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MOBILE PHASE EFFECTS IN REVERSED-PHASE CHROMATOGRAPHY

I. CONCOMITANT DEPENDENCE OF RETENTION ON COLUMN TEMPERATURE AND ELUENT COMPOSITION

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SUMMARY

Enthalpy–entropy compensation observed in reversed-phase chromatography affords an expression of the free energy change associated with the chromatographic process as a function of both the eluent composition and temperature. It takes a simple form and reveals the existence of a non-compensating enthalpy residue in the absence of heat capacity effects when the free energy change is linear in solvent composition. Statistical analysis confirmed that the resulting three-parameter equation suffices to transform retention data for a solute obtained at a given temperature and solvent composition to another set of experimental conditions. With arylalkanes investigated so far, the parameters exhibit a linear relationship with the carbon number of the solutes. These findings suggest the possibility of constructing a hydrophobic index system for characterization of biological substances on the basis of retention data.

INTRODUCTION

During the short history of reversed-phase chromatography (RPC) with hydrocarbonaceous bonded phases a great deal of evidence has accumulated that by using aqueous methanol or acetonitrile as the eluent, the free energy for the chromatographic process is linear in the solvent composition measured by volume fraction[1–3]. Whereas such a behavior falls short of a rigorous theoretical explanation so far, the usually observed linear dependence of the free energy on the reciprocal absolute temperature[4] is expected in the absence of heat capacity effects[5].

The Gibbs free energy for the solute–stationary phase interaction, ΔG^0, is related to the natural logarithm of the retention (capacity) factor, k, which is readily obtained experimentally. Denoting ln k by κ_e we can express the relationship by

$$\kappa_e = -\frac{\Delta G^0}{RT} + \Phi \qquad (1)$$

where R is the gas constant, T is the absolute temperature and Φ is the natural logarithm of the so-called phase ratio in the column. Eqn. 1 can be written with the appropriate enthalpy, ΔH^0, and entropy, ΔS^0 as

$$\kappa_e = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} + \Phi \tag{2}$$

Fig. 1 illustrates typical linear relationships between the logarithm of retention factor and the reciprocal temperature as well as the volume fraction of water in the binary eluent containing acetonitrile, for toluene on an octadecyl-silica column. In practice either column temperature or eluent composition, or both, are varied in order to obtain optimal chromatographic results.

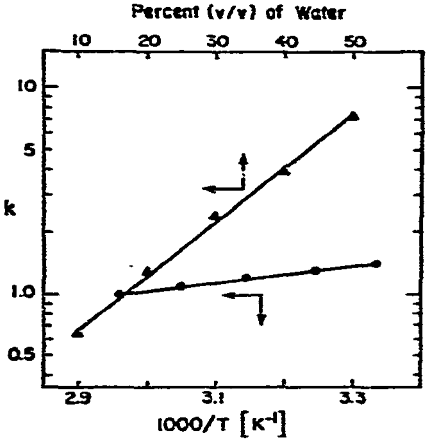

Fig. 1. Plots of the retention factor of toluene on a logarithmic scale against the reciprocal temperature and the composition of the acetonitrile–water mixture used as the mobile phase in reversed-phase chromatography with octadecyl-silica as the stationary phase. In the experiments at different temperatures the eluent contained 80% (v/v at 25°C) acetonitrile and the temperature was maintained at 35°C when the solvent composition was changed.

This communication examines the possibility of expressing the retention factor of a given solute as a function of both column temperature and solvent composition in view of its well behaved dependence on the individual variables. An analytical function of this kind would not only contribute to our physicochemical understanding of RPC and related phenomena but also would facilitate handling of rapidly accumulating retention data.

THEORY

Enthalpy–entropy compensation upon changing solvent composition

To the best of our knowledge, no simple analytic function with a rigorous thermodynamic basis exists to establish a link between the effect of temperature and solvent composition on the retention of a solute in chromatography. Recent results[5], however, suggest that such a relationship can be established for RPC, as it was found that in RPC with hydroorganic eluents having different composition as far as the concentration of the organic component is concerned, enthalpy–entropy compensation[6–8] occurs. The observed behavior implies that the change in the enthalpy of solute binding to the stationary phase with changing solvent composition is proportional to a change in the corresponding entropy and the proportionality constant is the so-called compensation temperature. The results therefore could be represented as

$$\frac{\delta \Delta H^{0}(\varphi)}{\delta \varphi} = T_{C} \frac{\delta \Delta S^{0}(\varphi)}{\delta \varphi} \tag{3}$$

where $\delta\Delta H^{0}(\varphi)$ and $\delta\Delta S^{0}(\varphi)$ are incremental changes in enthalpy and entropy at solvent composition, φ, upon $\delta\varphi$ change in composition measured as volume fraction of organic modifier in the eluent and T_{C} is the compensation temperature. One can formally integrate eqn. 3 when the solvent composition, φ', changes from a reference composition, $\varphi = 0$, to a final composition, φ, as

$$\int_{\varphi'=0}^{\varphi'=\varphi} \frac{\mathrm{d}\Delta H^{0}(\varphi')}{\mathrm{d}\varphi'} = T_{C} \int_{\varphi'=0}^{\varphi'=\varphi} \frac{\mathrm{d}\Delta S^{0}(\varphi')}{\mathrm{d}\varphi'} \tag{4a}$$

to find that

$$\Delta H^{0}(\varphi) - \Delta H^{0}(0) = T_{C}\Delta S^{0}(\varphi) - T_{C}\Delta S^{0}(0) \tag{4b}$$

The dependence of the entropy of binding on solvent composition implicit in eqn. 2 can be eliminated by combination of eqns. 2 and 4b with the result that

$$\kappa_{e} = \frac{-\Delta H^{0}(\varphi)}{RT} + \frac{\Delta H^{0}(\varphi) - \Delta H^{0}(0)}{RT_{C}} + \frac{\Delta S^{0}(0)}{R} + \Phi \tag{5}$$

This expression relates the retention factor at a particular composition and temperature to the enthalpy of binding at that composition and the binding enthalpy and entropy at the reference composition, taken here to be neat water. However, it is of interest to establish a relationship between the enthalpy at any composition and that at the reference state.

Enthalpy as a function of solvent composition

There exists no obvious relationship between the enthalpy in water, $\Delta H^{0}(0)$, and that in a hydro-organic mixture having a composition, $\varphi, \Delta H^{0}(\varphi)$, mandated by thermodynamic considerations. However, it is clear that it must yield $\Delta H^{0}(0)$ when the solvent composition is $\varphi = 0$. Solvent dependence of the enthalpy may take many various forms. The simplest relationship is given by

$$\Delta H^{0}(\varphi) = \Delta H^{0}_{C}(0)\, \mathrm{f}(\varphi) \tag{6}$$

where $\Delta H^{0}_{C}(0)$ is the enthalpy with no organic component in the mobile phase and consists only of terms which manifest compensation behavior, *i.e.*, it is exclusively compensated, as indicated by subscript c. The solvent compensation function $\mathrm{f}(\varphi)$ relates the two enthalpies and it is unity for neat aqueous solutions.

When some portion of the enthalpy, $\Delta H_a^0(0)$, does not undergo compensation the relationship can be written in the simplest case as

$$\Delta H^0(\varphi) = \Delta H_a^0(0) + \Delta H_C^0(0)\,\mathrm{f}(\varphi) \qquad (7)$$

Eqn. 6 can be incorporated into eqn. 5 with the result that

$$\kappa_c = \frac{-\Delta H_C^0(0)\,\mathrm{f}(\varphi)}{RT} + \frac{\Delta H_C^0(0)\,[\mathrm{f}(\varphi) - 1]}{RT_C} + \frac{\Delta S^0(0)}{R} + \Phi \qquad (8a)$$

or the combination of eqns. 5 and 7 yields a more complicated relationship as

$$\kappa_c = \frac{-\Delta H_C^0(0)\,\mathrm{f}(\varphi) - \Delta H_a^0(0)}{RT} + \frac{\Delta H_C^0(0)\,[\mathrm{f}(\varphi) - 1]}{RT_C} + \frac{\Delta S^0(0)}{R} + \Phi \qquad (8b)$$

For eqns. 8a or 8b to be useful, it is necessary that both the compensation temperature, T_C, and the solvent compensation function, $\mathrm{f}(\varphi)$, be independent of solute properties at least for members of a given homologous series. It is not necessary, however, that it be invariant between homologous series, *e.g.*, between alkylbenzenes and carboxylic acids. The form of $\mathrm{f}(\varphi)$ can be determined by curve fitting of data obtained on the dependence of retention factor on solvent composition. It is useful to examine eqns. 8a and 8b to see the properties of the function. Upon differentiating either eqn. 8a or 8b with respect to the composition one finds

$$\frac{\mathrm{d}\kappa_c}{\mathrm{d}\varphi} = \frac{-\Delta H_C^0(0)}{R}\left(\frac{1}{T} - \frac{1}{T_C}\right)\frac{\mathrm{df}(\varphi)}{\mathrm{d}\varphi} \qquad (9)$$

A linear relationship between the logarithm of the retention factor, κ, and solvent composition, φ, is frequently observed and it has been suggested to be the general rule with hydro-organic eluents[2]. In this case, it is clear that $\mathrm{df}(\varphi)/\mathrm{d}\varphi$ is invariant and, therefore, $\mathrm{f}(\varphi)$ is given by in view of the definition of $\mathrm{f}(\varphi)$ in eqns. 6 or 7 as

$$\mathrm{f}(\varphi) = 1 + a\varphi \qquad (10)$$

where a is a constant and expected to have a negative value.

On the other hand, one group of workers[9] suggested a quadratic relationship for the dependence of κ on the solvent composition. In this case we can write for $\mathrm{f}(\varphi)$ that

$$\mathrm{f}(\varphi) = 1 + a\varphi + \beta\varphi^2 \qquad (11)$$

where a and β are constants.

For the dependence of κ, which is the decimal logarithm of the retention factor, k, on solvent composition and temperature both eqns. 8a and 8b yield by using eqn. 10 the relationship

$$\kappa = A_1\varphi(1 - T_C/T) + A_2/T + A_3 \qquad (12a)$$

and by using eqn. 11 the expression

$$\kappa = A_1\varphi(1 - T_C/T) + A_2/T + A_3 + A_4\varphi^2(1 - T_C/T) \tag{12b}$$

The significance of the parameters A_1, A_2, A_3 and A_4 in eqns. 12a and 12b is given in Table I.

TABLE I

PHYSICAL MEANING OF PARAMETERS A_1, A_2, A_3 AND A_4 IN EQNS 12a AND 12b

The parameters $\Delta H^0_c(0)$ and $\Delta H^0_n(0)$ are the compensating and non-compensating parts of the enthalpy associated with the reversible binding of solute by the stationary phase when no organic solvent is present in the mobile phase. R, T_C, Φ, α and β are the gas constant, compensation temperature, natural logarithm of column phase ratio and coefficients for the first- and second-order solvent dependence of the decimal logarithm of retention factor, κ, respectively.

Parameter	*Full enthalpy compensation according to eqn. 8a*	*Partial enthalpy compensation according to eqn. 8b*
A_1	$\alpha\Delta H^0_c(0)/2.3RT_C$	$\alpha\Delta H^0_c(0)/2.3RT_C$
A_2	$-\Delta H^0_c(0)/2.3R$	$-[\Delta H^0_c(0) + \Delta H^0_n(0)]/2.3R$
A_3	$\Delta S^0(0)/2.3R + \Phi/2.3$	$\Delta S^0(0)/2.3R + \Phi/2.3$
A_4	$\beta\Delta H^0_c(0)/2.3RT_C$	$\beta\Delta H^0_c(0)/2.3RT_C$

Model discrimination

Solvent compensation function. For the above analysis of experimental results the appropriate form of the solvent compensation function given in either eqn. 10 or 11 has to be chosen. Most conveniently the dependence of κ on solvent composition is examined and a linear or quadratic relationship is selected on the basis of a statistical test for the fit of experimental data to eqn. 12a or 12b, respectively.

Non-compensating enthalpy residue. Analysis of the results in Table I immediately shows that the coefficients A_1, A_3 and A_4 are the same for the two models of the enthalpy function given in eqns. 6 and 7. There is a difference, however, in the physical meaning of A_2. Furthermore, a unique relationship exists between A_1 and A_2 in each model. If the enthalpy consists of an exclusively compensated part given by eqn. 6 then A_2 is related to A_1 as

$$A_2 = - T_C A_1/\alpha \tag{13a}$$

However, if a non-compensating part exists as suggested by eqn. 7 then the corresponding relationship is given by

$$A_2 = \Delta H^0_n(0)/2.3R - T_C A_1/\alpha \tag{13b}$$

Comparison of eqns. 13a and 13b shows that a plot of A_2 *versus* A_1 obtained with several solutes will have non-zero intercept only if no full compensation occurs. Since the ratio of A_1 to A_2 is constant with total enthalpy–entropy compensation, examination of this ratio offers another test for choosing either eqn. 6 or 7 to express the dependence of κ on the solvent composition and temperature in a given column in RPC.

EXPERIMENTAL

A liquid chromatograph was assembled from a Model FR-30 pump (Knauer, West Berlin, G.F.R.), a Model 7010 sample injector (Rheodyne, Berkeley, Calif., U.S.A.) and a Model 770 variable wavelength UV detector (Schoeffel, Westwood, N.J., U.S.A.). The optical density of the effluent was monitored at 254 nm and chromatograms were recorded by using a Perkin-Elmer (Norwalk, Conn., U.S.A.) Model 56 strip-chart recorder. A 10-μm Partisil ODS 2-column (250 × 46 × 64 mm) was obtained from Whatman (Clifton, N.J., U.S.A.). Acetonitrile was "distilled-in-glass" from Burdick & Jackson Labs. (Muskegon, Mich., U.S.A.). Distilled water was prepared with a Barnstead distilling unit. *n*-Alkyl benzenes having C_0 to C_8 side-chain length were purchased from Aldrich (Milwaukee, Wisc., U.S.A.).

Isocratic elution was carried out with different acetonitrile–water eluents and the column temperature was maintained by circulating water through a water jacket from Model K2R-D thermostatted bath (Messgeräte Werk, Lauda, G.F.R.). A heat exchanger coil comprising 1 m of a 1 mm I.D. × 1.6 mm O.D. stainless-steel tubing was immersed in the temperature bath in order to bring the mobile phase to column temperature before entering the column.

Retention data were obtained with eluents having acetonitrile concentration from 40 to 90% (v/v) and the elution time of a peak obtained by injecting pure acetonitrile into the column was taken as t_0. The retention ratio, k, was calculated from the retention time, t_R, of the sample components as $k = (t_R - t_0)/t_0$. All k values subjected to statistical analysis were in the range from 0.2 to 100.

Benzene and eight *n*-alkylbenzenes up to *n*-octylbenzene were chromatographed at column temperatures from 25 to 65°C at 10 degrees intervals. At each temperature measurements were made with acetonitrile–water mixtures at 10% (v/v at 25°C) composition increments in the range specified above.

The compensation temperature, T_C, was taken as 625°K according to literature data for RPC[5]. The regression coefficients in eqns. 12a and 12b were calculated by multiple linear regression analysis and the statistical significance of regression coefficients was tested by using *t*-statistic. All calculations were carried out on an IBM 370/158 computer at Yale Computer Center.

RESULTS AND DISCUSSION

Analysis of the retention data obtained with nine *n*-alkylbenzene homologues on octadecyl-silica column with acetonitrile–water mixtures over a relatively wide temperature range showed linear Van 't Hoff plots and linear dependence of κ on the solvent composition. The results of multiple regression analysis are given in Table II which contains the parameters of eqn. 12a for all the sample components employed. The goodness-of-fit is illustrated by the scatter diagram in Fig. 2, which shows the correlation between κ values observed experimentally and calculated from eqn. 12a by using the parameters listed in Table II and 625°K for the compensation temperature.

As mentioned above, the ratio of the parameters A_2 to A_1, *vide* Table I, is constant only if the chromatographic system exhibits full enthalpy–entropy compensation. Examination of the ratios listed in Table II shows that A_2/A_1 is not invari-

TABLE II

PARAMETERS A_1, A_2 AND A_3 OBTAINED FOR *n*-ALKYLBENZENES BY FITTING THEIR RETENTION DATA TO EQN. 12a

The ratio of A_2/A_1 is also listed

Side Chain N_c	A_1	A_2	A_3	$\frac{A_2}{A_1}$
0	2.446	1253.0	−2.129	512.3
1	2.710	1497.1	−2.564	552.5
2	3.008	1683.3	−2.813	559.6
3	3.309	1859.6	−3.010	561.9
4	3.609	2027.1	−3.176	561.7
5	3.717	2234.5	−3.613	601.1
6	3.912	2504.6	−4.183	640.2
7	4.146	2686.0	−4.437	647.9
8	4.336	2855.3	−4.690	658.5

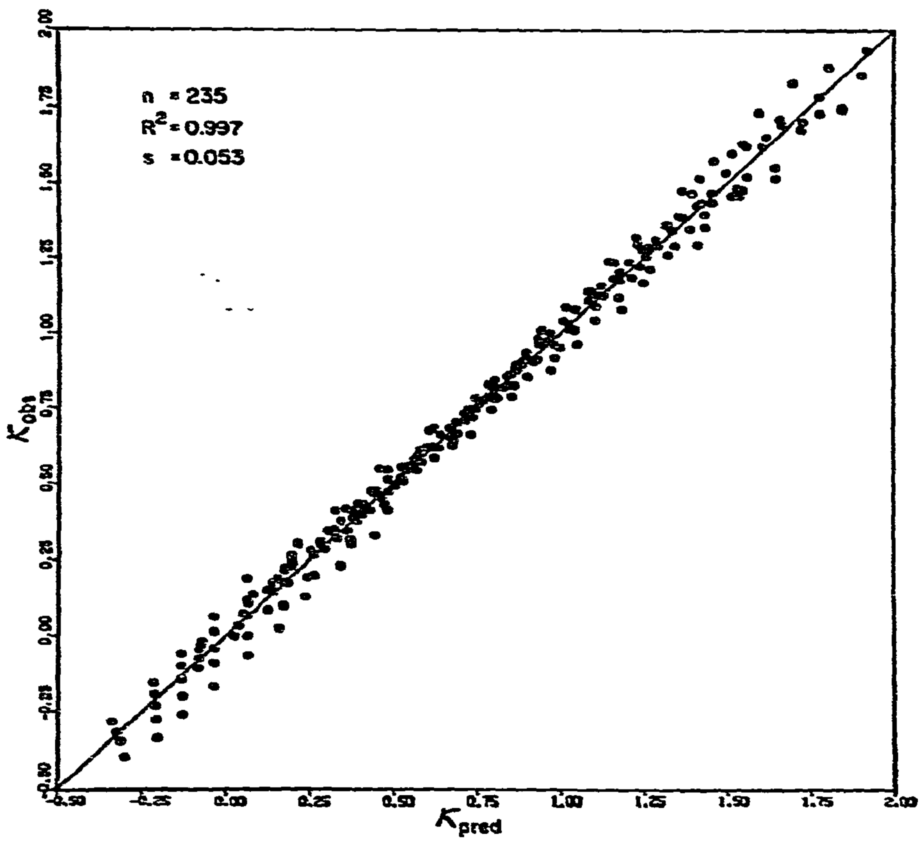

Fig. 2. Correlation between the calculated, κ_{pred}, and the measured, κ_{obs}, values of the logarithm of retention factor.

ant but increases with the carbon number of the solutes. This observation precludes the validity of the simple relationship given in eqn. 6 for binding enthalpy in hydro-organic mixtures that assumes full enthalpy–entropy compensation for the chromatographic system. Parameter A_1 is linearly dependent on parameter A_2 as shown in Fig. 3 with non-zero intercept. From this finding and the observed variation of A_2/A_1 we conclude that eqn. 7 is the simplest function appropriate to represent the dependence of enthalpy on solvent composition. Consequently there is a non-compensating enthalpy residue, ΔH°_π, which has a constant value of 3.8 kcal/mole for the chromatographic system under investigation with all solutes. Its value is compared to the compensating enthalpy, ΔH°_c, which ranges from −9.5 to −16.8 kcal/mole when the size of solute molecules increases from benzene to *n*-octylbenzene. The existence of an invariant ΔH°_π may be a significant finding of the present study and will be the subject of further investigation.

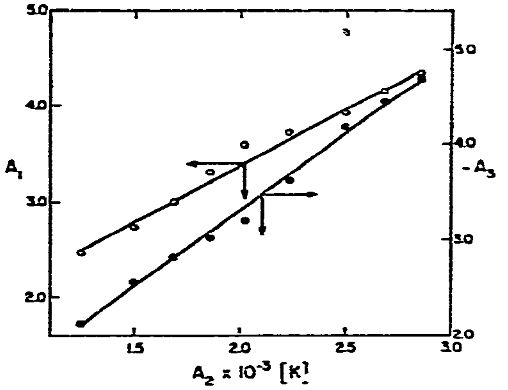

Fig. 3. Plots of the parameters A_1 and A_3 against parameter A_2 listed in Table II. The linear relationship between A_2 and A_3 indicates, *cf* Table I, a linearity between the enthalpy $\Delta H^\circ(0)$ and entropy $\Delta S^\circ(0)$ for eluite binding from neat aqueous eluent in reversed-phase system.

A further manifestation of linear free energy relationships in RPC is seen from the linear dependence of parameter A_3 on parameter A_2 as illustrated in Fig. 3. Table I shows that it is tantamount to a linearity between $\Delta H^\circ(0)$ and $\Delta S^\circ(0)$, that is, the enthalpy and entropy changes associated with the chromatographic process with neat aqueous eluent for various solutes. Such a behavior was also observed in our laboratory in a previous study on extrathermodynamic relationships in RPC[5] and is encouraging with respect to successful applications of the present approach.

Further support for the possible general use of eqn. 12a to predict the concomitant dependence of the retention factor on temperature and solvent composition can be gained from the effect of carbon number on the parameters of eqn. 12a as shown in Fig. 4. The results demonstrate that all parameters are linearly dependent on the carbon number of the side chain in the *n*-alkylbenzene molecules. This finding suggests linear free energy relationships between the parameter values and the structural elements of the solute molecules and the possibility of extending our approach to predict QSRR[10] under a wide range of conditions in RPC. Recent results[10], which indicate that retention data, κ, obtained on different reversed-phase columns by using

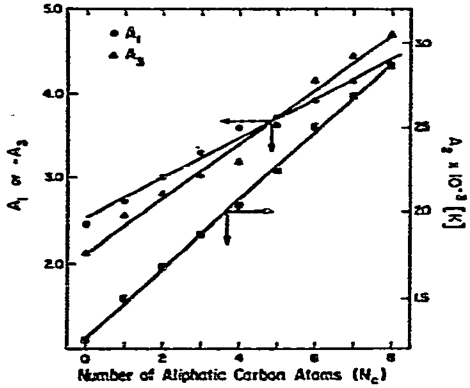

Fig. 4. Plots of the regression coefficients A_1, A_2 and A_3 in eqn 12a as functions of the carbon number of the side chain in *n*-alkyl benzenes.

the same eluent are collinear, also support our expectation that a generalized treatment of retention in RPC is possible.

In practice, one can reduce the retention factor of an eluite either by increasing the concentration of the organic component in a hydro-organic eluent or by elevating the temperature. The results presented above provide an explicit formulation of the effect of concomitant changes in temperature and composition of the mobile phase on chromatographic retention. They therefore afford the possibility of determining the operating temperature at a given solvent composition which is equivalent in its effect on the retention factor to another mobile phase composition at a different temperature.

The retention factor at a given temperature, T_1, and composition, φ_1, is identical to that obtained at another set of conditions, T_2 and φ_2, according to eqn. 12a if

$$A_1\varphi_1(1 - T_C/T_1) + A_2/T_1 = A_1\varphi_2(1 - T_C/T_2) + A_2/T_2 \tag{14}$$

This expression can be rearranged to find the temperature, T_2, to effect the same retention at a new composition φ_2. The temperatures required thus obtained as

$$\begin{aligned} T_2 &= \frac{T_1(A_2 - A_1\varphi_2 T_C)}{T_1 A_1\varphi_1(1 - T_C/T_1) + A_2 - A_1 T_1 \varphi_2} \\ &= \frac{A_2 - A_1\varphi_2 T_C}{(\kappa_1 - A_3) - A_1\varphi_2} \end{aligned} \tag{15}$$

where κ_1 is the logarithm of the retention factor, k, at mobile phase composition, φ_1, and temperature, T_1.

The predictive value of eqn. 15 is illustrated by the two chromatograms in Fig. 5. *n*-Alkylbenzenes were chromatographed with acetonitrile–water mixtures on an octadecyl-silica column. Fig. 5(a) shows the chromatogram obtained with 78% (v/v) of acetonitrile in the eluent at 10°C. The median peak of ethylbenzene has a retention factor of 2.0 under these conditions. Using eqn. 15 and the parameters listed in Table II, we calculated the eluent composition required to elute ethylbenzene with the same retention factor at 75°C. The predicted acetonitrile concentration is 72% (v/v). At 75°C a chromatographic run was performed with this eluent and the results are shown in Fig. 5(b). The retention factors of ethylbenzene are identical on the two chromatograms.

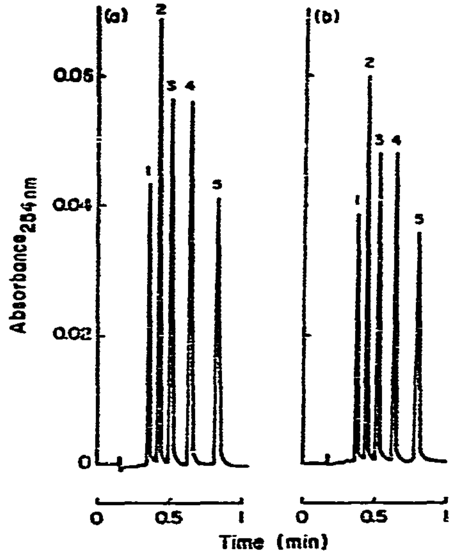

Fig. 5. Chromatogram of *n*-alkylbenzenes obtained by isocratic elution The choice of eluent was made by the use of eqn. 15 with the condition that the retention factor of ethylbenzene be 2 in each case and the operating temperatures be those given below. Parameters of Table II were used in the calculation. (a), 78% (v/v) acetonitrile at 10°C; (b), 72% (v/v) acetonitrile at 75°C. Flow-rate 1.0 ml/min. Column, 10-μm Partisil ODS-2, 250 × 4.6 mm. Sample components: 1 = benzene, 2 = toluene, 3 = ethylbenzene, 4 = propylbenzene, 5 = butylbenzene.

CONCLUSIONS

The preceding results reveal a simple, intrinsic relationship between the effect of solvent and temperature on chromatographic retention in RPC that arises from the previously observed extrathermodynamic enthalpy–entropy compensation. The analytical expression introduced here facilitates transformation of retention data obtained at a given temperature and eluent composition to another set of experimental conditions. Furthermore, the relationship offers an extrathermodynamic basis for a retention index system to catalogue retention data rapidly amassing in RPC. Such a system would be particularly useful to quantify the hydrophobic properties of biological molecules and to remove obstacles from utilizing the full potential of HPLC in the field.

Although three parameters of the above relationship have to be determined experimentally, analysis of retention data of arylalkanes showed that all parameters are linearly dependent on the carbon number. This finding encourages us to believe that these parameters are subject to linear free energy relationships with respect to structural elements of eluite molecules, so that upon establishing a library of the pertinent substituent parameters, retention in RPC could be calculated *a priori*. From this perspective the somewhat unexpected results of the present work may represent a major step forward establishing a rigorous treatment of chromatographic retention based on two-dimensional linear free energy relationships. Moreover, the finding of a non-compensating enthalpy residue invariant under a wide range of conditions should give rise to further research to establish its extrathermodynamic significance.

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