New Statistical Approach to a Gas Chromatography Retention Model: Application to Dichlorophenol Isomers

Yves Claude Guillaume* and Christiane Guinchard

Laboratoire de Chimie Analytique, Faculté de Médecine et Pharmacie, Place St. Jacques, 25030 Besancon, Cedex, France

Received: March 24, 1997; In Final Form: June 16, 1997[⊗]

The retention mechanism of a series of six positional isomers of dichlorophenol in gas chromatography was investigated using pure α-, *β*-, and *γ*-cyclodextrin (CD) stationary phases. For each CD, the values of enthalpy (ΔH) and entropy (ΔS) of transfer of an isomer from the gas to the CD stationary phase were determined. These data demonstrated the leading role of enthalpy according to entropy. Enthalpy−entropy compensation revealed that the retention mechanism was independent of both the chloro group position on the phenol ring and the size and shape of the CD cavity. A model based on the number of CD inclusion complexes with an isomer was also presented. This model explains current experimental data including the reverse elution order observed for certain pairs of isomers.

Introduction

Cyclodextrins (CDs), which are doughnut-shaped cyclic oligosaccharides consisting of six or more α-(1,4)-linked D-glucopyrannose units, are one of the well-known host molecules capable of forming an inclusion complex (host−guest complex) with a variety of organic molecules or so-called guest molecules.[1−4] The mechanism of separation of CDs and modified CDs is based on van der Waals interactions and hydrogen bonds,[5−6] changes in conformation,[7] steric interactions,[8−12] and size of the inclusion cavity.[13−19] Methylated cyclodextrins have been diluted in polar siloxanes to illustrate the role of the inclusion and determine the effects of dilution on resolution.[20] Modified cyclodextrins have also been evaluated to show the influence of diluting the phases on enantioselectivity[21−29] and the contribution of thermodynamic parameters to chiral resolution.[30] This study investigates the thermodynamic behavior and the selectivity of six positional isomers of dichlorophenol on undiluted α-, *β*-, and *γ*-CDs as stationary phases. The enthalpies (ΔH) and entropies (ΔS) of transfer of these compounds from the gas to the undiluted CD stationary phase were determined. To understand the dependence of ΔH and ΔS on the cyclodextrin cavity, a cavity model that takes into account the number of inclusion complexes was developed. This model was used to study the positional recognition mechanism and the temperature reversal order of certain pairs of isomers on α-, *β*-, or *γ*-CDs.

Experimental Section

Apparatus. The analyses were performed using a DI 200 gas chromatography apparatus with a flame ionization detector (Varian, Strasbourg, France) and a D 2500 chromato Integrator (Merck, Nogent-sur-Marne, France).

The three capillary columns used were 30 m long with a 0.25 mm i.d. and a film thickness of 0.25 *μ*m (Macherey-Nagel, Hoerdt, France). The columns were coated with α-CD (first column), *β*-CD (second column), and *γ*-CD (third column). The packing specifications reported by the manufacturer were the following:

(1) α-, *β*-, and *γ*-CDs were pure and undiluted in polar siloxanes.

(2) These CD pure stationary phases cancel out almost all the other possible interactions except those between the isomer molecule and CD.

The carrier gas was nitrogen with a flow rate of 1 mL/min and the column temperature from 135 to 250 °C with isothermal conditions.

Reagents. The chromatographed compounds were positional isomers: 2,3-dichloro- (1), 2,4-dichloro- (2), 2,5-dichloro- (3), 2,6-dichloro- (4), 3,4-dichloro- (5), and 3,5-dichloro- (6) phenols were obtained from Merck (Nogent-sur-Marne, France). The chemical structures of these compounds are given in Figure 1. For gas chromatographic investigation, the samples were dissolved in methanol to a final concentration of 3 mg/mL. Methane was used as a dead time marker. Each solute was injected, and the retention times were measured using a Merck D2500 chromato integrator. All the experiments were repeated three times.

Temperature Studies. Isomer retention factors were determined over the temperature range 135−250 °C. The chromatographic system was allowed to equilibrate at each temperature for at least 30 min prior to each experiment. To study this equilibration, the retention time of the compound 3,5-dichlorophenol was measured every 30 min for 7 h and again after 22, 23, and 24 h. The maximum relative difference in the retention times of this compound between these different measurements was always 0.1%, making the chromatographic system sufficiently equilibrated for use after 30 min.

Methods

Thermodynamic Relationships. isomer retention is usually expressed in terms of the retention factor k'_T determined at temperature T by the equation:[31]

$$\ln k'_T = -\Delta H/RT + \Delta S/R + \ln \phi \qquad (1)$$

where ΔH (ΔS, respectively) is the enthalpy (entropy, respectively) of transfer of the isomer from the gas to the CD stationary phase, T the column temperature, R the gas constant, and ϕ the phase ratio (volume of the stationary phase divided by the volume of the mobile phase). For a capillary column, the phase

[⊗] Abstract published in *Advance ACS Abstracts,* September 15, 1997.

S1089-5647(97)01037-7 CCC: $14.00

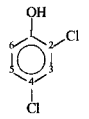
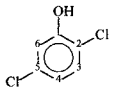
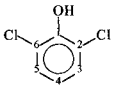
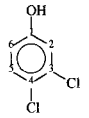
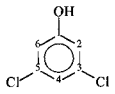
Compound No	Nomenclature	Chemical structure
(1)	2,3-dichlorophenol	
(2)	2,4- dichlorophenol	
(3)	2,5- dichlorophenol	
(4)	2,6- dichlorophenol	
(5)	3,4- dichlorophenol	
(6)	3,5- dichlorophenol	

Figure 1. Chemical structures: 2,3-dichlorophenol (1), 2,4-dichlorophenol (2), 2,5- dichlorophenol (3), 2,6-dichlorophenol (4), 3,4-dichlorophenol (5), and 3,5- dichlorophenol (6).

ratio is related to the film thickness (d_f) and the column diameter (d_c) via[31]

$$\phi = 4d_f/d_c \qquad (2)$$

Equation 1 shows that the plot of $\ln k'_T$ versus $1/T$ (called a Van't Hoff plot) has a slope of $-\Delta H/R$ and an intercept of $\Delta S/R + \ln \phi$. This provides a convenient way of calculating the thermodynamic constants ΔH and ΔS for the chromatographic system.

Results and Discussion

The retention factor of each of the six dichlorophenol isomers was determined for a wide range of column temperatures 135−250 °C. Twenty-four temperature values ($p = 24$) were included in this range. All the experiments were repeated three times. According to eq 1, Van't Hoff plots were obtained for all isomers. The coefficients of variance for the linear fits were in excess of 0.9800 ($= r^2$). The typical standard deviations of slope and intercept were, respectively, 0.005 and 0.02. Tables 1, 2, and 3 contain a complete list of the ΔH and ΔS values obtained for all isomers with, respectively, α-, β-, and γ-cyclodextrins as stationary phases. Negative ΔH obviously indicated that it was energetically favorable for the weak polar positional isomer to form an inclusion complex with the hydrophobic cavity of the CD. This isomer degree freedom loss, due to its cavity inclusion, would thus explain the negative ΔS values obtained. Also listed in Tables 1, 2, and 3 were $T\Delta S$ values at $T = 150$ °C (423 K). For every isomer evaluated

TABLE 1: ΔH, ΔS, and $T\Delta S$ Values for All Isomers in the α-Cyclodextrin Stationary Phase

isomer	r^2,[a] $p = 24$	ΔH (kcal·mol^{-1})	ΔS (cal·mol^{-1}·K^{-1})	$T\Delta S$[b] (kcal·mol^{-1})
2,3-dichlorophenol (1)	0.9986	−9.49	−9.97	−4.22
2,4-dichlorophenol (2)	0.9883	−9.77	−10.60	−4.48
2,5-dichlorophenol (3)	0.9893	−10.12	−11.25	−4.76
2,6-dichlorophenol (4)	0.9909	−10.01	−10.87	−4.60
3,4-dichlorophenol (5)	0.9969	−13.91	−16.61	−7.03
3,5-dichlorophenol (6)	0.9968	−14.79	−18.49	−7.82

[a] r^2: value for the linear fit of the Van't Hoff plot. [b] For $T = 423$ K.

TABLE 2: ΔH, ΔS, and $T\Delta S$ Values for All Isomers in the β-Cyclodextrin Stationary Phase

isomer[a]	r^2,[b] $p = 24$	ΔH (kcal·mol^{-1})	ΔS (cal·mol^{-1}·K^{-1})	$T\Delta S$[c] (kcal·mol^{-1})
(1)	0.9879	−9.64	−10.17	−4.30
(2)	0.9874	−9.80	−10.64	−4.50
(3)	0.9874	−10.28	−11.54	−4.88
(4)	0.9891	−10.02	−10.87	−4.59
(5)	0.9972	−14.47	−17.68	−7.48
(6)	0.9981	−14.69	−18.47	−7.81

[a] See the corresponding isomer in Table 1. [b] r^2 value for the linear fit of the Van't Hoff plot. [c] For $T = 423$ K.

TABLE 3: ΔH, ΔS, and $T\Delta S$ Values for All Isomers in the γ-Cyclodextrin Stationary Phase

isomer[a]	r^2,[b] $p = 24$	ΔH (kcal·mol^{-1})	ΔS (cal·mol^{-1}·K^{-1})	$T\Delta S$[c] (kcal·mol^{-1})
(1)	0.9882	−9.94	−10.43	−4.41
(2)	0.9868	−9.92	−10.52	−4.45
(3)	0.9858	−10.33	−11.25	−4.76
(4)	0.9883	−10.19	−10.87	−4.59
(5)	0.9965	−14.88	−18.10	−7.66
(6)	0.9963	−14.69	−17.98	−7.61

[a] See the corresponding isomer in Table 1. [b] r^2 value for the linear fit of the Van't Hoff plot. [c] For $T = 423$ K.

when ΔH was compared with $T\Delta S$ over the temperature range from 135 to 250 °C, the magnitude of ΔH was always greater than that of $T\Delta S$. This indicated that enthalpy played a greater role in the transfer of an isomer from the gas to the cyclodextrin stationary phase and therefore in the retention process than did entropy.

Small differences in ΔH and ΔS values were observed for various isomers with α-, β-, or γ-cyclodextrins. Enthalpy−entropy compensation is a term used to describe a compensation temperature that is system-independent for a class of similar experimental systems. Enthalpy−entropy compensation has been applied to chromatographic systems to evaluate the retention mechanism.[31−33] Equation 3 relates the compensation temperature Z to the capacity factor at some temperatures $T(k'_T)$:

$$\ln k'_T = -\Delta H/R(1/T - 1/Z) - \Delta G_Z/RZ + \ln \phi \qquad (3)$$

If a plot of $\ln k'_T$ versus $-\Delta H$ for a set of isomers is linear, then enthalpy−entropy compensation exists for the system, which means that the mechanism of retention is similar for all the cases evaluated.

The equation $\ln k'_{423K}$ against $-\Delta H$ (kcal·mol^{-1}) for the six isomers on the α-cyclodextrin stationary phase was

$$\ln k'_{423K} = -3.207 + 0.412(-\Delta H) \qquad r^2 = 0.9896 \qquad (4)$$

For β- and γ-cyclodextrin stationary phases, the coefficients of variance (r^2) for the linear fits were, respectively, 0.9913 and

0.9926. This high degree of correlation indicated that the retention mechanism on a α-, β-, or γ-cyclodextrin stationary phase was the same whatever the position of the chloro groups on the phenol ring. An alternative method for evaluating enthalpy−entropy compensation is to determine the compensation temperature as[34,35]

$$Z = \Delta H/\Delta S \tag{5}$$

The compensation temperature was determined for each isomer. For the α-, β-, and γ-cyclodextrin stationary phases, the values ranged from roughly 520 to 680 °C. These values were similar for a particular isomer on the α-, β-, and γ-cyclodextrin stationary phases. For example, the *Z* values determined for 3,5 dichorophenol on the α-, β-, and γ-cyclodextrin stationary phases were, respectively, 529, 522, and 543 °C. This comparison indicated that for this solute molecule family, the isomer retention was the same for α-, β-, and γ-cyclodextrin. The retention mechanism was, thus, independent of both the isomer molecular structure and the size and shape of the cyclodextrin. Therefore, an empirical model was developed to explain the present experimental data.

Cavity Model. Cyclodextrin stationary phase was considered to contain *N* cavities. As all the CD stationary phases were pure, the isomer molecule was only able to form, at random, *n* inclusion complexes (host−guest complexes). The number of free cavities n_f was therefore:

$$n_f = N - n \tag{6}$$

The cyclodextrin entropy due to the formation of the *n* inclusion complexes (IC) underwent only an ΔS_c variation; we called it "configuration" due to the distribution, at random, of *n*IC in the cyclodextrin. When *n*IC were created in the cyclodextrin, the probability of obtaining a cyclodextrin in which the *n*IC had a given arrangement was

$$N!/(n!n_f!) \tag{7}$$

The corresponding configuration entropy was

$$\Delta S_c = k_B \ln(N!/n!n_f!) \tag{8}$$

where k_B is the Boltzmann's constant equal to the ratio of the gas constant *R* and Avogadro number *N*:

$$k_B = R/N \tag{9}$$

It was hypothesized that the formation enthalpy ΔH_{IC} of only one inclusion complex was independent of its number *n*, thus independent of the cyclodextrin state. Thus, every host cavity in the cyclodextrin was surrounded by the same strength field. This was formalized by the fact that

$$n \ll n_f \tag{10}$$

Thus, the creation of a supplementary inclusion complex did not change the potential of each remaining free cavity.

At temperature *T*, the Gibbs free energy variation due to the creation of *n*IC in the cyclodextrin was

$$\Delta G = n\Delta H_{IC} - k_B T \ln(N!/n!n_f!) \tag{11}$$

The real Gibbs free energy of the cyclodextrin containing *n*IC was

$$G = G_p + n\Delta H_{IC} - k_B T \ln(N!/n!n_f!) \tag{12}$$

where G_p was the thermodynamic potential corresponding to both $n = 0$ and $n_f = N$.

As *n* and n_f were very high, eq 12, using eq 6, could be rewritten using the well-known Sterling approximation:

$$G = G_p + n\Delta H_{IC} - k_B T n \ln((n_f+n)/n) - k_B T n_f \ln((n_f+n)/n_f) \tag{13}$$

Using inequality 10, eq 13 could be rewritten as

$$G = G_p + n\Delta H_{IC} + k_B T n\,(\ln(n/n_f) - 1) \tag{14}$$

More quantitatively, the value of the optimal inclusion complex number was obtained by minimizing the real Gibbs free energy *G* of the cyclodextrin as a function of *n* giving

$$n \rightarrow (\partial G/\partial n) = 0 \tag{15}$$

Therefore, the inclusion complex number at equilibrium at temperature *T* was

$$n = n_f \exp(-\Delta H_{IC}/k_B T) \tag{16}$$

Introducing eq 9 into eq 16 leads to

$$n = n_f \exp(-N\Delta H_{IC}/RT) \tag{17}$$

The formation enthalpy of *N* inclusion complexes was equal to the molar enthalpy of transfer of the solute molecule from the gas to the cyclodextrin stationary phase

$$N\Delta H_{IC} = \Delta H \tag{18}$$

Introducing eq 18 into 17 leads to

$$n = n_f \exp(-\Delta H/RT) \tag{19}$$

Equations 17 and 19 lead to the following conclusion in accordance with our predictions:

(1) The number of inclusion complexes depends on its formation enthalpy. This confirmed the major role of enthalpy in comparison with entropy in the solute retention process.

(2) The formation of an inclusion complex was favored at low temperature. This confirmed the decrease in solute retention with an increase in temperature.

When considering the transformation

$$N(\text{cavities}) \rightarrow (N - n)(\text{free cavities}) + n(\text{IC}) \tag{20}$$

$n_f = N - n$ free cavities were in thermodynamic equilibrium with *n* inclusion complexes IC:

$$n_f(\text{free cavities}) \rightleftharpoons n(\text{IC}) \tag{21}$$

This equilibrium necessitated the Gibbs free energy ΔG_{IC} for the formation of only one inclusion complex. The expression relating *n* to n_f was, thus, the application of the action mass law to the chemical equilibrium 21. Therefore, the hypothesis made above in our simple model would not have been necessary if ΔH_{IC} in eq 11 had been changed to ΔG_{IC}. This result has been objectivized by Guggenheim's statistical work.[36] Equation 16 was then rewritten as

$$n = n_f \exp(-\Delta G_{IC}/k_B T) \tag{22}$$

Selectivity-Positional Recognition Mechanism. The selectivity, ξ, links the difference of affinity of two positional isomers in relation to the CD cavity. $\xi = 1$ obviously corresponds to

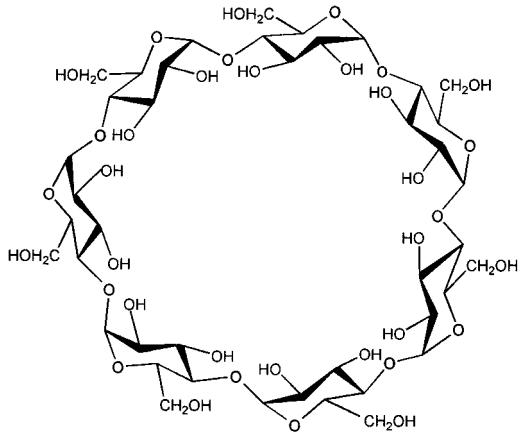


Figure 2. Chemical structure of β-cyclodextrin.

an identical affinity. This positional recognition mechanism for the dichlorophenol isomers on a CD stationary phase was based on the selective properties of CD that involved a combination of attractive (hydrogen bonding, van der Waals, electrostatic)[5,6] and repulsive (steric)[8−12] interactions. Cyclodextrins have torus-like macro rings built up from glucopyranose units. α-cyclodextrin consists of six glucopyranose units, β-cyclodextrin consists of seven glucose units, and finally γ-cyclodextrin comprises eight such units. Figure 2 shows, for example, the structure of β-cyclodextrin. For dichlorophenols, the less voluminous group attached to the phenol ring, OH, was included in the interior of the CD cavity and engaged with hydrogen bonds with the glycosidic oxygen bridges. The OH group steric hindrance was particulaly enhanced when one of either the chloro group was in the ortho position (or position 2 on the phenol ring) (Figure 1). Consequently, the 3,4-dichloro and the 3,5-dichloro isomers interacted more strongly than the 2,3-dichloro, the 2,4-dichloro, the 2,5-dichloro, and the 2,6-dichloro isomers with the hydrophobic cavity. The 3,4-dichloro and the 3,5-dichloro isomers were immobilized better than the other four. Thus, the ΔH and ΔS values were lower for the 3,4-dichloro and the 3,5-dichloro isomers than for the other four.

The number of inclusion complexes for two positional isomers of dichlorophenol called Nos. 1 and 2, were, respectively, n_1 and n_2 . If ΔS_{IC} was the entropy formation of only one inclusion complex, then

$$\Delta G_{IC} = \Delta H_{IC} - T\Delta S_{IC} \tag{23}$$

and

$$\xi = n_1/n_2 \tag{24}$$

Combining eqs 22, 23, and 24 leads to

$$\ln \xi = -\Delta(\Delta H_{IC})/k_B T + \Delta(\Delta S_{IC})/k_B \tag{25}$$

Combining eqs 9 and 25 leads to

$$\ln \xi = -N\Delta(\Delta H_{IC})/RT + N\Delta(\Delta S_{IC})/R \tag{26}$$

This latter equation is similar to the one obtained in gas chromatography where ξ is equal to the ratio of the capacity factor of solutes 1 and 2, k_1 and k_2:

$$\xi = n_1/n_2 = k_1/k_2 \tag{27}$$

$N\Delta(\Delta H_{IC})$ ($N\Delta(\Delta S_{IC})$, respectively) are the molar difference in the inclusion complex formation enthalpy $\Delta(\Delta H)$ (entropy $\Delta(\Delta S)$, respectively) of the two positional isomers. Thus, eq 26 yields

$$\ln \xi = -\Delta(\Delta H)/RT + \Delta(\Delta S)/R \tag{28}$$

Chromatographically, the particular temperature-dependent reversal of the elution order θ at which there is no separation between two adjacent isomers on the chromatogram corresponds to the same number of inclusion complexes created by the two isomers; thus, $\xi = 1$. Then eq 28 leads to

$$\theta = \Delta(\Delta H)/\Delta(\Delta S) \tag{29}$$

Equation 28 showed that ln ξ versus $1/T$ was a Van't Hoff plot. The coefficients of variance for the linear fits were in excess of 0.9200 ($= r^2$). The typical standard deviations of slope and intercept were respectively 0.003 and 0.01. $\Delta(\Delta H)$ and $\Delta(\Delta S)$ were, respectively, determined from the slope and intercept. θ was then calculated using eq 29. For θ, the two isomers have an identical affinity for the cavity. In the temperature range studied the elution reversal was detected for certain positional isomers on each of the α-, β-, and γ-CD stationary phases.

All the corresponding $\Delta(\Delta H)$, $\Delta(\Delta S)$, and θ values are summarized in Table 4.

TABLE 4: $\Delta(\Delta H)$, $\Delta(\Delta S)$, and Temperature-Dependent Reversal of the Observed Elution Order, θ, for Certain Pairs of Isomers on α-, β-, and γ-Cyclodextrin

cyclo-dextrin	isomer pair[a]	r^2,[b] $p = 24$	$\Delta(\Delta H)$ (kcal·mol^{-1})	$\Delta(\Delta S)$ (cal·mol^{-1}·K^{-1})	θ (°C)
α	(1)/(2)	0.9557	−0.388	−0.896	160
	(5)/(6)	0.9885	−0.896	−1.926	192
β	(1)/(3)	0.9716	−0.669	−1.419	198
	(3)/(4)	0.9937	−0.502	−1.204	144
γ	(4)/(3)	0.9274	−0.241	−0.587	137

[a] See the corresponding isomer in Table 1. [b] r^2 value for the linear fit of the corresponding Van't Hoff plot.

In conclusion, the thermodynamic data and the simple cavity model outlined above show:

(1) The major role of enthalpy according to entropy.

(2) The independence on both the type of CD (α-, β-, or γ-) and the chloro group position on the phenol ring with the retention mechanism.

(3) The importance of both attractive (hydrogen bonds) and repulsive (steric) interactions on the positional recognition mechanism.

(4) The existence of a simple relation between the selectivity of two positional isomers of dichlorophenol with the number of inclusion complexes created in the CD.

Glossary

CD:	cyclodextrin
d_c:	column diameter
d_f:	film thickness
G:	real Gibbs free energy of cyclodextrin containing n inclusion complexes
G_p:	Gibbs free energy of cyclodextrin containing no inclusion complex = its thermodynamic potential
IC:	inclusion complex
k'_T:	isomer retention factor at temperature T
k_B:	Boltzmann's constant
n:	inclusion complex number
n_f:	free cavity number in the cyclodextrin
N:	total cavity number in the cyclodextrin
$\mathcal{N}$:	Avogadro number

p: number of temperatures for which the experiments were carried out

r: correlation coefficients of Van't Hoff plots

Z: compensation temperature

ξ: selectivity between two isomer molecules

θ: temperature-dependent reversal of the elution order

ϕ: column phase ratio

ΔG_{IC}: Gibbs free energy of the formation of only one inclusion complex

ΔG_Z: molar Gibbs free energy of transfer of an isomer from the gas to the cyclodextrin phases at Z compensation temperature

ΔH_{IC}: enthalpy of formation of only one inclusion complex

ΔH: molar enthalpy of transfer of an isomer from the gas to the cyclodextrin phases

$\Delta(\Delta H)$: molar difference in the inclusion complex formation of two isomer molecules

ΔS_{IC}: entropy of formation of only one inclusion complex

ΔS: molar entropy of transfer of an isomer from the gas to the cyclodextrin phases

ΔS_c: configuration entropy of cyclodextrin containing n inclusion complexes

$\Delta(\Delta S)$: molar difference in the inclusion complex formation of two isomer molecules

Acknowledgment. We thank Nadia MORIN and Mireille THOMASSIN for their technical assistance.

References and Notes

(1) Saenger, W. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 344.
(2) Hinze, W. L. *Sep. Purif. Methods* **1981**, *10*, 159.
(3) Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry*; Springer-Verlag: New York, 1978.
(4) Szejtli, J. *Cyclodextrins and their inclusion complexes*; Akademiai Kiado: Budapest, Hungary, 1982.
(5) Kober, F.; Schomburg, G. *J. High Resolut. Chromatogr.* **1993**, *16*, 693.
(6) Schmarr, H. G.; Mosandl, A.; Kaunzinger, A. J. Microcolumn Sep. **1991**, *3*, 395.
(7) Wan, H.; Zhou, X.; Ou, Q. *J. Chromatogr. A* **1994**, *673*, 107.
(8) Steinborn, A.; Reinhardt, R.; Engewald, W.; Wyssuka, K.; Schulze, K. *J. Chromatogr. A* **1995**, *697*, 485.
(9) Lee, S. H.; See, Y. J.; Lee, K. P. J. *Korean Chem. Soc.* **1995**, *39*, 94.
(10) Krupcik, J.; Benicka, E.; Majek. P.; Skacani, I.; Sandra, P. *J. Chromatogr. A* **1994**, *665*, 175.
(11) Smith, I. D.; Simpson, C. F. *J. High Resolut. Chromatogr.* **1993**, *16*, 530.
(12) Kallenborn, R.; Oehme, M.; Vetter, W.; Parlar, H. *Chemosphere* **1994**, *28*, 89.
(13) Engewald, W.; Reinhardt, R.; Steinborn, A. *Pure Appl. Chem.* **1993**, *65*, 2253.
(14) Betts, T. J. *J. Chromatogr.* **1994**, *672*, 254.
(15) Schurig, V.; Grosenick, H. *J. Chromatogr.* **1994**, *666*, 617.
(16) Jaques, K.; Buda, W. M.; Pottie, M.; Van der Eychen J.; Vanderwalle, M.; Venema, A.; Sandra, P. J. *High Resolut. Chromatogr.* **1993**, *16*, 703.
(17) Betts, T. J. *J. Chromatogr. A* **1995**, *707*, 390.
(18) Smith, L. D.; Colin, F. *Anal. Proc.* **1993**, *80*, 479.
(19) Takeichi, T.; Toriyama, H.; Shimura, S.; Takayama, Y.; Morikawa, M. *J. High Resolut. Chromatogr.* **1995**, *18*, 179.
(20) Schmarr, H. G.; Mosandl, R.; Neukom, H. P.; Grob, K. *J. High Resolut. Chromatogr.* **1991**, *14*, 207.
(21) Maas, B.; Dietrich, A.; Karl, V.; Kaunzinger, A.; Lehmann, D.; Koepke, T.; Mosandl, A. *J. Microcolumn Sep.* **1993**, *5*, 421.
(22) Saturnin, C.; Tabacchi, R.; Saxer, A. *Chimia* **1993**, *47*, 221.
(23) Bicchi, C.; Damato A. A..; Manzin, V.; Galli, M. *J. Chromatogr. A* **1994**, *666*, 137.
(24) Dietrich, A.; Bigit, M.; Brand, G.; Volker, K.; Kaunzinger, A. M. *J. High Resolut. Chromatogr.* **1992**, *15*, 769.
(25) Wan. H.; Wang, Y.; Ou, Q.; Yu, W. *J. Chromatogr.* **1993**, *644*, 202.
(26) Casabianca, H.; Graff, J. B. *J. High Resolut. Chromatogr.* **1994**, *17*, 184.
(27) Reinhardt, R.; Engewald, W.; Georoeg, S. J. High Resolut. Chromatogr. **1995**, *18*, 259.
(28) Anon. *J. High Resolut. Chromatogr.* **1993**, *16*, 338.
(29) Dietrich, A.; Maas, B.; Mosandl, A. *J. Microcolumn Sep.* **1994**, *6*, 33.
(30) Dietrich, A.; Maas, B.; Mosandl, A. *J. High. Resolut. Chromatogr.* **1995**, *18*, 152.
(31) Guillaume, Y.; Guinchard, C. *Chromatographia* **1994**, *39*, 438.
(32) Guillaume, Y.; Guinchard, C. *Anal. Chem.* **1996**, *68*, 2869.
(33) Guillaume, Y.; Guinchard, C. *Anal. Chem.* **1997**, *69*, 183.
(34) Boots, H. M. J.; de Bokx, P. K. *J. Phys. Chem.* **1989**, *93*, 8243.
(35) Tchapla, A.; Heron, S.; Colin, H.; Guiochon, G. *Anal. Chem.* **1988**, *60*, 1443.
(36) Guggenheim G. *Application of Statistical Mechanics*; Clarendon Press: Oxford, 1996.