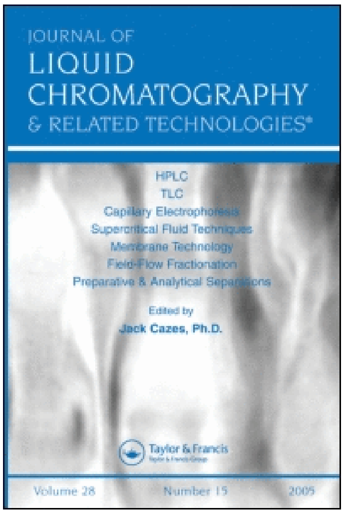

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Solute Retention in Column Liquid Chromatography. IV. Extension of the Langmuir Sorption-Isotherm Function to Include Stepped Variation of Retentions with Composition of Multicomponent Mobile Phases

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SOLUTE RETENTION IN COLUMN LIQUID CHROMATOGRAPHY. IV. EXTENSION OF THE LANGMUIR SORPTION-ISOTHERM FUNCTION TO INCLUDE STEPPED VARIATION OF RETENTIONS WITH COMPOSITION OF MULTICOMPONENT MOBILE PHASES

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ABSTRACT

Retentions are reported for substituted phenol solutes with a reverse-phase silica sorbent and acidified aqueous mobile phases containing 0-100% v/v tetrahydrofuran over the temperature range 10°-35°C. Curvature in plots of $1/k'_{(M)}$ against ϕ is substantial, although monotone, while those of $1/V'_{R(M)}$ against ϕ exhibit points of inflexion. All are shown to be described to well within experimental error by expansion of the Langmuir monolayer sorption isotherm relation presented and discussed previously in Parts I-III of this Series. Account is thereby taken of multilayer formation of sorbed mobile-phase constituents; the relation is then shown to describe all six BET isotherm Types of isocratic retention data, as well as retentions under conditions of gradient elution. Fully parabolic curve shapes for retentions derived from the literature are identified in addition that are not forecast or described by any other model heretofore put forth in column liquid chromatography, yet which are shown to be fitted nearly exactly also by the extended isotherm relation.

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INTRODUCTION

The mechanisms of retention and selectivity in "normal" as well as "reverse-phase" (rplc; i.e., derivatized-silica) modes of column liquid chromatography (lc) have yet to be clarified with detail sufficient to relate explicitly solute elution either with the make-up of the packing, the composition of the mobile phase, or the column temperature, as described and discussed in Parts I-III of this Series (1-3). To date, the most successful approach is that introduced by Scott and Kucera (4), wherein specific interactions are taken into account that occur in each phase, and which is said to be applicable both to adsorption and to partition chromatography. Their formulation leads, in the instance of binary mobile phases, to relations of the form:

$$\frac{1}{k'_{(M)}} = \frac{\phi_A}{k'_{(A)}} + \frac{\phi_S}{k'_{(S)}} \qquad (1)$$

where ϕ is a volume fraction, the subscripts referring to carrier components A or S, and where $k'_{(i)}$ (i = A, S, or M, the latter representing A + S) is the solute capacity factor with mobile phase i. However, eqn. 1 has proved to be inadequate in describing the retention behavior of many systems, insofar as it predicts that $k'_{(M)}{}^{-1}$ will vary linearly against ϕ_A whereas, more often than not, curvature is in fact observed. As a result, a modified form of eqn. 1 was introduced by Purnell, Wellington, and their coworkers (5), which is of the form:

$$\frac{1}{k'_{(M)}} = \phi_A \left\{ \frac{1}{k'_{(A)}} + \frac{b\ \phi_S}{1 + b'\ \phi_S} \right\} + \frac{\phi_S}{k'_{(S)}} \qquad (2)$$

where b and b' are system constants derived from analysis of the experimental data. The additional term in eqn. 2 arises directly from the Langmuir treatment of gas-solid adsorption, where b ≠ b' takes account of hysteresis in the sorption/desorption kinetics of liquid/solid systems pertinent to lc.

The isotherm equation, 2, has thus far been shown to provide excellent fits to all retention data examined to date, whether curved or linear (b and b' being set to zero in the latter instance), provided that whatever curvature is extant does not exhibit points of inflexion (1-3). However, the systems considered have in the main been comprised of silica adsorbent with binary non-electrolyte mobile phases over the compositional range pure solvent A to pure solvent S and, for a lesser number of reverse-phase systems, with somewhat more restricted mobile-phase compositions. We therefore examine in this work the retention characteristics of a number of substituted phenols with a reverse-phase sorbent and binary aqueous/organic mobile phases over the complete range of solvent composition, as well as over the temperature span 10^{o} to 35^{o}C. In addition, we develop further the isotherm relation, eqn. 2, in order to account for the observed retention phenomena. We then utilize data reported independently in the literature to test to what extent our modification describes other systems and in particular, retentions that vary in a stepped fashion with mobile-phase composition.

EXPERIMENTAL

Solvents. "HPLC"-grade tetrahydrofuran (THF) (Fisher Scientific) was employed as received and was the organic modifier (S) throughout this work. Demineralized and doubly-distilled water with reagent-grade acetic acid (Allied Chemical) comprised the aqueous component (A).

Mobile-Phase Preparation. Acetic acid at the level of 1% v/v was added to the water component of the mobile phase in order to diminish any interactions between residual unreacted or accessible silanol groups of the reverse-phase packing as well as to supress dissociation of the phenol solutes. Solutions of the aqueous blend with the organic modifier were prepared by volume just prior to use over the entire concentration range 0-100% THF in acidified water at intervals of 5% for the first 20%, and at intervals of 10% thereafter.

Solutes. Solutions of phenols in the mobile phase comprised the test samples, these being phenol (1), p-nitrophenol (2), 2,4-dinitrophenol (3), o-chlorophenol (4), o-nitrophenol (5), 2,4-dimethylphenol (6), and 2,4-dinitro-o-cresol (7). All were supplied by Chem Services.

Instrumentation. The liquid chromatograph employed in this work was a Varian Model 5000 with a Vari-Chrom variable wavelength UV detector, utilized at 254 nm. The injector was a Valco valve fitted with a 0.2-μl internal loop, whilst two identical columns were utilized, both being 25 cm x 4.5 mm i.d. packed with 5μ Spherisorb ODS (Jones Chromatography, Columbus, Ohio). Both the injector and column were immersed in a thermostated water bath, a Neslab Model RTE 8, which provided temperature control to at worst ± 0.03°C. The mobile phase was also brought to column temperature prior to entering the column.

Procedures. The column was allowed to equilibrate with each new mobile phase under constant flow until solute retention times remained constant. Retentions were then measured in triplicate. Data were taken over the entire solvent composition before changing to the next temperature. The flow rate, nominally 1 cm^3 min^{-1}, was monitored continuously with a thermostated (25°C) pipet. The column void volume V_A was taken as the average of the retention volumes of all solutes with 100% THF at each temperature (see later).

RESULTS AND DISCUSSION

Retention Volumes of the Test Solutes. The inverse raw retention volumes of all solutes at each temperature are presented in Table 1, where the subscripts A and S pertain to acetic acid + water and THF mobile phases, respectively. The averages of those with the latter carrier were taken at each temperature to correspond to V_A, and amounted consistently to ca. 2.1-2.2 cm^3.

Extension of Eqn. 2 to Account for Stepped Retentions. In contrast to our previous work with normal-phase (adsorption) systems and with rplc systems over the small volume fraction range $\phi = 0.2$ to 0.45, eqn. 2 was found in the present instance to provide poor fits to the experimental data at intermediate mobile-phase compositions. Further, curvature in the plots of $1/k'_{(M)}$ vs. $\phi_{modifier}$ extended over a far greater range than had heretofore been encountered. Moreover, in this work, plots of inverse raw retentions against the volume fraction of THF were found to be S-shaped, for which eqn. 2 fails to account entirely.

TABLE 1

Inverse Retention Volumes $V_{R(A)}^{-1}/cm^{-3}$ (Acetic Acid-Water Mobile Phase) and $V_{R(S)}^{-1}/cm^{-3}$ (THF Mobile Phase) for Test Solutes at 10°-35°C; and Fitted Parameters of Eqn. 3

Solute	T/°C	$V_{R(A)}^{-1}$/ cm^{-3}	$V_{R(S)}^{-1}$/ cm^{-3}	d	d'	e	e'
Phenol	10.0	0.0345	0.465	-0.447	-1.803	0.821	1.548
	15.0	0.0388	0.470	-0.451	-2.002	0.774	1.839
	20.0	0.0440	0.476	-0.429	-2.458	0.772	2.678
	25.0	0.0450	0.444	-0.453	-2.041	0.891	2.408
	30.0	0.0470	0.467	-0.369	-2.879	0.692	3.624
	35.0	0.0500	0.463	-0.453	-1.923	0.822	1.918
p-Nitrophenol	10.0	0.0090	0.468	-0.474	-2.528	0.779	2.277
	15.0	0.0166	0.488	-0.492	-2.479	0.799	2.280
	20.0	0.0140	0.484	-0.489	-2.526	0.827	2.394
	25.0	0.0160	0.452	-0.466	-2.445	0.857	2.430
	30.0	0.0150	0.467	-0.470	-2.516	0.861	2.603
	35.0	0.0190	0.490	-0.413	-3.095	0.705	3.401
2,4-Dinitrophenol	10.0	0.0075	0.474	-0.462	-2.722	0.750	2.353
	15.0	0.0080	0.481	-0.465	-2.733	0.765	2.424
	20.0	0.0100	0.482	-0.463	-2.743	0.780	2.453
	25.0	0.0120	0.453	-0.456	-2.725	0.822	2.618
	30.0	0.0130	0.467	-0.433	-2.825	0.776	2.772
	35.0	0.0140	0.473	-0.437	-2.899	0.776	3.088
o-Chlorophenol	10.0	0.0080	0.461	-0.460	-2.436	0.751	2.206
	15.0	0.0100	0.476	-0.479	-2.442	0.781	2.373
	20.0	0.0120	0.476	-0.476	-2.445	0.789	2.403
	25.0	0.0120	0.453	-0.456	-2.564	0.809	2.863
	30.0	0.0120	0.471	-0.412	-3.063	0.751	3.587
	35.0	0.0150	0.463	-0.515	-1.829	0.864	1.804
o-Nitrophenol	10.0	0.0125	0.453	-0.424	-2.367	0.640	2.040
	15.0	0.0130	0.470	-0.492	-2.385	0.642	2.281
	20.0	0.0140	0.468	-0.422	-2.306	0.652	2.127
	25.0	0.0160	0.451	-0.432	-2.201	0.710	2.088
	30.0	0.0170	0.465	-0.392	-2.722	0.636	3.193
	35.0	0.0180	0.454	-0.454	-1.636	0.719	1.392

TABLE 1 (Continued)

2,4-Dimethylphenol	10.0	0.0040	0.456	-0.440	-2.492	0.659	2.323
	15.0	0.0040	0.472	-0.474	-2.203	0.684	1.964
	20.0	0.0040	0.482	-0.441	-2.515	0.657	2.403
	25.0	0.0040	0.446	-0.431	-2.410	0.702	2.461
	30.0	0.0060	0.463	-0.441	-2.477	0.683	2.712
	35.0	0.0060	0.480	-0.436	-2.560	0.703	2.603
4,6-Dinitro-o-cresol	10.0	0.0040	0.456	-0.437	-2.565	0.620	2.184
	15.0	0.0040	0.467	-0.422	-2.693	0.605	2.366
	20.0	0.0040	0.478	-0.452	-2.539	0.659	2.124
	25.0	0.0040	0.448	-0.415	-2.644	0.643	2.385

To accomodate these observations, eqn. 2 was modified by the addition of a second term:

$$\frac{1}{k'_{(M)}} = \phi_A \left\{ \frac{1}{k'_{(A)}} + \frac{b\ \phi_S}{1 + b'\ \phi_S} + \frac{c\ \phi_S}{1 + c'\ \phi_S} \right\} + \frac{\phi_S}{k'_{(S)}} \qquad (3)$$

where c and c', as b and b', are derived from experiment, and where c is not set equal to c' so as to account for hysteresis.

Interestingly enough, the solutions to eqn. 3 for the systems studied here in fact turned out to be complex. However, straightforward substitution provides:

$$\frac{1}{k'_{(M)}} = \phi_A \left\{ \frac{1}{k'_{(A)}} + \frac{d\ \phi_S + e\ \phi_S^{\,2}}{1 + d'\ \phi_S + e'\ \phi_S^{\,2}} \right\} + \frac{\phi_S}{k'_{(S)}} \qquad (4)$$

where the following transformations have been made: d = (b + c); d' = (b' + c'); e = (bc' + cb'); and e' = (b'c'). For example, the roots of eqn. 3 for p-nitrophenol at 35°C are: b = -0.206 - 0.033i; b' = -1.548 - 1.007i; c = -0.206 + 0.033i; and c' = -1.548 + 1.007i. It is then a simple matter to confirm that those for eqn. 4 must then be: d = -0.413; d' = -3.095; e = 0.705; and e' = 3.401.

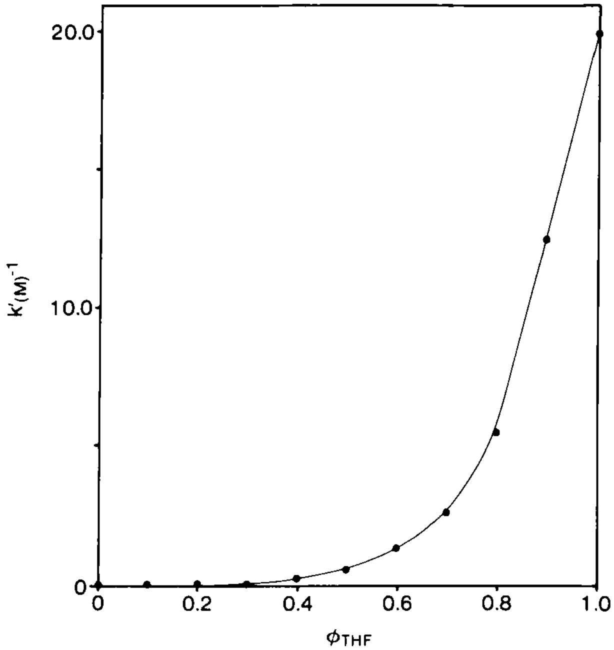

FIGURE 1. Plot of $k'^{-1}_{(M)}$ against ϕ_{THF} for 2,4-dimethylphenol solute at 15°C. Solid line constructed from eqn. 4 with constants of Table 1; solid circles: experimental data. Column: 25 cm x 0.46 mm i.d. containing 5μ Spherisorb ODS. Detector: UV at 254 nm.

Eqn. 3 is intuitively what would be written for multilayer formation while, by analogy, eqn. 2 would then be said to account only for monolayer sorption of a mobile-phase constituent on the surface of the stationary phase. There is some justification as well for not equating b with b' nor c with c' since it has been argued for some time (6) that surface-sorbed species can undergo what amount to second-order conformational changes in the manner in which they are sorbed, e.g., THF can conceivably be sorbed in a plane-parallel fashion, or on edge, or with the oxygen hydrogen-bonded to a hydroxyl group of silica or a free hydroxyl group in the case of reverse-phase silica. In addition, as Hubbard and his co-workers (7-9) have shown, multi-step conformational transformations can be brought about simply by altering the concentration of the sorbate present in the bulk

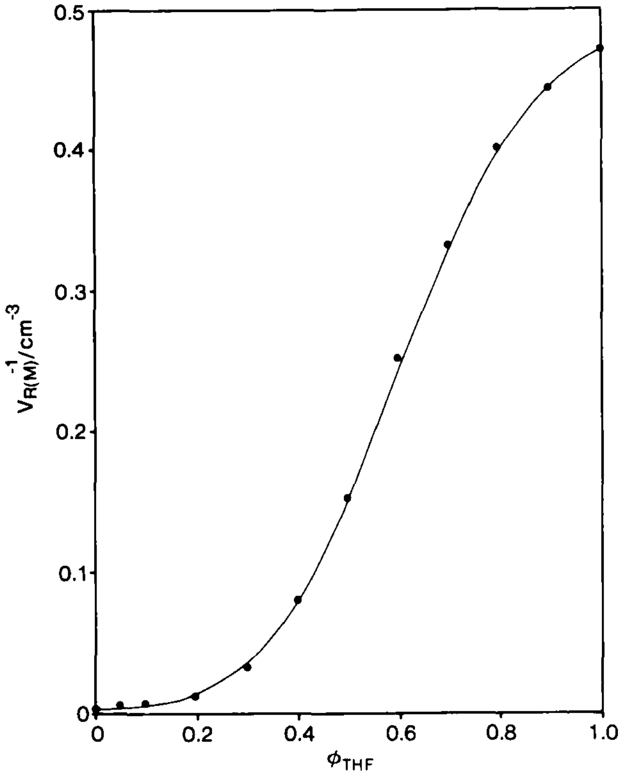

FIGURE 2. Plot of $V_{R(M)}^{-1}$ against ϕ_{THF} for o-chlorophenol solute at 15°C. Remaining conditions as in Figure 1.

liquid above the solid sorbent. Presumably, this would also alter the extent to which solutes can interact with sorbed mobile phase. Moreover, the conversion from one conformation to another is a smooth one and so, the retentions of solutes pass through smooth points of inflexion when plotted against mobile-phase composition, as opposed to exhibiting sharp discontinuities.

An example representative of the excellence of fit of the modified isotherm relation is shown in Figure 1, where the $k'_{(M)}{}^{-1}$ data for 2,4-dimethylphenol are plotted against ϕ_{THF}: the solid curve was generated via eqn. 4, the constants being calculated from the experimental data which are shown as solid circles.

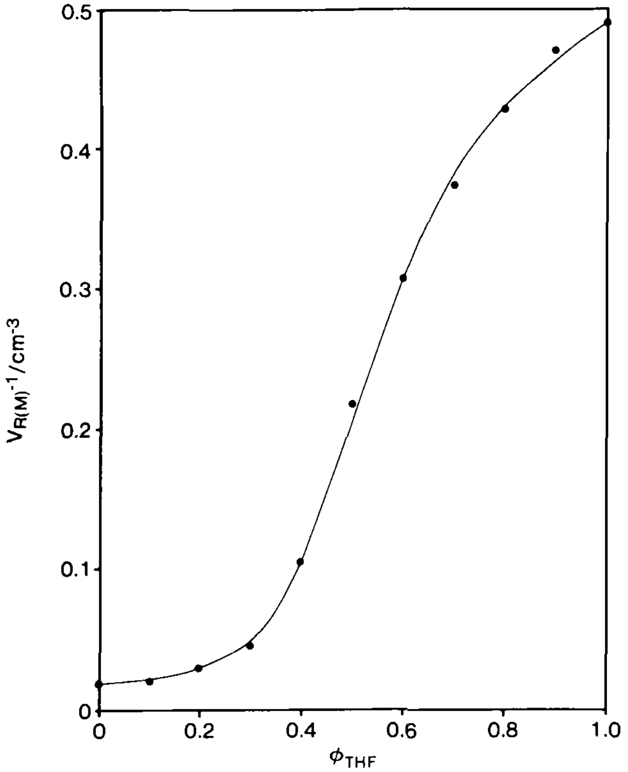

FIGURE 3. As in Figure 2; p-nitrophenol at 35°C.

Application of Eqns. 3 and 4 to Raw Retentions. An ever-present problem when using capacity factors is the need for accurate determination of the system dead volume V_A, this being especially critical at low retention volumes where small inaccuracies in retention measurements lead to large errors in calculated $1/k'_{(M)}$. To circumvent this difficulty, eqn. 4 was cast in terms of inverse uncorrected retention volumes, $1/V_R$. The resultant fitted values of d, d', e, and e' are reported in the fifth through eighth columns of Table 1 for all solutes studied at each temperature. The fits were found in every case to be excellent, examples of which are shown in Figures 2-4 for the solutes o-chlorophenol, p-nitrophenol, and phenol. As in Figure 1, the solid lines were constructed with the fitted parameters of

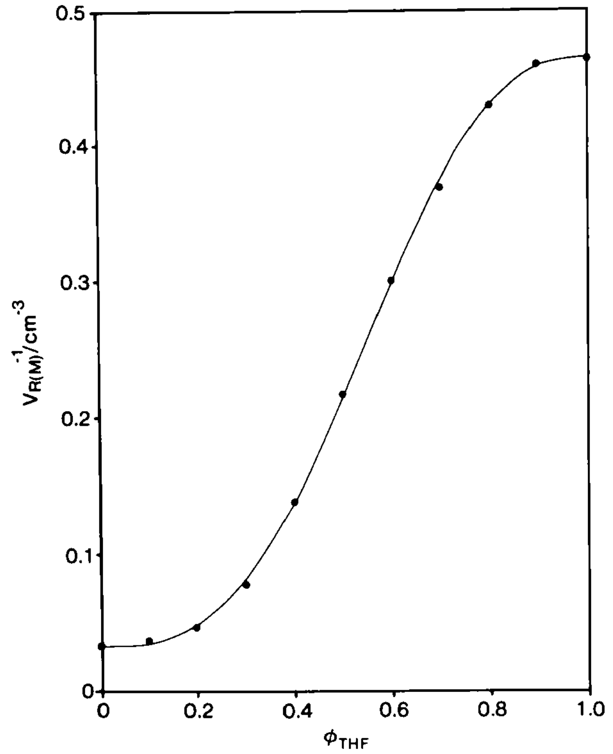

FIGURE 4. As in Figure 2; phenol at 35°C.

eqn. 4, while the solid circles correspond to the experimental data.

In order to provide a more exacting test of eqns. 3 and 4, the rplc retention data for the solute phenylalanine with water-acetonitrile, representative of the systems published recently by Hearn and Grego (10), were considered in terms both of capacity factors, for which a dead volume of 2.4 cm^3 was taken (11), as well as raw retention volumes. Their data covered only the first 80% of the volume fraction range and so, it was necessary to assign an arbitrary value of "ϕ_{ACN}" = 1 for an actual value of ϕ_{ACN} = 0.8 for fitting purposes. However, this presents no difficulty in assessing the goodness of fit of eqn. 4 since, as pointed out in Part III of this Series (3), an upper abscissa of hypothetical ϕ spanning the complete

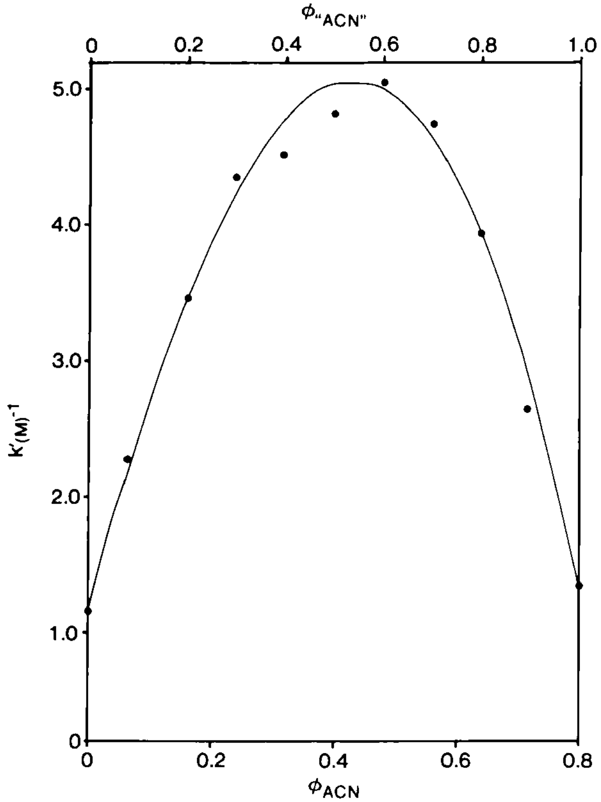

FIGURE 5. Plot of $k'^{-1}_{(M)}$ against "ϕ_{ACN}" (cf. Text) for phenylalanine solute with µBondapak C_{18} column at 18°C. Solid curve constructed from eqn. 4; circles represent experimental data taken from Ref. 10.

range ϕ = 0 to 1 can always be constructed in order to represent retention data, irrespective of the true range (or, indeed, identity) of the independent variable comprising the actual (lower) abscissa.

The results are presented in Figures 5 and 6 where the former, comprised of $1/k'_{(M)}$ against ϕ_{ACN}, is fitted by eqn. 4 equally as well as the latter in which use is made of ordinal $1/V_R$. Furthermore, each exhibits a new shape, namely fully parabolic, that has not previously been observed by us with rplc systems nor, for that matter, so far as we are aware, men-

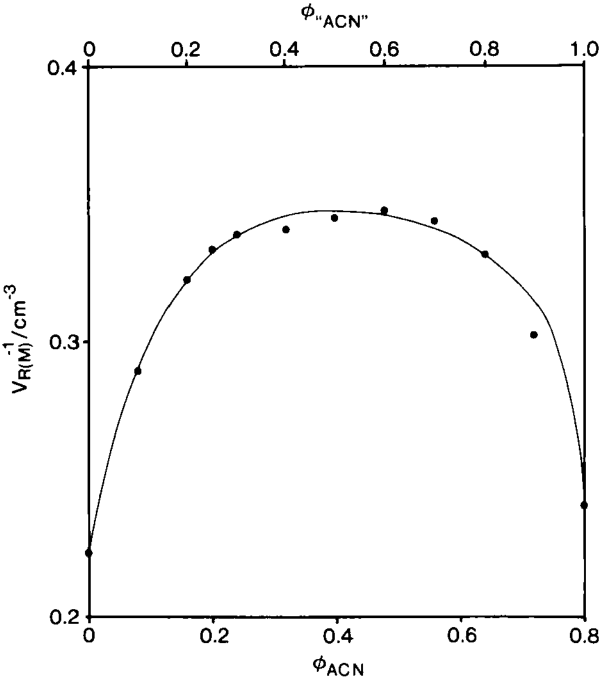

FIGURE 6. As in Figure 5 except ordinal $V_{R(M)}^{-1}$.

tioned as arising from any other data representation model or scheme. The figures therefore represent an important addition to the types of curves to which eqns. 3 and 4 apply, as well as allude to retention mechanism(s) heretofore considered as unimportant, or even impossible, in isothermal column liquid chromatography.

Schoenmakers, et al. (12) have also recently investigated some chromatographic systems similar to those of this study where, in their work, plots of ln $k'_{(M)}$ (M = water + methanol, acetonitrile, or THF) exhibited interesting maxima over the compositional range $\phi_{modifier} = 0$ to 0.1. The resultant data were then fitted to an equation of the form:

$$\ln k'_{(M)} = A\,\phi_A^2 + B\,\phi_A + C + D\,f^2(\phi_A) + E\,f(\phi_A) \quad (5)$$

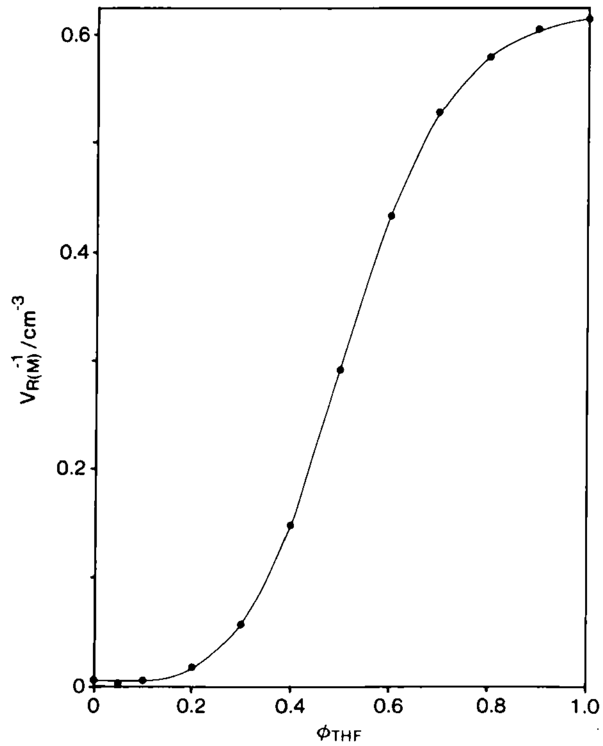

FIGURE 7. Plot of $V_{R(M)}^{-1}$ against ϕ_{THF} for p-chlorophenol solute with 5u Hypersil ODS column. Solid curve constructed from eqn. 4; circles represent experimental data taken from Ref. 12.

where the constants A, B, D, and E were said to relate primarily to the identity and solubility parameters of the constituents of the mobile phase; C = ln k'(water); and where the functions f were formulated so as to describe the variation of a mixed solubility parameter with mobile-phase composition. We show in Figure 7 their experimental data for p-chlorophenol replotted as V_R^{-1} against ϕ_{THF}, where the solid line was generated in accordance with eqn. 4 and where the solid circles are the observed inverse raw retentions (their reported value of V_A of 1.5 cm^3 was utilized in converting capacity factors to retention volumes). We see immediately that the retention behavior in fact differs little from that observed in this work,

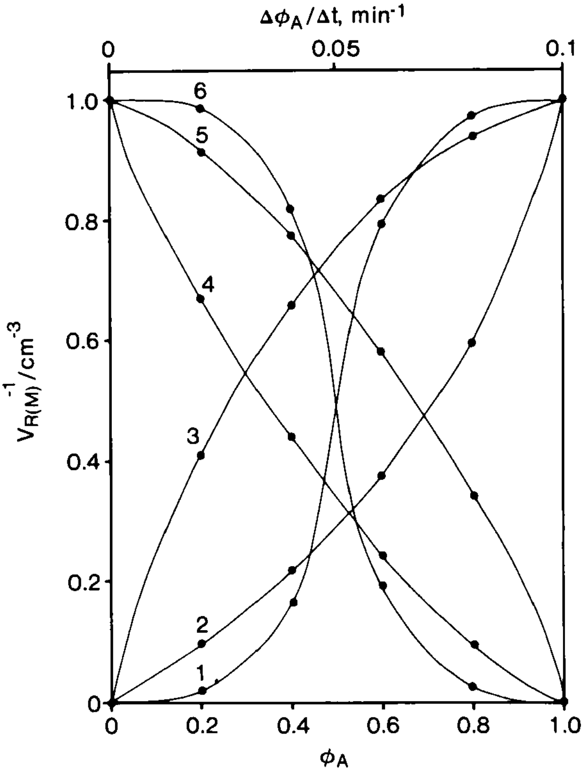

FIGURE 8. Hypothetical curve shapes arising in plots of $V_{R(M)}^{-1}$ against volume fraction of mobile-phase component A in isocratic mode of elution (lower abscissa) or under conditions of gradient elution (upper abscissa of $\Delta\phi_A/\Delta t$). Curves 1-3 represent BET isotherm Types V, III, I; curves 6, 5, and 4 are the respective mirror images reflected in the horizontal. Solid circles: arbitrary data points; solid curves then fitted with solid points and eqn. 4.

and that the inverse retentions exhibit a Type V isotherm (see below) as a function of mobile-phase composition.

Application of Eqns. 3 and 4 to Other Isotherm Types. The data representations shown in Figures 1-7 afforded by eqn. 4 (hence 3) are particularly gratifyingly in view of the complex shapes of the curves. The first of these corresponds, in the nomenclature of Brunauer, Emmett, and Teller (13), to a Type III sorption isotherm (observed commonly in our pre-

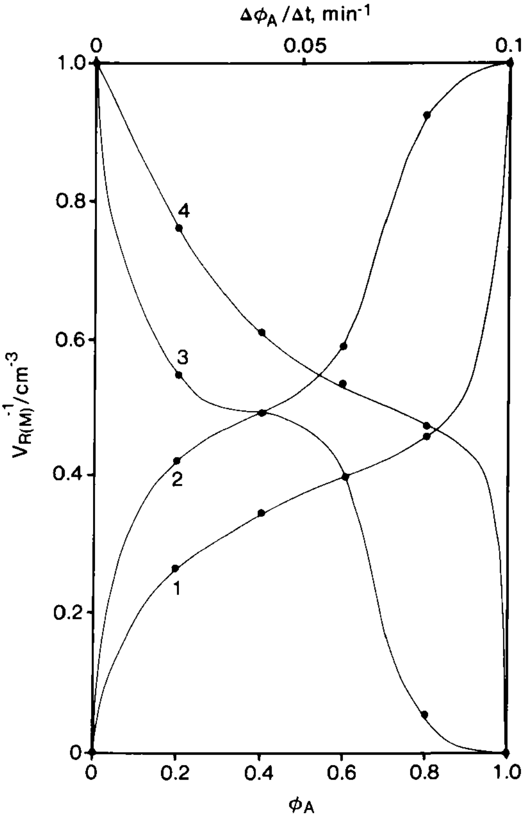

FIGURE 9. As in Figure 8; BET isotherm Types II, IV, and their mirror images.

vious work, as were systems exhibiting the zeroth, i.e., linear, Type 0 as well), while Figures 2-4 and 7 correspond to Type V (the behavior exhibited in Figures 5 and 6 have not been observed to our knowledge in gas-solid systems). There is therefore the possibility that eqn. 4 will describe the remaining isotherm Types I, II, and IV also, hypothetical examples of which are presented in Figures 8 and 9 along with their mirror reflections. The points (solid circles) were placed so as to produce the desired shapes, but the values of each were otherwise chosen purely at random. Following construction of the plots, eqn. 4 was then used to attempt to fit the data, the results constituting the solid curves. Once again we find that the two-term isotherm relation provides a near-exact description of the curve shapes.

The lower abscissas in Figures 8 and 9 have been cast as ϕ_A, but could equally depict some or other function of temperature; packing composition; or parameters related to alteration of the carrier phase during the course of a chromatographic run. For example, the upper abscissas have been labeled as $\Delta\phi_A/\Delta t$, that is, the plots might well represent inverse retentions observed under conditions of gradient elution. Optimization of the gradient program could then be carried out in the usual way with window diagrams and the computer algorithm described previously by Laub (3) in terms of "ϕ_A", where a perpendicular constructed from the optimal (hypothetical) "volume fraction" abscissa to the actual abscissa would yield the overall optimal gradient rate of change.

Finally, it is of some interest that the transformed version of eqn. 3, namely eqn. 4, bears at least superficial resemblance to the well-known isotherm relation of Brunauer, Emmett, and Teller when the substitution $\phi_A = 1 - \phi_S$ is made. The relation cannot therefore be dismissed as entirely empirical nor, at the same time, does its apparent universality constitute theoretical justification for its general form. Even so, we make the assertion that whatever theories are put forth to account for the stepped variation of retentions with composition of multicomponent mobile phases, each must reduce to eqns. 3 and 4 in the instances, at the least, of the very many varieties of systems now known to be described by these relations.

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3. Laub, R. J., **J. Liq. Chromatogr., 7**, 647, 1984. Two typographical errors require correction in this work: eqn. 4 refers of course to N_{req}, not N_{eff}; also, the statement following should read, "and where N and N_{eff} are related by...".

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