Thermodynamic Properties of the Solute Transfer from the Aqueous Acetonitrile Mobile Phase to the Stationary Phase Monitored by HPLC

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High-performance liquid chromatography is suitable for getting thermodynamic information about solute-solvent interactions. We used a squalane impregnated C_{18} phase as a presumably bulk-like stationary phase to secure a simple partition mechanism for solute retention in reversed phase liquid chromatographic system. We measured retention data of some selected solutes (benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, phenol, benzylalcohol, phenethylalcohol, benzylacetone, acetophenone, benzonitrile, benzylcyanide) at 25, 30, 35, 40, 45, and 50 °C in 30/70, 40/60, 50/50, 60/40 and 70/30 (v/v%) acetonitrile/water eluents. The van't Hoff plots were nicely linear, thus we calculated dependable thermodynamic values such as enthalpies and entropies of solute transfer from the mobile phase to the stationary phase based on more than four retention measurements on different days (or weeks). We found that the cavity formation effect was the major factor in solute distribution between the mobile and stationary phases in the system studied here. Our data were compared with some relevant literature data.

Introduction

Reversed phase liquid chromatography (RPLC) has been extensively used in separation science.[1] The retention mechanism is still a topic of controversy, but the partition mechanism seems to be more supported than the adsorption mechanism.[2-6]

There have been numerous studies of the temperature effect on solute retention in reversed phase liquid chromatography. The linear van't Hoff plots were observed in the typical RPLC system.[7-11] Nonlinear van't Hoff plots were also observed in some studies of temperature effects on solute retention in RPLC.[12-20] Cole and Dorsey[21] clearly showed that the phase transition of a stationary phase occurs at 20-30 °C when the ligand density is larger than 3.0 μmol/m^2 and that the phase transition temperature gets higher as the ligand density increases. The studies of temperature effects on solute retention in RPLC tend to expand the territory to a variety of systems.[22-31]

Most of such studies were, however, related to rather qualitative discussion such as changes of physical properties of the stationary phase on temperature variation, increasing or decreasing trends of the magnitudes of the thermodynamic quantities for a group of solutes, and their comparison among different stationary or mobile phases.

In the previous study,[32] we prepared a squalane impregnated C18 phase as a presumably bulk stationary phase, and obtained quantitative thermodynamic properties for the solute transfer from the aqueous methanol mobile phase to the squalane impregnated C18 stationary phase using five solutes (benzene, toluene, ethylbenzene, phenol, and acetophenone).

In this study, we obtained quantitative thermodynamic properties for solute transfer from the aqueous acetonitrile mobile phase to the squalane impregnated C18 phase using twelve solutes.

Experimental Section

Acetonitrile and water were purchased from Fisher (Pittsburg, PA, USA) and used without further purification. Benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, phenol, benzylalcohol, phenethylalcohol, benzylacetone, acetophenone, benzonitrile and benzylcynide were purchased from Aldrich (Milwaukee, IL, USA) and used as received. The chromatographic system we used was a Shimadzu (Tokyo, Japan) HPLC system composed of a LC-10AD, a SCL-10A system controller, a SIL-10A autoinjector, CTO-10AC column oven, a SPD-10A UV/VIS detector set at 254 nm and a Chromatopac C-R7A data system.

The column (4.6 mm I.D. × 250 mm) was home made and was packed with a squalane impregnated C18 stationary phase. The procedure of making the packing material and determining the phase ratio(ϕ) was reported in the previous study.[32]

The mobile phases used were acetonitrile/water mixtures (30/70, 40/60, 50/50, 60/40 and 70/30 v/v%) and the flow rate was fixed at 1.0 mL/min throughout. The temperature range was 25-50 °C. Sample solutions were prepared by dissolving the solutes in methanol. We used KNO_3 as a void volume marker. KNO_3 was dissolved in water and injected alone before and after the sample injection. The column was placed in the column oven and its temperature was controlled with an accuracy of ±0.1 °C. The solvent bottle and the transfer tubing (1 m, 1 mm I.D) between the pump and the injector were placed in the column oven, too. So the mobile phase was preheated before entering the pump.

The capacity factor data based on more than four indepen-

dent measurements on different weeks were used to calculate the thermodynamic properties of solute transfer.

Results and Discussion

As mentioned before, more than four independent measurements were made on different weeks for given mobile phases and temperatures. The data were handled as follows. First, from the van't Hoff plots (ln k' *vs.* 1/T) of each run, the enthalpy (ΔH^0) and entropy (ΔS^0) of solute transfer from the mobile to the stationary phase were obtained. Van't Hoff plots for a variety of solutes are all linear and the regression correlation coefficients are better than 0.998 in all cases.

The thermodynamic relationship between the capacity factor (k') and temperature has been well known.

$$\ln k' = -\Delta H^0/RT + \Delta S^0/R + \ln \phi$$

In the above equation, ϕ is the phase ratio. Typical examples of van't Hoff plots are shown in Figures 1 and 2. The averages and standard deviations of the solute transfer enthalpies and entropies based on more than four independent measurements are assembled in Tables 1 and 2. The standard deviations of the solute transfer enthalpies and entropies are mostly better than 5% and 15% for the worst case. ΔH^0 and ΔS^0 are both negative in all mobile phase compositions, thus the transfer of a solute from the mobile to the stationary phase is enthalpically favorable and entropically unfavorable as shown in the previous study.[32] ΔH^0 of various solutes are plotted against mobile phase composition in Figures 3 and 4.

ΔH^0 is the sum of the solute-stationary phase interaction enthalpy minus the solute-mobile phase interaction enthalpy and the cavity formation enthalpy in the stationary phase minus the cavity formation enthalpy in the mobile phase.

In terms of interaction enthalpy between a solute and a solvent, the solute, if polar, will prefer the mobile phase to the stationary phase, or if nonpolar, will have no particular preference to any phase, which is against the observation of this study that the stationary phase was favored by all the

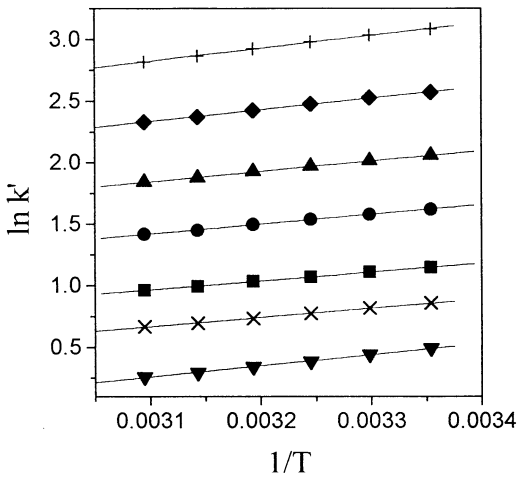

Figure 1. The van't Hoff plot for the data obtained in 50/50 (v/v %) acetonitrile/water at 25-50 °C. From the top, butylbenzene (+), propylbenzene (◆), ethylbenzene (▲), toulene (●), benzene (■), benzylacetone (×), benzylcyanide (▼).

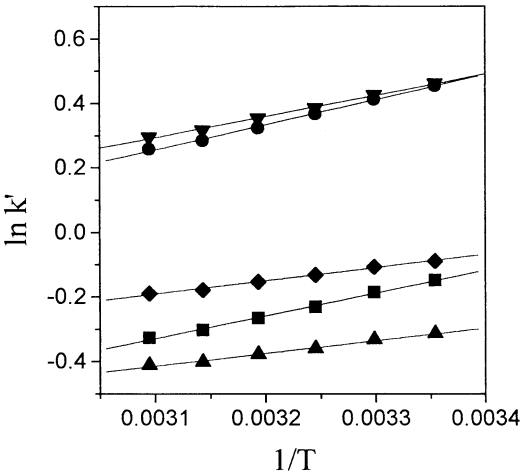

Figure 2. The van't Hoff plot for the data obtained in 50/50 (v/v %) acetonitrile/water at 25-50 °C. From the top, acetophenone (▼), benzonitrile (●), phenethylalcohol (◆), phenol (■), benzylalcohol (▲).

Table 1. The enthalpies of solute transfer from the mobile phase to the stationaryphase based on more than four independent retention measurements on diffrent days over the temperature range of 25-50 °C (unit: J/mol)

Solute	Mobile Phase				
	MeCN 30%	MeCN 40%	MeCN 50%	MeCN 60%	MeCN 70%
Benzene	-9413 ± 457	-8142 ± 153	-6297 ± 352	-5123 ± 313	-4017 ± 270
Toluene	-10879 ± 498	-8932 ± 157	-6873 ± 309	-5758 ± 278	-4886 ± 301
Ethylbenzene	-12223 ± 483	-9681 ± 156	-7360 ± 320	-6200 ± 263	-5521 ± 248
Propylbenzene	-14003 ± 575	-10859 ± 203	-8237 ± 327	-7091 ± 222	-6611 ± 232
Butylbenzene	-15411 ± 579	-11893 ± 317	-9146 ± 171	-7970 ± 86	-7634 ± 221
Phenol	-8932 ± 595	-7979 ± 252	-6447 ± 534	-5126 ± 536	-3315 ± 316
Acetophenone	-8113 ± 520	-7021 ± 66	-5405 ± 42	-4729 ± 47	-3988 ± 347
Benzylalcohol	-5245 ± 527	-4814 ± 259	-3923 ± 654	-3546 ± 530	-2745 ± 55
Phenethylalcohol	-5791 ± 575	-4876 ± 200	-4344 ± 435	-3437 ± 301	-2656 ± 492
Benzylacetone	-10004 ± 564	-8319 ± 84	-6179 ± 66	-5296 ± 85	-4605 ± 307
Benzonitrile	-10514 ± 608	-9144 ± 215	-7274 ± 580	-6079 ± 386	-4598 ± 342
Benzycyanide	-11923 ± 586	-10263 ± 169	-7669 ± 150	-6606 ± 387	-4741 ± 368

Table 2. The entropies of solute transfer from the mobile phase to the stationary phase based on more than four independent retention measurements on different days over the temperature range of 25-50 °C (unit : J/mol K)

Solute	Mobile Phase				
	MeCN 30%	MeCN 40%	MeCN 50%	MeCN 60%	MeCN 70%
Benzene	-16.3 ± 1.4	-16.8 ± 0.3	-16.6 ± 1.0	-16.2 ± 0.1	-15.1 ± 0.4
Toluene	-13.2 ± 0.4	-14.6 ± 0.5	-14.5 ± 0.7	-15.1 ± 0.8	-15.3 ± 0.1
Ethylbenzene	-11.8 ± 0.5	-12.5 ± 0.4	-12.6 ± 0.9	-13.6 ± 0.8	-15.4 ± 0.8
Propylbenzene	-10.7 ± 0.4	-11.2 ± 0.6	-11.3 ± 1.0	-13.1 ± 0.6	-15.8 ± 0.1
Butylbenzene	-9.2 ± 0.8	-9.5 ± 0.8	-9.4 ± 0.1	-12.4 ± 0.2	-16.5 ± 0.7
Phenol	-27.8 ± 1.9	-28.5 ± 0.7	-28.1 ± 1.4	-25.7 ± 1.9	-24.2 ± 2.8
Acetophenone	-17.7 ± 0.5	-19.9 ± 0.3	-20.9 ± 0.8	-21.1 ± 1.3	-22.2 ± 1.4
Benzylalcohol	-16.6 ± 0.7	-19.7 ± 0.8	-21.2 ± 1.4	-22.1 ± 1.8	-25.8 ± 1.9
Phenethylalcohol	-15.0 ± 0.8	-17.4 ± 0.6	-19.4 ± 1.4	-19.8 ± 1.6	-20.5 ± 0.8
Benzylacetone	-18.0 ± 0.5	-19.4 ± 0.2	-20.3 ± 1.3	-20.1 ± 0.8	-19.6 ± 0.4
Benzonitrile	-25.5 ± 1.9	-25.9 ± 0.6	-25.5 ± 1.2	-24.1 ± 1.3	-22.1 ± 1.4
Benzycyanide	-29.6 ± 1.8	-29.6 ± 0.4	-27.4 ± 1.2	-26.4 ± 1.4	-25.1 ± 2.0

solutes.

We should consider another enthalpy factor for solute distribution, that is, the cavity formation effect based on the solvophobic theory.[33-40] The solvophobic theory was, however, rather based on an adsorption-like retention model. The partition mechanism in the octadecyl bonded stationary phase seems to be generally accepted. Recently Tan and Carr[41] reanalyzed solvophobic driving forces in RPLC and found that retention on monomeric bonded phases with octyl chains or longer is dominated by a partition mechanism and that an adsorption-like mechanism contributes to retention in monomeric bonded phase with short bonded chains or with low surface coverage density.

The cavity formation enthalpy of the mobile phase is much larger than that of the stationary phase because there are only dispersive interactions in the stationary phase, while there exist dipole-dipole and hydrogen bond interactions in addition to the dispersive interaction in the mobile phase. For example let us compare the solubility parameters(δ) of the relevant solvents.[42] The cohesive energy density(heat of vaporization of a solvent divided by the molar volume) equals to δ^2. The cavity formation energy of a solute in the solvent is the product of δ^2 and the solute volume. Thus δ^2 is a measure of cavity formation enthalpy when we compare different solvents for a given solute. The δ values for water, methanol, acetonitrile, and dodecane are 23.53, 14.5, 12.11, and 7.84 $(cal/cm^3)^{1/2}$. Dodecane is regarded as a model for the stationary phase. We can note that δ^2 of aqueous methanol or acetonitrile will be much higher than that of dodecane. Therefore, the solute prefers the stationary phase to the mobile phase with respect to the cavity formation enthalpy.

Thus the cavity formation effect is dominant compared to the solute-phase interaction effect. The cavity formation enthalpy gets larger as the mobile phase gets more polar or the solute size gets bigger as shown in Figures 3 and 4.

The squalane impregnated C18 stationary phase is composed of approximately equal amounts of squalane and octadecyl ligands. The phase is of a narrow thickness and is rather viscous, so a solute in this phase will lose a portion of

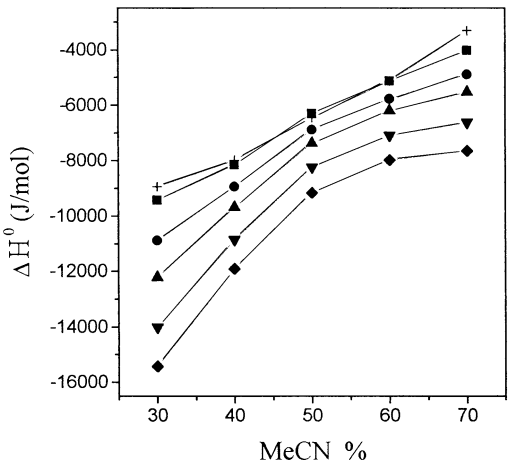

Figure 3. The plot of solute transfer enthalpies against acetonitrile volume fraction in the mobile phase. From the top, phenol (+), benzene (■), toulene (●), ethylbenzene (▲), propylbenzene (▼), butylbenzene (◆).

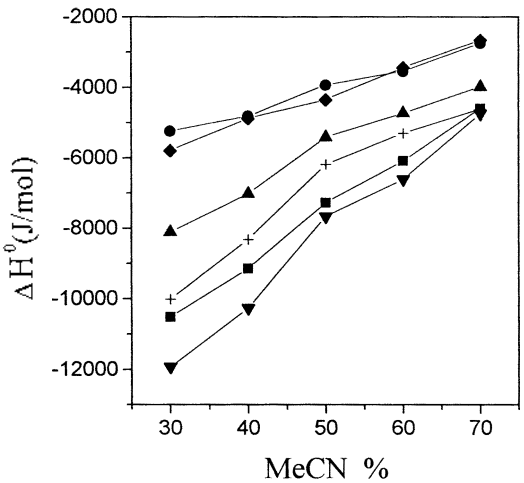

Figure 4. The plot of solute transfer enthalpies against acetonitrile volume fraction in the mobile phase. From the top, benzylalcohol (●), phenethylalcohol (◆), acetophenone (▲), benzylacetone (+), benzonitrile(■), benzylcyanide (▼).

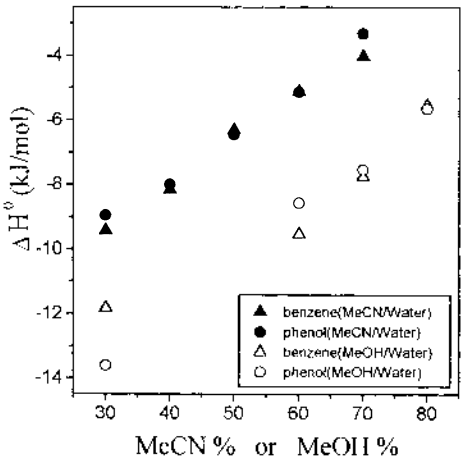

Figure 5. Comparison of ΔH^0 in the MeCN/water system of this study with ΔH^0 in the MeOH/water system.

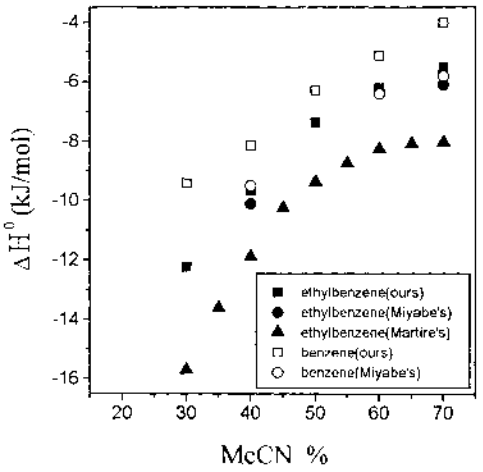

Figure 6. Comparison of literature ΔH^0 data with ours for benzene and ethylbenzene.

its freedom (entropy) compared to the solute in the mobile phase. Thus solute transfer entropies are exclusively negative (Tables 1 and 2).

Now let us compare our data with some relevant literature data (Figure 5 and 6). We mentioned that in the previous study[32] we had obtained quantitative thermodynamic properties for solute transfer from the MeOH (methanol)/water mobile phase to the squalane impregnated C18 phase. Thus we compared ΔH^0 data obtained in this study with those obtained in MeOH/water mobile phases (Figure 5). ΔH^0 in the MeOH/water system are more negative than those in the MeCN (acetonitrile)/water system as shown in Figure 5. This phenomenon can also be explained by the cavity formation effect. The cavity formation effect is larger in MeOH/water than in MeCN/water since methanol is more polar than acetonitrile.

Miyabe *et al.*[43] measured ΔH^0 values for benzene, toluene and ethylbenzene in 20-80% MeCN/water using a C18 stationary phase of large (45 μm) particle size. We compared ΔH^0 data obtained in this study(squalane impregnated C18 stationary phase) with those obtained by Miyabe *et al.*[43] and those obtained by Martire *et al.*[44] in Figure 6.

Martire *et al.* used a regular C18 stationary phase. We should note that in the previous study[32] where we measured ΔH^0 in the methanol/water system with the squalane-impregnated C18 stationary phase, our ΔH^0 values were rather in good agreement with Martire's ΔH^0 values and were considerably more negative than Miyabe's ΔH^0 values. We thought that Miyabe *et al.* had used a C18 phase with low ligand density to result in partially adsorption-like retention.

In this study where acetonitrile/water eluents were used, our ΔH^0 values are rather close to Miyabe's ΔH^0 values, and considerably less negative than Martire's ΔH^0 values (Figure 6).

If the argument we proposed in the previous report[32] is assumed to be valid, we may come to a conclusion that the squalane-impregnated C18 phase showed partially adsorption-like retention in the acetonitrile/water system and partition-like retention in the methanol/water system. We do not have any reasonable explanation for such observation at the present time. It might be related to higher wetting ability of acetonitrile to the stationary phase over methanol. Or the entropic effects should be considered altogether.

Otherwise, the discrepancies in solute transfer enthalpy among different stationary phases might be simply attributed to differences in ligand load, density of residual silanol groups, properties of base silica, etc. although it is unclear how such factors affect solute transfer enthalpies for nonpolar solutes that we chose for comparison purpose (Figure 6). We may assume that the effective stationary phase includes the bulk ligand phase (C18 + squalane) and the adsorbed mobile phase layer on it since the ligand phase has a large surface area. The more nonpolar component of the mobile phase will be preferentially adsorbed, and the amount of the adsorbed solvent will be different for different stationary phases, resulting in different solute transfer enthalpies. Such rationalization, however, will be valid only if the solute follows the adsorption mechanism. If the solute follows the partition mechanism, the solute enters in the bulk ligand phase, and the adsorbed mobile phase will not affect the solute transfer enthalpy. We will examine solute retention in a regular C18 stationary phase in both solvent systems to find better explanation for these results in the future study.

Conclusion

We measured thermodynamic properties of solute transfer with our chromatographic system using the squalane-impregnated C18 stationary phase and the acetonitrile/water mobile phase. The van't Hoff plots of ln k' *vs.* 1/T were nicely linear in our system. The transfer of a solute from the mobile phase to the stationary phase is enthalpically favorable and entropically unfavorable in general. The cavity formation has proven to be the major factor that governs the solute distribution between the mobile and stationary phases.

Our data were compared with some relevant literature data. Under similar chromatographic conditions, ΔH^0 values

of methanol/water system are more negative than those of acetonitrile/water system.

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References

1. Majors, R. E. *LC-GC* **1988**, *6*, 298.
2. Martire, D. E.; Bohem, R. E. *J. Phys. Chem.* **1983**, *87*, 1045.
3. Dill, K. A. *Phys. Chem.* **1987**, *91*, 1980.
4. Dill, K. A.; Naghizard, J.; Marqusee, J. A. *Annu. Rev. Phys. Chem.* **1988**, *39*, 425.
5. Dorsey, J. G.; Dill, K. A. *Chem. Rev.* **1989**, *89*, 331.
6. Ying, P. T.; Dorsey, J. G.; Dill, K. A. *Anal. Chem.* **1989**, *61*, 2540.
7. Grushka, E.; Colin, H.; Guichon, G. *J. Chromatogr.* **1982**, *248*, 325.
8. Tchapla, A.; Heron, S.; Colin, H.; Guichon, G. *Anal. Chem.* **1988**, *60*, 1443.
9. Yamamoto, F. M.; Rokushika, S.; Hatano, H. *J. Chromatogr. Sci.* **1989**, *27*, 704.
10. Issaq, H. J.; Jaroniec, M. *J. Liq. Chromatogr.* **1989**, *12*, 2067.
11. Sander, L. C.; Field, L. R. *Anal. Chem.* **1980**, *52*, 2009.
12. Hammers, W. E.; Verschoor, P. B. A. *J. Chromatogr.* **1983**, *282*, 41.
13. Morel, D.; Serpinet, J. *J Chromatogr.* **1980**, *200*, 95.
14. Morel, D.; Serpinet, J. *J. Chromatogr.* **1981**, *214*, 202.
15. Morel, D.; Serpinet, J. *J. Chromatogr.* **1982**, *248*, 231.
16. van Mitenburg, J. C.; Hammers, W. E. *J. Chromatogr.* **1983**, *268*, 147.
17. Kessaissia, Z.; Papier, E.; Donnet, J. B. *J. Colloid Interface Sci.* **1981**, *79*, 257.
18. Gilpin, R. K.; Squires, J. A. *J. Chromatogr. Sci.* **1981**, *19*, 195.
19. Yang, S. S.; Gilpin, R. K. *J. Chromatogr.* **1988**, *449*, 115.
20. Morel, D.; Serpinet, J.; Untz, G. *Chromatographia* **1984**, *18*, 611.
21. Cole, L. A.; Dorsey, J. G. *Anal. Chem.* **1992**, *64*, 1317.
22. Han, L.; Linert, W.; Gutmann, V. *J. Chromatogr. Sci.* **1992**, *30*, 142.
23. Lamparczyk, H.; Zarzycki, P. K. *J. Pharmac. Biomed. Anal.* **1995**, *13*, 543.
24. Sellergren, B.; Shea, K. J. *J. Chromatogr. A.* **1995**, *690*, 29.
25. Gilpin, R. K.; Ehtesham, S. E.; Gregory, R. E. *Anal. Chem.* **1991**, *63*, 2825.
26. Makela, M.; Pyy, L. *J. Chromatogr. A* **1995**, *699*, 49.
27. Gennaro, M. C.; Giacosa, D.; Abrigo, C.; Marengo, E. *J. Chromatogr. Sci.* **1995**, *33*, 260.
28. Goldberg, A. P. *Anal. Chem.* **1982**, *54*, 342.
29. Sentell, K. B.; Ryan, N. I.; Henderson, A. N. *Anal. Chim. Acta* **1995**, *307*, 203.
30. Bell, C. M.; Sander, L. C.; Wide, S. A. *J. Chromatogr. A* **1997**, *757*, 29.
31. McGuffin, V. L.; Chen, S. *J. Chromatogr. A* **1997**, *762*, 35.
32. Lee, C. S.; Cheong, W. J. *J. Chromatogr. A* **1999**, *848*, 9.
33. Smith, R. J.; Nicass, C. S.; Wainwright, M. S. *J. Liq. Chromatogr.* **1986**, *9*, 1387.
34. Djerki, R. A.; Laub, R. J. *J. Liq. Chromatogr.* **1987**, *10*, 1749.
35. Sadek, P. C.; Carr, P. W.; Doherty, R. M.; Kamlet, M. J.; Taft, R. M.; Abraham, M. H. *Anal. Chem.* **1985**, *57*, 2971.
36. Park, J. H.; Carr, P. W.; Abraham, M. H.; Taft, R. W.; Doherty, R. M.; Kamlet, M. J. *Chromatographia* **1988**, *25*, 373.
37. Kamlet, M. J.; Doherty, R. M.; Abraham, M. H.; Marcus, Y.; Taft, R. W. *J. Phys. Chem.* **1988**, *92*, 5244.
38. Horvath, C.; Melander, W. *J. Chromatogr. Sci.* **1977**, *15*, 393.
39. Horvath, C.; Melander, W.; Molnar, I. *J. Chromatogr.* **1976**, *125*, 129.
40. Sinanoglu, O. *Chem. Phys. Lett.* **1981**, *81*, 188.
41. Tan, L. C.; Carr, P. W. *J. Chromatogr. A.* **1997**, *775*, 1.
42. Riddick, J. A.; Bunger, W. B.; Sakano, T. K. *Organic Solvents: Physical Properties and Methods of Purification.* 4th Ed; John Wiley & Sons: New York, 1986.
43. Miyabe, K.; Takeuchi, S. *Anal. Chem.* **1997**, *69*, 2567.
44. Alvarez-Zepeda, A.; Barman, B. N.; Martire, D. E. *Anal. Chem.* **1992**, *64*, 1978.