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Calorimetry and liquid chromatography: determination of enthalpies of transfer from mobile phase to stationary phase

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Abstract

The enthalpies of solute transfer from an HPLC mobile phase to the stationary phase were directly determined by flow microcalorimetry, i.e., without preparation of van’t Hoff plots based on the temperature dependence of chromatographic retention data. Silica gel and hexane or binary mixtures of hexane and ethyl acetate were employed as the stationary phase and the mobile phases, and a homologous series of phthalates (dimethyl, diethyl, dipropyl, dibutyl, dipentyl, dioctyl and diphenyl phthalates) as the solutes. The flow system was contained with an isothermal microcalorimeter, set at 298 K, and included a reaction vessel (4 ml) into which a cylindrical cartridge (0.6 ml) was fixed. The phthalates dissolved in the solvent phases, equivalent to the mobile phases in the HPLC system, were flowed through the cartridge which contained silica gel and the generated power was recorded. With few exceptions negative values for the enthalpies of solute transfer were found; the values increased with increasing length of solute alkyl chain and with the content of ethyl acetate in the solvent phase. The enthalpies of solute transfer were also determined by a van’t Hoff method and no discrepancy between the calorimetric and chromatographically based values was observed.

Keywords: Enthalpies; Solute transfer; HPLC; Flow microcalorimetry; Phthalates

1. Introduction

Analyzing the retention data derived from high performance liquid chromatography (HPLC) with thermodynamic considerations is of importance in the estimation of retention mechanisms for analytes. Chromatographic retention depends upon the free energy (ΔG°) of the transfer process of a solute between the mobile phase and the stationary phase. The following expression [1,2] relates the chromatographic parameter, the capacity factor, k', to ΔG°, the enthalpy (ΔH°) and entropy (ΔS°) of transfer from the mobile phase to the stationary phase

$$\ln k' = -\frac{\Delta G^\circ}{RT} + \ln\phi = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln\phi$$

where R is the gas constant, T the absolute temperature and ϕ the phase ratio of the column, i.e., the volume of the stationary phase to that of the mobile phase. Linear plots of $\ln k'$ versus $1/T$, the van’t Hoff plot, indicate that the enthalpy and entropy are constant and the mechanism of the retention process is unchanged over the experimental temperature range. This provides a convenient way of calculating the thermodynamic constants for a chromatographic system provided that the column phase ratio is known; ΔH° can be evaluated from the slope and ΔS° from the intercept of the regression line.

On the basis of this theory, thermodynamic properties for solute transfer between mobile phases and stationary phases in reversed-phase HPLC have been investigated by measuring solute retention, at various different temperatures, for some homologous series of compounds, e.g., nonionic surfactants [3], fatty acids [4], alkylbenzenes [5], peptides [6] and low-molar-mass polystyrenes and polyesters [7]. The temperature dependence of chiral discrimination has also been investigated by normal- and reversed-phase HPLC [8–12]; these data were used to probe understanding of separation mechanisms.

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In the work reported in this paper, we determined the enthalpy of solute transfer from the mobile phase to the stationary phase in HPLC, for an homologous series of phthalates, by a flow microcalorimetric approach, i.e., direct determination without preparation of van't Hoff plots. This study was designed to explore whether the enthalpy determined indirectly by measuring the temperature dependence of the chromatographic retention data was consistent with that determined directly by calorimetry or not. There is an extensive literature (see for example [13]) on flow microcalorimetric studies of adsorption although there appears to be little related to chromatographic systems. In addition here has been concern expressed in the literature [14–19] about the essential conformity of microcalorimetric and van't Hoff derived enthalpy data.

Silica gel and hexane or binary mixtures of hexane and ethyl acetate were employed as the stationary phase and the mobile phase. In the calorimetric system silica gel with large particle size (250–500 μm), usually used for open column chromatography, was chosen because of the filter size limitation in the flow through cartridge. The resulting enthalpies were compared with those derived from a van't Hoff plots based study which were obtained from the separately performed HPLC experiments using a commercially available analytical column packed with silica gel (5 μm).

2. Experimental

2.1. Materials

Silica gel (pore size 22 Å, surface area 726–764 m^2/g), particle size ranging between 250 and 500 μm, used in the flow microcalorimetric studies was obtained from Sigma-Aldrich and used as supplied. Dimethyl, diethyl, dipropyl, dibutyl and diphenyl phthalate (more than 99%) were obtained from Sigma-Aldrich, and dipentyl (more than 99%) and dioctyl phthalate (more than 98%) from Fluka. 1,3,5-Tri-tert-butylbenzene was also obtained from Fluka. Hexane (water $<$ 0.005%) and ethyl acetate (water $<$ 0.05%), used as the solvent phases for flow microcalorimetry and as the mobile phases for HPLC, were of HPLC grade and obtained from Fluka; the same batches of solvents were used throughout the experiments reported. Hexane-ethyl acetate solvent systems (0.5, 1.0 and 2.0% in ethyl acetate) were prepared and used to explore the consequences of polarity/solvation effects on the study systems.

2.2. Flow microcalorimetry

A 2277 Thermal Activity Monitor (Thermometric AB) equipped with a 2250 perfusion unit was used as the flow microcalorimetric system. Silica gel (0.1–0.4 g) was placed inside a small cylindrical cartridge (0.6 ml) fitted with caps of 200 μm mesh at the both ends, and this was fixed within a 4 ml stainless reaction vessel. The solvent phases, hexane or binary mixtures of hexane and ethyl acetate, and the sample solutions prepared by dissolving the phthalates [0.01 mol/l, except for phenyl phthalate (0.005 mol/l) with low solubility] into the corresponding solvent phases were flowed through the cartridge containing the silica gel by an HPLC pump (Jasco, 880-PU). As the reference a 4 ml stainless vessel containing 4 ml of the corresponding solvent phase was used. The whole system was housed in a constant temperature room (21 ± 0.1 °C) and was operated at 298 K. A solvent container was thermostated at 298 K in a water bath by applying a heating thermostat (Coplay, ERWEKA E). Prior to the measurement, the system was conditioned by flowing the solvent phase until a stable baseline signal was obtained (>15 h); this procedure was always followed when new silica gel was packed into the cartridge. The measurement was started by replacing the solvent phase used for conditioning, flowing through the cartridge, by the sample solution. The power signal (ϕ, in Watts) was recorded every 15 s using the dedicated Digitam (Thermometric AB) software. Data analysis was performed using a graphics fitting program, ORIGIN (Microcal Software). At the end of the microcalorimetric experiment the silica gel to which phthalates were adsorbed was taken out of the cartridge, and the phthalates extracted with three different portions of 20 ml of ethyl acetate (ultrasonic bath; 100 W for 30 min each). The extracts were collected, filtered through a membrane filter (0.45 μm) and the filtrate was lyophilized. The residue was dissolved in 50 ml of a mixture of hexane and ethyl acetate (98:2, vol.) to be subjected to quantitative determination of solute by the HPLC procedure described below.

2.3. High performance liquid chromatography

Chromatographic measurements were carried out using a metering pump (Milton Roy, consta Metric III) and a variable UV detector (Varian, 2550) set at 254 nm. The analytical column (150 mm × 4.6 mm ID) packed with silica gel (5 μm, pore size 120 Å, surface area 170 m^2/g), Hypersil, was obtained from Thermo Hypersil-Keystone. The column and mobile phase container were thermostated in a water bath by a combination of a cooling (Neslab, FTC-350) and a heating thermostat (Grant, VF). The same solvents used as the solvent phases in the flow calorimetric experiments, i.e., hexane or binary mixtures of hexane and ethyl acetate, were used as the mobile phases. Injection samples were dissolved in the mobile phase at a concentration of 0.1 mg/ml and 5 μl portions of them were injected through a six-port sample injector (Rheodyne, 7125) fitted with a 5 μl sample loop. The flow rate of the mobile phases was 2.0 ml/min. Chromatograms were acquired using an analog to digital converter (Pico Technology, ADC-16). The capacity factor, k', was calculated using the equation, $k' = t_R/t_0 - 1$, where t_R is the retention time for the analyte and t_0 the retention time for an unretained compound; in this study t_0 was obtained from injection of 1,3,5-tri-tert-butylbenzene. For the quantitative determination of phthalates adsorbed onto silica gel in the calorimetric experiments, HPLC was performed, at 298 K, using a mixture of hexane and ethyl acetate (98:2, vol.) as the mobile phase.

3. Results and discussion

3.1. Optimization of flow microcalorimetric conditions

Flow rate is a pivotal factor for flow microcalorimetry because it influences not only the total measurement time but also the heat exchange efficiency; a fast flow rate is favourable for a quick power response, however, it is undesirable for efficient heat exchange in the reaction vessel. Fig. 1 shows the power–time curves obtained by flowing a hexane solution of diethyl phthalate (0.01 mol/l) into the cartridge which contains 0.1 g of silica gel (250–500 μm, pore size 22 Å, surface area 726–764 m^2/g), at 298 K, at various flow rates between 0.10 and 0.30 ml/min. The shapes of the power–time peaks became sharper as the flow rate increased; the peak response returned to the base line value within 2 h at a flow rate of more than 0.25 ml/min. It was clear that the observed power was generated by adsorption of diethyl phthalate onto the silica gel, i.e., transfer of diethyl phthalate from the hexane phase to the silica gel phase. The peak area, corresponding to the enthalpy of solute transfer, remained unchanged [-4451 ± 76 mJ (mean ± S.D., $n = 5$)] over the investigated flow rates. Note that this is, therefore, an exothermic process. The enthalpies generated by blank measurements which were performed using an empty cartridge for each phthalate were very small and hence have been neglected. The flow rate was, therefore, set at 0.25 ml/min throughout the present study. However, the enthalpy of solute transfer decreased on flowing binary mixtures of hexane with a small content (between 0.5 and 2.0%) of ethyl acetate (see next section). In order to obtain measurable output (Joules) for accurate determination even on using mixtures of hexane and ethyl acetate, the amount of silica gel used in the present study was varied from 0.1 to 0.4 g (maximum amount for the cartridge used).

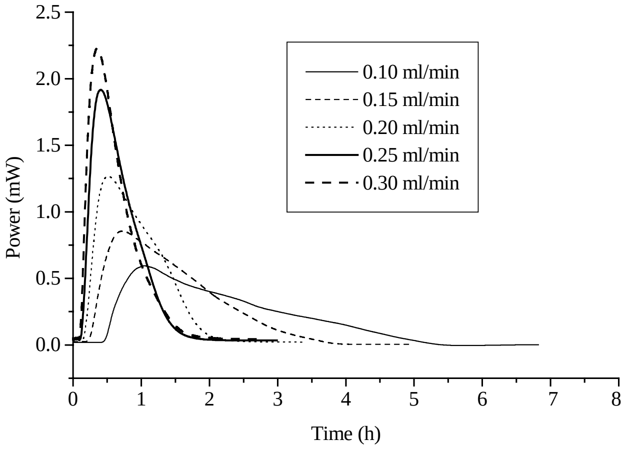

Fig. 1. Power–time curves for solute transfer from a hexane phase to the silica gel phase for diethyl phthalate at various flow rates (298 K).

3.2. Measurement of enthalpy of solute transfer by flow microcalorimetry

Table 1 summarizes the amount of silica gel placed in the cartridge, that of the phthalates adsorbed onto the silica gel and the total and molar enthalpies of solute transfer, from each solvent phase to the silica gel phase, for dimethyl, diethyl, dipropyl, dibutyl, dipentyl, dioctyl and diphenyl phthalates. The molar enthalpies, calculated by dividing the total enthalpies by the molar amounts of phthalates adsorbed onto the silica gel, yield negative (exothermic) values except for dipentyl phthalate in a solvent phase of 2.0% ethyl acetate and dioctyl phthalate in that of 1.0 and 2.0% ethyl acetate. In the hexane phase (0% ethyl acetate), the values of the molar enthalpy decrease as the length of the phthalate alkyl chain increases, however, the opposite tendency was observed for all the binary solvent phases. The values also increase as the content of ethyl acetate in the binary solvent phase is increased. Phenyl phthalate showed a lower molar enthalpy than any other phthalate in all solvent phases. The obtained values were converted into per gram of silica gel; the number of moles of phthalates decreased and the enthalpies of the solute transfer increased with increasing the molecular size of phthalates (Table 2).

Fig. 2 shows a plot of the determined enthalpy change values for interaction of the solutes with silica gel as a function of alkyl chain length. Strikingly the data showed a liner relationship for dimethyl = 1 to dibutyl phthalate = 4, but of opposite trends, in hexane and the mixed solvents. The data for dipentyl = 5 and dioctyl phthalate = 8 suggests that the enthalpies of interaction may plateau. This observation of a change in behavior at around carbon number = 4–6 has been

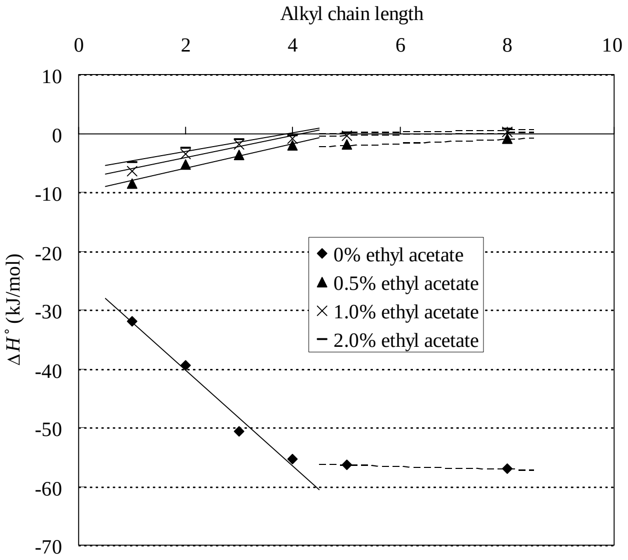

Fig. 2. Enthalpies of solute transfer as a function of alky chain length.

Table 1
Amount of the adsorbed phthalates and the enthalpies of the solute transfer, determined by calorimetry, from the study solvent phases to the silica gel phase

Ethyl acetate (vol.%)	Phthalate	Silica gel (mg)	Adsorbed phthalate (mg)	Adsorption	
				q (mJ)	ΔH° (kJ/mol)
0	C1	100	28.9	−4767.0	−32.00
	C2	100	25.1	−4445.3	−39.42
	C3	100	18.8	−3810.7	−50.73
	C4	100	18.1	−3599.0	−55.32
	C5	100	17.7	−3258.5	−56.31
	C8	100	17.3	−2523.5	−57.04
	Phenyl	100	19.2	−3592.4	−59.62
0.5	C1	200	49.7	−2164.9	−8.47
	C2	200	40.9	−967.7	−5.26
	C3	400	60.4	−881.4	−3.66
	C4	400	52.8	−388.3	−2.05
	C5	400	40.8	−260.1	−1.95
	C8	400	35.0	−83.5	−0.93
	Phenyl	400	64.5	−1751.6	−8.64
1.0	C1	300	56.6	−1870.2	−6.42
	C2	300	42.1	−665.4	−3.51
	C3	400	45.3	−331.1	−1.83
	C4	400	41.0	−121.8	−0.83
	C5	400	31.0	−33.0	−0.33
	C8	400	27.7	12.1	0.17
	Phenyl	400	55.3	−1209.8	−6.96
2.0	C1	400	61.9	−1606.6	−5.04
	C2	400	41.1	−481.2	−2.60
	C3	400	29.2	−115.2	−0.99
	C4	400	21.1	−25.4	−0.34
	C5	400	17.5	8.9	0.16
	C8	400	10.5	18.2	0.68
	Phenyl	400	34.2	−809.0	−7.52

Table 2
Amount of the adsorbed phthalates and the enthalpies of the solute transfer, converted into per gram of silica gel

Ethyl acetate (vol.%)	Phthalate	Adsorbed phthalate (mmol/g)	Enthalpy (mJ/g)
0	C1	1.490	−47.670
	C2	1.128	−44.453
	C3	0.751	−38.107
	C4	0.651	−35.990
	C5	0.579	−32.585
	C8	0.442	−25.235
	Phenyl	0.603	−35.924
0.5	C1	1.279	−10.824
	C2	0.920	−4.839
	C3	0.603	−2.204
	C4	0.474	−0.971
	C5	0.333	−0.650
	C8	0.224	−0.209
	Phenyl	0.507	−4.379
1.0	C1	0.972	−6.234
	C2	0.631	−2.218
	C3	0.453	−0.828
	C4	0.369	−0.304
	C5	0.253	−0.083
	C8	0.177	0.030
	Phenyl	0.435	−3.024
2.0	C1	0.797	−4.016
	C2	0.463	−1.203
	C3	0.292	−0.288
	C4	0.190	−0.064
	C5	0.143	0.022
	C8	0.067	0.045
	Phenyl	0.269	−2.023

Table 3
Comparison of desorption enthalpies with the adsorption enthalpies

Ethyl acetate (vol.%)	Phthalate	Adsorption ΔH° (mJ)[a]	Desorption ΔH° (mJ)	Recovery (%)
0	C1	−4760.2	−2256.3	47.4
	C2	−4442.9	−1986.0	44.7
	C3	−3806.0	−1674.6	44.0
	C4	−3610.3	−1527.2	42.3
	C5	−3351.1	−1360.5	40.6
	C8	−2523.4	−979.1	38.8
	Phenyl	−3599.8	−1284.4	35.7
0.5	C1	−2170.2	−2146.3	98.9
	C2	−952.8	−966.9	101.5
	C3	−888.3	−884.8	99.6
	C4	−389.6	−386.9	99.3
	C5	−256.9	−257.0	100.0
	C8	−82.4	−85.3	103.5
	Phenyl	−1744.9	−1740.0	99.7
1.0	C1	−1867.5	−1837.6	98.4
	C2	−667.7	−669.0	100.2
	C3	−330.4	−328.5	99.4
	C4	−119.8	−118.9	99.2
	C5	−33.4	−31.9	95.5
	C8	11.7	12.2	104.7
	Phenyl	−1200.9	−1202.1	100.1
2.0	C1	−1599.6	−1586.8	99.2
	C2	−479.9	−486.6	101.4
	C3	−115.3	−113.7	98.6
	C4	−23.9	−24.1	100.8
	C5	9.7	8.9	91.8
	C8	16.9	16.3	96.3
	Phenyl	−813.3	−818.2	100.6

[a] Difference in the adsorption enthalpies listed in Tables 1 and 2 are $<0.5\%$ for values of adsorption <-1000 mJ and range to $<10\%$ for adsorption enthalpies around 0 mJ. This reflects the error association with determination of small numbers of mJ for the adsorption process.

noted before [20] and is related to the freer rotation about C–C bonds which are more than four methylene groups distant from the "core" group—here the phthalate moiety. It is notable that changing the polarity/solvation characteristics of the mobile phase inverts the $\Delta(\Delta H^\circ)$ sign and values.

In order to determine the desorption enthalpy, i.e., enthalpy of solute transfer from the silica gel phase back to the solvent phase, the sample solution flowing through the cartridge was replaced, in another series of experiments, by the corresponding solvent phase after the measurement of the adsorption enthalpies. That is, the flow system was changed as follows; solvent phase (conditioning) → sample solution (adsorption) → solvent phase (desorption). In the hexane solvent phase (0% ethyl acetate), the power–time curves gave very broad endothermic peaks and the resulting enthalpies were less than 50% of the original adsorption enthalpies for each phthalate (Table 3). This suggests that solute transfer between a hexane phase and the silica gel phase is an irreversible process. It could therefore be presumed that the irreversible adsorption process would contribute to the significantly negative values for the enthalpies of transfer found for the hexane phase (see Tables 1 and 2). However, for the binary solvent phases at any mixing ratio, the desorption enthalpies agree very closely with the original adsorption enthalpies for each phthalate (Table 3). This result is based on the assumption that the enthalpy of desorption is equal but opposite in sign, to that of adsorption. No attempt was made to quantify the extent of desorption as the principal purpose of this work was to explore direct calorimetric determination of the enthalpies of adsorption. These data suggest that, in hexane, some significant solute association occurs with the silica gel (chemisorption) whereas in the mixed solvent systems the associative forces are more physisorptive in character (see next section).

3.3. *Comparison with chromatographic data*

The enthalpies of solute transfer were also determined by HPLC performed at five temperatures of 283, 288, 293, 298 and 303 K. In this experiment a commercially available analytical column packed with silica gel (5 μm, pore size 120 Å, surface area 170 m^2/g) was used. Linear van't Hoff behavior was observed in all cases (Fig. 3), suggesting that no changes in the transfer mechanism occurred over the investigated temperature range. The enthalpies calculated from the slopes of the resultant van't Hoff plots are listed, along with the correlation coefficients for the linear fit, in Table 4. For hexane (0% of ethyl acetate) as the mobile phase, chromatographic data could not be obtained because the affinity of each phthalate toward the stationary phase was so strong that none of them were eluted within 3 h.

Fig. 4 shows a graphical comparison of enthalpies of solute transfer determined by calorimetry and HPLC. No

Table 4
Summary of the enthalpies of the solute transfer, determined by van't Hoff method, from the solvent phases to the silica gel phase

Ethyl acetate (vol.%)	Phthalate	r^2[a]	ΔH° (kJ/mol)
0.5	C1	0.9940	−6.27
	C2	0.9954	−4.56
	C3	0.9935	−3.00
	C4	0.9966	−2.46
	C5	0.9823	−2.10
	C8	0.9868	−1.61
	Phenyl	0.9975	−6.72
1.0	C1	0.9941	−4.40
	C2	0.9980	−2.69
	C3	0.9951	−1.17
	C4	0.9942	−0.50
	C5	0.9917	−0.23
	C8	0.9832	0.23
	Phenyl	0.9977	−6.36
2.0	C1	0.9973	−4.35
	C2	0.9909	−2.51
	C3	0.9988	−1.13
	C4	0.9964	−0.41
	C5	0.9976	−0.09
	C8	0.9856	0.14
	Phenyl	0.9990	−7.04

[a] r^2 value for the linear fit of the van't Hoff plot.

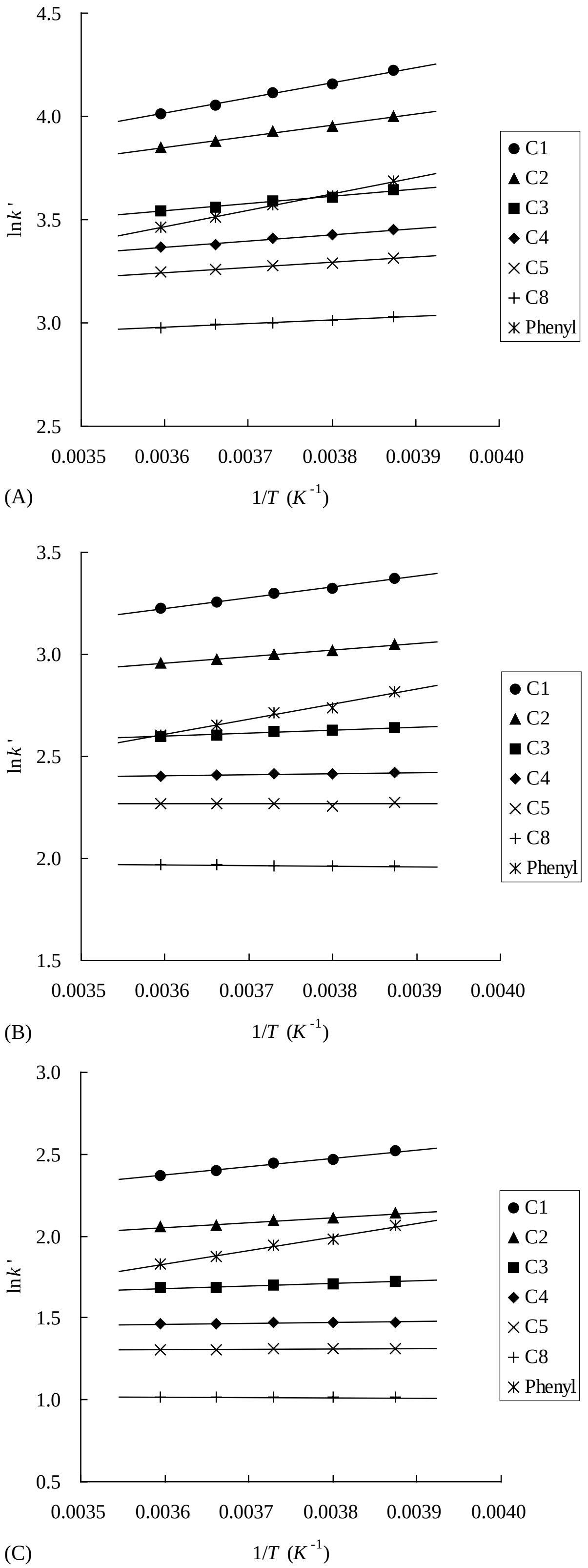

Fig. 3. van't Hoff plots for a homologous series of phthalates. (A) 0.5% ethyl acetate; (B) 1.0% ethyl acetate; (C) 2.0% ethyl acetate.

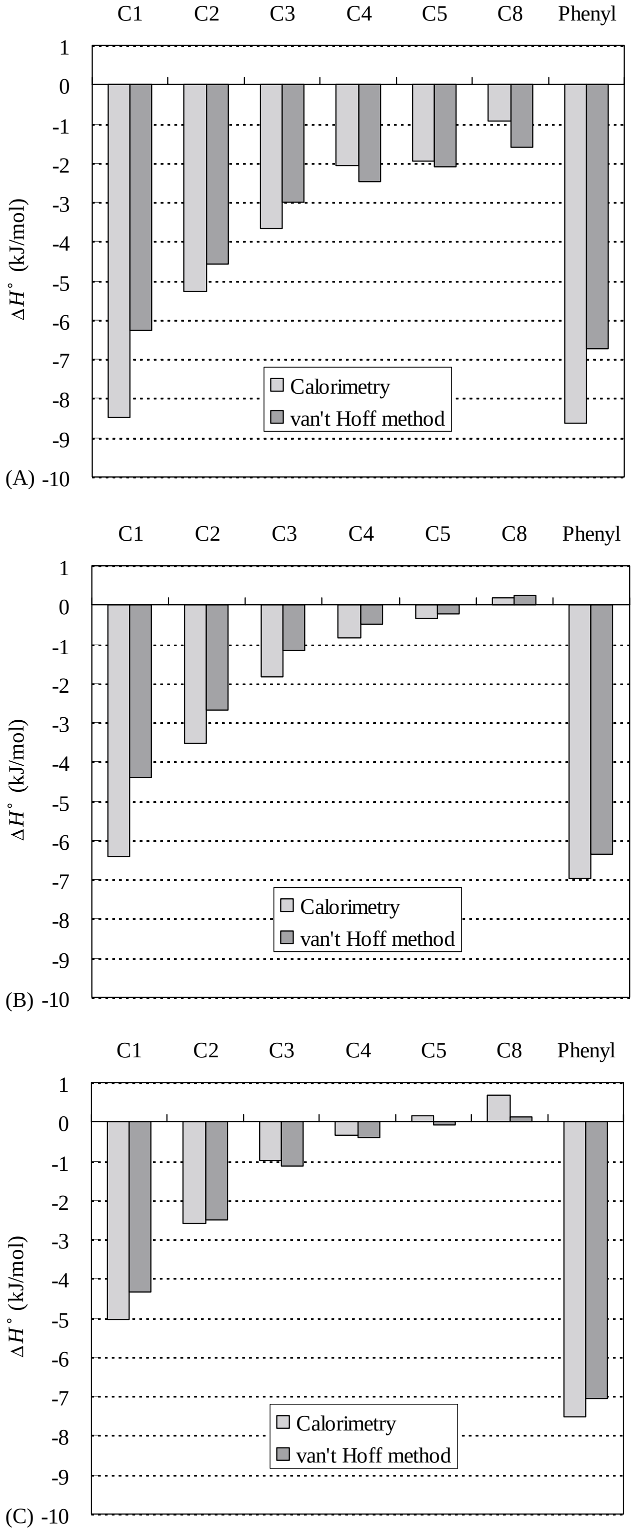

Fig. 4. Comparison of the enthalpies of solute transfer determined by the van't Hoff method with those determined by microcalorimetry: (A) 0.5% ethyl acetate, (B) 1.0% ethyl acetate, (C) 2.0% ethyl acetate.

significant discrepancies were observed between these two methods for the studied reaction system. Although the particle sizes, pore size and surface area of the silica gel used in the microcalorimetric and chromatographic experiments were different, it can be presumed that the difference in adsorption mechanism on surface active sites, i.e. silanol groups, responsible for the adsorption of the phthalates is negligibly small. From the results described above, the enthalpy of solute transfer calculated from van't Hoff plots could be successfully supported by the microcalorimetric approach.

4. Conclusion

In the past few years the equivalence of reaction enthalpies determined directly by titration calorimetry and indirectly by measuring the temperature dependence of the equilibrium constant, i.e., the van't Hoff method has been called into question [14–18]. However, Horn et al. [19] concluded that when the experimental setup and analysis are correctly performed, no statistically significant discrepancies between the methods exist. In the present study the enthalpies of the solute transfer in HPLC were determined directly by flow calorimetry and indirectly by measuring the temperature dependence of the values of k' in the chromatography. The consistency of the values of enthalpy determined by the both methods supported the views of Horn et al. [19]. We believe these results are a partial experimental justification for the generally used procedure, based not on calorimetric approach but chromatographic retention data, for the estimation of enthalpy of solute transfer in HPLC. These data and their sensitivity to mobile phase solvent composition suggest that more detailed and direct study of enthalpies of adsorption may be useful in understanding separation processes. In particular separation of chiral compounds could be explored. It is possible that the conclusions noted above represent an outcome only for some simple systems where the adsorption phenomena are dominated by direct solute–solid interactions. They may, possibly, not hold for all systems. However, the procedures described here allow exploration of a range of behaviors that may be shown by these systems.

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