22], size[23] and size distribution[24]. Chemical composition of the silica substrate undoubtedly affects retention behavior. Engelhardt and Muller[3] have shown that reversed-phase separations of amines are improved by pretreating the substrate with a base prior to bonded phase synthesis. Separations of phenols are enhanced by acid pretreatment of the silica. Furthermore, some evidence suggests that the presence of trace elements in the silica matrix may adversely affect chromatographic performance[25,26].

Although pore size and structure are important considerations in size-exclusion chromatography, these parameters have received little attention in the study of bonded-phase systems. Because most solutes chromatographed under reversed-phase conditions are of low molecular weight, size-exclusion retention mechanisms are seldom considered important. Most substrates currently used with bonded phases have pore diameters of 100 Å or less. Surface area values for such substrates are high, and this results in high load capacities. A limit of about 60 Å is usually imposed for C_{18} phases. The C_{18} alkyl chain, when fully extended, is about 24 Å long, and thus it can be envisioned that very small pores might limit surface modification reactions due to steric constraints[27]. Studies of the thermodynamic consequences of using very small-pore bonded sorbents are in progress[28].

In this work the effect of silica pore size on selectivity and retention is examined for a series of monomeric and polymeric alkyl C_{18} phases. Polycyclic aromatic hydrocarbon probes are used in the evaluation of the phases. Differences between wide- and narrow-pore substrates, modified with monomeric and polymeric C_{18} phases, are further studied by size-exclusion chromatography. In the remainder of this study, the effect of silica pretreatment on polymeric phase synthesis and column selectivity is examined for acid- and base-washed silicas.

EXPERIMENTAL*

Reagents

Silane reagents were purchased from Petrarch Systems (Bristol, PA, U. S.A.) and were used without further purification. Chromatographic grade solvents were used in all syntheses and LC separations. Mixtures of 16 polycyclic aromatic hydrocarbons (PAHs), Standard Reference Material (SRM) 1647 (National Bureau of Standards, Gaithersburg, MD, U.S.A.) and phenanthro[3,4-*c*]phenanthrene (PhPh),

---

* Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Bureau of Standards (NBS), nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

benzo[*a*]pyrene (BaP), and 1,2:3,4:5,6:7,8-tetrabenzonaphthalene (TBN) were used in column evaluation. 800-, 2000-, 4000-, 9000-, 17500-, 50000-, 111000-, 233000- and 867000-dalton polystyrene standards were used for column evaluation.

Silica substrates

Twenty-two different silica substrates were used in the preparation of the bonded phases evaluated in this study. Nominal pore diameters as quoted by the manufacturers ranged from 50 to 1000 Å (see Table I). In order of increasing pore size, the substrates used were Nucleosil (50 Å) (Macherey, Nagel & Co., Düren, F.R.G.), LiChrosorb (60 Å) (E. Merck, Darmstadt, F.R.G.), Polygosil (60 Å) (Macherey, Nagel & Co.), RSiL (60 Å) (Alltech, Deerfield, IL, U.S.A.), Vydac HS (60 Å) (discontinued product, Separations Group, Hesperia, CA, U.S.A.), Zorbax (60 Å) (experimental sample, DuPont, Wilmington, DE, U.S.A.), Econosphere (80 Å) (Alltech), RoSiL (80 Å) (Alltech), Partisil (85 Å) (Whatman, Clifton, NJ, U.S.A.), Vydac HS (90 Å) (Separations Group), LiChrosorb (100 Å) (E. Merck), LiChrospher (100 Å) (E. Merck), Zorbax (100 Å) (experimental sample, Dupont), Hypersil (120 Å) (Shandon, Sewickley, PA, U.S.A.), Zorbax (150 Å) (experimental sample, DuPont), Hypersil WP (300 Å) (pre-production sample, Shandon), LiChrospher (300 Å) (E. Merck), Protosil (300 Å) (Whatman), Zorbax (300 Å) (experimental sample, DuPont), Vydac TP (330 Å) (Separations Group), LiChrospher (500 Å), (E. Merck) and LiChrospher (1000 Å) (E. Merck).

TABLE I

PHYSICAL PROPERTIES OF SILICA SUBSTRATES

Silica	*Particle diameter (μm)*	*Particle shape**	*Pore diameter (Å)*	*Area* (m^2/g)	*Pore volume (ml/g)*	*Density* (g/cm^3)	*Background carbon (%)*	*pH*
Nucleosil	10	s	50	316				
LiChrosorb (60)	10	i	60	398	0.66	0.44	0.17	7.2
Polygosil	10	i	60	245	0.71	0.43	0.07	6.5
RSil	10	i	60	433	0.75	0.42	0.85	8.0
Vydac HS (60)	5	s	60	287	0.44	0.59	0.21	4.8
Zorbax (60)	10	s	60	432	0.50	0.57	0.16	5.6
Econosphere	5	s	80	171	0.52	0.62	0.22	9.0
RoSil	8	s	80	357	0.71	0.51	0.14	8.4
Partisil	10	i	85	429	0.72	0.44	0.27	4.9
Vydac HS (90)	10	s	90	423			0.82	4.2
LiChrosorb (100)	10	i	100	297	1.11	0.36	0.06	6.7
LiChrospher (100)	10	s	100	266			0.14	4.6
Zorbax (100)	7	s	100	139	0.45	0.66	0.08	3.8
Hypersil	10	s	125	149			0.08	8.2
Zorbax (150)	7	s	150	99	0.39	0.68	0.06	4.8
Hypersil WP-300	5	s	300	57			0.10	5.2
LiChrospher (300)	10	s	300	207			0.18	4.7
Protosil	10	i	300	257			0.11	4.6
Zorbax (300)	8	s	300	39	0.28	0.72	0.06	5.4
Vydac TP	10	s	330	82	0.73	0.50	0.38	4.1
LiChrospher (500)	10	s	500	59			0.06	8.6
LiChrospher (1000)	10	s	1000	32			0.05	8.1

* i = Irregular, s = spherical.

Specific surface area measurements were performed on each of the substrates with a Quantasorb BET surface analyzer. Multipoint BET measurements were made at three or more helium/nitrogen compositions within the range $0.1 \leqslant P/P_0 \leqslant 0.35$, and in all cases the linear regression fit parameters were better than 0.998. P Refers to the partial pressure of the adsorbate (nitrogen) in the flow mixture, and P_0 to the saturated vapor pressure of the adsorbate. The accuracy of the measurements was estimated by analyzing an alumina surface standard, SRM 2004 (Quantachrome, Syossett, NY, U.S.A.). An average of 8 replicate measurements (3 points per sample) gave a value of 237 ± 4 m^2/g, which compares favorably with the certified value for the standard, 239 ± 2.6 m^2/g. The relative pH of the substrates was investigated by adding 1 g of silica (as received from the manufacturer) to 10 ml of deionized water (pH 7 ± 0.5). The pH of the suspended slurry was measured immediately upon mixing. Carbon analyses were performed in the NBS Inorganic Analytical Research Division. For most of the bonded phases analyzed, triplicate measurements were made and the relative standard deviations of the measurements were better than 1%.

Column preparation

Monomeric phases were prepared by reaction with dimethyloctadecylchlorosilane under conditions intended to give maximum coverage[1]. Approximately 3 g of silica were added to a solution of 100 ml of carbon tetrachloride containing a 10-fold excess of silane (based on two bonded groups/nm^2). The slurry was refluxed for 4 h, filtered, washed, and dried. Polymeric phases were prepared by reaction with octadecyltrichlorosilane in the presence of water. A 10-ml aliquot of the silane reagent was added to 100 ml of carbon tetrachloride in a 500-ml vessel. The mixture was heated to boiling and 3.0 g of dry silica was added. After adding 0.5 ml water, the slurry was refluxed for 4 h. At the conclusion of the reaction, the silica was filtered, washed and dried in the usual manner[1]. Phases were not endcapped. All columns were prepared from 25 cm × 4.6 mm tubing.

Silica pretreatment

The effect of acid and base pretreatment was studied for Polygosil silica. A 3.5-g sample of Polygosil was refluxed in 100-ml of 1.0 *M* hydrochloric acid for 75 min. The treated silica was filtered and washed until the pH of the filtrate was equal to that of distilled water. Less rigorous conditions were employed for aqueous base pretreatment. A second sample of Polygosil was suspended in 0.1 *M* sodium hydroxide solution for 3 min and then filtered and washed. Both silicas were dried at 150°C under reduced pressure for 2 h. Monomeric and polymeric phases were synthesized on each substrate as described above.

Chromatography

Separations were performed with a liquid chromatograph consisting of two reciprocating piston pumps, a 20-μl loop injector, solvent programmer, and a 254-nm UV detector. For size-exclusion studies, a refractive index detector was used with toluene as the mobile phase. Separations of SRM 1647 were made with a linear gradient; 40–100% acetonitrile in water over 45 min, at a flow-rate of 2 ml/min. The three-component PAH mixture was chromatographed isocratically at 85% acetonitrile/water.

RESULTS AND DISCUSSION

Substrate parameters

The pore size studies carried out in this work were performed on a series of silica substrates with pore diameters ranging from 50–1000 Å (see Table I). A number of physical parameters vary markedly among these silicas. Specific surface areas were measured for each of the substrates using the multipoint BET technique[29]. Surface areas varied from 32–433 m^2/g. In the course of this work, several lots of Vydac silica were analyzed. The lot to lot variation in surface area for this one substrate was approximately 12%. Differences between the measured values and the nominal values quoted by the manufacturers were often much larger, sometimes approaching 50%. The pore volume and packing density of selected substrates were measured by preparing silica columns with tared column blanks. The pore volume was taken as the difference in retention volume of toluene and a high-molecular-weight polystyrene standard using tetrahydrofuran as the mobile phase. The packing density of each substrate was calculated from the weight difference between the packed silica column and the column blank. Solvent was removed from the column by flushing with supercritical carbon dioxide at 70°C. Finally, the carbon background of the unbonded silica was measured for each of the substrates. Measurable carbon backgrounds were observed for each silica. RSiL and Vydac HS silicas had the highest residual carbon values, Vydac TP and Partisil silicas intermediate backgrounds, while most of the remaining silicas had only trace levels of carbon. While the chromatographic effect of residual carbon has not been explored, it is clear that an accurate assessment of bonded-phase surface coverage must include correction for background carbon, at least when substrates with high background levels or low phase loadings are employed. The pH measurements are reported for information value only. Engelhardt and Muller[3] have demonstrated the utility of this measurement, especially when acidic or basic solutes are to be chromatographed.

Bonded-phase syntheses

Two types of C_{18} phases were prepared on the substrates: monomeric and polymeric. Reaction conditions were carefully controlled for the two phase types so as to produce phases as similar as possible among the different pore size silicas. Table II lists percent carbon loadings and phase coverages for the columns.

Phase coverage values for the polymeric phases were about twice those for the monomeric phases. The highest coverages for polymeric phase syntheses were obtained with wide pore substrates. A plot of polymeric phase coverage versus nominal pore diameter is shown in Fig. 1. The curve shown is plotted only for the four Zorbax substrates which are all manufactured under similar conditions[30]. Although the relationship of phase coverage to pore size is not unequivocal, it is apparent that a general trend toward lower phase coverages exists for the smaller pore substrates. The variation that we have observed in surface coverage among different substrates of the same pore diameter suggests that factors other than pore size, such as pore size distribution, also affect phase loading. These variations cannot be accounted for by irreproducibility of the bonding process. In past work polymeric phase syntheses were found to give highly reproducible phase coverages and column selectivities for syntheses carried out on a single lot of silica, and also for multiple lots of the same silica when corrections were made for the surface area of each individual lot[1].

TABLE II

COLUMN PROPERTIES

α = Selectivity factor; k' = capacity factor.

No.	*Silica*	*Phase type*	*Pore diameter (Å)*	*Area (m^2/g)*	*Carbon loading (%)*	*Surface coverage ($\mu mol/m^2$)*	$\alpha_{TBN/BaP}$	k'_{BaP}
23	Zorbax (300)	monomeric	300	39	2.16	2.31	2.12	3.46
09	Vydac TP	monomeric	300	96	5.80	2.53	2.00	1.57
74	Zorbax (100)	monomeric	100	139	8.30	2.76	1.97	4.56
76	Zorbax (150)	monomeric	150	99	5.00	2.22	1.82	2.34
79	Zorbax (60)	monomeric	60	432	11.32	1.26	1.78	3.18
101	Zorbax (60)	polymeric	60	432	17.70	2.57	1.33	8.42
99	Zorbax (100)	polymeric	100	139	10.05	3.92	1.25	8.32
63	RSil	polymeric	60	433	22.22	3.39	1.21	10.93
84	Econosphere	polymeric	80	171	11.50	3.69	1.18	8.55
71	Partisil	polymeric	85	429	21.91	3.49	0.97	11.11
83	LiChrosorb (100)	polymeric	100	297	19.82	4.41	0.97	9.41
62	LiChrosorb (60)	polymeric	60	398	22.00	3.81	0.93	12.68
39	LiChrospher (1000)	polymeric	1000	32	3.16	4.72	0.92	1.56
82	RoSil	polymeric	80	357	20.10	3.72	0.90	12.61
38	LiChrospher (500)	polymeric	500	59	5.38	4.54	0.89	2.77
86	Polygosil	polymeric	60	245	17.08	4.34	0.86	9.93
103	Zorbax (300)	polymeric	300	39	4.28	5.35	0.86	4.21
85	Vydac HS (90)	polymeric	90	423	23.98	3.92	0.84	13.28
105	Zorbax (150)	polymeric	150	99	8.89	4.77	0.83	9.88
77	Hypersil	polymeric	120	149	13.53	5.26	0.79	11.73
40	Nucleosil	polymeric	50	316	18.38	3.74	0.75	10.71
37	LiChrospher (100)	polymeric	100	266	19.48	4.78	0.71	11.42
33	LiChrospher (300)	polymeric	300	207	16.43	4.83	0.71	6.57
61	Vydac HS (60)	polymeric	60	288	16.11	3.38	0.69	13.60
32	Hypersil	polymeric	300	57	5.71	5.03	0.66	4.93
28	Protosil	polymeric	300	257	20.06	5.17	0.65	13.07
27a	Vydac TP	polymeric	330	94	9.15	4.99	0.62	6.77

The source of the differences of bonding chemistry for silicas from different manufacturers remains elusive. One can speculate that the surface activity or silanol concentration of each silica may vary depending on the steps involved in the silica synthesis. If such differences are responsible for differences in the nature of the bonding, then some type of pretreatment of the silica might be advised to obtain better phase reproducibility between silicas. Acid pretreatment procedures have been proposed that would increase the concentration of surface silanols[31,32]. In the current work, with the exception of the silica pretreatment study involving Polygosil, no attempt was made at silica pretreatment so that any changes that might occur in pore diameter or structure would be avoided.

Bonded-phase selectivity differences

Two mixtures of PAHs were used in the evaluation of the phases. PAH probes were chosen to avoid complications from solute–silanol interactions. The primary retention mechanism for these solutes is assumed to be hydrophobic retention. The

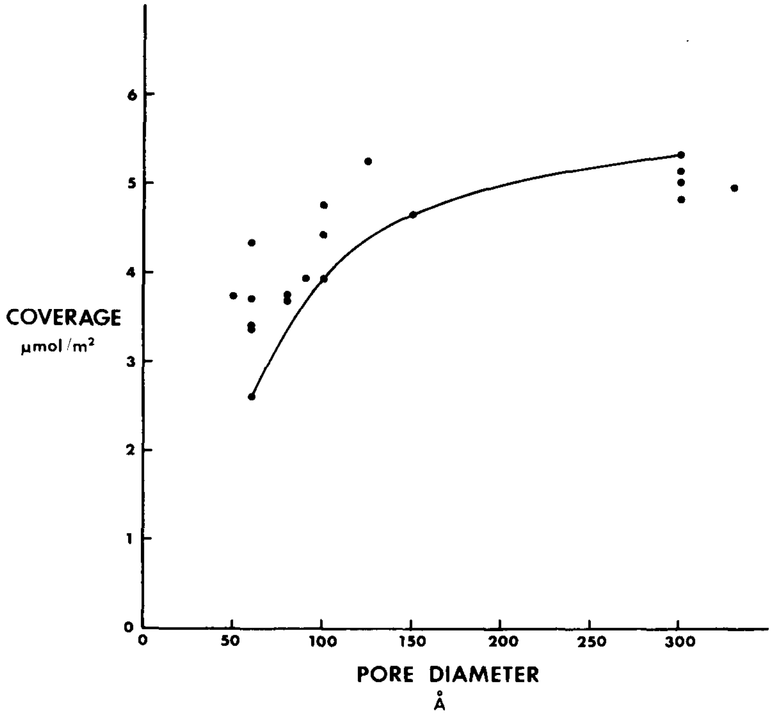

Fig. 1. Bonded-phase surface coverage, plotted as a function of nominal pore diameter for polymeric phase syntheses. The curve shown is for 60, 100, 150 and 300 Å pore diameter Zorbax substrates.

three-component PAH mixture containing BaP, PhPh and TBN has been shown to be of value in distinguishing between polymeric and monomeric C_{18} phases[1].

Representative separations of the two mixtures for monomeric phases are illustrated in Fig. 2. Four columns are shown: monomeric phases, prepared on 60, 100, 150 and 300 Å pore diameter substrates. To eliminate complications from differences in substrate properties (among materials from different manufacturers), these columns were prepared on a single silica type: Zorbax. One trend is apparent. With the exception of the 60 Å Zorbax phase, retention decreases with increasing pore diameter for the monomeric phases. This trend is expected since the surface area of the large-pore-diameter substrates is less than for the small-pore substrates. Retention is lower than expected for the 60 Å phase probably because of the lower than normal phase loading for this substrate (see Table II). The selectivities among monomeric phases toward PAHs do not, however, change significantly with pore size. The elution order of the compounds in SRM 1647 and the three-component mixture is constant. Resolution of each mixture is best with the smaller pore silicas, simply because retention is greater.

More significant differences are observed with polymeric phases (Fig. 3), in which column selectivity does change as a function of pore diameter. Unlike the monomeric phases, the best separations occur on larger pore diameter substrates. Components 9 and 10, benz[*a*]anthracene and chrysene, are unresolved on the 60 Å

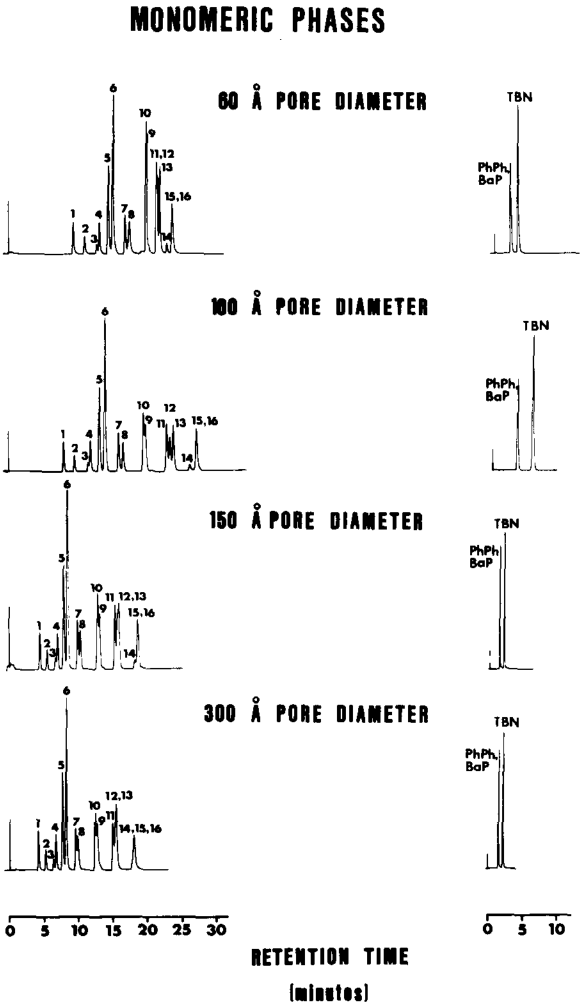

Fig. 2. Separation of 16 polycyclic aromatic hydrocarbons (SRM 1647) on representative monomeric phases, prepared on Zorbax substrates. The separation was performed by using gradient elution, 40–100% acetonitrile in water over 45 min at 2 ml/min. The three-component mixture was chromatographed isocratically with acetonitrile–water (85:15). Peaks: 1 = naphthalene; 2 = acenaphthylene; 3 = acenaphthene; 4 = fluorene; 5 = phenanthrene; 6 = anthracene; 7 = fluoranthene; 8 = pyrene; 9 = benz[*a*]anthracene; 10 = chrysene; 11 = benzo[*b*]fluoranthene; 12 = benzo[*k*]fluoranthene; 13 = benzo[*a*]pyrene; 14 = dibenz[*a*,*h*]anthracene; 15 = benzo[*ghi*]perylene; 16 = indeno[1,2,3-*cd*]pyrene; PhPh = phenanthrophenanthrene; TBN = tetrabenzonaphthalene; BaP = benzo[*a*]pyrene.

polymeric column. Partial separation occurs on the 100 Å column, and baseline separation on the 150 Å and 300 Å columns. Similar changes occur for components 3 and 4 (acenaphthene and fluorene), and 15 and 16 (benzo[*ghi*]perylene and indeno[1,2,3-*cd*]pyrene). Even more dramatic changes in selectivity occur for the three component "phase type" test mixture. The elution order on the 60 and 100 Å polymeric phase columns is PhPh, BaP, and TBN. On the larger pore substrates, the order of

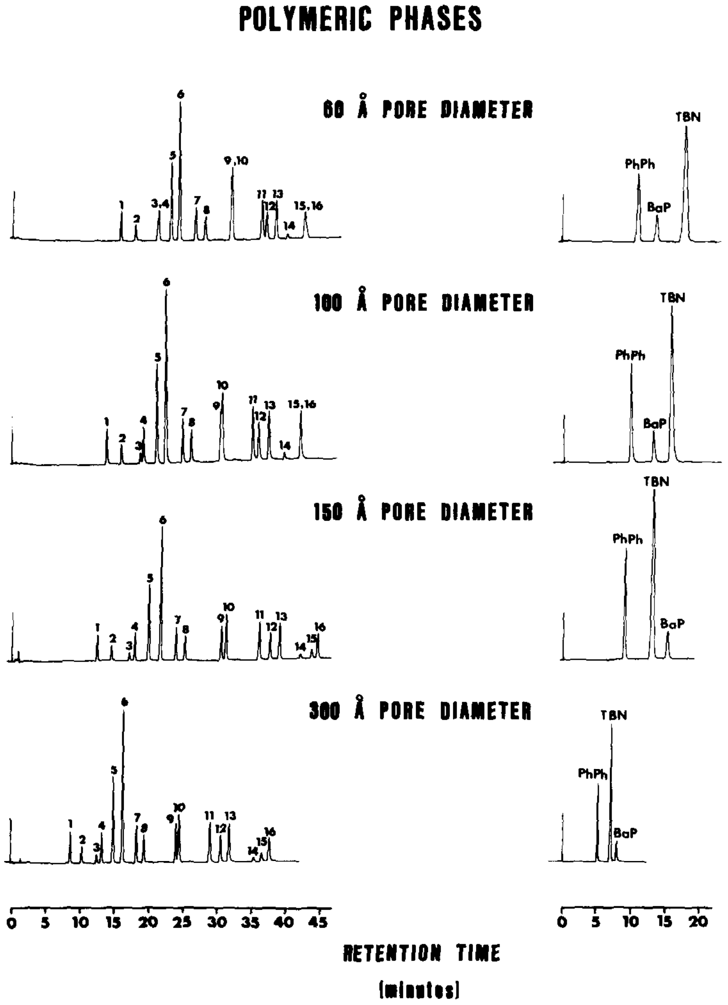

Fig. 3. Separation of SRM 1647 on polymeric phases, prepared on substrates with varying pore diameter. Chromatographic conditions and peak identification as in Fig. 2.

elution is PhPh, TBN, and BaP. In previous studies the elution order of these solutes was found to be dependent on phase type, *i.e.*, monomeric, polymeric or oligomeric[1]. The 60 and 100 Å polymeric phases in this study exhibit atypical retention behavior, and actually have selectivity toward PAHs similar to monomeric and oligomeric phases.

The basis for selectivity differences among C_{18} polymeric phases, prepared on different pore size substrates, cannot adequately be explained by a size-exclusion separation mechanism. PAH molecules with molecular weights of about 400 or less are too small to undergo significant size differentiation in 60–300 Å diameter pores. Furthermore, changes in column selectivity do not occur among the monomeric phases of different pore size. Selectivity changes would be expected on monomeric and polymeric phases alike if the origin of the differences were size exclusion. Finally, the trends observed among the polymeric phases are contrary to those expected for size exclusion: separation of PAHs improves with increasing pore diameter. Maximum resolution of small solutes would be predicted to occur for small pore diameter substrates. These arguments suggest that the makeup of the bonded phase itself might be altered as a consequence of pore size.

Differences in column selectivity among the polymeric phases can be explained in terms of a size exclusion mechanism limiting reaction during bonded phase synthesis. For the synthesis of monomeric C_{18} phases, phase coverage should be relatively insensitive to substrate pore size as long as the pores are large enough to allow silane molecules to diffuse inside the pore network and react with the surface. A limit is reached for very small pore substrates where surface modification might result in pore blockage. Also, Berendsen *et al.*[27] have suggested that phase coverage may be reduced for small pore substrates due to steric effects of long alkyl chains attached to a curved surface. Except in such limiting cases, the phases produced should be similar, and independent of pore size.

For polymeric surface modification, a mixture of two species exists in solution: silane monomer and silane polymer molecules. These species are each reactive and can covalently bond to the silica. Because silane polymer molecules are larger than monomers, differences in the types of bonded phases produced occur as a function of pore size (see Fig. 4). Unger[32] has stated that the diffusion and kinetics of reaction of silane molecules may be affected by pore diameter. Thus, silane molecules of different molecular weight can be expected to react with the substrate at different rates within the pore network. In the limiting case, a monomeric phase would result for a small pore substrate, if the silane polymer was excluded and only the monomer was accesssible to the pore network (see Fig. 4A). In practice, a competition exists between the silane monomer and polymer molecules which is limited by the accessible surface area of the silica. The resulting polymeric phase probably consists of monomeric C_{18} chains interspersed with C_{18} polymers. Differences among polymeric syntheses performed on substrates of different pore diameter can be attributed to the degree of reaction of polymer molecules with the silica surface. Because of the size of the polymer molecules, the mobility of these species is limited within small pores. Using an analogy to size exclusion chromatography, during a given period of time, more of the pore volume will be accessible to the small monomeric silane molecules than to the larger polymeric silane molecules. This trend is strongest for small pore substrates. With increasing pore size, a larger fraction of the pore volume is available to the silane polymers and increased polymeric phase character can result (Fig. 4B). Competition of the silane polymer molecules for reaction at the silica surface is most effective with large-pore substrates (Fig. 4C). Hence, the polymeric nature of bonded-phase sorbents increases with increasing pore diameter. It should be noted that, regardless of pore size, the exterior surface of the silica particles will be modified with a polymeric layer. While the exterior surface area of silica particles is usually small compared to the area originating from the pores, the significance of the externally bonded polymeric phase on retention and selectivity is as yet unknown.

The phase resulting from such a reaction is essentially a mixture of monomeric and polymeric surface groups. In this model, the surface of a lightly loaded polymeric phase has isolated polymeric groups with surrounding regions modified by mono-

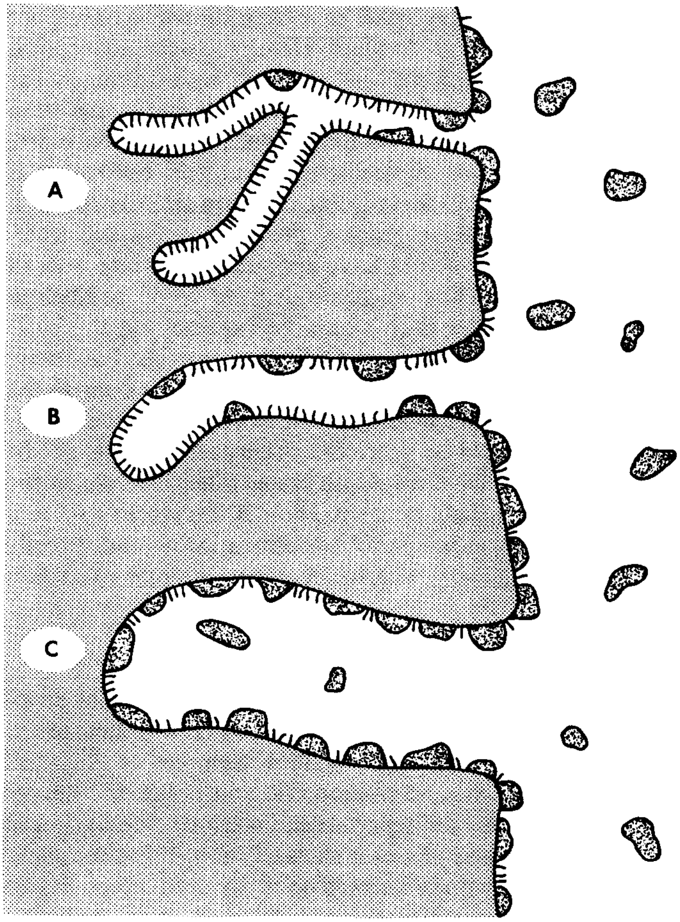

Fig. 4. Schematic representation of a size-exclusion mechanism that may limit polymeric coverage during phase synthesis.

meric C_{18} chains. For heavier polymeric phases, the number of polymeric groups attached to the surface increases, and the distance between the groups decreases. If PAH solutes interact differently with the polymeric C_{18} groups than with monomeric C_{18} chains, this model could account for the changes in selectivity observed as a function of polymeric phase loading.

Not all of the substrates examined in Table II follow the trend of reduced polymeric behavior with decreasing pore size. Notable exceptions are Nucleosil (50 Å), Polygosil (60 Å), Vydac HS (60 Å) and LiChrospher (100 Å) substrates. The corresponding polymeric phase columns prepared from the substrates (columns 40, 86, 61, and 37, respectively) exhibit higher selectivity towards PAHs than were observed for other small-pore substrates. Furthermore, the LiChrospher (500 Å) and (1000 Å) substrates demonstrated slightly lower PAH selectivity than expected, as evidenced by the selectivity factor, $\alpha_{TBN/BaP}$.

Although the source of these anomalies is not known, several explanations can be proposed. At least two of the silica substrates listed have been shown to have pore diameters significantly different from the nominal values. Using nitrogen adsorption and desorption isotherm measurements, pore size distributions were obtained for Nucleosil and Polygosil[33]. Both substrates were found to have larger pores than expected, which accounts for the observed shifts in column selectivity. Furthermore, the pore network in silica does not consist of monodisperse channels, but rather is made up of a range of pore sizes. It can be expected that the pore size distribution will affect the nature of bonding and chromatographic properties of a substrate. Substrates with wide pore size distributions would have appreciable surface area in pores larger and smaller than the mean. Thus, such a substrate could produce a bonded phase with more polymeric character than would be expected on the basis of its average pore size. Pore shape may also influence sorbent properties. Polymeric phase loadings might well be expected to be reduced for substrates with constricted pore openings. Such "ink bottle" shaped pores would permit passage of silane monomers, but entrance of larger molecules would be restricted. Alternatively, it might be suggested that the chemical properties of the various substrates differ, at least at the silica surface. For example, the surface silanol concentration is known to decrease as silica is heated above 200°C[32]. Variation in silanol concentration would directly affect bonded phase coverage. Other chemical properties may vary among substrates as well.

Effect of silica pretreatment

Experiments were carried out to investigate ways in which the chemical properties of silica substrates might be altered. The effect of acid and base pretreatment of silica on phase synthesis was examined for Polygosil silica. Samples of Polygosil were treated with acidic and basic solutions, and monomeric and polymeric phases were synthesized on the resulting substrates (see experimental section). Separation of PhPh, TBN and BaP on each of the polymeric phases is shown in Fig. 5. Pretreatment was observed to have a significant effect on the surface coverage and selectivity of the phases prepared. Surface coverage values for the base-washed, untreated, and acid-washed substrates were determined to be 3.92, 4.48, and 4.52 μmol/m^2, respectively. The greatest polymeric phase selectivity (largest selectivity factor $\alpha_{TBN/BaP}$) was observed for the silica pretreated by acid reflux. The base-rinsed silica phase had significantly different selectivity than the untreated silica phase. Similar trends were observed for pretreated Vydac TP silica. The shift in selectivity was away from the polymeric trend. In contrast to the polymeric phases, no significant differences in retention or selectivity were observed among monomeric phases prepared on pretreated silica. This suggests that acid/base pretreatment indirectly influences column selectivity of polymeric phases by altering phase synthesis (see below). Changes in selectivity are probably not due to changes in solute–substrate interactions, because monomeric phase retention behavior was unaffected by pretreatment. Measurement of the specific surface areas of the acid- and base-treated substrates indicated very little change in surface area, suggesting that the pretreatment procedures had little physical effect on pore structure. The untreated silica surface area was measured at 245 m^2/g, while the surface areas for the acid and base treated Polygosil were 253 and 250 m^2/g, respectively. Thus, it is doubtful that either pretreatment procedure caused changes in pore size sufficient to alter bonding characteristics.

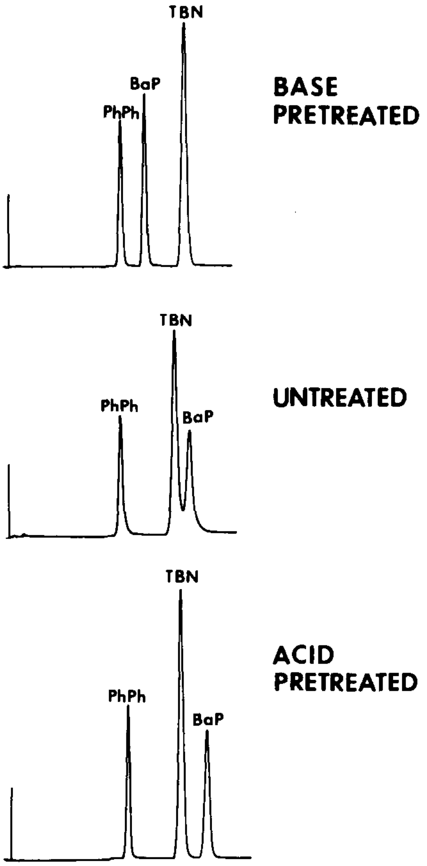

Fig. 5. The effect of silica pretreatment on phase selectivity. Polymeric phases were prepared on acid-treated, base-treated and untreated Polygosil substrates (see text).

An alternative explanation can be proposed in terms of the reactivity of the silica surface. Acid and base pretreatment of silica may affect the surface according to eqns. 1 and 2.

$$\text{(silica)–Si–OH} + \text{NaOH} \rightarrow \text{(silica)–Si–O}^{-}\text{Na}^{+} \quad (1)$$

$$\text{(silica)–Si–OH} + \text{HCl} \rightarrow \text{(silica)–Si–OH(HCl)} \quad (2)$$

Reaction of sodium hydroxide with the silica surface results in the abstraction of silanol protons and the formation of sodium silicate. Below a pH of 2, hydrochloric acid may add to silanols as in eqn. 2. Both acids and bases can initiate siloxane bond hydrolysis, with subsequent silanol production (eqn. 3).

$$\text{(silica)–Si–O–Si–(silica)} \rightarrow \text{(silica)–Si–OH HO–Si–(silica)} \quad (3)$$

Thus, pretreatment procedures may increase the number of silanols at the silica surface.

The reactivity of acid-treated silica has been addressed by Unger[32]. He states that "hydrochloric acid evolved [from chlorosilanes] may be preferentially adsorbed at the unreacted surface sites, impeding further attack by the reactant". Reactions of chlorosilanes with the sodium silanol salt, however, is energetically favored over reaction at untreated silanol sites due to the formation of an ionic salt by-product. Because the silane polymerization reaction is initiated after the addition of the silane to the silica slurry, monomeric phase modification may occur for a brief period. With the base-treated silica, reaction with the monomer would occur faster than with the acid-treated silica, and so would lead to a phase with greater monomeric character. Because the acid-treated silica is less reactive, fewer silanols may react initially and make more sites available for reaction with the silane polymer.

A combination of surface activity and pore size effects could explain much about the characteristics of the phases listed in Table II. The variations in column selectivity observed for polymeric phases synthesized on different substrates of the same pore diameter might thus be due to differences in the surface activity of each silica. This would vary with the manufacturing processes used to make the substrates. While general methods are well known for the preparation of chromatographic grade silica[32], specific procedures are often proprietary. The acid/base pretreatment experiment described above illustrates the need for details about the synthesis for each substrate used.

Size-exclusion studies

Differences between monomeric and polymeric C_{18} phases were further characterized by exclusion chromatography. Monomeric and polymeric phases were each prepared on intermediate- and wide-pore substrates (Polygosil and Vydac TP silicas, respectively). The columns were operated in a size-exclusion mode by using toluene as the mobile phase. A series of polystyrene standards ranging in molecular weight from 800 to 867,000 daltons were chromatographed. The permeation volume (V_m) was determined by injection of benzene, and the pore volume (V_p) from the difference between the permeation and exclusion (V_e) volumes. The resulting calibration curves for the smaller and wide-pore substrates are shown in Figs. 6 and 7. Significant changes in the size-exclusion properties of the sorbents are apparent after surface modification. For the narrow-pore substrate, both the monomeric and polymeric C_{18} phases cause a substantial reduction in pore volume. The monomeric phase reduces pore volume by 7%, while with the polymeric phase, pore volume is reduced 43% (see Table III). Correspondingly, a lower exclusion limit is observed for the polymeric phase than for the unmodified silica.

A less marked change was observed for the wide-pore substrate. Only a 3% pore volume reduction was measured for the monomeric phase, while a 15% reduction occurred with the polymeric phase. Changes in molecular weight exclusion limits were not observed. Interestingly, as shown in Figs. 6 and 7, the absolute exclusion volume decreases with surface modification. If similar packing characteristics are assumed for each of these substrates, this indicates that a reduction in the interparticle space occurs as a result of the bonded process. This is interesting because it has been estimated that less than 1% of the total surface area of a silica particle is due to the external surface (most of the surface area is within the pores). It would seem that the external surface is not a negligible factor in the study of bonded phases.

Following the size-exclusion studies on the narrow- and wide-pore substrates,

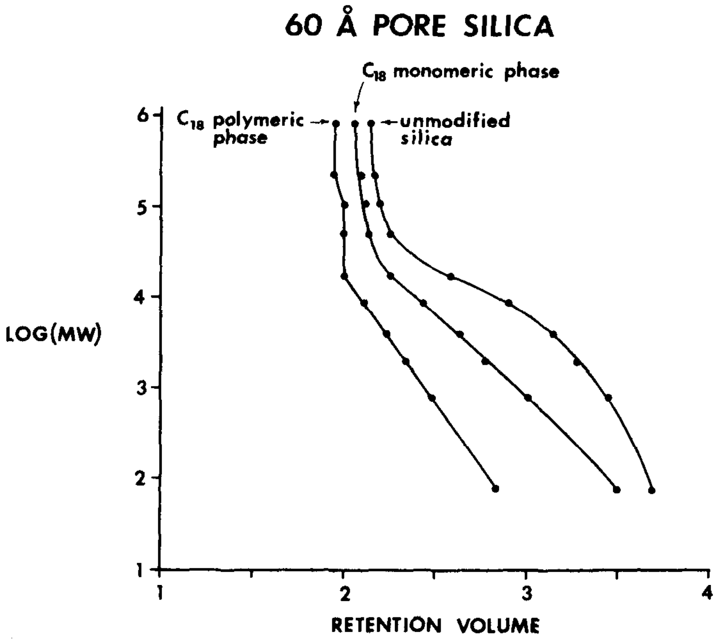

Fig. 6. Polystyrene calibration curves for unmodified, monomeric and polymeric C_{18} Polygosil silica. MW = Molecular weight. Retention volumes are in ml.

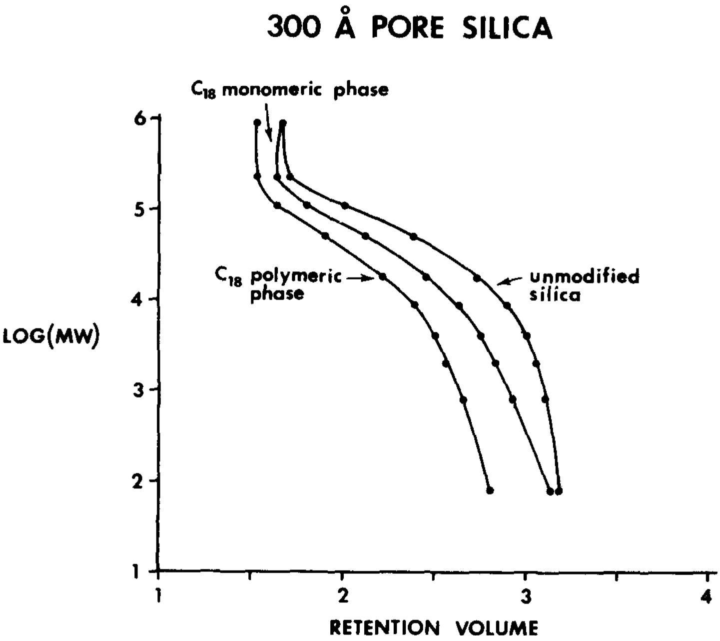

Fig. 7. Polystyrene calibration curves for unmodified, monomeric and polymeric C_{18} Vydac TP silica. Retention volumes are in ml.

TABLE III

SIZE-EXCLUSION PROPERTIES OF MODIFIED AND UNMODIFIED SUBSTRATES

Volumes listed are for 25 cm × 4.6 mm columns.

Substrate	V_m *(ml)*	V_e *(ml)*	V_p *(ml)*	*Volume fraction bonded phase**	*Particle porosity:* $V_p/(V_{tube} - V_e)$	*Packing density* *(g/cm³)*
Polygosil unmodified	3.69	2.14	1.55		0.77	0.45
Polygosil monomeric	3.50	2.06	1.44	7%	0.69	0.50
Polygosil polymeric	2.84	1.95	0.89	43%	0.40	0.66
Vydac TP unmodified	3.18	1.67	1.51		0.61	0.50
Vydac TP monomeric	3.14	1.67	1.47	3%	0.59	0.56
Vydac TP polymeric	2.81	1.53	1.28	15%	0.49	0.59

* $[V_{p(silica)} - V_{p(phase)}]/V_{p(silica)}$.

each column was carefully unpacked and the silica collected. This silica was then dried and weighed. These values, along with the column tubing volumes were used to calculate substrate densities. The "packing density" in Table III refers to the weight of the sorbent contained in the column, divided by volume of the column. These measurements were used to calculate substrate porosities, which are listed in Table III. The packing density can be seen to increase with bonded-phase loading. Since most of the bonded phase is contained within the pores of the substrate, no "extra room" is needed to accommodate the alkyl chains within the chromatographic tube. The bonded substrate particles are heavier than unbonded particles simply because of the mass of the alkyl chains within the pores.

Silane polymer analysis

To gain a better understanding of polymeric phase syntheses, an experiment was designed to characterize the makeup of a polymeric phase reaction slurry. The conditions for a polymeric phase synthesis were duplicated for this experiment, with one exception: no silica was present in the solution. A white precipitate formed immediately upon addition of water to the silane and carbon tetrachloride. Analysis of the reaction mixture by size-exclusion chromatography revealed the presence of a high-molecular-weight component. The elution volume for this component corresponded to a polystyrene standard of molecular weight 5000. The concentration increased rapidly with time, but leveled off after about 60 min (see Fig. 8). After the initial addition of water to the solution, the white precipitate slowly dissolved over a period of about 10 minutes, leaving a clear solution. Water remained in the mixture as a separated phase. Because size-exclusion chromatography achieves separation through differences in hydrodynamic volume, the exact molecular weight of the polymer cannot be determined by direct comparison with the polystyrene standards. These experiments did however disprove the existence of a very-high-molecular-weight polymer (greater than 5000 daltons) in solution.

The dissolution of the silane polymer over time raises the question of how this affects bonded phase synthesis. The above experiment was repeated and the solution was refluxed for 15 min. To this clear solution was added 3.5 g of Vydac TP silica.

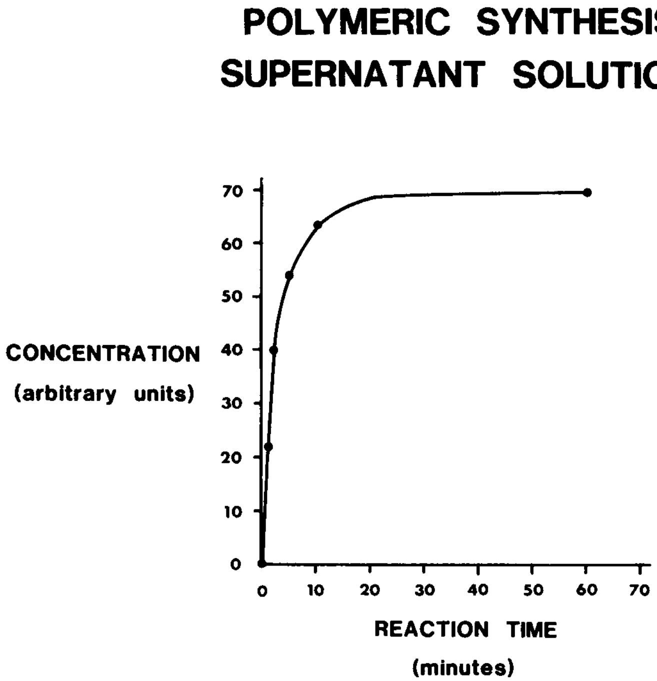

Fig. 8. Silane polymer concentration in solution, plotted as a function of time after the initial addition of water to the reaction mixture.

The slurry was then refluxed for 4 h. In effect, this bonded-phase synthesis is identical to the procedure normally used, with the exception that silane polymer solution was refluxed for a short time prior to the addition of silica. Interestingly enough, the column produced from this synthesis exhibited solute retention and selectivity nearly identical to Vydac monomeric phases. Thus, polymeric phase formation probably occurs during the first few minutes of reaction after the addition of water to the slurry. The white precipitate that forms clearly has an important role in the polymeric phase synthesis, since the phases produced after this precipitate dissolves are monomeric in nature. It can be envisioned that the precipitate might easily coat the exterior of the silica particles, but entry within the pore network would remain restricted. To a limited extent, the size-exclusion model of polymeric phase formation might still apply, even though the low-molecular-weight silane polymer that is formed in solution over time apparently does not affect the characteristics of the bonded phase synthesized. This could explain why phases produced on some of the small-pore silica substrates had more monomeric character than similar phases on wide-pore substrates. Obviously, differences in surface activity between substrates complicate any analysis of reaction mechanisms and make a definitive explanation of the situation difficult. A second possibility exists for the formation of the polymeric phase: polymerization may occur at the silica surface by monomer addition to other silane monomers bonded to the surface. Neither reaction scheme has yet been proven, and it is possible that each occurs concurrently.

CONCLUSIONS

Although phase reproducibility is readily controlled for a given silica substrate, the results of this study clearly demonstrate that large variations in phase loading and column performance may result when syntheses are performed on different substrates. Two parameters have been observed to affect bonded phase syntheses: substrate pore diameter and silica pretreatment. Differences in column selectivity resulting from these effects are most pronounced with polymeric syntheses. In general, the relative polymeric phase character decreases for phases synthesized on narrow-pore (compared to wide-pore) substrates. Similarly, phases with low polymeric character result after basic silica pretreatment. Other substrate parameters which have not been considered in this study may further influence phase characteristics. Until all of these effects are better understood, it may be necessary to specify the sources of silica substrates in order to describe bonding schemes adequately.

ACKNOWLEDGEMENTS

We thank Barry Diamondstone (Inorganic Analytical Research Division, NBS) for performing the numerous carbon analyses on the bonded-phase sorbents, and Mary Ellen Goffredo and Debbie Johnson (Shandon Southern Instruments, Inc.), Frederic Rabel (Whatman Chemical Separations, Inc.), Richard Stout (E. I. duPont de Nemours and Co.), and Terry Yates (The Separations Group) for providing samples of narrow- and wide-pore silicas.

REFERENCES

1 L. C. Sander and S. A. Wise, *Anal. Chem.*, 56 (1984) 504–510.
2 S. A. Wise and W. E. May, *Anal. Chem.*, 55 (1983) 1479–1485.
3 H. Engelhardt and H. Müller, *J. Chromatogr.*, 218 (1981) 395–407.
4 C. Gonnet, C. Bory and G. Lachatre, *Chromatographia*, 16 (1982) 242–246.
5 M. Zakaria and P. R. Brown, *J. Chromatogr.*, 255 (1983) 151–162.
6 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 142 (1977) 213–232.
7 J. L. DiCesare and M. W. Dong, *Chromatogr. Newsl.*,10 (1982) 12–19.
8 B. Shaikh and J. E. Tomaszewski, *Chromatographia*, 17 (1983) 675–678.
9 G. Schomburg, A. Deege, J. Köhler and U. Bien-Vogelsang, *J. Chromatogr.*, 282 (1983) 27–39.
10 A. P. Goldberg, *Anal. Chem.*, 54 (1982) 342–345.
11 G. E. Berendsen and L. de Galan, *J. Chromatogr.*, 196 (1980) 21–37.
12 H. Hemetsberger, W. Maasfeld and H. Ricken, *Chromatographia*, 9 (1976) 303–310.
13 N. Tanaka, K. Sakagami and M. Araki, *J. Chromatogr.*, 199 (1980) 327–337.
14 C. H. Lochmüller and D. R. Wilder, *J. Chromatogr. Sci.*, 17 (1979) 574–579.
15 S. A. Wise, L. C. Sander and W. E. May, *J. Liquid Chromatogr.*, 6 (1983) 2709–2721.
16 M. C. Hennion, C. Picard and M. Caude, *J. Chromatogr.*, 166 (1978) 21–35.
17 P. Spacek, M. Kubin, S. Vozka and B. Porsch, *J. Liquid Chromatogr.*,3 (1980) 1465–1480.
18 C. Dewaele, P. Mussche and M. Verzele, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 5 (1982) 616–620.
19 C. H. Lochmuller and D. B. Marshal, *Anal. Chim. Acta*, 142 (1982) 63–72.
20 M. Verzele and P. Mussche, *J. Chromatogr.*, 254 (1983) 117–122.
21 H. Müller and H. Engelhardt, in I. Molnar (Editor), *Practical Aspects of Modern High Performance Liquid Chromatography*, Walter de Gruyter, New York, 1983, pp. 25–39.
22 M. Verzele, J. Van Dijck, P. Mussche and C. Dewaele, *J. Liquid Chromatogr.*, 5 (1982) 1431–1448.
23 D. E. Damagalska and C. R. Loscombe, *Chromatographia*, 15 (1982) 657–659.
24 C. Dewaele and M. Verzele, *J. Chromatogr.*, 260 (1983) 13–21.

25 M. Verzele, M. de Potter and J. Bhysels, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 151–153.
26 M. Verzele and C. Dewaele, *J. Chromatogr.*, 217 (1981) 399–404.
27 G. E. Berendsen, K. A. Pikaart and L. de Galan, *J. Liquid Chromatogr.*, 3 (1980) 1437–1464.
28 D. E. Martire, G. A. Oweimereen and L. C. Sander, unpublished results.
29 S. J. Gregg and K. S. W. Sing, *Adsorption, Surface Area and Porosity*, Academic Press, London, 2nd ed., 1982.
30 R. Stout, personal communication, June 1984.
31 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 2nd ed., 1979.
32 K. K. Unger, *Porous Silica*, Elsevier, Amsterdam, New York, 1979.
33 L. C. Sander, S. A. Wise and W. Robbins, unpublished results.