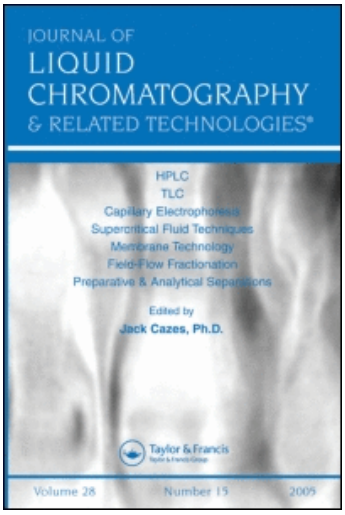

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ABSTRACT

Novel methods to use molecular mechanics (MM) in modeling solute-receptor interactions are developed that are pertinent to computational studies of dynamic separation or sensing processes. Specifically, a grid search MM modeling approach is used to study separation behavior in cyclodextrin (CD)-modified capillary electrophoresis (CE). Laboratory CE separations of di-substituted naphthalene compounds are accomplished employing an anionic, single isomer carboxymethyl-CD as a running buffer additive. Computational work involved the docking of these solutes into the cavity of this CD using a systematic grid search to probe the interaction space. It was discovered that the size of incremental changes in position and dimensional freedom in defining the grid are significant parameters. At each grid position

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the configuration of the atoms in the complex is altered in a process to minimize energy.

It was discovered that comprehensive minimization involving many iterations of the process is extremely important in locating true low energy conformers. Interaction energy contours, involving translation and rotation of the solute into the CD cavity, are produced that graphically reveals the strength of the CD-solute interaction. Increasing the number of minimization iterations from 100s to1000s resulted in remarkable changes in those contours, and improved the correlation between computationally determined and experimental distribution coefficients. The correct ordering of distribution coefficients was achieved with a few approaches, but with the comprehensive minimization approach experimental and computational coefficients were generally within a factor of 4 of each other. Moreover, the comprehensive minimization approach resulted in many points in the grid search, micro-positional states, that minimized to a single conformer. Consideration of the degeneracy of these states is also shown to be significant.

INTRODUCTION

The computational molecular modeling studies reported herein were performed to augment separations using cyclodextrin (CD)–modified capillary electrophoresis. The use of this mode of separation is based primarily on (i) high separation efficiency, (ii) the fact that the phases (CDs) that are used to selectively modify solute migration rates are fluid components in the running buffer, (iii) and there many possible CDs. Points ii and iii serve to simplify the modeling approaches taken, as complications arising from bonding the CD phases to solid supports (e.g., orientation and distortion effects) are avoided and, moreover, the CDs can be quickly changed to customize the separation. When combinations of CDs are employed, we refer to the technique as cyclodextrin distribution capillary electrophoresis (CDCE).

With the CDCE technique, solutes migrate through the capillary based on their distribution between the running buffer (rb) and neutral CDs, all moving with electroosmotic flow (EOF); any charged CDs in the system are migrating at velocities determined by EOF and their intrinsic mobilities. The characteristics and advantages of CDCE have been discussed and illustrated in our previous reports.[1-4] The effective mobility of a neutral solute, μ_{eff}, is given by Equation 1

$$\mu_{eff} = f_{rb}\mu_{eof} + f_{CD1}\mu_{CD1} + f_{CD2}\mu_{CD2} + f_{CD3}\mu_{CD3} + \quad (1)$$

where the fs and μs are the mole fractions associated with each phase and the mobilities of those phases, respectively.

Macrocycle reagents often exhibit unique molecular recognition properties and, as such, are important reagents in chemical separations. The number of papers describing the uses of these reagents for extraction, HPLC, and electrophoresis is very extensive. Some representative reports in connection with CE techniques include the use of crown ethers,[5,6] calixarenes,[7-9] and antibiotics.[10-12] A pertinent point is that the molecular architecture of many macrocycles may be computationally modeled and synthetically adjusted to probe solute recognition. Moreover, in many cases, combinations of the macrocycles may be employed with predictable effects on separations (see Equation 1).

The macrocycles that have received the greatest attention are the CDs.[13-21] Native CDs are neutral, cylindrical-shaped, macrocyclic sugar molecules that posses a hydrophobic cavity and a hydrophilic exterior. Cavity diameters for these chiral reagents vary depending on the number of glucopyranose units (6, 7, and 8 units of α-CD, β-CD, and γ-CD, respectively) in the structure. Native CDs have been derivatized with a wide variety of neutral and ionizable functional groups (dozens of forms are commercially available). Inclusion complex formation between guest solutes and the cavity of the CD host is influenced by geometry, size, and physiochemical properties of the solute and CD.

In separation processes (including CDCE), the solutes are transported through the system in discrete steps involving rapid association – disassociation with phases or reagents in the separation system. The complexity and dynamics of these processes complicates modeling. Nevertheless, various attempts and approaches to model separations have been reported.[7,12-15,22-34] These approaches to modeling separations can be put into different categories. Statistical fitting methods, such as employed by Khaladi for categorizing surfactants for micellar electrokinetic capillary chromatography,[23,26] represent one category. In these methods, quantitative structure-property relationships are developed using descriptors (molecular polarity, H-bonding capabilities, etc.) for the system.

Coefficients that indicate the importance of the descriptors are derived via regression analysis using laboratory experiments to generate the necessary data. In another approach, Vigh and coworkers developed a charged resolving agent model that is effective in predicting enantiomeric separation performance in CD-modified CE.[24] This model requires prior knowledge of the enantiomer-CD inclusion constant.

An important type of modeling utilizes applied theory methods.[28] These methods have the potential to provide information on relative values of inclusion constants. This is the approach taken in this work for modeling CD-modified CE separations of neutral, achiral compounds. In this type of modeling, semi-empirical quantum mechanics or empirical force fields (molecular mechanics, MM, or molecular dynamics, MD) are used for computer simulations of the separation

system. The empirical force field approach is computationally faster and more amenable to the multi-atom systems that are often encountered in chemical separations. These methods have been applied, most often, to model chiral separations.[7,13-15,22-32,34] Some of these efforts involve simply depicting conformers of hosts and visually rationalizing separation behavior.

In the simplest sense, MM predicts the most energetically favorable conformations of a molecule. This is done by considering the molecule as a collection of atomic nuclei held together by elastic bonds having differing force constants. The forces that hold the atoms together are described by potential energy functions of bond lengths, bond angles, and non-covalent bonding interactions. The potential energy functions are calculated with respect to an "ideal" structure of the molecule in question. The sum of these energies is the energy of the molecule in a given configuration. The geometry of the nuclei in the molecule can be altered (configuration changed) and the energy recalculated in pursuit of energetically favorable conformers. Significant to separations, the energy of a complex (non-covalent interacting species, such as a host and solute guest) also can be estimated and geometrically minimized.

In this work, our goal is to develop reliable methods of finding solute-CD conformers and conformer energies using grid search procedures developed in our laboratory. We do not focus on the use of MM methods to probe the specific modes of molecular recognition. Rather, the intention of this work was to develop a molecular modeling application that is useful in predetermining separation behavior in our system. The grid search is performed in conjunction with commercial MM modeling software and force fields. We have also attempted to determine the most appropriate way to use this conformer energy data in calculations and analysis to correlate to experimental findings. We model a relatively simple system involving docking several disubstituted naphthalene compounds into a single-isomer charged CD, carboxymethyl-β-cyclodextrin, degree of substitution 1 (CM-β-CD-1). After experimenting with numerous approaches using MM modeling, we have developed methods that yield fair to good correlations between calculated interaction energies for the solute-CM-β-CD-1 combinations and observed retention characteristics.

EXPERIMENTAL

Separations

The solutes, 1,5-dinitronaphthalene (1,5DNN), 1,8-dinitronaphthalene (1,8DNN), 2,7-dinitronaphthalene (2,7DNN), 1,5-dimethylnaphthalene (1,5DMN), and 1,5-dihydroxynaphthalene (1,5DHN), were obtained from Aldrich Chemical

Co. (Milwaukee, WI), and the CM-β-CD-1 was obtained from Regis Technologies, Inc. (Morton Grove, IL).

Separations were performed using a Hewlett Packard automated Capillary Electrophoresis system (HP^{3D}CE) with a diode array detector interfaced to a Pentium I personal computer. Fused silica capillaries were obtained from Polymicro Technologies Inc. (Phoenix, AZ). The running buffer used was a 10 mM CM-β-CD-1 and 25 mM phosphate solution, adjusted to a pH of 5.0 with an Orion Model SA 520 pH meter.

The samples were prepared by dissolving the solute in a running buffer-methanol mixture (20% v/v) to improve solubility. The capillary (50 μm i.d. x 360 μm o.d.) was cut to a length of 48 cm with the coating removed at 39.5 cm for detection. Injection was accomplished by the application of 10 mbar of pressure to the inlet vial for 6 seconds.

The separations were performed in positive polarity mode at 15 kV. Detector signals were recorded at 205 and 254 nm, and the data was collected using the Hewlett-Packard Chemstation Software. Further details concerning the CDCE separations of these dinitronaphthalene compounds can be found in a previous report.[2]

Molecular Modeling

The modeling experiments were performed using the Sybyl 6.6 molecular modeling software[35] and run on a Silicon Graphics Octane workstation with dual 270 MHz MIPS processors and 768 MB RAM. The solute molecules were constructed with the SKETCH feature in Sybyl. The CM-β-CD-1 molecule was built by using SKETCH to modify a β-CD structure obtained from the Cambridge Chrystallographic Data Center's structural database. The molecules were then each minimized in a water environment using the Sybyl MAXIMIN2 function with the Tripos force field and the Geistiger-Hückel charge calculation method before being stored on the hard drive.

A macro program was written in Sybyl Programming Language to achieve the grid search docking for each solute with the CM-β-CD-1. This program systematically translated and rotated the solute into the cavity of the CD as shown in Figure 1. The centers of mass were used to help visually align the solute and CD prior to docking. The solute was then translated from –5.0 Å out of the CD cavity to 0 Å in increments of –0.1 Å. At each of these translational positions, the solute was rotated 360 degrees, in increments of 5 degrees. The energy of the solute-CD complex was minimized at each step and recorded to an output file along with the translational and rotational coordinates.

Three different types of docking experiments were performed with the solutes, differing in the way that the energy of the complex was minimized. In

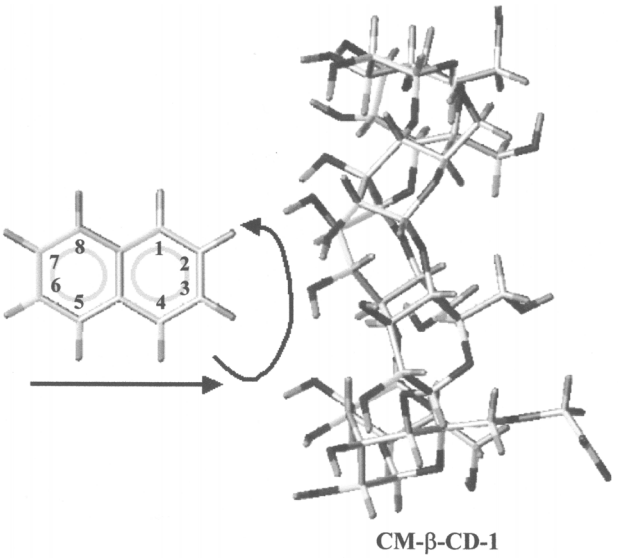


Figure 1. Depiction of the translation and rotation of the solute during the docking sequence. The solute is translated toward the CD at 0.1 angstroms and rotated with increments of 5 degrees at each step.

the first experiment (referred to as'100 min'), 100 minimization iterations were used at each point. Energy minimization was achieved using the Tripos Force Field with the dielectric constant (ε) set to 1. After running the macro program, the output file was imported into Sigma Plot[36] where the energy was plotted versus the translational and rotational positions. The data was then imported into Microsoft Excel for analysis. Using the 3-D energy contour plots and data generated by the first docking experiment, the translational and rotational positions of the solutes that produced the most favorable (lowest energy) complex were determined. This information was used to write the docking programs for the second type of experiment ('250 min'). In this procedure, the selected docking positions were probed, more in-depth, by changing the rotational increment from 5 degrees to 1 degree. Also, the energy of the complex was more extensively minimized by increasing the number of minimization iterations to 250. All other minimization parameters remained the same.

In the third type of experiment, the solutes were docked with the CD as in experiment 1, but at each point the complex was 'comprehensively' minimized. The programs were written so that the minimization process would not be completed until the energy could not be minimized any further (typically ~ 2500 iterations). This experiment was performed with ε set to 20 for all solutes.

RESULTS AND DISCUSSION

MM calculations function by determining a structure that corresponds to a minimum of energy. A force field is used in which the sum of several energy contributions constitutes the total energy, E, of the molecule. The Tripos force field that is available with the Sybyl 6.6 software was used in this work, and is represented as:

$$E = \Sigma \text{ Estr} + \Sigma \text{ Ebend} + \Sigma \text{ Eoop} + \Sigma \text{ Etors} + \Sigma \text{ Evdw} + \Sigma \text{ Eele} \qquad (2)$$

where

Estr = energy of a bond stretched or compressed from its natural bond length
Ebend = energy of bending bond angles from their natural values
Eoop = energy of bending planar atoms out of the plane
Etors = torsional energy due to twisting about bonds
Evdw = energy due to Van der Waals non-bonded interactions
Eele = energy due to electrostatic interactions.[35]

The absolute energy value determined by the force field is not significant, but it can be used to find the important relative energies of two or more structures.

The program uses a minimization procedure to rearrange the structure to one of lower energy. These iterative procedures move the atomic coordinates of the structure in steps until a minimum of energy is reached. Minimization generally fails to produce the global minimum; a local minimum close to the starting coordinates is almost always the result. One approach to determining the global minimum, is to systematically use different sets of starting coordinates.[35]

Minimization is achieved in this work by utilizing the MAXIMIN2 function in the Sybyl 6.6 software. MAXIMIN2 is a two step function that first uses a non-derivative based procedure, called the Simplex Method,[37] on the structure until the maximum force on any atom drops below a value assigned by the user. Next, the atomic coordinates of all the atoms are simultaneously altered based on the first derivative of the energy equation with respect to the degrees of freedom.[35] This is done using the Powell Method,[38] which belongs to the Conjugate Gradient family of minimization methods.[37] Figure 2 shows a typical plot of the energy of the CD-solute complex versus the number of minimization iterations. The application of the Simplex Method can be seen in the first 10 iterations as the energy rapidly decreases. The Powell Method then fine tunes the complex structure and takes the minimization process the rest of the way. As shown in the figure, important minimization activity was found to occur even after 4000 iterations, so the comprehensive minimization experiments were extended well beyond that in many cases.

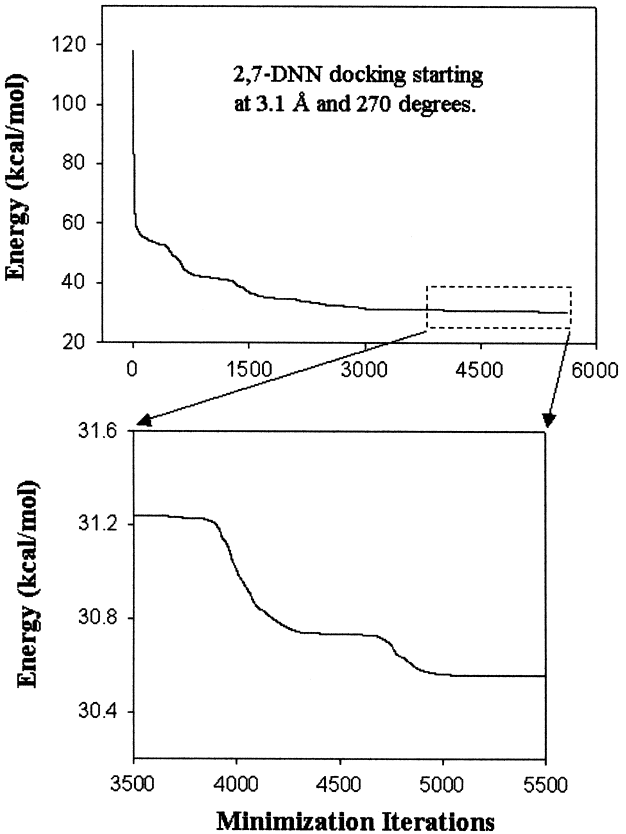


Figure 2. Typical plot of the cd-solute complex energy vs. the number of minimization iterations.

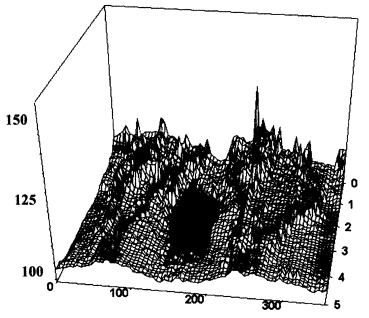
Figure 1 provides a depiction of the basic structure of the CD and solutes used in this study. The CM-β-CD-1 was chosen for this work for many reasons. First, for it to be able to separate neutral solutes as a buffer additive, the CD needs a charge associated with it in order to have an electrophoretic mobility. Second, the CM-β-CD-1 is a single isomer CD so the solutes only need to be modeled with one CD structure. Third, the dimensions of the CM-β-CD-1 cavity are such that it can accommodate each solute only lengthwise, thereby, limiting the orientations to consider. Finally, this CD is available from a commercial vendor.

The solutes were also chosen for specific purposes, as well. First, they will only fit into the CD cavity one way that will produce complexes low enough in energy to be significant. It was found through other docking experiments, that

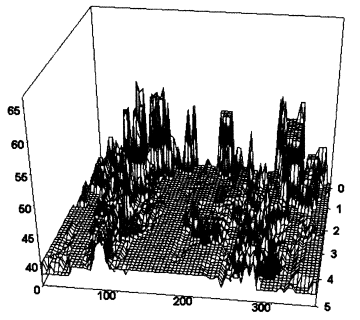
when the solutes were rotated 90 degrees and docked "flat" into the CD cavity, no conformations were detected with significant favorable interaction energies. Second, all of the solutes chosen also exhibit similar symmetry, and since they are rotated 360 degrees while docking lengthwise into the cavity the need to dock each one twice is eliminated. Finally, the solutes are hydrophobic in nature, making them well suited to forming complexes in the hydrophobic cavity of the CD. The DNNs (1,5-, 1,8, and 2,7-DNN) were chosen to determine if different substitutional isomers could be effectively modeled, while the DXNs (1,5-DNN, -DMN, and -DHN) were chosen to probe the selectivity the CD exhibits toward different functional groups.

Figure 3 provides several 3-dimensional contour plots resulting from grid search docking experiments. These plots serve as maps of the CD-solute interaction energy versus translation and rotation and contain important information, such as which docking positions form the best, energetically favorable conformers. The plots generated in this work were initially attempted in a solvent environment using the "solvate" function in Sybyl, wherein the complex was surrounded with a sphere of >150 water molecules prior to minimization. However, this introduced two major problems which caused the solvating approach to ultimately be abandoned. First, the amount of computing time needed to minimize each point in the grid search was prohibitively long. Second, the minimized energy was plagued by local minima problems, which obscured any features of the plot, making it appear like noise. It was also found, that increasing the number of minimization iterations did not ameliorate this problem. In fact, even after 100,000 minimization iterations of the solvated complexes, the global energy minimum had not yet been found.

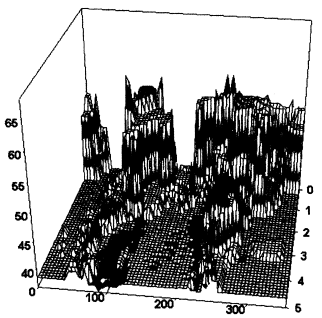
An energy plot of 1,5-DNN docking from the '250 min' experiment is shown in Figure 3a. One striking feature of this plot, is the presence of two energy "ridges" at about 90 and 270 degrees. This is a result of the CD structure being elliptical after minimization. The elliptical CD accommodates the solute well at the angles between the ridges, and favorable energies of the resulting complex are clearly shown. Another noteworthy aspect of this plot, is that every point in the docking grid has a different energy value. This was also found to be the case with the '100 min' experiments, and suggests that a different conformation is present at each and every one of the docking locations (for a total of 3600 different conformations) for these two types of experiments. Figure 3b shows 1,5-DNN docking under comprehensive minimization conditions, and illustrates that a much smaller number of conformations are found in this type of experiment. Many of the points in Figure 3a. that were individual conformations, can be seen minimizing to the same conformer and energy in Figure 3b. This phenomenon is, again, clearly illustrated in Figure 4, which shows graphical screen shots of 1,5-DHN at two different starting locations in the docking grid, but arriving at the same preferred conformer upon comprehensive minimization. This is evidence



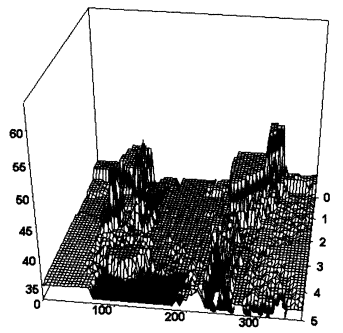
a. 1,5-DNN; 250 min iterations.



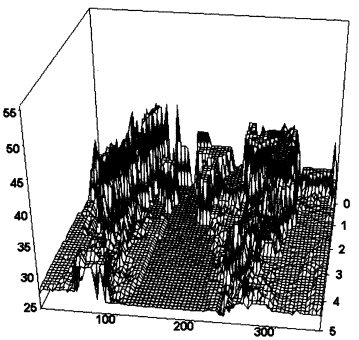
b. 1,5-DNN; Comprehensive min.



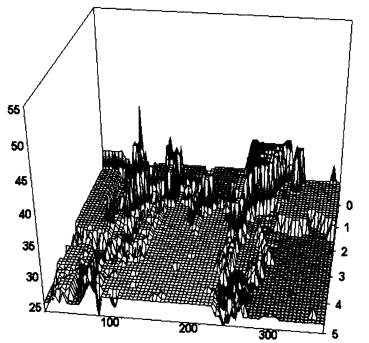
c. 1,8-DNN; Comprehensive min.



d. 2,7-DNN; Comprehensive min.



e. 1,5-DMN; Comprehensive min.



f. 1,5-DHN; Comprehensive min.

Figure 3. Plot of the solutes rotation (x-axis, degrees) vs. translation (y-axis, angstroms) vs. energy (z-axis, kcal/mol) from docking with the CD. a) 1,5-DNN, 250 min, and b) 1,5-DNN, c) 1,8-DNN, d) 2,7-DNN, e) 1,5-DMN, and f) 1,5-DHN all comprehensive minimization.

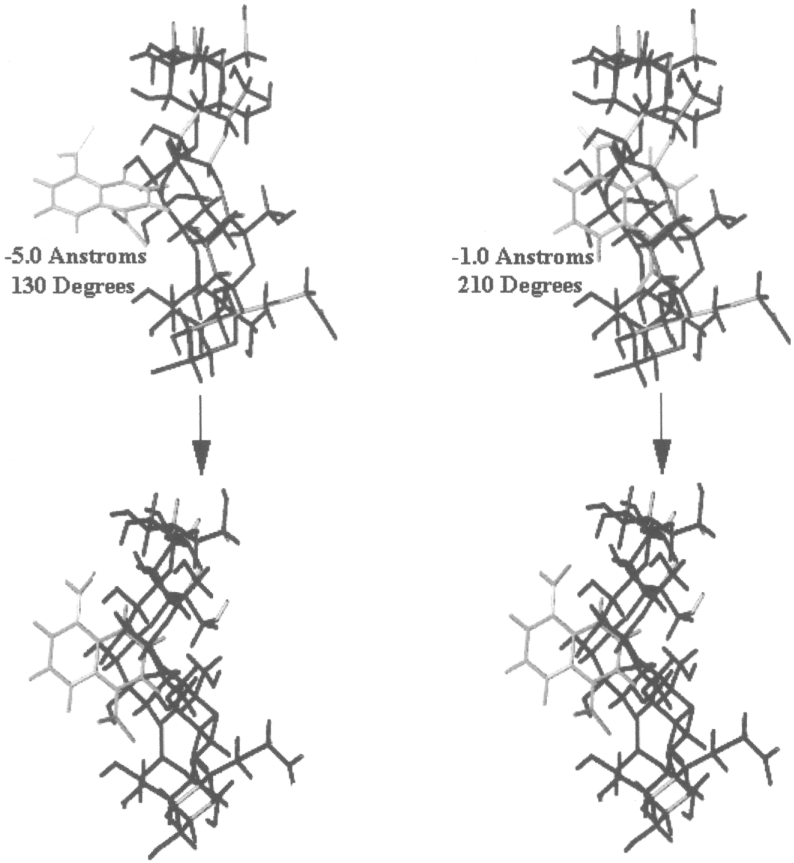


Figure 4. Depiction of 1,5-DHN starting at two different docking points and minimizing to the same conformer.

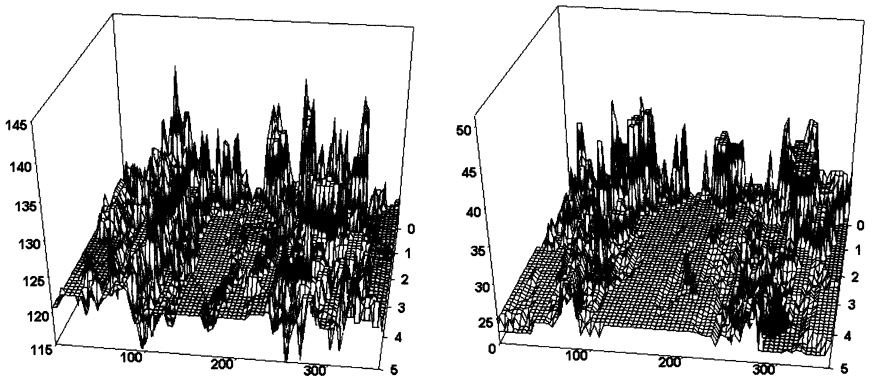
that the comprehensive minimization experiment is finding real conformations while the '100 min' and '250 min' experiments may be experiencing a multitude of local minima problems.

Figures 3c and 3d show comprehensive minimization energy plots of 1,8-DNN and 2,7-DNN, respectively. Immediately noticeable, is the fact that most of the 2,7-DNN conformers are lower in energy than the 1,8-DNN conformers. This is consistent with the experimental CDCE findings that 2,7-DNN has a better inclusion constant with CM-β-CD-1 than does 1,8-DNN.[2] It is also apparent, in the plots, that the starting docking locations of 2,7-DNN that minimize to a certain conformer, are bigger and fewer in number than those in the 1,8-DNN docking. This can be attributed to the fact that 2,7-DNN is relatively long and slender,

with the functional groups on the ends of the naphthalene framework. It can more freely rotate and move around inside the CD cavity during minimization than the wider 1,8-DNN. This effect can again be seen, where the smaller, more compact 1,5-DHN (Figure 3f), more easily rearranges its position in the CD cavity than the bulky 1,5-DMN (Figure 3e).

The comprehensive minimization experiments were performed at a ε value of 20. The value of ε influences the electrostatic energy term (E_{ele}) of the Tripos force field.[35] By increasing ε from the default value of 1, the contribution of the electrostatic energy term in the force field is decreased, simulating the shielding effect the solvent molecules might have between the solute and CD. At many docking points where the solute is deep inside the CD cavity, there is insufficient space for solvent molecules to fit between two interacting species, and ε is effectively 1. Conversely, when the solute is far away from the CD, ε may be better represented by a value of 80 (that of water). To study the effect that changing the dielectric constant has on the comprehensive model, some of the solutes were docked into CM-β-CD-1 with ε = 1, 20, 40, and 80.

As shown in Figure 5, the conformations found when the dielectric = 80 were fewer and from larger docking grid areas than the conformers from docking with ε set to 1. This can be attributed to the fact that at high dielectric values, the solute is shielded enough from restraining forces within the CD cavity to rearrange and move about relatively freely. It was found by looking at the individual electrostatic energy terms from many docking points and other data in this experiment, that a ε of 20 provides a good balance by preventing dominating electrostatic contributions when ε = 1, and excessive shielding effects when ε =



a. 1,5-DNN; ε = 1 **b. 1,5-DNN; ε = 80**

Figure 5. Plot of the solutes docking with different dielectric constants (ε). a) 1,5-DNN; ε=1, b) 1,5-DNN; ε=80.

80. This value of ε = 20, was also determined to be appropriate by Christensen and coworkers.[39] Ideally, we would like to include the solvent in the modeling system to more realistically mimic the actual molecular environment.

As discussed earlier, however, several problems make this approach unfeasible at this time, and we must currently rely on the more simplistic method of manipulating the dielectric constant to attempt to account for solvent effects. Given that all of the solutes are so similar in nature, it is believed that much of the role that the solvent plays in this environment would be the same for each solute, and this more simplistic treatment may be justified for our work here.

To determine the energies of the conformers found in all the docking experiments, the CD and solute's energy before docking is subtracted from the computed energy of each conformer. This allows for comparisons between solutes by accounting for the different starting energies of each solute. Next, the energies, ε_i, from all docking starting positions, i, with a degree of degeneracy, g_i, are entered into Equation 3

$$\langle e \rangle = \frac{\Sigma g_i e_i \exp -\left(e_i / RT\right)}{\Sigma g} \tag{3}$$

to give an average solute-CD interaction energy <e>. In calculations using Equation 3, the gas constant R, is multiplied by room temperature, T = 298 K; any temperature increases in CE applications due to Joule heating are ignored. As an example of data generated for Equation 3, Table 1 shows the top 5 conformers contributing to the calculated <e> of 1,5-DHN.

Degeneracy, g_i, in this work, is defined as the number of micropositional (translational and rotational) starting points that minimize to a given conformer and e_i. This approach may be compared to that used by Stillinger and Weber to map the "quenching" of molecular positions to relative minima on the potential energy hyperspace of a many-body system.[40] The absolute value of g_i is somewhat arbitrary, as smaller incremental changes in starting positions when per-

Table 1. Top Five Conformers and Percent Contribution to <e> for 1,5-DHN

Region	Energy	# Points	Contribution
1	−36.95 kcal/mol	40	41 %
2	−34.82	701	18
3	−36.45	18	8
4	−35.32	117	7
5	−36.39	14	5

Note: <e> = −36.03.

forming the grid search, will yield larger g_i values. However, the relative magnitudes of g_i between different conformers should remain approximately constant for a given solute-CD system, when increments are reduced. Conversely, the relative degeneracies of micropositional states that quench to the conformers, could differ if higher dimensionality (e.g., off-axis translations of the solutes as they insert into the CD cavity) is considered. We have limited our considerations to the two dimensional plateaus, shown in Figures 3 and 5, to curtail computing time. While adding dimensionality could change relative values of g_i, it is not expected to change the conformers that were located. This was confirmed with an experiment, wherein, a solute was moved in the x and y dimension as it was inserted (z-dimension) and rotated into the CD cavity. The added dimensionality did not change the located conformers, although it did add greatly to the computational time. It is interesting to note, that for the '100 min' and '250 min' cases, incremental changes in translation in the grid search must be reduced to $< 10^{-3}$ angstroms before any degeneracy is observed. This further illustrates the importance of performing the comprehensive minimization.

A goal of this work, is to develop MM modeling methods that can be exploited in future studies to estimate f-values (see Equation 1) for various solute-CDCE systems. The f-values can be calculated with knowledge of distribution coefficients ($K_d = [solute]_{CD} / [solute]_{rb}$) and the concentrations of the CDs. The ability to predict mobility for CDCE systems using experimentally determined K_d values was demonstrated in prior work.[2] With the MM modeling approach, it may be possible to make predictions and optimize separations (achieve desired selectivity with regard to μ_{eff} values for sample mixtures) without actual laboratory experiments. Success in this work should not require the exact predictions of the mobilities of the solutes in a sample. It is only necessary to correctly predict the CDCE system from possible choices that will work best for a given separation. Experimental fine-tuning would most likely be needed. An additional advantage of the MM modeling approach, is that one can evaluate unavailable systems and, thus, guide the synthesis of new macrocycle reagents. We have already used elements of this approach to guide the synthesis of a new single isomer CD for separations of the DNNs.[41]

Equation 4 is employed to convert <e> to K_d. The CM-β-CD-1 concentration used most often in our studies is 10 mM. Because the solutes are injected as dilute solutions, the free concentration of CD in the region of solute bands may be assumed to be this concentration.

$$<e> = - RT \ln ([solute]_{CD} / [solute]_{rb} \cdot [CD]) = - RT \ln (K_d / [CD]) \quad (4)$$

The rigorous use of Equation 4 requires an equivalency between <e> and the free energy change associated with the exchange of solute between running buffer and CD. Although this equivalency is not strictly valid, implicit in <e> val-

ues are the various potential energy terms listed in Equation 2 and entropy information represented by g_i. The solutes used in this case all exhibit similar symmetry and, thus, differences in the entropies of the free solutes may not be extremely large. Moreover, we normalize our <e> values based on the experimentally determined K_d of one of the solutes (2,7-DNN). In this regard, it is only necessary that values of K_d obtained via Equation 4, scale with true values of K_d.

Table 2 compares experimentally determined K_d values with the values obtained using five different computational approaches. The five approaches are '100 min' and '250 min' using Equation 1 (although g_i values are all 1) and comprehensive minimization, averaging the 5 best conformer e_i values, using Equation 1 with g_i assumed to be equal to 1, and using Equation 1 and employing the observed g_i values. Since we are using only one CD and normalizing to match the <e> of 2,7-DNN to its experimental K_d determined from an actual separation (see Figure 6), it is possible to ignore [CD] in Equation 4 (i.e., we can assume <e> = -RT ln K_d in this particular experiment).

With prior knowledge of the mobility of CM-β-CD-1, it is possible to determine capacity factors for the peaks seen in Figure 6.[2] These capacity factors, and the phase ratio for the system, were used to determine the experimental K_d values shown in Table 2. Comprehensive minimization with a simple averaging of conformer energies (Approach 1), or using Equation 3 but assuming $g_i = 1$ (Approach 2), yields the incorrect elution order, as seen in the table. When consideration is made of all the statistically accessible micropositional states that can quench into the observed conformer energies (Approach 3), the correct elution order is observed and the relative magnitudes of K_d values among the solutes are reasonably close to the experimental values (note: an error in energy of only 0.3 kcal/mol results in a 2-fold change in K_d). The contribution of conformer 2 (region 2) from Table 1 (conformers for 1,5-DHN) is very small relative to con-

Table 2. Distribution Coefficients, K_d, from CE Experiment and MM Modeling Approaches

Solutes (in order)	Experimental	'100 min'	'250 min'	Comprehensive Minimization Approach 1	Approach 2	Approach 3
1,5-DNN	28	0.035	9.6	260	21	11
1,8-DNN	36	0.18	11	130	18	20
2,7-DNN	54	54	54	54	54	54
1,5-DMN	80	2000	2.4×10^4	2.4×10^4	1800	570
1,5-DHN	410	4.9×10^5	3.6×10^4	2.7×10^4	2700	1900

Approach 1 - <e> = Mean of the energies of the five best conformers.
Approach 2 - <e> from Equation 3, g_i assumed to be 1.
Approach 3 - <e> from Equation 3, actual g_i values used.

former 3, if g_i values are ignored. Conversely, conformer 2's contribution to determining <e> is larger than conformer 3 when degeneracy is considered. Thus, Approaches 2 and 3 are decidedly different. The improvement in correlation with consideration of g_i, may indicate that molecular rearrangements (quenching) occurs rapidly relative to solute-CD exchange rates. The '100 min' and '250 min' cases also consider all the available grid search data (as Approach 3) and yield the correct elution order. However, the range of K_d values is enormous compared to the experimental values (especially for '100 min'). The 18 grid starting positions for region 3 (Table 1) were probed under '100 min' and '250 min' conditions with $\varepsilon = 20$. Each microposition yielded a unique energy when minimized (-8.1 to - 12.5 kcal/mol for the former and -14.4 to -23.8 kcal/mol for the latter). These energies do not seem to represent the true overall interaction energy (even in the relative sense) as well as the comprehensive minimization case. The predicted migration times that result from comprehensive minimization, Approach 3, are shown in Figure 6.

Although it is risky to extrapolate to other solute-macrocycle systems, these studies indicate the effectiveness of the grid search approach to studying the strength of solute-CD interactions as they influence migration behavior in CD-modified CE. It was found to be very important, to extensively geometri-

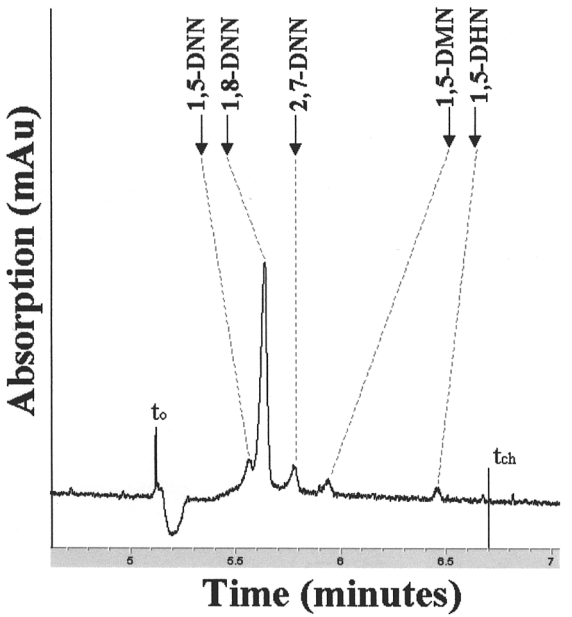


Figure 6. Predicted peak elution times based on modeling calculations are shown in arrows on the experiment electropherogram.

cally minimize solute-CD configuration to find reliable conformers. Further, it is important to weight the conformers based on the interaction space that can quench to the conformer. The data in Table 2, shows a stronger correlation between experimental and computational K_ds for isomers of nitrosubstituted naphthalenes than for changes in the nature of the substitute. This may reflect the need to fine tune the energy terms (see Equation 2) and/or study the use of different force fields to improve correlations. The addition of higher degrees of grid search dimensionality may also improve this work. Considerable research is still required if the ultimate goal of computationally manipulating selectivity for multiple CD systems is to be achieved, although significant progress has been made toward that goal.

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