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Abstract

The theory and use of the "three-phase" model in enantioselective gas–liquid chromatography utilizing a methylated cyclodextrin/polysiloxane stationary phase is presented for the first time. Equations are derived that account for all three partition equilibria in the system, including partitioning between the gas mobile phase and both stationary-phase components and the analyte equilibrium between the polysiloxane and cyclodextrin pseudophase. The separation of the retention contributions from the achiral and chiral parts of the stationary phase can be easily accomplished. Also, it allows the direct examination of the two contributions to enantioselctivity, i.e., that which occurs completely in the liquid stationary phase versus the direct transfer of the chiral analyte in the gas phase to the dissolved chiral selector. Six compounds were studied to verify the model: 1-phenylethanol, α-ionone, 3-methyl-1-indanone, *o*-(chloromethyl)phenyl sulfoxide, *o*-(bromomethyl)phenyl sulfoxide, and ethyl *p*-tolylsulfonate. Generally, the cyclodextrin component of the stationary phase contributes to retention more than the bulk liquid polysiloxane. This may be an important requirement for effective GC chiral stationary phases. In addition, the roles of enthalpy and entropy toward enantiorecognition by this stationary phase were examined. While enantiomeric differences in both enthalpy and entropy provide chiral discrimination, the contribution of entropy appears to be more significant in this regard. The three-phase model may be applied to any gas–liquid chromatography stationary phase involving a pseudophase.

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Theory and Use of the Pseudophase Model in Gas−Liquid Chromatographic Enantiomeric Separations

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The theory and use of the "three-phase" model in enantioselective gas−liquid chromatography utilizing a methylated cyclodextrin/polysiloxane stationary phase is presented for the first time. Equations are derived that account for all three partition equilibria in the system, including partitioning between the gas mobile phase and both stationary-phase components and the analyte equilibrium between the polysiloxane and cyclodextrin pseudophase. The separation of the retention contributions from the achiral and chiral parts of the stationary phase can be easily accomplished. Also, it allows the direct examination of the two contributions to enantioselctivity, i.e., that which occurs completely in the liquid stationary phase versus the direct transfer of the chiral analyte in the gas phase to the dissolved chiral selector. Six compounds were studied to verify the model: 1-phenylethanol, α-ionone, 3-methyl-1-indanone, *o*-(chloromethyl)phenyl sulfoxide, *o*-(bromomethyl)phenyl sulfoxide, and ethyl *p*-tolylsulfonate. Generally, the cyclodextrin component of the stationary phase contributes to retention more than the bulk liquid polysiloxane. This may be an important requirement for effective GC chiral stationary phases. In addition, the roles of enthalpy and entropy toward enantiorecognition by this stationary phase were examined. While enantiomeric differences in both enthalpy and entropy provide chiral discrimination, the contribution of entropy appears to be more significant in this regard. The three-phase model may be applied to any gas−liquid chromatography stationary phase involving a pseudophase.

Separations involving micelles or cyclodextrins (aka pseudophase separations[1]) are often highly specific. Indeed, the complex combination of hydrophobic, electrostatic, and steric interactions of a solute with a micelle or cyclodextrin molecule cannot be duplicated by any traditional pure or mixed solvent system.[2,3] Moreover, the partitioning or binding of compounds to micelles or cyclodextrins is an important phenomenon in many other areas

of study, such as membrane mimetic chemistry,[4] catalysis,[5,6] enzyme modeling,[7] chromatography,[8−10] spectroscopic analysis,[11] emulsion polymerization,[12] and over the last 20 years, enantiomer separations.[13−17] Since the initial reports by Armstrong and co-workers on the use of micellar and cyclodextrin pseudophases in separations,[1,8−10,13−17] there has been constant development of this area in both chromatography and capillary electrophoresis (CE).[18−21] In liquid chromatography (LC), there are three solute equilibria: between the bulk solvent and the stationary phase; solvent and the pseudophase; plus pseudophase and the stationary phase. These equilibria can be characterized either as partition coefficients or as binding constants.[8−10,22] This "three-phase" model provided a thermodynamic description of retention in these systems. The pseudophase model was extended to capillary electrophoresis using micelles, cyclodextrins, and other additives.[23] Either a two- or three-phase model is used in CE depending on whether one or two pseudophase components are added to the running buffer, respectively. Despite the tremendous impact and utility of pseudophase separations in LC and CE, the pseudophase model has not been used in gas chromatography.

One goal of this work was to apply the model to gas−liquid chromatography (GLC). This might seem illogical at first, since the pseudophase in all past separations of this type is part of a liquid mobile phase (for HPLC) or running buffer (for CE).

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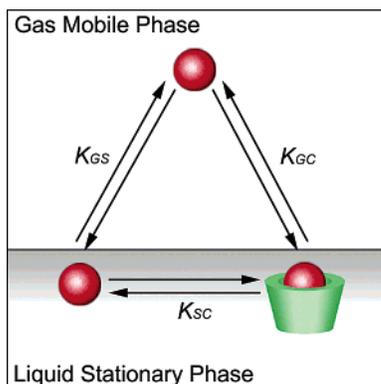
Figure 1. Three-phase model showing the equilibria implicated in gas chromatography when using dissolved cyclodextrins in a liquid polysiloxane stationary phase.

Obviously nonvolatile cyclodextrins, surfactants, etc., cannot be part of the carrier gas/mobile phase in GLC. However, GLC using dissolved cyclodextrins as chiral stationary phases must have three equilibria that control the separation, i.e., the partition equilibrium of the solute chromatographed between the gas mobile phase and the cyclodextrin in the stationary phase (K_{GC}), the equilibrium of the solute between the gas mobile phase and the bulk liquid component of the stationary phase (often a polysiloxane) (K_{GS}), and the equilibrium of the solute between the cyclodextrin dissolved in the stationary phase and that in the bulk liquid stationary phase (K_{SC}). Previously, several studies have been published that describe complexation and inclusion GLC using a two-equilibria model (retention increment concept).[24,25] However, the complete separation must be characterized by three partition coefficients (Figure 1). It is apparent that this system also can be described by a three-phase model, in a manner somewhat analogous to the original LC model.[8,10] Furthermore, this model provides a more accurate description of GLC enantiomeric separations for these specific systems, in that it can (for the first time) deconvolute the interactions that occur exclusively within the stationary phase from those that occur directly between the gas phase and the cyclodextrins. Thermodynamic data, generally obtained using a two-phase model[24,26] and retention increment concept,[24,25] may also be obtained using this method and may provide information regarding the process that drives the solute mobile−stationary phase exchange.

EXPERIMENTAL SECTION

Materials. The chiral analytes studied were 1-phenylethanol, α-ionone, and 3-methyl-1-indanone, all purchased from Aldrich (Milwaukee, WI), and *o*-(chloromethyl)phenyl sulfoxide, *o*-(bromomethyl)phenyl sulfoxide, and ethyl *p*-tolylsulfonate, which were prepared according to Anderson et al.[27] These chiral analytes were dissolved in methylene chloride (Fisher Scientific, Pittsburgh, PA) with concentrations of ∼2−5 ppm. The cyclodextrin used was heptakis-2,3,6-trimethyl-β-cyclodextrin (PM-β-CD) from Advanced

Separation Technologies (ASTEC, Whippany, NJ).[28] The coating solution consisted polysiloxane OV-1701 (14% cyanopropyl phenyl dimethyl polysiloxane) from Supelco (Bellefonte, PA) in dichloromethane. Dimethylphenyl deactivated polar fused-silica capillary columns (0.25-mm i.d.) were purchased from Supelco.

Methods. All capillary columns were coated by the static coating method. A 44-mg sample of OV-1701 polysiloxane was weighed and dissolved in 10 mL of dichloromethane. Increasing weights of permethylated cyclodextrin were added to the solution to produce a concentration range of 5.8−38.2% (w/w). The 0.25-mm-i.d. capillary was filled with the solution and sealed on both ends. The capillary was immersed in a 40 °C thermostatic bath, and one of its ends was opened and connected to a vacuum pump. Following the coating process, the coated columns were flushed with dry helium gas overnight and then conditioned from 30 to 150 °C at 1 °C/min. Evaporation of dichloromethane produced the deposition on the capillary internal wall of a regular polysiloxane film. The progress of the dichloromethane meniscus was watched and adjusted to be ∼1 cm/s. Overall, ∼3 h was needed to prepare a 10-m column. This procedure produces a film thickness of 0.28 μm. The exact characteristics of the columns are listed in Table 1. Column efficiency was tested using naphthalene at 100 °C. All columns had efficiencies between 2700 and 3400 plates/m. The stationary-phase enantioselectivity was evaluated daily using 1-phenylethanol at 100 °C.

The partial specific volume of PM-β-CD was determined using a pycnometer. A set amount of cyclodextrin was added to the pycnometer, which was then filled to the mark with hexane. The difference in weight between the known volume of pure hexane and the same volume including cyclodextrin was used to calculate the partial specific volume of the cyclodextrin.

A Hewlett-Packard 5890 model II gas chromatograph (GC) was used for all separations. Split injection was utilized with a split ratio of 100:1 and a flow rate of 1.0 mL/min. The flow rate was determined using a soap bubble flowmeter at the outlet of the GC column. Flame ionization detection was employed with injection and detection temperatures of 250 °C. Helium was used as the carrier gas. Methane was used to determine the dead volume of the column. All separations were performed under isothermal conditions.

Derivation of Partition Coefficient Relationships. Separations in gas−liquid chromatography using derivatized cyclodextrins as chiral dissolved components in achiral stationary-phase liquids are the result of three equilibria: i.e., the partition equilibria of the solutes chromatographed between the mobile phase and the cyclodextrin in the stationary phase; mobile phase and the bulk polysiloxane of the stationary phase; cyclodextrins in the stationary phase and the polysiloxane of the stationary phase, which are characterized by the partition coefficients K_{GS}, K_{GC}, and K_{SC}, respectively (Figure 1). The partitioning of the analyte from the mobile phase to the stationary phase is a surface adsorption process, in which the solute adsorbs to the surface of the polysiloxane or to a cyclodextrin at the gas−liquid interface. Upon adsorption, the analyte may then distribute itself throughout the volume of the stationary phase. The likelihood of a solute molecule partitioning directly from the mobile phase to either the polysiloxane or a cyclodextrin is regulated by the relative coverage

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Table 1. Physicochemical Characteristics of the Eight Capillary Columns[a]

	cyclodextrin (% w/w)							
	0	5.8	7.9	12.5	22.4	24.9	30	38.2
column length (m)	10.85	8.23	11.22	6.95	8.00	10.48	8.18	10.35
V_s (mm³)	2.39	1.82	2.48	1.54	1.76	2.32	1.81	2.29
m_s (mg)	2.39	1.84	2.52	1.58	1.83	2.42	1.90	2.43
st phase density (g/cm³)	1.000	1.012	1.016	1.024	1.041	1.045	1.052	1.063
mass cyclodextrin (mg)[b]	0.00	2.55	3.48	5.50	9.86	10.96	13.20	16.81
mass OV-1701 (mg)[b]	44.00	44.00	44.00	44.00	44.00	44.00	44.00	44.00
approx molar fraction CD	0.000	0.670	0.734	0.814	0.887	0.897	0.913	0.930
approx molar fraction OV-1701	1.000	0.330	0.266	0.186	0.113	0.103	0.087	0.070
concn cyclodextrin (M)	0.000	0.039	0.052	0.080	0.133	0.146	0.170	0.206
approx concn OV-1701 (M)	0.0200	0.0191	0.0188	0.0182	0.0170	0.0167	0.0162	0.0154

[a] For all columns, the capillary internal diameter is 0.25 mm, the stationary-phase film thickness is ~0.28 μm, and the geometrical phase ratio $\phi = V_s/V_m$ is 0.0045. The experimental phase ratios used in our calculations (V_s/(dead volume)) were 2−40% higher depending on column length, temperature, and mobile-phase inlet pressure since gases are compressible. [b] Masses weighted and dissolved in 10 mL of dichloromethane, the solution used to coat the columns.

of the two on the surface of the stationary phase. However, in accordance with the traditional definition of partition coefficients, the total volume of polysiloxane or cyclodextrin in the stationary phase was used in the calculations for the K_{GS} and K_{GC} partition coefficients in this study, despite the fact that an adsorption step is initially involved.[8] These equilibrium expressions may then be described by the following equations:

$$S_{(g)} \xrightleftharpoons{K_{GS}} S_{(Si)} \qquad K_{GS} = C_S^{Si}/C_S^G \qquad (1)$$

$$S_{(g)} \xrightleftharpoons{K_{GC}} S_{(CD)} \qquad K_{GC} = C_S^{CD}/C_S^G \qquad (2)$$

$$S_{(Si)} \xrightleftharpoons{K_{SC}} S_{(CD)} \qquad K_{SC} = C_S^{CD}/C_S^{Si} \qquad (3)$$

where S is the analyte in the gas and liquid polysiloxane phases and the cyclodextrin pseudophase; C_S^G, C_S^{Si}, and C_S^{CD} are the concentrations of the analyte in the gas and polysiloxane phases and cyclodextrin pseudophase, respectively; and K_{GS}, K_{GC}, and K_{SC} are the respective partition coefficients. Based on these equilibria, a relationship between K_{GS}, K_{GC}, and K_{SC} is found:

$$K_{GC} = K_{GS}K_{SC} \qquad (4)$$

The measured apparent partition coefficient (K) obtained through experimental gas chromatography can be defined as

$$K = \frac{(m_S^{Si} + m_S^{CD})/(V_{Si} + V_{CD})}{m_S^G/V_G} \qquad (5)$$

where m_S is the mass of the analyte in each respective phase and V is the volume of each phase. This expression is analogous to the equations previously used to describe micelle− and cyclodextrin−water−octanol partition coefficients.[29,30] V_G is equivalent to the void volume of the column, which was calculated using the void time and the column flow rate ($V_G = t_m F$). The total volume of the stationary phase (V_S) is simply obtained by $V_S =$

$V_{Si} + V_{CD} = L\pi[R^2 − (R − R_a)^2]$, where L is the length of the column, R is the radius of the column, and R_a is the thickness of the stationary phase. R_a is calculated using $R_a = R/(2c)$[31] with c being the ratio of the solvent volume (dichloromethane) to the stationary-phase volume (CD and polysiloxane) in the coating solution. Using the partition coefficient between the bulk polysiloxane liquid and the cyclodextrin,

$$K_{SC} = \frac{(m_S^{CD}/V_{CD})}{(m_S^{Si}/V_{Si})} \qquad (6)$$

the following equation can be produced by solving for m_S^{CD} in eq 6 and substituting this into eq 5:

$$K = (m_S^{Si}/V_{Si})\left[K_{SC}\left(\frac{V_{CD}}{V_{CD} + V_{Si}}\right) − \left(\frac{V_{CD}}{V_{CD} + V_{Si}}\right) + 1\right]\{m_S^G/V_G\}^{-1} \qquad (7)$$

A relationship between the concentration of cyclodextrin (C_{CD}) and the volumes of the cyclodextrin and polysiloxane phases exists involving the partial specific volume of the cyclodextrin (v_p):

$$C_{CD}v_p = V_{CD}/(V_{CD} + V_{Si}) \qquad (8)$$

The partial specific volume of PM-β-CD used in all calculations in this experiment was 1.122 L/mol. This value was determined as stated in the Experimental Section. Finally, by substituting eq 8 into eq 7 one obtains

$$K = v_p K_{GS}(K_{SC} − 1)C_{CD} + K_{GS} \qquad (9)$$

Plotting K versus C_{CD} allows all three partition coefficients to be determined. K_{GS} can be found directly from the y-intercept, while the $K_{SC} = \text{slope}/(y\text{-int.}v_p) + 1$. K_{GC} may then simply be calculated

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Table 2. Calculated Partition Coefficients for the Studied Compounds

compound	enantiomer[a]	T (°C)	$K_{GS} \pm SD^b$	$K_{GC} \pm SD^b$	$K_{SC} \pm SD^b$
o-(chloromethyl)phenyl sulfoxide	1	130	3190 ± 150	21500 ± 300	6.8 ± 0.3
	2		3170 ± 160	23900 ± 100	7.5 ± 0.4
1-phenyl-1-ethanol	1	100	690 ± 40	17700 ± 900	25.6 ± 0.6
	2		600 ± 70	20800 ± 500	34.5 ± 2.7
α-ionone	1	120	3150 ± 110	11100 ± 300	3.5 ± 0.1
	2		3110 ± 100	13200 ± 400	4.2 ± 0.1
3-methyl-1-indanone	1	130	1380 ± 60	8020 ± 210	5.8 ± 0.3
	2		1380 ± 40	9250 ± 180	6.7 ± 0.1
o-(bromomethyl)phenyl sulfoxide	1	130	5180 ± 290	38100 ± 200	7.4 ± 0.5
	2		5120 ± 330	43600 ± 300	8.5 ± 0.7
ethyl p-tolylsulfonate	1	115	7880 ± 430	43800 ± 300	5.6 ± 0.4
	2		7860 ± 480	48300 ± 800	6.1 ± 0.5

[a] Based on the elution order of the enantiomers [b] SD, standard deviations of the calculated partition coefficients (determined from four independent measurements)

using eq 4.[29,30] Alternately, the experimental partition ratio (k') may be used in place of K, producing a y-intercept of k'_{GS} and a slope of $v_p k'_{GS}(K_{SC} - 1)$. From these data, it is possible to obtain definitive information about the interactions that each enantiomer experiences when undergoing an enantiomeric separation by GC. Additionally, one can pinpoint the particular interactions/equilibria that contribute to both retention and enantioselectivity.

Alternatively, the association of the analyte in the bulk liquid polysiloxane phase with a cyclodextrin dissolved in the stationary phase can be given in terms of a binding constant.[26] The 1:1 complexation of the analyte with cyclodextrin may be described by the equilibrium

$$S_{(Si)} + CD_{(Si)} \xrightleftharpoons{K_{eq}} S \cdot CD_{(Si)} \qquad K_{eq} = \frac{[S \cdot CD]}{[S][CD]} \qquad (10)$$
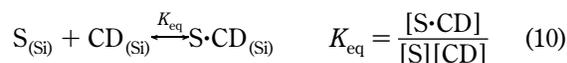
where K_{eq} is the equilibrium binding constant. Using eqs 10 and 8, the relationship between K_{SC} and K_{eq} is found to be

$$K_{eq} = K_{SC} v_p \qquad (11)$$

at relatively low cyclodextrin concentrations (at high [CD], the activity of the CD should be used in place of the concentration). It is important to note that these equations are completely valid for any GC experiment involving a pseudophase (e.g., micelle, polymer, protein, cyclodextrin, nanoparticle, etc.). Indeed, it is the only method currently known that provides both the association constant of an analyte in the gas phase to a ligand in the condensed phase and the liquid-phase association of the two molecules, from the same experiment.

RESULTS AND DISCUSSION

Analyte Partitioning. Using eq 9, K was plotted against the cyclodextrin concentration in the stationary phase. Figure 2 shows examples of the types of plots that are produced. Good correlations were obtained for these plots ($R^2 > 0.95$), particularly considering that each point in the plot corresponds to a separate column prepared with a different concentration of the cyclodextrin derivative. The corresponding calculated partition coefficients are shown in Table 2. K_{eq}, the binding constant of the analyte dissolved in the bulk polysiloxane to the dissolved cyclodextrin pseudophase,
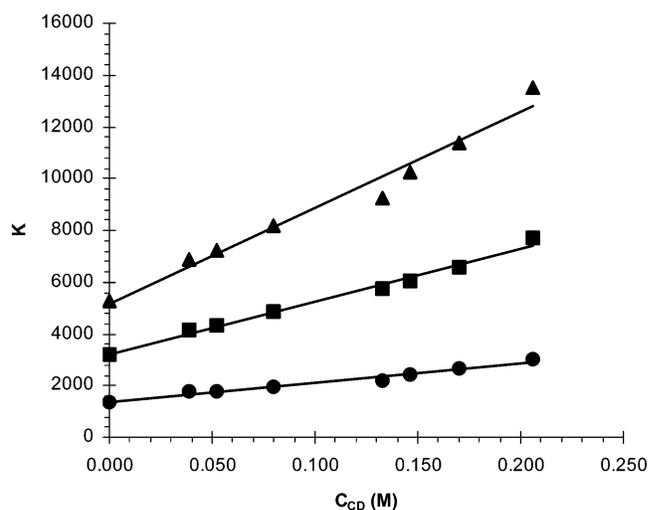

Figure 2. Plots of eq 9 for the first eluted enantiomer of o-(bromomethyl)phenyl sulfoxide (■), o-(chloromethyl)phenyl sulfoxide (▲), and 3-methyl-1-indanone (●).

Table 3. Calculated Binding Constants for the Studied Compounds between the Polysiloxane Phase and Bound to Cyclodextrin

compound	enantiomer[a]	T (°C)	$K_{eq} \pm SD^b$
o-(chloromethyl)phenyl sulfoxide	1	130	7.6 ± 0.3
	2		8.5 ± 0.4
1-phenyl-1-ethanol	1	100	28.7 ± 0.6
	2		38.7 ± 3.0
α-ionone	1	120	4.0 ± 0.1
	2		4.8 ± 0.1
3-methyl-1-indanone	1	130	6.5 ± 0.3
	2		7.6 ± 0.1
o-(bromomethyl)phenyl sulfoxide	1	130	8.2 ± 0.5
	2		9.6 ± 0.8
ethyl p-tolylsulfonate	1	115	6.2 ± 0.4
	2		6.9 ± 0.6

[a] Based on the elution order of the enantiomers. [b] SD, standard deviations of the calculated partition coefficients (determined from four independent measurements).

was calculated using eq 11, and the results are shown in Table 3. The standard deviations of these data, based on four independent measurements, are generally <5%. For all the analytes in this study, the K_{GS} values for both enantiomers are statistically
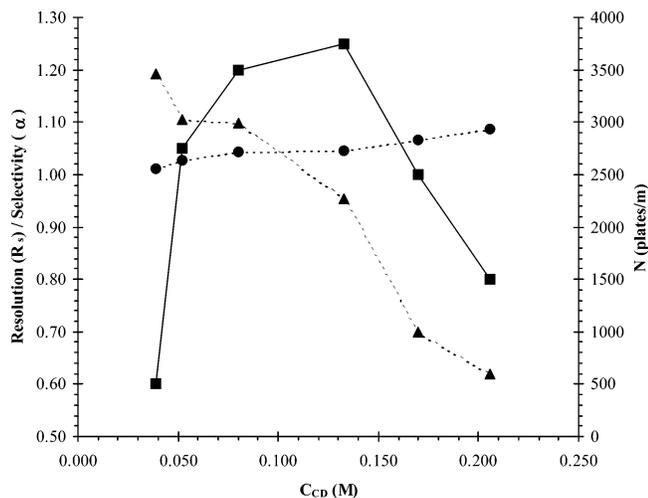
Figure 3. Effect of stationary-phase cyclodextrin concentration on selectivity (●), resolution (■), and efficiency (▲) of the enaniosepration of α-ionone.

Table 4. Calculated Polysiloxane and Cyclodextrin Coverage on the Surface of the Stationary Phase for the Eight Columns in This Study

cyclodextrin % (w/w)	total surface area (mm²)	polysiloxane surface area (mm²)	cyclodextrin surface area (mm²)	polysiloxane area/ cyclodextrin area
0	15 300	15 300	0.0	n/a
5.8	12 400	11 900	500	23.8
7.9	15 700	14 800	900	16.4
12.5	10 900	9 900	1000	9.9
22.4	12 100	10 300	1800	5.7
24.9	14 900	12 500	2400	5.2
30.0	12 300	10 000	2300	4.3
38.2	14 800	11 400	3400	3.4

equivalent within the margin of error. This trend is expected since the polysiloxane stationary phase is an achiral environment and cannot discriminate between enantiomers. However, each analyte's enantiomers have differing K_{GC}, K_{SC}, and K_{eq} values, indicating that the cyclodextrin in the stationary phase is controlling the chiral separation. Furthermore, by definition, the second eluting enantiomer always produces a higher K_{GC}, K_{SC}, and K_{eq}.

The data in Table 2 also indicate several other interesting aspects about cyclodextrin-based GC chiral stationary phases (which may be true for other types of CSPs as well). Clearly the association/partitioning of the solute to the chiral selector portion of the stationary phase (K_{GC}) is much greater than its association/partitioning to the bulk achiral portion of the stationary phase (K_{GS}). Hence, the cyclodextrin has a relatively greater effect on retention than the polysiloxane component of the stationary phase. This fact permits lower concentrations of cyclodextrins to be used in the stationary phase while still maintaining their effectiveness, since the solutes preferentially partition to the cyclodextrin over the bulk polysiloxane. Indeed, it may be this preferential partitioning to the chiral selector over the achiral matrix that helps make the entire class of cyclodextrin and polysiloxane CSPs so effective in GC. Figure 3 shows the effect of methylated cyclodextrin concentration in the stationary phase on the measured enantioselectivity, resolution, and efficiency for the separation of α-ionone enantiomers. Generally, the selectivity of the enantioseparation increases with the cyclodextrin concentration. However, as reported by Schurig et al.,[32] it is likely that a maximum selectivity could be reached at a higher cyclodextrin concentration than was examined in this study. Generally, a maximum peak-to-peak separation may be obtained at a defined cyclodextrin concentration. Beyond this point, each solute enantiomer experiences too long of a residence time in the cyclodextrin. The cyclodextrin may therefore no longer effectively discriminate between the solute moieties, resulting in a loss of enantioselectivity.[20] The 38.2% w/w cyclodextrin was the highest concentration examined here, as columns with higher cyclodextrin concentrations are difficult to produce due to the limited solubility of methylated cyclodextrin

in the bulk liquid polysiloxane. Despite the increasing enantioselectivity, the efficiency of the separations decreases steadily with increasing cyclodextrin in the stationary phase due to poor mass transfer. Therefore, by balancing these two factors, a maximum resolution may be obtained at relatively low cyclodextrin concentrations. As shown in Figure 3, the optimum resolution for α-ionone enantiomers was at ~0.130 M cyclodextrin.

Stationary-Phase Surface Coverage. As stated earlier, it is important to note that the partitioning of the analyte directly between the gas mobile phase and the cyclodextrin (K_{GC}) may only occur with cyclodextrins at the gas−liquid interface. Furthermore, the probability of a solute molecule in the mobile-phase partitioning directly to either the polysiloxane or a cyclodextrin is controlled by their relative stationary-phase surface coverage. As a result, it may be useful to approximate the polysiloxane and cyclodextrin coverage on the surface of the stationary phase that is available for analyte partitioning. To accomplish this, the total surface area of the stationary phase exposed to the gas mobile phase was calculated by

$$A = L2\pi(R - R_a) \qquad (12)$$

The relative coverage of the polysiloxane and the methylated cyclodextrin differs for each column, correlating directly to the level of cyclodextrin dissolved in the stationary phase. Using the percent volume of cyclodextrin present in the stationary phase, an approximation of the stationary-phase surface area occupied by cyclodextrin molecules may be calculated. Table 4 shows the calculated polysiloxane and cyclodextrin coverage area for each of the prepared columns. Clearly, the partitioning kinetics of the analyte is greatly affected by the relative surface coverage of the polysiloxane and cyclodextrin. Therefore, as the cyclodextrin area increases, analyte partitioning to this pseudophase (K_{GC}) must play a more significant role.

Thermodynamic Study. A thermodynamic examination was carried out to study the magnitude of the free energies for each equilibrium and to determine the contribution of enthalpy and entropy toward the chiral recognition of enantiomers by the stationary phase. The free energies relating to each individual partition coefficient may be easily calculated using

$$\Delta G = -RT \ln K \qquad (13)$$

(32) Jung, M.; Schmalzing, D.; Schurig, V. *J. Chromatogr.* **1991**, *552*, 43−57.

Table 5. Gibbs Free Energies Corresponding to the Individual Partition Coefficients[a]

compound	enantiomer[b]	T (°C)	K_{GS}	ΔG_{GS} (J/mol)	K_{SC}	ΔG_{SC} (J/mol)	K_{GC}	ΔG_{GC} (J/mol)
o-(chloromethyl)phenyl	1	130	3190	−27 000	6.8	−6 400	21 500	−33 400
sulfoxide	2		3170	−27 000	7.5	−6 770	23 900	−33 800
1-phenyl-1-ethanol	1	100	690	−20 300	25.6	−10 100	17 700	−30 400
	2		600	−19 900	34.5	−11 000	20 800	−30 900
α-ionone	1	120	3150	−26 300	3.5	−4 130	11 100	−30 500
	2		3110	−26 300	4.2	−4 720	13 200	−31 000

[a] Column 12.5% w/w CD with $\%_{CD}$ = 8.9% v/v and $\%_{Si}$ = 91.1% v/v. [b] Based on the elution order of the enantiomers.
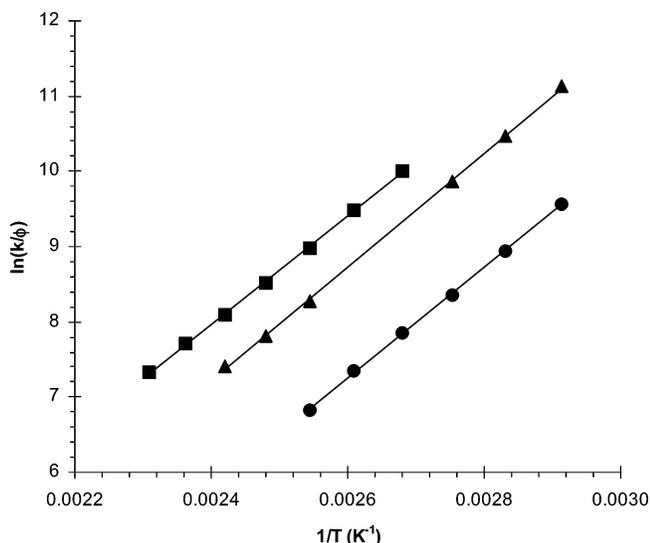

Figure 4. Van't Hoff plots for the first eluted enantiomer of o-(chloromethyl)phenyl sulfoxide (■), α-ionone (▲), and 1-phenyl-1-ethanol (●).

These values are shown in Table 5 and provide useful information regarding the thermodynamic process that drives the solute mobile−stationary-phase exchange. In addition, the relationship between all three free energies ($\Delta G_{GS} + \Delta G_{SC} = \Delta G_{GC}$) can be confirmed.

The enthalpy and entropy contributions to the free energy of the global solute mobile−stationary-phase exchange are typically studied by preparing van't Hoff plots using the following equation:

$$\ln\frac{k}{\phi} = \frac{\Delta S}{R} - \frac{\Delta H}{R}\cdot\frac{1}{T} \qquad (14)$$

A plot of $\ln(k/\phi)$ versus $1/T$ allows ΔS, ΔH, and ΔG for each enantiomer to be obtained.[33] The working range of temperatures was selected to provide enantioresolution of both enantiomers. This equation produces straight lines with regression coefficients (R^2) higher than 0.9995 for each studied enantiomer (Figure 4). Furthermore, by performing separate K_{GS}, K_{GC}, and K_{SC} determinations at varying temperatures and applying these values to eq 15 ($K = k/\phi$), it should be possible to determine ΔS, ΔH, and ΔG for each partition coefficient. While only the global thermodynamic properties of the solute mobile−stationary-phase exchange were examined in this work, using this model, it is

theoretically possible (for the first time) to determine the contribution of ΔS and ΔH to ΔG for each individual partition exchange.

Alternatively, an adaptation of eq 13 may be used to relate the partition coefficients to the free energy of the global analyte mobile−stationary-phase exchange

$$\Delta G = -RT\ln(\%_{CD}K_{GC} + \%_{Si}K_{GS}) \qquad (15)$$

where the percent factors are the volume fractions of the cyclodextrin and polysiloxane components in the stationary phase, respectively. Therefore, a comparison can be made between the ΔG values obtained using the van't Hoff plot (eq 15) and the free energies calculated using the partition coefficients (eq 16). Table 6 shows the values of ΔG calculated using both techniques, as well as the contributions of ΔS and ΔH to the free energy. Excellent correlation (within 2%) between the two free energy calculations exists, indicating the validity of eq 9.

Generally, the difference between the ΔG values of enantiomers ($\sim$0.1−0.4 kJ/mol) is relatively small when compared to the free energy of the global solute−stationary-phase exchange ($\sim$20−30 kJ/mol). This 2 orders of magnitude difference demonstrates that these enantioselective interactions can be delicate and difficult to obtain. It is apparent from the measured enthalpy and entropy values (Table 6) that ΔH facilitates the free energy of transfer of solutes to the stationary phase while ΔS has the opposite effect. However, the magnitude of the entropic contribution ($T\Delta S$) to the free energy is roughly 50% that of the enthalpic contribution, resulting in a net negative free energy. Despite this trend, enantiorecognition benefits from both the enantiomeric differences in entropy and enthalpy, as shown by the ratio of the enantiomers' enthalpies and entropies in Table 6. Overall, the entropy term provides more differentiation between enantiomers than the enthalpy term for all three of the analytes studied. However, as discussed by Schurig,[25,34] the selectivity of the system, in terms of free energy, is temperature dependent as defined by

$$-\Delta_{R,S}(\Delta G) = -\Delta_{R,S}(\Delta H) + T\Delta_{R,S}(\Delta S) \qquad (16)$$

Because a stronger analyte−cyclodextrin complex has less disorder, the contributions of $-\Delta_{R,S}(\Delta H)$ and $\Delta_{R,S}(\Delta S)$ compete for determining the selectivity, $-\Delta_{R,S}(\Delta G)$. At the isoenantioselective temperature (T_{iso}), the two contributions balance one another completely, resulting in a net $-\Delta_{R,S}(\Delta G)$ of zero.

(33) Berthod, A.; Li, W.; Armstrong, D. W. *Anal. Chem.* **1992**, *64*, 873.

(34) Schurig, V. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 747−765.

Table 6. Comparison of the Gibbs Free Energies Obtained Using van't Hoff Plot Regression and Solute Partition Coefficients[a]

compound	enantiomer[b]	T (°C)	ΔH (J/mol)	$\Delta H_2/\Delta H_1$	ΔS (J/mol K)	$\Delta S_2/\Delta S_1$	ΔG (J/mol)	K_{GS}	K_{GC}	ΔG (J/mol)
o-(chloromethyl)phenyl sulfoxide	1	130	−60 000	1.024	−77.9	1.041	−28 700	3190	21 500	−28 400
	2		−61 500		−81.1		−28 800	3170	23 900	−28 600
1-phenyl-1-ethanol	1	100	−60 900	1.038	−98.0	1.056	−24 300	690	17 700	−23 900
	2		−63 200		−103.5		−24 600	600	20 800	−24 200
α-ionone	1	120	−62 700	1.040	−90.4	1.065	−27 100	3150	11 100	−27 000
	2		−65 200		−96.3		−27 300	3110	13 200	−27 100

[a] Column 12.5% w/w CD with %$_{CD}$ = 8.9% v/v and %$_{Si}$ = 91.1% v/v. [b] Based on the elution order of the enantiomers.

Therefore, at temperatures below T_{iso}, the selectivity of the system is influenced more heavily by $-\Delta_{R,S}(\Delta H)$, while at temperatures above T_{iso}, $\Delta_{R,S}(\Delta S)$ provides a greater contribution.[25,34]

CONCLUSIONS

For the first time, the "three-phase" model has been applied to gas−liquid chromatography utilizing a cyclodextrin pseudophase. Important information regarding the mechanism of chiral recognition in cyclodextrin GLC may be obtained. With this model, one can assess the relative importance of analyte partitioning to/between the three phases in regard to both enantioselectivity and retention. Generally, analyte partitioning to the cyclodextrin pseudophase was greater than to the bulk liquid polysiloxane, resulting in the cyclodextrin having a more significant effect on retention. This may be one of the more important factors that control the overall effectiveness of any CSP that is composed of distinct chiral and achiral components. The analyte partitioning between the polysiloxane and cyclodextrin components of the stationary phase was several orders of magnitude smaller than the equilibria from the gas mobile phase. As expected, enantio-recognition was isolated only to equilibria involving the cyclodextrin component of the stationary phase. The thermodynamic studies indicated that the entropy was pivotal in the chiral recognition process. Further van't Hoff plots of the three individual partition coefficients may eventually allow the thermodynamic components of each to be isolated.