# Exothermodynamic Relationships in Liquid Chromatography

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Exothermodynamic relationships in reversed-phase and hydrophobic interaction chromatography, with the temperature, organic modifier or salt concentration, and carbon number of the eluite and of the stationary phase ligate as the operating variables, are classified and the links between various linear free energy relationships established. Starting from Martin's relationship based on group additivity, we arrive at two linear free energy relationships: one between the logarithmic retention factors, $\kappa$, obtained on two different columns with eluites of close structural similarity and the other between $\kappa$ and the logarithmic octanol–water partition coefficient. Molecular interpretation of classical enthalpy–entropy compensation is offered by the combination of van't Hoff's relationship with linear exothermodynamic relationships between thermodynamic quantities on one hand and properties of the eluite, the eluent, or the stationary phase on the other. Thus, the compensation temperature is expressed by the enthalpy and entropy changes per unit of the above properties. Furthermore, the criterion for the invariance of the compensation temperature is set within the framework of dual compensation. A generalized compensation model is developed to extend the concept of enthalpy–entropy compensation to phenomena involving compensation by any two chromatographic variables by drawing analogies between the dependence of $\kappa$ on the reciprocal temperature and on other operating variables, such as the organic modifier concentration in the eluent. The existence of 12 different compensation parameters is revealed, each marking the common intersection point of linear plots of free energy versus variables of the retention process. The compensation model leads to 12 three-parameter equations, each describing the retention behavior in reversed-phase chromatography as a function of two chromatographic variables. The family of exothermodynamic relationships encompasses most characteristic features of chromatographic retention and is expected to facilitate the organization, interpretation, and prediction of retention data.

## 1. Introduction

Several theoretical studies have enhanced our understanding of the fundamental principles underlying retention in reversed-phase chromatography (RPC)[1–6] and hydrophobic interaction chromatography (HIC).[7–10] The results shed light on the effect of temperature, organic modifier or salt concentration, molecular structure of the eluite, and alkyl chain length of the stationary phase on eluite retention. In many cases, linear relationships are observed between the logarithmic retention factors and the operating variables that are outside the formal structure of thermodynamics. Such exothermodynamic relationships have widely been used for data analysis in chromatography. A. J. P. Martin[11] introduced the first such exothermodynamic relationship by expressing the additivity of the free energy increments of structural elements of the eluite molecules. This explained the remarkable power of chromatography in separating two or more large molecules with only very slight structural differences. It was of great importance for the development of chromatography to understand that the difference in the free energy change for two compounds that differ in a structural element is proportional to the corresponding free energy change for that structural element but not the rest of the molecule.

Exothermodynamic relationships have also been widely employed in the field of organic and biophysical chemistry to explicate the role of molecular structural parameters in equilibrium and rate processes.[12] A number of specific representa-tions of linear free energy relationships (LFER) have been applied independently for data analysis, and perhaps the most popular among such empirical correlations is the Hammett equation[13] that describes the influence of meta and para substituents on the rate or equilibrium constant of a process involving a reactive aromatic compound. Its applicability was later extended to include the reactivities of aliphatic substances by the Taft equation.[14] A similar approach correlating molecular structure with biological activity has made substantial advances with the formulation of quantitative structure–activity relationships (QSAR).[15,16] This has led to the use of octanol–water partition coefficients for characterizing the hydrophobicity of environmental and pharmaceutical substances. Enthalpy–entropy compensation (EEC)[12] is another exothermodynamic relationship manifested by a linear dependence of the enthalpy change on the corresponding entropy change, upon changing an experimental variable of the process under investigation. Traditionally, it has been extensively used as a diagnostic tool for the mechanistic identity of various processes involving small molecules and proteins in aqueous media[17] as well as in specialized areas such as food reactions.[18] Recently, similar linear exothermodynamic relationships between heat capacity and enthalpy or entropy changes have also been established for protein denaturation, and they have been attributed to the linear dependence of the thermodynamic quantities on a single molecular property, such as the change in nonpolar water-accessible surface area upon transfer.[19]

Since the early works of Martin, a number of exothermodynamic relationships have been introduced in chromatography

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for the analysis, interpretation, and prediction of retention data as well as for the determination of hydrophobic parameters. For instance, plots of logarithmic retention factor against the carbon number of the eluite molecules and against the organic modifier concentration are employed to organize chromatographic retention data.[2,20-24] Empirical relationships between the slope and intercept of logarithmic retention factor versus organic modifier concentration plots are often used to estimate retention in RPC.[2,25,26] Correlations between logarithmic retention factors in chromatography and octanol−water partition coefficients have been developed for hydrophobicity measurements by chromatography and for QSAR applications.[27-29] Enthalpy−entropy compensation has been also found useful for the diagnosis of the mechanism of retention processes, such as reversed-phase and normal-phase chromatography,[30] ion-pair chromatography,[31,32] ion-exchange chromatography of alkali metal and alkaline earth metal ions with surface sulfonated resins,[33,34] gas chromatography,[35] and hydrophobic interaction chromatography.[36] The mechanism of interaction of enantiomers with cyclodextrin-derivatized gas chromatographic chiral stationary phases[37] as well as with cyclodextrins in capillary zone electrophoresis[38] has also been examined within the framework of EEC.

Thus far, almost all the above-mentioned exothermodynamic relationships have been used individually for specific applications. The goal of this study is to organize and present various exothermodynamic relationships in liquid chromatography in a systematic fashion and to delineate the architecture and commonality of such relationships, thus providing a unified framework for the interpretation and analysis of a large body of retention data. This serves to establish links between various forms of linear free energy relationships and thus to develop criteria for their existence. Such criterion will be tested by analyzing retention data in the literature and comparing the results obtained with the predictions of exothermodynamic relationships. Furthermore, by drawing analogies, the concept of enthalpy−entropy compensation will be expanded to develop a generalized model describing compensation behavior involving any two chromatographic variables.

## 2. Retention Thermodynamics in Chromatography

In this work, we shall investigate the effect on eluite retention of the four major operating variables in chromatography: temperature, the molecular structure of the eluite, composition of the eluent, and the stationary phase proper. The effect of temperature on eluite retention in liquid chromatography is based on the Gibbs−Helmholtz equation and therefore has a sound thermodynamic basis. In contradistinction, the relationships between logarithmic retention factors and other chromatographic variables, such as the changes in molecular structure, composition of the eluent, or the stationary phase, lie outside the formal structure of thermodynamics. We discuss in this section the basic thermodynamic relationships before moving to exothermodynamic relationships in the following sections.

The magnitude of eluite retention in linear elution chromatography is measured under isocratic condition by the retention factor, $k'$, that is evaluated directly from the chromatogram as

$$k' = \frac{t_R - t_0}{t_0} \tag{1}$$

where $t_R$ is the retention time of the eluite under consideration and $t_0$ is the elution time for an inert tracer. The logarithmic retention factor, $\kappa$, is related to the equilibrium constant, $K$, or

the Gibbs free energy change, $\Delta G°$, associated with eluite transfer from the mobile to the stationary phase as

$$\kappa = \ln k' = \ln K + \ln \phi = -\frac{\Delta G°}{RT} + \ln \phi \tag{2}$$

where $\phi$ is the phase ratio of the column, $T$ is the temperature, and $R$ is the universal gas constant. Then, the dependence of logarithmic retention factor of the eluite on temperature is obtained by evoking the Gibbs−Helmholtz relation

$$\Delta G° = \Delta H° - T\Delta S° \tag{3}$$

and combining with eq 2 as

$$\kappa = -\frac{\Delta H°}{RT} + \frac{\Delta S°}{R} + \ln \phi \tag{4}$$

where $\Delta H°$ and $\Delta S°$ are the standard enthalpy and entropy changes for the eluite transfer from the mobile to the stationary phase. When the heat capacity change upon binding the eluite to the stationary phase is zero, i.e., $\Delta H°$ and $\Delta S°$ are temperature-invariant, and $\phi$ is independent of the temperature, then plots of $\kappa$ against $1/T$, the so-called *van't Hoff plots*, are linear according to eq 4. In this case the enthalpy change associated with the retention can be evaluated from the slopes and the entropy changes from the intercepts provided the phase ratio of the column is known or can be estimated. Analysis of most RPC retention data obtained with small molecules in a wide range of organic modifier concentration usually yields linear van't Hoff plots.[30,39,40] Since the logarithmic retention factor is equivalent to the retention free energy change at a particular temperature, plots of $\kappa$ against one of the chromatographic variables will be collectively referred to as *free energy plots*.

Processes predominantly involving hydrophobic interactions often exhibit nonlinear van't Hoff plots.[41] For instance, calorimetric data obtained with the transfer of nonpolar solids,[42] liquids,[43] and gases[44,45] in water yield curved van't Hoff plots, indicative of a large negative heat capacity change. Since the retention in RPC is believed to be governed by hydrophobic interactions, we expect the retention data to yield curved van't Hoff plots when neat aqueous solution is used as the eluent. Indeed, with water-rich eluent systems RPC retention data of benzene[46] and small peptides[47] exhibits nonlinear van't Hoff plots that are characteristics of the hydrophobic effect. With water-lean eluents, the high organic modifier content in the eluent, however, causes the van't Hoff plots of most RPC retention data to be linear. Other cases of nonlinearity have also been observed with some RPC systems and this was attributed to secondary equilibria[48] or changes in the stationary-phase structure.[49-51]

The characteristic features of the hydrophobic effect are more pronounced in HIC, which employs aqueous salt solution to modulate eluite retention. Analysis of retention data with dansyl amino acids on HIC columns has revealed nonlinear van't Hoff plots that are indicative of significant heat capacity changes accompanying the retention process.[52] The changes in the heat capacity, $\Delta C_p°$, of the process leads to variations in $\Delta H°$ and $\Delta S°$ that can be evaluated by using the integrated form of Kirchoff's law. Assuming that the heat capacity change is invariant with temperature, the temperature dependence of the logarithmic retention factor may be expressed as[52]

$$\kappa = \frac{\Delta C_p°}{R}\left(\frac{T_H}{T} - \ln\frac{T_S}{T} - 1\right) + \ln \phi \tag{5}$$

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where $T_S$ and $T_H$ are the temperatures at which $\Delta S°$ and $\Delta H°$ are zero, respectively. Equation 5 was successfully employed to evaluate the heat capacity changes and other thermodynamic quantities from the van't Hoff plots.[52]

In the following, we discuss exothermodynamic relationships that describe the effect of eluite molecular structure, composition of the eluent, and the carbon number of the stationary-phase ligate on eluite retention.

## 3. Exothermodynamic Relationships between Thermodynamic Quantities and Molecular Structure

We examine first empirical relationships between the retention free energy change and the eluite molecular structure followed by the extension of such relationships to those involving other thermodynamic quantities, such as changes in enthalpy, entropy, and heat capacity.

**3.1. Linear Free Energy Relationships.** For the analysis and interpretation of chromatographic retention, various free energy relationships have been introduced. They involve the molecular structure either explicitly as in the relationship between $\kappa$ and the eluite carbon number or implicitly as in that between $\kappa$ values obtained for a set of eluites on two stationary phases. Our goal is to organize such exothermodynamic relationships and establish criterion for their existence.

*3.1.1. Martin's Relationship.* The fundamental form of all LFERs involving the molecular structure expresses the additivity of incremental free energy changes $\Delta G_j°$ assigned to each structural element $j$ of a substance containing $z$ such elements as

$$\Delta G° = \sum_{1}^{z} \Delta G_j° \tag{6}$$

where $\Delta G°$ is the free energy change of a thermodynamic process. Equation 6 is quite general and applicable to any substance but leads to other formulations of LFER when restricted to a set of structurally closely related substances that may be subject to the same mechanism of interaction. For molecules such as homologues that have varying number of reoccurring structural elements, for instance, the methylene groups, eq 6 can be written as

$$\Delta G° = a_{gn}N + b_{gn} \tag{7}$$

where $N$ and $a_{gn}$ are the number and free energy change of the reoccurring structural element and $b_{gn}$ is the free energy change due to all nonreoccurring elements.

Another LFER based on the properties of the eluite molecule is given by

$$\Delta G° = a_g \Delta A_{np} + b_g \tag{8}$$

and has been employed for the analysis of processes driven by the hydrophobic effect. Here, $a_g$ is the free energy change normalized with respect to the nonpolar surface area, $\Delta A_{np}$, and $b_g$ represents free energy contribution by polar groups. For a set of homologues, eq 8 can be derived from eq 7.[53] Equation 8 has been used successfully in thermodynamic studies on protein folding,[19] octanol−water partitioning,[54] and transfer of various groups of small molecules into water.[55,56]

Often, more than one molecular property may govern the thermodynamics of an equilibrium process. For instance, in protein folding or in the HIC retention of proteinaceous eluites on mildly hydrophobic stationary phases, both nonpolar and polar interactions of the proteins with the solvent molecules or

with the stationary-phase ligates may contribute to the energetics of the process. In the case of such dual mechanism of interaction, eq 6 can be modified to yield

$$\Delta G° = a_g \Delta A_{np} + b_g' \Delta A_p + c_g \tag{9}$$

where $\Delta A_p$ is the polar surface area of the proteinaceous substance, $b_g'$ is the free energy change per unit polar surface area, and $c_g$ is a constant that does not depend on the molecular property.

Martin[11] introduced eq 6 in chromatography to explain its remarkable power in separating closely related biopolymers, e.g., peptides and proteins that differ only in a single amino acid. Equation 6 has also been employed in RPC to derive functional group contributions for a wide range of substituent groups on a range of parent structures in order to predict the retention of simple substituted compounds from parent structures.[57]

Combining eqs 2 and 7, we can express for homologous series the logarithmic retention factor as a function of methylene group or carbon number as

$$\kappa = a_n N + b_n \tag{10}$$

where at a fixed temperature the constants $a_n$ and $b_n$ are equivalent to $a_{gn}$ and $b_{gn}$, respectively. Equation 10 is widely used in the literature for the retention in RPC of homologous series, such as alkylbenzenes and aliphatic acids, ketones, alcohols, and amines.[20] Furthermore, it has found a large number of other applications, such as the determination of column dead volume,[21,58] the evaluation of solvent eluotropic strength,[59,60] and optimization of separations.[61] Whereas such plots depart from linearity when $N$ is less than 3, examples of nonlinear $\kappa - N$ free energy plots have also been reported in the literature. RPC retention data obtained with eluites having carbon number greater than that of the ligate has revealed a discontinuity in such plots at a point corresponding to the ligate carbon number.[22,23]

Similarly, the linear relationship obtained by combining eq 8 with eq 2 expresses the dependence of $\kappa$ on the nonpolar surface area of the eluite, and it has been employed for the analysis of retention data in both RPC[1,6,62] and HIC.[36]

*3.1.2. $\kappa-\kappa$ Relationships.* A linear exothermodynamic relationship for the retention of a set of eluites on two stationary phases has been established for comparing the energetics of eluite retention in RPC employing different columns.[63] According to this relationship, plots of $\kappa$ obtained with a set of eluites on one column against that obtained with the same set of eluites on another column are linear if the energetics of eluite retention on the two columns are similar. Such $\kappa-\kappa$ plots serve as useful diagnostic tools for the mechanism of complex chromatographic process over a broad range of conditions.[63]

The $\kappa-\kappa$ relationship does not explicitly involve the eluite molecular structure. However, it is evident that linear $\kappa-\kappa$ plots are likely to be observed with a set of eluites that exhibit a linear dependence of the free energy changes on the molecular structure. For instance, the logarithmic retention factors on two different columns, A and B, may be expressed as

$$\kappa_A = -\frac{\Delta G_A°}{RT} + \ln \phi_A \tag{11}$$

and

$$\kappa_B = -\frac{\Delta G_B°}{RT} + \ln \phi_B \tag{12}$$

**TABLE 1: Free Energy Change per Unit Nonpolar Surface Area, $a_g$, at 25 °C for the Retention of Aliphatic Homologues on Various Alkyl Silica and Styrenic Stationary Phases in Reversed-Phase Chromatography at Different Volume Fractions of Methanol in Water[a]**

| RPC stationary phases | $a_g$ (J mol$^{-1}$ Å$^{-2}$) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.0 | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 | 0.7 | 0.8 | 0.9 | 1.0 |
| C-22 | −105.5 | | −97.6 | | −75.5 | | −50.8 | | −30.9 | | |
| C-18 | −102.6 | −100.9 | −93.0 | −84.8 | −75.3 | −64.6 | −53.7 | −43.7 | −34.6 | −26.2 | −17.2 |
| C-14 | −106.0 | | −98.6 | | −75.0 | | −50.8 | | −30.9 | | |
| C-10 | −102.9 | | −95.0 | | −69.8 | −58.6 | −47.1 | −37.4 | −27.0 | −20.7 | −13.4 |
| C-8 | −99.0 | | −90.4 | | −68.2 | | −43.6 | | −28.7 | −19.0 | −10.1 |
| C-6 | −99.5 | | −87.5 | | −64.7 | | −40.7 | | −22.5 | | |
| C-4 | −82.6 | | −63.6 | −53.8 | −46.4 | −39.0 | −27.1 | −19.7 | −14.8 | −9.6 | |
| C-1 | −73.7 | | −69.8 | | −50.8 | | −27.0 | | −15.1 | | |
| PS-DVB | −148.5 | −139.8 | −123.8 | −111.9 | −101.1 | −84.6 | −70.9 | −55.6 | −40.1 | −30.9 | −15.5 |

[a] The data was compiled from a large body of references listed in ref 6.

where $\kappa_A$ and $\kappa_B$ correspond to the logarithmic retention factors of eluites on the two columns at otherwise identical operating conditions. When a set of closely related eluites is subject to identical retention mechanism on two stationary phases, then according to eq 8 we obtain that

$$\Delta G_A^\circ = a_{g,A}\Delta A_{np} + b_{g,A} \qquad (13)$$

and

$$\Delta G_B^\circ = a_{g,B}\Delta A_{np} + b_{g,B} \qquad (14)$$

Combining eqs 11−14 yields

$$\kappa_A = \alpha\kappa_B + \beta \qquad (15)$$

where

$$\alpha = a_{g,A}/a_{g,B} \qquad (16)$$

and

$$\beta = \ln\phi_A - \alpha\ln\phi_B + \frac{\alpha b_{g,B} - b_{g,A}}{RT} \qquad (17)$$

Thus, eqs 13 and 14 establish the criterion for the existence of $\kappa-\kappa$ relationships in liquid chromatography. When the retention data obtained with a common set of eluites satisfies eqs 13 and 14 on columns A and B, respectively, $\kappa-\kappa$ plots are linear according to eq 15. When data obtained with a set of eluites does not satisfy either or both equations, $\kappa-\kappa$ plots are nonlinear. Equation 15 was employed to analyze data obtained on various column pairs with a common set of eluites under otherwise identical conditions.[63] It was concluded that the retention data exhibited *homoenergetic* (identical) behavior when $\alpha = 1$, i.e., $\kappa-\kappa$ plots for data obtained on two columns were linear with a slope of unity. On the other hand, when linear $\kappa-\kappa$ plots were obtained with a slope $\alpha \neq 1$, *homoenergetic* (similar) behavior was observed. In contradistinction, nonlinear plots reflected *heteroenergetic* (dissimilar) retention behavior.

Equation 16 expresses $\alpha$ in terms of the free energy change per unit nonpolar surface area associated with eluite retention on two columns and provides a physicochemical interpretation of homo- and homoenergetic behavior. In a recent study,[6] $a_g$ values in RPC at 25 °C were evaluated from a large body of literature data obtained with a set of homologues on various stationary phases in the entire range of the organic modifier concentration. These values are compiled in Table 1 for the retention data with methanol−water mixtures. It is seen that at a fixed eluent condition $a_{g,A} = a_{g,B}$ for the RPC retention on

**TABLE 2: Typical $\alpha$ Values for Some RPC Column Pairs at Various Volume Fractions of Methanol in Water Obtained by Using Eq 16 and $a_g$ Data Listed in Table 1**

| column pairs A, B | $\alpha$ | | | | |
|---|---|---|---|---|---|
| | 0.0 | 0.2 | 0.4 | 0.6 | 0.8 |
| C-18, C-22 | 0.97 | 0.95 | 1.00 | 1.06 | 1.12 |
| C-18, C-10 | 1.00 | 0.98 | 1.08 | 1.14 | 1.28 |
| C-18, C-8 | 1.04 | 1.03 | 1.10 | 1.23 | 1.20 |
| C-18, C-4 | 1.24 | 1.46 | 1.62 | 1.98 | 2.34 |
| C-18, C-1 | 1.39 | 1.33 | 1.48 | 1.99 | 2.29 |
| C-18, PS-DVB | 0.69 | 0.75 | 0.75 | 0.76 | 0.86 |

various alkyl-bonded phases with ligate carbon number higher than 8. On the other hand, at the same operating conditions different values of $a_g$ are obtained with homologues on poly-(styrene−divinylbenzene) stationary phases as well as on siliceous alkyl-bonded stationary phases with ligate carbon number less than 8. From data in Table 1, $\alpha$ was evaluated for some column pairs at different methanol concentrations using eq 16, and these values are listed in Table 2. It is seen that in a wide range of methanol concentration, $\alpha$ is close to unity for the first three column pairs involving stationary phases with long alkyl chain lengths but different from unity for column pairs involving the styrenic stationary phase or siliceous stationary phases with short alkyl chain lengths. This strongly supports the earlier finding that the retention of various sets of homologues follows homoenergetic behavior on most alkyl-bonded phases with medium or long chain ligates but homoenergetic behavior on stationary phases with short alkyl chains or adamantyl ligates.[63] In contradistinction, it has been shown that retention data on column pairs obtained with eluites of wide ranging polarity in eluents rich in organic modifier exhibits heteroenergetic behavior.[63] This is expected since polar interactions between eluites and surface silanols are significantly enhanced in eluents rich in organic modifier so that retention in RPC is no longer governed solely by hydrophobic interactions. As a result, eluites under investigation either do not exhibit any correlation with the molecular property or conform to eq 9 instead of eq 8. In neither case is the $\kappa-\kappa$ relationship linear.

Another $\kappa-\kappa$ relationship similar to eq 15 was established for correlating retention data obtained with a set of eluites on a column employing two different eluent conditions and used to investigate the effect of mobile-phase additives on peptide retention in RPC.[64] Our analysis in this case expresses the slope $\alpha$ of linear $\kappa-\kappa$ plots by the ratio of the retention free energy change per unit nonpolar surface area for one eluent condition to that for another eluent condition. Data presented in Table 1 indicates that RPC retention of a set of nonpolar eluites on a column employing two different eluent conditions will generally

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exhibit homoeonergetic behavior since $a_g$ values vary significantly with the organic modifier concentration.

*3.1.3. Correlation between RPC Retention and Octanol–Water Partitioning.* Partitioning between water and an immiscible nonpolar solvent is widely used to measure the hydrophobicity of compounds for QSARs to correlate structure with biological activity or environmental fate.[15,16] However, the experimental determination of partition coefficients by the shake-flask technique has a number of disadvantages, so there has been an intensive search for alternative methods. The early works of Martin and co-workers[65,66] have shown that chromatographic retention may also be the source of quantitative information on hydrophobicity. Since then, numerous studies[29,67–70] have described the correlation between logarithmic retention factors in chromatography and logarithmic octanol–water partition coefficients for a given solute series.

Following an analysis similar to that employed for establishing $\kappa$–$\kappa$ relationships in the previous section, we shall derive the relationship between logarithmic retention factor in RPC and logarithmic octanol–water partition coefficient by employing linear relationships between the free energy change and the nonpolar surface area. When a set of nonpolar substances is subject to a dominant mechanism of interaction in octanol–water partitioning, then we may express the free energy of partitioning as

$$\Delta G^\circ = -RT\pi = a_{g,ow}\Delta A_{np} + b_{g,ow} \tag{18}$$

where $\pi$ is the logarithmic octanol–water partition coefficient, $a_{g,ow}$ is the free energy change associated with octanol–water partitioning per unit nonpolar surface area, and $b_{g,ow}$ is the free energy change due to polar interactions. Equation 18 has been employed to analyze partition data for various homologous series,[54] and the average value of $a_{g,ow}$ may be taken as $-91$ J mol$^{-1}$ Å$^{-2}$.[71] Combining eqs 2, 8, and 18 we obtain

$$\kappa = A\pi + B \tag{19}$$

where

$$A = a_g/a_{g,ow} \tag{20}$$

and

$$B = \ln\phi + \frac{Ab_{g,ow} - b_g}{RT} \tag{21}$$

Equations 8 and 18 establish the criterion for the existence of linear $\kappa$–$\pi$ plots that are obtained when the susbtances under investigation are subject to essentially the same mechanism in RPC and in octanol–water partitioning. Data for those substances in the set that do not conform to eq 8, eq 18, or both will be outliers in $\kappa$–$\pi$ plots This is interpreted as the result of their being subject to another mechanism of interaction.

To test the validity of eq 20, we analyzed data obtained with RPC retention and octanol–water partitioning from the literature. Eleven sets of pharmaceutical and environmentally relevant substances listed in Table 3 were investigated. Table 3 also lists the range of $\kappa$ values obtained with octadecylated silica using different methanol–water mixtures, the range of $\pi$ values, and the sources of the data. Plots of $\kappa$ against $\pi$ were prepared, and $A$ was evaluated from the slopes of the linear plots according to eq 19 in the entire range of the methanol concentration. We also evaluated $A$ from $a_g$ values listed in Table 1 for the retention on octadecylated silica and $a_{g,ow}$ value

**TABLE 3: Summary of Data for Octanol–Water Partitioning and for RPC Retention Employing Octadecylated Silica and Methanol–Water Eluents That Is Analyzed in the Present Investigation To Evaluate *A* from Linear $\kappa$–$\pi$ Plots**

| no. | substances | methanol concn (% v/v) | symbol in Figure 1 | ref |
|---|---|---|---|---|
| 1 | alkylbenzenes | 0–95[a] | ◆ | 70 |
| 2 | benzene derivates | 30–90 | ● | 72, 73 |
| 3 | polycyclic aromatic hydrocarbons (PAH) | 90 | □ | 74 |
| 4 | pesticides, aromatic hydrocarbons, chlorinated compounds | 75 | + | 75 |
| 5 | phenylureas | 70 | △ | 76 |
| 6 | pyridazinones | 60 | ■ | 77 |
| 7 | barbiturates | 50 | ⊙ | 78 |
| 8 | hydantoins | 50 | ▲ | 78 |
| 9 | 1,4-benzodiazepines | 0[a] | ⊕ | 79 |
| 10 | polychlorobiphenyls (PCB) | 0[a] | ▼ | 80 |
| 11 | pyridazinones | 0[a] | × | 77 |

[a] Extrapolated values for water-rich eluents.
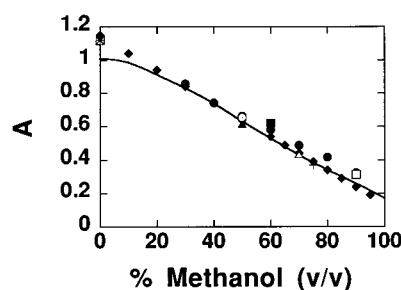


**Figure 1.** Dependence of the *A* parameter, *vide* eqs 19 and 20, on the methanol concentration. The solid line represents *A* values obtained by using eq 20 and data listed in Table 1 for octadecylated silica. The symbols represent *A* values calculated from slopes of linear $\kappa$ versus $\pi$ plots of data from the references listed in Table 3.

of $-91$ J mol$^{-1}$ Å$^{-2}$ by using eq 20. Figure 1 illustrates the dependence of *A* on the methanol concentration in the eluent thus obtained by using the above two methods. It is seen that *A* increases with decreasing methanol concentration in the eluent and approaches the value of unity when neat aqueous eluent is used. This is expected since the magnitude of $a_g$ associated with RPC retention increases with the methanol content in the eluent and approaches the value of $a_{g,ow}$ in neat water, as seen from data presented in Table 1. Furthermore, the *A* values obtained by the two methods are in excellent agreement as seen in Figure 1. The finding confirms that good linear correlation between RPC retention and octanol–water partitioning is likely to be observed with a set of substances that conform to linear exothermodynamic relationships between free energy change and a dominant molecular property.

**3.2. Additivity of Group Contributions to Other Thermodynamic Quantities.** Assuming that eq 6 can be written not only for the free energy but also for the entropy, enthalpy, and heat capacity change, which accompany chromatographic retention, we obtain

$$\Delta S^\circ = \sum_1^z \Delta S_j^\circ \tag{22}$$

$$\Delta H^\circ = \sum_1^z \Delta H_j^\circ \tag{23}$$

and

$$\Delta C_p^\circ = \sum_1^z \Delta C_{p,j}^\circ \tag{24}$$

Such group-contribution schemes have been frequently employed to relate thermodynamic quantities for processes involving hydrophobic interactions to molecular structure.[81,82]

In a similar way, eq 7 can be also written for the entropy, enthalpy, and heat capacity changes associated with the retention of a set of homologous eluites in terms of the number of reoccurring structural elements as

$$\Delta S^\circ = a_{sn} N + b_{sn} \tag{25}$$

$$\Delta H^\circ = a_{hn} N + b_{hn} \tag{26}$$

and

$$\Delta C_p^\circ = a_{cn} N + b_{cn} \tag{27}$$

where $a_{sn}$, $a_{hn}$, and $a_{cn}$ are the entropy, enthalpy, and heat capacity changes of the retention per reoccurring structural units, whereas $b_{sn}$, $b_{hn}$, and $b_{cn}$ represent thermodynamic quantities for the nonreoccurring structural elements. Equations 25 and 26 have abundant experimental support with RPC retention data. For instance, plots of retention enthalpy and retention entropy against the number of methylene groups or the carbon number of homologous eluites are linear.[40,83] Equation 27, however, may not hold for the eluite retention in RPC employing water-lean eluents since the heat capacity change is negligible.

Furthermore, the dependence of entropy, enthalpy, and heat capacity changes for the retention on the nonpolar surface area of the eluites can be expressed by the same relationship as eq 8 for the change in retention free energy. Indeed, analysis of HIC retention data obtained with dansyl amino acids on different columns has yielded linear plots of the enthalpy, entropy, and heat capacity changes against the nonpolar surface area of dansyl amino acids.[36]

## 4. Exothermodynamic Relationships between Thermodynamic Quantities and Other Chromatographic Variables

In addition to the temperature and the molecular structure, other variables may also be used to analyze the retention process by assuming that the retention free energy change is a linear function of such independent variables. In the following, we shall discuss the effect of organic modifier or salt concentration as well as the carbon number of the alkyl ligate of the stationary phase on the changes in the retention free energy, entropy, and enthalpy.

**4.1. Effect of Organic Modifier or Salt Concentration.** As a first approximation, the retention free energy change in RPC may be considered to depend linearly on the organic modifier concentration. With notations similar to that in eq 10, we can write for the dependence of the retention free energy change on the organic modifier concentration, $\varphi$, that

$$\Delta G^\circ = a_{g\varphi} \varphi + b_{g\varphi} \tag{28}$$

where $a_{g\varphi}$ and $b_{g\varphi}$ represent the slope and intercept of linear plots of the free energy change against the organic modifier concentration. Combining eq 28 with eq 2, we obtain a linear

relationship between the logarithmic retention factor and the organic modifier concentration in RPC as

$$\kappa = \kappa_w - S\varphi \tag{29}$$

where $\kappa_w$ is the measure of eluite retention with neat aqueous eluent and $S$ is a constant for a given eluite. Both $S$ and $\kappa_w$ are equivalent to $a_{g\varphi}$ and $b_{g\varphi}$ in eq 28 at a fixed temperature. Although experimental RPC data obtained in a wide range of organic modifier concentrations yields nonlinear $\kappa-\varphi$ plots,[2] it has been well-established that over a limited range of $\varphi$ eq 29 accurately describes the retention behavior in RPC.[24,84] It is noted that dual retention mechanism, i.e., retention due to an interplay of hydrophobic and silanophilic interactions, may lead to U-shaped plots as exhibited by the RPC retention data obtained with protonated bases.[85]

In a way similar to eqs 25 and 26, we introduce the following relationships to express entropy and enthalpy changes in terms of the organic modifier concentration as

$$\Delta S^\circ = a_{s\varphi} \varphi + b_{s\varphi} \tag{30}$$

$$\Delta H^\circ = a_{h\varphi} \varphi + b_{h\varphi} \tag{31}$$

where $a_{s\varphi}$ and $a_{h\varphi}$ are the entropy and enthalpy changes normalized with respect to the organic modifier concentration and $b_{s\varphi}$ and $b_{h\varphi}$ are constants. Indeed, entropy and enthalpy changes for the retention of alkylbenzenes in RPC employing methanol−water mixtures exhibit a linear dependence on the methanol concentration in a wide range according to eqs 30 and 31, respectively.[86] However, the study reveals that with alkylbenzenes in acetonitrile−water eluent system eqs 30 and 31 are not valid in the entire range of the acetonitrile concentration, thus indicating a different mechanism.

Unlike that in RPC, retention in HIC is modulated by changing the salt concentration in the eluent. Assuming a linear dependence of retention free energy change in HIC on the molal salt concentration, $m$, in the eluent as

$$\Delta G^\circ = a_{gm} m + b_{gm} \tag{32}$$

we obtain a linear relationship between logarithmic retention factor and molal salt concentration as

$$\kappa = \kappa_w + Cm \tag{33}$$

where $a_{gm}$ and $b_{gm}$ represent the slope and intercept of linear plots of the free energy change against the molal salt concentration, $\kappa_w$ is the hypothetical logarithmic retention factor that would be obtained with a given chromatographic system in the absence of the salt, and $C$ is a constant that depends on the contact surface area upon binding. Experimental data[7,87−91] for protein retention with cosmotropic salts, which do not preferentially bind to the protein, obtained at sufficiently high salt concentrations where the mechanism of retention is governed predominantly by hydrophobic interactions, lends support to eq 33. However, deviations from eq 33 have been observed with the retention of eluites employing chaotropic salts,[92,93] and this may be attributed to the preferential binding of the salt to the protein surface.[94,95]

Entropy and enthalpy changes may also vary linearly with the molal salt concentration so that exothermodynamic relationships similar to those represented by eqs 25 and 26 could be

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established for $m$ as

$$\Delta S^\circ = a_{sm}m + b_{sm} \tag{34}$$

$$\Delta H^\circ = a_{hm}m + b_{hm} \tag{35}$$

where $a_{sm}$, $a_{hm}$ and $b_{sm}$, $b_{hm}$ represent the slopes and intercepts of linear plots of entropy and enthalpy against the molal salt concentration. Since significant heat capacity effects are observed for the eluite retention in HIC, we may also write an expression for the retention heat capacity change in terms of molal salt concentration as

$$\Delta C_p^\circ = a_{cm}m + b_{cm} \tag{36}$$

where $a_{cm}$ and $b_{cm}$ represent the slopes and intercepts of linear plots of heat capacity change against the molal salt concentration. However, such relationships are yet to be tested in HIC.

**4.2. Effect of Stationary Phase.** The role of stationary phase in the retention is quite complex and not well understood in both RPC and HIC. The retention factor is determined by the equilibrium constant and the phase ratio according to eq 2. Since the chromatographic surface available for binding to eluites is believed to be heterogeneous, the measured retention factors represent average values. In one limiting case, the retention factor depends linearly on the size and/or concentration of the ligates at the chromatographic surface.[96] This may occur when the phase ratio is proportional to these values and the equilibrium constant remains the same. In the other limiting case, $\kappa$ linearly depends on the size of the ligate at least in a certain carbon number range when hydrophobic interactions dominate the retention process. This occurs when the phase ratio is essentially unchanged and the logarithmic equilibrium constant is a linear function of the carbon number of the ligate. Experimental data in the literature does not lend itself to unambiguous interpretation. For instance, it has been shown that plots of both $k'$ and $\kappa$ against carbon load of the stationary phase are linear, and this indicates that the increase in eluite retention with the carbon load can be due to an increase in either the phase ratio or the equilibrium constant.[96,97]

For simplification, we assume that the phase ratio remains essentially unchanged when the retention free energy changes linearly with the ligate carbon number, $N_l$, as

$$\Delta G^\circ = a_{gn_l}N_l + b_{gn_l} \tag{37}$$

so that we may write in a certain ligate carbon number range the following relationship

$$\kappa = a_{n_l}N_l + b_{n_l} \tag{38}$$

where $a_{gn_l}$ and $b_{gn_l}$ denote the slope and intercept of linear plots of retention free energy change against ligate carbon number plots and $a_{n_l}$ and $b_{n_l}$ represent the same for linear $\kappa - N_l$ plots. Experimental retention data[98-100] obtained with nonpolar eluites on various bonded phases by employing methanol−water eluents appears to support eq 38 in a small range of ligate carbon number. At higher ligate numbers, however, the retention levels off.[98] It is noted that heterogeneity in the stationary phase may be the cause for additional mechanisms of interaction. For instance, in the case of structurally complex or polar eluites, steric effects or silanophilic interactions may also affect their retention behavior so that $\kappa - N_l$ plots are nonlinear and eq 38 does not hold.

Since the ligate chain length is expected to have an effect on the eluite retention similar to that of eluite carbon number, the
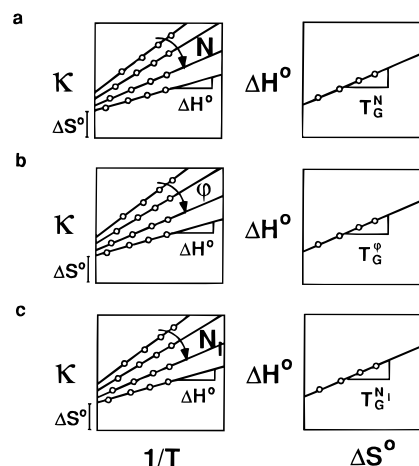


**Figure 2.** Graphical illustration of the van't Hoff and the enthalpy versus entropy plots when parameter $P$ is represented by: (a) eluite carbon number, $N$, (b) organic modifier concentration, $\varphi$, or (c) ligate carbon number of the stationary phase, $N_l$.

enthalpy and entropy changes can be considered linearly dependent on the stationary-phase ligate number in RPC and expressed in a way similar to eqs 25 and 26 as

$$\Delta S^\circ = a_{sn_l}N_l + b_{sn_l} \tag{39}$$

$$\Delta H^\circ = a_{hn_l}N_l + b_{hn_l} \tag{40}$$

where $a_{sn_l}$, $a_{hn_l}$ and $b_{sn_l}$, $b_{hn_l}$ represent the slopes and intercepts of linear plots of entropy and enthalpy against the ligate carbon number.

## 5. Enthalpy−Entropy Compensation

Enthalpy−entropy compensation is an exothermodynamic relationship between the enthalpy and the entropy change that is expressed by the compensation equation

$$\Delta H^\circ = T_G^P \Delta S^\circ + \Delta G_{T_G^P} \tag{41}$$

where $T_G^P$ is the compensation temperature, $\Delta G_{T_G^P}$ is the free energy change at the compensation temperature, and the superscript $P$ denotes the particular experimental parameter chosen to obtain a set of $\Delta H^\circ - \Delta S^\circ$ pairs from van't Hoff plots for a given process.[53] As illustrated in Figure 2, the parameter $P$ can be a molecular property such as the number of reoccurring structural elements in a group of molecules ($N$), an eluent property such as the organic modifier concentration ($\varphi$) or the molal salt concentration ($m$), or a stationary-phase property such as the carbon number of the alkyl ligates ($N$). Linear $\Delta H^\circ - \Delta S^\circ$ plots in Figure 2 according to eq 41 have been considered as sufficient evidence for compensation to occur. A caveat is in place, however, because EEC may not only be due to phenomena of interest but also to statistical effects associated with the determination of $\Delta H^\circ$. It has been shown that when plots of $\Delta G^\circ$ against $\Delta H^\circ$ are used, statistical compensation is minimized.[101,102] Chemical reactions and equilibrium processes having similar compensation temperatures are considered fundamentally related and called isokinetic and isoequilibrium processes.[12]

**5.1. Molecular Property as the Parameter.** In this section, EEC is examined in reversed-phase chromatography by choosing an eluite molecular property $N$ as the parameter to obtain a set of $\Delta H^\circ - \Delta S^\circ$ pairs from van't Hoff plots, as illustrated in

Figure 2a. Retention of a set of eluites exhibit EEC when plots of $\Delta H°$ against $\Delta S°$ are linear according to eq 41 with the slope given by $T_G^N$.

It has been shown that retention processes involving a set of structurally closely related eluites, such as homologues, exhibit EEC.[35] Assuming that these eluites are subject to the same retention mechanism according to eqs 7, 25, and 26, and comparing with eq 41 where $N$ is chosen as the parameter, we obtain an expression for $T_G^N$ in terms of enthalpy and entropy changes per unit nonpolar surface area as

$$T_G^N = a_{hn}/a_{sn} \tag{42}$$

and for $\Delta G_{T_G^N}$ that

$$\Delta G_{T_G^N} = b_{hn} - T_G^N b_{sn} \tag{43}$$

Equations 42 and 43 provide a molecular interpretation of the compensation temperature and the free energy change at that temperature. Thus, the occurrence of EEC with molecular property as the parameter requires linear dependence of enthalpy and entropy changes on the molecular property for the eluites under investigation, according to eqs 25 and 26.

Examining compensation phenomenon in chromatography, we divided them earlier into two groups: classical and nonclassical EEC.[10,53] Chromatographic retention such as in RPC, which yields linear van't Hoff plots according to eq 4, exhibits classical compensation behavior, i.e., plots of $\Delta H°$ against $\Delta S°$ are linear with slope $T_G^N$ that is independent of the temperature. When the retention process involving structurally closely related eluites yields nonlinear van't Hoff plots according to eq 5, nonclassical EEC is observed, i.e., plots of $\Delta H°$ against $\Delta S°$ obtained at different temperatures are linear with the slope $T_G^N$ depending on the temperature.

To investigate classical EEC in liquid chromatography with eluite molecular property as the parameter, we combine eqs 4 and 41 to obtain the free energy compensation equation

$$\kappa = \left(\frac{1}{T_G^N} - \frac{1}{T}\right)\frac{\Delta H°}{R} + \kappa_{T_G^N} \tag{44}$$

where the logarithmic retention factor, $\kappa_{T_G^N}$, at temperature, $T_G^N$, is given by

$$\kappa_{T_G^N} = \frac{-\Delta G_{T_G^N}}{RT_G^N + \ln \phi} \tag{45}$$

Linear $\kappa - \Delta H°$ plots, according eq 44, are indicative of EEC due to the intrinsic similarity between the retention mechanisms with the eluites under investigation. Such free energy compensation plots minimize statistical compensation effects and have been employed for testing EEC.[100,101] It was found that RPC retention data obtained with structurally closely related eluites at different temperatures conforms to eq 44 and the compensation temperature $T_G^N$ is constant.[30] Further, combining eqs 26 and 44 we obtain for the dependence of the logarithmic retention factor on both the temperature and the number of methylene groups

$$\kappa = \left(\frac{1}{T_G^N} - \frac{1}{T}\right)\frac{(a_{hn}N + b_{hn})}{R} + \kappa_{T_G^N} \tag{46}$$

Upon differentiating both sides of eq 46 with respect to $N$ we

obtain that

$$\frac{\partial \kappa}{\partial N} = \left(\frac{1}{T_G^N} - \frac{1}{T}\right)\frac{a_{hn}}{R} \tag{47}$$

and setting the left-hand side (LHS) of eq 47 to zero yields $T = T_G^N$. Therefore, all the van't Hoff plots of the eluites under investigation intersect at a single point when the retention process exhibits EEC and the coordinates of this intersection point are $T_G^N$ and $\kappa_{T_G^N}$.

Rearrangement of eq 46 gives us a three-parameter equation for the combined dependence of logarithmic retention factor on temperature and molecular property as

$$\kappa = A_{TN}N\left(\frac{1}{T_G^N} - \frac{1}{T}\right) + \frac{B_{TN}}{T} + C_{TN} \tag{48}$$

where the three parameters, $A_{TN}$, $B_{TN}$, and $C_{TN}$, are given by

$$A_{TN} = a_{hn}/R \tag{49}$$

$$B_{TN} = -b_{hn}/R \tag{50}$$

$$C_{TN} = b_{hn}/(RT_G^N) + \kappa_{T_G^N} \tag{51}$$

Equation 48 describes the change in the retention of various structurally closely related eluites with change in temperature provided $T_G^N$ does not change appreciably and, to our best knowledge, has not been described in the literature.

Nonclassical compensation behavior was examined in HIC with a set of dansyl amino acids and the compensating process exhibited three characteristic temperatures, namely, isoentropic, $T_S^{N*}$, isoenthalpic, $T_H^{N*}$, and isoenergetic, $T_G^{N*}$, temperatures that mark common intersection points of the respective plots of entropy, enthalpy and free energy change against temperature.[10,53] It was shown that $T_G^N$ of classical EEC is a particular case of $T_G^{N*}$ in the absence of heat capacity effects and both $T_G^N$ and $T_G^{N*}$ represent temperatures where the eluites under consideration coelute. In view of the treatment of nonclassical EEC in the literature, in this study we shall restrict ourselves to classical compensation phenomena.

**5.2. Eluent Property as the Parameter.** EEC is examined here in reversed-phase chromatography by choosing the organic modifier concentration, $\varphi$, as the parameter, $P$, to obtain a set of $\Delta H° - \Delta S°$ pairs. EEC will occur when the plots are linear according to eq 41. Following the treatment in the previous section, we shall derive an expression for $T_G^\varphi$ that represents the slope of the $\Delta H°$ against $\Delta S°$ plot of an eluite at different eluent conditions. Assuming eqs 30 and 31 to be valid in the range of the organic modifier concentration investigated and comparing with eq 41 in which $\varphi$ stands for $P$, we obtain that

$$T_G^\varphi = a_{h\varphi}/a_{s\varphi} \tag{52}$$

which establishes the criterion for EEC in RPC with the organic modifier concentration as the parameter.

Combining eqs 4 and 41, with $\varphi$ as the parameter, we obtain another free energy compensation equation as

$$\kappa = \left(\frac{1}{T_G^\varphi} - \frac{1}{T}\right)\frac{\Delta H°}{R} + \kappa_{T_G^\varphi} \tag{53}$$

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where

$$\kappa_{T_G^\varphi} = \frac{-\Delta G_{T_G^\varphi}}{R T_G^\varphi} + \ln\varphi \tag{54}$$

Equation 53 is analogous to eq 44 and precludes statistical compensation effects and therefore, a better suited for testing compensation behavior than is eq 41.[101,102] An analysis similar to that presented above for $T_G^N$ reveals that $T_G^\varphi$ marks the common intersection point of van't Hoff plots of data obtained with an eluite at different organic modifier concentrations. Thus, $T_G^\varphi$ is the temperature at which the eluite under investigation has the same retention factor, $\kappa_{T_G^\varphi}$, in the whole range of the organic modifier concentrations.

By combining eqs 31 and 53, we establish a three-parameter equation for the combined dependence of the logarithmic retention factor on the temperature and mobile-phase composition as

$$\kappa = A_{T\varphi}\varphi\left(1 - \frac{T_G^\varphi}{T}\right) + \frac{B_{T\varphi}}{T} + C_{T\varphi} \tag{55}$$

provided $T_G^\varphi$ is invariant for the system under investigation. In eq 44, $A_{T\varphi}$, $B_{T\varphi}$, and $C_{T\varphi}$ are constants similar to $A_{TN}$, $B_{TN}$, and $C_{TN}$ in eq 48. A similar three-parameter equation was also developed from EEC in a somewhat different way and successfully used to describe the effect of mobile-phase composition and temperature in RPC[48] and to transform retention data obtained at a given temperature and eluent composition to another set of experimental conditions.[103,104]

**5.3. Dual Compensation with Molecular and Eluent Property.** EEC is used as a diagnostic tool for the mechanism of retention in liquid chromatography and the invariance of compensation temperature reflects the mechanistic identity of such processes. It is intriguing to establish a criterion for the invariance of compensation temperatures. RPC offers a suitable multivariate system not only to investigate the relationship between $T_G^N$ and $T_G^\varphi$ but also to examine their dependence on other chromatographic variables. Thus, by obtaining RPC retention data at different temperatures with a set of structurally related eluites and different eluent conditions, we can examine EEC with either the molecular or the eluent property as described in the previous two sections. Furthermore, we can also investigate dual compensation behavior with both molecular and eluent property together.

In RPC, retention data obtained with a set of homologues usually conforms to eqs 4, 10, and 28. Then, eqs 7, 25, 26, 28, 30, and 31 are expected to hold as well for the retention system so that $a_{sn}$, $b_{sn}$, $a_{hn}$ and $b_{hn}$ can be expressed in terms of the organic modifier concentration as and
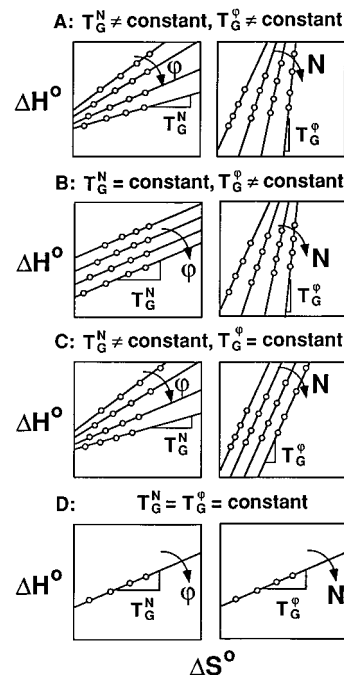
$$a_{sn} = a_{s1}\varphi + a_{s2} \tag{56}$$

$$b_{sn} = b_{s1}\varphi + b_{s2} \tag{57}$$

$$a_{hn} = a_{h1}\varphi + a_{h2} \tag{58}$$

$$b_{hn} = b_{h1}\varphi + b_{h2} \tag{59}$$

where $a_{s1}$, $a_{s2}$, $b_{s1}$, $b_{s2}$, $a_{h1}$, $a_{h2}$, $b_{h1}$, and $b_{h2}$ are constants. Comparing eqs 25, 26, 30, 31, 42, 52, and 56–59, we obtain



**Figure 3.** Graphical illustration of four cases of dual-compensation behavior observed with eluite molecular property, $N$, and eluent property, $\varphi$, as parameter, $P$: (a) $T_G^N$ and $T_G^\varphi$ are given by eqs 60 and 61, (b) $T_G^N$ is invariant with $\varphi$ and given by eq 62, $T_G^\varphi$ is given by eq 61, (c) $T_G^\varphi$ is invariant with $N$ and given by eq 63, $T_G^N$ is given by eq 60, and (d) both compensation temperatures, $T_G^N$ and $T_G^\varphi$, are invariant and equal.

for $T_G^N$ and $T_G^\varphi$ that

$$T_G^N = \frac{a_{h1}\varphi + a_{h2}}{a_{s1}\varphi + a_{s2}} \tag{60}$$

and

$$T_G^\varphi = \frac{a_{h1}N + b_{h1}}{a_{s1}N + b_{s1}} \tag{61}$$

Four different kinds of compensation behavior are identified and illustrated in Figure 3 depending on the relationship between the constants $a_{s1}$, $a_{s2}$, $b_{s1}$, $b_{s2}$, $a_{h1}$, $a_{h2}$, $b_{h1}$, and $b_{h2}$. In general, $T_G^N$ and $T_G^\varphi$ depend on $\varphi$ and $N$ according to eqs 60 and 61 so that linear plots of $\Delta H^\circ$ against $\Delta S^\circ$ have different slopes, as shown in Figure 3a.

The second kind of compensation behavior occurs when $a_{h2}/a_{h1} = a_{s2}/a_{s1}$ so that $T_G^N$ is invariant with the organic modifier concentration and given by

$$T_G^N = a_{h1}/a_{s1} = a_{h2}/a_{s2} \tag{62}$$

Then, plots of $\Delta H^\circ$ against $\Delta S^\circ$ obtained with a set of eluites at a fixed eluent condition are linear with slope $T_G^N$ independent of the organic modifier concentration as shown in Figure 3b. On the other hand, plots of $\Delta H^\circ$ against $\Delta S^\circ$ obtained with an eluite at different organic modifier concentrations are linear with slope $T_G^\varphi$ that varies with $N$ according to eq 61.

When $b_{h1}/a_{h1} = b_{s1}/b_{s2}$, the third type of compensation behavior is observed as illustrated in Figure 3c. Then

$$T_G^\varphi = a_{h1}/a_{s1} = b_{h1}/b_{s1} \tag{63}$$

so that $\Delta H^\circ - \Delta S^\circ$ plots obtained with an eluite at different

organic modifier concentrations are linear with slope $T_G^{\varphi}$ that is invariant with $N$. On the other hand, such linear plots obtained with a set of eluites at a fixed organic modifier concentration have different slopes, $T_G^N$, that vary with $\varphi$ according to eq 61.

Figure 3d illustrates the last type of compensation behavior when $T_G^N = T_G^{\varphi}$, i.e., both eqs 62 and 63 hold. In this case, enthalpy and entropy data obtained with either $N$ or $\varphi$ as the parameter lies on the same straight line.

To elucidate the physical significance of the ratios $a_{h2}/a_{h1}$, $a_{s2}/a_{s1}$, $b_{h1}/a_{h1}$, and $b_{s1}/a_{s1}$, which jointly establish the criterion for the compensation temperature to be invariant, we obtain from eqs 25, 26, and 56−59 the combined dependence on $N$ and $\varphi$ of entropy and enthalpy changes as

$$\Delta S° = (a_{s1}\varphi + a_{s2})N + (b_{s1}\varphi + b_{s2}) \qquad (64)$$

and

$$\Delta H° = (a_{h1}\varphi + a_{h2})N + (b_{h1}\varphi + b_{h2}) \qquad (65)$$

A function linear in two variables always yields an intersection point when the function is plotted against one of the variables, with the other as the parameter. By differentiating eqs 64 and 65 with respect to $N$ and setting the LHS to zero, we find that $-a_{s2}/a_{s1}$ and $-a_{h2}/a_{h1}$ are the organic modifier concentrations that mark the common intersection points of plots of $\Delta S°$ and $\Delta H°$ against $\varphi$ obtained for different homologues, respectively. For convenience, we shall refer to them as isoentropic, $\varphi_S^N$, and isoenthalpic, $\varphi_H^N$, organic modifier concentration since the entropy and enthalpy changes attain a common value for all eluites at these concentrations. Similarly, by differentiating eqs 58 and 59 with respect to $\varphi$ and setting the LHS to zero, we obtain $N_S^{\varphi} = -b_{s1}/a_{s1}$ and $N_H^{\varphi} = -b_{h1}/a_{h1}$ that mark the common intersection points of plots of $\Delta S°$ and $\Delta H°$ against $N$ plots obtained at different eluent conditions, respectively. We shall refer to $N_S^{\varphi}$ and $N_H^{\varphi}$ as isoentropic and isoenthalpic carbon number, respectively.

We analyzed the thermodynamic data[86] obtained with alkylbenzenes in RPC employing methanol−water mixtures and C-18 bonded stationary phase in order to examine the effect of dual enthalpy−entropy compensation on the retention process. Figure 4a,b illustrates the plots of $\Delta H°$ and $\Delta S° + R \ln V_S$, where $V_S$ is the volume of the stationary phase,[86] against the carbon number of five alkylbenzenes at various methanol concentrations. It is seen that the plots are linear according to eqs 25 and 26. Furthermore, extrapolation of linear plots yields a common intersection point in both cases, thus establishing the parameters, $N_S^{\varphi}$ and $N_H^{\varphi}$. From the plots in Figure 4 we obtain slightly different values of $N_S^{\varphi}$ and $N_H^{\varphi}$ so that $T_G^{\varphi}$ varies with the molecular structure according to eq 61. We rearrange the retention data presented in Figure 4a,b and plot both $\Delta H°$ and $\Delta S° + R \ln V_S$ against the methanol concentration for each alkylbenzene in Figure 5a,b. Such plots are linear according to eqs 30 and 31 and intersect upon extrapolation at the methanol concentrations $\varphi_S^N$ and $\varphi_H^N$, respectively. Both $\varphi_S^N$ and $\varphi_H^N$, at which the respective entropy and enthalpy changes for all eluites attain a common value, are found to be the same with a value of 117% so that $T_G^N$ is invariant for the system according to eq 63.

Since the experimental data under investigation yields the same values for $\varphi_S^N$ and $\varphi_H^N$ but slightly different values for $N_S^{\varphi}$ and $N_H^{\varphi}$, we expect to observe dual-compensation behavior illustrated in Figure 3b. This is confirmed by preparing two types of enthalpy versus entropy plots as shown in Figure 6. In
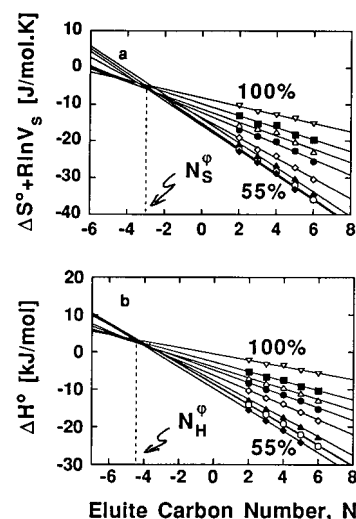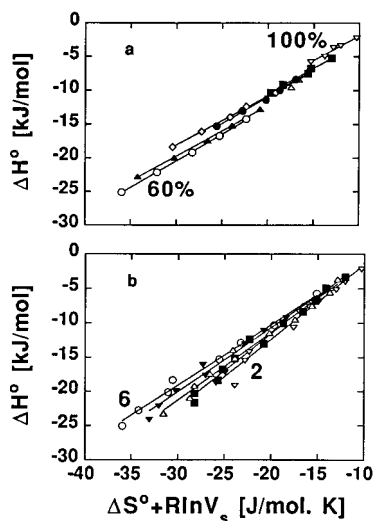


**Figure 4.** Plots of (a) entropy and (b) enthalpy changes against eluite carbon number, $N$, for the RPC retention of alkylbenzenes on octadecylated silica at various methanol concentrations in the eluent. At the isoentropic, $N_S^{\varphi}$, and isoenthalpic, $N_H^{\varphi}$, carbon numbers, which mark the common intersection points of the linear plots upon extrapolation, the entropy and enthalpy changes attain the same value. Data taken from ref 86.
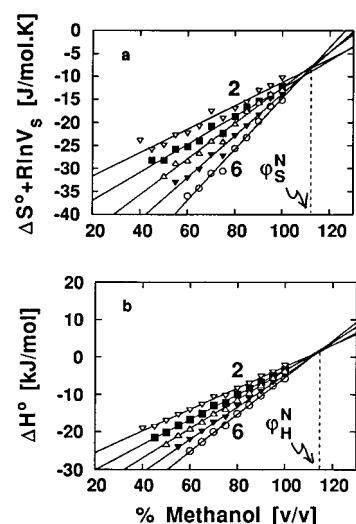


**Figure 5.** Plots of (a) entropy and (b) enthalpy changes against methanol concentration in the eluent, $\varphi$, for the RPC retention of various alkylbenzenes on octadecylated silica. At the isoentropic, $\varphi_S^N$, and isoenthalpic, $\varphi_H^N$, organic modifier concentrations, which mark the common intersection points of the linear plots upon extrapolation, the entropy and enthalpy changes attain the same value. Data taken from ref 86.

Figure 6a, $\Delta H°$ values obtained with alkylbenzenes are plotted against the corresponding $\Delta S° + R \ln V_S$ values at each mobile-phase composition. It is seen that the plots are linear with similar slopes. By fitting eq 41 to the linear plots in Figure 6a, we evaluated $T_G^N$ at various methanol concentrations from the slopes. The values thus obtained and listed in Table 3 are found to be similar in magnitude; i.e., $T_G^N$ is invariant with $\varphi$. An average value of 755 K for $T_G^N$ is calculated for the data presented in Table 4 and this compares well with the $a_{h1}/a_{s1}$ or $a_{h2}/a_{s2}$ value of 815 K obtained by fitting eqs 64 and 65 to the retention data. The good agreement between $T_G^N$ and $a_{h1}/a_{s1}$ or $a_{h2}/a_{s2}$ values confirms the validity of eq 62. Thermodynamic data in Figure 6a was replotted in Figure 6b with $\varphi$ as the parameter; i.e., $\Delta H°$ values obtained at different mobile-phase composition were plotted against the corresponding $\Delta S° + R$

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**Figure 6.** Plots of enthalpy change against entropy change obtained (a) with a set of alkylbenzenes at each methanol concentration in the eluent and (b) with each alkylbenzene at different methanol concentrations in the eluent. Data taken from ref 86.

**TABLE 4: Compensation Temperature $T_G^N$ Obtained from the Slopes of Linear $\Delta H°$ versus $\Delta S°$ Plots in Figure 6A**

| methanol concn (% v/v) | $T_G^N$ (K) | methanol concn (% v/v) | $T_G^N$ (K) |
|---|---|---|---|
| 100 | 727 | 75 | 717 |
| 90 | 759 | 65 | 749 |
| 85 | 755 | 60 | 795 |
| 80 | 781 | | |

**TABLE 5: Compensation Temperature $T_G^\varphi$ Obtained from the Slopes of Linear $\Delta H°$ versus $\Delta S°$ Plots in Figure 6b**

| carbon number, $N$ | $T_G^\varphi$ (K) | carbon number, $N$ | $T_G^\varphi$ (K) |
|---|---|---|---|
| 2 | 1086 | 5 | 936 |
| 3 | 1074 | 6 | 880 |
| 4 | 988 | | |

ln $V_S$ values for each eluite. Again the plots are linear, but they have different slopes. The $T_G^\varphi$ values evaluated for each eluite from the slopes of the linear plots by fitting eq 41 are listed in Table 5. As seen $T_G^\varphi$ appears to depend on $N$ and approaches the value of $T_G^N$ with increasing $N$ according to eq 61.

From the above analysis, it is concluded that $T_G^N$ is invariant for the system under investigation when the isoentropic, $\varphi_S^N$, and isoenthalpic, $\varphi_H^N$, organic modifier concentrations are the same. Similarly, $T_G^\varphi$ is invariant for the system under investigation when the isoentropic, $N_S^\varphi$, and isoenthalpic, $N_H^\varphi$, carbon numbers are the same.

**5.4. Other Compensation Temperatures.** In this section, we shall briefly examine EEC when either the alkyl ligate carbon number of the stationary phase in RPC or the molal salt concentration in HIC is chosen as the parameter.

In RPC, the logarithmic retention factor as well as the entropy and the enthalpy change frequently depend linearly on the carbon number of alkyl ligates.[98-100] Comparing eqs 39 and 40 to eq 41, we obtain an expression for compensation temperature, $T_G^{N_l}$, similar to eq 42 in terms of the enthalpy and entropy changes per unit ligate carbon number. Following an analysis similar to that given in section 5.2, it can be shown that $T_G^{N_l}$ marks the common intersection point of linear van't Hoff plots for the retention of an eluite on stationary phases
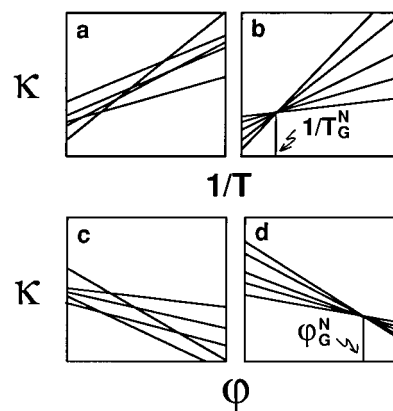


**Figure 7.** Graphical illustration of typical plots of the logarithmic retention factor against the reciprocal temperature and the organic modifier concentration for the RPC retention of two sets of eluites. (a) van't Hoff plots with eluites of diverse chemical structure, (b) van't Hoff plots with a set of structurally closely related eluites, (c) $\kappa$ versus $\varphi$ free energy plots with eluites of diverse chemical structure and (d) $\kappa$ versus $\varphi$ free energy plots with a set of structurally closely related eluites.

with varying chain length of the alkyl ligates. Furthermore, by combining eqs 4 and 41, when $N_l$ is the parameter of choice, we obtain a three-parameter equation similar to eqs 48 and 55 that describes eluite retention in RPC as a function of both the temperature and the carbon number of the stationary-phase ligate.

Since eluite retention in HIC yields nonlinear van't Hoff plots, nonclassical compensation behavior is expected to occur in HIC when $m$ is chosen as the parameter to obtain a set of $\Delta H°-\Delta S°$ pairs from van't Hoff plots. Following the analysis presented in a previous study,[53] we obtain three corresponding characteristic temperatures, namely, isoentropic, $T_S^{m*}$, isoenthalpic, $T_H^{m*}$, and isoenergetic, $T_G^{m*}$, temperatures that mark common intersection points of the respective plots of entropy, enthalpy, and free energy change against temperature with $m$ as the parameter. The meaning of these temperatures is similar to that of $T_S^{N*}$, $T_H^{N*}$ and $T_G^{N*}$ of nonclassical EEC analysis, which are obtained with $N$ as the parameter and are well-established in the literature.[19,53,105]

## 6. Generalized Compensation with Variables Other than the Temperature

In this section, we shall develop a generalized compensation model for reversed-phase chromatography by drawing analogies between various linear free energy plots and by establishing other compensation parameters similar to $T_G^P$ in EEC.

Analysis of retention data in the literature yields two distinct types of van't Hoff plots for a set of eluites under investigation as shown schematically in Figure 7a,b. RPC retention data obtained with eluites of diverse chemical structure yields linear van't Hoff plots in a wide range of temperature according to eq 4, and the lines intersect each other at different points as seen in Figure 7a. In contradistinction, van't Hoff plots of retention data of structurally closely related eluites exhibits a common intersection point due to the manifestation of enthalpy−entropy compensation, as seen from the illustration in Figure 7b. Strikingly analogous behavior is observed when retention data from the literature is analyzed in terms of the corresponding $\kappa-\varphi$ free energy plots. Figure 7c illustrates typical $\kappa-\varphi$ free energy plots that are linear with most eluent systems according to eq 29 and intersect at different points when eluites with diverse chemical structure are investigated. In contradistinction,
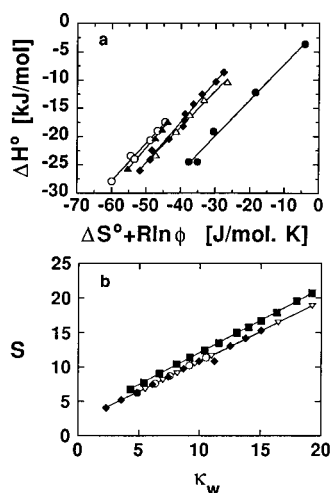
**Figure 8.** Plots of (a) enthalpy change against entropy change and (b) $S$ against $\kappa_w$ for the RPC retention of a set of structurally closely related eluites on octadecylated silica employing methanol−water mixtures. Data taken from references cited in Tables 6 and 7. Symbols are listed in (a) Table 6 and (b) Table 7.

**TABLE 6: Compensation Temperature $T_G^N$ Obtained from the Slopes of Linear $\Delta H°$ versus $\Delta S°$ Plots According to Eq 41 When $N$ Is Chosen as Parameter $P$ for the Retention of Various Homologous Substances on Octadecylated Silica with Methanol−Water Mixtures**

| no. | substances | methanol concn (% v/v) | $T_G^N$(K) | symbol in Figure 8a | ref |
|---|---|---|---|---|---|
| 1 | alkylbenzenes | 40 | 714 | ◆ | 40 |
| 2 | alkylbenzenes | 55 | 633 | △ | 107 |
| 3 | benzene derivatives | 60 | 637 | ● | 39 |
| 4 | alkylbenzenes | 80 | 639 | | 108 |
| 5 | alkanal DNPHs | 80 | 665 | ○ | 83 |
| 6 | 2-alkanone DNPHs | 80 | 708 | ▲ | 83 |
| 7 | substituted benzoates | 80 | 639 | | 108 |
| 8 | alkylbenzenes | 85 | 609 | | 109 |
| 9 | chlorobenzenes | 85 | 603 | | 109 |
| 10 | chlorotoluenes | 85 | 603 | | 109 |
| 11 | chloronaphthalenes | 85 | 633 | | 109 |
| 12 | chlorobiphenyls | 85 | 598 | | 109 |

the schematic in Figure 7d illustrates a common intersection point in $\kappa$−$\varphi$ plots, similar to that in van't Hoff plots shown in Figure 7b, with structurally closely related eluites as revealed by several studies.[24,59,100,106] In this light, we may express the logarithmic retention factor as a function of the organic modifier concentration for all eluites in a set of structurally closely related substances as

$$\kappa = (\varphi_G^N - \varphi)S + \kappa_{\varphi_G^N} \qquad (66)$$

where $\varphi_G^N$ is the organic modifier concentration at which the logarithmic retention factor, $\kappa_{\varphi_G^N}$, of all the eluites are the same.

Further parallelism between van't Hoff and $\kappa$−$\varphi$ free energy plots becomes evident when the corresponding slopes are plotted against the intercepts, i.e., when $\Delta H°$ is plotted against $\Delta S°$ and $S$ is plotted against $\kappa_w$, for a set of structurally closely related eluites. Plots of $\Delta H°$−$\Delta S°$ with $N$ as the parameter, $P$, of RPC retention data from sources listed in Table 6 are shown in Figure 8a. The plots are linear with similar slopes indicating EEC. The corresponding compensation temperatures, $T_G^N$, were evaluated from the slopes according to eq 41 and also listed in Table 6. It is seen that $T_G^N$ values are invariant in a wide range

**TABLE 7: Compensation Concentration, $\varphi_G^N$, Obtained from the Slopes of $S$−$\kappa_w$ Plots According to Eq 66 for the RPC Retention at Room Temperature of Various Substances on Octadecylated Silica Employing Methanol−Water Mixtures**

| no. | substances | $\varphi_G^N$% (v/v) | symbol in Figure 8b | ref |
|---|---|---|---|---|
| 1 | alkylbenzenes | 114 | ▽ | 70 |
| 2 | alkylbenzenes | 108 | ■ | 59 |
| 3 | alkylbenzenes | 109 | ○ | 69 |
| 4 | alkylbenzenes | 116 | ◆ | 109 |
| 5 | chlorobiphenyls | 118 | | 109 |
| 6 | halogenated benzenes | 130 | | 69 |
| 7 | methylbenzenes | 122 | | 69 |
| 8 | pharmaceuticals | 113 | | 110 |
| 9 | nitroanilines | 136 | | 110 |
| 10 | herbicides | 139 | | 76 |
| 11 | nicotinate esters | 125 | | 111 |
| 12 | nitro-PAHs | 122 | | 112 |
| 13 | acetanilide derivatives | 114 | | 113 |

of the methanol concentration, in good agreement with our finding in section 5.3. Similarly, the $S$−$\kappa_w$ plots of RPC retention data from sources listed in Table 7 exhibit linearity with almost identical slopes, as seen in Figure 8b. This linearity is well-documented in the literature and $S$−$\kappa_w$ relationships have been employed for data analysis.[109,24] Combining eqs 29 and 66, we obtain the following relationship between $S$ and $\kappa_w$

$$S = \frac{1}{\varphi_G^N}\kappa_w - \frac{\kappa_{\varphi_G^N}}{\varphi_G^N} \qquad (67)$$

The $\varphi_G^N$ values obtained by fitting eq 67 to the retention data in Figure 8b are listed in Table 7 and show that the $\varphi_G^N$ values are almost the same for the different classes of eluites under investigation.

Thus, the common intersection points in both the van't Hoff and the $\kappa$−$\varphi$ free energy plots of structurally closely related substances in Figs. 7b,d, and the parallelism between $\Delta H°$−$\Delta S°$ and $S$-$\kappa_w$ plots in Figure 8, allow us to apply the concept of classical EEC, with the variable $1/T$ and the parameter $N$, also to the analysis of data by using the variable $\varphi$ and the parameter $N$. Furthermore, the analogy may be extended to any variable and parameter of interest so that the concept of EEC includes compensation effects when the retention free energy change depends linearly on both the variable and the parameter under consideration. This approach provides a generalized compensation model to test compensation behavior in RPC with various chromatographic variables.

Combinations of a variable and a parameter from a set of four involving the temperature, the eluite carbon number, the organic modifier concentration, and the ligate carbon number, yield 12 cases of compensation for retention in RPC. They are listed in Table 8 under case $XP$ where $X$ denotes the variable and $P$ the parameter. For instance, case $(1/T)N$ in Table 8 stands for the choice of $1/T$ as the variable and $N$ as the parameter $P$. In this case, retention data is obtained at different temperatures to yield linear van't Hoff plots, and from the slopes and the intercepts of such plots, the enthalpy and entropy changes are evaluated for each eluite according to eq 4. The $\Delta H°$−$\Delta S°$ pairs obtained with a set of structurally closely related eluites are then plotted according to the compensation equation, eq 41, to confirm EEC. Alternatively, the logarithmic retention factor may be plotted against the enthalpy change according to the free energy compensation equation, eq 43, to reveal the common

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**TABLE 8: Classification of Compensation Phenomena in RPC on the Basis of the Van't Hoff Equation and Other Free Energy Relationships**[a]

| Case $XP$ | Eqn. | Slope | Intercept | Compensation eqn. | Intersection at | Free energy compensation eqn. |
|---|---|---|---|---|---|---|
| $\dfrac{1}{T}N$ | 4 | $\dfrac{-\Delta H^\circ}{R}$ | $\dfrac{-\Delta S^\circ}{R}+\ln\phi$ | $\Delta H^\circ = T_G^N \Delta S^\circ + \Delta G_{T_G^N}$ | $\dfrac{1}{T_G^N},\ \kappa_{T_G^N}$ | $\kappa = \left(\dfrac{1}{T_G^N}-\dfrac{1}{T}\right)\dfrac{\Delta H^\circ}{R}+\kappa_{T_G^N}$ |
| $\dfrac{1}{T}\varphi$ | 4 | $\dfrac{-\Delta H^\circ}{R}$ | $\dfrac{-\Delta S^\circ}{R}+\ln\phi$ | $\Delta H^\circ = T_G^\varphi \Delta S^\circ + \Delta G_{T_G^\varphi}$ | $\dfrac{1}{T_G^\varphi},\ \kappa_{T_G^\varphi}$ | $\kappa = \left(\dfrac{1}{T_G^\varphi}-\dfrac{1}{T}\right)\dfrac{\Delta H^\circ}{R}+\kappa_{T_G^\varphi}$ |
| $\dfrac{1}{T}N_l$ | 4 | $\dfrac{-\Delta H^\circ}{R}$ | $\dfrac{-\Delta S^\circ}{R}+\ln\phi$ | $\Delta H^\circ = T_G^{N_l}\Delta S^\circ + \Delta G_{T_G^{N_l}}$ | $\dfrac{1}{T_G^{N_l}},\ \kappa_{T_G^{N_l}}$ | $\kappa = \left(\dfrac{1}{T_G^{N_l}}-\dfrac{1}{T}\right)\dfrac{\Delta H^\circ}{R}+\kappa_{T_G^{N_l}}$ |
| $N\dfrac{1}{T}$ | 10 | $a_n$ | $b_n$ | $a_n = \dfrac{-1}{N_G^T}b_n - \dfrac{\kappa_{N_G^T}}{N_G^T}$ | $N_G^T,\ \kappa_{N_G^T}$ | $\kappa = (N - N_G^T)a_n + \kappa_{N_G^T}$ |
| $N\varphi$ | 10 | $a_n$ | $b_n$ | $a_n = \dfrac{-1}{N_G^\varphi}b_n - \dfrac{\kappa_{N_G^\varphi}}{N_G^\varphi}$ | $N_G^\varphi,\ \kappa_{N_G^\varphi}$ | $\kappa = (N - N_G^\varphi)a_n + \kappa_{N_G^\varphi}$ |
| $NN_l$ | 10 | $a_n$ | $b_n$ | $a_n = \dfrac{-1}{N_G^{N_l}}b_n - \dfrac{\kappa_{N_G^{N_l}}}{N_G^{N_l}}$ | $N_G^{N_l},\ \kappa_{N_G^{N_l}}$ | $\kappa = (N - N_G^{N_l})a_n + \kappa_{N_G^{N_l}}$ |
| $\varphi N$ | 29 | $-S$ | $\kappa_w$ | $S = \dfrac{1}{\varphi_G^N}\kappa_w - \dfrac{\kappa_{\varphi_G^N}}{\varphi_G^N}$ | $\varphi_G^N,\ \kappa_{\varphi_G^N}$ | $\kappa = (\varphi_G^N - \varphi)S + \kappa_{\varphi_G^N}$ |
| $\varphi\dfrac{1}{T}$ | 29 | $-S$ | $\kappa_w$ | $S = \dfrac{1}{\varphi_G^T}\kappa_w - \dfrac{\kappa_{\varphi_G^T}}{\varphi_G^T}$ | $\varphi_G^T,\ \kappa_{\varphi_G^T}$ | $\kappa = (\varphi_G^T - \varphi)S + \kappa_{\varphi_G^T}$ |
| $\varphi N_l$ | 29 | $-S$ | $\kappa_w$ | $S = \dfrac{1}{\varphi_G^{N_l}}\kappa_w - \dfrac{\kappa_{\varphi_G^{N_l}}}{\varphi_G^{N_l}}$ | $\varphi_G^{N_l},\ \kappa_{\varphi_G^{N_l}}$ | $\kappa = (\varphi_G^{N_l} - \varphi)S + \kappa_{\varphi_G^{N_l}}$ |
| $N_l N$ | 37 | $a_{n_l}$ | $b_{n_l}$ | $a_{n_l} = \dfrac{-1}{N_{lG}^N}b_{n_l} - \dfrac{\kappa_{N_{lG}^N}}{N_{lG}^N}$ | $N_{lG}^N,\ \kappa_{N_{lG}^N}$ | $\kappa = (N_l - N_{lG}^N)a_{n_l} + \kappa_{N_{lG}^N}$ |
| $N_l\dfrac{1}{T}$ | 37 | $a_{n_l}$ | $b_{n_l}$ | $a_{n_l} = \dfrac{-1}{N_{lG}^T}b_{n_l} - \dfrac{\kappa_{N_{lG}^T}}{N_{lG}^T}$ | $N_{lG}^T,\ \kappa_{N_{lG}^T}$ | $\kappa = (N_l - N_{lG}^T)a_{n_l} + \kappa_{N_{lG}^T}$ |
| $N_l\varphi$ | 37 | $a_{n_l}$ | $b_{n_l}$ | $a_{n_l} = \dfrac{-1}{N_{lG}^\varphi}b_{n_l} - \dfrac{\kappa_{N_{lG}^\varphi}}{N_{lG}^\varphi}$ | $N_{lG}^\varphi,\ \kappa_{N_{lG}^\varphi}$ | $\kappa = (N_l - N_{lG}^\varphi)a_{n_l} + \kappa_{N_{lG}^\varphi}$ |

[a] Case $XP$ represents the variable $X$ and parameter $P$ employed to investigate compensation phenomena by the corresponding equation listed above. $X$ and $P$ are the reciprocal temperature, $1/T$, or the eluite carbon number, $N$, or the organic modifier concentration, $\varphi$, or the ligate carbon number, $N_l$. The slopes, intercepts, and intersection points are for plots of $\kappa$ versus variable $X$ with $P$ as the parameter. Compensation occurs when slope versus intercept plots according to the compensation equation and/or $\kappa$ versus slope plots according to the free energy compensation equation are linear. So far compensation and free energy compensation equations only for cases $(1/T)N$[30,83,109] and $\varphi N$[24,106] have been used in practice. Those for the rest of the cases are first proposed here

intersection point as shown in Figure 7b. Two other cases of EEC, i.e., when $1/T$ is the variable, are $(1/T)\varphi$ and $(1/T)N_l$ in Table 8 with $\varphi$ and $N_l$ as the respective parameters.

Other cases in Table 8 represent the use of a variable other than the temperature to recognize compensation in RPC. For example, case $\varphi N$ entails $\varphi$ as the variable with $N$ as the parameter. Then, in the case of compensation $\kappa-\varphi$ free energy plots are linear for each eluite with the slope, $S$, and the intercept, $\kappa_w$, evaluated according to eq 29. Using a set of structurally closely related substances, $S-\kappa_w$ pairs are obtained and plotted according to the compensation equation, eq 67, to confirm compensation by linear plots. Alternatively, logarithmic retention factors may be plotted against $S$ values according to eq 66 to reveal, in the case of compensation, the common intersection point as shown in Figure 7d.

The set of equations in Table 8 manifests a system of exothermodynamic relationships represented by LFERs and compensations that apply to chromatographic retention. Table 8 also lists the coordinates of the common intersection points

of linear free energy plots when compensation occurs in RPC. Thus, the compensation temperature, $T_G^N$, is the temperature at which the van't Hoff plots of a set of structurally closely related eluites intersect at one point, i.e., the logarithmic retention factor, $\kappa_{T_G^N}$, is the same for all eluites. In a strikingly similar fashion, the $\kappa-\varphi$ linear free energy plots for structurally closely related eluites intersect at a point given by $\varphi_G^N$ and the corresponding logarithmic retention factor, $\kappa_{\varphi_G^N}$, as shown in Figure 7b,d. By analogy with the compensation temperature, $T_G^N$, we shall refer to $\varphi_G^N$ as the compensation concentration of the organic modifier. In our search for experimental support of common intersection points in various linear free energy plots, we analyzed a large body of retention data from the literature. It lead to several linear free energy plots with common intersection points that are depicted in Figures 9 and 10. Figure 9 illustrates 8 out of 12 linear free energy plots in RPC employing methanol−water mixtures whereas Figure 10 illustrates 4 out of 12 linear free energy plots in RPC employing acetonitrile−
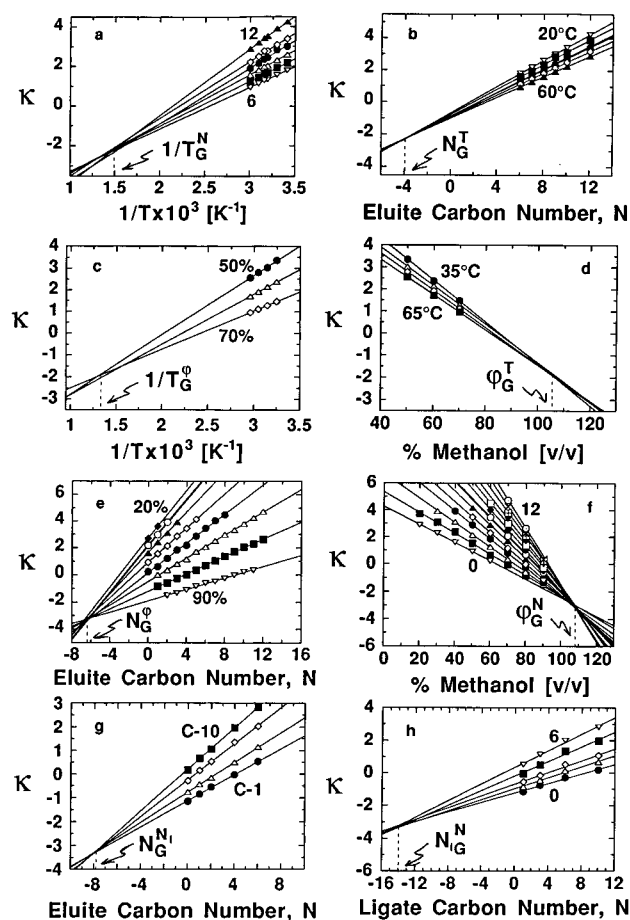
**Figure 9.** Illustration of eight free energy plots in RPC with methanol−water mixtures and their specific common intersection points. (a) van't Hoff plots with eluite carbon number, $N$, as parameter $P$; $n$-alkanal dinitrophenylhydrazones on octadecylated silica with 80% (v/v) methanol in water from ref 83. (b) $\kappa$ versus $N$ free energy plots with temperature as parameter $P$; $n$-alkanal dinitrophenylhydrazones on octadecylated silica with 80% (v/v) methanol in water from ref 83. (c) van't Hoff plots with methanol concentration, $\varphi$, as parameter $P$; 1-nitro-2-methylnaphthalene on octadecylated silica from ref 112. (d) $\kappa$ versus $\varphi$ free energy plots with temperature as parameter $P$; 1-nitro-2-methylnaphthalene on octadecylated silica from ref 112. (e) $\kappa$ versus $N$ free energy plots with methanol concentration, $\varphi$, as parameter $P$; alkylbenzenes on octadecylated silica at 25 °C from ref 59. (f) $\kappa$ versus $\varphi$ free energy plots with eluite carbon number, $N$, as parameter $P$; alkylbenzenes on octadecylated silica at 25 °C from ref 59. (g) $\kappa$ versus $N$ free energy plots with ligate carbon number, $N_l$, as parameter $P$; alkylbenzenes on alkyl-silica stationary phases with 70% (v/v) methanol in water at 27.5 °C from ref 100. (h) $\kappa$ versus $N_l$ free energy plots with eluite carbon number, $N$, as parameter $P$; alkylbenzenes on alkyl-silica stationary phases at 70% (v/v) methanol in water at 27.5 °C from ref 100.

water mixtures. With the exception of cases $\varphi N$ (*vide* Figure 9f) and $NN_l$ (*vide* Figure 9g), the other six plots in Figure 9 and four in Figure 10 are from data sets that were published without recognizing that there is a compensation particular to the retention process. As seen, each of the plots exhibits a common intersection point as predicted by our generalized compensation model. The results confirm the finding that RPC retention simultaneously obeys the linear relationships in eqs 4, 10, 29, and 37 with common intersection points in linear free energy plots, each exhibiting different forms of compensation.

The equations in Table 8 and the graphical rendition of the exothermodynamic relationships in Figure 11 show the 12 cases of compensation for chromatographic retention. As mentioned
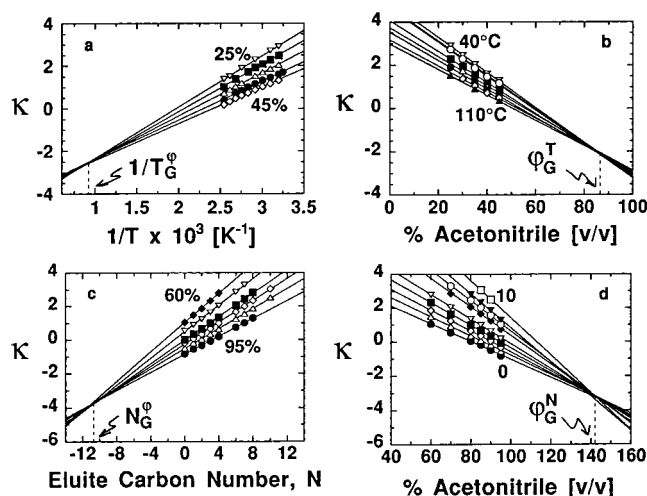


**Figure 10.** Illustration of four free energy plots in RPC with acetonitrile−water mixtures and their specific common intersection points. (a) van't Hoff plots with the acetonitrile concentration, $\varphi$, as parameter $P$; nitrobenzene on poly(styrene−divinylbenzene) stationary phase from ref 114. (b) $\kappa$ versus $\varphi$ free energy plots with the temperature as parameter $P$; nitrobenzene on poly(styrene−divinylbenzene) stationary phase from ref 114. (c) $\kappa$ versus $N$ free energy plots with acetonitrile concentration, $\varphi$, as parameter $P$; alkylbenzenes on octadecylated silica from ref 115. (d) $\kappa$ versus $\varphi$ free energy plots with eluite carbon number, $N$, as parameter $P$; alkylbenzenes on octadecylated silica from ref 115.
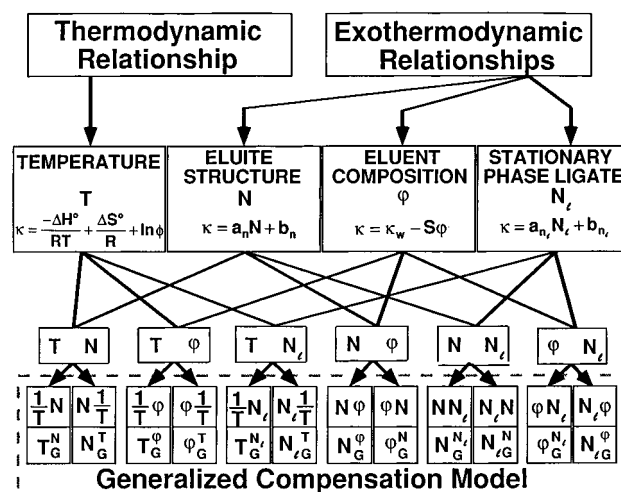


**Figure 11.** Major features of linear free energy relationships and compensation effects in reversed-phase chromatography as illustrated by their "family tree". The generalized compensation model is obtained by combining two of the four free energy relationships shown above for the temperature, the eluite structure, the eluent composition, and the stationary-phase ligate. The 12 specific cases of the generalized compensation model correspond to those given in Table 8 by the general code, case $XP$, where $X$ and $P$ are either the reciprocal temperature, $1/T$, or the eluite carbon number, $N$, or the organic modifier concentration, $\varphi$, or the ligate carbon number, $N_l$. For each case the corresponding compensation parameter, at which the linear free energy plots intersect, is also shown. Enthalpy−entropy compensation is a special case of generalized compensation when the van't Hoff relationship is considered in combination with any other LFER.

earlier, classical enthalpy−entropy compensation[53] is a particular case of the generalized compensation model when the temperature is the variable and the van't Hoff equation is employed to express the relationship between retention free energy change and temperature. These are cases $(1/T)N$, $(1/T)\varphi$, and $(1/T)N_l$ shown in Table 8 and Figure 11 with $T_G^N$, $T_G^\varphi$ and $T_G^{N_l}$ as the compensation parameters that mark common intersection points of the van't Hoff plots. It is noteworthy that we have not found

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**TABLE 9: Three-Parameter Equations of the General Form, $\kappa = \pm A_{XP}P(X - X_G^P) + B_{XP}N + C_{XP}$, Based on Compensation Phenomena in RPC[a]**

| code | three-parameter equation |
|------|--------------------------|
| $(1/T)N$ | $\kappa = A_{TN}N((1/T_G^N) - (1/T)) + B_{TN}/T + C_{TN}$ |
| $(1/T)\varphi$ | $\kappa = A_{T\varphi}\varphi((1/T_G^\varphi) - (1/T)) + B_{T\varphi}/T + C_{T\varphi}$ |
| $(1/T)N_l$ | $\kappa = A_{TN_l}N((1/T_G^N) - (1/T)) + B_{TN}/T + C_{TN}$ |
| $N(1/T)$ | $\kappa = (A_{NT}/T)(N - N_G^T) + B_{NT}N + C_{NT}$ |
| $N\varphi$ | $\kappa = A_{N\varphi}\varphi(N - N_G^\varphi) + B_{N\varphi}N + C_{N\varphi}$ |
| $NN_l$ | $\kappa = A_{NN_l}N_l(N - N_G^{N_l}) + B_{NN_l}N + C_{NN_l}$ |
| $\varphi N$ | $\kappa = A_{\varphi N}N(\varphi_G^N - \varphi) + B_{\varphi N}\varphi + C_{\varphi N}$ |
| $\varphi(1/T)$ | $\kappa = (A_{\varphi T}/T)(\varphi_G^T - \varphi) + B_{\varphi T}\varphi + C_{\varphi T}$ |
| $\varphi N_l$ | $\kappa = A_{\varphi N_l}N_l(\varphi_G^M - \varphi) + B_{\varphi N_l}\varphi + C_{\varphi N_l}$ |
| $N_lN$ | $\kappa = A_{N_lN}N(N_l - N_{lG}^N) + B_{N_lN}N_l + C_{N_lN}$ |
| $N_l(1/T)$ | $\kappa = (A_{N_lT}/T)(N_l - N_{lG}^T) + B_{N_lT}N_l + C_{N_lT}$ |
| $N_l\varphi$ | $\kappa = A_{N_l\varphi}\varphi(N_l - N_{lG}^\varphi) + B_{N_l\varphi}N_l + C_{N_l\varphi}$ |

[a] They relate the logarithmic retention factor to the chromatographic variable $X$ with parameter $P$. each of such pairs represent a case $XP$ where $X$ and $P$ are the reciprocal temperature, $1/T$, eluite carbon number, $N$, organic modifier concentration, $\varphi$, or the ligate carbon number, $N_l$. Only the three-parameter equation for the dependence of logarithmic retention factor on temperature and mobile phase composition (case $(1/T)\varphi$) has previously been described in the literature.[48,103,104]

the latter two cases mentioned in the literature, so that the present study may prompt the exploration of these kinds of exothermodynamic relationships. Figure 9a,c illustrates the first two cases of EEC. Due to paucity of retention data, the third case could not be examined. Out of the remaining nine cases, five are illustrated in Figure 9 so that four cases in all have remained unexplored with methanol−water mixtures.

A few free energy compensation plots in RPC retention were published in the literature, and the linear plots with common intersection points were considered as curiosities or "rather unexpected" results. According to our classification, they were cases $N_G^\varphi$, $T_G^N$, and $N_G^{T\,116,117}$ and case $N_G^{N_l}$.[100] Only in one instance, with cases $N_G^\varphi$ and $\varphi_G^N$, have the authors recognized that the common intersection point is the result of combining two linear relationships for the retention factor.[118] An interesting justification for the observed common intersection point in the case $N_G^\varphi$ was provided by using a "model of interaction indices", which represents another linear free energy relationship for the dependence of RPC retention on the eluent composition, instead of eq 29 in the present work.[59]

The generalized compensation model may also be employed to derive a number of three-parameter equations for the description of the retention behavior as a function of any two chromatographic variables of interest. Table 9 lists such three-parameter equations for each of the 12 cases identified in Table 8. It shows that a general form of the three-parameter equation is established that describes the combined dependence of the logarithmic retention factor in RPC on both $X$ and $P$ as

$$\kappa = \pm A_{XP}P(X - X_G^P) + B_{XP}N + C_{XP} \tag{68}$$

where $A_{XP}$, $B_{XP}$, and $C_{XP}$ are the three parameters and $X_G^P$ is the characteristic compensation parameter of the system that is invariant. Two particular cases, i.e., $X = 1/T$, $P = N$, and $X = 1/T$ $P = \varphi$, of eq 68 were described in sections 5.1 and 5.2. With the knowledge of $X_G^P$, eq 68 can be used to transform retention data obtained at one set of experimental conditions to another and thus to facilitate prediction of retention behavior.

## 7. Conclusion

The until now enshrouded interconnectedness of various exothermodynamic relationships in reversed-phase and hydro-

phobic interaction chromatography is revealed by this study. For instance, the linear relationship between retention free energy change and the molecular structure of the eluite sets a criterion for the linear relationships between the logarithmic retention factors, $\kappa$, measured on two RPC columns as well as for the linear relationships between $\kappa$ and the logarithmic octanol−water partition coefficient. A "family tree" of linear free energy relationships and compensation effects, such as enthalpy−entropy compensation, is shown in Figure 11 to summarize the linear exothermodynamic relationships pertinent to reversed-phase chromatography.

The fundamental four free energy relationships are those that describe the effect of the temperature, the eluite molecular structure, the composition of the eluent, and the structure of the functional groups at the stationary-phase surface on the retention free energy change. Only the effect of temperature on retention rests on a sound thermodynamic basis; therefore, the dependence of the retention free energy change on the other three chromatographic variables is described by linear exothermodynamic relationships. The 12 pairwise combinations of any two out of the four free energy relationships at the bottom of Figure 11 represent specific cases of the generalized compensation model with linear free energy plots that have common intersection points determined by the pertinent compensation parameters. This model gives rise to 12 three-parameter equations, as shown in Table 9, that can be used to describe the retention in RPC as a function of two chromatographic variables. By introducing new relationships between thermodynamic quantities and chromatographic variables, we not only set the criterion for compensation to occur in RPC but also provide a molecular interpretation of various compensation parameters. The dependence of compensation parameters on other chromatographic variables is investigated within the framework of the dual-compensation model in order to establish the conditions that lead to invariant values of compensation parameters.

The present study provides a framework of exothermodynamic relationships to serve as tools for the diagnosis, analysis, organization, and interpretation of retention data in RPC and HIC. The significance of this work, however, goes beyond the treatment of chromatographic data. It provides a hermeneutics of exothermodynamic relationships by virtue of the interconnectedness of LFERs and compensation. Thus, it is expected to be applicable to multivariate equilibrium and rate processes at large.

## List of Symbols and Abbreviations

$a_{cn}$ = heat capacity change of a reoccurring structural element

$a_g$ = free energy change per unit nonpolar surface area of the eluite

$a_{g,A}$ = free energy change per unit nonpolar surface area in RPC employing column A

$a_{g,B}$ = free energy change per unit nonpolar surface area in RPC employing column B

$a_{g,ow}$ = free energy change per unit nonpolar surface area in octanol−water partitioning

$a_{gn}$ = free energy change of the reoccurring structural element

$a_{g\varphi}$ = free energy change per unit concentration of the organic modifier

$a_{hn}$ = enthalpy change of a reoccurring structural element

$a_{h\varphi}$ = enthalpy change per unit concentration of the organic modifier

$a_{h1}$ = constant in eq 58

$a_{h2}$ = constant in eq 58

$a_n$ = logarithmic retention factor of a reoccurring structural element or the logarithmic methylene group selectivity

$a_{n_l}$ = logarithmic retention factor per unit carbon number of the ligate

$a_{sn}$ = entropy change of a reoccurring structural element

$a_{s\varphi}$ = entropy change per unit concentration of the organic modifier

$a_{s1}$ = constant in eq 56

$a_{s2}$ = constant in eq 56

$A$ = Ratio of the free energy change per unit nonpolar surface area in RPC to that in octanol−water partitioning according to eq 20

$A_{XP}$ = one of the three parameters in eq 68 with $X$ as the variable and $P$ as the parameter

$b_{cn}$ = heat capacity change due to all nonreoccurring structural elements

$b_g$ = free energy change of polar groups

$b_{g,A}$ = free energy change of polar groups obtained with column A

$b_{g,B}$ = free energy change of polar groups obtained with column B

$b_{g,ow}$ = free energy change of polar groups in octanol−water partitioning

$b_{gn}$ = free energy change due to all nonreoccurring structural elements

$b_{g\varphi}$ = free energy change in neat aqueous solution

$b'_g$ = free energy change per unit polar surface area of the eluite

$b_{hn}$ = enthalpy change due to all nonreoccurring structural elements

$b_{h\varphi}$ = enthalpy change in neat aqueous solution

$b_{h1}$ = constant in eq 59

$b_{h2}$ = constant in eq 59

$b_n$ = logarithmic retention factor of a reoccurring structural element

$b_{n_l}$ = logarithmic retention factor when the ligate carbon number is zero

$b_{sn}$ = entropy change due to all nonreoccurring structural elements

$b_{s\varphi}$ = entropy change in neat aqueous solution

$b_{s1}$ = constant in eq 57

$b_{s2}$ = constant in eq 57

$B$ = constant in eq 19

$B_{XP}$ = one of the three parameters in eq 68 with $X$ as the variable and $P$ as the parameter

$c_g$ = free energy change that is independent of the eluite molecular structure

$C$ = logarithmic retention factor per unit molal salt concentration

$C_{XP}$ = one of the three parameters in eq 68 with $X$ as the variable and P as the parameter

$k'$ = retention factor

$K$ = equilibrium constant for the retention process

$m$ = molal salt concentration

$N$ = eluite carbon number or the number of reoccurring structural elements

$N_l$ = carbon number of the alkyl ligate of the stationary phase

$N_H^\varphi$ = carbon number of a hypothetical eluite that has the same enthalpy change at all organic modifier concentrations

$N_S^\varphi$ = carbon number of a hypothetical eluite that has the same entropy change at all organic modifier concentrations

$P$ = chromatographic parameter, such as the temperature, the eluite carbon number, the organic modifier concentration, or the ligate carbon number

$R$ = universal gas constant

$t_o$ = elution time for an inert tracer

$t_R$ = retention time of the eluite

$S$ = logarithmic retention factor per unit organic modifier concentration

$T$ = temperature

$T_G^P$ = compensation temperature with $P$ as the parameter for obtaining $\Delta H° - \Delta S°$ pairs

$T_G^P*$ = isoenergetic temperature with $P$ as the parameter for obtaining $\Delta H° - \Delta S°$ pairs

$T_H$ = temperature at which enthalpy change is zero

$T_H^P*$ = isoenthalpic temperature with $P$ as the parameter

$T_S$ = temperature at which entropy change is zero

$T_S^P*$ = isoentropic temperature with $P$ as the parameter

$X$ = chromatographic variable, such as the temperature, the eluite carbon number, the organic modifier concentration or the ligate carbon number

$X_G^P$ = compensation parameter with $X$ as the variable and $P$ as the parameter

$z$ = total number of structural elements in a substance

$\Delta A_{np}$ = nonpolar surface area of the eluite

$\Delta A_p$ = polar surface area of the eluite

$\Delta C_p°$ = standard heat capacity change associated with eluite transfer from the mobile to the stationary phase

$\Delta C_{p,j}°$ = heat capacity change for the structural element $j$ of an eluite

$\Delta G°$ = standard free energy change associated with eluite transfer from the mobile to the stationary phase

$\Delta G_A°$ = free energy change of an eluite on column A

$\Delta G_B°$ = free energy change of an eluite on column B

$\Delta G_j°$ = free energy change for the structural element $j$ of an eluite

$\Delta G_{T_G^P}$ = free energy change at the compensation temperature $T_G^P$

$\Delta G_{X_G^P}$ = free energy change at the compensation parameter $X_G^P$

$\Delta H°$ = standard enthalpy change associated with eluite transfer from the mobile to the stationary phase

$\Delta H_j°$ = enthalpy change for the structural element $j$ of an eluite

$\Delta S°$ = standard entropy change associated with eluite transfer from the mobile to the stationary phase

$\Delta S_j°$ = entropy change for the structural element $j$ of an eluite

*Greek Letters*

$\alpha$ = ratio of the free energy change per unit nonpolar surface on column A to that on column B according to eq 16

$\beta$ = constant in eq 15

$\phi$ = phase ratio of the column

$\phi_A$ = phase ratio of column A

$\phi_B$ = phase ratio of column B

$\varphi$ = organic modifier concentration

$\varphi_H^N$ = organic modifier concentration at which all eluites have the same enthalpy change

$\varphi_S^N$ = organic modifier concentration at which all eluites have the same entropy change

$\kappa$ = logarithmic retention factor

$\kappa_A$ = logarithmic retention factor on column A

$\kappa_B$ = logarithmic retention factor on column B

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$\pi$ = logarithmic octanol−water partition coefficient

$\kappa_w$ = logarithmic retention factor obtained with neat aqueous solution

$\kappa_{T_G^P}$ = logarithmic retention factor at the compensation temperature $T_G^P$

$\kappa_{X_G^P}$ = logarithmic retention factor at the compensation parameter $X_G^P$

*Acronyms*

EECE = enthalpy−entropy compensation

HIC = hydrophobic interaction chromatography

HPLC = high-performance liquid chromatography

LFER = linear free energy relationship

QSAR = quantitative structure−activity relationship

RPC = reversed-phase chromatography

## References and Notes

(1) Horváth, Cs.; Melander, W. R.; Molnár, I. *J. Chromatogr.* **1976**, *125*, 129.

(2) Schoenmakers, P. J.; Billiet, H. A. H.; de Galan, L. *J. Chromatogr.* **1979**, *185*, 179.

(3) Martire, D. E.; Boehm, R. E. *J. Phys. Chem.* **1983**, *87*, 7, 1045.

(4) Geng, X.; Regnier, F. E. *J. Chromatogr.* **1984**, *296*, 15.

(5) Dill, K. A. *J. Phys. Chem.* **1987**, *91*, 1, 1980.

(6) Vailaya, A.; Horváth, Cs. *J. Phys. Chem.* **1997**, *101*, 5875.

(7) Melander, W. R.; Corradini, D.; Horváth, Cs. *J. Chromatogr.* **1984**, *317*, 67.

(8) Arakawa, T. *Arch. Biochem. Biophys.* **1986**, *248*, 101.

(9) Barford, R. A.; Kumosinski, T. F.; Parris, N.; White, A. E. *J. Chromatogr.* **1988**, *458*, 57.

(10) Vailaya, A.; Horváth, Cs. *Ind. Eng. Chem. Res.* **1996**, *35*, 5, 2964.

(11) Martin, A. J. P. *Biochem. Soc. Symp.* **1949**, *3*, 4.

(12) Leffler, J.; Grunwald, E. *Rates and Equilibria of Organic Reactions*; Wiley-Interscience: New York, 1963.

(13) Hammett, L. P. *Physical Organic Chemistry*; McGraw-Hill: New York, 1940.

(14) Taft, R. W. J. In *Steric Effects in Organic Chemistry*; Newman, M. S., Ed.; Wiley: New York, 1959; p 556.

(15) Collander, R. *Acta Chem. Scand.* **1951**, *5*, 774.

(16) Hansch, C. In *Drug Design*; Ariens, E. J., Ed.; Academic Press: New York, 1971; Vol. 1, p 271.

(17) Lumry, R.; Rajender, S. *Biopolymers* **1970**, *9*, 1125.

(18) Labuza, T. H. *Food Technol.* **1980**, *34*, 67.

(19) Lee, B. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 5154.

(20) Melander, W. R.; Horváth, Cs. *Chromatographia* **1982**, *15*, 86.

(21) Krstulovic, A. M.; Colin, H.; Guiochon, G. *Anal. Chem.* **1982**, *54*, 4, 2438.

(22) Tchapla, A.; Colin, H.; Guiochon, G. *Anal. Chem.* **1984**, *56*, 6, 621.

(23) Tchapla, A.; Héron, S.; Lesellier, E. *J. Chromatogr. A* **1993**, *656*, 81.

(24) Valkó, K.; Snyder, L. R.; Glajch, J. L. *J. Chromatogr. A* **1993**, *656*, 501.

(25) Jandera, P. *J. Chromatogr.* **1984**, *314*, 13.

(26) Hafkenscheid, T. L.; Tomlinson, E. *J. Chromatogr.* **1983**, *264*, 47.

(27) Chen, B.-K.; Horváth, Cs. *J. Chromatogr.* **1979**, *171*, 15.

(28) Chen, B.-K.; Horváth, Cs.; Bertino, J. R. *J. Med. Chem.* **1979**, *22*, 2, 483.

(29) Braumann, T. *J. Chromatogr.* **1986**, *373*, 191.

(30) Melander, W. R.; Campbell, D. E.; Horváth, Cs. *J. Chromatogr.* **1978**, *158*, 215.

(31) Riley, C. M.; Tomlinson, E.; Jefferies, T. M. *J. Chromatogr.* **1979**, *185*, 197.

(32) Riley, C. M.; Tomlinson, E.; Hafkenscheid, T. L. *J. Chromatogr.* **1981**, *218*, 427.

(33) de Bokx, P. K.; Boots, H. M. J. *J. Phys. Chem.* **1989**, *93*, 3, 8243.

(34) Boots, H. M. J.; de Bokx, P. K. *J. Phys. Chem.* **1989**, *93*, 3, 8240.

(35) Li, J.; Carr, P. W. *J. Chromatogr. A* **1994**, *670*, 105.

(36) Vailaya, A.; Horváth, Cs. *Biophys. Chem.* **1996**, *62*, 81.

(37) Berthod, A.; Li, W.; Armstrong, D. W. *Anal. Chem.* **1992**, *64*, 4, 873.

(38) Ma, S.; Horváth, Cs. *Electrophoresis* **1997**, *18*, 873.

(39) Knox, J. H.; Vasvari, G. *J. Chromatogr.* **1973**, *83*, 181.

(40) Grushka, E.; Colin, H.; Guiochon, G. *J. Chromatogr.* **1982**, *248*, 325.

(41) Tanford, C. *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, 2nd ed.; Wiley-Interscience: New York, 1980.

(42) Murphy, K. P.; Gill, S. J. *Thermochim. Acta* **1990**, *172*, 11.

(43) Gill, S. J.; Wadsö, I. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 2955.

(44) Dec, S. F.; Gill, S. J. *J. Solution Chem.* **1984**, *13*, 27.

(45) Dec, S. F.; Gill, S. J. *J. Solution Chem.* **1985**, *14*, 827.

(46) Cole, L. A.; Dorsey, J. G.; Dill, K. A. *Anal. Chem.* **1992**, *64*, 4, 1324.

(47) Purcell, A. W.; Aguilar, M. I.; Hearn, M. T. W. *J. Chromatogr.* **1992**, *593*, 103.

(48) Melander, W. R.; Nahum, A.; Horváth, Cs. *J. Chromatogr.* **1979**, *185*, 129.

(49) Morel, D.; Serpinet, J. *J. Chromatogr.* **1980**, *200*, 95.

(50) Morel, D.; Serpinet, J. *J. Chromatogr.* **1981**, *214*, 202.

(51) Cole, L. A.; Dorsey, J. G. *Anal. Chem.* **1992**, *64*, 4, 1317.

(52) Haidacher, D.; Vailaya, A.; Horváth, Cs. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 2290.

(53) Vailaya, A.; Horváth, Cs. *J. Phys. Chem.* **1996**, *100*, 0, 2447.

(54) Yalkowsky, S. H.; Valvani, S. C. *J. Med. Chem.* **1976**, *19*, 9, 727.

(55) Hermann, R. B. *J. Phys. Chem.* **1972**, *76*, 6, 2754.

(56) Chotia, C. *Nature (London)* **1974**, *248*, 338.

(57) Smith, R. M. *J. Chromatogr. A* **1993**, *656*, 381.

(58) Berendsen, G. E.; Schoenmakers, P. J.; de Galan, L.; Vigh, G.; Varga-Puchony, Z.; Inczédy, J. *J. Liq. Chromatogr.* **1980**, *3*, 1669.

(59) Colin, H.; Krstulovic, A. M.; Gonnord, M.-F.; Guiochon, G.; Yun, Z.; Jandera, J. *Chromatographia* **1983**, *17*, 9.

(60) Colin, H.; Guiochon, G.; Yun, Z.; Diez-Masa, J. C.; Jandera, J. *J. Chromatogr. Sci.* **1983**, *21*, 179.

(61) Colin, H.; Guiochon, G.; Diez-Masa, J. C. *Anal. Chem.* **1981**, *53*, 3, 146.

(62) Smith, R. M. *J. Chromatogr.* **1981**, *209*, 1.

(63) Melander, W. R.; Stoveken, J.; Horváth, Cs. *J. Chromatogr.* **1980**, *199*, 35.

(64) Corradini, D.; Kalghatgi, K.; Horváth, Cs. *J. Chromatogr. A* **1996**, *728*, 225.

(65) Martin, A. J. P.; Synge, R. L. M. *Biochem. J.* **1941**, *35*, 1359.

(66) Consden, R.; Gordon, A. H.; Martin, A. J. P. *Biochem. J.* **1944**, *38*, 224.

(67) Haggerty, W. J.; Murrill, E. A. *Res. Dev.* **1974**, *25*, 30.

(68) Nahum, A.; Horváth, Cs. *J. Chromatogr.* **1980**, *192*, 315.

(69) Hammers, W. E.; Meurs, G. J.; de Ligny, C. L. *J. Chromatogr.* **1982**, *247*, 1.

(70) Harnisch, M.; Mockel, H. J.; Schulze, G. *J. Chromatogr.* **1983**, *282*, 315.

(71) Vailaya, A.; Horváth, Cs. *J. Chromatogr.*, in press.

(72) Schoenmakers, P. J.; Billiet, H. A. H.; de Galan, L. *J. Chromatogr.* **1981**, *218*, 261.

(73) Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley: New York, 1979.

(74) Ruepert, C.; Grinwis, A.; Govers, H. *Chemosphere* **1985**, *14*, 279.

(75) McDuffie, B. *Chemosphere* **1981**, *10*, 73.

(76) Braumann, T.; Weber, G.; Grimme, L. H. *J. Chromatogr.* **1983**, *261*, 329.

(77) Braumann, T.; Grimme, L. H. *J. Chromatogr.* **1981**, *206*, 7.

(78) Hafkenscheid, T. L.; Tomlinson, E. *Int. J. Pharm.* **1983**, *16*, 225.

(79) Hulshoff, A.; Perrin, J. H. *J. Chromatogr.* **1976**, *129*, 263.

(80) Sherblom, P. M.; Eganhouse, R. P. *J. Chromatogr.* **1988**, *454*, 37.

(81) Cabani, S.; Gianni, P.; Mollica, V.; Lepori, L. *J. Solution Chem.* **1981**, *10*, 563.

(82) Ooi, T.; Oobatake, M.; Némethy, G.; Scheraga, H. A. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 3086.

(83) Vigh, G.; Varga-Puchony, Z. *J. Chromatogr.* **1980**, *196*, 1.

(84) Snyder, L. R.; Dolan, J. W.; Gant, J. R. *J. Chromatogr.* **1979**, *165*, 3.

(85) Nahum, A.; Horváth, Cs. *J. Chromatogr.* **1981**, *203*, 53.

(86) Alvarez-Zepeda, A.; Barman, B. N.; Martire, D. E. *Anal. Chem.* **1992**, *64*, 4, 1978.

(87) Melander, W. R.; Horváth, Cs. *Arch. Biochem. Biophys.* **1977**, *183*, 200.

(88) El Rassi, Z.; Horváth, Cs. *J. Liquid Chromatogr.* **1986**, *9*, 3245.

(89) Fausnaugh, J. L.; Regnier, F. E. *J. Chromatogr.* **1986**, *359*, 131.

(90) Katti, A.; Maa, Y.-F.; Horváth, Cs. *Chromatographia* **1987**, *24*, 646.

(91) Melander, W. R.; El Rassi, Z.; Horváth, Cs. *J. Chromatogr.* **1989**, *469*, 3.

(92) Miller, N. T.; Karger, B. L. *J. Chromatogr.* **1985**, *326*, 45.

(93) Szepesy, L.; Horváth, Cs. *Chromatographia* **1988**, *26*, 13.

(94) Arakawa, T.; Timasheff, S. N. *Biochemistry* **1982**, *21*, 1, 6545.

(95) Arakawa, T.; Timasheff, S. N. *Biochemistry* **1984**, *23*, 3, 5912.

(96) Melander, W. R.; Horváth, Cs. In *High Performance Liquid Chromatography−Advances and Perspectives*; Horváth, Cs., Ed.; Academic Press: New York, 1980; Vol. 2, p 113.

(97) Hemetsberger, H.; Kellermann, M.; Ricken, H. *Chromatographia* **1977**, *10*, 726.

(98) Karch, K.; Sebastian, I.; Halász, I. *J. Chromatogr.* **1976**, *122*, 3.

(99) Hennion, M. C.; Picard, C.; Caude, M. *J. Chromatogr.* **1978**, *166*, 21.

(100) Berendsen, G. E.; de Galan, L. *J. Chromatogr.* **1980**, *196*, 21.

(101) Krug, R. R.; Hunter, W. G.; Grieger, R. A. *J. Phys. Chem.* **1976**, *80*, 0, 2335.

(102) Krug, R. R.; Hunter, W. G.; Grieger, R. A. *J. Phys. Chem.* **1976**, *80*, 0, 2341.

(103) Melander, W. R.; Horváth, Cs. *Chromatographia* **1984**, *18*, 353.

(104) Melander, W. R.; Chen, B.-K.; Horváth, Cs. *J. Chromatogr.* **1985**, *318*, 1.

(105) Murphy, K. P.; Privalov, P. L.; Gill, S. J. *Science* **1990**, *247*, 559.

(106) Tan, L. C.; Carr, P. W. *J. Chromatogr. A* **1993**, *656*, 521.

(107) Issaq, H. J.; Jaroniec, M. *J. Liq. Chromatogr.* **1989**, *12*, 2067.

(108) Jinno, K.; Ozaki, N. *J. Liq. Chromatogr.* **1984**, *7*, 877.

(109) Opperhuizen, A.; Sinnige, T. L.; van der Steen, J. M. D. *J. Chromatogr.* **1987**, *388*, 51.

(110) Valkó, K. *J. Liq. Chromatogr.* **1987**, *10*, 1663.

(111) Reymond, D.; Chung, G. N.; Mayer, J. M.; Testa, B. *J. Chromatogr.* **1987**, *391*, 97.

(112) Robbat, A., Jr.; Liu, T. *J. Chromatogr.* **1990**, *513*, 117.

(113) Baty, J. D.; Sharp, S. *J. Chromatogr.* **1988**, *437*, 13.

(114) Chen, H.; Horváth, Cs. *AMI* **1993**, *1*, 213.

(115) Hanai, T.; Hubert, J. *J. Chromatogr.* **1984**, *290*, 197.

(116) Möckel, H. J.; Höfler, F.; Melzer, H. *J. Chromatogr.* **1987**, *388*, 285.

(117) Möckel, H. J. *J. Chromatogr. A* **1994**, *675*, 13.

(118) Czok, M.; Engelhardt, H. *Chromatographia* **1989**, *27*, 5.