Journal of Chromatography, 505 (1990) 79–97
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 22 261

Physicochemical model of retention for capillary supercritical fluid chromatography

DEBRA R. LUFFER, WILHELM ECKNIG[a] and MILOS NOVOTNY*
Department of Chemistry, Indiana University, Bloomington, IN 47405 (U.S.A.)

SUMMARY

The solvating ability of a supercritical fluid mobile phase depends on density and temperature, which in turn govern solute retention. A model is proposed for capillary supercritical fluid chromatography that relates the logarithm of the capacity factor to the logarithm of the reduced density by an empirical equation. This relationship takes into account molecular weight and shape of solute and mobile phase, reduced density, reduced temperature, free energy of solute transfer between phases and film thickness of the stationary phase. Ultimately, each mobile phase and stationary phase combination can be described by a "phase constant" that seems to be dictated by the relative polarizability of the phases. This model has so far been proven valid for a representative group of polyaromatics.

INTRODUCTION

Unlike with gas chromatography (GC) and liquid chromatography (LC), where various retention models have been extensively developed for years, supercritical fluid chromatography (SFC) suffers from the lack of useful models for solute retention. Physicochemical models with a firm theoretical basis covering a variety of analytically useful separation conditions in SFC are necessary not only to separate and delineate the mobile phase and stationary phase contributions to retention, but also to offer insight into the interactions between the two phases and the solutes[1–4]. In modern SFC, the practice of separations is clearly far ahead of the theory to explain them.

Over the years, several models have been suggested to explain retention. Schoenmakers[5] proposed a rigorous thermodynamic model requiring the use of the Lee and Kesler equation of state[6], which covers a wide range of reduced pressures and temperatures. He then evaluated his model using data found in the literature by Van Wasen *et al.*[7]. However, the model does not yield a quantitative estimate for the capacity factor, k', and it requires the use of complex algebraic equations.

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[a] On leave from the Academy of Sciences of the German Democratic Republic, Central Institute of Physical Chemistry, Rudower Chaussee 5–8, Berlin 1199, G.D.R.

The most popular model proposed to date has been the assumption of a linear log k' vs. ρ (density) relationship, ascribed to by many researchers in the field[7–13]. However, in the cases where plots of log k' vs. ρ over a wide density range are shown, the curves are clearly non-linear. One common cause of this misrepresentation is that many of the curves are plotted over a very narrow range of densities[10,13], which is misleading.

Martire and Boehm[14] presented a unified theory of GC, LC and SFC in partition chromatography based on a fluid-lattice model. With respect to SFC, they showed that the reduced temperature and reduced density are the natural descriptors for the effect of the mobile phase on retention. This is logical since the reduced parameters are the only way to relate completely dissimilar fluid phases. The reduced density was believed to be involved in a quadratic relationship describing retention. The authors derived an equation to explain the retention of a series of n-alkanes and then attempted to fit literature experimental data to their model with moderate success. However, the main drawback of the quadratic relationship was that the coefficients were specific for a particular set of solutes in a specific system, and could not be further generalized to other SFC systems.

Although there exist various theoretical treatments of retention in SFC, there is a dearth of complete, informative experiments available to confirm or refute any claims made in the literature. The aim of this study was to investigate retention of a series of polyaromatics on all combinations of four mobile phases and three stationary phases of varying polarity. It was also hoped that from this database would emerge a logical, empirical relationship to adequately describe retention in SFC.

THEORY

The solubility in fluid systems at low density, and consisting of weak intermolecular interactions, can be described[15] by a linear relationship between the logarithm of the fraction of the solute that is present in the mobile phase, X_M, and the density at constant temperature, ρ_T by

$$\log X_M = b\rho_T + c \tag{1}$$

where b and c are coefficients dependent on the properties of the fluid at that particular temperature. The quantity X_M, which is also referred to as the solute distribution in the mobile phase, is defined by

$$X_M = n_M/(n_M + n_S) \tag{2}$$

where n_M and n_S are the moles of solute in the mobile phase and stationary phase, respectively.

The relationship in eqn. 1 has been corrected by a higher order term in order to compensate for deviations from linearity, so that

$$\log X_M = a\rho_T^2 + b\rho_T + c \tag{3}$$

where a is another constant dependent on the fluid and temperature. Eqn. 3 is a valid correction to eqn. 1, but it has been found that at very low pressures the correction becomes unnecessary. However, Franck[15] predicted that at higher densities, solubility in the fluid phase would require an empirical logarithmic relationship in order to account for the stronger intermolecular interactions between solvent and solute. This is described by

$$\log X_{\mathrm{M}} = b' \log \rho_T + \mathrm{C}' \tag{4}$$

where b' and c' are once again constants that depend on the fluid and temperature. In order to establish the validity of eqn. 4 as model of solvation in SFC, it is necessary to identify the factors that contribute to the coefficients b' and c'.

Importantly, eqn. 4 must be further modified so that it can be used to describe any supercritical fluid in SFC, and thereby allow the different fluids to be directly compared with one another. This is accomplished by replacing the density with the reduced density at constant temperature, $\rho_{\mathrm{R}(T)}$, which is defined by

$$\rho_{\mathrm{R}(T)} = \rho_T/\rho_{\mathrm{c}} \tag{5}$$

where ρ_{c} is the critical density of the fluid of interest. Eqn. 4 can now be rewritten in its new form

$$\log X_{\mathrm{M}} = b' \log \rho_{\mathrm{R}(T)} + c'' \tag{6}$$

It should be noted that the alteration of the abscissa scale by a constant amount will produce the same slope as in eqn. 4, but the intercept will change, as indicated by the new constant, c''.

At this point, eqn. 6 must take on a form that relates solubility to retention in SFC. By definition,

$$k' = X_{\mathrm{S}}/X_{\mathrm{M}} = n_{\mathrm{S}}/n_{\mathrm{M}} \tag{7}$$

where X_{S} is the solute distribution ratio in the stationary phase (*cf.*, eqn. 2), and k' is the capacity factor, which can be related to retention by

$$k' = (t_{\mathrm{R}} - t_0)/t_0 \tag{8}$$

where t_{R}, t_0 are solute and void-volume retention times, respectively. The logarithmic dependence is simply introduced by

$$\log k' = \log X_{\mathrm{S}} - \log X_{\mathrm{M}} \tag{9}$$

Eqn. 9 is the first and most important step in actually separating the mobile phase from the stationary phase contribution to retention. If we substitute eqn. 6 into eqn. 9, this results in

$$\log k' = \log X_{\mathrm{S}(T)} - c''_{(\mathrm{T})} - b' \log \rho_{\mathrm{R}(T)} \tag{10}$$

where all the terms in the above equation are dependent on temperature. Eqn. 10 is the first step in evaluating the relationship between mobile phase density and retention in capillary SFC. In reality, if the concentration of solute in the mobile phase is varied due to a corresponding density increase, the concentration in the stationary phase must also vary, although not to the same extent as the former. The major assumption being made is that X_S exhibits its own distinct density dependence, similar to that of X_M (*cf.*, eqn. 6); *i.e.*, log X_S is linearly related to log $\rho_{R(T)}$ by

$$\log X_S = a' \log \rho_{R(T)} + d'' \tag{11}$$

Hence a combination of eqn. 10 with eqn. 11 results in

$$\log k' = d''_{(T)} - c''_{(T)} + (a' - b') \log \rho_{R(T)} \tag{12}$$

The immediate consequence of assuming this linear variation of log X_S with log $\rho_{R(T)}$ is the introduction of a new constant, $d''_{(T)}$, which is analogous to the $c''_{(T)}$ term for the mobile phase, where both constants are dependent on temperature, but independent of density. It-is evident from eqn. 12 that a straight line should be obtained from a plot of log k' *vs.* log $\rho_{R(T)}$. This implies that the slope of such a curve will contain a contribution from the variation of X_S with reduced density, denoted by the constant, a’. In practice, the slope of the log-log plot is found to be a negative value; this implies that a’ $\ll b'$ in the temperature and density range studied. Unfortunately, it may not be possible to specifically distinguish the mobile phase contribution from that of the stationary phase. For the sake of simplicity, in the remainder of the paper the (a’ — 6’) term in eqn. 12 will be referred to as e’ in the following manner:

$$\log k' = d''_{(T)} - c''_{(T)} + e' \log \rho_{R(T)} \tag{12}$$

EXPERIMENTAL

Apparatus

The instrument used for all experiments was a Model 602 capillary and packed-column SFC system (Lee Scientific, Salt Lake City, UT, U.S.A.). The syringe pump assembly has a capacity of 175 ml and is surrounded by a cooling jacket through which cold water is circulated in order to fill with the desired mobile phase. The cooling water was supplied by an RTE-110 refrigerated bath circulator (Neslab Instruments, Portsmouth, NH, U.S.A.). The water temperature was maintained between 5 and 10°C during the filling process; however, the bath was shut off when the fill was complete in order to allow expansion of the mobile phase at room temperature.

A pressure-relief valve system and fluid manifold system containing basic alumina (80-200 mesh, Brockman activity I) was placed between the gas cylinder and the pump in order to trap organics, particles and water. In addition, a charcoal filter was situated prior to the alumina trap in order to further ensure the trapping of organic impurities.

Injections were performed by a pneumatically operated injection valve (Valco, Houston, TX, U.S.A.) with a 200-nl sample loop. A variable length (6 to 8 cm) of 10 μm I.D. fused silica was used to split the sample upon injection. The split ratio was approximately 10:1. Injection time ranged between 0.06 and 0.10 s.

A flame ionization detection (FID) system was used for all experiments except those performed with SF_6. This is due to the fact that upon combustion, SF_6 produces HF, which eventually erodes the collector electrode of the detector. To circumvent this problem, an ultraviolet detector (UVIDEC-100 II, Jasco, Easton, MD, U.S.A.) was placed prior to the FID system and configured in the following way: the end of the 50 μm I.D. column was removed from the oven through an existing exit port and attached by epoxy glue to approximately 2 cm of 250 μm I.D. fused silica that had a l-cm section of its polyimide coating burned away. The other end of this detector cell was connected by epoxy glue to the distal end of a 50 μm I.D. frit restrictor (Lee Scientific) which was also brought outside of the oven by way of the same exit port in the oven side. The frit end of the restrictor sat inside the heated FID system (325°C) as in all of the experiments with other mobile phases, except the flame was not lit. This arrangement is desirable because the column and restrictor configurations are essentially the same for both detection strategies. The absorbance at 254 nm was observed for all injections.

Three 50 μm I.D. capillary columns were obtained from Lee Scientific. The columns and their specifications are summarized in Table I. The four mobile phases chosen were supercritical fluid grade carbon dioxide, nitrous oxide, sulfur hexafluoride (Scott Specialty Gases; Plumsteadville, PA, U.S.A.) and xenon (Air Products, Shelbyville, IN. U.S.A.).

TABLE I

50 μm I.D. COLUMNS USED IN THESE STUDIES

Column	*Stationary phase*	*Film thickness (pm)*	*Length (m)*
A	SB-Methyl-100%	0.50	10
B	SB-Biphenyl-30%	0.15	15
C	SB-Cyanopropyl-50%	0.15	10

The solutes studied were naphthalene, biphenyl, fluorene, phenanthrene, fluoranthene, pyrene, *para*-terphenyl and chrysene. All were obtained from Aldrich (Milwaukee, WI, U.S.A.). The two polyphenyls were included in order to verify the assumption that their retention is governed by a different mechanism than that of the polynuclear aromatic hydrocarbons. The solvent used to dilute these compounds was methylene chloride (Mallinckrodt, Paris, KY, U.S.A.). The concentration of each solute was between 4 and 10 mg/ml.

Procedures

The procedures consisted of replicate injections (usually three) of a mixture of the solutes at the desired temperature and density. Two sets of data were collected for every mobile phase and stationary phase combination. One set consisted of fixing the density and investigating retention as a function of temperature. The other set involved varying the density under constant-temperature conditions.

The mobile phase filling procedure was straightforward for all gases except xenon. In this instance, the xenon gas had to be trapped in a stainless-steel coil configuration by immersing the coils in liquid nitrogen for several minutes. Subsequently, the tank valve was closed, the liquid nitrogen removed, and the coils allowed to warm up to room temperature. In this way, the xenon was forced through a one-way valve into the cooled pump housing. The procedure was repeated five or six times, or until the pressure in the pump read 45 atm. At this time, the cooling water was shut off and the xenon allowed to expand to room temperature. This method of filling generated an initial till-pressure of approximately 70 atm, as opposed to approximately 20 atm without the trap.

The densities of CO_2 and N_2O were provided directly by the Lee Scientific Software, which utilizes the theory of corresponding states for all calculations. However, since no such data were available for Xe and SF_6, this entailed the use of an iterative program based on the Lee-Kesler equation of state for a compressible fluid[6]. The densities calculated with this program for CO_2 and N_2O agreed with those from the instrument software to within 5%.

All data were collected by a Perkin-Elmer (Norwalk, CT, U.S.A.) Sigma 10 integrator. The peak retention times were used to calculate capacity factors for each solute. The dead volume retention time (t_0) was assumed to be the elution time of the solvent, methylene chloride.

RESULTS AND DISCUSSION

Slope

Only a few studies in the literature have given the log k' *vs.* log ρ_R model any credence[7,9,16]. A first step in the evaluation of the proposed log-log relationship is to examine the experimental evidence to see if other models can account for the observed behavior. Hence, for each set of data at constant temperature, the log k' vs. ρ plot was constructed in order to test the linearity of retention with density. Fig. 1 is

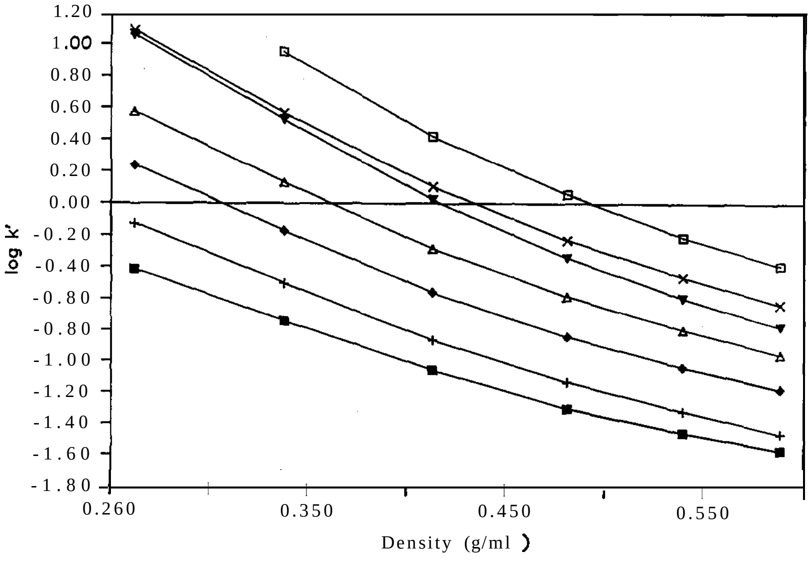

Fig. 1. Retention behavior with density: nitrous oxide and column B. ■ = Naphthalene; + = biphenyl; ● = fluorene; △ = phenanthrene; × = pyrene; ▼ = *p*-terphenyl; □ = chrysene.

a representative example of such a plot; it is clear that deviations from linearity occur. Furthermore, the very shape of the curve appears to suggest a logarithmic dependence. It should be noted that this same curve shape was found for every plot of this type.

A quadratic dependence was also considered as a means of explaining the non-linearity of the curve. The model would only be valid in the event that a minimum in the curve exists at high densities[14]. To date, no experimental data points have been found in the literature that pass through a minimum in the curve, although the minimum may exist at an "imaginary" density. No minimum was detected for any of the solutes studied in our laboratory; invariably, retention continued to decrease as density was increased.

Fig. 2 is a log-log plot of the same data as in Fig. 1. These results were encouraging in that the log-log relationship was more successful than the other two models in producing a straight-line relationship between retention and density, even though the densities do not span a very wide range. It is important to point out that the lines connecting the data points in Fig. 2 arise from the linear regression calculated for the data sets, with a correlation coefficient of 0.999 or better. Although Fig. 2 represents retention for seven solutes at a particular temperature on a specific mobile phase-stationary phase combinaton, similar plots were obtained for 8 solutes at 4 other temperatures, on 11 other mobile phase-stationary phase systems; in total, 480 such straight line log-log relationships were produced during the course of this work. However, it was still deemed necessary to attach physical and chemical significance to the slopes and intercepts of these log-log plots rather than to adopt the model by default.

The log-log relationship proposed by Franck[15] and extended to eqn. 6, predicts a monotonic increase in solute solubility with fluid density. Hence, the choice of this model must be justified in light of recent evidence that at very high fluid densities, the

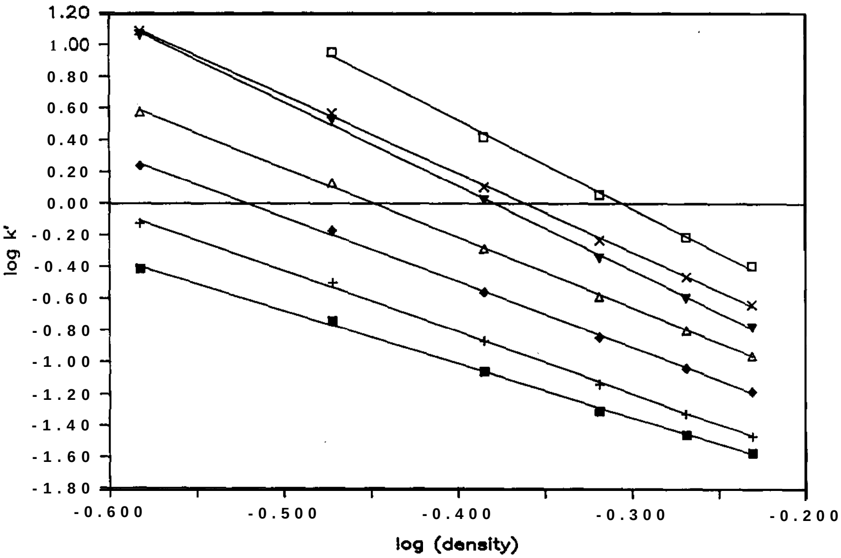

Fig. 2. Retention behavior with log (density): system and solutes as in Fig. 1

TABLE II

EXAMPLES OF SLOPES AND INTERCEPTS OBTAINED FROM THE LOG-LOG PLOT (EQN. 12)

Solute	*Column A and CO_2*				*Column B and N_2O*				*Column C and SF_6*			
	Slope		*Intercept*		*Slope*		*Intercept*		*Slope*		*Intercept*	
	Temperature (K)		*Temperature (K)*		*Temperature (K)*		*Temperature (K)*		*Temperature (K)*		*Temperature (K)*	
	398	*473*	*398*	*473*	*398*	*423*	*398*	*423*	*423*	*523*	*423*	*523*
Naphthalene	-2.77	-1.77	-0.759	-1.03	-3.06	-2.77	-1.37	-1.45				
Biphenyl	-3.14	-2.02	-0.625	-0.936	-3.56	-2.93	-1.22	-1.32	-2.22	-0.884	-0.813	-1.25
Fluorene	-3.39	-2.21	-0.441	-0.762	-3.75	-3.26	-0.918	-1.05	-2.18	-0.926	-0.407	-0.856
Fluoranthene	-4.04	-2.65	-0.102	-0.473	-4.46	-4.01	-0.406	-0.587	-2.31	—	+0.346	—
Phenanthrene	-3.67	-2.42	-0.279	-0.631	-4.08	-3.61	-0.676	-0.832	-2.30	-0.955	-0.0472	-0.506
Pyrene	-4.08	-2.77	-0.0453	-0.445	-4.55	-4.05	-0.328	-0.505	-2.48	-0.965	+0.390	-0.0724
Chrysene	-4.63	-3.12	+0.133	-0.306	-5.24	-4.50	-0.0515	-0.239				

final predominance of repulsive forces may “squeeze” the solute out of the fluid phase[14,17]. This premise is further supported by the discovery that the infinite-dilution partial molal volume of solute in a high-density fluid ultimately becomes positive[18]. For the purpose of modeling retention in SFC, it is safe to say that we will not be examining densities that are high enough to exhibit this phenomenon, i.e. $\rho_R > 1.6$ (ref. 18). In addition, it can be assumed that the high mobile phase velocity associated with a very high density would cause elution of most low-molecular-weight solutes with the void volume, thereby masking any expected repulsive force interaction.

Table II contains a very small representation of data obtained from a linear regression of a log k' vs. log ρ_R plot. The trends shown in this table were observed for all systems studied and are discussed in detail here.

As temperature is increased for a given solute, the slope increases while the intercept decreases. As solute molecular weight increases at a given temperature, the slope decreases while the intercept increases. However, the most striking feature of the data was that when the slope is divided by the solute molecular weight at a given temperature the result is roughly constant for a pseudo-homologous series of polyaromatics (phenanthrene, pyrene and chrysene). It should be noted that a different constant was obtained for the polyphenyls, biphenyl andp-terphenyl, which supports the belief that the two groups are governed by very different modes of retention based on shape. Furthermore, it is clear from Fig. 2 that the slopes of the curves for these compounds deviate significantly from those of the polyaromatic compounds.

In order to prove that these trends were not restricted to our results, we sought comparable systems in the literature as well. Notably, Yonker *et al.*[3] produced retention data at different temperatures for chrysene in CO_2 on an SE-54 stationary phase (column D in Table III). Not surprisingly, the slopes calculated from their data followed the very same trends as ours; in addition, the values for the slopes were in the same range as those calculated for our stationary phases at similar temperatures. The calculated slopes are summarized in Table III.

TABLE III

COMPARISON OF LOG k' *VS.* LOG ρ_R (CO,) SLOPES WITH LITERATURE VALUES FOR CHRYSENE

Temperature (K)	*Column*			
	A	*B*	*c*	*D (ref. 3)*
373	−5.07	-5.14	-5.38	-4.88 (380.2 K)
398	-4.63	-4.84	-4.90	-4.39 (400.4 K)

It was soon discovered that, in addition to the solute molecular weight, a “shape factor” would be required to account for the distinctly different retention times of the positional isomers, fluoranthene and pyrene. For this reason, the Van der Waals volume (b_v term) was introduced, which could account not only for the size of the solute, but for the shape as well. Since these values are not commonly found in the literature, they had to be estimated based on a procedure detailed by Edward[19] and Bondi[20]. The calculated values (b_v^{sol}) for the eight solutes are shown in Table TV.

TABLE IV

CALCULATED VAN DER WAALS VOLUMES

Based on procedure outlined in refs. 19 and 20.

Solute	*Volume (ml/mol)*
Naphthalene	14.29
Biphenyl	90.60
Fluorene	95.79
Phenanthrene	83.46
Fluoranthene	109.82
Pyrene	109.06
p-Terphenyl	132.84 (max)
Chrysene	125.18

It seemed logical that if size and shape of the solute were vital, then the same should be true for the mobile phase. Therefore, the molecular weight of the mobile phase was included in addition to its Van der Waals volume. These b_v values are tabulated for most gases[21]; however, an approximation from the Van der Waals equation of state was adopted for b_v that makes use of the critical values of the individual mobile phases?

$$b_v = R_1 T_c / 8 P_c \tag{13}$$

where T_c, P_c are the critical temperature and pressure of the given mobile phase, respectively, and R_1 is the gas constant in units of l atm/mol K.

At this point, the slope from eqn. 12, e', was found to be proportional to the following expression:

$$\frac{\mathrm{MW}^{\mathrm{sol}}\ R_1\ T_c}{\mathrm{MW}^{\mathrm{mob}}\ b_v^{\mathrm{sol}}\ 8P_c} \tag{14}$$

where $\mathrm{MW}^{\mathrm{sol}}$ and $\mathrm{MW}^{\mathrm{mob}}$ are the molecular weight of the solute and mobile phase, respectively. Eqn. 14 represents the status of the empirical equation using only our constant temperature data, and so far lacks a suitable proportionality constant.

The next step was to look at the constant density experiments involving the variation of temperature, which involved a plot of $\ln k'$ *vs.* l/T. This is a variation of the Van 't Hoff plot from which enthalpy and entropy change are obtainable from slope and intercept, respectively:

$$AH = -(\mathrm{slope})R_2 \tag{15}$$

$$\mathrm{AS} = (\mathrm{intercept} + \ln \beta)R_2 \tag{16}$$

where R_2 is the gas constant in units of cal/mol K, and β is the phase ratio, defined by:

$$\beta = V_M / V_S \tag{17}$$

where V_M and V_S are the volumes occupied by the mobile and stationary phase, respectively. For these systems, the enthalpy and entropy values describe solute transfer *from* the mobile phase *to* the stationary phase. It should be noted that the *AS* values were meaningful only if swelling was neglected and the phase ratio was known. The calculated phase ratios were 25 for column A, and 83 for both columns B and C.

Obviously, the thermodynamics of solute transfer play a crucial role in retention in SFC[23]. In order to verify this fact, *AH* and *AS* were combined for each solute on each system by

$$AG = AH - TAS \tag{18}$$

where *AG* is the free energy of solute transfer from mobile to stationary phase. Table V contains *AH* and *AS* values that exemplify the trends noted for all systems studied. The corresponding *AG* values calculated for two different temperatures are also included in Table V. The following is a summary of the trends observed for *AG*.

(a) For a given solute at constant temperature, *AG* increases (i.e., its absolute value decreases) as density increases. This is explained by the increasing solvating ability of the mobile phase, which makes solute transfer into the stationary phase less favorable.

(b) For a given solute at constant density, *AG* increases as temperature increases. This may be explained by the increase in thermal energy imparted to the system.

(c) At constant temperature and density, *AG* decreases as the solute molecular weight increases. This indicates a lower solubility of the larger. heavier molecules in the mobile phase. In addition, one would expect more negative *AG* values for compounds that are retained longer and would, therefore, have larger partition coefficients.

It was necessary to prove that *AG* is included in the e' term of eqn. 12, by assuming that this slope is proportional to log *K*, were *K* is the partition coefficient, defined by:

$$K = k'\beta \tag{19}$$

Proving that *AG* is included in the slope was accomplished by combining eqn. 19 with the following relationship:

$$\log K = \frac{-AG}{2.303R_2T} \tag{20}$$

whereby the combination of the two equations results in the following:

$$\frac{-AG}{2.303R_2T} = \log k' + \log \beta \tag{21}$$

Subsequently, eqn. 21 was combined with eqn. 12 to yield

$$\frac{-AG}{2.303R_2T} = d''_{(T)} - c''_{(T)} + e' \log \rho_{R(T)} + \log \beta \tag{22}$$

TABLE V

THERMODYNAMICS OF SOLUTE TRANSFER AS A FUNCTION OF TEMPERATURE AND REDUCED DENSITY FOR COLUMN B AND CO_2

Solute	*AH (kcal/mol) and AS (cal/mol K)*				$\Delta G_{(T)}$ *(kcal/mol)*			
	ρ_R = 0.6547		ρ_R = 0.8712		*T* = 398 *K*		*T* = 448 *K*	
	AH	*AS*	*AH*	*AS*	ρ_R = *0.6547*	ρ_R = *0.8712*	ρ_R = *0.6547*	ρ_R = *0.8712*
Naphthalene	—	—	-5.41	-9.69	—	-1.55	—	-1.07
Biphenyl	-7.24	-11.7	-6.19	-10.8	-2.60	-1.91	-2.02	-1.37
Fluorene	-7.42	-10.6	-6.62	-10.4	-3.21	-2.48	-2.69	-1.97
Fluoranthene	-9.02	-11.4	-8.00	-11.1	-4.49	-3.57	-3.92	-3.02
Phenanthrene	-8.15	-10.9	-7.22	-10.6	-3.81	-3.01	-3.27	-2.49
Pyrene	-9.14	-11.2	-8.13	-11.0	-4.68	-3.75	-4.12	-3.20
Chrysene	-10.6	-13.1	-8.99	-11.6	-5.23	-4.36	-4.78	-3.78

Finally, eqn. 22 was rearranged to the following form:

$$-\Delta G_{(T)} = (2.303R_2T)\,[d''_{(T)} - c''_{(T)} + \log\,\beta] + (2.303R_2T)e' \log\,\rho_{R(T)} \qquad (23)$$

Hence, a plot of $-\Delta G_{(T)}$ *versus* log $\rho_{R(T)}$ should result in a straight line with slope $(2.303R_2T)e'$, at a given temperature, ***T.*** Based on eqns. 21-23, thee’ value obtained in this manner should be identical to that obtained from the log *k' vs.* log $\rho_{R(T)}$ plot. The results for three different systems at three nominal temperatures are shown in Table VI; the agreement is very good, even striking in some cases considering that the ***AG*** values were obtained from a completely different set of experiments (constant density) than those obtained from eqn. 12 (constant temperature).

TABLE VI

COMPARISON OF e’ VALUES OBTAINED FROM THE SLOPE OF $-\Delta G_{(T)}$ *VS.* LOG $\rho_{R(T)}$ (EQN. 23) WITH THOSE OBTAINED FROM THE SLOPE OF LOG *k' VS.* LOG $\rho_{R(T)}$ (EQN. 12)

Solute	*e’*					
	Column A and CO_2, *T = 373 K*		*Column B and* N_2O, *T = 448 K*		*Column B and CO,. T = 398 K*	
	Eqn. 23	*Eqn. 12*	*Eqn. 23*	*Eqn. 12*	*Eqn. 23*	*Eqn. 12*
Naphthalene	-2.89	-3.00	—	− 2.70	_	-3.00
Biphenyl	-3.31	-3.41	—	-3.17	-3.38	-3.31
Fluorene	-3.69	—	-2.78	-2.96	-3.50	-3.53
Fluoranthene	-4.43	-4.42	-3.58	-3.75	-4.24	-4.23
Phenanthrene	-3.90	-4.01	-3.22	-3.26	-3.79	-3.85
Pyrene	-4.38	-4.47	-3.63	-3.67	-4.29	-4.28
Chrysene	-5.20	-5.07	-4.24	-4.21	-4.99	-4.84

The free energy can now be incorporated into the expression for the slope, *e'*, by multiplying eqn. 14 by the right-side of eqn. 20 to yield

$$\frac{AG \quad R_1 T_c\, \mathrm{MW}^{\mathrm{sol}}}{8P_c \quad 2.303R_2T \quad \mathrm{MW}^{\mathrm{mob}}\; b_v^{\mathrm{sol}}} \qquad (24)$$

This expression produces a value which is directly proportional to the experimental value of the slope for any solute in any mobile phase at any temperature, except it does not account for the density dependence of the free energy change. As stated earlier, it was not surprising that ***AG*** is density dependent; however, the key was to quantify this dependence and include it in the expression in eqn. ***24.*** Ultimately, it was determined that the product of the free energy and the reduced density, ***AG*** ρ_R, is a constant for a given system at a given temperature. Consequently, eqn. 24 was further revised to include this dependence:

$$e' = f\,\frac{AG \quad \rho_R\, R_1\, \mathrm{MW}^{\mathrm{sol}}}{8P_c \quad 2.303R_2T_R \quad \mathrm{MW}^{\mathrm{mob}}\; b_v^{\mathrm{sol}}} \qquad (25)$$

where T_R is the reduced temperature, T/T_c.

A dimensional analysis of eqn. 25 reveals that all the units cancel, leaving the constant, f, as a dimensionless proportionality constant that is characteristic of the mobile phase-stationary phase combination, Hence, we call this value the "phase constant", although we are currently unsure of the exact factors that give rise to it. The average values of the phase constant for the polyaromatic group of solutes, standard deviation and number of points used in the determinations, are listed in Table VII. The values for both CO_2 and N_2O were determined from data sets consisting of 5 temperatures, 3 densities and the series of phenanthrene, pyrene, fluoranthene and chrysene. Although fluoranthene was not expected to fit into the pseudo-homologous series of polyaromatics, it consistently exhibited a comparable phase constant and was included in the calculation of the average f value. The values for xenon were calculated from 3 temperatures, 2 densities and all 4 solutes. For SF_6 with column A, the data set consisted of 5 temperatures, 1 density and all 4 solutes. The combination of SF_6 and column B only consisted of one solute (phenanthrene) at one density because of instrumental difficulties. Finally, for SF_6 with column C, the set consisted of 4 temperatures, 3 densities and 2 solutes, phenanthrene and pyrene. It is apparent that the values for all systems are within a comparable range, except for xenon. The unique results for xenon may be related to the fact that it is a monoatomic molecule of unusually high polarizability[24,25].

TABLE VII

AVERAGE "PHASE CONSTANT" (f) CALCULATED FOR SOLUTES IN THE POLYAROMATIC SERIES OF PHENANTHRENE, PYRENE, FLUORANTHENE AND CHRYSENE

Mobile phase	*Column*	*Phase constant ± S.D.*	*n*
CO_2	A	1.58 ± 0.08	60
	B	1.31 ± 0.06	60
	C	1.34 ± 0.05	55
N_2O	A	1.70 ± 0.09	60
	B	1.55 ± 0.07	48
	C	1.53 ± 0.06	45
Xe	A	4.43 ± 0.35	24
	B	3.87 ± 0.16	24
	C	4.44 ± 0.23	21
SF_6	A	1.50 ± 0.06	20
	B	1.22 ± 0.06	5
	C	1.13 ± 0.17	24

As mentioned earlier, it is very likely that the effect of density on X_S is included in the a' term in eqn. 11. Since there are no stationary phase factors explicitly included in eqn. 25, it follows that f contains factors related to the amount and composition of the stationary phase coated on the column wall. An interesting point to note, with respect to CO_2 and N_2O, is that the phase constants on the biphenyl-containing (column B) and cyanopropyl-containing (column C) stationary phases are not significantly different from one another. This may be proof that the polarity and/or the polarizability of the stationary phase is one of the dominant factors in the phase constant, accompanied by the specific interaction between a given mobile phase and a given stationary phase. However, in the case of xenon, it seems that the f values for columns A and C are comparable, although the standard deviations are substantial. In this particular case, it may be assumed that xenon undergoes a unique interaction with all three stationary phases due to its size and polarizability.

Another dominant factor implicit in the phase constant, f, may be the swelling of the stationary phase caused by uptake of the mobile phase[26–28]. Specifically, since column A has a larger film thickness than the two more polar columns, the difference between the respective phase constants may indeed be a reflection of swelling effects, or simply a greater sample capacity exhibited by the phase of greater film thickness. Closer examination of the values in Table VII reveals that the ratio of f for column A to that of column B is between 1.1 and 1.2 for all four mobile phases. This may be related to the fact that column B only contains 30% as much unswollen stationary phase as column A. Further investigation is clearly warranted in this area.

The precision in the constant for a given system is surprisingly good, considering the numerous sources of error associated with the development of this empirical equation. First, there is a large uncertainty associated with the determination of AG from the Van ‘t Hoff plot due to the expected error in the slope and intercept from the linear regression. In addition, AG is dependent on knowing the true value of β, which is assumed to be invariant with temperature and density for a thin-film capillary column[26].

Other sources of error in the phase constant can be attributed to the estimation of the Van der Waals volume of the solute, and an error in the calculation of the “true” critical density, which is an iterative process based on an equation of state, and may not be truly representative of the experimental value. Finally, there exists a source of experimental error in maintaining a constant density. Although the conditions remain relatively stable, there will always be fluctuation around the desired value, which will detract from the precision of the resulting retention data.

Table VIII demonstrates that the other solutes, naphthalene, biphenyl and fluorene, do not produce the same constant and must belong to different series. This is only surprising in the case of naphthalene, which should fit into the polyaromatic series of Table VII. This anomalous behavior is attributed to an exaggerated error in peak retention for the smallest molecule, which often coelutes with the solvent peak. Another possible source of error is the use of methylene chloride for the determination of t_0, because even this small, volatile molecule will exhibit some retention during elution. A more accurate measure of t_0 may be obtained from extrapolation of homologous series retention measurements or alternatively, from an injection of methane gas[29].

Examination of the “phase constants” in Table VIII reveals that most of the calculated values are between 1.75 and 2.45, with the exception of the results for xenon. Once again, it is apparent that those f values are considerably higher than those of the other systems, *i.e.,* between 5 and 8. Unfortunately, there are not enough values on any given system to permit a detailed comparison of behavior based on the combination of mobile phase and stationary phase.

Intercept

We have so far ignored the intercept of the log k' vs. log $\rho_{R(T)}$ plot. The intercept

TABLE VIII

AVERAGE "PHASE CONSTANT" (f) CALCULATED FOR OTHER SOLUTES STUDIED

Mobile phase	*Column*	*Solute*	Phase constant ± *S.D.*	*n*
CO_2	A	Naphthalene	2.22 ± 0.14	12
		Biphenyl	2.13 ± 0.11	12
		Fluorene	1.95 ± 0.05	8
	B	Naphthalene	2.45 ± 0.23	5
		Biphenyl	2.13 ± 0.12	15
		Fluorene	1.75 ± 0.04	15
	C	Fluorene	2.05 ± 0.12	12
N_2O	A	Naphthalene	2.32 ± 0.06	12
		Biphenyl	2.28 ± 0.06	12
		Fluorene	2.11 ± 0.14	15
	B	Fluorene	2.06 ± 0.19	15
SF_6	A	Naphthalene	2.45 ± 0.08	5
		Biphenyl	2.21 ± 0.10	5
		Fluorene	1.85 ± 0.05	5
	C	Biphenyl	2.31 ± 0.23	12
		Fluorene	1.63 ± 0.20	12
Xe	A	Naphthalene	7.25 ± 1.22	6
		Biphenyl	6.53 ± 0.85	6
		Fluorene	5.51 ± 0.44	6
	B	Fluorene	5.30 ± 0.26	6

TABLE IX

COMPARISON OF LOG k'_0 OBTAINED EXPERIMENTALLY AND FROM THE INTERCEPT OF THE LOG-LOG PLOT FOR TWO SOLUTES AT TWO TEMPERATURES

Solute	*Mobile phase*	*Column*	*398 K*		*423 K*	
			Experimental	*Intercept*	*Experimental*	*Intercept*
Pyrene	CO_2	A	-0.045	-0.092	-0.25	-0.17
		B	-0.14	-0.14	-0.37	-0.33
		C	-0.17	-0.15	-0.42	-0.37
	N_2O	A	-0.16	-0.22	-0.36	-0.31
		B	-0.33	-0.34	-0.53	-0.51
		C	-0.37	-0.32	-0.63	-0.57
Fluorene	CO_2	A	-0.48	-0.44	-0.62	-0.54
		B	-0.81	-0.80	-0.98	-0.96
		C	-0.94	-0.90	-1.14	-1.05
	N_2O	A	-0.58	-0.50	-0.69	-0.64
		B	-0.94	-0.92	-1.10	-1.05
		C	—	-1.05	—	-1.21

contribution should theoretically come from the solubility of the solute in the mobile phase and the stationary phase, and perhaps other unknown contributions. In this way, the intercept can be isolated quite easily when $\rho_R = 1$, i.e. when $\rho = \rho_c$; at this point, log k' should equal the intercept of the log-log plot. Therefore, we refer to this value as the "critical-density point", log k'_0. Martire and Boehm[14] proposed a similar concept in their study of retention in SFC; however, their term, called $\ln K^0$, represented the "zero-density point", corresponding to ideal GC. We believe that our interpretation reflects the true situation in SFC. In order to verify the assumption, a series of injections was performed at the estimated critical density, i.e., at several points on the critical isotherm, for several systems. Table IX contains the comparison of measured and linearly extrapolated log k'_0 values for two nominal solutes, pyrene and fluorene, although similar results were found for all solutes studied. The agreement is strikingly good, and any discrepancies between the two methods are believed to be due to variations in pump pressure during the run, and from the error in choosing the critical density. The final form of the empirical equation is presented here, combining intercept and slope terms derived above:

$$\log k' = \log k'_0 + f \frac{A\,G \quad \rho_R\, R_1\, \mathrm{MW}^{\mathrm{sol}}}{8P_c \;\; 2.303R_2 \;\; T_R \;\; \mathrm{MW}^{\mathrm{mob}} \;\; b_v^{\mathrm{sol}}} \log \rho_{R(T)} \tag{26}$$

where

$$\log k'_0 = d''_{(T)} - c''_{(T)} \tag{27}$$

at a given point on the critical isotherm. The value obtained for the intercept is normally negative at high temperatures, but often positive at low temperatures, depending on the molecular weight of the solute. This is very interesting because it implies that the relative contributions of d'' and c" are dependent on solute size as well as temperature. From these investigations, it was found that elution of the largest solute (chrysene) at low temperatures consistently produces the most positive intercept, whereas elution of the smallest solute (naphthalene) at higher temperatures produces the most negative intercept (see Table II). These observations can be rationalized by considering that the heavier molecules exhibit low solubility in the mobile phase, especially at low temperature, and therefore possess a dominant d'' term. Conversely, the lighter solutes will be more soluble in the mobile phase, and therefore possess a dominant c" term. Identification of the d'' and c" constants can be made by considering the definition of log k'_0 (*cf.*, eqn. 9) where

$$\log k'_0 = \log n_{S(0)} - \log n_{M(0)} \tag{28}$$

Eqn. 28 is, by definition, simply the amount of solute dissolved in each phase at a given point on the supercritical isotherm, and the subscript (0) denotes the critical density condition. The final equation that describes polyaromatic solute retention results from the substitution of eqn. 28 into eqn. 26.

CONCLUSION

A log k' vs. log ρ_R relationship was chosen to describe retention in SFC. The resultant empirical equation is a comprehensive and potentially useful model of retention. Although this model has only been proposed for a capillary column system, it seems likely that the theory would be valid for packed columns as well. Experimental data obtained with polynuclear solutes are in excellent agreement with eqn. 26, which indicates that the actual retention of a group of chemically related compounds may be predictable. In addition, the investigation of the polyphenyl compounds demonstrated that different classes of compounds indeed exhibit different retention mechanisms, as one would expect. Importantly, although most of the SFC practiced today is done with density programming, this does not represent a fundamental problem in our derivation. If the variation of density with time is known, then the prediction of retention under programming conditions should essentially be a simple mathematical problem.

As yet, the main drawback of our equation is that the "phase constant", f, must be pre-determined for a given mobile phase-stationary phase combination because of the presently unknown factors that give rise to it. In addition, since the equation has been developed for a specific group of polyaromatics, it is unknown at this time how the equation should be modified to describe the retention of other compound classes. There is also a minor difficulty in adapting the equation to modified mobile phases, because the critical temperature, pressure and density, must be known in order to use the equation. Hence, there is a need to develop algorithms that are capable of determining the critical conditions of mixed mobile phases with reasonable accuracy.

Finally, it must be pointed out that the theory cannot be logically extrapolated to GC or LC, as it is by no means an attempt at providing a unified view of chromatography; however, the derived equations make sense in the generalized concept of SFC and will be continually developed in order to encompass a broader range of solutes and phases in order to be truly useful. For example, work is now underway to test this theory on the retention of polar compounds.

ACKNOWLEDGEMENTS

We thank Michal Roth, Czechoslovak Academy of Sciences, Brno, Czechoslovakia, for writing the density calculation algorithm and for helpful suggestions with respect to this manuscript. In addition, we thank Daniel Martire for verifying the derivations. We gratefully acknowledge the scientific agreement between the Academy of Sciences of the G.D.R. and the U.S. National Academy of Sciences enabling one of us (W.E.) to perform research at Indiana University. This research was supported by the Office of Naval Research, and the National Science Foundation, Grant No. CHE-8605935.

REFERENCES

1 C. R. Yonker, B. W. Wright, R. C. Petersen and R. D. Smith, *J. Phys. Chem.*, 89 (1985) 5526–5530.
2 B. W. Wright, H. T. Kalinoski and R. D. Smith, *Anal. Chem.*, 57 (1985) 2823-2829.
3 C. R. Yonker, D. G. McMinn, B. W. Wright and R. D. Smith. *J. Chromatogr.*, 396 (1987) 19-29.

4 C. R. Yonker and R. D. Smith, in B. A. Charpentier and M. R. Sevenants (Editors), *Supercritical Fluid Extraction and Chromatography,* American Chemical Society, Washington, DC, 1988, Ch. 9, p. 161.
5 P. J. Schoenmakers, *J. Chromutogr., 315 (1984) 1-18.*
6 B. I. Lee and M. Ci. Kesler, *AIChE J.*, 21 (1975) 510.
7 U. van Wasen, I. Swaid and G. M. Schneider, Angew. *Chem., Int.* Ed. *Engl.*, 19 (1980) 575-587.
8 S. T. Sie, W. van Beersum and G. W. A. Rijnders, *Sep. Sci.*, 1 (1966) 459490.
9 P. A. Peaden and M. L. Lee, *J. Liq. Chromaiogr., 5* (Suppl. 2) (1982) 179-221.
10 T. L. Chester and D. P. Innis, *J. High Resolut. Chromatogr. Chromatogr. Commun., 8 (1985) 561-568.*
11 D. R. Gere, R. Board and D. McManigill, *Anal.* Chem., 54 (1982) 736-740.
12 G. M. Schneider, Angew. *Chem., Int. Ed. Engl.*, 17 (1978) 716-727.
13 D. Bartmann and G. M. Schneider, *J.* Chromutogr., 83 (1973) 135-145.
14 D. E. Martire and R. E. Boehm, *J. Phys.* Chem., 91 (1987) 2433-2446.
15 E. U. Franck, *Z. Phys. Chem.* N.F., 6 (1956) 345-355.
16 E. Stahl, W. Schilz, E. Schutz and E. Willing, *Angew. Chem., Int. Ed. Engl.*, 17 (1978) 731-738.
17 K. P. Johnston, D. H. Ziger and C. A. Eckert, *Ind. Eng.* Chem. *Fundam.*, 21 (1982) 191-197.
18 C. A. Eckert, D. H. Ziger, K. P. Johnston and S. Kim, *J. Phys.* Chem., 90 (1986) 2738-2746.
19 J. T. Edward, *J. Chem. Educ.*, *47 (1970)* 261-270.
20 A. Bondi, *J. Phys.* Chem., 68 (1964) 441-451.
21 R. C. Weast (Editor), *Handbook of Chemistry and Physics,* CRC Press, Boca Raton, FL, 64th ed., 1983, p. D-191.
22 G. M. Barrow, *Physical Chemistry,* McGraw-Hill, New York, 1979, p. 56.
23 C. R. Yonker ad R. D. Smith, *J. Chromutogr., 351 (1986) 211-218.*
24 R. D. Smith, S. L. Frye, C. R. Yonker and R. W. Gale, *J. Phys. Chem.*, 91 (1987) 3059-3062.
25 S. B. French and M. Novotny, *Anal.* Chem., 58 (1986) 164-166.
26 S. R. Springston, P. David, J. Steger and M. Novotny, *Anal.* Chem., 58 (1986) 997-1002.
27 C. R. Yonker and R. D. Smith, *Anal.* Chem., 61 (1989) 1348-1353.
28 J.-J. Shim and K. P. Johnston, *AIChE J., 35 (1989) 1097-1 106.*
29 D. E. Martire, personal communication, September 1989.