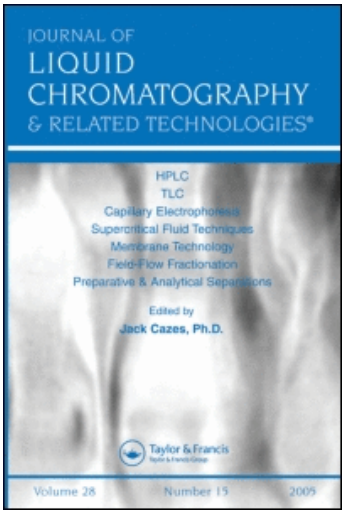

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High Performance Liquid Chromatographic Evaluation of a Low-Temperature Glassy Carbon Stationary Phase

C. T. Rittenhouse[a]; S. V. Olesik[a]
[a] The Ohio State University, Columbus, OH

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC EVALUATION OF A LOW-TEMPERATURE GLASSY CARBON STATIONARY PHASE

C. T. Rittenhouse, S. V. Olesik

The Ohio State University
100 W Eighteenth Ave
Columbus, OH 43210

ABSTRACT

A low temperature glassy carbon (LTGC) stationary phase coated on porous glassy carbon and zirconium oxide is chromatographically evaluated and compared to an uncoated porous glassy carbon stationary phase. The curing temperature of the LTGC was chosen to produce a surface having a reversed phase type retention mechanism. The LTGC coating of the porous glassy carbon reduced retention due to dispersive interactions while maintaining a similar retention mechanism as the untreated porous glassy carbon phase. The LTGC-coated zirconia produced an efficient column with classical reversed-phase retention behavior.

INTRODUCTION

The most commonly-used stationary phases are based on bonded silanes or polysiloxane polymers supported on silica gel surfaces. These materials are not typically suitable for separations performed at high or low pH.

The search for more stable stationary phases as well as alternative nonpolar stationary phases and supports for chromatographic separations is continuous. A surface containing only carbon atoms is often considered the nonpolar surface of choice due to its uniform chemical nature. Kieselev and his associates initiated and established the use of graphitic materials, such as carbon blacks, as adsorbents in gas chromatography.[1] Graphitic carbon black adsorbents are now used routinely for the separation of structural isomers of compounds by gas chromatography. These materials cannot be readily used in high performance liquid chromatography (HPLC) because of inadequate mechanical stability and surface area.

Glassy carbon was first prepared by Yamada and Sato in 1962 by high temperature pyrolysis of phenolic resins.[2] Glassy carbon is macroscopically amorphous, but contains the regional microstructures of graphite. Glassy carbon is less dense than graphite, which implies structural voids. These voids are apparently not connected since, glassy carbon is impermeable to gases. Glassy carbon is particularly well suited as an adsorbent or stationary phase material for HPLC because of its high mechanical, excellent chemical stability and high surface area (150-200 m^2/g).[3]

In 1982 Knox and Gilbert described the production and use of a glassy carbon adsorbent they call "porous glassy carbon" or "porous graphitic carbon" (PGC).[4] This material is produced by coating suitable phenolic resins on the surface of porous silica gel particles, polymerizing the resin in the pores, pyrolyzing the resin in N_2 (or Ar), dissolving the silica particle (template) and then heating the polymerized resin to 2000-2800°C to produce particles of "porous glassy carbon." These particles are now commercially available as Hypercarb.

The retention mechanism and the selectivity of solutes on porous glassy carbon in HPLC and SFC were studied by us[5-9] and others.[10-13] Glassy carbon, like graphite, is a hydrophobic, highly-polarizable solid. In reversed phase HPLC, it can be classified as an adsorbant where the glassy carbon surface acts as a Lewis base toward polar solutes and is involved in π-π interactions and dispersive interactions with aromatic solutes. Porous glassy carbon (PGC) has the advantage of extreme pH stability. The highly ordered planar surface, on the molecular level, produces unique structural selectivity, with an amorphous macro structure that forms the porous particles necessary for high surface area. The homogeneous surface has fewer chromatographic active sites and provides good chromatography for basic compounds.

A method of producing glassy carbon at low temperatures (200-800°C) was recently reported.[14,15] Poly-(phenylene diethynyl) compounds are first synthesized and then slowly heated to produce glassy carbon. The heat treatment of these oligomers results in small observable mass loss that allows the production of continuous low temperature glassy carbon (LTGC) films. Since poly-(phenylene diethynyl) oligomers are soluble in organic solvents, such as methylene chloride, standard coating procedures used to produce open tubular columns with polysiloxane stationary phases should be applicable for the production of open tubular columns with LTGC films.

Applications of LTGC-coated silica chromatographic stationary phases in HPLC and SFC were recently demonstrated.[6-8,16] LTGC stationary phases have been produced with a range of retention mechanisms. If the LTGC is cured at low processing temperatures (i.e., 200°C), then its surface specificity is similar to bonded phenyl polysiloxane stationary phases.[16] As the processing temperature is increased, then conjugation within the LTGC increases and the dipolarity / polarizability of a solute becomes more important. At processing temperatures near 550°C, the LTGC has chemical specificity and retention very similar to that of PGC. At higher processing temperatures the importance of analyte dipolarity / polarizability on retention seems to increase further than that possible with PGC,[16] although this requires further study.

PGC has the disadvantage of being very retentive, and higher molecular weight solutes may not elute. Large molecule mobile phase additives, such as p-terphenyl, have been used to reduce retention and improve peak shapes.[3,4] The retentivity of LTGC increases with processing temperature. The retentivity of LTGC processed at 550°C is similar to that of PGC. At lower temperatures the retentivity is markedly decreased.

Because the LTGC can be produced at low temperatures (200-600°C), a variety of chromatographic support materials are possible. For example, silica, alumina, zirconia and PGC particles are all potential supports. To date, silica gel is the only LTGC support material studied. Zirconium oxide is stable over the entire (0 - 14) pH range and its use has been demonstrated in RP-HPLC.[11,17-22] Zirconia offers significant advantages to silica gel and is a worthy choice to investigate. The hydrophobic surface of PGC should be an advantage when coating with LTGC monomers dissolved in hydrophobic solvents and should result in a more uniform coating than a polar support. This paper compares the chromatographic characteristics of PGC (produced by the Knox method), LTGC coated on zirconia particles, and LTGC coated on PGC particles.

The final processing temperature of the LTGC in these studies was 400°C to supply retentivity and selectivity characteristics approximately in the middle of the range available for LTGC. The solvatochromic comparison method was used to evaluate the relative chromatographic selectivity of the three different column types studied.

MATERIALS

Reagents and Chemicals

Table 1 lists the test solutes (Aldrich Chemical Co.) used in this study. The 60:40 water: acetonitrile mobile phase was prepared using purified water (Millipore, Milford MA) and HPLC grade acetonitrile (Fisher Scientific, Fairlawn, NJ).

The oligomer precursor was synthesized in-house according to the procedure previously reported.[14] The 5 μm zirconia was obtained from Keystone Scientific (State College, PA) and 5.5 μm Hypercarb® were obtained from Shandon HPLC (Runcorn, UK). For each type of chromatographic support, 100 mg of particles were slurried in 2 mL of a 10 mg/mL solution of oligomer in methylene chloride for 5 minutes, then the slurry was sonicated briefly (5 min.) under a vacuum. The coated particles were isolated by filtration without rinsing, and placed in the fluidized bed apparatus; argon flow was introduced and they were slowly heated at a rate of 1°C/ min over the temperature range of 50-400°C. The LTGC was cured at 400°C for 1 hr then cooled under argon to room temperature.

Columns

The particles were packed into 0.51 mm x 10 cm x 1/16" O.D. columns of Silcosteel tubing (Restek Corp., Bellefonte, PA, USA). A slurry of the PGC packing materials was prepared by sonicating the required amount of packing in 1.5 mL HPLC grade acetonitrile for approximately 30 minutes. The slurry was then transferred by syringe from the vial to a 4.6 mm id x 5 cm stainless steel reservoir connected directly to the head of the column. The reservoir connector was machined to form a funnel-like taper to the head of the column. The column was placed into a sonicator bath while the slurry was forced into the column at 4000 psi, using a syringe pump, for at least one hour. After one hour the column was allowed to depressurize to ambient before being removed.

Table 1

Solvatochromic Regression Data

Solute	$V/100^{24}$	π^{*24}	β^{24}	α^{24}	PGC	Log Ret. Factor LTGC/ Zirconia	LTGC/ PGC
1,3,5 trimethylbenzene[A]	0.769	0.43	0.13	0	1.564	0.284	1.052
3 xylene	0.668	0.51	0.12	0	1.122	0.060	0.738
benzene	0.491	0.59	0.10	0	0.228	-0.458	0.022
biphenyl	0.920	1.18	0.20	0	2.094	0.610	1.668
bromobenzene	0.624	0.79	0.06	0	0.941	-0.019	0.616
butylbenzene[A]	0.883	0.49	0.12	0	1.596	0.503	1.150
chlorobenzene	0.581	0.71	0.07	0	0.777	-0.125	0.331
ethylbenzene[A]	0.687	0.53	0.12	0	0.907	-0.010	0.564
iodobenzene	0.671	0.81	0.05	0	1.212	0.154	0.864
naphthalene	0.753	0.70	0.15	0	1.808	2.053	1.384
pentylbenzene	0.981	0.47	0.12	0	2.007	0.697	1.475
propylbenzene	0.785	0.51	0.12	0	1.252	0.218	0.865
toluene	0.592	0.55	0.11	0	0.658	-0.212	0.425
2 chloroaniline	0.652	0.83	0.40	0.25	0.636	-0.432	0.217
3 chlorophenol	0.626	0.77	0.23	0.69	0.613	-0.497	0.248
3 cresol[A]	0.634	0.68	0.34	0.58	0.305	-0.666	-0.050
3 phenyl 1 propanol	0.830	0.95	0.55	0.33	0.314	-0.760	-0.127
4 chlorophenol	0.626	0.72	0.23	0.67	0.599	-0.508	0.228
4 cresol	0.634	0.68	0.34	0.58	0.329	-0.666	-0.036
N ethylaniline[A]	0.758	0.82	0.47	0.17	0.849	-0.228	0.420
N methylaniline	0.660	0.73	0.47	0.12	0.471	-0.458	0.122
aniline	0.562	0.73	0.50	0.26	-0.125	-0.896	-0.144
benzanol[A]	0.634	0.98	0.52	0.39	-0.163	-1.028	-0.502
phenol	0.536	0.72	0.33	0.61	-0.132	-1.080	-0.416
N,N diethylaniline[A]	0.948	0.86	0.43	0	1.442	0.334	0.953
acetophenone	0.690	0.90	0.49	0.06	0.499	-0.550	0.210
anisole	0.630	0.73	0.32	0	0.546	-0.378	0.214
benzonitrile[A]	0.590	0.90	0.37	0	0.378	-0.586	0.005
butyrophrenone[A]	0.886	0.85	0.49	0.06	1.295	-0.067	0.742
hexanophenone[A]	1.092	0.83	0.49	0.06	2.161	0.461	1.461
methylbenzoate	0.736	1.14	0.52	0	0.908	-0.299	0.453
nitrobenzene[A]	0.631	1.01	0.30	0	0.756	-0.038	0.375
phenotole	0.727	0.69	0.30	0	0.956	-0.154	0.539
propiophenone[A]	0.788	0.87	0.49	0.06	0.966	-0.280	0.471
valerophenone	0.984	0.83	0.49	0.06	1.702	0.182	1.078
hexane	0.648	-0.04	0	0			

[A] Solutes used in hexane mobile phase experiment.

The zirconia packing was slurried with isopropanol to inhibit rapid settling of the denser particles, but otherwise handled the same. A 10 cm x 0.51 mm column should contain about 50 mg of the PGC packing and about 70 mg of the zirconia coated packing.

Equipment

The chromatographic system consists of an LC-2600 syringe pump (ISCO, Inc., Lincoln NE, USA), W-series high pressure injection valve with 200 nL loop (Valco Instruments, Houston TX), and Spectroflow 757 UV detector (Kratos Analytical Inst., Ramsey, NJ, USA) operated at 210 nm. The detector cell was prepared from 100 μm id fused silica with the polyimide coating removed.

The column temperature was maintained at 50°C with an HP 5890 GC oven (Hewlett-Packard, Avendale, PA, USA). The chromatographic data were collected with EZchrom Chromatography Data System V6.0 (Scientific Software, Inc., San Ramon, CA, USA) and analyzed by Peak Fit (Jandel Scientific, San Rafael, CA, USA).

RESULTS AND DISCUSSION

Loading Linearity

To achieve optimum efficiency in elution chromatography, the linear portion of an isotherm should be used. The variation of the retention factor (or capacity factor, k) with amount of solute injected was studied. Test solutes for the loading study were chosen for their different chromatographic properties.

The solute concentration ranges were chosen so that 0.08-1.64 μg was injected; the ethylbenzene concentration was slightly less due to solubility limits. The same volume (200 μL) of each sample solution was injected.

Figure 1 contains plots of the retention factor (k) versus the amount of solute injected. The tested amounts all gave constant retention factors and the subsequent chromatographic tests were performed by injecting approximately 0.2 μg of solute, well within the established linear loading range.

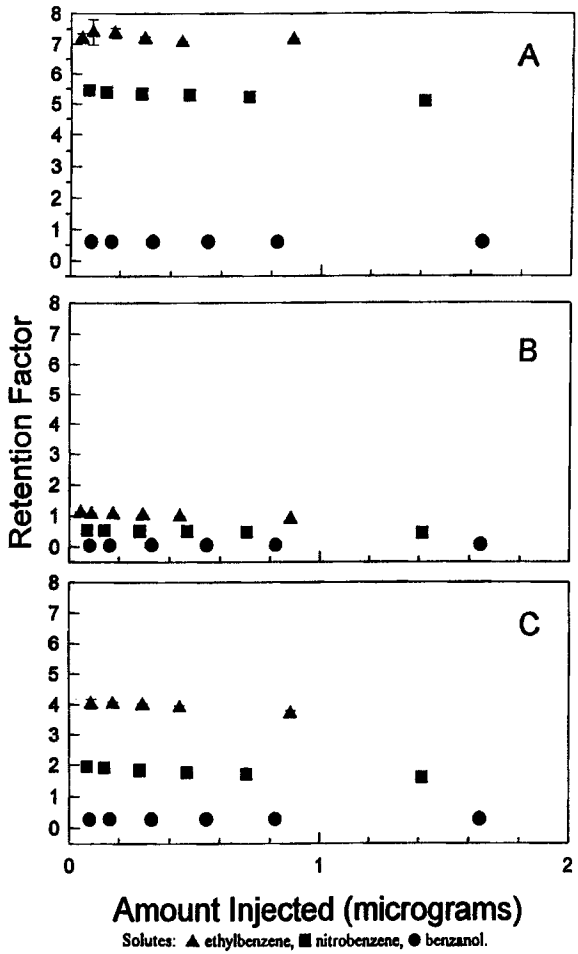

Figure 1. Loadability of stationary phases; A- PGC, B- LTGC-coated zirconia, C- LTGC-coated PGC. Solutes: ▲ ethylbenzene, ■ nitrobenzene, ● benzanol. Error bars are within the symbols if not visible.

Solvatochromic Comparisons Method

Many chromatographic processes have been investigated via the solvatochromic comparison method.[17,23-28] With this method (equation 1) a linear free energy model is used as a general equation to relate solute retention factors, log k, to the molecular level interactions occurring in solution and at

the stationary phase.[24]

$$\log k = SP_o + M(\delta_m^2 - \delta_s^2)\frac{V_2}{100} + S(\pi_s^* - \pi_m^*)\pi_2^* + A(\beta_s - \beta_m)\alpha_2 + B(\alpha_s - \alpha_m)\beta_2 \quad (1)$$

The solute properties (subscripted *2*) are multiplied by the difference in the complementary properties of the competing mobile and stationary phases, subscripted *m* and *s*, respectively. The first term, SP_0, is the intercept. The second term is a measure of the free energy needed to create space in the solvent for a solute molecule, where δ, the Hildebrand solubility parameter, measures the solvent's cohesiveness.[29] To a first approximation, V, the van der Waals molar volume of the solute is proportional to the solute's dispersive interactions with the stationary phase. The molar volume is divided by 100 so the values are of the same magnitude as the other parameters. The last three terms are Kamlet-Taft terms, α and β are measures of H-bond donor ability, H-bond acceptor ability, and π^* measures dipolarity / polarizability.[30-32] The coefficients of equation 1 (SP_0, M, S, A, B) are unknown, but can be determined by multifactor regression analysis.

In the studies described herein, the retention factors of the test solutes are measured for each stationary phase while the mobile phase conditions are held constant. Under these conditions, equation 1 simplifies to Equation 2.

$$\log k = SP_o + m\frac{V_2}{100} + s\pi_2^* + a\alpha_2 + b\beta_2 \quad (2)$$

The new coefficients, *m*, *s*, *a* and *b*, contain the information relating to the chemical nature of the mobile and stationary phases and also the original model coefficients. The sign of the coefficient represents whether the solute property is an exoergic or endoergic factor in the retention process.

The regression of the log k for the same solute set on various stationary phases under the same chromatographic conditions will give model coefficients that differ because of the stationary phase properties. Therefore a comparison of these coefficients shows the differences in the retention processes of the stationary phases under the mobile phase conditions and should give insight into the chemical nature of the stationary phase. The integrity of the data were determined using established guidelines for multivariable linear regression analyses.[33] A cross correlation of the independent parameters produces a maximum correlation coefficient of 0.598

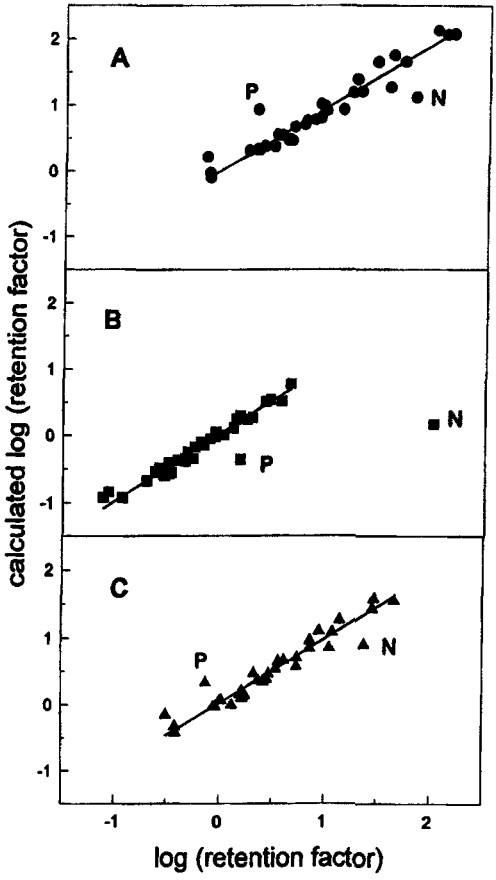

Figure 2. Solvatochromic regression plots of the solutes observed vs calculated log (retention factor) for the stationary phases; A- PGC, B- LTGC-coated zirconia, C- LTGC-coated PGC. The outliers phenyl propanol and naphthalene are not included in the regression but are designated P and N.

for β and π^* indicating that no significant correlative relationships exist between the independent variables. The F-test at the 95% confidence level was used to indicate whether the relationships in model equation was statistically significant. All models described here passed the F-test. The t-statistic of the coefficient was evaluated at the 95% confidence level to test for statistical significance.

The multivariable regression analysis was initially performed with all the independent variables. The significance of each independent parameter was evaluated at the 95% confidence level; those not meeting the requirements were omitted from the model.[34] The same data were also analyzed via the stepwise backward multiple regression analysis where one independent parameter was added to the model equation at a time in the order of most significant. Those independent parameters not improving the precision of the fitted equation were removed. The model equations from both regression methods for a given column and data set were the same.

The data used for the regression analysis are listed in Table 1. The chosen solute set consists of a range of solvatochromic parameter values to determine the significance of the corresponding interactions. Figure 2 illustrates the ability of the model to predict the retention factors for each column. Naphthalene and 3-phenyl-1-propanol are significant outliers in the LTGC-coated zirconia data (Figure 2B). These solutes are also outliers in the other data sets and are shown on all plots. These data were excluded from all regression analyses to maintain consistency between data sets. The omission of naphthalene and 3-phenyl-1-propanol improved the precision of the model equations in all cases. The retention models evaluate thermodynamic interactions of the solute with no consideration given to the shape of the molecule or its orientation to the stationary phase. The shape selectivity of chromatographic adsorbant stationary phases[13] is the most likely cause for the outliers.

Table 2 lists the summary of the regression results for the present study. Figure 3A is a plot of the table data comparing the coefficient values. The statistics show an excellent fit of the data using the solvatochromic model, Equation 2.

The results show that dispersive interactions, i.e., the m coefficient that scales with solute size, is the most important parameter in solute retention on all three supports. In all cases, the sign of the m coefficient is positive which shows as solute size increases so does its retention. A solute's ability to accept a hydrogen bond (b coefficient) was the next most important variable. In all cases the sign of the b coefficient was negative which indicates decreased retention with increasing H-bond accepting ability of the solutes. The s coefficient (dipolarity / polarizability) and the a coefficients (hydrogen bond donating ability) were of lesser importance but still affected solute retention on PGC and LTGC-PGC to approximately the same magnitude, but with opposite effects. Increasing π^* causes increased retention and increasing solute hydrogen bond donor ability, α, decreased retention. Interestingly, for the LTGC-coated zirconia the s coefficient (dipolarity / polarizability) was not

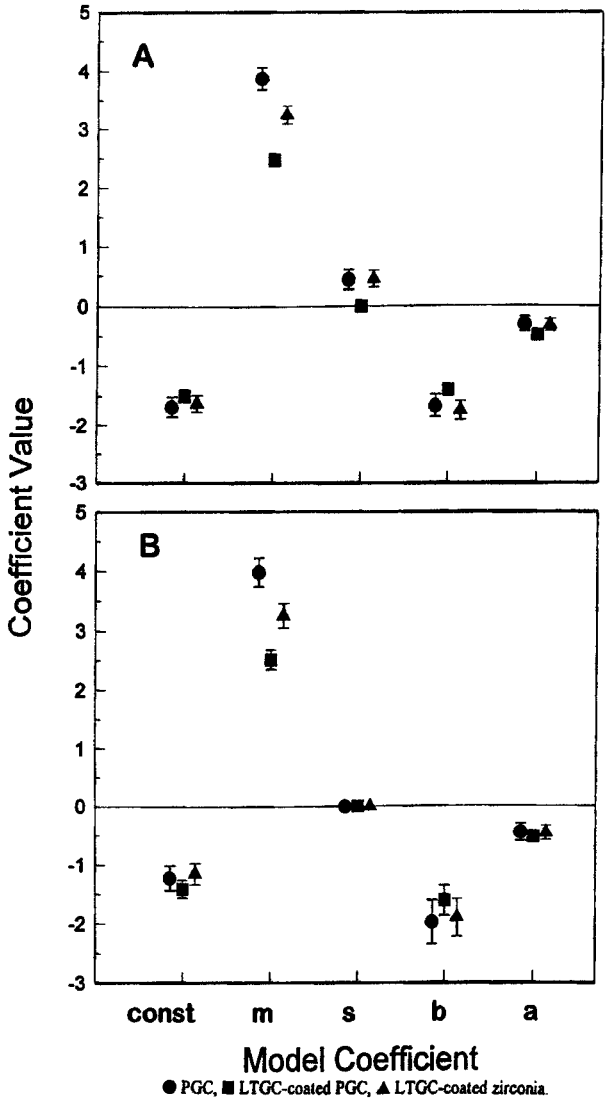

Figure 3. Comparison of the coefficient values from the solvatochromic regression using the aqueous mobile phase. ● PGC, ■ LTGC-coated zirconia, ▲ LTGC-coated PGC.

Solute groupings: A- all solutes, B- hydrogen bonding solutes. Error bars are within the symbols if not visible.

Table 2

Solvatochromic Model Coefficients using Water/Acetonitrile Mobile Phase with all Solutes

Column	Constant (s)[a]	m (s)	s (s)	b (s)	a (s)	Statistics N[b]	R^{2c}	F[d]
PGC	-1.68 (0.17)	3.88 (0.18)	0.44 (0.16)	-1.67 (0.19)	-0.30 (0.12)	33	0.957	157
LTGC-coated zirconia	-1.49 (0.08)	2.48 (0.11)	-[e]	-1.40 (0.09)	-0.48 (0.07)	33	0.972	332
LTGC-coated PGC	-1.62 (0.14)	3.26 (0.16)	0.45 (0.14)	-1.75 (0.16)	-0.32 (0.10)	33	.0960	166

[a] Standard Deviation. [b] Number of solutes. [c] Square of multiple correlation coefficient. [d] Calculated F value, all are statistically significant at the 95% confidence level. [e] not significantly correlated.

statistically significant; while the *a* coefficient (hydrogen bond donating ability of the solute) was even more significant than observed with PGC or LTGC-coated PGC.

The individual regression coefficients for PGC were compare to those from the LTGC-coated supports and tested for equality using the t-test at the 95% confidence level.[35] The *m* coefficients (dispersive interactions) were statistically different for all three supports and decreased in the order of PGC> LTGC-coated PGC > LTGC-coated zirconia. In general, the retentivity of the LTGC phases are less than that of the PGC mainly through a decrease in dispersive interactions; however, the *s* values determined for the PGC and the LTGC-coated PGC phases are statistically the same. These results suggest the LTGC coating does not diminish the dipolar / polarizability solute interactions with the PGC support. Statistically the *s* coefficient is 0 for the LTGC-coated zirconia which indicates the lack of solute dipolarity / polarizability interactions of the LTGC coating. The uncoated zirconia was found to have negligible retention of these solutes in an aqueous 10% acetonitrile mobile phase and would be expected to have no solute dipolar / polarizability interactions under the current conditions.[36]

Table 3

Solvatochromic Regression Coefficients using Water/Acetonitrile Mobile Phase with Nonpolar Solutes

Column	Constant (s)[a]	m (s)	s (s)	b (s)	a (s)	Statistics N[b]	R^{2c}	F[d]
PGC	-1.62 (0.18)	3.43 (0.21)	0.51 (0.15)	-[e]	-[e]	12	0.969	141
LTGC-coated zirconia	-1.58 (0.090)	2.44 (0.14)	0.16 (0.08)	-1.22 (0.52)	-[e]	12	0.983	150
LTGC-coated PGC	-1.632 (0.19)	2.98 (0.23)	0.47 (0.16)	-[e]	-[e]	12	0.954	93

[a] Standard Deviation. [b] Number of solutes. [c] Square of multiple correlation coefficient. [d] Calculated F value, all are statistically significant at the 95% confidence level. [e] not significantly correlated

These data suggest that the LTGC coating (processed to 4000C) itself doesn't enhance or participate in π^* interactions. The *a* and *b* coefficients were statistically the same for all three supports.

A previous study of C_{18} bonded phase RP-HPLC revealed that solute size is the major factor in retention followed by its hydrogen bond acceptor ability with a minor dependence upon solute dipolarity / polarizability.[23] The same observations can be made here with the addition of a minor dependence upon the solute's hydrogen bond donor ability, especially in the case of the zirconium coated LTGC.

The data were also analyzed by solute class to highlight the differences in specific stationary phase interactions. The solutes were divided into the classes of nonpolar, hydrogen bond donors and hydrogen bond acceptors. The nonpolar compounds contain only aromatic solutes with aliphatic or halogen groups, and the results are presented in Table 3. The hydrogen bond donor solutes all contain donable protons and some have hydrogen bond acceptor sites as well. The hydrogen bond acceptor solutes had no donable protons. The hydrogen bond donor and acceptor solute data were combined and correlated

Table 4

Solvatochromic Model Coefficients using Water/Acetonitrile Mobile Phase HBD and HBA Solutes

Column	Constant (s)[a]	m (s)	s (s)	b (s)	a (s)	Statistics N[b]	R^{2c}	F[d]
GPC	-1.22 (0.22)	3.98 (0.24)	-[e]	-1.99 (0.37)	-0.46 (0.14)	21	0.956	125
LTGC-coated zirconia	-1.41 (0.15)	2.52 (0.17)	-[e]	-1.62 (0.26)	-0.53 (0.10)	21	0.954	121
LTGC-coated PGC	-1.15 (0.19)	3.26 (0.20)	-[e]	-1.91 (0.32)	-0.47 (0.12)	21	0.954	119

[a] Standard Deviation. [b] Number of solutes. [c] Square of multiple correlation coefficient. [d] Calculated F value, all are statistically significant at the 95% confidence level. [e] not significantly correlated.

because separate analysis were yielding strong correlations with weak data set parameters, i.e. those with small ranges. The combined data set yields more conclusive information the statistical results are presented in Table 4. A comparison of the coefficient values determined for the hydrogen bond forming and nonpolar solutes are plotted in Figures 3B and 4A.

Only those observations which are unique to the solute classes are discussed. The retention of the nonpolar solutes on the LTGC-coated zirconia is strongly dependent upon solute dipolarity / polarizability interactions with the stationary phase. There is a strong stationary phase hydrogen bond donor interaction with the nonpolar solutes. The negative value of *b* indicates a decrease in retention and therefore a repulsive effect on the nonpolar solutes. The standard error is large. however the large *b* indicates a strong interaction. The hydrogen bonding solute retentions have no correlation with dipolarity / polarizability interactions with any stationary phase, which is surprising since the π^* values for the solute set cover a good range.

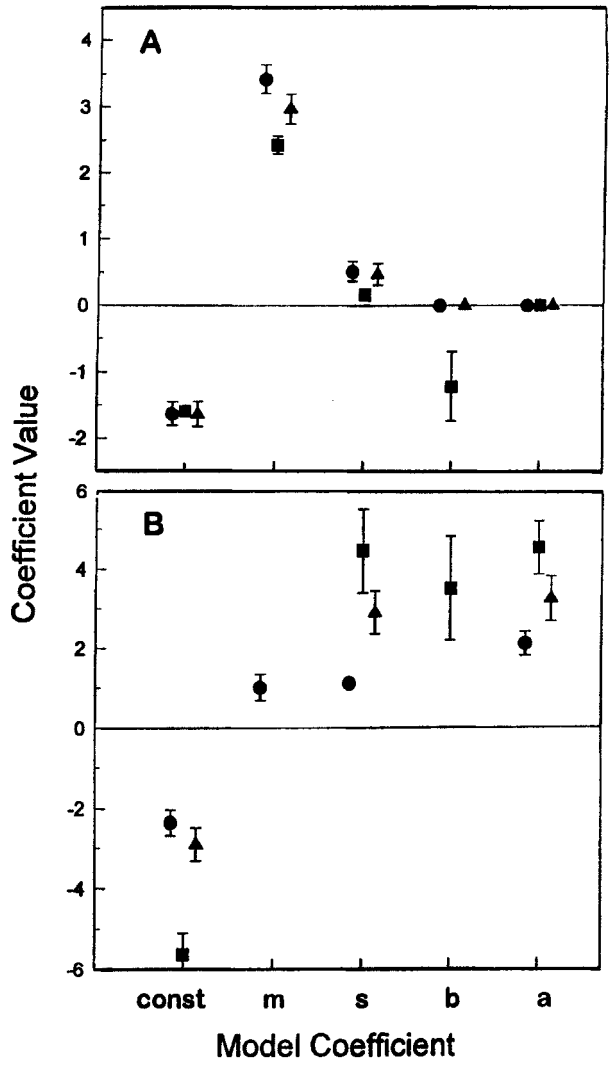

Figure 4. Comparison of the coefficient values from the solvatochromic regression of the stationary phases; ● PGC, ■ LTGC-coated zirconia, ▲ LTGC-coated PGC. Using: A- the nonpolar solutes with aqueous mobile phase, B- selected solutes with hexane mobile phase. Error bars are within the symbols if not visible.

The solvatochromic analysis of the entire and individual solute sets indicate the LTGC-coated PGC phase has the same retention mechanisms as the PGC phase with the exception of less dependence on solute dispersive interactions. Comparison of the PGC and the LTGC-coated PGC reveal no significant active site interactions are caused by the LTGC coating under the test conditions. The LTGC-coated zirconia results suggest much weaker dipolar / polarizable interactions with the nonpolar solutes which was not apparent in the analysis of the entire solute data set. Evidence of stationary phase hydrogen bond donor interactions with nonpolar solutes indicate stationary phase active sites capable of donating a hydrogen bond. It can be concluded that the strong dependence on solute β with LTGC-coated zirconia must be due to the presence of the zirconia.

Hexane Mobile Phase

The Hildebrand solubility parameter, δ, for hexane is very small relative to the aqueous acetonitrile mobile phase used earlier.[37] Unlike the aqueous mobile phase, hexane has a very large dispersive interaction with the solutes and a dispersive shielding effect on the stationary phase. The solvatochromic parameters for hexane, Table 1, indicate the absence of any hydrogen bonding ability. The negative π^* value shows there are slight repulsive dipolarity / polarizability interactions on the solute molecules. Chromatographic probing using a hexane mobile phase should enhance solute hydrogen bond interactions with the stationary phase.

The columns were flushed over night with acetonitrile at a column temperature of 80°C and then allowed to equilibrate with hexane for several hours. Both solvents were dried with a molecular sieve for at least three days. The column temperature was reduced to 50°C for the experiment. Each column was evaluated with a set of 13 solutes representative of those listed in Table 1 using hexane as the mobile phase.

The multiple correlation parameters were all 0.94 or greater which is a good fit for such a small diverse data set, Figure 5. Table 5 and Figure 4B lists the coefficients of the regression for comparison.

Only PGC retains a dependence upon dispersive interactions in the hexane mobile phase agreeing with the strong dispersive interactions observed in the reversed-phase system.

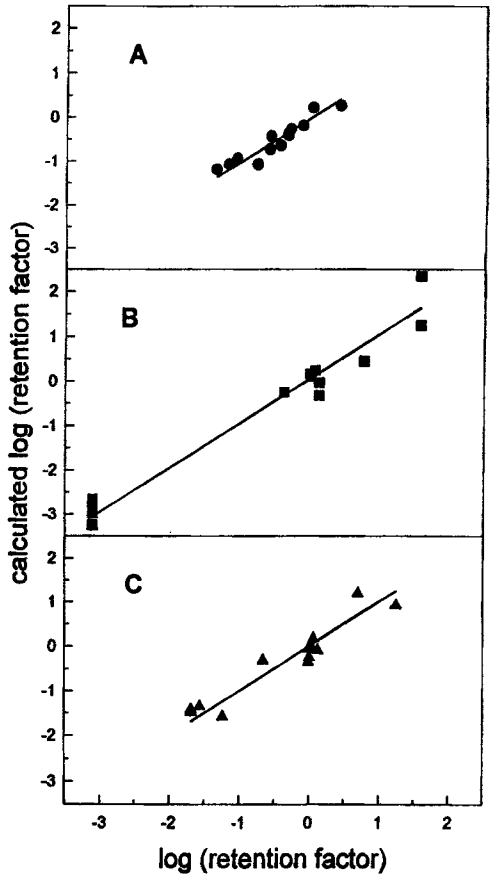

Figure 5. Solvatochromic regression plots using hexane mobile phase. A- PGC, B- LTGC-coated zirconia, C- LTGC-coated PGC.

The retention on LTGC-coated zirconia in hexane is strongly dependent upon the solute dipolarity / polarizability, hydrogen bond acceptor, and hydrogen bond donor interactions. Both PGC and LTGC-coated PGC had positive s and a coefficients, and b was statistically 0. The absence of solute hydrogen bond acceptor interactions (i.e., $b = 0$) with the PGC and LTGC-coated PGC stationary phases indicates there was no adsorbed water on the stationary phases.

Table 5

Solvatochromic Model Coefficients using Hexane Mobile Phase

Column	Constant (s)[a]	m (s)	s (s)	b (s)	a (s)	Statistics N[b]	R^{2c}	F[d]
PGC	-2.35 (0.33)	1.02 (0.33)	1.12 (0.28)	-[e]	2.11 (0.31)	13	0.896	26
LTGC-coated zirconia	-5.62 (0.53)	-[e]	4.47 (1.07)	3.51 (1.30)	4.54 (0.68)	13	0.964	81
LTGC-coated PGC	-2.89 (0.42)	-[e]	2.91 (0.54)	-[e]	3.24 (0.57)	13	0.881	37

[a] Standard Deviation. [b] Number of solutes. [c] Square of multiple correlation coefficient. [d] Calculated F value, all are statistically significant at the 95% confidence level. [e] not significantly correlated.

High *s* values for all stationary phases can be attributed to the absence of dipolarity / polarizability competition with the mobile phase. However, the ordering of the *s* values (LTGC-coated zirconia > LTGC-coated PGC > PGC) is not readily explained, as it is contrary to the aqueous mobile phase results.

The stationary phase hydrogen bond acceptor strength is greatest for the LTGC-coated zirconia and least for the PGC. The oxide sites of the glassy carbon edge planes could cause these hydrogen bond acceptor interactions with the solutes. Greater solute hydrogen bond acceptor interaction with the LTGC-coated PGC may indicate more oxide formation during the polymer curing. The LTGC-coated zirconia has both strong hydrogen bond donor and hydrogen bond acceptor interactions with the solutes. This observation agrees with the nonpolar solutes - aqueous mobile phase observations. The stationary phase hydrogen bond donor interactions are therefore most likely due to the zirconia. The increased hydrogen bond acceptor interactions could also be from the zirconia. The presence of surface oxides on the zirconia is a known cause of chromatographic active sites.[21] Surface oxides in normal phase HPLC can cause polarization of large solutes.[38] This type of solute interaction should manifest as an increase in the *s* coefficient as was observed here.

This data is useful for discerning the surface of the stationary phases, however it is important to note that the "hexane mobile phase active sites" would most likely have little effect upon the reversed-phase retention mechanism.

Efficiency

The chromatographic efficiency of each column was determined by measuring the reduced plate height (h) for three chromatographically different test solutes, used previously in the loading study, over a range of mobile phase flow rates. Figures 6A through 6C show the results of this efficiency study.

The efficiency behavior between the columns varied. Both the LTGC-coated columns have much shallower slopes contrasted to the PGC column indicating faster mass transfer kinetics. The LTGC-coated zirconia column has the highest efficiency and a different ordering of the solute curves versus the other columns. That is, nitrobenzene yields the lowest efficiency curves with both the PGC and the LTGC-coated PGC phases, but ethylbenzene is the least efficient curve of the LTGC-coated zirconia plots. This switch in order of efficiency may be due to different retention mechanisms between the columns; however the order of analyte retention was the same for all three columns.

Scanning electron microscopy (SEM) was performed on the packing materials to further compare the packing materials. The range in particle diameters (2-8 μm) was substantial for PGC particles. The LTGC-coated PGC particles had the same size distribution as the PGC. Because the range of particle dimensions was so large for the PGC, the film thickness of the LTGC was not discernable. However, the uncoated particles had markedly smoother topology which indicated that the PGC particles were clearly coated with LTGC. Micrographs of both the PGC and the LTGC-coated PGC particles unexpectedly showed several broken shells of hollowed out spheres. The broken shells and the deeper pore structure of the LTGC-coated PGC would contribute to the lower column efficiency observed. The LTGC-coated zirconia also appeared smooth with a more uniform particle size distribution.

Homolog vs Log Retention Factor

The linear dependence of log k versus homolog carbon number, i.e., the number of repeating methylene units, is conventionally used to demonstrate reversed-phase retention behavior.[39] The first few homologs have been shown to deviate from linearity especially on adsorbant phases.[40]

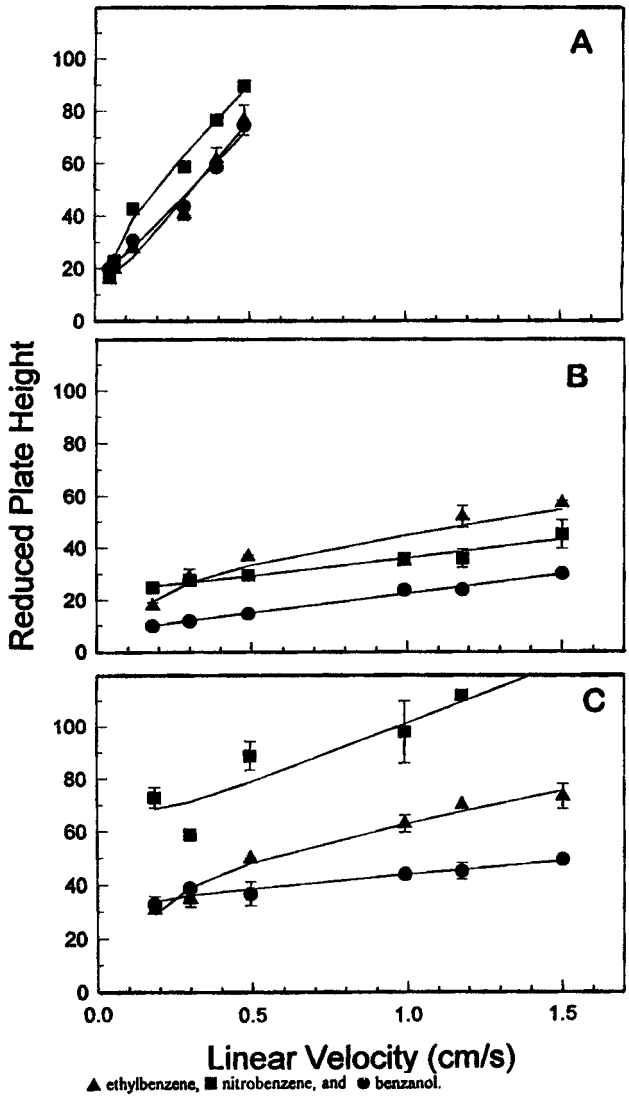

Figure 6. Reduced plate height vs linear velocity for ▲ ethylbenzene, ■ nitrobenzene, and ● benzanol. A- PGC, B- LTGC-coated zirconia, C- LTGC-coated PGC. Error bars are within the symbols if not visible.

Alkylbenzene and alkylphenone homologs were used in this study because they were previously used to characterize the selectivity on a carbon-clad zirconia support.[40]

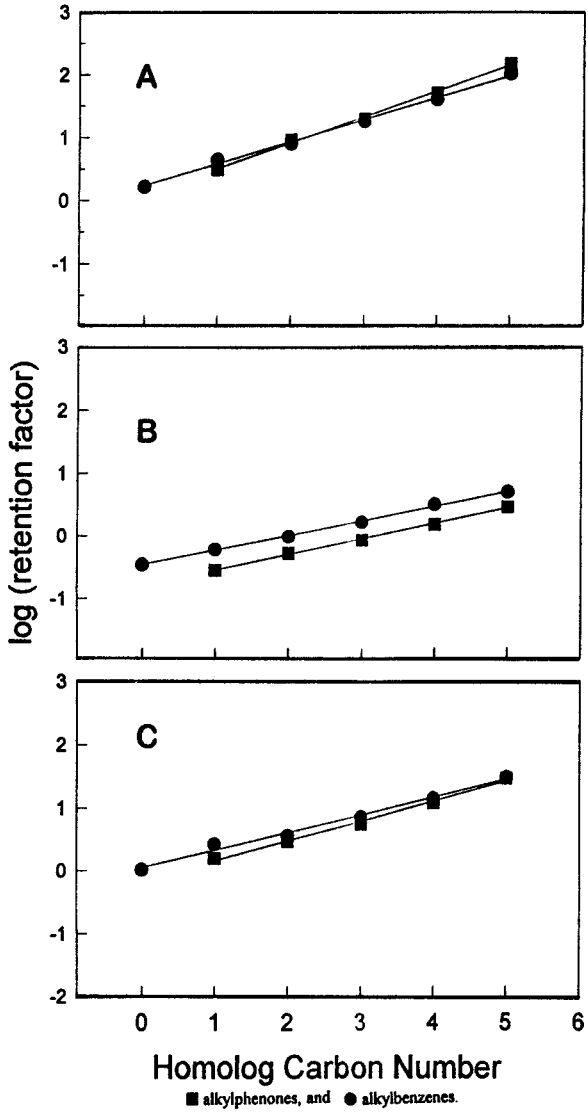

Figure 7. Log (retention factor) vs homolog carbon number for ■ alkylphenones, and ● alkylbenzenes. A- PGC, B- LTGC-coated zirconia, C- LTGC-coated PGC. Error bars are within the symbols if not visible.

Table 6

Stationary Phase	Alkylbenzene Slope (s)	Alkylbenzene Intercept (s)	Alkylphenone Slope (s)	Alkylphenone Intercept (s)
PGC	0.34 (0.011)	0.25 (0.03)	0.41 (0.012)	0.11 (0.042)
LTGC-coated PGC	0.28 (0.01)	0.06 (0.04)	0.31 (0.01)	-0.15 (0.05)
LTGC-coated zirconia	0.23 (0.01)	-0.46 (0.02)	0.25 (0.01)	-0.80 (0.02)

Figure 7A is a plot of the log (retention factor) for the alkylphenone and alkylbenzene homologs versus carbon number on the PGC column. The plot shows a crossover at carbon number 2 where the alkylphenones are more retained at carbon numbers greater than 2. The intercepts for both lines are positive as listed in Table 6.

Figure 7B is the homolog linearity plot of the LTGC-coated zirconia. The regression lines are almost parallel as indicated by the slopes demonstrating consistent selectivity throughout the tested range of carbon numbers. Both intercepts are negative with the alkylphenone being less retained.

The homolog plot of the LTGC-coated PGC stationary phase (Figure 7C) shows a crossover at 5 carbon numbers with the alkylbenzenes being more retained throughout the range 0 to 5 carbon numbers.

The intercepts confirm previous observations on retentivity with PGC being the most retentive and LTGC-coated zirconia being the least retentive. The differences in the slope between the three columns is very interesting.

The slope of the lines for the alkylphenones homolog was greater than that of the alkylbenzenes for all three columns. The difference in slope values between the alkylbenzene and the alkyl phenones decreased in the order of PGC> LTGC-coated PGC> LTGC-coated zirconia.

With conventional chemical-bonded reversed-phase supports, the alkylphenone homologs are retained less than the alkylbenzenes and the selectivity for a methylene group (slope) in the alkylbenzene and alkylphenones

is very similar. The selectivity of the LTGC-coated zirconia is very similar to that of conventional reversed phase supports. Marked differences between the slopes of alkybenzene and alkylphenone homologs were previously observed by Carr and coworkers[40] for a carbon-cladded surface. When a polybutadiene film was coated over the carbon-claded surface or the surface was exposed to hydrogen, the differences in the slopes decreased substantially. The cause of the different slopes was then attributed to active sites on the carbon cladded surface that were removed by exposure to hydrogen or covered with the polybutadiene. It stands to reason that active sites also cause the biggest difference in slopes with PGC and the LTGC coating diminished the problem.

The presence of active sites that polar compounds adhere to would also explain the difference in observed efficiency between the LTGC-coated zirconia and the LTGC-coated PGC or PGC. For purposes of comparison, the efficiency was also markedly lower for the alkylphenone homologs than the alkylbenzene homologs on PGC or LTGC-coated PGC; while the efficiency was approximately the same for the two homologous series on the LTGC-coated zirconia.

SUMMARY

The LTGC-coated PGC had less reverse phase retentivity than the uncoated PGC through decreased solute-stationary phase dispersive interactions. The both LTGC-coated phases had faster mass transfer kinetics than the PGC. The LTGC-coated zirconia had the lowest reversed phase retentivity and the highest efficiency column with good mass transfer, characteristic of its pellicular-like structure. The selectivity of the LTGC-coated zirconia was most like conventional reversed phase HPLC.

The LTGC-coated phase on either support provides advantages over the PGC phase and the choice support would depend upon the specific application. Further improvements in the chromatographic behavior and utility of the LTGC stationary phases can be accomplished through studies in the type and size of support, the coating amount, coating process, the curing temperature, and the chromatographic retention mechanism.

REFERENCES

1. A. A. Kiselev, Russ. J. Phys. Chem., **36**, 13 (1960).

2. S. Yamada, H. Sato, Nature, **193**, 261 (1962).

3. J. H. Knox, B. Kaur, **High-Performance Liquid Chromatography**, R. A. Hartwick, B. J. Brown, eds., (1989) pp. 189-222.

4. J. H. Knox, B. Kaur, G. R. Millward, J. Chromatogr., **352**, 3-25 (1986).

5. Y. Cui, S. V. Olesik, Anal. Chem., **63**, 1812-1819 (1991).

6. T. M. Engel, S. V. Olesik, Anal. Chem., **63**, 1830-1838 (1991).

7. T. M. Engel, S. V. Olesik, Anal. Chem., **62**, 1554-1560 (1990).

8. T. M. Engel, S. V. Olesik, J. High Res. Chromatogr., **14**, 99-102 (1991).

9. W. Larkins, Jr., S. V. Olesik, J. Microcol. Sep., **5**, 543-550 (1993).

10. B. J. Bassler, R. Kaliszan, R. A. Hartwick, J. Chromatogr., **461**, 139-147 (1989).

11. T. P. Weber, P. W. Carr, Anal. Chem., **62**, 2620-2625 (1990).

12. R. Kaliszan, K. Osmialowski, B. J. Bassler, R. A. Hartwick, J. Chromatogr., **499**, 333-334 (1990).

13. J. Kríz, E. Adamcová, J. H. Knox, J. Hora, J. Chromatogr., **663**, 151-161 (1994).

14. M. R. Callstrom, T. X. Neenan, R. L. McCreery, D. C. Alsmeyer, J. Am. Chem. Soc., **112**, 4954-4956 (1990).

15. N. L. Pocard, C. C. Alsmeyer, R. L. McCreery, T. X. Neenan, M. R. Callstrom, K. Mater. Chem., **2**, 771-784 (1992).

16. T. M. Engel, S. V. Olesik, M. R. Callstrom, M. Diener, Anal. Chem., **65**, 3691-3700 (1993).

17. T. P. Weber, PhD University of Minnesota at Minneapolis, 1991.

18. T. P. Weber, P. T. Jackson, P. W. Carr, Anal. Chem., **67**, 3042-3050 (1995).

19. U. Trudinger, G. Muller, K. K. Unger, J. Chromatogr., **535**, 111-125 (1990).

20. D. A. Hanggi, N. R. Marks, LC - GC, **11**, 128-138 (1993).

21. J. A. Blackwell, P. W. Carr, J. Chromatogr., **549**, 59-75 (1991).

22. M. P. Rigney, T. P. Weber, P. W. Carr, J. Chromatogr., **484**, 273-291 (1989).

23. P. C. Sadek, P. W. Carr, R. M. Doherty, M. J. Kamlet, R. W. Taft, M. H. Abraham, Anal.Chem., **57**, 2971-2978 (1985).

24. P. W. Carr, R. M. Doherty, M. J. Kamlet, R. W. Taft, W. Melander, C. Horváth, Anal. Chem., **58**, 2674-2680 (1986).

25. J. E. Brady, D. Byorkman, C. D. Herter, P. W. Carr, Anal.Chem., 56, 278-283 (1984).

26. J. H. Park, P. W. Carr, M. H. Abraham, R. W. Taft, R. M. Doherty, M. J. Kamlet, Chromatographia, **25**, 373-381 (1988).

27. L. R. Snyder, P. W. Carr, S. C. Rutan, J.Chrom., **656**, 537-547 (1993).

28. S. Pedigo, L. D. Bowers, J.Chrom., **499**, 279-290 (1990).

29. M. J. Kamlet, R. M. Doherty, M. H. Abraham, Y. Marcus, R. W. Taft, J.Phys. Chem., **92**, 5244-5255 (1988).

30. M. J. Kamlet, R. W. Taft, J. Am. Chem. Soc., **98**, 2886-2894 (1976).

31. M. J. Kamlet, J. L. Abboud, R. W. Taft, J. Am. Chem. Soc., **99**, 6027-6038 (1977).

32. M. J. Kamlet, R. W. Taft, J. Am. Chem. Soc., **98**, 377-383 (1976).

33. M. Charton, S. Clementi, S. Ehrenson, O. Exner, J. Shorter, S. Wold, S. Quant. Struct. Acta. Relat., **4**, 29 (1985).

34. J. H. Zar, **Biostatistical Analysis**, Prentice-Hall, Inc. Englewood, N.J. (1974) pp. 263-268.

35. J. H. Zar, **Biostatistical Analysis**, Prentice-Hall, Inc. Englewood, N.J. (1974) pp. 228-230.

36. C. T. Rittenhouse, S. V. Olesik [Unpublished] (1996) .

37. L. R. Snyder, J. J. Kirkland, **Introduction to Modern Liquid Chromatography**, John Wiley & Sons, Inc. New York (1979) pp. 259

38. L. R. Snyder, **Principles of Adsorption Chromatography**, Marcel Dekker, Inc. New York (1968) pp. 22-24.

39. C. F. Poole, S. K. Poole, **Chromatography Today**, Elsevier, Amsterdam (1991) pp. 395

40. T. P. Weber, P. W. Carr, E. F. Funkenbush, J. Chromatogr., **519**, 31-52 (1990).

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