

Effect of phase ratio on van't Hoff analysis in reversed-phase liquid chromatography, and phase-ratio-independent estimation of transfer enthalpy

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Abstract

In analysis of the thermodynamics of the transfer of a solute from the mobile phase to the stationary phase in reversed-phase liquid chromatography, it is nearly always assumed that the phase ratio is constant. This type of analysis is typically performed by applying a form of the van't Hoff equation, which relates the retention factor to temperature via the enthalpy and entropy of transfer. When non-linear van't Hoff plots are observed, it is often assumed that the enthalpy and entropy of transfer change with temperature. However, when the possibility of a change in the phase ratio is considered, it becomes apparent that non-linear van't Hoff behavior may or may not be due to changes in enthalpy or entropy. In this work, we present mathematical evidence that phase ratio changes, if they occur, can cause deviations from linearity in a van't Hoff plot. We also show that the phase ratio influence can be eliminated by considering the molecular difference between two solutes instead of the solutes themselves. The resulting selectivity van't Hoff plots may be linear, even when the van't Hoff plots of the two solutes are non-linear. In such cases, temperature-dependent phase ratio changes, and not necessarily changes in the transfer enthalpy, may be responsible for the curved van't Hoff plots of the individual solutes. In addition, we present chromatographic evidence that different solutes may “see” different thermodynamic phase ratios. It is clear that the concept of a phase ratio in reversed-phase chromatography is not nearly as well defined as a phase ratio in a bulk system like a liquid–liquid extraction.

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1. Introduction

1.1. Limitations of the van't Hoff equation in chromatography

Chromatographic retention is often used to calcu-

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late the partial molar enthalpy of transfer of a solute from the mobile phase to the stationary phase. The transfer enthalpy can then be used to characterize or compare various stationary phases using a particular mobile phase. Typically, the transfer enthalpy is obtained by invoking a form of the van't Hoff equation:

$$\ln k = \frac{-\Delta H^{\circ}}{RT} + \frac{\Delta S^{\circ}}{R} + \ln \Phi \quad (1)$$

where k , ΔH° , ΔS° , R , T , and Φ are the retention factor for the solute, the standard partial molar enthalpy of transfer, the standard partial molar entropy of transfer, the gas constant, the absolute temperature, and the phase ratio (that is, the volume of the stationary phase, V_S , divided by the volume of the mobile phase, V_M), respectively [1,2]. The procedure involves plotting $\ln k$ against $1/T$ (often called a *van't Hoff plot*), then setting the slope equal to $-\Delta H^\circ/R$, and solving for ΔH° . If the plot is not a straight line, it is often presumed that ΔH° varies with temperature; ΔH° is then evaluated from the slope at any particular $1/T$ value. This is valid if Φ is constant with respect to temperature.

Let us consider two questions regarding the interpretation of van't Hoff plots: If linearity is observed, does it imply that ΔH° is constant with temperature (or does curvature imply that ΔH° changes with temperature)? Is the phase ratio constant and does it have any influence on the curvature or slope of a van't Hoff plot?

We will consider these questions using the general case of the distribution of a solute between two immiscible phases, each one an open system but together constituting a closed system [3]. Our process involves the transfer of solute from its standard state in the mobile phase to its standard state in the stationary phase. Note that standard states must be defined at each temperature considered. We will also assume that ΔH° , ΔS° , and Φ are temperature-dependent. The slope of a van't Hoff plot is given by the partial derivative of Eq. (1) with respect to $1/T$:

$$\begin{aligned} \text{slope} &= \frac{\partial \ln k}{\partial(1/T)} \\ &= \frac{-\Delta H^\circ}{R} - \frac{1}{RT} \left(\frac{\partial(\Delta H^\circ)}{\partial(1/T)} \right) + \frac{1}{R} \frac{\partial(\Delta S^\circ)}{\partial(1/T)} \\ &\quad + \frac{\partial \ln \Phi}{\partial(1/T)} \end{aligned} \quad (2)$$

If the slope equals $-\Delta H^\circ/R$ as is widely assumed, then the last three terms on the right must sum to zero at all temperatures. We will show that the slope does not equal $-\Delta H^\circ/R$ when Φ is not constant with T . We will also show how to make Φ -independent determinations of ΔH° for molecular differences between two probe molecules.

1.2. Theory

A transfer of solute from the mobile phase to the stationary phase is associated with a reduction in the Gibbs energy of the mobile phase and an increase in the Gibbs energy of the stationary phase. In each of the mobile and stationary phases, the change in Gibbs energy equals the chemical potential of the solute in that phase times the quantity of solute reduced or increased, respectively, for transfers of infinitesimally small amounts of solute at constant temperature and pressure. The sum of the change in both phases is identical to the change in the total Gibbs energy for the overall system. From the definition of the Gibbs energy [4,5], we can substitute $\Delta G^\circ/T = \Delta H^\circ/T - \Delta S^\circ$, where ΔG° is the standard partial molar Gibbs energy of transfer of the solute (that is, the standard chemical potential for the stationary phase minus that for the mobile phase), into Eq. (1). Then, taking the constant-pressure partial derivative of $\ln k$ with respect to $1/T$ and applying the Gibbs–Helmholtz [4,5] equation in the form:

$$\left(\frac{\partial(\Delta G^\circ/T)}{\partial(1/T)} \right)_P = \Delta H^\circ \quad (3)$$

where P is pressure, we find for a van't Hoff plot at constant pressure

$$\text{slope} = \left(\frac{\partial \ln k}{\partial(1/T)} \right)_P = \frac{-\Delta H^\circ}{R} + \left(\frac{\partial \ln \Phi}{\partial(1/T)} \right)_P \quad (4)$$

Therefore, the terms containing the partial derivatives of enthalpy and entropy in Eq. (2) must sum to zero at constant pressure [4,5]. This still leaves the partial derivative of the phase ratio with respect to $1/T$, and this term will only be zero if the phase ratio is constant with respect to temperature.

The restriction of constant pressure in Eq. (4) may be removed for many liquid and solid systems since pressure effects on Gibbs energy can be ignored as long as the phases involved are essentially incompressible. Thus, Eq. (4) may be applied to high-performance liquid chromatography (HPLC) over a range of pressure if the phases are not significantly compressible under the test conditions. This is usually a reasonable assumption with typical HPLC conditions, but compression effects on retention can

be demonstrated at pressures within the realm of HPLC [6]. Eq. (4) may also be applied to open-tubular (ot) supercritical fluid chromatography (SFC) if the mobile phase is kept at a low reduced density (that is, using a high reduced temperature and low reduced pressure) so that the pressure drop from the column inlet to the outlet is insignificant compared to the average pressure. However, significant pressure drops may result in otSFC at high reduced density [7] thereby introducing errors into the apparent transfer enthalpy derived from Eq. (4). Strictly, Eq. (4) may be applied to gas chromatography (GC) and to packed-column SFC only in the limit as the flow-rate approaches zero so that the pressure is constant over the length of the column. This results because both of these techniques have compressible mobile phases and significant pressure drops under normal operating conditions. However, the error in applying Eq. (4) to GC without a pressure-drop correction will often be negligible compared to experimental uncertainties since the transfer enthalpy in GC is dominated by the enthalpy of solution of the solute in the stationary phase. The errors that would be encountered in packed-column SFC, where there is a significant pressure-dependent enthalpy of solution for solutes in the mobile phase, are not expected to be negligible.

It is clear from Eq. (4) that whenever Φ is constant with temperature, the slope of a van't Hoff plot equals $-\Delta H^\circ/R$, even if the plot is not linear. Thus, ΔH° can be evaluated from the slope at any temperature, even if the van't Hoff plot is curved, as long as Φ is constant. The converse is equally clear: in a chromatographic experiment there will be no observable indication of the constancy of Φ since a curved plot could result from variation in either ΔH° or Φ , and a linear plot could result from mutually compensating changes in both ΔH° and Φ . Therefore, a determination of ΔH° from a van't Hoff plot of a solute should only be trusted if there is good reason to believe that the phase ratio is constant, or else if the "thermodynamic phase ratio" (as actually accessed by the solute) is somehow independently measured and an appropriate correction is made.

The definition of the stationary phase volume for bonded-phase columns is the subject of debate, with opinions ranging from the molecular volume of the bonded moieties [8–10] to the volume of a solvation

layer or interphase perhaps containing some non-moving "mobile" phase molecules [11–16]. The concept of a "Gibbs Dividing Surface" has also been used to delineate the boundary between the stationary and mobile phases [12]. Yun et al. further described this concept in their thermodynamic study of chromatographic retention and adsorbed mobile phase components on the bonded-phase surface [13]. Kazakevich et al. studied excess adsorption isotherms of reversed-phase organic modifiers on reversed-phase packing surfaces and reported significant amounts of the strong mobile phase component adsorbed onto collapsed stationary-phase chains on the packing surface [14]. Alvarez-Zepeda and Martire also employed surface excess isotherm data to determine V_M [15,16]. Their model defined V_M as the volume-fraction-weighted average of the retention times of isotopically labelled mobile phase components. Some parts of both the stationary phase and the mobile phase are inaccessible to some solutes, either because of steric effects or charge exclusion [11].

Evidence also exists for a "phase transition" of the bonded stationary phase chains at a temperature similar to the melting temperature of neat octadecane [17–19]. These conclusions were based on differential scanning calorimetry (DSC) experiments [17] and on breaks or non-linearities in a van't Hoff plot [18,19]. However, spectroscopic studies seem to indicate that a gradual change in the stationary phase conformation, rather than an abrupt transition, actually occurs [20]. Since a phase transition is defined as a discontinuity in the physical state of a substance, one would expect that a discontinuity, rather than a bend or non-linearity, would be seen in a van't Hoff plot if a phase transition were occurring.

In reversed-phase HPLC with bonded stationary phases (RPLC), it is often *assumed* that the phase ratio is constant, more specifically that the stationary phase volume is constant. Yet, there are numerous reports of bonded phase collapse, particularly for octadecylsilyl (ODS)-bonded-phase columns used with high concentrations of water in the mobile phase [21,22], in contrast to the permanently collapsed chains described by Kazakevich et al. [14]. In addition, temperature can have an effect on stationary phase conformation [20,23–26]. Changes in these parameters can change the volume of the

stationary phase, or perhaps more properly stated, the volume of the stationary phase accessible to solute molecules, which is the important thermodynamic quantity [11]. Therefore, the effective or thermodynamic phase ratio is difficult if not impossible to define in a physical sense, but reflects the behavior of individual solutes in the system. Although we are unable to define what this phase ratio is, our point is that it is not necessarily constant as conditions are changed and different solutes are examined.

Cole et al. assumed the phase ratio of an ODS-bonded-phase column was constant, and calculated transfer enthalpy values for benzene from the slope of van't Hoff plots using a mobile phase of 95% water and 5% 1-propanol [27]. Their method assumed a stationary phase volume equal to the molecular volume of the stationary phase alkyl chains, as calculated by the method of Sentell and Dorsey [10]. This assumes that the entire stationary phase chain is accessible to every solute molecule. For illustration, let us interpret the same data differently, this time assuming that the transfer enthalpy is constant and that the phase ratio changes with temperature. Fig. 1 shows the relative phase ratio thus calculated as a function of temperature for a

constant partial molar transfer enthalpy of -7.11 kJ/mol and a constant partial molar transfer entropy of 19.9 J/mol-K. (These enthalpy and entropy values are within the range of values calculated by Cole and Dorsey using this data set.) The resulting relative phase ratio changes are of reasonable values, particularly if the adsorbed mobile phase is part of the stationary phase; Alvarez-Zepeda and Martire showed changes around 30% in the ratio of the adsorbed solvent volume to the mobile phase volume with a temperature change from 35 to 55 °C [15]. However, there is no justification to believe, without further evidence, that either of the extreme assumptions we have discussed (constant stationary phase volume or constant transfer enthalpy) is correct. For retention data to be generally useful for estimating transfer enthalpy, we need an estimation procedure that is independent of phase ratio.

1.3. Transfer enthalpies of molecular differences

Methylene selectivities have been calculated by numerous researchers as another means of characterizing solute transfer in chromatographic columns under specific mobile-phase–stationary-phase con-

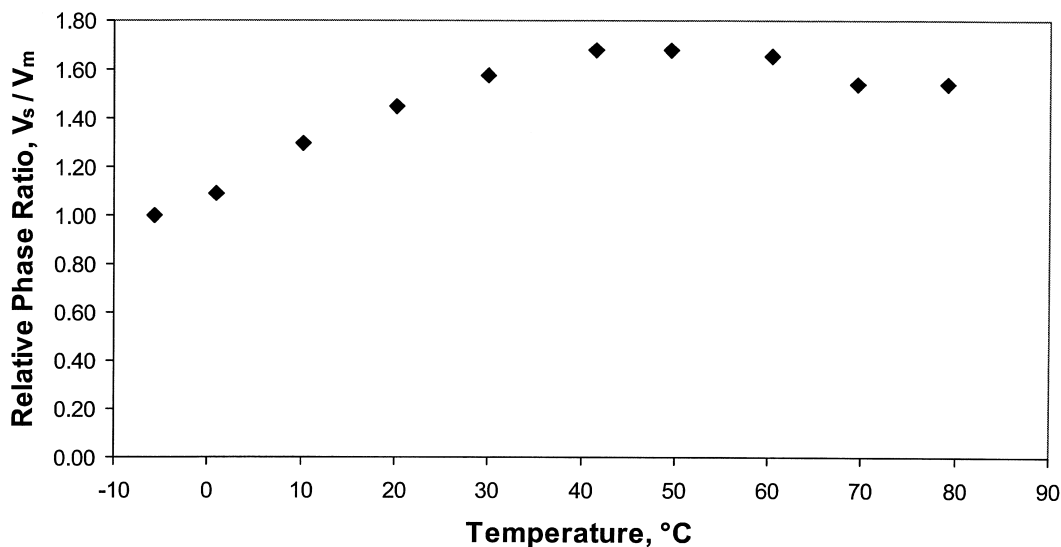


Fig. 1. Apparent phase ratio, relative to the value at -5 °C, as a function of $1/T$, derived from data in Ref. [27]. In this representation, the values of the enthalpy and entropy of transfer of benzene from the mobile phase to the stationary phase are held constant, at -7.11 kJ/mol and 19.9 J/mol-K, respectively, and the phase ratio is allowed to vary. This data interpretation contrasts to that in Ref. [27], in which the phase ratio was assumed to be constant and the enthalpy and entropy of transfer were assumed to change with temperature.

ditions [28–34]. This is accomplished by comparing the retention of two solutes differing structurally by one methylene unit, or by chromatographically analyzing a homologous methylene series and taking the slope of a plot of $\ln k$ vs. homolog number as the natural log of the methylene selectivity.

Differences other than methylene selectivity have also been studied. Lee and Cheong [35] performed experiments that used chromatographic selectivity to evaluate interactions between polar functional groups and mobile phases. Sentell and Dorsey [31], in their study of Dill's lattice model of retention [8,9], examined two types of "phenyl" selectivity, especially as it relates to stationary phase bonding density. Phenyl selectivity was taken as a measure of shape selectivity, and the results obtained were consistent with the Dill model for retention. Carr and coworkers compared the partitioning influence of several different polar functional groups attached to benzene, using both chromatographic retention and partitioning in bulk solutions [36].

We propose expanding and applying this approach of examining chromatographic selectivity to any molecular difference between two probe solutes. When compared to using single probes, this difference method has the advantage of being independent of phase ratio changes as long as the probes are similar enough to access the same phase volumes.

For example, if $\ln k$ values for members of an oligomeric series follow a particular trend with respect to temperature, then the molecular unit representing the difference between adjacent series members should follow the same trend as a molecule of structure similar to the molecular difference. For oligomers, this molecular difference will resemble the monomer from which the series was built. By writing Eq. (1) for each of two adjacent series members and taking the difference, we find:

$$\begin{aligned} \ln \alpha &= \ln k_{i+1} - \ln k_i \\ &= \frac{(-\Delta H_{i+1}^{\circ} + \Delta H_i^{\circ})}{RT} + \frac{(\Delta S_{i+1}^{\circ} - \Delta S_i^{\circ})}{R} \end{aligned} \quad (5)$$

where α is the separation factor (also called the selectivity) between adjacent series members, and i is the degree of polymerization of the first oligomer of the pair. By taking the partial derivative of Eq. (5) with respect to $1/T$, or by applying Eqs. (2) and (3),

as we did earlier, to the separate $\ln k$ expressions going into Eq. (5), we can deduce that the slope of a plot of $\ln \alpha$ against $1/T$ is:

$$\begin{aligned} \text{slope} &= \frac{\partial \ln \alpha}{\partial (1/T)} = \frac{-(\Delta H_{i+1}^{\circ} - \Delta H_i^{\circ})}{R} \\ &= \frac{-\Delta H_d^{\circ}}{R} \end{aligned} \quad (6)$$

where ΔH_d° is the partial molar enthalpy of transfer added to oligomer i with the addition of one more unit to the oligomeric chain.

Eq. (6) is independent of the phase ratio because any temperature-dependent change in the phase ratio affects the k values for the individual probes by exactly the same factor at that particular temperature if both probes have access to the same stationary phase volume. However, if the probes do not have access to the same stationary phase volume, then the partial derivatives of the phase ratio for each of the probes may not cancel and we may be left with some degree of phase-ratio dependence in the slope. Eq. (6) is not restricted to oligomers, but applies in general to any two probes with access to the same stationary phase volume. We might want to consider ΔH_d° a virtual enthalpy since the molecular difference characterized by this method does not actually exist as a solute.

If two such probes individually produce curved van't Hoff plots, but if a linear $\ln \alpha$ plot results over the same temperature range, this would suggest that there is a constant transfer enthalpy for their molecular difference. If the molecular difference were similar to the structure of the smaller probe, then the results would suggest that the van't Hoff curvature, at least for the smaller probe, is caused by a changing thermodynamic phase ratio rather than by factors affecting the probe transfer enthalpy.

2. Experimental

2.1. Instrumentation

All experiments were performed on a Gilson SF3 chromatography system (Gilson, Middleton, WI, USA) operated in HPLC mode. This instrument consists of a Model 308 primary pump, a Model 306

slave pump, a Model 811C dynamic mixer, a Model 831 column oven, a Model 821 pressure regulator, a Model 119 UV detector, and a Model 234 auto-sampler. The pressure regulator was not used for these HPLC experiments; effluent from the detector was sent directly to the waste collector. The auto-sampler was equipped with a Rheodyne (Rohnert Park, CA, USA) Model 7010 injection valve with a 10- μ L sample loop. The analytical column was a Discovery C₁₈ (Supelco, Bellefonte, PA, USA), 50 \times 4.6 mm, with 5 μ m particle diameter and 180 Å mean pore diameter. Data collection was performed with Gilson's Unipoint software. For experiments above ambient temperature, the column was heated in the Model 831 oven. For subambient-temperature experiments, the column was immersed in a Model RTE-111 temperature-controlled bath (Neslab, Newington, NH, USA).

2.2. Chemicals

Water was purified using a Milli-Q water purification system (Millipore, Milford, MA, USA). HPLC-grade methanol was obtained from EM Science (Gibbstown, NJ, USA). Test solutes included benzene, biphenyl, toluene, propylparaben, and butylparaben from various sources. All test solutes were dissolved in methanol.

2.3. Methods

Chromatographic measurements were made over a variety of temperatures from -5 to 80 °C with typical intervals of 10 °C. Retention factors were calculated from retention times, corrected for the extra-column volumes, using uracil as a void time marker. Data were analyzed using Microsoft Excel 2000 (Microsoft, Redmond, WA, USA). Benzene–biphenyl experiments were performed using a mixture of water–methanol (40:60, v/v) as the mobile phase. Benzene–toluene and propylparaben–butylparaben experiments used water–methanol (60:40, v/v) as the mobile phase.

3. Results and discussion

Our first set of experiments examined if changes

in the phase ratio could be the cause of curvature in the van't Hoff plot of benzene. Van't Hoff plots for benzene, biphenyl, and their selectivity are shown in Fig. 2. Since the molecular difference between benzene and biphenyl is a phenylene group (an aromatic, C₆H₄ ring), which only differs from benzene in the loss of two hydrogen atoms, the phenylene selectivity should give a reasonable indication of benzene partitioning independent of phase ratio effects.

There are at least two reports of linear van't Hoff behavior of benzene and substituted benzenes using bulk-phase octanol–water partitioning experiments [37,38]. However, the benzene and biphenyl plots in Fig. 2 are clearly non-linear. The *apparent* benzene transfer enthalpy calculated from the slope of the benzene plot, assuming constant phase ratio, ranges from approximately -12 kJ/mol at the high-temperature end of the range to about $+2$ kJ/mol at the low-temperature end. The *apparent* transfer enthalpy of biphenyl ranges from about -20 to -5 kJ/mol. In contrast, the difference between the biphenyl and benzene curves, that is the phenylene selectivity plot, is linear with a squared correlation coefficient (r^2) exceeding 0.995. Since, according to Eq. (6), this plot is presumably independent of phase ratio, the linearity suggests that the transfer enthalpy for phenylene is constant. Calculating its value from the slope we get -8.61 kJ/mol. Since the benzene retention van't Hoff plot is influenced by the phase ratio, while the phenylene selectivity plot is not, it can be inferred that the non-linearity in the benzene retention plot is predominantly the result of changes in the phase ratio rather than changes in the transfer enthalpy.

A similar experiment was performed using benzene and toluene as test solutes. Because these solutes differ by one methylene group, the selectivity between them should yield a phase-ratio-independent measure of the hydrophobic interactions affecting separation. As in the previous experiment, the van't Hoff plots of the individual solutes are curved. These are shown in Fig. 3. The *apparent* transfer enthalpy for toluene, derived assuming a constant phase ratio, ranges between approximately -21 and 0 kJ/mol, and that of benzene is between approximately -17 and $+0.4$ kJ/mol. (The mobile phase is lower in methanol, now 40%, but the previous benzene–bi-

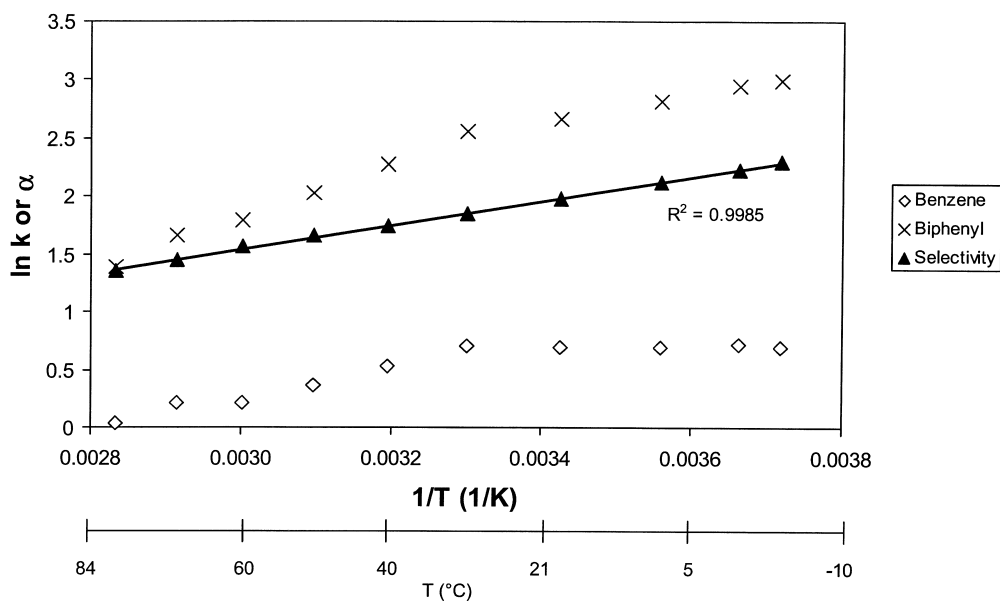


Fig. 2. Van't Hoff plots of benzene, biphenyl, and their difference, which is the phenylene selectivity. The phase-ratio-dependent plots of benzene and biphenyl are non-linear, while the phase-ratio-independent plot of phenylene selectivity is linear. This suggests a phase-ratio dependence in the van't Hoff plots of benzene. Experimental conditions: water–methanol (40:60) mobile phase, ODS stationary phase, at a flow-rate of 1.00 mL/min.

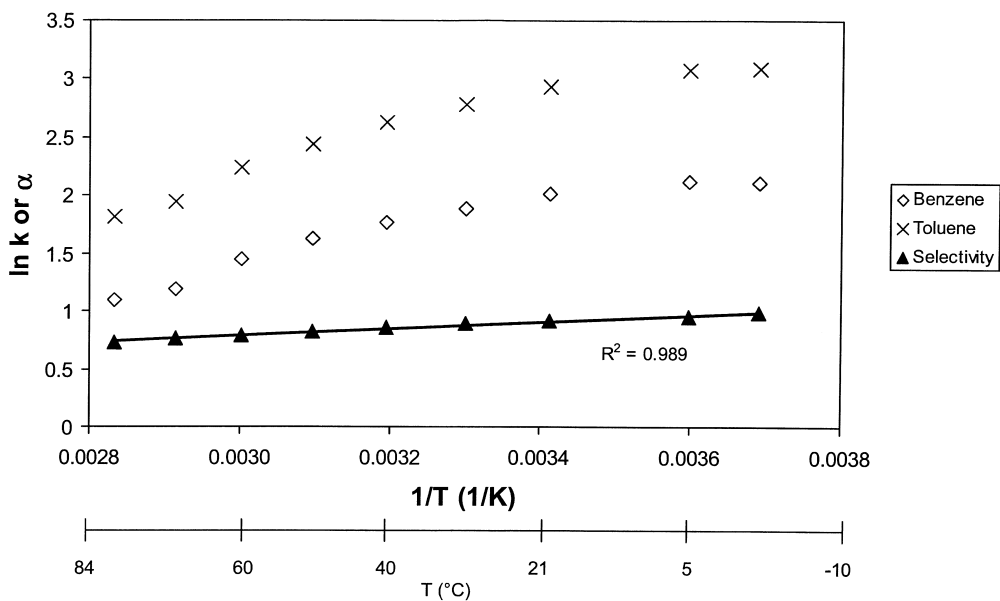


Fig. 3. Van't Hoff plots of benzene, toluene, and their difference, which is the methylene selectivity. As in Fig. 2, the phase-ratio-dependent plots of benzene and toluene are non-linear, while the phase-ratio-independent methylene selectivity plot is linear. Again, this suggests a phase-ratio dependence in the van't Hoff plots of benzene and possibly toluene. Experimental conditions: water–methanol (60:40) mobile phase, ODS stationary phase, at a flow-rate of 1.00 mL/min.

phenyl experiment used 60% methanol. As we might expect, the apparent transfer enthalpy for benzene is somewhat higher in magnitude when the methanol content of the mobile phase is lowered.) In contrast to the curved retention plots, the methylene selectivity plot was linear ($r^2 = 0.99$) yielding a constant methylene transfer enthalpy of -2.48 kJ/mol over the temperature range examined. As before, this suggests that the non-linearities in the retention plots are predominantly the result of changes in the phase ratio with temperature, not a result of changes in the transfer enthalpy of either solute.

Benzene, biphenyl, and toluene are low-polarity solutes that are thought to partition into the alkyl chains of the ODS phase [8,39]. Different retention behavior may be expected of more polar solutes since the partitioning of solutes with hydrophilic functional groups from an aqueous–alcoholic mobile phase into the hydrophobic stationary phase will be influenced by polar interactions, particularly hydrogen bonding. The van't Hoff plots for propyl- and butylparaben are both highly linear, as shown in Fig. 4. Both solutes' van't Hoff plots have squared linear

correlation coefficients exceeding 0.99 over the temperature range studied. The apparent transfer enthalpy for propylparaben is -21.23 kJ/mol, and that for butylparaben is -23.57 kJ/mol. The methylene selectivity is also linear and yields a methylene transfer enthalpy of -2.33 kJ/mol.

Ranatunga and Carr estimated the transfer enthalpy of benzene between water–methanol and hexadecane (representing an octadecyl-bonded phase) using headspace GC in a thermodynamic cycle: the transfer free energies from water–methanol to gas, and from hexadecane to gas, were determined, and the transfer free energy from water–methanol to hexadecane was then obtained by difference [40]. They calculated an overall transfer enthalpy for benzene from water–methanol (40:60) to hexadecane of -6.9 kJ/mol. This compares with our value of -8.61 kJ/mol for phenylene using our method. They also calculated the transfer enthalpy of a methylene group from water–methanol (60:40) to hexadecane of -2.78 kJ/mol, which compares to our values of -2.33 and -2.48 kJ/mol. Ranatunga and Carr justified using hexadecane to represent

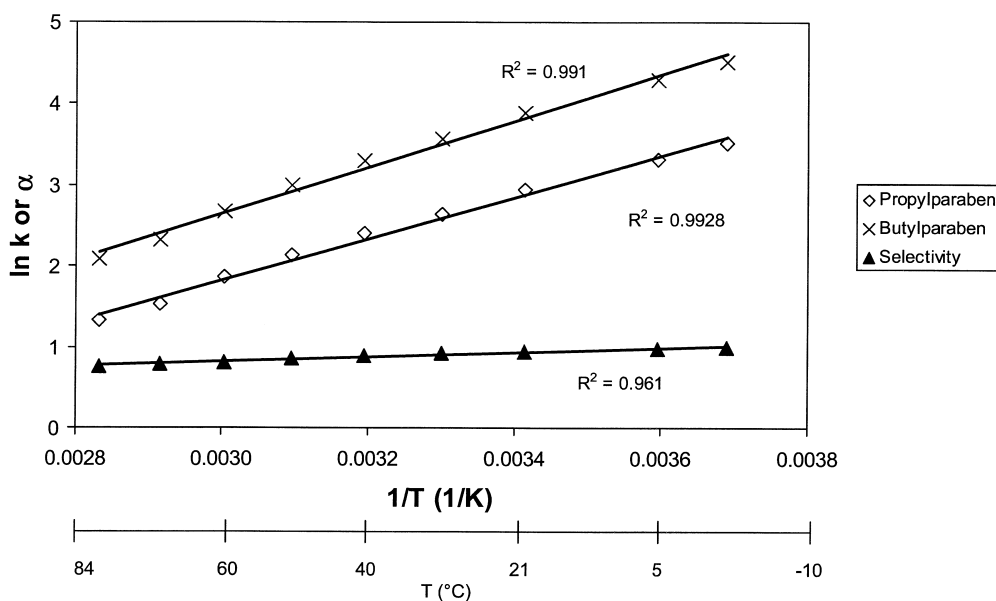


Fig. 4. Van't Hoff plots of propylparaben, butylparaben, and their difference, which is the methylene selectivity. In contrast to the non-polar solutes in Figs. 2 and 3, these solutes, as well as the methylene selectivity, exhibit linear van't Hoff behavior. This suggests that the thermodynamic phase ratio seen by these solutes is constant over the entire temperature range, and that the retention mechanism of these semi-polar solutes is different than that of the non-polar solutes benzene, biphenyl, and toluene. Experimental conditions are the same as in Fig. 3.

bonded octadecane based on the similarities of methylene selectivities for the two systems, so it is not surprising that the methylene enthalpies of the two methods more closely agree than do the benzene transfer enthalpy from their experiment and the phenylene transfer enthalpy from ours.

One explanation commonly given for van't Hoff curvature cites the hydrophobic effect [27], which states that solubility of a non-polar solute in a polar solvent should be at a minimum [41,42], and thus the reversed-phase retention should be at a maximum [43], around 25 °C. This is the temperature where the formation of a solute cavity in the polar mobile phase is most entropically unfavorable [42]. Since the transfer entropy changes with temperature according to this explanation, the enthalpy must also change since the two quantities are related through the constant-pressure heat capacity [4,5]. However, if the enthalpy were to change, this should also be reflected in phase-ratio-independent measurements, such as selectivity van't Hoff plots. Our selectivity van't Hoff plots (Figs. 2–4) suggest that the enthalpies of transfer for the phenylene and methylene functional groups are constant over the temperature range studied (–5 to +80 °C). This further suggests that the curvature seen in the non-linear van't Hoff plots in these examples is due to changes in the thermodynamic phase ratio, rather than to changes in the transfer enthalpy and entropy of the solutes. It also suggests that benzene and toluene see a different part of the stationary phase than do propyl- and butylparaben.

As demonstrated by Sentell and Dorsey [39], small, non-polar solutes are retained by partitioning into the alkyl chains of the stationary phase. If the temperature is changed, the stationary phase alkyl chains may change conformation, and all the thermodynamic properties could change. However, the retention mechanism may be different for more polar solutes such as the parabens in our example, or for the strongly retained but hexadecane-insoluble dye described by Carr et al. [36]. The preferred configuration of these solutes with the stationary phase may leave the hydrophilic functional groups available to interact with the mobile phase while the solute is retained. It appears that for non-polar solutes such as benzene, which more fully partition into the alkyl chains of the stationary phase, the thermodynamic

phase ratio changes with temperature. However, for a semi-polar solute like propylparaben, which may not fully partition into the stationary-phase alkyl chains, the thermodynamic phase ratio does not change with temperature. This model is consistent with the observations of Carr et al. [36] and with the model proposed by Tchaplal and co-workers in their study of methylene selectivity [30], where they stated that retention is due to partitioning of the alkyl chain of a solute lengthwise into the stationary phase.

Concern has been raised that this approach may fail if the solutes are so chemically similar that their ΔH° values exhibit essentially the same behavior with respect to temperature. For example, what if the two probes were not oligomers but were positional isomers? Would the ΔH° behavior of highly similar individual probes be canceled in the derivation of Eq. (6) leaving it useless to interpret the van't Hoff behavior? What would the slope of $\ln \alpha$ vs. $1/T$ mean in this situation?

To answer this, let us consider several, specific circumstances under the requirement that the two probes have access to the same stationary phase volume: (1) How would Eq. (6) apply if two solutes were so similar that their ΔH° and ΔS° values were essentially identical to each other, respectively, at all temperatures? In this case, whether the ΔH° values change with temperature or are constant with temperature, we see from Eq. (5) that $\ln \alpha$ would be zero at all temperatures, α would be unity at all temperatures, the solutes would coelute at all temperatures, and the slope of $\ln \alpha$ vs. $1/T$ [given by Eq. (6)] would be zero at all temperatures. (2) How would Eq. (6) apply if the ΔH° values for the two solutes were different but change in a mutually similar fashion with respect to temperature so that their difference (ΔH_d°) is constant and non-zero? In this case, the slope given by Eq. (6) would be constant and non-zero. This would indicate that the selectivity of the two solutes is constant with temperature. (3) How would Eq. (6) apply if the solute ΔH° values were dissimilar with respect to temperature so that their difference were not constant with temperature? In this case, the slope given by Eq. (6) would vary with temperature.

Therefore, in some cases, the slope given by Eq. (6) may be zero, but it would be because the values of ΔH° for the probes are equal, not because the

ΔH° dependence of the probes is removed in the derivation of Eq. (6). It appears that this approach is valid for cases involving real molecular differences, such as a methylene or phenylene difference between two molecular probes. By extension, this approach also appears valid for simple combinations of molecular differences, such as when the difference in two probes represents the relocation of a group to make a positional isomer, or when the difference between the probes is due to the complete replacement of one or more groups by molecularly dissimilar groups. The approach seems valid regardless of the similarity or differences in the ΔH° behavior of the probes as long as the requirement that the two probes effectively access the same stationary phase volume is met.

We must keep in mind that there could be some additional steric artifacts in this approach since the molecular difference between two probes cannot necessarily access the volumes that are unavailable to the probes themselves, but the molecular analog of the difference could be small enough to access some of these volumes. Therefore, small-pore stationary phases explored with two, large probes may not produce exactly the same results as a (small) molecule resembling the difference between the probes. In addition, if the probes are close to the dimensions of the stationary phase pores, the larger probe may be excluded from more stationary-phase volume than the smaller probe. Therefore, interpretation of the results of this method should be done cautiously until we gain more experience.

4. Conclusions

RPLC is considerably more complex than the discrete, two-phase mechanism on which partition chromatography is commonly modeled. The concept of a phase ratio is somewhat tenuous in RPLC, especially when it is compared to the phase ratio in liquid–liquid extraction where the volume of the interphase between the two bulk phases is thermodynamically negligible. However, in RPLC, one of the phases behaves almost entirely as an interphase. This makes definition and determination of phase volumes difficult, particularly for the stationary phase.

The molecular volume of the ODS chains is often

taken as the stationary phase volume, and it is usually assumed to be constant. However, in the complex interphase where RPLC retention occurs, this approach may not be valid. It appears that the accessible volume of the stationary phase is solute dependent, and that parts of the stationary phase may change volume with temperature. Conformation of the stationary phase, steric restrictions on solute diffusion into the pores of the stationary phase supporting material, and solute-dependent differences in interactions with the ODS chains can all contribute to different solutes “seeing” different phase ratios. Determining stationary phase volume by a percent-carbon method, and the mobile phase volume by a gravimetric method, as is commonly done in HPLC studies, may not yield the proper thermodynamic values.

Chromatographic van't Hoff plots must be interpreted carefully. Enthalpy values, derived from the slopes of such plots without regard to effective or thermodynamic phase-ratio changes that may occur, should not be trusted, particularly if the plot is curved (although the approach would be perfectly valid if the thermodynamic phase ratio were known as a function of temperature for the solutes under examination). The molecular difference method is expected to give a reliable transfer enthalpy if the two probes used encounter the same thermodynamic phase ratio.

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