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Selectivity in Capillary Electrochromatography Using Native and Single Isomer Anionic Cyclodextrin Reagents

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Separations of naphthalene compounds that differ in position of substitution and type of substituent were accomplished using cyclodextrin distribution capillary electrochromatography. Separation systems composed of running buffers containing mixtures of native neutral and single isomer anionic cyclodextrins (CDs) were employed yielding efficiencies of approximately 200 000 plates/meter. Solute migration rates and relative orders can be readily modified by changing CD types and concentrations. Experiments were performed to determine distribution coefficients between each of the CDs used in these studies and an aqueous running buffer. For this work, naphthalene-CD cavity inclusion is assumed to be the principal mode of interaction. The distribution coefficients for carboxymethyl-*β*-cyclodextrin (CM-*β*-CD), degree of substitution 1, were 10−70% larger than those for native *β*-CD and 75−1800% larger than those for *γ*-CD. The CM-*β*-CD was singly charged and yielded a narrow elution window. Nevertheless, baseline resolution was achieved for several substituted naphthalene compounds using CM-*β*-CD in conjunction with *β*-CD or *γ*-CD. Under certain conditions, the *γ*-CD system yielded an elution order that differed from that of the *β*-CD system. Heptakis-(2,3-dimethyl-6-sulfato)-*β*-CD with its −7 charge produced a much larger elution window. The extensive substitution with sulfonic groups at the truncated bottom of the CD seemed to inhibit inclusion as the distribution coefficients for the naphthalene compounds were generally more than an order of magnitude smaller than those for CM-*β*-CD. Moreover, there was evidence that this sulfato-CD interacted with both the capillary wall and neutral *β*-CD. This work differs from prior uses of CDs in that relatively complicated mixtures of neutral, achiral compounds are separated using combinations of recently developed single-isomer CDs as running-buffer additives. The single-isomer CDs, as opposed to most highly complex derivatized CD products, facilitate predictions of separation performance for multicomponent samples. In this manner, the ability to use knowledge of distribution coefficients to predict elution characteristics for a ternary CD system is demonstrated.

Capillary electrophoresis (CE) has been established as a powerful technique for separating an unusually large variety of compounds. This versatility is due in part to the ease and speed with which the CE running buffer can be altered with a variety of reagents to influence the migration rates of injected compounds. Included among these additives are a variety of macrocycle reagents such as cyclodextrins (CDs), crown ethers,[1] calixarenes,[2] antibiotics,[3] etc. Since their first reported use in CE by Terabe and co-workers,[4] CDs have received the greatest attention.

Cyclodextrins are composed of a number of D(+)-glucose units connected by α-(1,4)-linkages. They are doughnut-shaped with all the glucose units in a largely undistorted chair conformation. The interior cavity of the CD is relatively nonpolar and can accommodate many classes of compounds. Inclusion complex formation between guest compounds and the CD host is influenced by the physiochemical properties of both the guest and host. In addition to these unique characteristics, these chiral CDs are moderately water soluble and can be derivatized with a wide variety of functional groups. These properties render CDs immensely useful as reagents in CE.

CDs have been used in many different CE modes. In the native (neutral) form they are used extensively to enantioselectively moderate the migration rates of charged chiral compounds.[5] Similarly, neutral chiral compounds have been separated using derivatized charge CDs.[5] While our efforts have included chiral separations,[6,7] the focus has been largely on the uses of CDs for separations of multicomponent mixtures of neutral, achiral compounds. Initially, this involved employing neutral CDs (native or derivatized) in conjunction with surfactants in micellar electrokinetic capillary electrophoresis (MEKC).[8,9] In this instance, the CD selectively modifies migration rates by reducing the interactions of neutral solutes with the charged MEKC micellar phase.

There is also a mode of CE in which the running buffer contains two different CDs, one neutral and the other charged.

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This approach was first realized by Lurie and co-workers[10] and later by other researchers for separations of charged[11,12] or neutral[13] chiral compounds. The use of such dual CD systems for separations of neutral achiral compounds was first reported in 1995.[14−17] The technique was dubbed cyclodextrin distribution capillary electrochromatography (CDCE) to indicate the mode of separation involves a differential distribution of solutes between CDs that exhibit different intrinsic mobilities. The CDCE technique offers distinct advantages over MEKC, with the most significant being a higher level of selectivity.[15,16] This selectivity results from the unique molecular recognition properties of CDs; properties which vary significantly among the native and especially among the derivatized forms of the CDs. Derivatization can occur at the 2- or 3-position secondary −OH groups on the face of the CD or at the 6-position primary −OHs on the truncated bottom of the macrocycle.

An intriguing feature of this selectivity characteristic of CDCE is that it raises the possibility of rationally designing separation systems using simple to moderately complex combinations of CDs. If the CDs do not interact with each other (a provision that the work presented herein questions for certain systems), then the effects of the CDs on solute migration can be predicted. The predictions require knowledge of the relative strength of interaction between the guest (sample solute) and hosts (CDs) in the CDCE system and the migration properties of the hosts. Earlier work was complicated by the enormous complexity of commercially available derivatized CDs. The presence of a wide and uncharacterized range of CDs with regard to degree of substitution (DS) and position of substitution in these products makes general predictions of migration behavior difficult and specific studies of molecular recognition impossible.[16,18] Chen and co-workers developed a model for describing the migration behavior using systems containing derivatized CDs that possess a wide range of CD products.[19] Their successful model requires that the composition of the multi-isomer, multi-DS product remain constant. Unfortunately, derivatized CDs from different syntheses or commercial lots cannot be expected to have the same composition. Nevertheless, their theoretical approach is similar to that employed in this work. Recently, single-isomer CD products have been synthesized in the laboratory[18,20,21] and made commercially available (see Experimental Section). This has opened the way for the work presented herein.

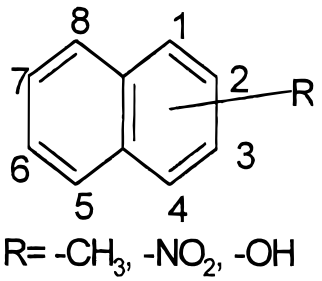

Figure 1. Structures of substituted naphthalene test solutes.

The strength of interaction between solute and CD can be marked using inclusion constants which can be determined by spectroscopic,[22] chromatographic,[23] or CE methods.[24,25] The object of the selectivity studies presented herein are similar to that pursued by Gareil and co-workers[11] and Surapaneni and co-workers[13] for separations of pairs of enantiomers using a dual-CD systems. We differ in that our CDCE systems involve a greater number of CDs and our samples are multicomponent and achiral. The theoretical approach is also somewhat different and makes extensive use of experimentally measured distribution coefficients (*K*s). Inclusion constants are not determined using any of the methods mentioned above, but large *K* values should translate into large inclusion constants. The ability to use experimentally determined distribution coefficients to predict selectivity (solute mobility) is demonstrated.

EXPERIMENTAL SECTION

Materials. 1,3-dinitronaphthalene (1,3-DNN), 1,5-dinitronaphthalene (1,5-DNN), 1,8-dinitronaphthalene (1,8-DNN), 1,5-dimethylnaphthalene (1,5-DMN), and 1,5-dihydroxynaphthalene (1,5-DHN) were obtained from Aldrich Chemical Co. (Milwaukee, WI). Mestyl oxide (MO) was also obtained from Aldrich. 2,7-Dinitronaphthalene (2,7-DNN) was obtained from Fluka Chemical Co. (Ronkonkoma, NY). A depiction of the test solute structures is provided in Figure 1. β-Cyclodextrin (β-CD) was obtained from Sigma Chemical Co. (St. Louis, MO), Carboxymethyl-β-Cyclodextrin, degree of substitution (DS) 1 (CM-β-CD), and the sodium salt of heptakis (2,3-dimethyl-6-sulfato)-β-Cyclodextrin (HDMS-β-CD) were obtained from Regis Technologies, Inc. (Morton Grove, IL). γ-CD was obtained from CTD, Inc. (Gainesville, FL). CE buffers were prepared with distilled, deionized water and reagent-grade NaH_2PO_4 and adjusted to the required pH with H_3PO_4. Phthalic acid was used for the determination of the elution time of the charged cyclodextrin (t_{ch}) and was obtained from Eastman Kodak Chemical Co. (Rochester, N. Y.).

Apparatus and Methods. All CE experiments were performed using a Hewlett-Packard automated Capillary Electrophoresis system (HP^{3D}CE) interfaced to a HP Pentium I personal computer. Fused silica capillaries (50 μm i.d. × 360 μm o.d.) were obtained from Polymicro Technologies Inc. (Phoenix, AZ). The

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capillaries were cut to a total length of 48 cm, and the capillary coating was removed at 39.5 cm for purposes of performing detection.

Procedure for Preparation of Running Buffer. The running buffers used in the separations were a 25 mM phosphate buffer at pH 5.0 for the CM-β-CD systems and at pH 7.5 for the HDMS-β-CD systems. The lower pH was chosen for the CM-β-CD systems to allow for a larger elution window. However, because of the low capacity of the buffer at pH 5.0, the pH should be monitored closely. The CDs were dissolved at concentrations between 2 and 10 mM. The pH of the solutions was adjusted using an Orion model SA520 pH meter. Before each pH measurement, the pH meter was calibrated using a standard solution. To remove the dissolved gases in the running buffer, the solution is first sonicated and then filtered using a 0.22-μm nylon filter. Finally, it is placed under vacuum conditions for approximately 10 min. The solutions were stored in the refrigerator because it was observed that after about 2 days standing at room temperature the buffer would degrade due to microorganism growth. It was found that by refrigerating the solutions, the buffers containing γ-CD, β-CD, and CM-β-CD were stable for about 3 days. However, the HDMS-β-CD buffer solutions could not be used after about 36 h even with refrigeration.

Sample Preparation. The samples were prepared by dissolving the test solutes in either the running buffer or in a running buffer/methanol (20−40 v/v%) mixture. The sample solutions contained the solutes at concentrations ranging from 10^{-6} to 10^{-5} M. Some solutes were present near their solubility limit; methanol was used to increase the solubility of the sample in solution. Methanol was not used in the running buffer.

Separation Conditions. It is well-known that migration times in CE can be irreproducible due to changes in electroosmotic flow (μ_{eo}). With the addition of CDs to the running buffer, which can adsorb to the capillary walls, the problem is augmented. To minimize fluctuations in μ_{eo}, a special flush procedure was developed and applied before each injection. First, the capillary is flushed with 0.1 M NaOH for 5 min. Next, a solution of 0.1 M NaOH and 5% SDS (w/w) is run through the capillary for 15 min. This is followed by flushing for 2 min with distilled water and then filling the capillary with buffer solution.

Injection was achieved by the application of 10 mbar of pressure for 6 s to the inlet buffer vial. The applied potential for all CE experiments was 15 kV. UV absorbance detection was performed at wavelengths ranging from 205 to 254 nm for all the test solutes. All experiments were conducted at room temperature (24 ± 1 °C). Since distribution coefficients are temperature-dependent, studies need to be perform at a relatively constant temperature. This also applies to maintaining constant electrophoretic parameters (currents, voltages, etc.) that may also influence capillary temperature.

Experiments conducted to determine distribution coefficients (*K*s) can be classified into two groups. *Experiment 1:* These experiments are performed to determine the *K* values of the test solutes with charged CD. The running buffer solutions contained only charged CDs. Capacity factors (k') were calculated for each test solute for charged CD concentrations normally ranging from 2 to 10 mM. *Experiment 2:* To determine the *K* values of the test solutes with the neutral CDs, the concentration of charged CD was kept constant (typically 8 mM) and the concentration of neutral CD was varied from 2 to 10 mM. The k' values were obtained for each test solute. Peaks were assigned to the individual test solutes using a combination of spiking experiments and predetermined ratios of peak heights at different detection wavelengths.

Indirect Detection for the Determination Mobility of CDs, t_0, and t_{ch}. The mobilities of the charged CDs were determined via an indirect detection method. A 20 mM solution of phthalic acid was prepared in the running buffer without the CDs. A 10 mM sample of the charged CD and MO was prepared using this running buffer as the solvent and injected under the same electrophoretic conditions as for other experiments. The normal positive going peak for MO (at 254 nm) was used to mark the void time of the system (t_0). The migration times of the charged CDs (t_{ch}) were determined with indirect detection (at 214 nm) on the basis of a negative-going peak. The center of mass of these skewed peaks was used to estimate t_{ch}. The migration times of MO and charged CD are used to calculate μ_{eo} and CD apparent mobility (μ_{app}), respectively. The electrophoretic mobility of the charged CD ($\mu_{elec,cd}$) is calculated as $\mu_{app} - \mu_{eo}$.

The beginning of a solvent-related, negative-going baseline disturbance (see electropherograms presented later) was used to mark t_0 for each separation. The t_0 value establishes μ_{eo}, and by using the previously determined $\mu_{elec,cd}$, it is possible to determine t_{ch} for that separation (see Results and Discussion section).

Safety. Because almost all the solutes used in this work are severe irritants and their effects on living organisms are not known, caution was exercised while working with them. Disposable gloves were worn, and waste sample solutions were disposed of properly.

RESULTS AND DISCUSSION

Theoretical Considerations. The effective mobility of a solute in CDCE can be predicted with knowledge of distribution coefficients (K = solute concentration in CD phase/solute concentration in running buffer (rb) phase), phase volumes (*V*s), and effective mobilities of the phases in the system. In the case of a ternary CD system, the effective mobility of a neutral solute is given by eq 1

$$\mu_{sol} = f_{rb}\,\mu_{eo} + f_{cd1}\,\mu_{cd1} + f_{cd2}\,\mu_{cd2} + f_{cd3}\,\mu_{cd3} \qquad (1)$$

where the μs are mobilities and the fs are mole fractions in a particular phase. The value of f_{cd1} is given by eq 2 (similar expressions can be written for the other *f*s)

$$f_{cd1} = \frac{N_{cd1}}{N_{rb} + N_{cd1} + N_{cd2} + N_{cd3}} = \frac{K_{cd1}\,V_{cd1}}{1 + K_{cd1}\,V_{cd1} + K_{cd2}\,V_{cd2} + K_{cd3}V_{cd3}} \qquad (2)$$

where the *N*s are solute molar amounts in the phases. V_{rb} is assumed to be 1 and unchanged with addition of low concentrations of CDs. SYBYL 6.5 molecular modeling software (Tripos Inc., St. Louis, MO) was used to determine V_{cd}, the approximate

Table 1. CD Cavity Volumes and Mobilities

CD[b]	cavity dimensions[a] ($cm \times 10^{-8}$) avg radius	depth	vol_{cav}[c] (cm^3)	V_{cd}/V_{rb}[d] (per millimol per liter)	μ_{cd}($cm^2/Vs \times 10^{-4}$)
β-CD	4.0	6.0	3.0×10^{-22}	1.8×10^{-3}	~5−7 (same as μ_{eo})
γ-CD	5.25	6.0	5.6×10^{-22}	3.3×10^{-3}	~5−7 (same as μ_{eo})
CM-β-CD	4.0	6.0	3.0×10^{-22}	1.8×10^{-3}	−1.2
HDMS-β-CD	3.5	7.5	2.7×10^{-22}	1.6×10^{-3}	−4.2

[a] Distances are from atom centers from minimized solvated structures that define dimensions. [b] All the β-CDs are elliptical with the greatest effect being for the CM-β-CD, D. S. 1. The HDMS-β-CD appears more closed off at the truncated bottom relative to the other CDs. [c] Volumes computed as π x average cavity radius^2 × cavity depth. [d] V_{cd}/V_{rb} is the factor needed to convert CD concentration (in mM) to volume phase ratio.

volume of the CD (see Table 1). The mobility of the solute is then given by eq 3.

$$\mu_{sol} = \frac{\mu_{eo} + K_{cd1} V_{cd1} \mu_{cd1} + K_{cd2} V_{cd2} \mu_{cd2} + K_{cd3} \mu_{cd3}}{1 + K_{cd1} V_{cd1} + K_{cd2} V_{cd2} + K_{cd3} V_{cd3}} \quad (3)$$

Solute migration times can be calculated to simulate a separation with knowledge of solute mobility. Systems with greater numbers of CDs yield more complicated versions of eq 3. However, CDCE systems that employ multiple-isomer, derivatized CDs can be complex insofar as predicting solute mobility.[16,18]

The distribution coefficients shown in eq 3 are calculated from k' values, which are experimentally determined for CDCE in a manner analogous to MEKC.[26] In the case of a simple binary CDCE system with neutral cd1 and charged cd2, the observed k' is given by eq 4 where the Cs are solute concentrations in the

$$k'_{obs} = \frac{C_{cd2} V_{cd2}}{C_{rb} V_{rb} + C_{cd1} V_{cd1}} = \frac{t_m - t_0}{t_0(1 - t_m/t_{cd2})} \quad (4)$$

phases, t_0 is assumed to be the migration time of neutral cd1, t_{cd2} is migration time of charged cd2 (see Experimental Section), and t_m is solute migration time.

Two experiments are performed to determine K_{cd1} and K_{cd2}. In Experiment 1, neutral V_{cd1} is held at zero and the V_{cd2} is varied by changing cd2 concentration. A plot of k'_{obs} versus V_{cd2}/V_{rb} has a slope equal to K_{cd2} (see eq 4). In Experiment 2, charged V_{cd2} is held constant and V_{cd1} is varied. Equation 4 can be modified to eq 5.

$$1/k'_{obs} = \frac{V_{rb}}{K_{cd1} V_{cd2}} + \frac{K_{cd1} V_{cd1}}{K_{cd2} V_{cd2}} \quad (5)$$

A plot of $1/k'_{obs}$ versus V_{cd1}/V_{cd2} has the slope K_{cd1}/K_{cd2}. The K_{cd2} values from Experiment 1 can then be used to determine K_{cd1}. By applying eq 3, the mobility of a solute can be predicted for combinations of CDs (charged and neutral) from experimental conditions and experimentally determined K values. This treatment assumes solutes form simple 1-to-1 complexes with CDs and the CDs act independently.

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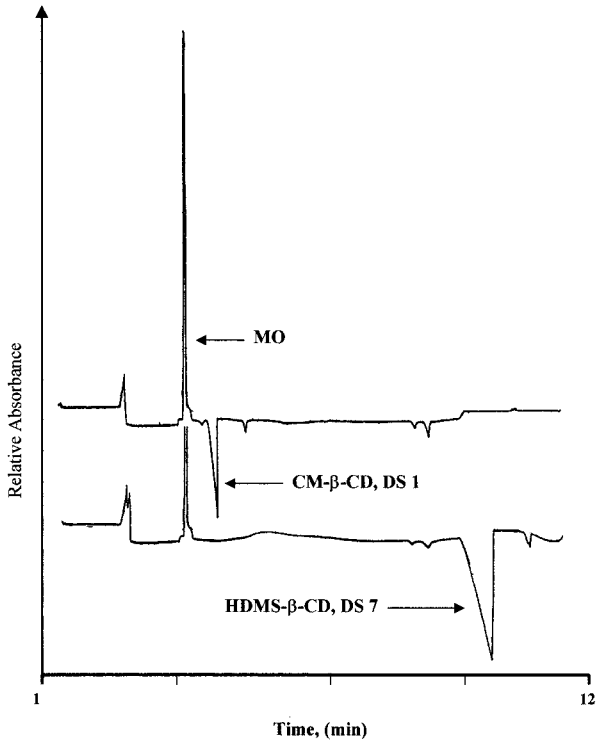

Figure 2. Indirect UV detection electrophorograms for determination of the mobilities of CM-β-CD and HDMS-β-CD. Running buffer: 20 mM *o*-pthalic acid in 25 mM phosphate buffer at pH 5.0. Capillary: 50-μm i.d., 39/48 cm effective/total length, uncoated fused silica. Detection wavelength: 254 nm. Applied potential: 15 kV.

The above treatment requires knowledge of t_0 and t_{cd2}. Since the use of CDs can result in modifications of μ_{eo}, the values of t_0 and t_{cd2} must be determined for each individual separation. Initially, injections of mixtures of MO, to mark t_0, and the charged CD in question are performed to establish the mobility of the CD. The indirect detection conditions described in the Experimental Section are used for this determination (see Figure 2 for electropherograms from these injections). For the purposes of this work, it was assumed that inclusion of the background electrolyte into the CD either did not occur or, if inclusion occurs, charged CD mobility is not appreciably altered. Similarly, it is assumed that inclusion of the relatively low molecular weight test solutes does not alter the mobility of the charged CD. Separations using running buffers containing CDs are performed under the same conditions (same field, E, and effective column length, L), and the value of t_0 is marked by a solvent baseline disturbance. The value of t_{cd2} is determined from t_0 using eq 6 where the constant

$$t_{cd2} = t_0/(1 - C\,t_0) \quad (6)$$

$C = \mu_{cd2,elec}\,E/L$ is determined from the injection of the MO−cd2 mixture (see Appendix for derivation). The mobilities of the anionic CDs that appear in Table 1 are roughly in proportion to the charge/mass ratios of the CDs.

The error associated with determining k' values can be quite large in this work, particularly when solutes elute near the ends of the elution window, i.e., with very small or large k' values. Equation 6 can be modified and differentiated to yield eq 7 (see Appendix for derivation).

$$\frac{\delta k'}{k'} = -\frac{(1 + k')^2}{k'\,C\,t_0^{\,2}}\,\delta t_0 \quad (7)$$

This equation provides the relative random error in determining k' values. The broad solvent baseline disturbance that is used to mark t_0 and determine μ_{eo} in these studies probably represents the largest element of uncertainty. This baseline disturbance results from the samples containing an appreciable amount of methanol (see Experimental Section). An alternate method of employing an external μ_{eo} marker was used successfully by Williams and Vigh.[27] That method was not employed in this work because it was felt that in some cases the marker would coelute with a test solute in the multicomponent sample mixture. Figure 3 is a plot of the relative uncertainty in determining $k'(\delta k'/k')$ as a function of k'. An uncertainty due to random error in measuring t_0 of 0.05 min is assumed, and the value of C is 0.046 (typical value for CM-β-CD, DS 1). At k' values less than 0.5 or greater than 10, the relative error can exceed 20%. Replicate ($n = 8$) injections of the test solutes using a running buffer containing 10 mM CM-β-CD yielded an experimental average CV in k' values of 13% (with a high value of 49%). Most of the separations to determine k' values were performed in duplicate. Plots to determine K values exhibited regression constants ranging from 0.97 to 0.99. The distribution coefficients are reported in this work with two significant figures, although uncertainties due to random errors in these determinations are greater than ±1 unit in the last significant digit.

In addition to random errors associated with determining k', systematic errors can occur due to association of the t_0 marker with CDs in the system. Szolar, Brown, and Luong noted errors in assigning μ_{eo} using a methanol marker of 0−8% when anionic CD concentrations were increased to values of 25 mM.[17] If one assumes that the over estimation of t_0 grows to 3% as HDMS-β-CD concentration is increased to 10 mM (the highest used in these studies), the treatment in Experiment 1 will underestimate $K_{HDMS-\beta-CD}$ by a factor of less than 1.5 (see below for values obtained without considering overestimation). An appreciable magnitude of systematic error can also result from errors in assigning phase volumes for the CDs (see Table 1). The K values obtained in this work correspond to inclusion complexation constants which are a little on the high side of normal values. This may be accounted for simply by the manner in which phase volumes are assigned. While these potential systematic errors in K values are significant in an absolute sense, our goal is to demonstrate and study selectivity in CDCE and to illustrate that CE-determined K values can be used to predict separation selectivity for more complex CDCE systems. In these instances, relative K values are most important. The systematic errors discussed above will produce constant errors that do not alter relative values of distribution coefficients.

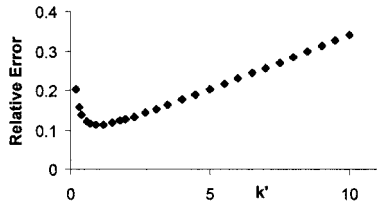

Figure 3. Plot of $\delta K'/K'$ versus K'. Equation 7 with $C = 0.046$, $t_0 = 3.5$ min, and $\delta t_0 = 0.05$ min.

Illustrations of Selectivity. Three dual-CD systems were investigated: β-CD or γ-CD with CM-β-CD, DS 1, and β-CD with HDMS-β-CD. Certain complications that arose in this work are discussed below. Changes in μ_{eo} can occur due to running buffer viscosity effects[25] and sorption of running buffer components on the surface of the capillary.[28] The CD concentrations used in these studies were sufficiently low so as to cause negligible changes in viscosity. Conversely, there was evidence of CD−capillary wall interactions. At acidic pHs (5.0 typically used), the CM-β-CD systems required rinsing (see Experimental Section) after each injection or a significant and continuous increase in t_0 was observed with each subsequent injection. The situation was much more severe with HDMS-β-CD, and neutral running buffer pH (7.5 typically used) was needed, in addition to the rinses, to obtain fairly reproducible t_0 values. It seems surprising that a CD with a −7 charge would interact with a surface that exhibits an effective negative charge. Silanol densities for SiO_2 surfaces of 5×10^{14}/cm^2 have been reported.[29] This corresponds to average silanol spacing of less than 5 Angstroms. Thus, simultaneous interaction of the HDMS-β-CD with multiple silanol groups is possible. The interactions can be attractive hydrogen bonding with the sulfato groups, which are known to be strong proton acceptors,[30] or Coulombic repulsion if the silanol group is deprotonated. Deprotonation of surface silanols on silica occurs over a wide range of pH. Apparently the attractive forces dominate at acidic pHs. At neutral pH, the percentage of ionized silanols is large enough for the Coulombic repulsions between the silica surface and the HDMS-β-CD to reduce surface interactions to an acceptable level. Interactions of sulfato-CDs with the surface of fused silica capillaries, and concomitant changes in μ_{eo}, have been noted by Vigh and co-workers.[31]

Solute interactions with CDs can involve several different elements of molecular recognition, e.g., hydrophobic interactions within the cavity, polar interactions (hydrogen bonding, general dipole, π-system) with secondary hydroxyl groups or other substitutes on the lip of the CD, size and/or shape recognition, and chiral recognition. Six test solutes were chosen for these

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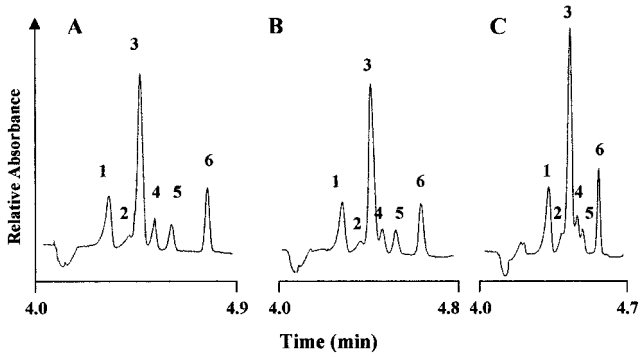

Figure 4. Effect of β-CD concentration on separations of test solutes. **1**, 1,3-DNN; **2**, 1,5-DNN; **3**, 1,8-DNN; **4**, 2,7-DNN; **5**, 1,5-DMN; **6**, 1,5-DHN. Running buffer: 25 mM phosphate buffer at pH 5.0 with **A**, 2 mM β-CD/8 mM CM-β-CD; **B**, 4 mM β-CD/8 mM CM-β-CD; **C**, 8 mM β-CD/8 mM CM-β-CD. Other conditions as in Figure 2 except detection wavelength of 225 nm.

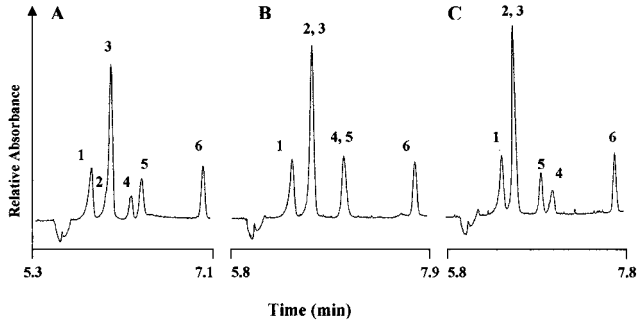

Figure 5. Effect of γ-CD concentration on separations of test solutes. CD concentrations: **1**, 1,3-DNN; **2**, 1,5-DNN; **3**, 1,8-DNN; **4**, 2,7-DNN; **5**, 1,5-DNN; **6**, 1,5-DHN. **A**, 2 mM γ-CD/8 mM CM-β-CD; **B**, 4 mM γ-CD/8 mM CM-β-CD; **C**, 8 mM γ-CD/8 mM CM-β-CD. Other conditions as in Figure 4.

studies. Four of the solutes involve different patterns of substitution on naphthalene with moderately bulky $-NO_2$ groups. Differences in size, shape, and the ability to weakly hydrogen bond (as acceptor) as well as dipole interactions are expected to be important in determining selectivity. Two other test solutes assume the 1,5-substitution pattern but involve $-CH_3$ substitution (eliminating the possibility of hydrogen bonding and dipole interactions) and $-$OH substitution (creating the possibility of strong hydrogen bonding interactions).

Figures 4 and 5 are CDCE separations of the six test solutes used in these studies using β-CD/CM-β-CD and γ-CD/CM-β-CD systems, respectively. Among the DNNs, the elution order is 1,3-, 1,5-, 1,8-, and 2,7-DNN. The importance of hydrophobic interactions is indicated by the greater retention of 2,7-DNN which has the greatest aspect ratio of these solutes and is less likely to show polar interactions upon inclusion. The 1,5-DMN and 1,5-DHN show greater affinity for these CDs. Possibly this is due to strong hydrophobic and hydrogen-bonding interactions, respectively. As shown in Table 2, inclusion into CM-β-CD and the β-CD have distribution coefficients that are fairly similar (the former being only slightly larger), although slight differences in selectivity were observed. As expected, increasing the ratio of neutral to anionic CD concentrations (the CD phase ratio) results in a steady reduction in k'. The differences in selectivity are not great enough to cause changes in elution order as the CD phase ratio is changed (see Figure 4). The effect of the γ-CD on retention is smaller than

Table 2. Determination of Distribution Coefficients from k' Measurements

	experiment 1		experiment 2[b]	
solute	$K_{CM-\beta-CD}$	$K_{HDMS-\beta-CD}$	$K_{\beta-CD}$	$K_{\gamma-CD}$
1,3-DNN	29	5	17	7
1,5-DNN	47	2	30	*a*
1,8-DNN	65	8	40	29
2,7-DNN	96	2	66	4
1,5-DMN	130	5	100	41
1,5-DHN	490	20	445	46

[a] $K_{\gamma-CD}$ for 1,5-DNN was not obtained due to a detectability limitation for the solute. [b] Experiment 2 was performed for the CM-β-CD system. The apparent interaction of HDMS-β-CD with the neutral CD precluded Experiment 2 with the sulfato-CD.

that of the β-CD. Presumably this is a result of greater cavity size and a less "snug" hydrophobic fit. In fact, the elongated 2,7-DNN has such a weak interaction with the γ-CD that as the CD phase ratio is increased the elution order for 2,7-DNN and 1,5-DMN reverses (see Figure 5).

Although not demonstrated with electropherograms, the interaction of these solutes with the HDMS-β-CD is considerably weaker (Table 2). Despite the larger elution window, the weakness of these interactions and the observed selectivity prohibited baseline resolution. Under most CDCE conditions using HDMS-β-CD, only two or three peaks were observed, near the front of the elution window, for the six test solutes. On the basis of the much larger K values for β-CD relative to HDMS-β-CD, one would expect that increasing the β-CD/HDMS-β-CD phase ratio would cause all the solutes to rapidly converge on t_0. Surprisingly, this was not observed.

One possible explanation is the occurrence of hydrogen-bonding interactions between the sulfato groups of the anionic CD and the secondary $-$OH groups of the neutral CD. Thus, the effect of the "capped" β-CD in reducing k' is diminished. Possible evidence of this inter-CD interaction is given by the aforementioned apparent HDMS-β-CD$-$capillary wall interactions. Further, Terabe and co-workers use running buffers that contain urea to minimize undesirable hydrogen bonding for additives such as CDs.[28] When we added 1.0 M urea to the running buffer the effect of increasing the phase ratio on reducing k's was greater, although still less than expected. Finally, a molecular modeling experiment was performed using the SYBYL 6.5 system. Information on our general approach to molecular mechanics modeling can be found elsewhere.[7,9,16,32] In this particular experiment, the sulfato end of HDMS-β-CD was positioned above the top of a β-CD molecule. The former CD was then translated toward the latter CD in increments and rotated at each increment. This was performed in a molecular modeling mode that allows inspection of dynamic (intermolecular) hydrogen bonding.[32] Although actual hydrogen-bonding energies are not accessible with the system, the formation of these bonds is seen (on the monitor of the work station employed) as dashed lines. When the centers of mass of the CDs were separated by 7 angstroms as many as 23 bonds are seen between sulfato oxygen atoms of the HDMS-β-CD and secondary $-$OH groups on the β-CD. This experiment was performed in a

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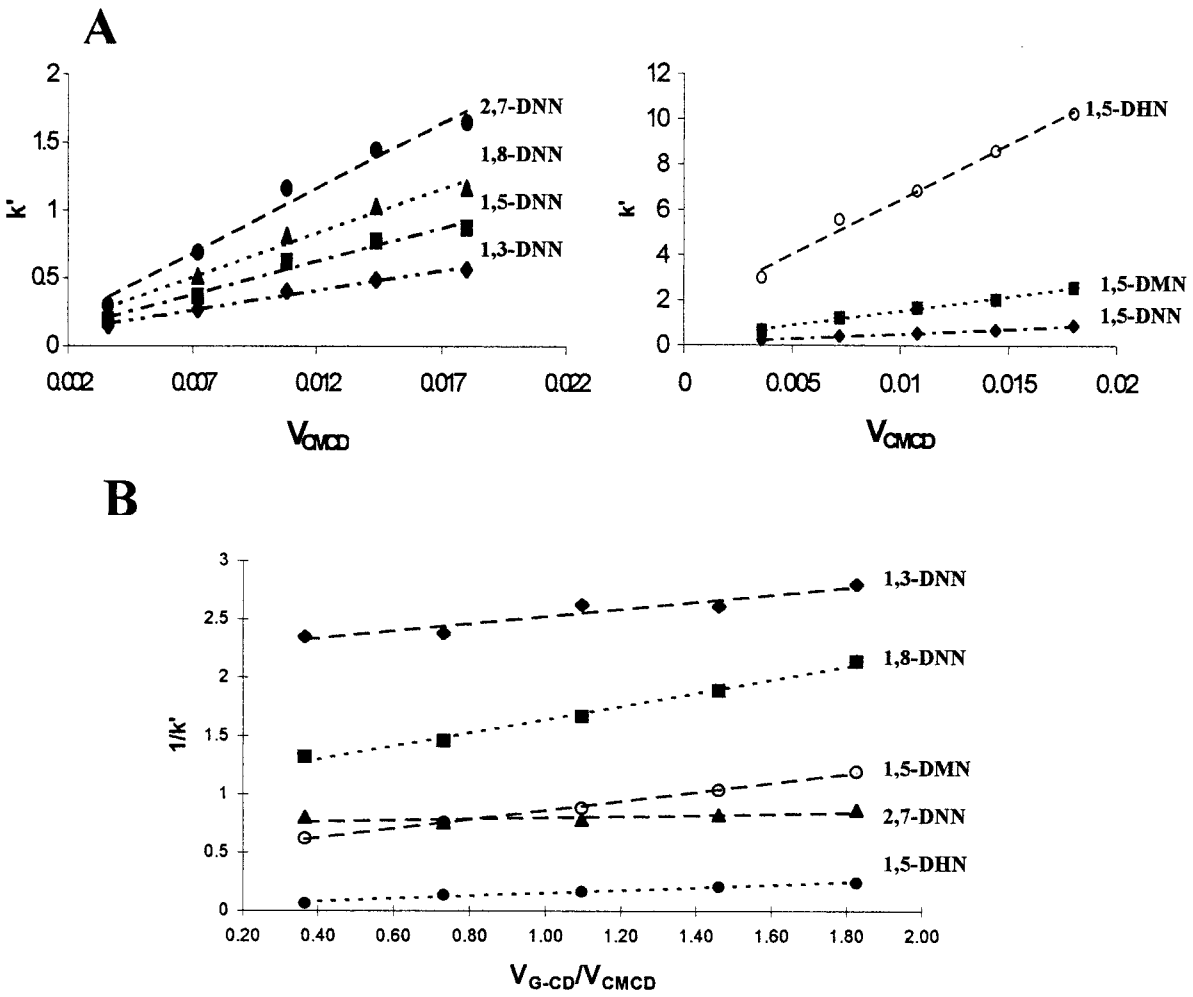

Figure 6. (A) Graphs of k' vs $V_{CM-\beta-CD}$, Experiment no. 1 and (B) Graph of $1/k'$ vs $V_{\gamma-CD}/V_{CM-\beta-CD}$, Experiment no. 2.

sterile environment (no solvent molecules) and with an effective dielectric constant of 1. To make the experiment more meaningful, it was repeated using the carboxymethyl equivalent of HDMS-β-CD (i.e., the 7 sulfato groups were replaced by carboxymethyl groups) as the charged CD. Under the best conditions, only 16 hydrogen bonds could be observed. Our conclusion from these various experiments is that the CDs are probably not acting independently for CDCE systems that employ the HDMS-β-CD.

Determination of Distribution Coefficients. Examples of plots of k' versus volume ratio of a charged CD (CM-β-CD) to the running buffer (i.e., the results of Experiment 1) are shown in Figure 6 A. Slopes of these plots are the distribution coefficients (K_{cd2} in eq 4) for the solutes with the charged CD. Similarly, Figure 6B shows a plot of $1/k'$ versus neutral CD (γ-CD) to charged CD (CM-β-CD). In the case of this Experiment 2, slopes can be used to compute the distribution coefficients (K_{cd1} in eq 4) for the solutes with the neutral CD. Good linearity and, in the case of Figure 6B, unique selectivity effects are seen in these plots. The K values for all the solute-CD systems tested are shown in Table 2. The missing data for 1,5-DNN is due to a detectability problem. The 1,5-DNN is barely detectable with our instrumentation even near its solubility limit. The selectivity trends cited in the above section are clearly evident in the data in this table. The elution order of two of the six solutes can be changed by simply altering the type of cd1 and/or its concentration. It is possible that reductions in K values for HDMS-β-CD, relative to the CM-β-CD, are exaggerated due to the association of the t_0 marker, methanol, with the former CD. Nevertheless, actual separations using the HDMS-β-CD clearly suffer from poor retention (low capacity factors, k's) which produced inadequate resolution. The buffer concentration is considerably higher than the CD concentrations that are employed. Nevertheless, it should be noted that experiments used to generate the distribution coefficients in Table 2 involve running buffers with different CD concentrations and ionic strengths. The same is true when comparing normal running buffers to those containing background electrolyte for indirect detection. Kenndler and co-workers have developed relationships that indicate that the effect of ionic strength on mobility can be substantial.[33]

Prediction of Separation Selectivity. The distribution coefficients in conjunction with eq 3 (or an expanded version) should be useful in predicting separation selectivity for moderately complex CDCE systems. As an initial illustration of the reliability of the predictive model, two CDCE systems were generated using neutral β- and γ-CD with charged CM-β-CD. The concentrations of the CDs were as follows: (Case 1) 2 mM β-CD, 6 mM γ-CD, and 8mM CM-β-CD; (Case 2) 6 mM β-CD, 2 mM γ-CD, 8 mM CM-β-CD.

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Table 3. Comparison of Predicted and Experimental Mobilities, cm²/(V sec)

	case 1 2/6/8 mM β-CD/γ-CD/CM-β-CD		case 2 6/2/8 mM β-CD/γ-CD/CM-β-CD	
solute	observed ($\times 10^{-4}$)	predicted ($\times 10^{-4}$)	observed ($\times 10^{-4}$)	predicted ($\times 10^{-4}$)
EOF	4.35		4.54	
1,3-DNN	4.07	4.09	4.27	4.29
1,8-DNN	3.99	3.98	4.16	4.18
2,7-DNN	3.86	3.85	4.07	4.11
1,5-DMN	3.86	3.86	4.07	4.11
1,5-DHN	3.60	3.68	3.91	4.02
CM-β-CD	3.38		3.57	

The calculated and experimentally observed mobilities are shown in Table 3. The 1,5-DNN solute is omitted due to the aforementioned detectability problem. There is good agreement between predicted and observed mobilities. In most cases the relative differences are <2%. The error is slightly greater for the late-eluting solutes. This may be due to greater random error in assigning k' values as expressed in Figure 3. The experimentally observed μ_{eo} is used with eq 3 to obtain the predicted values. Our intuition based on prior work was that the two cases might show a reversal of the elution order of the 2,7-DNN and 1,5-DMN. In fact, the prediction is for coelution in Case 2, and this was observed. The prediction in Case 1 was for the 2,7-DNN to migrate slightly ahead of 1,5-DMN. However, the predicted relative difference in solute mobility of 0.01/3.85 ($\Delta\mu/\mu_{ave}$), at an efficiency (N) of $\sim 10^5$ plates, would yield only a resolution [using $R_s = (N/16)^{0.5}(\Delta\mu/\mu_{ave})$] of about 0.2.[34] Indeed there was no evidence of a separation between these components in the electropherogram. In this case, experimental knowledge of K values permits accurate predictions.

In conclusion, combinations of neutral (β- and γ-CD) and anionic single-isomer CDs are added to CE running buffers and successfully used to separate various naphthalene compounds. Excellent selectivity is observed for systems involving CM-β-CD, DS 1, despite a limited elution range. In other work, HDMS-β-CD was shown to function well in separations of charged or highly polar compounds, particularly chiral compounds.[21] However, in this work it was shown to be less useful when used in combination with other CDs for electrochromatographic separations of moderately hydrophobic naphthalene compounds. Experimentally determined distribution coefficients are used to permit fairly accurate predictions of separation behavior. Future work will involve the development of simplex optimization methods and molecular mechanics modeling methods as allied tools in optimizing cyclodextrin distribution capillary electrochromatographic separations.

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APPENDIX

The migration time of cd2 (the charged CD) is given by (i)

$$t_{cd2} = \frac{L}{(\mu_{eo} - \mu_{cd2,elec})\, E} \qquad \text{(i)}$$

where $\mu_{cd2,\ elec}$ is the absolute value of the anodic electrophoretic mobility of cd2.

Dividing numerator and denominator by $\mu_{eo}\, E$ and using the relationship $\mu_{eo} = L/(t_0\, E)$ yields (ii) which is equivalent to eq 6.

$$t_{cd2} = \frac{t_0}{1 - (\mu_{cd2,elec}\, E/L)t_0} \qquad \text{(ii)}$$

Equation 6 can be rearranged and inserted into eq 4 to eliminate t_{cd2} from k' as seen in (iii).

$$k' = \frac{t_m - t_0}{t_0 - t_m + C\, t_0\, t_m} \qquad \text{(iii)}$$

The derivative $\delta k'/\delta t_0$ will depend on t_m, t_0, and C. But eq iii can be used to eliminate any one of these at the expense of introducing k'. Differentiation gives (iv).

$$\frac{\delta k}{\delta t_0} = -\frac{C t_m^{\ 2}}{(t_0 - t_m + C\, t_0\, t_m)^2} \qquad \text{(iv)}$$

Using eq (iii) and squaring yields (v).

$$(1 + k')^2 = \frac{(C\, t_0\, t_m)^2}{(t_0 - t_m + C\, t_0\, t_m)^2} \qquad \text{(v)}$$

Comparing the right-hand sides of eqs iv and v, produces (vi).

$$\frac{\delta k'}{\delta t_0} = -\frac{(1 + k')^2}{C\, t_0^{\ 2}} \qquad \text{(vi)}$$

Dividing both sides of eq vi by k' and rearranging yields eq 7.

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