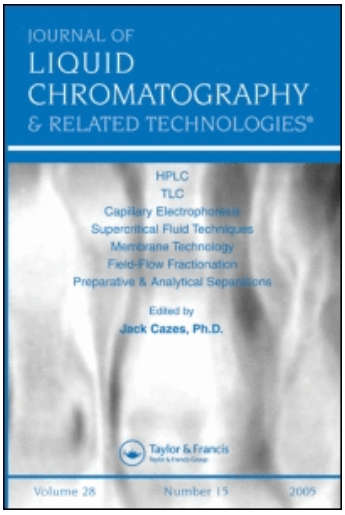

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CHARACTERIZATION OF LC STATIONARY PHASES BY INVERSE SIZE EXCLUSION CHROMATOGRAPHY

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THEORY

The most efficient use of a porous particulate stationary phase can only be made when the material in the column is fully characterized. While complete characterization includes both chemical and physical aspects, the macro structure provides the framework for the microscopic chemical interactions. The physical analysis is therefore critically important for a more complete calibration of the chromatographic effects.

Several reliable methods exist for characterizing the internal physical structure of porous materials (1-4). Only one of these (4), inverse size exclusion, is suitable for swollen gels. It has the added feature that it permits the use of conventional liquid chromato-

graphic apparatus to carry out the characterization. So far, the technique has been applied to porous stationary phases that were designed for size exclusion LC. Here we wish to explore the possibility of a broader application to atationary phases designed for enthalpic partitioning.

Our approach is based upon a previous finding that interactive partitioning can be treated empirically using the following expression (5)

$$\ell nD_i = -(g_0 + g_1 L_i) + \frac{\bar{V}i}{RT}\left[(\delta_i - \delta_m)^2 - (\delta_i - \delta_s)^2\right] \qquad (1)$$

where D is the distribution coefficient, δ refers to solubility parameter, $\bar{V}$ is molar volume, and the subscripts refer to solute i, liquid carrier m, and intermal or interactive phase S. The equation consists of two parts: an initial anenthalpic contribution based on Giddings theory (6) of size exclusion according to the surface area g_1 and the mean projected solute size L, and (b) and enthalpic contribution based on the solubility factors. In the past (4) Equation 1 was applied under the condition $\delta_m = \delta_s$ where the enthalpic contribution vanishes.

We have explored conditions that may help make equation 1 a more general guide to stationary phase characterization. An important limitation should be recognized at the outset. It is already known (5) that enthal-

pic liquid-gel partitioning is strongly affected by solvent effects. This solvation effect manifests itself through the empirical value of δ_s which was found to be intermediate between δ_m and δ_s°, where the latter refers to the unsolvated interactive phase, and much closer to δ_m than to δ_s°. This effect seems to constrict our approach to the consideration of two options: making the overall interactive term vanish, or holding it constant.

The condition $\delta_m = \delta_s$ predicts vanishing interaction, i.e., the entire solubility parameter term in Equation 1 becomes zero. It is easily shown that $\delta_i = (\delta_s + \delta_m)/2$ has the same effect but the practical advantage of this is not clear. Next, consider probe solutes whose δ_i values are approximately constant, so that the δ-terms then be replaced by

$$-\ln D_i = -(g_0 + g_1 L_i) + g_2 \bar{V}_i \qquad (2)$$

Since L_i and $\bar{V}_i$ are nearly parallel, except for a small conformational contribution, Equation 2 can be seen to lead to a condition where the g_1 and g_2 terms would be confused and, hence, to inaccurate values of g_1. This sort of pitfall would need to be avoided. Another alternative is to exploit a strong solvent: solute or solvent: substrate masking effect (7). This was shown (8) to block the adsorption of coronene by crosslinked PSDVB

network. One disadvantage with this is the difficulty in measuring the exact size of the solvated probe molecules. It appears that difficulties arise whenever the interactive effect is allowed to happen.

Since macroporous gels consist of both large and small pores, preferential desorption from the latter microporous phase is expected when $\delta_i \rightarrow \delta_m$ and $\delta_m \rightarrow \delta_s^\circ$. This would cause solute rejection from the microporous structure and the size exclusion effect should be simplified. If the theory holds, the value of D_i becomes equal to D_i(micropores) $\longrightarrow$ 0 plus D_i(macropores) where the latter is simply:

$$\ln D_i(\text{macropores}) = -(g_0 + g_1 L_i) \qquad (3)$$

where g_1 becomes characteristic of the macropores and the micropore contribution is negligible. It follows (4) that the pore size of the macropore channels is obtained using

$$d_{\text{macropore}} = 4/g_1(\text{desorption}) \qquad (4)$$

Two other properties of the macroporous phase may also be accessible. The porosity P is obtained for the value of D that corresponds to a solute size L equivalent to that of the solvent (9).

$$P = D(L_i = L_{\text{solvent}}) \qquad (5)$$

Finally, the pore surface area per unit volume of pores (g_1) can be adjusted to approach the more conventional pore area, based on matrix volume, SA

$$SA\left(\frac{m^2}{cm^3}\right) = 10^4 g_1 \left(\frac{P}{1+P}\right) \qquad (6)$$

expressed in square meters of pore area per cubic centimeter of the bulk volume of the stationary phase.

We examined the application of these equations to two different interactive columns and a macroporous PSDVB stationary phase (100 A μStyragel, Waters). This latter material tends to exhibit maximum swelling when immersed in a solvent to like polarity. Because this material can be compressed, or deswollen, it and other swelling gels have seemed difficult to pack reproducibly. A variety of packing procedures for the gels have been recommended. Moore (10) and Altegelt (11) advised the use of mild conditions. Dawkins (12) packed gels in a partly deswollen state. Balanced density slurries have also been used (13). We used Dawkins procedure with varying amounts of deswelling.

EXPERIMENTAL

Chemicals and Standards

Reagent grade tetrahydrofuran (THF) was distilled over sodium to remove stabilizer and peroxides. The distilled solvent was stored under nitrogen at 5° until

needed. Reagent grade methanol was used without further purification. Solute standards included two normal alkanes (C_5 and C_{16}) as well as seven polystyrene (PS) standards (MW = 600, 2100, 4000, 10000, 20400, 37000, and 220,000 obtained from Pressure Chemical Company in Pittsburgh, Pennsylvania).

Apparatus

The equipment used included a solvent pump (Waters Associates Inc., Model 60000A), six port injection valve (Rheodyne Model 7120 with 100 μl injection loop), refractive index detector (Laboratory Data Control) and a 254 nm ultraviolet detector (Waters Model 440). The data collection system was built around a Wang 2200 desk calculator which allowed data to be collected at one point per second with resolution of the detector output to one part in 256.

Columns and Packing Procedures

Two bonded phase interactive partition columns, μBondapack-C_{18} and μBondapak-NH_2 (both silica gel substrates) were obtained from Waters Associates, Inc. Their calculated internal volumes (V_C) were each 3.6 ml. These columns came prepacked and were used without any modifications. In measurements using the 100A μStyragel, one empty glass (0.635 x 12.9 cm) and one empty steel (0.4 x 30 cm) column were used to pack the material. This packing was done in the following manner.

First, fine particles were removed by six repeated sedimentations in methanol. The methanol was removed in an oven and the THF was added to form a slurry. Depending upon the particular column being packed (see Table I), a percentage of methanol was added to the slurry to partially deswell the gel. For the glass columns, the slurry was poured through an upper reservoir column into the lower receiving column. Connection to the pump was made at the head of the upper column, and fluid of the same composition as the slurry was passed through both columns under the flow rates and maximum pressures given in Table I. Packing was stopped when the formed bed rose above the bottom column. This column was then removed and connected to the full LC apparatus for the calibration. The steel columns were packed in essentially the same manner except that a steel slurry reservoir (Micromeritics) was used to hold the slurry below the receiving column. Packing, now in the upward direction, proceeded as before, under the conditions given in Table I. Each column was packed using the same sample of gel.

Calibration Procedures

The calibration of each column involved measurement in triplicate of the retention volumes of several probe solutes. The experimental conditions were as follows.

TABLE I

Packing conditions of flow rate, maximum pressure, and percent methanol are given for both glass (G) and steel (S) columns. Twelve experiments are listed.

Column	Flow Rate (ml/min)	Percent MeOH	Max PSI
1G	0.0*	0	0*
2G	2.0	10	500
3G	2.0	15	500
4G	2.0	25	500
5G	9.9	0	1000
6G	9.9	0	1500
7S	0.5	0	200
8S	0.5	0	200
9S	2.0	8	600
10S	2.0	20	2000
11S	9.9	0	2000
12S	9.9	0	2000

*This column was packed by gravity sedimentation.

Using a flow rate of 0.5 ml/min, the THF solvent was pumped through the column until a stable baseline was observed on the UV detector. At this point, polystyrene probe solutes (0.05% w/v in THF) were each sequentially injected. The injection order was from

largest polymer to smallest, and the process was repeated three times, for both of the interactive columns. From the UV detector response, retention volumes were calculated electronically using the automated data collection system and the Wang 2200 desk calculator. The void volume was taken from the retention volume of the excluded 220K PS peak. A similar procedure was followed for each of the variously packed GPC columns containing the 100 A μStyragel.

The calibration of the μStyragel was done immediately after packing was completed. The THF (δ = 9.1) carrier is closely matched to the gel polarity (14), δ(polystyrene-co-divinylbenzene) = 9.1, so the partitioning effect should be negligible. Using an RI detector, the baseline was monitored while the THF solvent was pumped through the system at a flow rate of 0.5 ml/min. When a stable baseline was reached, that indicated that the methanol used in the packing had been removed. This process also allowed the gel to re-swell to the limits dictated by its expansibility and packing constraints. Calibration followed this equilibration. The test solutes were the two normal alkanes pentane, hexadecane and an excluded polystyrene standard (MW = 10000, Pressure Chemical Co., 0.05% in THF). The calibration measurements were again obtained in triplicate for each of the test solutes.

Results

For each solute injected, symmetrically shaped peaks recorded. The retention volume was taken as the difference between the injection time and the location of the chromatographic peak maxium. Using this approach, the automated data analysis system yielded retention volumes precise to within 1%. From the retention volumes, distribution coefficients (D) were calculated using $D = (V_R - V_O)/V_S$ where V_O was the retention volume of an excluded solute and V_S was calculated from $V_C - V_O$. Then for each column, a set of data points containing paired values of $-\ln D$ and L was analyzed using least squares. This yielded values for the slope g_1 and intecept for Equation 3. As an example of this process, the raw results and computed data for the two interactive columns are given in Table II. A plot of $-\ln D$ against L for these columns is given in Figure I. From the linear data shown in Figure 1, we obtained the final results for these two columns using Equations 4, 5, and 6. The results are given in Table II.

The twelve packing tests of 100A μStyragel were treated in the same manner as the interactive columns. However, in this case, we wished to examine the changes in packing characteristics that varied with the overall efficiency of each column. So, in addition to the pore

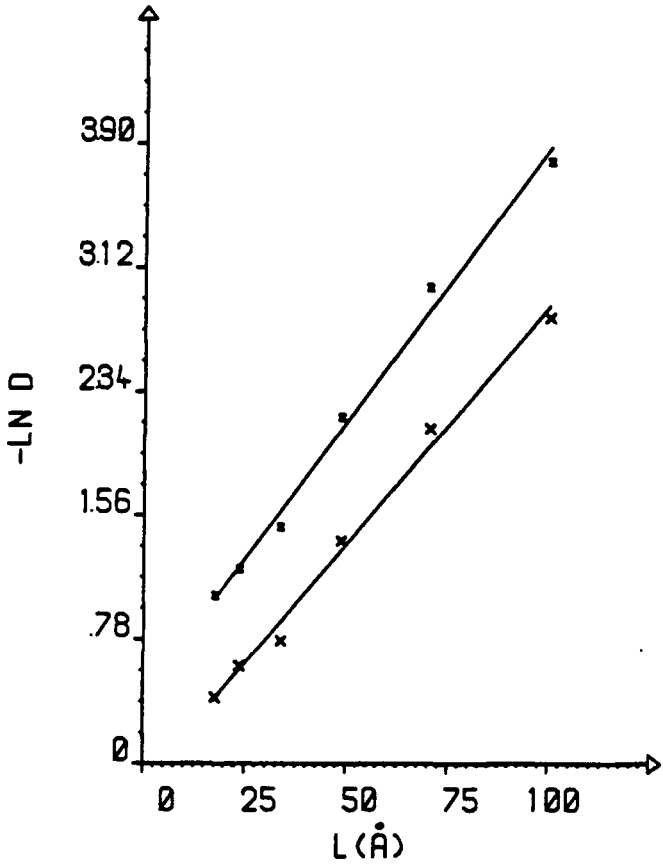

Figure 1. These plots are for μBondapak C-18 (above) and μBondapak-NH_2 (below). The linear relationship between ln D and solute size, L, is predicted by the Giddings, Freeman and Poinescu treatment of a monomodal pore size distribution in the stationary phase.

size, pore area and volume, we also calculated the resolution (between C_5 and C_{16}), and the plate height (from C_5). These latter values are found from the raw chromatographic data using conventional definitions. The data are presented in Table III.

TABLE II

Raw chromatographic results and computed D values for two interactive columns

PS Solute (MW)	L(A) (4)	μBondapak-C_{18} V (ml) R	D	μBondapak-NH_2 V (ml) R	D
600	18	2.14	0.348	2.96	0.659
2.1K	24	2.02	0.294	2.74	0.538
4.0K	34	1.87	0.226	2.60	0.462
10.0K	49	1.62	0.113	2.21	0.247
20.4K	70.7	1.48	0.050	1.98	0.120
37.K	100.4	1.42	0.0226	1.87	0.060
Column Parameters					
V_C (measured)			3.58 ml		3.58 ml
V_O (from 220K MW PS)			1.37 ml		1.76 ml
V_S (from $V_C - V_O$)			2.21 ml		1.82 ml
Void Fraction Estimate V_O/V_C			0.38		0.50
Calibration Results					
g_O			0.424		-0.116
g_1			0.0345		0.0299
d_{pore} (A)			116.		134.
Porosity, P			0.56		0.98
Surface Area, SA (m^2/cm^3)			120.		150.

DISCUSSION

The pore size determinations for both interactive columns agree to within 3% of the provided nominal values

TABLE III

The varying characteristics of 100Å Styragel are given in this table. Both the internal pore characteristics, d_{pore}, porosity P, and surface area S.A., as well as the column packing characteristics, plate height (from C_5) and resolutuon R (from C_5/C_{16}) are presented here. The "G" stands for glass while the "S" refers to the steel columns.

Column	d_{pore} (Å)	P	S.A. (m^2/cm^3)	Plate Height (cm)	R
1G	32	0.54	440	0.022	1.50
2 G	28	0.57	520	0.015	2.96
3G	31	0.21	230	0.038	1.36
4G	33	0.22	220	0.030	1.36
5G	31	0.54	250	0.028	1.43
6G	30	0.55	470	0.019	1.89
7S	35	0.34	290	0.029	1.30
8S	40	0.37	260	0.025	1.27
9S	30	0.26	280	0.042	1.51
10S	30	0.35	350	0.12	0.68
11S	27	0.54	530	0.017	2.90
12S	31	0.47	410	0.019	2.25

of 125+ 5 A (15). The porosity and surface area for the C_{18} column, while not verified for accuracy, appear as reasonable values. However, the NH_2 column results

seem less acceptable. This is particularly true for the porosity value which approaches 100% and must be in error. Another value which seems interesting is the void volume fraction (V_O/V_C). Values for spherical particles have been reported as 0.38 (16). However, we find a value closer to 0.50. This is a relatively high void volume, but that may not be unusual for a high speed packing technique.

Our results for the 100 A μStyragel demonstrate a more detailed usefulness of the inverse GPC technique. Consider the results in Table III for the two highest resolution columns (2 and 11). Both of these columns have the two smallest pore sizes, their porosities are both large, with the best column having the largest internal porosity of the entire set, and both of these columns have large surface areas. This type of information is easily obtained and of practical interest. While we do not have enough data to draw general conclusions about packing techniques, it is clear that inverse LC does show promise as a way to explore the factors which influence resolution in packed columns.

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