Journal of Chromatography A, 666 (1994) 493–503
Elsevier Science B.V., Amsterdam

CHROM. 25 819

Review

Theoretical studies of brush-type chiral stationary phases

Kenny B. Lipkowitz

Department of Chemistry, Indiana University–Purdue University at Indianapolis, Indianapolis, IN 46202 (USA)

ABSTRACT

Atomistic molecular modeling techniques are described. A review of these methods directed toward understanding the origins of enantioselective binding on brush-type stationary phases used in chiral chromatography is presented.

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1. INTRODUCTION

How do chiral surfaces used in chromatography discriminate between enantiomeric analytes? Where does this enantiodiscrimination take place on the chiral stationary phase (CSP)? What are the intermolecular forces holding the transient analyte–CSP complex together, and are those the same forces responsible for chiral selection? These and other questions have been the focus of ongoing research in separation science for over two decades (see refs. 1–8 for recent books). To some researchers these questions are raised with the goal of understanding how nature works (pure science). To others, these questions are posed with the goal of being able to separate enantiomers for some other purpose or to make improvements in CSP separability (technology). Irrespective of who raises these questions, they are fundamental questions that need to be answered in both science and technology. To date, most of the answers to such questions have been answered experimentally. More recently, such questions have been addressed with theoretical or computational means as (i) an adjunct to experiment or (ii) a technique that can provide information not amenable to experimentation. The purpose of this chapter is to review computational studies that have been used to help understand chiral recognition in chromatography. In keeping with the theme of this issue honoring Professor Pirkle, and to provide a focus, we describe here computational studies on brush-type CSPs. A

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book chapter on modeling enantiodifferentiation in chromatography, covering other types of CSPs, will soon appear [9].

2. MODELING

A model is a likeness or a representation of something else. There are two broad categorizations of chemical models: macroscopic and microscopic, the latter of which is concerned with atomic detail and, accordingly, is sometimes called atomistic or molecular modeling. One can further partition molecular modeling into statistical fitting methods used, for example in quantitative structure–activity relationship studies, and, applied theory or simulation. Both methods consider atomic information and both are atomistic modeling techniques. In chiral chromatography most atomistic modeling has been the applied theory type which serves as the focus of this review.

The computational tools used in molecular modeling include quantum mechanics, empirical force fields as used in molecular mechanics, molecular dynamics and Monte Carlo simulations, and computer graphics. A brief description of each follows.

In quantum mechanics the objective is to describe the spatial positions of electrons and nuclei. In molecular orbital (MO) theory, the most common implementation of quantum mechanics, the electrons flow around the nuclei until they reach a "self consistent field", that is, until the attractive and repulsive forces between all the particles (electrons and nuclei) are in a steady state and the energy is as low as it can get. Once self consistency is reached one can incrementally move one or more nuclei and repeat the self consistent field (SCF) calculation again to see if the final energy has gone up or down when compared to the first SCF calculation. In an iterative procedure one moves nuclei around followed by SCF calculations until the energy can no longer be lowered. This is called energy minimization or geometry optimization and results, ideally, in the molecule finding its lowest energy shape with equilibrium bond lengths, bond angles and so on.

These SCF calculations are typically done two ways; *ab initio* [10] and semi-empirically [11]. The semi-empirical methods are faster and, because they have been suitably parameterized to give reliable structures and energies, they have been used in theoretical studies of chiral chromatography. As we shall see, several research groups have used these method to elucidate the shapes CSPs can adapt as well as to compute the differential binding energies of analytes to those CSPs.

Molecular mechanics is a non-quantum mechanical way of computing structures, energies and some properties of molecules [12]. In this approach electrons are not explicitly included in the calculations; rather, they are implicitly treated. This makes molecular mechanics over an order of magnitude faster than most semiempirical SCF MO methods and consequently this method is well suited for studying large molecules like biopolymers.

Molecular mechanics (MM) is synonymous with empirical force fields (EFFs) [13]. Empirical force fields are a collection of potential energy functions that serve as a recipe for reproducing a molecule's potential energy surface. The potential energy surface in turn dictates the molecule's shape. In molecular mechanics it is assumed that the nuclei are held together by sticky, harmonic forces (much like springs interconnecting masses) and we describe those forces with potential energy functions.

Energy minimization of molecular structure (using either quantum mechanics or molecular mechanics) results in a structure that is at its equilibrium position and which is motionless, *i.e.* it has no "temperature." Molecules are actually dynamical; they move and change their shape as time progresses. Molecular dynamics (MD) calculations give us information about the variation in structure and energy of a molecule over a given time period [14]. The forces between atoms can be computed by quantum mechanics or with empirical force fields. Because EFFs can be as accurate as quantum mechanics for many needs like prediction of molecular structure, energies, vibrational spectra and some properties like molecular dipoles and heats of formation, many scientists have adopted molecular mech-

anics as their computational tool of choice, especially because it is a fast method.

MD methods account for kinetic and potential energy which is to be contrasted with MM which only has potential energy. As the atoms move about they tend to stay near their equilibrium positions which correspond to the bottom of a potential energy well. If the energy well is not very deep or the temperature is high the system can climb up and out of the well into another. Molecular dynamics calculations are referred to as simulations because they simulate the motions of particles over a time period. The time period of most simulations is usually very short (in the order of 50–500 ps), because these calculations are very computer intensive. Consequently the simulations can be somewhat misleading because this view of what the molecule is doing is so short. Nonetheless this method is a very useful tool for assessing the dynamic aspects of how molecules interact.

Monte Carlo simulations use the same kinds of empirical force fields as above [15]. One starts with a collection of particles whose interaction energies are computed. Then randomly (hence the connection with Monte Carlo) one selects a particle and moves it to a different position. The energy at that new position is computed and compared to the previous energy. If this new position (called a configuration) is of lower energy we accept it and if it is of higher energy than the previous configuration we accept it with some probability, depending on how much higher in energy it is. The particles are again randomly moved, the system's energy is recomputed and accepted or rejected in an iterative procedure to provide a large number of low-energy configurations (*i.e.* the most important ones) that serve as the microstates for statistical averaging. Ultimately one computes averaged values that can be compared to experimental results which are themselves averaged values.

After modeling a process or a structure, the results can be visualized with computer graphics [16]. These pictures may be graphs, simple lines representing bonds connecting points which represent atoms, or they may be highlighted, depth-queued images outstripping the best hand-held, plastic, space-filling models. The computationally intense aspect of modeling generates an enormous amount of data; graphics or visualization renders it manageable and assimilable.

The purpose of all these simulations is to reproduce (model) the results of an experiment and then extract information from that simulation which is not amenable to experimentation. This gives new insights about intermolecular interactions which can be used to help guide experimentation. We now review what has been done in the area of modeling enantiodifferentiation on brush-type CSPs.

3. MODELING BRUSH-TYPE CSPs

As analytes migrate through the column they encounter solvated CSP, displace solvent and form the corresponding solvated diastereomeric complexes. Rather than compute ΔG for Eq. 1 and ΔG for Eq. 2 to obtain $\Delta\Delta G$, the differential free energy of binding, we recognize that by an enantiomeric relationship, where analyte $A^R = A^S$ in an unbound state, the left hand sides of both equilibria are identical. Consequently one need only compute the energies of the two diastereomeric complexes to determine which analyte is more tightly bound and, accordingly, has the longer retention time of the column.

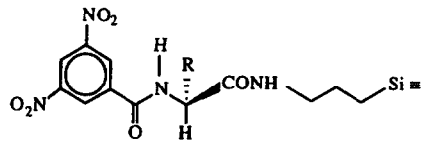
$$\mathrm{CSP}^R + A^R \rightleftharpoons \mathrm{CSP}^R \cdot A^R \quad (1)$$

$$\mathrm{CSP}^R + A^S \rightleftharpoons \mathrm{CSP}^R \cdot A^S \quad (2)$$

There are many assumptions made when doing these calculations. These include assuming the rate of complex formation is the same for *R vs. S* analyte and that only the relative stabilities of the complexes are important; complete neglect of mobile phase additives, ions and solvent, although we know that diastereomers have differential solvation free energies and experimentally we can find reversal in retention orders depending on solvent; elimination or truncation of the spacer chain connecting the CSP to the silica surface even though it is known that the length and method of attachment to the packing material is important; neglecting the packing (usually silica gel) altogether. Hence, all modeling done to date (published that is) are in the

"gas phase" and often using CSP *analogues* rather than the actual CSP.

Brush-type CSPs are not rigid, lattice-like structures. Rather, they are flexible. One can envisage a CSP having at least two conformational states; one of low energy and heavily populated and the others of higher energy and less populated. It is conceivable that a less populated conformer is not only doing most of the binding but is also most responsible for the chiral recognition. The first modeling work done was to assess the distribution of conformational states accessible to these CSPs since the templating ability of these CSPs depend on their conformational potential energy surfaces.



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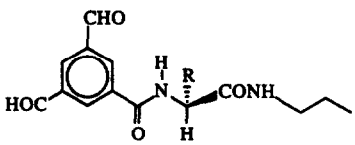

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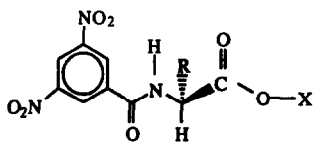
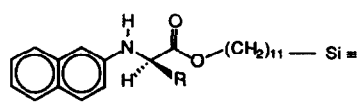


3

Conformational analysis was first done on Pirkle's dinitrobenzoyl (DNB) propylamide phase **1**, modeled as **2** where R = Me. The spacer chain had been truncated to a propyl group and, because the EFF parameters for nitroaromatics had not yet been developed, the NO_2 groups were replaced with formyl groups. The multidimensional potential energy surface (PES) for analogue **2** was computed with MM2 and five minimum energy conformations were located [17]. The interconversion pathway between the lowest energy structures was described and the templating ability of **1** was discussed. This work was extended to the ionic Pirkle CSPs modeled as **3** where X = H (the free carboxylic acid) and where $X = \oplus H_3N-CH_3$ to mimic the propylammonium spacer [18]. The *R* groups considered were methyl, phenyl, isopropyl, isobutyl and *tert.*-butyl. The molecular stereodynamics of these CSPs were assessed with semiempirical molecular orbital methods and with two EFFs. These CSPs were found to have two important conformational manifolds, syn and anti, that can play a role in chiral recognition, and, the conformational potential energy surfaces of these systems were found to be somewhat flat, allowing for rapid conformational interconversions. That work was then extended [19] to covalent analogues of **3** where X = methyl. The second generation Pirkle phase, naphthylamine **4** (whose potential energy surface was modeled as **5**), was then considered using quantum and molecular mechanics [20].



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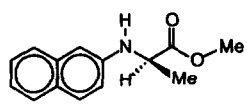

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The upshot of all this is that a great deal of time was spent evaluating the conformational attributes of these CSPs because it was deemed important to understand the possible shapes they can adopt when analytes bind. In all cases more than one conformation of CSP exists indicating that, when modeling analyte binding, one should include more than one shape of CSP.

The next step in modeling is to determine the possible conformations of the analyte molecules and then somehow bring the CSP and analyte molecules together and compute their interaction energies. This is the hard part of modeling.

One approach involved using only the most stable shape of CSP and the most stable shape of analyte. These conformational states were located as described above. The position of analyte with respect to CSP is represented in a spherical coordinate system (r, Θ, Φ). An origin and three orthogonal axes on the CSP were selected. An origin and a set of axes on the analyte were then selected in a way that allows for systematic sampling of all orientations and positions of the analyte with respect to the CSP. The distance, r, between origins and the latitude Θ and the longitude Φ between origins precisely defines where the analyte is with respect to the CSP. In essence what is being done is rolling the analyte over the Van der Waals surface of the CSP looking of the lowest energy binding region, in addition to finding the most stable orientation of the two molecules with respect to each other. Because of the large number of orientations to be sampled, MM was used to compute the intermolecular energies. Furthermore, since empirical force fields are not as reliable as desired, it was decided that the R and S analytes would be treated in an identical manner. This way, if MM underestimates, say, hydrogen bonding and overestimates electrostatics, the errors should be nearly the same for both R and S analytes. This cancellation of errors should result in small but meaningful energy differences between diastereomeric complexes.

Using this philosophy the binding of 2,2,2-trifluoro-1-(9-anthryl)ethanol to an R-phenylglycine DNB analogue of **1** was examined [21]. It was found that the S analyte has the lower binding energy (which agrees with experimental retention orders) and that the energy differences between R and S binding (0.34 kcal mol^{-1}; 1 kcal = 4.1868 kJ) agrees nicely with the experimental separability factor, $\alpha = 1.33$. More important, though, was that the mode of R *vs.* S analyte binding was found to be different than that proposed by Pirkle. Using the same types of interactions Pirkle used in his chiral recognition model, *e.g.* π-stacking, steric repulsions and hydrogen bonding, an alternative explanation was developed [21].

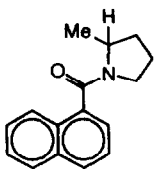
Of the numerous assumptions made in those studies, the ones about using only a single shape of selector and selectand, and, of comparing enthalpies rather than free energies were most disconcerting. Accordingly, Lipkowitz *et al.* [22] developed a protocol for computing the enantioselective binding of analytes to CSPs that overcame those problems. In this procedure they try to account for all important shapes of CSP, all important shapes of analyte, and, all important orientations of the two molecules. Also, rather than simply consider just the lowest-energy structures of the competing diastereomeric complexes they compute a statistical mechanics averaged interaction energy, $\bar{E}$, as in Eq. 3.

$$\bar{E} = \sum_{h=1}^{l}\sum_{i=1}^{m}\left(\frac{e^{-E_{CSP,h}/kT}}{\sum_{h'=1}^{l} e^{-E_{CSP,h'}/kT}}\right) \cdot \left(\frac{e^{-E_{A,i}/kT}}{\sum_{i'=1}^{m} e^{-E_{A,i'}/kT}}\right) \cdot \sum_{j=1}^{n} g_{hij}\left(\frac{e^{-\epsilon_{hij}/kT}}{\sum_{j'=1}^{n} e^{-\epsilon_{hij}/kT}}\right) \quad (3)$$

The terms within the parentheses are simply probabilities and the first term is the probability of finding the CSP in a particular conformation, the second term is the probability that the analyte is in a particular conformation and the last term is the probability that the two molecules are positioned and oriented in a particular way with respect to each other. The reader should note, too, that since one can locate all the minima on the complex's intermolecular potential energy surface one can derive the entropy of the system. Therefore $\bar{E}$ is actually a good representation of the macroscopic free energy of interaction. For a definition of all symbols and a derivation of this equation, the reader is referred to the original literature.

Using this protocol a reevaluation of the binding of R and S 2,2,2-trifluoro-1-(9-anthryl)-ethanol on the R-phenylglycine DNB Pirkle phase was undertaken. The results reaffirmed earlier work [22]. Then, in two subsequent papers [23,24], the protocol was tested on a broad set of analytes (**6–11**) binding to CSP

analogues 2 (R = phenyl) and 5. Because it was possible to compute the differential free energies of binding[a], it was also possible to compute the corresponding separability factors, α. Having demonstrated the modeling protocol to be reliable the authors began extracting information from the simulations.



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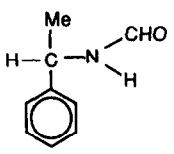

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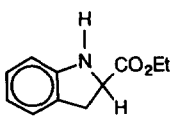

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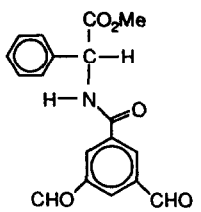

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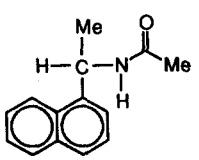

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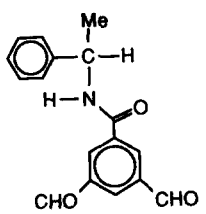

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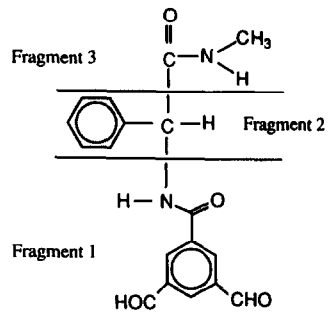
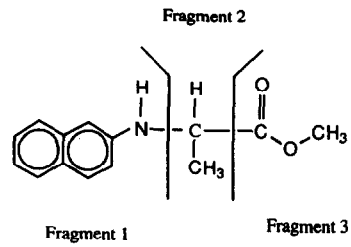
First they considered the binding site on the CSP. They were able to conclude that the binding sites are the same indicating that it is not *where* the analyte binds that is important but rather *how* it binds that is important. Next they considered the stereodifferentiation process itself. They developed an energy partitioning scheme that allowed them to divide the total binding enthalpy into molecular fragments constituting the CSP. Generally what they find is that for both CSPs, fragments 1 and 3 are most responsible for discrimination.

[a] The applicability of this computational method to other problems in chiral recognition has been demonstrated [25].



CSP2



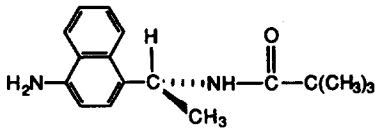
CSP4

Concurrent with this were studies by Topiol *et al.* [26] of enantioselective binding of *R* and *S*-N-(3,5-dinitrobenzoyl) leucine N-propyl amide ("DNB"), with *S*-N-(2-napthyl) alinate ("NAP"). The most stable structures of each of these two molecules were obtained with an EFF and then the relative position of the two molecules in the complex was determined by a limited search strategy. Several low energy structures were obtained with the EFF method that have intermolecular π-stacking and hydrogen bonding interactions similar to those proposed by Pirkle and Pochapsky. However, Pirkle and Pochapsky claim that chiral discrimination in these systems (via a "three-point" model) is because the *SR* complexes cannot have the same three interactions as the more stable *SS* complexes. The results of Topiol *et al.* show that the *SR* complexes can. The most significant and widely debated issues identified from Topiol *et al.*'s work is that the model posited by Pirkle to explain the mechanism for chiral discrimination in the systems studied is not a "three-point" model

because the three interactions described emanate from only two bonds of the stereogenic center. This raises the possibility, suggested in refs. 26–29, that the same three interactions may be present in both diastereomeric complexes.

Topiol and Sabio [26] also used semi-empirical molecular orbital methods to understand the origins of enantioselectivity. He divided the molecules into smaller entities that would allow him to assess how much π-stacking energy and how much hydrogen bonding energy contributed to the stabilization of each complex. In a later paper [27] full geometry optimization using the AM1 Hamiltonian resulted in a larger distance between the centroids of the aromatic rings. The results of this paper confirm Topiol *et al.*'s earlier prediction [26] that the same three primary interactions found in the *SS* complex are found in the *SR* complex but to a lesser extent. Even higher quality quantum mechanical treatments of the π-stacking was reported in a following paper [28] resulting in the same conclusion.

The same molecules described above were also examined by Sabio and Topiol [29] with molecular dynamics. The main thrust of Topiol's MD paper is that the primary interactions of the *SS* complex are similar to those in the *SR* complex (same conclusions as above) and that these interactions are conserved during the MD simulation even though other conformational changes take place. Two results from his study are: the lowest energy structure favors the *SR* complex, and the Boltzmann averaged structure favors the *SR* complex. These results are inconsistent with experiment. However, the author repeatedly stresses that the energy difference responsible for the chiral separation, and the reversal of orders, is well within the error limits of methodology. Actually, the reversal of the Boltzmann weighted data is not due to the MD simulations, but rather, from using weighted data taken from the minimized energies. It is stressed that the closeness of the energy is the meaningful result. Overall, though, Topiol [26–29], like Lipkowitz [19–24], finds similar interactions in the two competing diastereomeric complexes that differ only in the magnitude of interaction which takes place.



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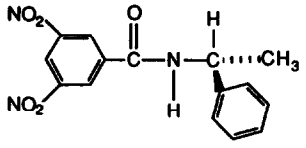

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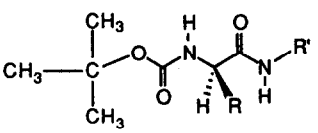
Two other research groups have also been very concerned with how best to sample configurations for Boltzmann weighting. They are Still and Rogers at Georgia and Däppen, Karfunkel and Leusen at Ciba-Geigy. Däppen *et al.* [30][a] have determined than the *R* enantiomer of **13** is bound tighter to **12**, which is tethered to silica by the amino group, than the *S* enantiomer. Because they had experimental $\Delta\Delta H$ values they focused their efforts on computing enthalpies rather than free energies. They began by carrying out a conformational analysis of **12** and **13** with semiempirical molecular orbital methods and with an EFF. The low energy structures were then used to construct starting points for the binary complexes. They considered two docking strategies. One is to use a systematic grid search and the other is to use docking maneuvers that would take advantage of well established binding motifs found between the two molecules in the complex. Three binding motifs were considered to be important: hydrogen bonding, π-stacking and dipole stacking of the two amide groups. They express confidence in this approach to building the complexes because all the low energy structures have at least one of these three binding motifs. Däppen *et al.*'s approach is a good one and they were able to successfully model the enantioselective

[a] A preliminary study, using semiempirical molecular orbital theory to derive the conformations of their "PNEA" chiral phase and phenylethylamine 3,5-DNB analyte, along with an analysis of selectivity on chiral *vs.* achiral CSPs attributable to π-acid–π-base interactions, should be read [31].

binding of **13** to CSP analogue **12** and then make a prediction of what structural modification to **12** would result in enhanced selectivity. An excellent description of how to do these motif-based searches can be found in ref. 32.

Still and Rogers have synthesized chiral stationary phases by bonding *tert.*-butyloxy carbonyl (BOC) derivatives of amino acids to a butyl spacer on silica and then examined their ability to discriminate between *R* and *S* 2,2,2-trifluoro-1-(9-anthryl) ethanol. The modeling involved CSP analogue **14** where R = CH_3 (alanine), R = isopropyl(valine), and R′ = different length *n*-alkyl chains [33]. Using the MM2 force field Still and Rogers assessed the distribution of conformers of the analyte and the CSP analogues. Four docking strategies were employed in this study and only the most stable structures from their conformational analysis were used except in the case of BOC-D-valine-N′-*n*-propylamide where two conformers of similar energy were used.



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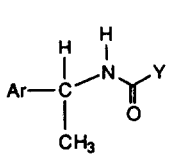
The docking strategies were based on results from NMR chemical shifts, and so, is an example of motif-based docking. The first strategy involved maximizing the interactions between the carbonyl oxygen of the BOC group and the hydroxyl hydrogen of the analyte along with the interaction of the protected amine's hydrogen with the analyte's anthryl ring. Different orientations did not have large interaction energies for either assumed points of interaction so three other motif-based docking maneuvers were employed until low energy structures were found.

For analyte binding to the BOC-D-alanine-N′-*n*-propylamide CSP the *S* enantiomer was found to be favoured by 0.05 kcal mol^{-1}. This is inconsistent with experimental retention orders but is consistent with the small energy difference observed experimentally ($\alpha = 1.02$). The 0.05 kcal mol^{-1} energy difference is within the uncertainty of the force field used. When analyte binds to the BOC-D-valine-N′-*n*-propylamide CSP the *R* enantiomer is favored by 0.18–0.52 kcal mol^{-1} depending on which conformer of CSP was used in the docking. The authors concluded that the valine phase would be more effective than the alanine and that the *R* analyte would be eluted later than *S* on the valine phase. Both predictions agree with experiment.

Still and Rogers then began assessing the origins of enantioselectivity. They examined the energy of interaction between parts of the analyte with the CSP. This is the same partitioning method Lipkowitz used except that Still and Rogers consider the most stable structures while Lipkowitz averages over many structures. The largest difference, and thus the most discriminating fragment, is the anthryl ring and not the oxygen of the analyte even though this atom is contributing heavily to the formation of the complex. A similar treatment allowed them to determine the most discriminating parts of the valine CSP. Eventually they reassessed their sampling strategy and examined alternative ways of computing interaction energies [34].

The system Still and Rogers focused on was the *R*-phenylglycine DNB Pirkle phase that Lipkowitz had earlier studied but replacing the N-methyl with an N-propyl group to better represent the spacer chain. Three aminoethanes, **15–17**, whose retention orders and separability factors are known were examined. In all cases the *S* enantiomer was longer retained on the *R* CSP. In an ensuing paper [35] they extended the computational study to consider how the dielectric of the medium affects the conformer populations, discussed modeling of different size spacer linkages, and, provided far more detail of the structures of the docked species. The authors also demonstrated that relying only on the weighted average enthalpy terms did not always agree with those based on free energies nor with experimental data; entropy must be considered.



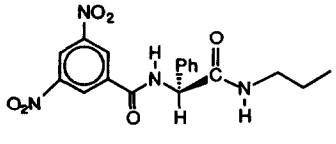
15 Ar = 1-naphthyl, Y = Me $\alpha = 1.86$

16 Ar = 1-naphthyl, Y = OMe $\alpha = 1.52$

17 Ar = 1-phenyl, Y = Me $\alpha = 1.15$

Lipkowitz and his group also modeled this system [36]. The enantioselective binding of the same analyte to Still and Rogers' BOC-D-Val chiral stationary phase was carried out using a grid searching strategy. It was found that the enantiomer with the *R* configuration is longer retained and that the separation factor, α, was slightly overestimated. It was found that both enantiomers bind to the same general region around the CSP but that the intermolecular potential energy surfaces are much flatter than in the Pirkle system. Also, the BOC group was determined not to be most responsible for chiral recognition as proposed by Still and Rogers. Rather, the amide group on the spacer is most enantiodifferentiating. Finally, in an attempt to understand why the separations are insensitive to solvent polarity (the k values decrease but α is invariant to polar modifiers) an analysis of diastereomer solvation was undertaken. Fully 3/4 of the BOC-D-Val CSP's surface was found to be hydrocarbon in spite of the CSP having two amides and an ester functionality. These polar functional groups seem to be hidden under an umbrella of aliphatic hydrocarbon atoms preventing polar solvents from interacting with CSP. Eventually the concern of neglecting solvent when modeling enantioselective binding precipitated a full study of the differential solvation energies of weakly bound, non-ionic diastereomers as found in chiral chromatography [37].

Several other groups have also used molecular modeling to predict chiral separations on brush-type CSPs. Of particular note are the papers of Norinder and Sundholm [38,39]. These scientists considered monopole–monopole, monopole–dipole and dipole–dipole interactions in their calculation of the electrostatic part of the total interaction energy. They considered **18** as a CSP analogue for **1** (R = phenyl), **19** as a CSP analogue for **4** (R = methyl) and **20** as an analogue for CSP **21**.



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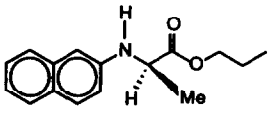

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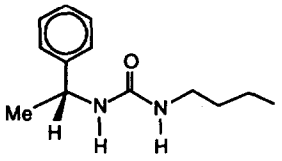

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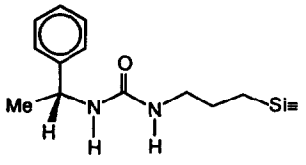


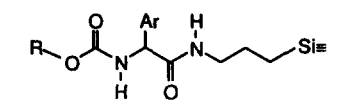
21

Using an empirical force field they assessed the allowed conformational states of CSP analogs and of analyte. The lowest energy structure of each was then selected to provide a set of starting structures for geometry optimization. Their results agree with the observed retention orders but the ΔH values do not correlate with the observed separation factors, α. Better agreement may be found using Boltzmann averaged enthalpies rather than the energy of only the most stable complex and, as Lipkowitz [22] and Rogers [34] point out, a true free energy that includes an entropy term should be considered. Still, because the retention orders were correctly predicted, Norinder and Sundholm were able to examine the structural similarities and differences of the diastereomeric complexes that are presumed to result in chiral recognition. It should be noted that this is one of the better treatments of electrostatic effects in the EFF computation of diastereomeric complexes. A shortcoming of most empirical force fields is that they deal with only "classical", well characterized interactions of the electrostatic type but omit or misrepresent other effects like π-facial hydrogen bonding described by Still and Rogers above.

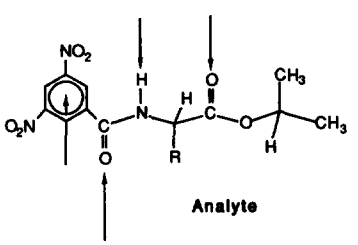
In another paper the authors extended their work to two other derivatives of the drug alaproclate binding to CSP analogue **20** [39]. Of these two derivatives one yields small separations (α =

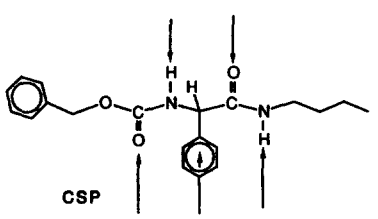
1.06) indicating very small differential binding energies while the other can not be separated at all. Their modeling results indicate small energy differences which are consonant with experiment but predict the wrong retention order for the analyte that was resolved. These small energy differences are well within the limits of the force field which, therefore, is not reliable enough to make such predictions unless special precautions are taken.

Finally, Kruger *et al.* [40] examined the resolution of a variety of amino acid derivatives on CSPs **22–24**; the first two of which are D-phenylglycine derivatives and the last an L-1-naphthylglycine derivative. The analytes included 3,5-dinitrobenzoyl amino acid 2-propyl esters and N-acyl amino acid 2-propyl esters which, depending on the derivatization pattern of the amino acid, adsorbed to the CSP by two different mechanisms resulting in opposite elution orders. One of these mechanisms clearly indicated the hydrogen bonding of the analyte's amide as a binding site, and, that $\pi-\pi$ interactions between aromatic rings was important. The binding sites on the analytes are indicated by arrows above and the complementary sites on the CSP are depicted below.



22 Ar = phenyl; R = benzyl
23 Ar = phenyl; R = tert-butyl
24 Ar = 1-naphthyl; R = tert-butyl





To better understand the CSP–analyte binding they computed the energies of the complexes using an EFF. No description of a conformational analysis nor of the docking procedure was given. The authors appear to have used the motif-based docking strategy and, with computer graphics, manually moved the molecules around until the energies of the minimized structures agreed with experimental elution orders.

Although the structures of the complexes and a discussion of the types of intermolecular bonding is presented, the validity of these results cannot be evaluated because no energy differences were reported for comparison with experiment. Nonetheless their modeling is consistent with elution orders and they do propose viable retention mechanisms elucidated by theory.

4. CONCLUSIONS

Atomistic molecular modeling has been successfully implemented in several disciplines in the chemical sciences to make predictions and to gain new insights. Application of these computational methods in separation science has been, in the author's opinion, less successful. Of the many published studies, it is common to find the wrong retention order predicted by theory. At scientific meetings one hears that some scientists can compute the correct order of analyte retention by simple docking and energy minimizing but other examples attempted that way are not consonant with experiment.

One problem is that the computational tools needed to successfully model enantioselective binding are only now being developed to accommodate much of what has been omitted from most calculations, *e.g.* solvent, ions, etc. Furthermore, a statistically averaged interaction energy for the transient, diastereomeric complexes is needed but is rarely computed. This requires *a priori* information, *e.g.*, motif-based searches, or large statistical samplings of positions and orientations accounting for all probable conformers of both selector and selectand. The best approach is to carry out full molecular simulations rather than simple energy minimizations. Those who have done this have generally had more success than those who have not

adequately sampled conformational and configurational space.

The future for molecular simulation of chiral recognition in separation science is bright. The proliferation of hardware and software needed to be successful has been astonishing during the past three years. These tools are now available and, with some care, one can carry out meaningful simulations to answer questions that are important to both the science and technology of chiral chromatography.

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