Accurate measurements of solubility and thermodynamic transfer quantities using reversed-phase liquid–liquid chromatography

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ABSTRACT

A reversed-phase high-performance liquid chromatographic system, consisting of polydimethylsiloxane coated on non-porous glass beads as the stationary phase and pure water as the mobile phase, was used to measure the absolute solubility and the temperature dependence of the solubility of a series of alkylbenzenes in water in the temperature range 0–80°C. The system and the method of analysis provide accurate values of the molar free energy, enthalpy, entropy and reasonable values of the heat capacity of transfer of the alkylbenzenes from their own liquids into water. The thermodynamic data were analysed in terms of the Flory–Huggins theory giving combinatorial and non-combinatorial, *i.e.*, interactional, contributions to the free energy of transfer. All the data were found to agree very well with literature values. The success of the system is attributed to the liquid nature of the stationary phase, the low surface area-to-volume ratio offered by the support material chosen and the availability of the absolute value of the volume of the stationary phase to calculate the phase ratio.

INTRODUCTION

In reversed-phase high-performance liquid chromatography (RP-HPLC), the stationary phase is less polar than the mobile phase, which is often a mixture of water and an organic solvent (modifier). Chromatographic retention is due to the equilibrium distribution of a probe molecule between the stationary phase and the mixed mobile phase. This retention is expressed in terms of a capacity factor, k', relating the retention volume to the hold-up volume in the system. This in turn is related to the partitioning of the probe between the stationary phase and the mobile phase. Therefore, thermodynamics under equilibrium conditions can be applied to chromatographic retention [1–4]. Liquid and gas chromatography have in fact been shown to be very powerful tools for obtaining thermodynamic functions of mixtures [5,6]. However, extracting thermodynamic information is often hampered by the lack of knowledge of the volume of the stationary phase required to calculate the partition coefficient. Hence one has to be content with the dependence of a relative quantity, *e.g.*, the capacity factor, k', on parameters such as the mixing ratio of water and organic modifier in the mobile phase or the temperature. In the first case, for weak interactions between the probe molecules and the molecules in the mixed mobile phase, log k' is linearly dependent on the composition of the mixed phase. However, this dependence has been found to become non-linear as the water content of the mobile phase is increased [2]. Less attention has been paid to pure aqueous systems, which are of interest in this work. The advantage of using temperature as a parameter is that the enthalpy change can be obtained directly from k' for the process of

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transferring a probe molecule between the stationary phase and the mobile phase. However, absolute values of the free energy and the entropy of transfer require a knowledge of the total volume of the stationary phase in order to relate the capacity factor to the partition coefficient through the phase ratio.

In this paper we show that it is indeed possible to obtain meaningful and absolute values of all the thermodynamic transfer functions and also the solubility, by virtue of a high-molecular mass stationary phase, which is a non-polar liquid, and an accurate independent measurement of the phase ratio. These thermodynamic data for hydrocarbons at infinite dilution in pure water can be used to examine hydrocarbon–water interactions.

The interaction of non-polar parts of molecules with water is an important factor in several common physico-chemical processes, such as micellization of surfactants and protein denaturation. These interactions are usually determined by measuring the solubility of alkyl derivatives in water. The model compounds often contain one polar part, which makes only a minor contribution to the solubility of a homologous series in dilute aqueous solution [7]. However, there are experimental problems associated with measuring the solubility of even these alkyl derivatives in water owing to their low aqueous solubility. These problems are largely circumvented by the use of chromatography.

This study extends our previous work [8] on the transfer of toluene and ethylbenzene from a non-polar liquid to water by measuring benzene and propylbenzene on the same HPLC set-up, which consists of water as the mobile phase and a non-polar liquid, viz., polydimethylsiloxane (PDMS), coated on non-porous glass beads as the stationary phase. The volume of the polydimethylsiloxane stationary phase is characterized by elemental analysis such that the phase ratio and thereby the free energy, entropy and enthalpy are obtained for the transfer process.

EXPERIMENTAL

Chemicals and column preparation

The stationary phase consisted of a polymer coated on non-porous glass beads (30–60 μm)

from Werner Glass (Stockholm, Sweden). The polymer was PDMS, a secondary standard used as received from Aldrich (M ca. 600 000). The polymer is a liquid over the whole temperature range investigated, i.e., 0–80°C. The fact that the polymer is very hydrophobic together with the high molecular mass prevents bleeding of the stationary phase. The PDMS was deposited on the non-porous glass beads by first dissolving it in toluene and mixing the solution with the glass beads for 15 min, and then evaporating the toluene in a Rotavapor. The glass beads, used as the support material, were precleaned by washing with water and 95% ethanol to remove organic material before drying at 110°C overnight to remove traces of water.

The coated beads were slurried in water containing about 10% of chromatographic-grade methanol in order to improve the dispersion. The slurry was packed with this water–methanol mixture as the packing fluid in a Magnus Scientific Instruments slurry packer at ca. 350 atm (1 atm = 101 325 Pa). The packing process was complete after 2 l of packing solution had passed through the column, then the methanol was rinsed out with another 2 l of deaerated water, equivalent to about 200 column dead volumes.

The water used in the above processes was purified with a modified Millipore Milli-Q purification system. The feed water was purified by the following steps: decalcination, prefiltration with activated charcoal, reverse osmosis, treatment with two mixed-bed ion exchangers, activated charcoal, an in-line filter (0.2 μm), an Organex cartridge and finally filtration through a 0.2-μm cationic nylon filter. All purification units were Millipore products, except the final filter, which was obtained from Zetapore.

The probe liquids were chromatographic-grade benzene and propylbenzene from Merck and Aldrich. A saturated solution of the probe in water was mixed daily, and the water-rich part was diluted 2–4-fold with water before use. Sodium nitrate was added to this diluted solution to mark the column hold-up or dead volume. A knowledge of the exact concentration of the probe sample was not necessary for our purposes once it had been verified that the retention volumes were independent of probe concentration.

Characterization of the stationary phase

In order to obtain the phase ratio, it is necessary to determine the volume of the liquid polymer in the stationary phase. The initial mass of the polymer on the glass beads unfortunately could not be used as a measure of the amount of PDMS deposited on the beads as a certain amount of the polymer was lost both in the preparation of the packing and during the packing process with methanol–water. The amount was instead determined by elemental analysis, performed by MikroKemi (Uppsala, Sweden), of 200-mg samples of the column material removed after the experiment. This was found to be a more accurate method of determining the amount of PDMS in the column than the differential thermal analysis method used in our previous work on toluene and ethylbenzene [8]. We therefore re-measured the packing of the previous column (No. 3) and recalculated the resulting thermodynamic quantities for toluene and ethylbenzene to be presented here together with the results for benzene and propylbenzene.

Four different columns were used for retention measurements of benzene, toluene, ethylbenzene and propylbenzene. For benzene we joined columns 1 and 2 in series in order to obtain large enough retention volumes that could be measured accurately. Column 3 was used for toluene and ethylbenzene and column 4 for propylbenzene. The total amounts of PDMS in columns 1 + 2, 3 and 4 were found to be 0.0819 ± 0.0009, 0.0264 ± 0.0003 and 0.00105 ± 0.00001 g, respectively. The resulting thickness of the layer of polymer on the glass bead support material was calculated to be 800–1400 Å for columns 1 + 2, 500–800 Å for column 3 and 10–20 Å for column 4. Both the volume of polymer used and the resulting thickness decreased as the alkyl chain length increased to ensure reasonable retention times. The phase ratio, i.e., the ratio of the volume of the stationary to that of the mobile phase in the chromatographic system, was calculated to be 0.048, 0.036 and 0.0023 for columns 1 + 2, 3 and 4, respectively.

Analysis of the column performance gave about 400 theoretical plates for columns 1 + 2 and 3 and 30 theoretical plates for column 4. This corresponds to a height equivalent to a theoretical plate (HETP) of 0.8 mm for columns

1 + 2 and 3 and 1.8 mm for column 4. The high HETP value for the latter column is probably due to dispersion effects caused by the pure, non-coated glass beads (see below).

Apparatus and chromatographic procedure

Stainless-steel columns with 0.5-mm end frits of various lengths and widths were used to obtain reasonable retention times for both benzene and propylbenzene. Two 4.6 mm I.D. columns were used in series for benzene, one 5 cm (No. 1) and one 25 cm long (No. 2). For propylbenzene, a 4 cm × 2.1 mm I.D. column (No. 4) was used and packed with 50% cleaned, non-coated glass beads. A reference column was packed with only cleaned glass beads in order to verify that there was no interaction of the propylbenzene with the glass surface. Stainless-steel tubing of 3-ml volume was inserted between the water source and the injector to ensure a well thermostated mobile phase. The stainless-steel tubing from the injector to the column was 1/16 in. O.D. (1 in. = 2.54 cm) and 0.01 mm I.D. and that from the column to the detector was 1/16 in. O.D. and 0.007 mm I.D., and was insulated in order to reduce any local temperature effects of the laboratory. The latter tubing was kept as short as possible in order to minimize peak spreading. The column temperature was controlled by regulating a water-bath to ±0.25°C. The 20-μl loop of a Rheodyne Model 7125 injector and part of the injector itself were immersed in the water-bath. The loop was flushed with several volumes of the temperature-equilibrated probe solution before filling and finally left for a period of time in order to come to temperature equilibrium before injection. The eluate was detected with a Waters Model 410 Millipore refractive index meter. A pressure of 50 ± 15 atm from an LKB Model 2150 HPLC mini-pump ensured a constant flow-rate of 1.00 ± 0.005 ml/min. The eluate was collected in a beaker and weighed continuously in order to determine the retention volume. The detector output was recorded on a chart recorder and the peak maxima of the probe and marker were recorded [9]. The primary information was obtained as the difference in retention volumes of the probe and the salt marker, $V^N = V^R - V^{MK}$

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(a full list of the symbols used is given at the end of the paper).

Treatment of experimental data

In chromatography, the most commonly measured parameter is the capacity factor, defined as

$$k' = (V_R - V^{MK})/V^{MK} = V^N/V^{MK} \tag{1}$$

where V^N is the net retention volume, i.e., the difference between the retention volume of the probe, V^R, and the retention volume of a marker, V^{MK}, e.g., a non-retarding molecule such as a salt, giving the mobile phase hold-up volume.

The retention mechanism in our system involves both absorption of the probe molecules into the stationary polymer liquid and adsorption at the polymer–water interface. The net retention volume can therefore be written as

$$V^N = K_c V^P + K_{ads} A^P \tag{2}$$

where V^P is the total volume of the stationary polymer phase, A^P is the total interfacial area of the polymer being exposed to the water and K_c and K_{ads} are the partition coefficients of the probe for the absorption and adsorption processes, respectively. In our system the adsorption term was found to be negligible, i.e.,

$$K_c \approx V^N/V^P = k'/\phi \tag{3}$$

where ϕ is the phase ratio. This was shown by measuring the net retention volume as a function of polymer loading, V^P, with an approximately constant polymer surface area, A^P, shown in Fig. 1 in ref. 8. The straight line passing through the origin indicates the negligible importance of the last term in eqn. 2. This result is a consequence of the experimental design in using non-porous, and relatively large, glass beads as the support for the PDMS polymer, thus giving a minimum surface-to-volume ratio of the polymer stationary phase.

The future usefulness of eqn. 3 depends on the correctness of the following three assumptions:
(i) The LC system is operating at equilibrium, i.e., the partition coefficient is established quickly in comparison with the length of the column. This assumption was checked by verifying that
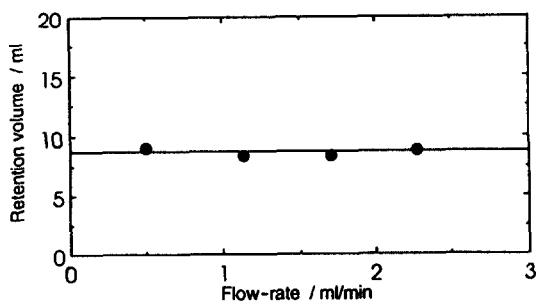

Fig. 1. Retention volume of toluene as a function of flow-rate.

the retention volume of a probe is independent of flow-rate, as shown in Fig. 1.
(ii) The partitioning is constant throughout the column. This assumption is true as long as we can verify that we are operating in the linear portion of the partition equilibrium. We found that the retention volume of a probe is independent of probe concentration (Fig. 2 in ref. 8), thus ensuring infinite probe dilution conditions, i.e., the system is operating in the Henry's law range.
(iii) The stationary and mobile liquid phases are mutually immiscible such that they can be considered as a pure water and a pure PDMS phase. This ensures that one is determining the difference in behaviour of two binary mixtures (probe–PDMS and probe–water) as opposed to two ternary mixtures. The correctness of this assumption was not checked, but the precaution was taken of using a very high-molecular-mass polymer. Also, no changes in retention volume were detected as a function of time, which would be the case if the polymer had a slight solubility in the water (bleeding).

We shall now link the partition coefficient to meaningful thermodynamic quantities.

THERMODYNAMIC BACKGROUND

The fundamental quantity in eqn. 3 is the equilibrium partition coefficient, K_c, describing the partitioning of the probe (component 1) between the two immiscible phases, in our sys-

tem the water mobile phase (W) and the non-polar polymer stationary phase (P). The partition coefficient, K_c, is at any temperature defined as the ratio of the probe molar concentration $[1]^i$ in either phase i. The concentration is thus expressed in moles of probe (n_1) per unit volume, i.e., n_1^P/V^P for the polymer phase and n_1^W/V^W for the aqueous phase, and the partition coefficient is

$$K_c = \frac{[1]^P}{[1]^W} = \frac{n_1^P/V^P}{n_1^W/V^W} \qquad (4)$$

where V^P and V^W are the volumes of the polymer and water phase, respectively.

From fundamental thermodynamics, the partition ceofficient, K_c, defined using molar concentrations of the probe, is obtained by defining the activity coefficient of component 1, γ_1, as

$$\gamma_1 = \frac{a_1}{[1]} \qquad (5)$$

where a_1 is the activity. Thus, the chemical potential is expressed as

$$\frac{\mu_1^i - \mu_1^0}{RT} = \ln a_1^i = \ln [1]^i + \ln \gamma_1^i, \qquad (6)$$

valid for both the polymer and aqueous phase, i.e., $i = P$ or W. The standard state, μ_1^0, throughout this work refers to the pure components. At equilibrium between the two phases we obtain

$$\ln \gamma_1^W - \ln \gamma_1^P = \ln \frac{[1]^P}{[1]^W} = \ln K_c$$

$$= \frac{\Delta_P^W G_c}{RT} = \ln \frac{V^R - V^{MK}}{V^P} \qquad (7)$$

where we have defined the molar free energy of transfer (using molar concentrations), $\Delta_P^W G_c$, in terms of the difference of the logarithm of the activity coefficients of the probe at infinite dilution in the two liquids. Another definition of the activity coefficient in eqn. 5 is possible using the volume fraction as the concentration variable [8,10], rendering K_φ and a free energy of transfer, $\Delta_P^W G_\varphi$, as

$$\frac{\Delta_P^W G_\varphi}{RT} \equiv \ln \frac{\varphi_1^P}{\varphi_1^W} = \ln K_\varphi$$

$$= \ln \frac{V_R - V^{MK}}{V^P} + \ln \frac{\bar{V}_1^P(\infty)}{\bar{V}_1^W(\infty)} \qquad (8)$$

where $V_1^P(\infty)$, and $V_1^W(\infty)$ are the partial molar volumes of the probe at infinite dilution in the polymer and water phase, respectively.

Eqns. 7 and 8 suffer from the drawback, however, that the free energies defined contain both information from the difference in molecular interaction that the probe experiences in the two different phases and information from the difference in molecular sizes. It is of interest to isolate the molecular interaction from other contributions to the transfer functions in order to obtain information on solute–solvent interactions. This can be accomplished using the Flory–Huggins expressions for the chemical potential where the activity coefficient is split up into a combinatorial part, stemming from the difference in molecular sizes, and an interactional or non-combinatorial, part [11]. Hence we can take advantage of this expression in order to eliminate the combinatorial contribution to the entropy of mixing molecules of different sizes. In the Flory–Huggins theory, the chemical potential is expressed as

$$\frac{\Delta\mu_1^i}{RT} = \frac{\mu_1^i - \mu_1^0}{RT} = \ln \varphi_1^i + (1 - \varphi_1^i)\left(1 - \frac{\bar{V}_1^i}{\bar{V}_i}\right)$$

$$+ \chi_1^i(1 - \varphi_1^i)^2 \qquad (9)$$

which is applied to both the polymer and aqueous phase, i.e., $i = P$ or W. In the equation, $\bar{V}_1^i$ is the partial molar volume of the probe in the solvent phase i, $\bar{V}_i$ is the molar volume of the solvent i and χ_1^i is the interaction parameter between the probe and solvent molecules. χ_1^W and χ_1^P represent the residual chemical potential in excess over the combinatorial contribution. We note in passing that $\bar{V}_P$ is strictly not defined due to the polydispersity of the polymer. However, this is not a problem when using very high-molecular mass polymers, because then the $\bar{V}_1^P/\bar{V}_P$ ratio is negligibly small, irrespective of the precise value of $\bar{V}_P$.

When equilibrium conditions prevail between the polymer and aqueous phase we are able to define a non-combinatorial free energy of transfer, $\Delta_P^W G_\varphi'$ in terms of the difference in χ_1^i parameters. In other words,

$$\frac{\Delta_P^W G_\varphi'}{RT} \equiv \chi_1^W - \chi_1^P = \ln\frac{\varphi_1^P}{\varphi_1^W} + \frac{\bar{V}_1^W(\infty)}{\bar{V}_W}$$

$$= \ln\frac{V_R - V^{MK}}{V^P} + \ln\left[\frac{\bar{V}_1^P(\infty)}{\bar{V}_1^W(\infty)}\right] + \frac{\bar{V}_1^W(\infty)}{\bar{V}_W} \tag{10}$$

Eqn. 10 is derived under the assumption of (i) equilibrium between the two phases, (ii) infinite dilute concentration of the probe and (iii) an infinitely high molecular mass of the PDMS polymer.

For the sake of clarity, we repeat that in our notation the non-combinatorial quantity denoted by $\Delta_P^W G_\varphi'$ is extracted from experimental retention volumes using a combinatorial contribution calculated according to the Flory–Huggins theory on a volume fraction basis.

The relationship between the non-combinatorial free energy of transfer, $\Delta_P^W G_\varphi'$, and the free energy of transfer, $\Delta_P^W G_\varphi$, defined by eqn. 8, is then

$$\Delta_P^W G_\varphi' = \Delta_P^W G_\varphi + \frac{\bar{V}_1^W(\infty)}{\bar{V}_W} \tag{11}$$

Pressure effects

Since HPLC operates at elevated pressures, one needs to consider the effect of pressure on the thermodynamic information obtained. This was pioneered by Locke and co-workers [4,12]. The $\mu_1^0(T, P)$ of eqn. 6 can be expressed at a standard pressure, P^*, of 1 atm as

$$\mu_1^0(T, P) = \mu_1^*(T, P^*) + (P - P^*)\bar{V}_1^0 \tag{12}$$

where $\bar{V}_1^0$ is the molar volume of the pure probe assuming that $(\partial\bar{V}_1^0/\partial P)_T = 0$. Following the development given in refs. 4 and 12, eqn. 12 can be applied to the polymer (P) and water (W) phases at equilibrium conditions, where $\Delta\mu_1^P(T, P) = \Delta\mu_1^W(T, P)$, to give

$$\frac{\Delta_P^W G(P)}{RT} = \frac{\Delta_P^W G(P^*)}{RT}$$

$$+ \frac{(P - P^*)}{RT}[\bar{V}_1^W(\infty) - \bar{V}_1^P(\infty)] \tag{13}$$

Note that the column pressure, P, is the mean pressure in the column, assuming a linear pressure gradient, as discussed in ref. 12. As eqn. 13 is applied at infinite dilution of the probe, $\bar{V}_1^0$ was replaced with $\bar{V}_1^i(\infty)$, i.e., the partial molar volume of the probe in the solvent i at infinite dilution. Eqn. 13 shows explicitly the effects operating the HPLC system above atmospheric pressure in the term $[(P - P^*)/RT][\bar{V}_1^W(\infty) - \bar{V}_1^P(\infty)]$.

Transfer of the probe to PDMS

In order to compare our chromatographic results with solubility measurements we need to fill in the missing gap, i.e., the transfer of the probe from the pure probe liquid to the PDMS polymer, illustrated below:
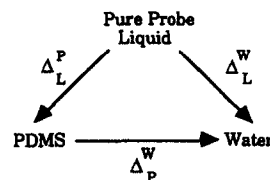
Thus $\Delta_L^W G = \Delta_L^P G + \Delta_P^W G$. The standard free energy of transfer of the probe from the pure liquid to the PDMS solution can be experimentally determined from vapour pressure or osmotic pressure measurements as well as GLC. The relative vapour pressure of the probe is related to its chemical potential according to

$$\frac{(\mu_1^P - \mu_1^0)}{RT} = \ln a_1^i = \ln\frac{p_1^P}{p_1^0}$$

$$= \ln\varphi_1^P + \left[1 - \frac{\bar{V}_1^P(\infty)}{\bar{V}_P}\right](1 - \varphi_1^P)$$

$$+ \chi_1^P(1 - \varphi_1^P)^2 \tag{14}$$

The free energy of transfer of the probe from the pure probe liquid to liquid PDMS, where $[\bar{V}_1^P(\infty)/\bar{V}_P] \approx 0$, and at the limit of infinite dilution, $\varphi_1^P \to 0$, is then

$$\frac{\Delta_L^P G_\varphi}{RT} = \ln\frac{1}{\varphi_1^P} = \chi_1^P + 1 \tag{15}$$

The corresponding non-combinatorial free energy of transfer is

$$\frac{\Delta_L^P G_\varphi'}{RT} = \chi_1^P \qquad (16)$$

It is now possible to express the free energy of transfer from the pure probe to water according to the schematic triangle above, i.e., from eqns. 8 and 15 we have

$$\frac{\Delta_L^W G_\varphi}{RT} = \ln\left(\frac{\varphi_1^P}{\varphi_1^W}\right) + \chi_1^P + 1$$

$$= \ln \frac{V^R - V^{MK}}{V^P} + \ln\left[\frac{\bar{V}_1^P(\infty)}{\bar{V}_1^W(\infty)}\right]$$

$$+ \chi_1^P + 1 + \frac{P-1}{RT}[\bar{V}_1^W(\infty) - \bar{V}_1^P(\infty)] \quad (17)$$

The corresponding non-combinatorial free energy is obtained from eqns. 10 and 16:

$$\frac{\Delta_L^W G_\varphi'}{RT} = (\chi_1^W - \chi_1^P) + \chi_1^P$$

$$= \ln\left(\frac{V^R - V^{MK}}{V^P}\right) + \frac{\bar{V}_1^W(\infty)}{\bar{V}_w} + \chi_1^P$$

$$+ \ln\left[\frac{\bar{V}_1^P(\infty)}{\bar{V}_1^W(\infty)}\right]$$

$$+ \frac{P-1}{RT}[\bar{V}_1^W(\infty) - \bar{V}_1^P(\infty)] \quad (18)$$

Solubility

One of the purposes of this work was to relate the thermodynamic quantities measured chromatographically to literature data on the solubility, in particular for the homologous series of alkylbenzenes. Solubility measurements can be considered as a transfer of the probe from the pure probe liquid, L, to water, W, and therefore we apply eqn. 9 to this system. At the solubility limit, the chemical potential of the hydrocarbon probe is equal to the chemical potential of the pure probe, provided that water has a limited solubility in the probe liquid, which is the case for hydrocarbons. The result, at the limit of infinite dilution of the probe in water, is then

$$\frac{\Delta_L^W G_\varphi}{RT} = \ln K_\varphi = -\ln[\varphi_1^W(\text{sat})]$$

$$= \left[1 - \frac{\bar{V}_1^W(\infty)}{\bar{V}_w}\right] + \chi_1^W \qquad (19)$$

where $\varphi_1^W(\text{sat})$ is the saturation limit of the probe in water expressed in volume fraction. In terms of non-combinatorial quantities, the standard free energy of transferring the probe from its own liquid to water can thus be obtained from solubility measurements, viz.,

$$\frac{\Delta_L^W G_\varphi'}{RT} = -\ln[\varphi_1^W(\text{sat})] - \left(1 - \frac{\bar{V}_1^W(\infty)}{\bar{V}_w}\right)$$

$$= \chi_1^W \qquad (20)$$

As most solubility measurements in the literature are given in mole fractions, we convert eqns. 19 and 20 to

$$-\ln x_1(\text{sat}) = \frac{\Delta_L^W G_\varphi}{RT} + \ln\left[\frac{\bar{V}_1^W(\infty)}{\bar{V}_w}\right] + 1 \qquad (21a)$$

$$-\ln x_1(\text{sat}) = \frac{\Delta_L^W G_\varphi'}{RT} + \ln\left[\frac{\bar{V}_1^W(\infty)}{\bar{V}_w}\right]$$

$$+ 1 - \frac{\bar{V}_1^W(\infty)}{\bar{V}_w} \qquad (21b)$$

It is now possible to compare the solutibility, in terms of ln $[x_1(\text{sat})]$, and the results from the chromatographic experiments. Combining eqn. 17 or 18 with eqn. 19 or 20, respectively, we obtain

$$-\ln[x_1(\text{sat})] = \ln\left[\frac{\bar{V}_1^W(\infty)}{\bar{V}_w}\right] + \chi_1^P + 1$$

$$+ \ln\left(\frac{V^R - V^{MK}}{V^P}\right) + \ln\left[\frac{\bar{V}_1^P(\infty)}{\bar{V}_1^W(\infty)}\right]$$

$$+ \frac{P-1}{RT}(\bar{V}_1^W(\infty) - \bar{V}_1^P(\infty)] \qquad (22)$$

Note that eqn. 22 is independent of the route, i.e., through $_L^W G_\varphi$ or $_L^W G_\varphi'$.

RESULTS AND DISCUSSION

Primary data

Table I shows the equilibrium partition coefficient, K_c, and the calculated non-combinatorial free energy at the experimental temperatures. For each probe, the $\Delta_L^W G_\varphi'$ data were plotted against temperature and fitted to a third-degree polynomial. The $T\Delta_L^W S_\varphi'$ data were obtained

TABLE I

PARTITION COEFFICIENT AND NON-COMBINATORIAL FREE ENERGY OF TRANSFER OF THE PROBE FROM THE PURE LIQUID TO WATER AT THE EXPERIMENTAL TEMPERATURES

Benzene			Toluene			Ethylbenzene			Propylbenzene		
T (°C)	K_c	$\Delta G'_\varphi$ (kJ/mol)	T (°C)	K_c	$\Delta G'_\varphi$ (kJ/mol)	T (°C)	K_c	$\Delta G'_\varphi$ (kJ/mol)	T (°C)	K_c	$\Delta G'_\varphi$ (kJ/mol)
0.1	89.4	22.2	2.6	297.4	27.2	2.6	911.6	31.6	1.6	3017.4	36.1
4.9	92.6	22.7	3.8	302.6	27.3	3.6	931.4	31.8	5.1	3153.0	36.7
10.0	95.0	23.2	5.5	307.2	27.5	5.5	934.3	32.0	10.1	3204.4	37.5
20.0	98.0	24.1	9.3	324.8	28.1	9.3	939.9	32.5	15.2	3331.1	38.3
25.0	97.8	24.5	9.8	311.8	28.0	9.8	949.1	32.5	20.1	3206.6	38.9
30.0	96.6	25.0	15.2	322.3	28.7	15.3	949.7	33.2	24.8	3185.0	39.5
39.3	92.9	25.6	20.1	307.2	29.1	20.1	927.8	33.7	29.9	3231.5	40.2
50.1	87.0	26.4	26.2	300.8	29.6	26.2	904.3	34.4	39.9	3056.8	41.5
60.3	80.3	27.1	35.0	285.6	30.4	35.0	850.8	35.3	50.0	2636.9	42.5
69.8	72.1	27.5	45.2	268.0	31.3	45.0	784.2	36.3	60.4	2295.6	43.5
80.1	64.4	28.1	55.0	244.6	32.0	55.0	708.5	37.2	70.2	1918.5	44.3
89.4	56.6	28.4	65.0	215.8	32.7	65.0	610.3	37.9	80.1	1570.6	45.0
			75.0	201.2	33.5	75.0	567.5	38.9	89.8	1373.9	45.9
			85.0	168.9	34.0	85.0	459.6	39.4			

from $\partial(\Delta_L^W G'_\varphi)/\partial T$ and $\Delta_L^W H'_\varphi$ was found using the fundamental equation $\Delta_L^W G'_\varphi = \Delta_L^W H'_\varphi - T\Delta_L^W S'_\varphi$. Finally, the heat capacity, $\Delta_L^W C'_{p_\varphi}$ ($= \partial \Delta_L^W H'_\varphi/\partial T$), was also calculated. The results are given in Table II for the experimental range 0–80°C; the values listed were calculated at convenient temperature intervals.

Importance of terms

It is of interest to investigate the different contributions to $\Delta_L^W G'_\varphi$ in eqn. 18. Table III shows the magnitude and sign, at 25°C, of each term, and its estimated error, as it appears on the right-hand side of eqn. 18.

The first term is equal to $RT \ln K_c$ according to eqn. 3 and varies between *ca.* 11 and 20 kJ/mol on going from benzene to propylbenzene. This term consists of the primary chromatographic data whereas all other terms were calculated from data collected from literature.

The second term, *i.e.*, $RT \ln [\bar{V}_1^W(\infty)/\bar{V}_W]$ was found to vary from 12 to 20 kJ/mol on going from benzene to propylbenzene. This is a substantial quantity and is of the same sign and size as the first term, involving the partition coeffi-

cient. Hence we conclude that $\Delta_L^W G_\varphi$ from eqn. 8, as opposed to the interactional free energy, $\Delta_L^W G'_\varphi$ from eqn. 10, *i.e.*, the free energy that is corrected for the combinatorial contribution, represents only about half of the interactional free energy. It is therefore essential to analyse the data in such a way as to take account of the combinatorial contributions arising from the size difference of the molecules if one wants the interactional, or non-combinatorial, contribution to the free energy of transfer. The $\bar{V}_1^W(\infty)/\bar{V}_W$ ratio was calculated in the following manner. First, the $\bar{V}_1^W(\infty)$ values were taken from Makhatadze and Privalov [13] for benzene and toluene in the temperature range 5–80°C. Second, the ratio $\bar{V}_1^W(\infty)/\bar{V}_1$ was calculated and found to be constant and equal to 0.913 for both benzene and toluene over the entire experimental temperature range. We therefore assume that this ratio has the same value also for ethylbenzene and propylbenzene. This factor was then multiplied by $\bar{V}_1/\bar{V}_W$ for which data are available from density measurements in the literature.

The third term in Table III, containing the χ_1^P parameter, is the non-combinatorial part of the free energy of transfer of the probe from its pure liquid to PDMS, and has previously been de-

TABLE II

NON-COMBINATORIAL THERMODYNAMIC QUANTITIES, $\Delta_L^W X'_\varphi$, FOR THE PURE PROBE LIQUID (L) TO WATER (W) TRANSFER, OBTAINED BY APPLYING FLORY–HUGGINS EXPRESSIONS ON EXPERIMENTAL HPLC DATA, *I.E.*, WITH THE USE OF EQN. 18, AND THE CONVENTIONAL THERMODYNAMIC QUANTITIES, $\Delta_L^W X_x$, OBTAINED FROM EQNS. 23 AND 24

Compound	Temperature (°C)	$\Delta G'_\varphi$ (kJ/mol)	$T\Delta S'_\varphi$ (kJ/mol)	$\Delta H'_\varphi$ (kJ/mol)	$\Delta C'_{p\varphi}$ (kJ/mol)	ΔG_x (kJ/mol)	$T\Delta S_x$ (kJ/mol)
Benzene	0	22.2	−28.9	−6.7	243	17.8	−22.0
	5	22.7	−28.2	−5.5	243	18.2	−21.2
	10	23.2	−27.5	−4.3	243	18.6	−20.3
	15	23.6	−26.7	−3.1	243	18.9	−19.5
	20	24.1	−26.0	−1.9	243	19.2	−18.6
	25	24.5	−25.2	−0.7	242	19.6	−17.7
	30	24.9	−24.4	0.5	241	19.8	−16.8
	40	25.7	−22.8	2.9	239	20.4	−14.9
	50	26.4	−21.1	5.3	237	20.8	−13.1
	60	27.0	−19.3	7.7	234	21.2	−11.2
	70	27.6	−17.6	10.0	230	21.5	−9.3
	80	28.0	−15.8	12.3	225	21.7	−7.4
Toluene	0	26.9	−32.4	−5.5	299	21.0	−23.7
	5	27.5	−31.5	−4.0	292	21.4	−22.6
	10	28.0	−30.6	−2.6	283	21.8	−21.6
	15	28.6	−29.8	−1.2	274	22.2	−20.6
	20	29.1	−28.9	0.1	265	22.5	−19.7
	25	29.5	−28.1	1.4	255	22.8	−18.7
	30	30.0	−27.3	2.7	245	23.1	−17.8
	40	30.9	−25.8	5.0	223	23.7	−16.1
	50	31.7	−24.5	7.1	199	24.2	−14.5
	60	32.4	−23.4	9.0	174	24.6	−13.1
	70	33.1	−22.5	10.6	146	25.0	−12.0
	80	33.7	−21.8	11.9	116	25.3	−11.1
Ethylbenzene	0	31.3	−36.3	−5.0	275	26.8	−33.7
	5	31.9	−35.5	−3.6	273	27.4	−32.0
	10	32.5	−34.8	−2.3	271	27.9	−30.4
	15	33.1	−34.1	−0.9	268	28.4	−28.8
	20	33.7	−33.3	0.4	266	28.9	−27.2
	25	34.3	−32.6	1.7	263	29.4	−25.6
	30	34.8	−31.8	3.0	260	29.8	−24.1
	40	35.8	−30.2	5.6	253	30.5	−21.2
	50	36.7	−28.7	8.1	245	31.1	−18.5
	60	37.6	−27.1	10.5	236	31.7	−16.0
	70	38.4	−25.6	12.8	226	32.1	−13.8
	80	39.1	−24.1	15.0	215	32.5	−11.9
Propylbenzene	0	35.8	−46.3	−10.5	463	23.8	−25.4
	5	36.6	−44.9	−8.2	453	24.3	−24.6
	10	37.4	−43.4	−6.0	443	24.7	−23.8
	15	38.2	−42.0	−3.8	432	25.1	−22.9
	20	38.9	−40.5	−1.7	420	25.5	−22.1
	25	39.6	−39.1	0.4	408	25.9	−21.2
	30	40.2	−37.8	2.4	395	26.2	−20.3
	40	41.4	−35.1	6.2	367	26.9	−18.6
	50	42.5	−32.7	9.8	337	27.4	−16.8
	60	43.4	−30.4	13.0	304	27.9	−15.0
	70	44.3	−28.5	15.8	268	28.3	−13.2
	80	45.1	−26.8	18.3	230	28.7	−11.5

TABLE III

COMPARISON OF THE MAGNITUDE AND SIGN OF THE TERMS, AT 25°C, CONTRIBUTING TO $\Delta_L^W G_*'$ IN EQN. 18

All values in kJ/mol.

Compound	$RT \ln \dfrac{V^R - V^{MK}}{V^P}$	$RT\left[\dfrac{\bar{V}_1^W(\infty)}{\bar{V}_w}\right]$	$RT\chi_1^P$	$RT \ln \dfrac{\bar{V}_1^P(\infty)}{\bar{V}_1^W(\infty)}$	$(P-1)[\bar{V}_1^W(\infty) - \bar{V}_1^P(\infty)]$
	(±0.14)	(±0.02)	(±0.02)	(±0.003)	(±0.003)
Benzene	11.44	11.19	1.90	0.090	−0.065
Toluene	14.28	13.36	1.90	0.090	−0.065
Ethylbenzene	16.99	15.38	1.90	0.090	−0.065
Propylbenzene	20.13	17.53	1.90	0.090	−0.065

termined [5,14] for benzene, toluene and ethylbenzene at two temperatures, 25 and 55°C. Although χ_1^P might be expected to increase with molecular size, it showed no discernible size dependence in this selection of probe sizes. Analysis of published data shows that $\chi = 1.9/RT$. Within the Flory–Huggins theory for polymer solutions based on a lattice model, $\chi = z\,\Delta w/kT$, where z is the coordination number and Δw is the molecular contact interaction energy. Thus, z and Δw were found to be approximately independent of temperature and probe size for all the probes in this study. Thereby the correction term, $RT\chi$, amounts to 1.90 kJ/mol, which is only ca. 2% of the free energy of transfer in eqn. 18. This is an important finding since the HPLC set-up, with the PDMS polymer coating, for the transfer from pure probe liquid to water is as such a good approximation even without taking into account the contribution from the transfer of the probe from its own liquid to PDMS. This is probably a consequence of the very hydrophobic properties of PDMS, such that the interaction between the probe molecules and the polymer is primarily due to dispersion interactions which are small and relatively independent of temperature in comparison with the probe–water interaction.

Compared with the first three terms, the last two terms are minor and serve only as very small correction terms. As for the fourth term, the ratio of $\bar{V}_1^W(\infty)$, from ref. 13, and $\bar{V}_1^P(\infty)$, determined using the Prigogine–Flory theory for non-polar polymer solutions in ref. 15, was

found to be constant, independent of probe (benzene and toluene) or temperature, and equal to 1.037. This value differs from that previously published by us [8], 1.13, which was found to be in error. The ratio was, however, still found to be constant for these two probes and were also used for ethylbenzene and propylbenzene. The correction term, $RT \ln [\bar{V}_1^P(\infty)/\bar{V}_1^W(\infty)]$ amounts to 0.090 kJ/mol, which is only ca. 0.4% of the free energy of transfer defined in eqn. 18.

We find that the last term in Table III, the pressure contribution at our HPLC operating conditions of ca. 50 atm and with the standard pressure $P^* = 1$ atm, amounts to ca. −0.065 kJ/mol. Hence this contribution to the free energy of transfer is very small, as predicted by Locke and co-workers [4,12], and amounts to ca. 0.3% of the total free energy of transfer. We note that the last two terms almost completely cancel and that the net difference is of the same order of magnitude (or less) as the error in the total free energy of transfer. The last two terms were therefore ignored in the calculation of the non-combinatorial thermodynamic transfer functions, shown in Table II and in the calculation of solubilities (see below).

Thermodynamic transfer functions

Fig. 2 shows the Van 't Hoff plot for the benzene data, i.e., where ln K_c is plotted as a function of the inverse of temperature. First we note that the function has a pronounced maxi-
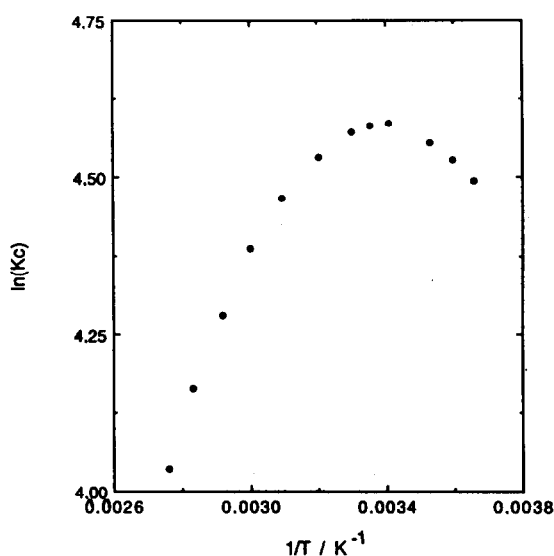
Fig. 2. Van 't Hoff plot for the partition coefficient, K_c, as obtained from the chromatographic retention of benzene.

mum corresponding to a point where the enthalpy of transfer is zero and where the solubility of the probe in water is at a minimum. We also note that the slope is not constant at any temperature, in contrast to what is normally found is reversed-phase systems with hydrocarbon-bonded silica as the stationary phase and water with organic modifier as the mobile phase. The unusual temperature behaviour in Fig. 2 has previously been successfully analysed in terms of a solute-induced solvent interaction, often described as water structuring, around the hydrophobic solute surface [8,16]. Hence we conclude that the difference in our results from those normally obtained with reversed-phase chromatography is that (i) the commonly used organic modifier affects the water in such a way that it does not structure on coming into contact with a hydrophobic surface and/or (ii) the hydrocarbon-bonded silica may not be a good reference in the sense that it may not act as a hydrophobic reference liquid for the transfer of the probe (solute) molecules [17]. We would also like to comment on the general consensus that a linear Van 't Hoff plot indicates an invariant separation mechanism [18,19]. Analysis of the curve in Fig. 2 for the four probes shows that in order to understand, or predict, the behaviour in the

figure only one mechanism need be assumed for the whole temperature range [8,16], despite the fact that $\Delta_L^W H_\varphi'$, i.e., the slope of the plot, is not constant with temperature (see Fig. 3).

We return to Table II in order to discuss the thermodynamic quantities of the probe transfer from the pure probe liquid to water. Table II reveals that the free energy of transfer is large and positive and increases only slightly with temperature, whereas the enthalpy and entropy of transfer are both negative at low temperature and more strongly temperature dependent, increasing with temperature. The enthalpy of transfer crosses zero at about room temperature, having positive values at higher temperatures. The heat capacity is typical of aqueous solutions of non-polar molecules, i.e., abnormally large and positive and decreasing with temperature [13].

Analysis of the results over small temperature intervals around room temperature is misleading, since this is where the $\Delta_L^W H_\varphi'$ is around zero. This observation forms the basis for the common misinterpretation that the hydrophobic effect is entropy driven. It can be seen from Table III that the enthalpy is in fact strongly temperature dependent and gives a large contribution to the free energy at temperatures other than around room temperature. An analysis of the results in Table III has been published previously [8] and an in-depth analysis of the hydrophobic effect will be presented elsewhere [16]. Further, we note that the data given in Table III shows an enthalpy–entropy compensation, a fact that is closely related with the creation, or destruction, of order in a system [8,20].

The values of the enthalpy and the temperature dependence, listed in Table III, compare very well with literature values [21] at 25°C, as shown in Fig. 3. The data in Fig. 3 afford an accurate estimate of the magnitude and temperature dependence of the heat of transfer. Further, the heat capacity of transfer at 25°C also compares reasonably well with literature values [13,21]: 244 vs. 238 or 225 ± 5 J/mol K for benzene, 256 vs. 305 or 236 ± 13 J/mol K for toluene, 262 vs. 318 ± 13 J/mol K for ethylbenzene and 413 vs. 391 ± 25 J/mol K for propylbenzene. The closeness in magnitude of the heat capacity data
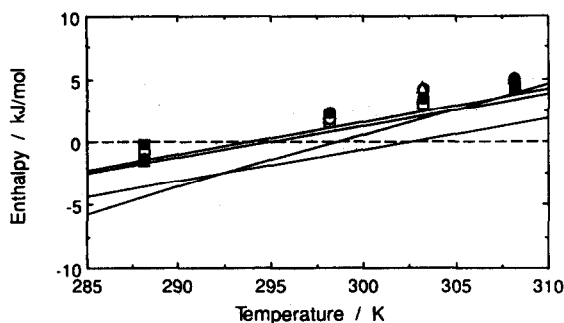
Fig. 3. Enthalpy for the transfer of probe molecules from the pure liquid to water, symbols as in Fig. 4, together with literature data from ref. 21.
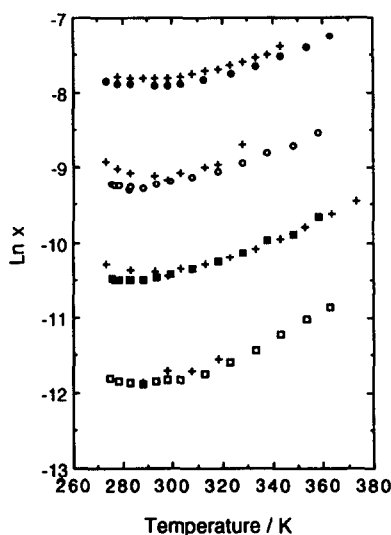

Fig. 4. Calculated values of the solubilities according to eqn. 22 as a function of the temperature. ● = Benzene; ○ = toluene; ■ = ethylbenzene; □ = propylbenzene; + = Literature data from ref. 22.

is remarkable considering that it is a second derivative with respect to temperature of the free energy, which has a rather low curvature. Hence the data clearly show that liquid–liquid chromatography is indeed a very powerful method for obtaining thermodynamic data for non-polar molecules in water. We note that the values for toluene and ethylbenzene in Table II differ from those previously published [8] for two reasons. The first is that in the previous paper we published $\Delta_L^W G_\varphi$ and not $\Delta_L^W G_\varphi'$ values as are shown here; the second is that this work involved a much more reliable analysis of the absolute amount of PDMS deposited on the column glass beads.

Solubilities

As seen in eqn. 22, it is possible to use the chromatographic data to calculate the solubilities of the probe molecules in water, or in any other suitable mobile phase. This is very interesting especially for strongly hydrophobic molecules where accurate and direct determinations are experimentally difficult owing to their extremely low solubility. Fig. 4 displays the calculated solubility, using eqn. 22, for the benzene to propylbenzene series together with literature values [22] as a function of temperature. It is apparent from Fig. 4 that the liquid–liquid chromatographic method accurately predicts both the absolute solubility and its temperature dependence for hydrocarbons in water.

We also note that in predicting the solubilities it is essential to report on what correction terms

are taken into account. For example, omitting the χ_1^P term, i.e., only studying the transfer from the stationary polymer phase to water instead of the transfer from pure probe liquid to water, will shift the predicted solubility curves by ca. 0.8 units, but the temperature dependence will not be altered. Such a shift would be dramatic if the solubilities are compared on a linear, instead of a logarithmic, scale.

We also emphasize the importance of choosing a realistic model in order to obtain the interactional free energy of transfer from solubility measurements. Unfortunately, the most common way to obtain $\Delta_L^W G$ from solubility measurements is through the equation

$$\Delta_L^W G_x = -RT \ln x_1(\text{sat}) \tag{23}$$

Using eqn. 23 implies the adoption of the regular solution model, i.e., a model where it is assumed that the solute and solvent molecular sizes are the same. The entropy is further obtained from

$$\Delta_L^W S_x = \frac{\Delta_L^W H - \Delta_L^W G_x}{T}$$

$$= \frac{\Delta_L^W H + RT \ln x_1(\text{sat})}{T} \tag{24}$$

We calculated $\Delta_L^W G_x$ from the following equation:

$$\frac{\Delta_L^W G_x}{RT} = \frac{\Delta_L^W G_\varphi'}{RT} + \ln\left[\frac{\bar{V}_1^W(\infty)}{\bar{V}_W}\right] + 1 - \frac{\bar{V}_1^W(\infty)}{\bar{V}_W}$$

(25)

which is obtained from eqns. 23 and 21b. The entropy of transfer was obtained from the first derivative of $\Delta_L^W G_x$ with respect to temperature. Values of $\Delta_L^W G_x$ and $\Delta_L^W S_x$ thus obtained are also given in Table III. We note that $\Delta_L^W S_x$ is larger than $\Delta_L^W S_\varphi'$. Further, plots of $\Delta_L^W S_x$ *versus* temperature reveal that $\Delta_L^W S_x$ is zero at temperatures around 130–160°C and becomes positive at higher temperatures. This temperature, T_s, where $\Delta_L^W S_x$ is zero, has been used in the analysis of the hydrophobic effect [23]. Such treatments should be used with caution as they are based on the assumption of equal-sized molecules and, hence, $\Delta_L^W S_x$ necessarily contains both intermolecular information and contributions from the combinatorial entropy of mixing [24]. We therefore strongly advocate the use of eqn. 20 instead of eqn. 23 in order to obtain meaningful thermodynamic information.

CONCLUSIONS

The agreement with literature data, in Fig. 4, shows that the primary retention data are of excellent quality. Liquid–liquid chromatography is indeed a method worth further exploiting in order to obtain high-quality thermodynamic and solubility data. Not only is the experimental set-up composed of readily available and relatively inexpensive equipment, it is also extremely flexible. The experimental parameters, temperature, mobile phase, stationary phase and probe molecules, can all be easily and quickly altered, affording a wide range of potential applications. This has been demonstrated using formamide as the mobile phase [25]. The success of this particular HPLC set-up lies in the balance between having a small enough thickness of the deposited polymer layer, such that it maintains equilibrium conditions, and large enough so as to ensure a liquid behaviour of the polymer, and in the use

of a non-porous support material so that the effect of probe adsorption is negligible.

The availability of a phase ratio, as a result of an independent and exact determination of the volume of polymer in the column, is the cornerstone of the method. It is through the phase ratio, which appears in the equilibrium partition coefficient, that we are able easily to calculate absolute values for the probe transfer free energy, entropy and enthalpy and the solubility in water.

Finally, we emphasize the use of non-combinatorial quantities in $\Delta_L^W G_\varphi'$ and $\Delta_L^W S_\varphi'$ instead of the commonly accepted $\Delta_L^W G_x$ and $\Delta_L^W S_x$ quantities. This treatment gives meaningful interactional parts of the free energy that can be used in further analysis of the hydrophobic effect [16].

SYMBOLS

1	Probe
a_1^i	Activity of the probe in solvent phase i
$[1]^i$	Molar concentration of the probe in solvent i
A^P	Interfacial area of the polymer stationary phase
i	Solvent phase
K_c	Equilibrium partition coefficient on a molar concentration basis
K_φ	Equilibrium partition coefficient on a volume fraction basis
K_{ads}	Adsorption partition coefficient
k'	Capacity factor
MK	Unretained marker for the column dead volume
n_1^i	Moles of probe in solvent phase i
P	Polymer (PDMS) stationary phase
P	Pressure
P^*	Standard pressure = 1 atm
R	Retained probe
V^i	Volume of the solvent phase
$\bar{V}_1$, $\bar{V}_i$	Molar volume of the probe and solvent phase, respectively
$\bar{V}_1^i$	Partial molar volume of probe in solvent phase i
$\bar{V}_1^i(\infty)$	Partial molar volume of the probe at infinite dilution in phase i
W	Water mobile phase

χ_1^i Flory–Huggins interaction parameter of the probe in solvent phase i

$\Delta_P^W X$ Transfer quantity, where $X = G = $ free energy, $H = $ enthalpy, $S = $ entropy, $C_p = $ heat capacity at constant pressure

$\Delta_P^W X_\varphi'$ Flory–Huggins non-combinatorial transfer quantities, where X is as above

$\Delta_P^W X_x$ Regular solution, mole fraction quantities, where X is as above

φ_1^i Volume fraction of the probe in solvent phase i

γ_1^i Activity coefficient of the probe in solvent phase i

ϕ Phase ratio

μ_1^i Chemical potential of the probe in solvent phase i

μ_1^0 Chemical potential of the pure probe

$x_1(\text{sat})$ Solubility limit of the probe, expressed in mole fraction

REFERENCES

1 D.C. Locke, *Adv. Chromatogr.*, 14 (1976) 87.
2 Cs. Horváth and W.R. Melander, in E. Heftmann (Editor), *Chromatography: Fundamentals and Applications of Chromatographic and Electrophoretic Methods*, Part A, Elsevier, Amsterdam, 1983, Ch. 3, p. A27.
3 P. Alessi, *Cron. Chim.*, 62 (1980) 3.
4 D.C. Locke, *Adv. Chromatogr.*, 8 (1969) 47.
5 D.R. Lloyd, T.C. Ward, H.P. Schreiber and C.C. Tizana, *Inverse Gas Chromatography—Characterization of Polymers and Other Materials* (*ACS Symposium Series*, No. 391), American Chemical Society, Washington, DC, 1989.
6 W.R. Summers, Y.B. Tewari and H.P. Schreiber, *Macromolecules*, 5 (1972) 12.
7 F. Franks, in F. Franks (Editor), *Water—A Comprehensive Treatise*, Vol. 4, Plenum Press, New York, 1975, p. 6.
8 R. Silveston and B. Kronberg, *J. Phys. Chem.*, 93 (1989) 6241.
9 J.Å. Jönsson, *Chromatographic Theory and Basic Principles*, Marcel Dekker, New York, 1987, p. 6.
10 D. Patterson, Y.B. Tewari, H.P. Schreiber and J.E. Guillet, *Macromolecules*, 4 (1971) 356.
11 L.R. DeYoung and K.A. Dill, *J. Phys. Chem.*, 94 (1990) 801.
12 D.C. Locke and D.E. Martire, *Anal. Chem.*, 39 (1967) 921.
13 G.I. Makhatadze and P.L. Privalov, *J. Chem. Thermodyn.*, 20 (1988) 405.
14 M. Galin, *Macromolecules*, 10 (1977) 1239.
15 R.S. Chahal, W.P. Kao and D. Patterson, *J. Chem. Soc., Faraday Trans. 1*, 69 (1973) 1834.
16 M. Costas, B. Kronberg and R. Silveston, submitted for publication.
17 K.A. Dill, *J. Phys. Chem.*, 91 (1987) 1980.
18 W. Melander, D.E. Campbell and Cs. Horváth, *J. Chromatogr.*, 158 (1978) 215.
19 R.J. Laub and S.J. Madden, *J. Liq. Chromatogr.*, 8 (1985) 187.
20 D. Patterson and M. Barbe, *J. Phys. Chem.*, 80 (1976) 2345.
21 S.J. Gill, N.F. Nichols and I. Wadsö, *J. Chem. Thermodyn.*, 8 (1976) 445.
22 D.G. Shaw (Editor), *IUPAC Solubility Data Series*, Analytical Chemistry Division Commission on Solubility Data, Pergamon Press, Oxford, 1989, Vol. 37, pp. 63–183 and 369–442, and Vol. 38, pp. 59–89 and 201–210.
23 P.L. Privalov and S.J. Gill, *Adv. Protein Chem.*, 39 (1988) 191.
24 B. Kronberg, M. Costas and R. Silveston, in preparation.
25 M. Sjöberg, R. Silveston and B. Kronberg, *Langmuir*, 9 (1993) 973.