Elucidation Of Chiral Recognition Mechanisms Of Solutes By Amylose Tris\[(s)-Alpha-Methylbenzylcarbamate\] Sorbent

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[(S)-ALPHA-METHYLBENZYLCARBAMATE] SORBENT

For the degree of  Doctor of Philosophy

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Head of the Graduate Program  Date
ELUCIDATION OF CHIRAL RECOGNITION MECHANISMS OF SOLUTES BY AMYLOSE TRIS[(S)-α-METHYLBENZYLCARBAMATE] SORBENT

A Dissertation

Submitted to the Faculty

of

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by

Hung-Wei Tsui

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of

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West Lafayette, Indiana
To

My family
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ABSTRACT


Enantioselective separations of chiral molecules are important in various chemical fields, such as pharmaceuticals and agrochemicals industries. Polysaccharide-based sorbents have been widely used in chiral liquid chromatography. The recognition mechanisms which determine their enantioselectivities are not completely understood.

In this dissertation, the chiral recognition mechanisms of a widely used commercial sorbent, amylose tris[(S)—methylbenzylcarbamate], for benzoin (B) enantiomers were first studied. The HPLC data for benzoin with pure n-hexane as the mobile phase have been obtained. The behavior of sorbent-solute-hexane systems can be interpreted by considering only sorbent solute two-component interactions. Infrared (IR) spectra showed evidence of substantial hydrogen bonding (H-bonding) interactions in the pure polymer phase, and additional H-bonding interactions between AS and benzoin. Density Functional Theory (DFT) was used to model the chain-chain and chain-benzoin H-bonding or other interactions. From high performance liquid chromatography (HPLC), and IR data, and DFT and molecular simulations, the observed enantioselectivities were inferred to be due primarily to two strong H-bonds, of the kind (AS) CO … HO (R-
benzoin) and (AS) NH … OC (R-benzoin) and one strong H-bond (AS) CO … HO (S-benzoin) for S-benzoin. Three additional solutes containing the same functional group, O= C-C-OH, as benzoin were studied: ethyl lactate (EL), methyl mandelate (MM), and pantolactone (PL). IR, DFT, and molecular simulations lead to a general hypothesis for the chiral recognition mechanism for these solutes. The mechanisms for these systems involve a non-enantioselective strong, or “leading”, H-bonding interaction and an enantioselective weaker, or “secondary”, H-bonding interaction, which is affected by geometrical restrictions. There is one or more additional interactions which determine the overall enantioselectivity. A new measure of molecular rigidity was developed with MD simulations. The solute with the small rigidity or high flexibility has the lowest enantioselectivities.

The adsorption mechanisms of the chiral solutes were also probed macroscopically by using retention factor data for hexane-alcohol modifier solutions and stoichiometric displacement models. The models were used to explain the slope of the plot of the logarithms of the solute retention factor versus the molar concentration of a competitive modifier in an inert solvent. In previous models the slope was inferred to be equal to the total number of the modifier molecules displaced from the sorbent and from the solute-modifier complex upon adsorption of a solute molecule, and were presumed to be generally greater than 1. Nonetheless, for the four chiral solutes studied, with increasing IPA concentration C\textsubscript{1\textsuperscript{0}}, it was discovered that slopes (B) smaller than 1 were possible, at concentrations from 0.13 to 1.3 M. The slopes were slightly more than 1 at higher concentrations. Such data cannot be explained by any previously available model.
To address this problem and make effective use of the data in elucidating the solute-sorbent interactions, five monovalent simple solutes, acetone, cyclohexanone, benzaldehyde, phenylacetaldehyde, and hydrocinnamaldehyde, were chosen for study. The results of IR and DFT simulations showed clear evidence of IPA aggregation with average aggregation number n=3. A new thermodynamic retention model was developed to take into account IPA aggregation. Such aggregation phenomena affect the slopes significantly and lead to a significant reduction in the IPA monomer concentrations, which affects the IPA-sorbent binding, the IPA-solute complexation, and the slope. This discovery and the new models allow an accurate and reliable interpretation of the data in terms of alcohol displacement from the sorbent and the solute, and allow the determination of the number of the interaction sites of the solute with the sorbent.

For the above four chiral solutes, a new more complex multivalent retention model is developed. It accounts for alcohol aggregation, multivalent solute adsorption, multivalent solute-alcohol complexation, alcohol adsorption, and solute intra hydrogen-bonding which was also found to be important for these four solutes. The limiting slope LS at a very high ("infinite") IPA concentration is predicted to be equal to the value of (x+y)/n, where y is the average number of the complexation binding sites, x is the adsorption binding sites, and n is the average alcohol aggregation number in hexane solution. The model was found to fit well the HPLC data. The estimated y-values correlate fairly well with the number of the solute functional groups, suggesting that y can be estimated from the inspection of the solutes molecular structures. Moreover, the x-values can be estimated from the values of the limiting slopes and the numbers of solute functional groups. The same values of the binding sites are found for the most R- and S-
enantiomers. The results suggest that the effective number of the binding sites are the same for enantiomers of each solute. The binding equilibrium constants were found to be significantly different for the two enantiomers, suggesting that S-enantiomers, which were predicted to be non-H-bonded, simply bind with the sorbent more weakly. Overall, the results of these models provide additional insights and complement the mechanistic studies done for these systems.
CHAPTER 1. INTRODUCTION

1.1 Background

Many biomolecules — nucleic acids, proteins, polysaccharides, lipids, and many drug molecules — are chiral enantiomers, or stereoisomers that are mirror images of each other. A 50:50 mixture of chiral enantiomers is called a “racemate”. The enantiomers have identical physical and chemical properties. The human body contains, however, numerous chiral sites, which show stereo-specific interactions with only one enantiomer, and may metabolize each enantiomer by separate pathways to produce different pharmacological activities. One enantiomer may be safe and therapeutically effective, while the other may be toxic, because of slow metabolism and accumulation in internal organs.

Many drugs derived from natural products are enantiomers. As products of synthetic chemistry, many chiral drugs have been used as racemates until recent years. Examples include Prozac, used for treating depression, and Thalidomide, one enantiomer of which was found to be teratogenic to the offspring of some users. After 1992, FDA guidelines for racemate drugs require rigorous testing of each enantiomer for its pharmacological activity. There is a growing trend to develop single enantiomer drugs. In 2001, 69% of all newly licensed or late-stage development products were single enantiomers. Worldwide sales of single-enantiomer drugs exceeded $150 billion per year.
in 2002. Examples of single-enantiomer blockbuster drugs with 2002 sales of $1 to 8 billion are for cardiovascular (Lipitor, Zocor), depression (Paxil, Zoloft), gastrointestinal, and respiratory diseases. “Chiral switching” has been used by companies to maintain a competitive advantage (Rouhi 2004).

To produce pure chiral enantiomers one needs to use enantiomer-specific synthesis or to produce a racemate mixture and separate the enantiomers. Significant advances have been made in the enantiomer-specific synthesis area. One racemate separation method, asymmetric crystallization, is often not feasible and cannot achieve yields higher than about 90%. Adsorptive separations are generally applicable, and they can achieve high purity (>99%) and high yield (99%) if used in simulated moving bed (SMB) processes (Broughton and Gerhold 1961; Broughton 1984; Ganetsos and Barker 1993; Gattuso et al. 1996; Schulte et al. 1996; Francotte and Richert 1997; Miller et al. 1999; Juza et al. 2000; Huthmann and Juza 2002). For chiral molecules, “upstream processing” costs are 30 to 50% of the total production cost, and “downstream processing” costs, mainly separation and purification processes, are higher, 50 to 70% of the total production cost (Agranat and Caner 1999; Srinivas et al. 2001).

The enantioselectivity, the adsorption capacity (grams or moles sorbed per kg of sorbent), the sorbent particle size, and the column configuration are the four key elements which control the efficiency and the cost of analytical and preparative chiral chromatography processes. The enantioselectivity of a sorbent, or the “enantioresolution” of the solute, is the ratio of the retention factors (k), or the equilibrium adsorption constants of the enantiomers. High enantioselectivity and moderate binding constants favor high productivity (measured as kg of product/kg sorbent per day) and low solvent
consumption (Francotte and Richert 1997; Thomas and Raymond 1998; Satinder 2000; Subramanian 2000; Franco and Minguillon 2001; Francotte 2001; Maier et al. 2001). Chiral Stationary Phases (CSPs) with a small sorbent particle size (5-10 µm), for which high operating pressures (50-200 atm) are needed, have been used to reduce peak spreading due to intraparticle diffusion, and to improve resolution in analytical chromatographic separations. CSPs with particle sizes of 20 to 30 µm have been used in preparative batch chromatography or in simulated moving bed (SMB) processes. The latter processes have an order of magnitude higher productivity and much lower solvent requirements than batch chromatography (Lee et al. 2005). Equipment costs are about 70% of the high-pressure SMB separation costs (ca. $100/kg). The Wang group at Purdue developed design methods based on the concepts of “standing concentration waves”, which can be used to help ensure high purity (99%) and high yield (99%) in medium to low pressure SMB processes (Ma and Wang 1997; Xie et al. 2000; Y. Xie et al. 2001; Mun et al. 2003). These methods have been incorporated in a genetic algorithm program used to optimize particle size, column length, column configuration, and zone flow rates for SMB processes (Lee et al. 2005). Sufficiently high enantioselectivities are the key for the successful application of such methods.

Many different types of chiral adsorbents − proteins, macrocyclic antibiotics, cyclodextrins, “Pirkle” phases (low molecular weight brush-type monolayers chemically attached to the particles surfaces), ligand exchange phases, and polysaccharide phases − have been developed over the past two decades (Pirkle 1997; Pirkle and Liu 1996; Snyder et al. 1997). The key suppliers of these CSPs are the Daicel and Akzo Nobel companies.
Derivatized-amylose or cellulose-based polymeric CSPs, or polysaccharide (PS)-based phases, are quite effective CSPs. They have been used in over 50% of all analytical and preparative chiral separations of low molecular weight (<500 Daltons) compounds (Ward and Ward 2010). Amylose or cellulose polymers without any derivatization (Figure 1.1) have usually unfavorable enantioselectivities for separating most chiral solutes, and derivatization is needed. Usually thin PS films are deposited inside porous silica particles. Moreover, commercial CSPs with the polymer covalently attached to the silica surfaces became available recently (Ikai et al. 2007; Ikai et al. 2008). Many literature articles on these CSPs have focused on separation results (mostly based on chromatography) by using various chiral compounds, or sorbents, or solvents. Excellent review papers on polysaccharide-based CSPs have focused on the development of these CSPs and their applications, and have covered various classes of chiral molecules (Ikai et al. 2008; Lammerhofer 2010; Ward and Ward 2010; Chankvetadze 2012). Other papers have also discussed the chiral recognition, or chiral discrimination, mechanisms of these CSPs based on information from several techniques (Yashima et al. 1994). In recent years, many important papers have been published on the elucidation of molecular-level recognition mechanisms of these CSPs.

The reasons why many derivatized polysaccharide phases can separate a wide range of chiral molecules remain somewhat unclear, despite many important empirical and mechanistic studies. Many experiments are often needed to screen many sorbents, solvents, and operating temperatures, in order to discover conditions for a favorable enantioselectivity for an analytical or preparative separation. Moreover, effective PS-based chiral sorbents can be quite costly, up to $20,000/kg. For reducing the number of
such experiments and the material, equipment, and solvent costs for chiral separations, fundamental understanding of the molecular recognition mechanisms is crucial. This understanding may help develop better, and less expensive CSPs, formulate appropriate sorbent and solvent screening strategies, and suggest molecular structures with promising or improved enantioselectivity properties.

Three derivatised amylose or cellulose PS polymers have been studied extensively by our group at Purdue University. One is ADMPC, or simply AD, or amylose tris(3,5-dimethylphenylcarbamate) (see Figure 1.1). Another is ASMBC, or simply AS, amylose tris((S)-α-methylbenzylcarbamate). Comparison of the behavior of AS to that of AD, can provide insights on the role of the side chain on the enantioselectivity. CDMPC, or simply OD, is cellulose tris(3,5-dimethylphenylcarbamate). Comparison of the AD properties with the OD properties provides insights on the role of the polymer backbone. When these polymers are used in commercial columns, they are then called Chiralpak AD, Chiralpak AS, and Chiralcel OD, respectively (Kasat et al. 2006A; 2006B; 2007; 2008A; 2008B; 2010; Tsui et al. 2011).

For two chiral molecules, PPA (norephedrine) and MEph (methyl ephedrine), the molecular structures of which are shown in Figure 1.1, the enantioselectivities $\alpha$ (S was used in several previous publications), at 25°C in 10% IPA in hexane, depend strongly on the polymer used (see Table 1.1). For PPA only AD has a substantial value of $\alpha=2.4$. For MEph only OD has a substantial value of $\alpha=2.1$. The polymer side chains have several potential solute-binding or solute-interacting sites, e.g. the C=O and N-H groups for H bonds, the phenyl groups for $\pi-\pi$ interactions, and the CH$_3$ groups for hydrophobic
Figure 1.1 Molecular structures of amylose, cellulose, side chains R₁ and R₂, along with those of PPA and MEph chiral solutes. AD is amylose with R₁ side chains, AS is amylose with R₂ side chains, and OD is cellulose with R₁ side chains.
interactions. Moreover, they contain nm-sized cavities (nano-cavities) of considerable steric complexity (Figure 1.2), which may allow selective inclusion, by accommodating, or favoring energetically, one enantiomer but not the other, to help achieve substantial enantioselectivity. It is a challenge to understand how the enantioselectivity depends on specific steric, hydrogen bonding (H-bonding), hydrophobic, or electrostatic interactions, or on other factors.

Chiral selectivity requires stereo-specific interactions. One established hypothesis, or model, for chiral selectivity involves the presence of adjacent sites for three-point interactions (Figure 1.3), which favor one enantiomer interaction energetically or sterically (Easson and Stedman 1933). This idea has been evidently used in developing commercial chiral sorbents (Okamoto and Ikai 2008; C. Yamamoto and Okamoto 2004). The possible mechanisms of chiral discrimination for three-(or sometimes four)-point interactions have been reviewed (Pirkle and Liu 1996; Booth, Wahnon, and Wainer 1997; Davankov 1997; Pirkle 1997; Wainer 2001) Such interactions may involve the formation of inclusion complexes in a cavity or the formation of chiral cavities, with specific binding and steric restrictions (Lammerhofer 2010). Cyclodextrin-based sorbents or other materials have been studied by groups led by Armstrong, Lindner, Lipkowitz, Okamoto, and others (Feibush 1998; Wainer 1987), and were reviewed by Lammerhofer (2010). The molecular interactions involved in various chiral recognition mechanisms have been modeled with molecular simulations.
Figure 1.2 Structure of AD polymer (see Figure 1.1), as predicted from MD simulations of a 12-mer. The backbone atoms are shown with a stick representation. The side chains are shown with a line representation. One cavity is shown with a ball-and-stick representation.
Figure 1.3 Schematic representation of the three-point interaction model for chiral discrimination.
1.2 **Thesis Objectives**

The chiral recognition mechanisms of polysaccharides-based sorbents are difficult to determine because of their complex structures and other factors. Although many recent studies have aimed at understanding at a fundamental and molecular level the reasons for having a significant enantioselectivity, the solute-sorbent interaction mechanisms and the chiral recognition mechanisms have not been completely understood. Elucidating such mechanisms is the major objective of this dissertation. Specific objectives include the elucidation of mechanisms of solute-sorbent interaction in pure hexane, and solute-sorbent, solute-alcohol, and alcohol-sorbent interactions in hexane-alcohol mobile phases.

1.3 **Thesis Synopsis**

1.3.1 **Infrared Spectroscopy and Molecular Simulations of a Polymeric Sorbent and Its Enantioselective Interactions with Benzoin Enantiomers**


Retention factors, $k_R$ and $k_S$, and enantioselectivities, $\alpha \equiv k_R/k_S$, of AS sorbent for benzoin (B) enantiomers were measured for various isopropanol (or IPA)/n-hexane compositions of the High Performance Liquid Chromatography, or HPLC, mobile phase. Novel results for pure n-hexane show values of $k_R = 106$, $k_S = 49.6$, and $\alpha = 2.13$. With some IPA from 0.5 to 10 vol.%., with $\alpha = 1.8$ to 1.4, the retention factors were smaller. IR spectra showed evidence of substantial hydrogen bonding, or H-bonding, interactions in the pure polymer phase, and additional H-bonding interactions between AS and benzoin.
Density Functional Theory (DFT) was used to model the chain-chain and chain-benzoin H-bonding and other interactions. They were also used to predict fairly well the IR wavenumber shifts caused by the H-bonds. DFT simulations of IR bands of NH and C=O allowed for the first time the predictions of the relative intensities and the relative populations of H-bonding strengths. Molecular Dynamics (MD) simulations were used to model a single 12-mer polymer chain. MD simulations predicted the existence of various potentially enantioselective cavities, two of which are sufficiently large to accommodate one benzoin molecule. Then “docking” studies of benzoin in AS with MD, Monte Carlo (MC), and MC/MD simulations were done to probe the AS-B interactions. The observed enantioselectivities are predicted to be primarily due to two H-bonds, of the kind (AS) CO … HO (R-benzoin) and (AS) NH … OC (R-benzoin) and two π-π (phenyl-phenyl) interactions for R-benzoin, and one H-bond (AS) CO … HO (S-benzoin) and one π-π interaction for S-benzoin. The MC/MD predictions are consistent with the HPLC and IR results.

1.3.2 Chiral Recognition Mechanism of Anyloin-Containing Chiral Solutes by Amylose Tris[(S)-α-methylbenzylcarbamate]


Four solutes containing acyloin O=C-C-OH which has a hydroxyl group on the α-position of a carbonyl group were studied: ethyl lactate (EL), methyl mandelate (MM), benzoin (B), and pantolactone (PL). The observed retention factors $k_R$ and $k_S$ and enantioselectivities ($\alpha = k_R/ k_S$) were determined in n-hexane and in hexane-IPA solutions.
IR and DFT simulations of the interactions of these solutes with the side chains of the polymer lead to a general hypothesis for the chiral recognition mechanism for these solutes. A strong H-bond forms as the primary, or “leading”, non-enantioselective interaction, or “anchor” point, between the solute OH group of each enantiomer and the sorbent C=O group. A weaker H-bond forms preferably for the R-enantiomer between the solute C=O groups and the sorbent NH groups. The S-enantiomer, is prevented from forming such a bond for steric restrictions. A third interaction may involve the O groups of the phenyl groups of the solutes. IR shows evidence of an intra H-bond for all four solutes. The retention factors increase with increasing strength of the inter H-bond, and with decreasing strength of the intra H-bond. The enantioselectivities correlate with the molecular rigidity or flexibility, as determined from the distribution of the torsion angles of the acyloin group. The enantioselectivity was higher for the more rigid molecules. Simulations of left-handed AS with 200 n-hexane molecules indicate no effect of hexane on H-bonds in AS. Monte Carlo (MC) and MD “docking” simulations of AS with these solutes reveal certain chiral cavities which can lead to chiral discrimination. The results support the proposed mechanism.

1.3.3 Retention Models and Interaction Mechanisms of Acetone and Other Carbonyl-Containing Molecules with AS Sorbent  


The stoichiometric displacement models developed in the literature have been widely used for understanding the adsorption mechanisms of solutes in various
chromatography systems. The models were used to explain the linear plots of the logarithms of the solute retention factor versus the molar concentration of a competitive modifier in an inert solvent. The slope of the linear plot was inferred to be the total number of the modifier molecules displaced from the sorbent and from the solute-modifier complex upon adsorption of a solute molecule. The slopes reported in the literature were generally greater than 1. In this study, we determined the retention factors of five monovalent solutes, acetone, cyclohexanone, benzaldehyde, phenylacetaldehyde, and hydrocinnamaldehyde, on a AS sorbent, as a function of the concentration of a polar modifier isopropanol (IPA) in n hexane (an inert solvent). Each solute has one C=O functional group, which can form an H-bond with a sorbent NH group and the OH group of IPA. The slopes, from 0.25 to 0.45, of the log-log plots are less than 1, which cannot be explained by the literature displacement models. The results of IR and DFT simulations show clear evidence of acetone-IPA complexation and IPA aggregation with average aggregation number n=3. A new thermodynamic retention model is developed to take into account IPA aggregation, IPA-solute complexation, and competitive adsorption. Dimensionless group analysis indicates that aggregation of IPA can lead to slopes B below 1, even at high IPA concentrations. The model parameters (IPA aggregation number and equilibrium constants) are estimated from the retention factors at different IPA concentrations. The retention model and the parameters are further validated with dynamic chromatography simulations. The results show that the aggregation leads to a significant reduction in the IPA monomer concentration, which affects the IPA-sorbent binding and the IPA-solute complexation. As a result, the slope of the log-log plot at a high IPA concentration approaches 1/n without complexation, or 2/n with complexation.
The variations of B between the five achiral solutes can be due to different strengths of solute-IPA complexation. Hence, the complexation and aggregation of the polar modifier in the mobile phase must be accounted for in the retention models used in the interpretation of the retention factors and the adsorption mechanisms.

1.3.4 Effect of Alcohol Modifier on the Retention Factors of Chiral Solutes with AS Sorbent: Modeling and Implications for the Interaction Mechanism

(See Chapter 6)

Various displacement models in the literature have been widely used for understanding the adsorption mechanisms of solutes in various chromatography systems. The models were used for describing the often-observed linear plots of the logarithms of the retention factor versus the logarithms of the polar modifier concentration $C_I^0$. The slopes of such a plot was inferred to be equal to the number of the displaced modifier molecules upon adsorption of one solute molecule, and were generally found to be greater than 1. In this study, the retention factors of four structurally related chiral solutes, ethyl lactate (EL), methyl mandelate (MM), benzoin (B), and pantolactone (PL), were measured for the amylose tris[(S)-α-methylbenzylcarbamate] sorbent, or AS, as a function of the concentration of isopropanol (IPA) in n-hexane. With increasing IPA concentration $C_I^0$, the slopes increase from less than 1, at a concentration range from 0.13 to 1.3 M, to slightly more than 1 at higher concentrations. Such slopes cannot be explained by the conventional retention models. It was found previously for monovalent solutes, such slopes can only be explained when the aggregation of the mobile phase modifier, isopropyl alcohol, was accounted for. A new retention model is presented here,
accounting for alcohol aggregation, multivalent solute adsorption, multivalent solute-alcohol complexation, alcohol adsorption, and solute intra hydrogen-bonding, which occur for these four solutes. The slope is found to be controlled by three key dimensionless groups, the fraction of the sorbent binding sites covered by IPA, the fraction of the solute molecules in complex form, and the fraction of the IPA molecules in aggregate form. The limiting slope at a very high IPA concentration is equal to the value of \( \frac{x+y}{n} \), where \( x \) is the number of the solute-sorbent binding sites and \( y \) is the number of the alcohol molecules in the solute-alcohol complex, and \( n \) is the alcohol aggregation number. The model was tested with the HPLC data of two sets of chiral solutes, one set of new data presented here and of one set of literature data by Gyimesi-Forrás et al. (2009). For these solutes, the values of \( x, y \), the retention factors in pure hexane, and the complexation equilibrium constants were estimated. For EL and PL, results of Infrared Spectroscopy, Density Functional Theory, and Molecular Dynamics simulations indicated strong solute-IPA complexation, consistently with the fitting results. The \( y \)-values correlated fairly with the solute functional groups, suggesting that \( y \) can be estimated from the inspection of the solute molecular structure. Hence, the new model has been shown to be more reliable than the previous models for estimating the numbers of the potential binding sites of multivalent solutes.
CHAPTER 2. MATERIALS AND METHODS

2.1 Materials

AS-polymer-coated silica beads, or ChiralPak AS, and semi-preparative ChiralPak AS columns, which were 100 mm long, with a 10 mm column diameter, and 20 μm particle diameters, were provided by Chiral Technologies (Exton, PA). HPLC-grade 2-propanol, or isopropanol, (IPA) was purchased from Mallinckrodt Chemicals (Phillipsburg, NJ). HPLC-grade n-hexane was purchased from EMD Chemicals (Gibbstown, NJ). 1,3,5-tri-tertbutylbenzene (TTBB), pantolactone racemate, R-pantolactone (R-PL), S-pantolactone (S-PL), benzoin racemate (B), (R)-benzoin (R-B), (S)-benzoin (S-B), methyl mandelate racemate, R-methyl mandelate, S-methyl mandelate, S-ethyl lactate, carbon tetrachloride (CCl₄), tetrahydrofuran (THF), methanol-OD, acetone, cyclo hexanone, benzaldehyde, phenylacetaldehyde, and hydrocinnamaldehyde were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Ethyl lactate racemate was purchased from MP Biomedicals (Solon, OH). R-ethyl lactate was purchased from Beta Pharma (Branford, CT).

Deuterated benzoin racemate (B-OD) was synthesized with a H- to D- exchange reaction, by dissolving B-OH racemate, or each of the enantiomers, in methanol-OD in a molar ratio of 3:100. The solution was stirred for at least three hours at 25 °C. The resulting mixture contained about 25 mol% B-OD and 75 mol% of B-OH.
2.2 HPLC: Apparatus and Procedures

An Agilent 1100 HPLC modular system was used. It consisted of a built-in variable wavelength detector (VWD), a Micro Vacuum Degasser, an autosampler, a binary pump, and a thermostatted column compartment for controlling the column temperature within 1 °C. A flow rate of 1.0 ml/min at 25 °C was used for all HPLC experiments. The mobile phase was a mixture of IPA and n-hexane. The pulse injection volume was 20 μL. The wavelengths used were 247 nm for benzoin enantiomers, 330 nm for acetone, 280 nm for cyclo hexanone, 249 nm for benzaldehyde, 260 nm for phenylacetaldehyde and hydrocinnamaldehyde, 219 nm for pantolactone, 247 nm for benzoin, methyl mandelate, and ethyl lactate, and 204 nm for IPA. The retention time $t_{ref}$ of a non-adsorbing solute, TTBB, was used as a reference. It was 5.5 min at the conditions of the experiments. The retention factors, $k$, were found from the equation

$$k_i \equiv \frac{(t_i - t_{ref})}{t_{ref}}$$

where $t_i$ is the retention time of the enantiomer $i$; $t_{ref}$ is the reference retention time, or “void time”, of a non-retained solute, TTBB; $t_{ref}$ was 5.5 min at the conditions of the experiments. The retention times were measured two or more times, and the averages are reported. The enantioselectivity $\alpha$ (S was used in several previous publications) is defined as

$$\alpha \equiv \frac{k_R}{k_S}$$
2.3 **IR Spectroscopy: Apparatus and Procedures**

A Nicolet Protégé 460 Fourier transform infrared spectrometer, equipped with an MCT detector cooled with liquid nitrogen, or a triglycine sulfate (DGTS) detector, was used to obtain all IR spectra reported in Chapter 3. The spectral contributions from water vapor and carbon dioxide were minimized by continuously purging the instrument’s sample chamber with dry air from a Balston purge gas generator. ATR spectra were collected with unpolarized incident light at 298 K using a custom-made accessory with a Si-ATR plate (Wilmad, NJ). The incident angle was typically 50°, with 7 reflections. All spectra were taken at intervals of about 1 cm\(^{-1}\) using Happ-Genzel apodization. The resolution and reproducibility are estimated to be better than ± 0.2 cm\(^{-1}\). Even though the Si-ATR plates have an absorption cut-off at about 1500 cm\(^{-1}\), reliable spectra were obtained down to 1000 cm\(^{-1}\) by subtraction of the blank spectrum. For enhancing the spectral accuracy and the signal/noise ratio, the spectra were averaged by collecting 256 scans.

The polymer used in Chapters 3-6 was obtained from the coated polymer beads by dissolving it in THF and separating the solutes from the silica particles by filtration. The THF solution was deposited on the ATR plates and then dried. The films were annealed in a vacuum oven for at least 1 h at 80 °C. Spectra of dry cast films were obtained first. Then, benzoin solutions in carbon tetrachloride (CCl\(_4\)) were deposited on the AS films, and the CCl\(_4\) was allowed to evaporate for at least 20 min.

A Nicolet Protégé 6700 Fourier transform infrared spectrometer, with a triglycine sulfate (DGTS) detector, was used to collect all spectra in Chapters 4-6 at room
temperature (ca. 22±2°C). A transmission BaF$_2$ cell was used with a path length of 1.0 mm. The spectra were taken at wavenumber intervals of about 1 cm$^{-1}$. To enhance the signal-to-noise ratio, the spectra were averaged by collecting at least 100 scans. The reproducibility of the wavenumbers is estimated to be better than ± 0.2 cm$^{-1}$.

2.4 Computational Methodology

2.4.1 DFT Computational Methodology

The Gaussian 03 program was used for the electronic structure calculations. The hybrid B3LYP (Becke, three-parameter, Lee-Yang-Parr) functional with the 6-311+g(d,p) basis set (triple-ζ level) was used. This functional yields similarly accurate predictions of energies, intermolecular geometries, and vibrational frequencies as those obtained by using the ab initio MP2 (Møller–Plesset perturbation theory) method. It includes a combination of Hartree-Fock exchange with a DFT-exchange correlation. The basis set includes polarization functions on hydrogen and other atoms, and diffuse functions with diffuse sp-shells for other atoms. It has been suggested for use in simulations of ethanol aggregates (González et al. 1999). The default setting of the convergence criteria in Gaussian was used for the calculations. The root-mean-squares of the density matrix elements were used to achieve convergence to eight decimal places for up to 128 Self-Consistent-Field (SCF) cycles. The density matrix converged to at least six decimal places. The energy converged to within 10$^{-6}$ hartree. The input geometries for the DFT calculations were first determined with Monte-Carlo (MC) and Molecular Mechanics (MM) simulations, with Consistent-Valence Force Field (CVFF). This functional provides fairly accurate predictions of molecular geometries, atomization energies, H-
bonding energies, and vibrational frequencies. DFT predictions are less accurate for calculations of $\pi-\pi$ interactions than for H-bonding interactions. A correction factor of 0.96 for all predicted wavenumbers was used. Predictions of wavenumber shifts are generally considered to be more accurate than predictions of absolute wavenumbers.

In Chapter 3, the calculations were done for (a) benzoin; (b) a model AS side chain, termed “S1” (it has 26 atoms); and (c) a simplified model of the side chain, “S2” (it has 13 atoms), which is the same as the molecule methyl N-methylcarbamate (MMC); see Figure 1. The structures and energies of these side chains at minimum energies were obtained within about two days of computations in a personal computer. The IR wavenumbers and intensities of B, S1, and S2 were obtained for the optimized structures. To estimate the strengths of H-bonded interactions between the side chains in the actual polymer phase, two chains in contact were modeled with DFT. For probing H-bond energies and IR wavenumbers of interacting chains, computations for a pair of side chains S1-S1 were too long. to reduce the computation time, computations were done for a simpler S2-S2 pair, which has fewer steric hindrance effects. The interactions between R-B or S-B with a single chain were modeled with DFT using either an S1 or an S2 chain. Each of these computations took about two weeks and one week, respectively. Although each of these calculations may not always lead to a configuration of global minimum energy, they provided some useful predictions.

In Chapter 4, the energies of the H-bonding interactions between each chiral solute with a single S2 side chain were determined for ranking the strengths of the H-bonds. In the first simulation, the chiral solute was placed with its OH group near the C=O group of the S2 side chain, which then acts as an H-bond acceptor. In the second
simulation, the chiral solute was placed with its C=O group near the NH group of the S2 side chain, which then acts as an H-bond donor. Each binding configuration was energy-minimized. Although each of these calculations may not lead to the configuration with the global minimum energy, it provides a useful prediction for helping test the postulated chiral recognition mechanism inferred from the IR data.

In Chapter 5, the calculations were done for single achiral solutes, single IPA molecules, and IPA aggregates. For the IPA aggregates, the reported binding configurations of alcohol aggregates were used as the possible initial geometries for structure optimization (González et al. 1999).

In Chapter 6, the energies of the H-bonding interactions between each chiral solute with an IPA molecule were determined. Each binding configuration was energy-minimized. These calculations were used for helping test the postulated binding mechanism.

2.4.2 Molecular Simulation Methodology

Since AS was reported to have the same pitch length as AD (Kasat et al. 2008), which forms a 4-fold left-handed helix (Ma et al. 2008; P. Zugenmaier and Steinmeier 1986), a left-handed 12-mer AS model with three four-fold unit cells was constructed. The Linked-Atom Least-Squares (LALS) package was used. The pitch length was chosen to be 1.46 nm, as found from XRD (Kasat et al. 2008). The backbone structure of the AS was fixed for all subsequent simulations. The energy of the resulting polymer (981 atoms), which has the shape of a rod with attached side chains, was first minimized using molecular mechanics (MM) simulations with the Discover Module from the Materials
Studio Modeling software (Accelrys), version 5.0. For finding the global minimum more reliably, MD simulations with the same Module were used. The consistent-valence force field (CVFF) was mostly used in both the MM and the MD simulations. Some comparisons with some other force fields were made. The CVFF force field uses a Morse potential for modeling bond stretching, Coulomb’s law for electrostatic interactions, and a Lennard-Jones function for van der Waals (vdW) interactions. Hydrogen bonding interactions are modeled as a combination of electrostatic and vdW interactions. With these features, these models may lead to somewhat accurate predictions of the molecular structures, but less accurate predictions of the H-bond energies and the IR wavenumber shifts (Dauber-Osguthorpe et al. 1988).

To explore as much as possible the potential energy surface, and allow the calculations to reach as close as possible the “equilibrium” minimum-energy configuration, by avoiding energy barriers, the MD simulations were done as follows. An NVT (number/volume/temperature) ensemble which was controlled by a Nose-Hoover thermostat was used (Nose 1991), with a time step of 1 fs. Simulations were first done at 500 K for 1 ns; then at 450 K for 1 ns; then at 350 K for 1 ns; and finally, at 298 K for 3 ns. The formation of hydrogen bonds was recognized from the ranges of the distances \( d \) between \( H \) and \( X \), and the angles \( \theta \) between \( X-H \) and \( H\cdots Y \), \( X-H\cdots Y \).

In Chapter 4, for studying solvent effects, a system of one AS polymer rod with 200 n-hexane molecules was used. A periodic boundary condition with a box of dimensions \( 3\times4\times7 \) nm was used. The simulations were done for 2 ns, to ensure reaching equilibrium. Twenty frames of the polymer/solvent structure after 2 ns were randomly used for the analysis. The thus predicted structural energies of AS can be separated into
valence energy, or intrinsic AS structural energies of the side chains, and non-bond energy, which is mainly the intermolecular interaction energy between the side chains. The non-bond energies can be further separated into two types, van der Waals energy and electrostatic energy.

In Chapters 3 and 4, for docking studies, the above 12-mer polymer model was used. For AS, AD, or CD, Kasat et al. also used a 12-mer (Kasat et al. 2008). For AD, Li et al. used a 36-mer (Li et al. 2010). In this dissertation, we focus on the central section of the 12-mer, containing monomers 5 through 8, to minimize possible chain end effects. In this section, there were cavities of sufficient sizes to accommodate one benzoin molecule. To avoid a bias in the selection of the cavities and of the initial solute orientations in the cavities, MC docking simulations were used before the MD simulations as done previously.18

The Sorption Module from Material Studio was used for the MC simulations. Since the predicted polymer structure changed little after equilibration, one snapshot of the AS structure was randomly chosen from the conformations near the equilibrium state.

During the simulations, the structures of the AS and the solute molecule were fixed, to search for the strongest interaction sites along the surfaces of the AS cavities. Van der Waals forces at distances of 0.5 nm or less were considered, to compensate for a possible overestimation of the π-π interactions with CVFF, and to improve the efficiency of searching for the binding sites. The simulations were performed with five temperature cycles, $10^5$ maximum loading steps, and $10^7$ production steps. The annealing search was automatically controlled by the program. At the end of the simulations, 20 frames with the lowest minimum energies were chosen as representing the most likely configurations.
The results of the solute and chain orientations and the configurations from the MC searching simulations were set as the initial values for subsequent MD simulations. Since polymer-polymer and polymer-polymer-solute interactions are not considered in these simulations, one may expect a qualitative agreement with enantioselectivity data, or, at best, a semi-quantitative agreement.

More detailed docking studies were done in Chapter 4. Since the PL molecule is quite rigid and has few possible conformations, it was used as the reference solute for the MC docking studies. For MM and EL, MC simulations are expected to take too long to converge because these solutes have many possible molecular conformations. For this reason, the same chiral cavity which was found for PL and B was used for MM and EL. The simulations for B are for the left-handed polymer backbone, and they are different from those done in Chapter 3, which were done for the right-handed polymer.

The structures of the AS and the PL enantiomers were first fixed at the equilibrium configuration, to search for the strongest interaction sites. The equilibrium structures, as first determined with MC/MD, were modified by using DFT predictions for the charges of the C=O and OH groups of each solute and for the C=O and NH groups of the polymer, and then more accurate estimates of the binding energies were calculated. Moreover, certain simulations showed unrealistically small distances (<1.7 Å) of H-bonds, as for (S-PL) OH with O=C-AS and (R-EL) OH with O=C (AS), probably because of errors introduced by the use of the classic CVFF force field parameters. When this was observed, the H-bond distances were fixed to the distances as predicted by DFT, adding also 0.03 Å to account for the temperature effects. The simulations were done for 300 ps. Twenty snapshots were exported for the analysis. Energy calculations were done
for distances of 5 Å, to explore the interactions only between the particular cavity and each solute.

Since polymer-polymer interactions, polymer-polymer-solute interactions, achiral binding sites, entropy effects, molecular rigidity, and intra H-bonding were not considered in these simulations, one may either expect a qualitative agreement with enantioselectivity data, or, at best, a semi-quantitative agreement.

In Chapter 4, for quantifying solute molecular rigidity or flexibility, MD simulations were done for R-PL, R-B, R-MM, and R-EL in vacuum. The charges of the H atom of the OH group and the O atom of the C=O group were removed in these simulations, to avoid the prediction of the formation of intra H-bonds which occur in practice (see Section 4.4). The simulations were done for 10 ns, to determine the dihedral torsion angle of the two planes formed by the two key functional groups, or the group of the four connected atoms O=C-C-O. Ten thousand snapshots of the structure were considered, for determining the distribution of the torsion angles.

In Chapter 6, for studying solute-solvent complexation effects, a system of one single solute with 150 IPA and 100 n-hexane molecules was used. The system was first energy-minimized using molecular mechanics (MM) simulations. The consistent-valence force field (CVFF) was used. The DFT-predicted electrostatic charges were used for the chiral solutes. A periodic boundary condition for a fluid system of density 0.7 g/cm³ was used. The MD simulations were done for 2 ns, to ensure reaching equilibrium. 400 frames of the system after 2 ns were randomly used for the radial distribution function (RDF), \( g(r) = \rho(r)/\rho_{av} \), analysis.
2.4.3 Dynamic Chromatography Simulations

The VERSE (VErsatile Reaction SEparation) simulation package developed at Purdue University by Wang and coworkers (Berninger et al. 1991), is a set of rate equations modeling the chromatographic process. The binding of IPA and acetone to AS is modeled with a Langmuir equilibrium adsorption isotherm, the value of which is the same as the one determined from the thermodynamic model. The complexation and aggregation were modeled as rate processes with a forward and a reverse rate constant. The ratios of these rate constants were the thermodynamic equilibrium constant as determined from the thermodynamic model. The values of the forward and the reverse rate constants were increased until the predicted retention times did not show any further change. Moreover, the effects of axial dispersion, mass transfer, and intraparticle diffusion were also taken into account in VRESE. The system of the equations is solved numerically. Details of the rate models and simulations have been discussed elsewhere, and have been tested with various experimental data (Berninger et al. 1991; Ma and Wang 1997; Xie et al. 2003; Wu et al. 1998; Mallmann et al. 1998; Xie et al. 2003; Lee et al. 2004; Whitley et al. 1991).

For the system of sorbent-acetone-hexane-IPA, the acetone-sorbent and the IPA-sorbent interactions were modeled by using the multicomponent Langmuir isotherm.

\[ \frac{C_L}{C_i} = \frac{a_i C_i}{1 + b_{AC} C_{AC} + b_i C_i} \]  \hspace{1cm} (2.1)

where \( C_i \) is the concentration of the AC or IPA in the mobile phase, \( C_L \) is the concentration of the adsorbed AC or IPA, and \( a_i \) (liquid volume per packing volume) and \( b_i \) (in M\(^{-1}\)) are constants.
In the pulse experiments, the concentration of acetone was quite small and was considered to be in the linear region of the Langmuir isotherm \( b_{AC} C_{AC} \ll 1 \). The equations allow for the addition of the generation or consumption terms due to reaction or aggregation for each species. The IPA aggregates and the acetone-IPA complex were treated as individual species and assumed to have no adsorption in the model. The open chain aggregate and the AC-IPA complex may also adsorb on the sorbent. The adsorption of the H-bonded complex should be less likely compared to the adsorption of solute or alcohol monomer, which would form stronger H-bonds. The same applies to potential adsorption of cyclical aggregates. For open-chain aggregates, some H-bonded adsorption may occur. The fact that the model as presented seems to work quite well for acetone and the other four achiral solutes (see Section 5.3), suggests that adsorption of these aggregates is negligible and that one does not need to account for it in the model. A mass action model with one value of the IPA aggregation number \( n \) was used. The n-hexane was assumed to be an inert species without any interactions with the solute or the sorbent. Some key simulation parameters are shown later in Section 5.6.
3.1 Introduction

(Most material in this chapter was published in Tsui, Hung-Wei, Jonathan N. Willing, Rahul B. Kasat, Nien-Hwa Linda Wang, and Elias I. Franses. 2011. The Journal of Physical Chemistry B 115: 12785-12800)

Polysaccharide (PS)-based sorbent materials, or chiral stationary phases (CSPs), especially derivatized amylose and cellulose polymeric CSPs, developed by Okamoto et al.,(Y. Okamoto and Yashima 1998) have been used widely for most analytical and preparative chiral separations of low molecular weight compounds (Lammerhofer 2010). Kasat et al. have studied the chiral discrimination of amylose tris-(3,5-dimethylphenylcarbamate), or ADMPC, or AD, and cellulose tris(3,5-dimethylphenylcarbamate), or CDMPC, or OD, which have the same side chain and a different backbone (2008; 2010). This thesis focuses on another widely used amylose-based CSP, amylose tris(S)-α-methylbenzylcarbamate, or ASMBC, or simply AS. It has an amylose backbone as AD, but a different side chain. A comparison between AS and AD may help elucidate further the mechanisms of chiral discrimination in this class of sorbents. A similar theme is shown here for benzoin with AS.
Figure 3.1 (A) Molecular structure of the S1 and S2 (or MMC) side chain models. (B) Molecular Mechanics 3D model of the molecular structure of a model with 3 unit cells and 12 monomer units. The amylose backbone atoms are shown with a stick representation. The side chains are shown with a line representation. The cavity A used for docking studies is shown with a ball-and-stick representation. (C) Detail of (B) showing cavity A formed by the following four side chains: chain C3 of monomer 4, chain C6 of monomer 6, chain C6 of monomer 7, and chain C2 of monomer 8. The cavity, shown with a ball-and-stick representation is used for docking studies. (D) Molecular structure of benzoin (the trans conformation is shown).
Kasat et al. used attenuated total reflection infrared spectroscopy, or ATR-IR, X-ray diffraction, or XRD, cross-polarization/magic-angle spinning, or CP/MAS, MAS solid-state nuclear magnetic resonance, or NMR, spectroscopy, and density functional theory, or DFT, to study the molecular environments in the polymers AD, OD, and AS (Kasat et al. 2007). They concluded that AS has the same backbone helical pitch length as AD. The side chains of AS have nonplanar conformations, which imply a shorter distance between polymer chains than in AD. They also reported the behavior of AD upon interacting with different solvents (Kasat et al. 2006). By using molecular mechanics (MM) and molecular dynamics (MD) simulations, with an 8-mer polymer rod model having a 4-fold helix, they inferred that the polymer rods contain many nm-sized cavities with intra-rod hydrogen bonds, H-bonds. Upon interacting with polar solvents, the polymer crystallinity and the side-chain mobility increase, and the distribution of the strengths of the H-bonding states of the AD changes significantly.

Kasat et al. studied the key interactions of phenylpropanolamine, or PPA, with AD, OD, and AS (Kasat et al. 2008). The enantioselectivities were quite different. AD showed a high enantioselectivity \( \alpha = k_R/k_S = 2.4 \), where \( k_R \) and \( k_S \) are the retention factors of the R and S enantiomers. AS showed no enantioselectivity (\( \alpha = 1.0 \)). OD had the reverse elution order and a low enantioselectivity (\( \alpha = 0.8 \)). Their ATR-IR results indicated that PPA causes different changes in the hydrogen-bonding of the amide groups of these polymers. They concluded that the enantioselectivity of AD for PPA is due to differences in the formation of H-bonds and in \( \pi-\pi \) (phenyl-phenyl) interactions between the sorbent and the solute.
Wirz et al. (2003) used polarization modulation ATR-IR spectroscopy with hexane in a flow cell and DFT calculations to study the chiral discrimination of ethyl lactate with AS. They concluded that the C=O groups of the (R)-ethyl lactate enantiomer form stronger hydrogen bonds with the polymer NH groups than those of the (S)-ethyl lactate. Such interactions were deemed to be crucial for the enantioselectivity. Wirz et al. (2008) also used a combination of High Performance Liquid Chromatography, or HPLC, and ATR-IR to probe changes in the H-bonding states of AS with pantolactone in 20 vol.% isopropanol, or IPA, in cyclohexane. Although no specific values of $\alpha$ were reported, the authors concluded that their observed enantioselectivity is due to stronger hydrogen bonding between the (R)-pantolactone C=O groups and the AS NH groups than those of the (S)-pantolactone.

Cass et al. (1997; 2003) reported a range of enantioselectivities of various chiral sulfoxides and N-arylamides, which have no asymmetric carbons but planar-torsion-based chiral centers. They used several PS-based CSPs, including AD and AS. The reported enantioselectivities were found, as expected, to depend on the type of the polysaccharide backbone and of the side chain, and the type and the composition of the mobile phase. For a series of amylose-based CSPs, Booth et al. (1997) concluded that the chirality of the amylose backbone affected the elution order, and that the chirality of the carbamate side chain affected the value of the enantioselectivity. Hu and Jiang (2009) used molecular simulations to model the flow and enantioseparation of phenylglycine enantiomers.

In a series of pioneering papers with AD, Wang et al. reported that the retention factors depend on the type and composition of the alcohol modifier in the mobile phase
(Wang and Chen 1999; Wang et al. 2000; Wenslow and Wang 2001; Wang and Wenslow 2003). The elution order could be reversed sometimes when different types of alcohol modifiers were used. The alcohol modifier may change the conformations and steric environments of the polymer cavities, the properties of which were considered to be important for the enantioselectivities. Ma et al. (2009) also reported that the polarity of the mobile phase may lead to conformational changes of the sorbent. In certain cases, a reversal of the elution order could be observed by changing the composition of certain hexane-alcohol mixtures. The idea of the chiral cavities being mostly relevant to the enantioselectivity was recently used for molecular simulations studies, or “docking” studies (Kasat et al. 2010; Kasat et al. 2008; Li et al. 2010). Li et al. used an AD polymer rod model to investigate the enantioselectivity of metalaxyl and benalaxyl enantiomers. No spectroscopic data are available for these systems. They concluded that the predicted energy differences of the complexes of the sorbent/(R)-solute and the sorbent/(S)-solute are due to the differences in the hydrogen bonding between the enantiomers and the sorbent sites in the cavities.

In a screening study of enantioselectivities of AS for various solutes at 25 °C in 10 vol.% IPA/n-hexane, we found that the enantioselectivity for benzoin (B) was quite high (α=1.8), and higher than those of several similar molecules, such as ethyl lactate, methyl mandelate, 1-phenyl-1-propanol, 1-phenyl-2-propanol, 2-phenyl-1-propanol, 2-amino-1,2-diphenylethanol, and others. For this reason, benzoin was chosen for systematic mechanistic study, and it is the main focus of this study. Benzoin is a simple model molecule with only a few functional groups, C=O, OH, and phenyls. The OH group allows for easier IR probing of the state of solute in contact with the polymer, as
shown below. Okamoto et al. had reported values of \( k_S = 4.29 \), \( k_R = 8.49 \), and \( \alpha = 1.98 \) for a home-made column of AS with benzoin at 25 °C, in a 10 vol.% IPA/n-hexane solvent (Okamoto et al. 1990). Muthupandi et al. also reported retention times of \( t_S = 8.533 \) and \( t_R = 13.350 \) min for the S and R enantiomers in 15 vol.% IPA in “hexanes”, which is a mixture of hexane isomers. Retention factors or \( \alpha \)-values were not reported (Muthupandi et al. 2009).

In Section 3.2, we report HPLC data for various concentrations of IPA in the n-hexane mobile phase. We present for the first time, to our knowledge, HPLC data with pure n-hexane. These data are important, to allow more direct comparisons of the data with enantioselectivities predicted with two-component polymer-solute simulations. It has been established from XRD and IR that, unlike hexane-alcohol mixtures, hexane does not change the H-bonding state of the polymer (Kasat et al. 2006). The new IR data allow the probing of the solute H-bonding state, since benzoin contains an OH group, which was modified to an OD group. The IR band of OD is shifted to an empty spectral zone. We present DFT simulation predictions not only of wavenumbers but also of relative intensities. This allows the quantitative prediction of the relative populations of different H-bonding states of the NH and C=O groups of AS. Such predictions allow unique insights in the AS molecular structure and comparisons to prediction of MD simulations.

We present MD simulations with various force fields for comparison. Even more importantly, we present novel results of MC and MC/MD docking simulations. The latter simulations have a higher predictive value than the MD simulations, because the locations of the solute in the chiral cavity are chosen without bias.
3.2 **HPLC Results of the Effects of Solvent Composition for the Retention Factors and Enantioselectivities**

The retention times and retention factors of R-benzoin (R-B) and S-benzoin (S-B) increase as the IPA concentration decreases from 10 to 0.5% (Figure 3.2 and Table 3.1). This trend is generally expected, because as the solvent becomes less polar, the solute H-bonding functional groups interact more with those of the polar sorbent, and have less competition from the OH groups of IPA. The values of the enantioselectivity $\alpha$ decrease from about 1.8 to about 1.4, and the enantiomer peaks are well separated.

The retention factors reported here for 10% IPA in n-hexane mobile phase differ from those previously reported by Okamoto et al. (1990), by 70 to 80%. The difference may be due to the use of different columns with different loading of polymer per unit volume of the column. Their reported enantioselectivity $\alpha$ of 1.98 differs less from our data (1.79).

For pure n-hexane as the mobile phase, the retention times and factors are much higher. Evidently, benzoin binds much more strongly to AS, because it does not form H-bonds with hexane. The hexane does not change the sorbent H-bonding state, as determined from IR and from the XRD spacings (Kasat et al. 2006). It can be argued that the AS-benzoin H-bonding interactions do not change in the presence of n-hexane. The enantioselectivity value of $\alpha= 2.13$ suggests that R-benzoin interacts with AS more strongly than S-benzoin. The R-benzoin either forms one H-bond which is much stronger than that of S-benzoin, or it forms an additional H-. The IR results below suggest that the latter possibility is more likely.
Figure 3.2 HPLC results of retention times of S- and R- benzoin enantiomers with Chiralpak AS beads for various concentrations of IPA in n-hexane, in vol.%, at 25 °C.
Table 3.1 Retention Factors ($k_R$ and $k_S$) and Enantioselectivities ($S$) of Benzoin Enantiomers with AS Polymer for Different vol.% of IPA in n-Hexane at 25°C; See Figure 3.2; the Retention Time of a Non-Adsorbing Reference Molecule, TTBB, was 5.5 Min under the Same Conditions

<table>
<thead>
<tr>
<th>% of IPA</th>
<th>$k_R$</th>
<th>$k_S$</th>
<th>$S$</th>
</tr>
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<tr>
<td>10</td>
<td>4.62</td>
<td>2.58</td>
<td>1.79</td>
</tr>
<tr>
<td>5.0</td>
<td>6.35</td>
<td>4.13</td>
<td>1.54</td>
</tr>
<tr>
<td>2.5</td>
<td>12.3</td>
<td>7.69</td>
<td>1.59</td>
</tr>
<tr>
<td>1.0</td>
<td>16.4</td>
<td>10.87</td>
<td>1.51</td>
</tr>
<tr>
<td>0.5</td>
<td>19.6</td>
<td>13.62</td>
<td>1.44</td>
</tr>
<tr>
<td>0</td>
<td>106</td>
<td>49.6</td>
<td>2.13</td>
</tr>
</tbody>
</table>
3.3 ATR-IR and DFT Results of the Interactions between Benzoin Enantiomers and AS Polymer

3.3.1 ATR-IR Spectra of AS, Benzoin, and AS/Benzoin Enantiomer Systems

The IR spectra of AS before and after absorption of R-B are shown in Figure 3.3; the spectrum of the benzoin racemate is also shown for comparison. The assignments will be discussed first, followed in Section 3.3.2 by a more detailed spectral analysis and interpretation of certain key bands.

The NH-stretching band of the side chains indicates three peaks. Peak 1 is a “shoulder” at ca. 3449 cm$^{-1}$. Peaks 2 and 3 overlap and have wavenumbers of ca. 3407 and 3310 cm$^{-1}$. Hence, there are three populations of NH groups. Peaks 2 and 3 correspond to medium and strongly-H-bonded groups. Peak 1 corresponds to either weakly H-bonded or non-H-bonded groups. The NH-stretching band of a similar molecule, methyl N-methylcarbamate, or MMC (or the model side chain S2), in the gas phase, where it is expected to be non-H-bonded, has one peak at 3460 cm$^{-1}$ in one reference (Randhawa et al. 1974), or 3474 cm$^{-1}$ in another reference (Maklakov et al. 1981). Hence Peak 1 at 3449 cm$^{-1}$ is more likely to arise from non-H-bonded groups. DFT calculations support this inference, predicting NH stretch wavenumbers of 3499 cm$^{-1}$ for non-H-bonded chain S2 or MMC, and 3495 cm$^{-1}$ for non-H-bonded chain S1 (Table 3.2).
Figure 3.3 ATR-IR spectra of pure AS, and of AS upon equilibration with R-benzoin (R-B) in CCl₄ and evaporation of CCl₄; also shown is the spectrum of solid benzoin racemate. The range between 2800 and 2000 cm⁻¹ has no peaks and is not shown. The vertical scales at the top figure (3600 cm⁻¹ to 2800 cm⁻¹ range) and the bottom figure (2000 cm⁻¹ to 1200 cm⁻¹ range) figures are different, for convenience in observing. The phenyl and methyl CH bands for AS and AS+R-B overlap. At this scale, the spectrum of AS+S-B looks similar to that of AS+S-B, and is not shown. For the difference spectra (AS+R-B)-(AS) and (AS+S-B)-(AS), see Figure 3.6.
Table 3.2 DFT Predictions of Wavenumbers $\nu_1$ and Intensities I of Certain IR Bands of Model Side Chains S1 and S2 (MMC), and of Benzoin. The Scaling Factor of 0.96 was Used for All Frequencies

<table>
<thead>
<tr>
<th>System #</th>
<th>Molecule</th>
<th>Band</th>
<th>$\nu_1$, cm$^{-1}$</th>
<th>Experiment, cm$^{-1}$</th>
<th>I</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S1</td>
<td>NH$_s$</td>
<td>3495</td>
<td>N/A</td>
<td>39</td>
<td>No H-bond</td>
</tr>
<tr>
<td>2</td>
<td>S2</td>
<td>NH$_s$</td>
<td>3499</td>
<td>3460$^{26}$, 3474$^{27}$</td>
<td>41</td>
<td>No H-bond</td>
</tr>
<tr>
<td>3</td>
<td>S1</td>
<td>C=O$_s$</td>
<td>1702</td>
<td>N/A</td>
<td>402</td>
<td>No H-bond</td>
</tr>
<tr>
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<td>S2</td>
<td>C=O$_s$</td>
<td>1713</td>
<td>1735$^{26}$, 1737$^{27}$</td>
<td>399</td>
<td>No H-bond</td>
</tr>
<tr>
<td>5</td>
<td>S1</td>
<td>NH$_b$</td>
<td>1471</td>
<td>N/A</td>
<td>373</td>
<td>No H-bond</td>
</tr>
<tr>
<td>6</td>
<td>S2</td>
<td>NH$_b$</td>
<td>1508</td>
<td>1525$^{26}$, 1520$^{27}$</td>
<td>253</td>
<td>No H-bond</td>
</tr>
<tr>
<td>7</td>
<td>S1</td>
<td>Ph$_s$</td>
<td>1572</td>
<td>1604</td>
<td>4</td>
<td>N/A</td>
</tr>
<tr>
<td>8</td>
<td>S1</td>
<td>Ph$_s$</td>
<td>1553</td>
<td>1585</td>
<td>2</td>
<td>N/A</td>
</tr>
<tr>
<td>9</td>
<td>B$_{\text{trans}}$</td>
<td>OH$_s$</td>
<td>3692</td>
<td>N/A</td>
<td>57</td>
<td>No H-bond</td>
</tr>
<tr>
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<td>B$_{\text{trans}}$</td>
<td>C=O$_s$</td>
<td>1656</td>
<td>N/A</td>
<td>196</td>
<td>No H-bond</td>
</tr>
<tr>
<td>11</td>
<td>B$_{\text{trans}}$</td>
<td>Ph$_s$</td>
<td>1576</td>
<td>1596</td>
<td>8</td>
<td>N/A</td>
</tr>
<tr>
<td>12</td>
<td>B$_{\text{trans}}$</td>
<td>Ph$_s$</td>
<td>1570</td>
<td>1578</td>
<td>1</td>
<td>N/A</td>
</tr>
<tr>
<td>13</td>
<td>B$_{\text{trans}}$</td>
<td>Ph$_s$</td>
<td>1557</td>
<td>1551</td>
<td>13</td>
<td>N/A</td>
</tr>
<tr>
<td>14</td>
<td>B$_{\text{trans}}$</td>
<td>Ph$_s$</td>
<td>1525$^{27}$</td>
<td>3474$^{27}$</td>
<td>106</td>
<td>Intra H-bond</td>
</tr>
<tr>
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<td>B$_{\text{cis}}$</td>
<td>OH$_s$</td>
<td>1626$^{27}$</td>
<td>1684$^{27}$</td>
<td>182</td>
<td>Intra H-bond</td>
</tr>
</tbody>
</table>
The bands at ca. 3100-2850 cm⁻¹ are due to CH stretches of the phenyl and the CH₃ groups. Since they overlap with some benzoin bands and do not change much upon absorption of benzoin, they are not thought to provide sensitive structural information, and they are not considered further. The amide I band, which is due mostly (ca. 80%) to C=O stretching (Bellamy 1975), is quite broad and shows three overlapping peaks at ca. 1732, 1711, and 1693 cm⁻¹. They indicate three populations of H-bonded C=O groups: free or weakly-H-bonded groups (Peak 1), medium-H-bonded groups (Peak 2), and strongly-H-bonded groups (Peak 3). The non H-bonded C=O band of MMC, which appears at 1735 cm⁻¹, is the basis for this inference. The DFT calculations predict that the wavenumber of the non-H-bonded C=O stretch band of MMC should be at 1713 cm⁻¹ (Table 3.2), which is within 2% of the data. Since the third peak overlaps with the C=O peak of benzoin at 1675 cm⁻¹, the use of difference spectra is needed (see below). Both the NH and the C=O stretch bands change upon the absorption of benzoin, indicating that the benzoin is incorporated into, or dissolved in, the polymer structure, and that the polymer H-bonding state changes upon the interaction of the sorbent with the benzoin. The amide II, or NH-bending (60%) and CN (40%) stretching band (Bellamy 1975), at ca. 1532 cm⁻¹, is quite strong and broad, and shows some changes upon benzoin absorption (Figure 3.3). The band at 1495 cm⁻¹ corresponds to a phenyl C-C vibration. The amide III band at 1245 cm⁻¹ arises from many complex vibrations, and is not analyzed further.

The bands at 1604 and 1585 cm⁻¹ (see Figure 3.3) are assigned to the symmetric and asymmetric C-C stretching vibrations of the AS phenyl groups (Kasat et al. 2007; Pawelka et al. 2003). These assignments are based on DFT simulations (#7 and 8 in
Table 3.2). As reported, the phenyl C-C stretching bands of AS are weak, because no polar group is directly attached to the phenyl groups, resulting in small instantaneous dipole moments during the vibration.\(^5\) Because these bands do not overlap with the respective phenyl C-C stretching bands of benzoin (which appear at 1596 and 1578 cm\(^{-1}\)), they are used to normalize the AS and B spectra in the determination of the difference spectra. The DFT wavenumber predictions are quite accurate, to within 2% of the data.

The wavenumber of non-H-bonded OH groups ranges normally from 3600 to 3400 cm\(^{-1}\). The wavenumber of benzoin OH groups in the gas phase (or in a nonpolar liquid phase such as carbon tetrachloride), where it is in the cis intramolecularly H-bonded conformation, is 3474 cm\(^{-1}\) (Pawelka et al. 2003). No data are available for trans non-H-bonded benzoin, because the preferred state is the H-bonded cis-state, with \(\Delta H = -14.76\) kJ/mol. Nonetheless, DFT calculations of non-H-bonded trans benzoin predict a shift of ca. -167 cm\(^{-1}\) when the non-H-bonded trans-benzoin is converted to a cis intra-H-bonded state. Hence, the predicted wavenumber of the non-physically existing trans non-H-bonded benzoin OH stretch should be around 3650 cm\(^{-1}\). The benzoin in the actual experimental solid state has much lower wavenumbers, ca. 3415 and 3378 cm\(^{-1}\) (Figure 3.3). All results indicate that this benzoin must be in the trans conformation with strong intermolecular H-bonds, as suggested previously (Pawelka et al. 2003). This inference has been confirmed further with single-crystal XRD results (Haisa et al. 1980), which show two populations of H-bonded trans molecules, with two different orientations in the elementary cell. (See Section 3.3.2 for a detailed spectral analysis.)
3.3.2  Detailed Spectral Analysis and DFT Calculations

Spectral deconvolution of the NH stretching and the C=O stretching bands reveals more accurately their wavenumbers and relative intensities (or peak areas) (Figure 3.4). The NH band for Peak 2 shows a shift of ca. -40 cm\(^{-1}\) from Peak 1, and the NH band for Peak 3 shows a shift of ca. -137 cm\(^{-1}\) from Peak 1 (Figure 3.4, top). The relative area intensities are 2%, 20%, and 78%, respectively. The intensity ratios of Peaks 2 and 3 versus Peak 1 are 10 and 39, respectively. The C=O peaks show shifts of -29 and -48 cm\(^{-1}\) (Figure 3.4, bottom). The relative area intensities are 19%, 74%, and 7% respectively.

To further interpret quantitatively these results in terms of relative group populations, DFT simulations were done for the NH groups of the S2 chains interacting with the C=O groups of the S2 chains, to describe the most probable inter-chain H-bonds in the polymer. The results (System 1, Table 3.3) show a wavenumber shift of -113 cm\(^{-1}\) and a large intensity enhancement by a factor of 12.5. Hence, the relative areas observed in Figure 3.4 do not represent the relative group populations. It appears that Peak 3, which has a shift of -137 cm\(^{-1}\), represents NH groups strongly bonded to C=O groups. If we use the intensity ratio R of 12.5 for Peaks 1 and 3, then the relative population for the groups of Peak 3 is 78/12.5\approx 6, or three times larger than the population for Peak 1. The predicted energy of this H-bond is quite high, -23.03 kJ/mol (see Appendix A). The predicted H-bond length d and angle \(\theta\) are 1.98 Å.
Figure 3.4 Deconvolution of the NH stretching band (top) and the amide I band (bottom) in the ATR-IR spectrum of dry AS polymer. The wavenumbers and area percentages of these peaks are indicated. The fit is good; $R^2=0.996$ (top) and 0.999 (bottom).
Table 3.3 DFT-Predictions of Wavenumber Shifts Δν (Expressed as Differences) and Intensity Changes (Expressed as Ratios) for Pairs of S1, S2, and Benzoin

<table>
<thead>
<tr>
<th>System #</th>
<th>Pair</th>
<th>Interactions</th>
<th>Band</th>
<th>Δν1, cm⁻¹</th>
<th>R=I/I₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S2 with S2</td>
<td>NH ↔ O=C</td>
<td>NH₁</td>
<td>-113</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C=O₂</td>
<td>-26</td>
<td>2.00</td>
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<td></td>
<td></td>
<td></td>
<td>NH₂</td>
<td>13</td>
<td>1.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NH ↔ O=C (fixed at 2.5Å)</td>
<td>NH₁</td>
<td>-34</td>
<td>4.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C=O₂</td>
<td>-17</td>
<td>1.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NH₂</td>
<td>3</td>
<td>1.57</td>
</tr>
<tr>
<td>2</td>
<td>S2 with S2</td>
<td>NH ↔ O</td>
<td>NH₁</td>
<td>-80</td>
<td>8.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NH₂</td>
<td>1</td>
<td>1.03</td>
</tr>
<tr>
<td>3</td>
<td>B with Bᵇ</td>
<td>OH ↔ O=C</td>
<td>OH₁</td>
<td>-183</td>
<td>14.7</td>
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<td></td>
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<td></td>
<td>C=O₂</td>
<td>-32</td>
<td>1.4</td>
</tr>
<tr>
<td>4</td>
<td>S1 with B</td>
<td>C=O ↔ HO</td>
<td>(S1) C=O₁</td>
<td>-34</td>
<td>1.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(B) OH₁</td>
<td>-192</td>
<td>13.1</td>
</tr>
<tr>
<td>5</td>
<td>S2 with B</td>
<td>C=O ↔ HO</td>
<td>(S2) C=O₁</td>
<td>-33</td>
<td>1.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(B) OH₁</td>
<td>-208</td>
<td>16.7</td>
</tr>
<tr>
<td>6</td>
<td>S1 with B</td>
<td>NH ↔ O=C</td>
<td>(S1) NH₁</td>
<td>-107</td>
<td>9.20</td>
</tr>
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<td></td>
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<td>(S1) NH₂</td>
<td>30</td>
<td>0.84</td>
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<td></td>
<td></td>
<td>(B) C=O₁</td>
<td>-16</td>
<td>1.42</td>
</tr>
<tr>
<td>7</td>
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<td>-90</td>
<td>12.6</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>(S2) NH₂</td>
<td>22</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(B) C=O₁</td>
<td>-14</td>
<td>1.71</td>
</tr>
<tr>
<td>8</td>
<td>S1 with B</td>
<td>NH ↔ OH</td>
<td>(S1) NH₁</td>
<td>-81</td>
<td>6.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(S1) NH₂</td>
<td>29</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(B) OH₁</td>
<td>-65ᵇ</td>
<td>5.05ᵇ</td>
</tr>
<tr>
<td>9</td>
<td>S2 with B</td>
<td>NH ↔ OH</td>
<td>(S2) NH₁</td>
<td>-90</td>
<td>9.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(S2) NH₂</td>
<td>12</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(B) OH₁</td>
<td>-18</td>
<td>0.84</td>
</tr>
</tbody>
</table>

ᵃ The frequencies were affected by the phenyl group of the S1 side chain model.

ᵇ Two H-bonds are formed between the two molecules.
and 169°, indicating a strong H-bond. The NH Peak 2 with Δν= -40 cm⁻¹ represents either NH groups H-bonded with medium strength H-bonds to C=O groups, or NH groups bonded to O atoms in the backbone or side chains, or to both. For the former case, a simulation with Δd fixed at 0.25 nm predicts an energy of -17.35 kJ/mol, a wavenumber shift of Δν = -33 cm⁻¹ (scaled as ca. 40×113/137), and an intensity enhancement R=4.67. Then the relative population of Peak 2 is predicted to be 20/4.67=4.3. Therefore, this approach predicts relative populations of 2:4.3:6, or about 1:2:3, for the three groups. A lower energy of the H-bond (S2) NH ↔ O (S2) was obtained from the DFT simulations. The DFT calculations for the H-bond (S2) NH ↔ O (backbone) predict that it has a similar binding strength as the (S2) C=O ↔ HN (S2). Upon H-bonding, the NH-bending band is predicted to have a positive wavenumber shift of 13 cm⁻¹. The predicted intensity enhancement is small about 1.3. This band is not as sensitive as the NH stretching band for probing the relative populations of the NH groups.

The simulations of the H-bonding between NH and C=O groups provide a wavenumber shift of -26 cm⁻¹ for the C=O groups. This value is close to the observed value of -29 cm⁻¹ for Peak 2. If Peak 2 corresponds to this H-bonded population, the predicted intensity ratio is 2, and the relative population of the C=O groups for Peak 2 versus those for Peak 1 is 2:1. It is unclear what is the origin of Peak 3, which represents a quite small relative population. Its wavenumber shift of -52 cm⁻¹ is quite large, making it unlikely that the predicted value represents Peak 2. No DFT predictions of such a high value are available. These results will be discussed further after the MD simulations, which include a large diversity of H-bonded states, and which will be used in docking
studies of AS with B. Nonetheless, the results are consistent with the NH stretch results. Probably, about $5/6 \approx 80\%$ of the NH groups form H-bonds with the C=O groups of the side chains and with some of the O groups of the backbone.

Since the OH band of benzoin overlaps with the NH band of AS, deuterated benzoin-OD was used to shift the hydroxyl band and help distinguish the two bands. The OH bands at 3415 and 3378 cm$^{-1}$ shift to 2531 and 2518 cm$^{-1}$ upon deuterium substitution for both enantiomers (Figure 3.5). These wavenumbers compare well to the values of 2532 and 2507 cm$^{-1}$ reported by Pawelka et al. (2003), who used benzoin-OD of higher purity of ca. 70\%. The shifts occur because the reduced mass of OD is about 90\% larger than the reduced mass of OH. The relative areas for each enantiomer are the same, 15 \pm 2\% for the medium-H-bonded and 85 \pm 2\% for the strongly-H-bonded OD groups. Upon absorption of B by AS, the OD band changes significantly, and the NH band changes little. This suggests that there is no H-D exchange for the AS polymer. Since the 2518 cm$^{-1}$ peak disappears, we infer that there is no pure solid benzoin left in the area examined by ATR (at the penetration length of about 1 \mu m above the ATR plate). Hence, all benzoin observed is absorbed by the AS sample and has a different spectral “signature”. The absorbed benzoin is also present in two populations. One population is medium-H-bonded at 2531 \pm 1 cm$^{-1}$, the same as for the pure solid benzoin, probably by coincidence. The other one, at 2508 cm$^{-1}$, corresponds to an even more strongly-H-bonded state than in pure benzoin. Moreover, upon benzoin absorption the relative areas of the two peaks change to 56 \pm 1\% and 44 \pm 1\%, indicating again the different state of
Figure 3.5 ATR-IR spectra of the OD stretching bands of R-benzoin and S-benzoin alone and with AS: top figure is R-B-OD (a) and AS+R-B-OD (b); bottom figure is S-B-OD (a) and AS+S-B-OD (b). The fits to the deconvolution spectra are good: $R^2=0.993$, $R^2=0.988$, $R^2=0.997$, and $R^2=0.990$, from top to bottom. The results for the two enantiomers relative areas are the same within experimental error of 2%.
H-bonding between benzoin and AS vs pure benzoin. The R and S- benzoin enantiomers interact similarly and strongly with AS.

This result is quite significant. Both enantiomers form an equally strong H-bond with the polymer. This implies that the additional interaction of R-benzoin inferred from the retention factors is not due to a stronger H-bond but to an additional bond, which is more probably an H-bond than a π-π interaction.

DFT simulations were done to predict the following: (a) the wavenumbers and intensities of various key bands of S1 and S2 chains and of benzoin (Table 3.2); (b) the shifts in the wavenumbers and intensity changes of the bands after H-bonding interactions (Table 3.3); and (c) the energies, lengths, and angles of these H-bonds. Several of these H-bonding interactions will now be described in detail, and the results will be summarized at the end of this section.

The simulations of OH of trans benzoin H-bonded to C=O of benzoin predict a high wavenumber shift (-183 cm\(^{-1}\), System 3 in Table 3.3), and a huge intensity ratio R=14.7. For C=O, the respective values are -32 cm\(^{-1}\) and 1.4. When one benzoin molecule forms two H-bonds with another benzoin molecule, the energy difference per H-bond is -23.78 kJ/mol. This energy difference is quite higher than the predicted energy difference (-14.76 kJ/mol) of an intra-molecular H-bond of cis benzoin. The H-bond energy of a B-OH group with an S1 C=O group is much larger (-33.31 kJ/mol) than the H-bond between a B-OH with a B-C=O. The predicted wavenumber shift is only slightly higher in absolute value, -192 vs. -183 cm\(^{-1}\). The fair predictions of wavenumber shifts
provide some confidence in the accuracy of the predictions of the H-bond energies, which are not directly measurable.

The interactions of single AS chains, model chain S1 (which has a benzene ring) and model chain S2 (which has no benzene ring) with benzoin were also simulated with DFT. When either chain hydrogen bonds to benzoin, its C=O wavenumber shifts by 34 cm\(^{-1}\). This shift is higher than the shift of 26 cm\(^{-1}\) of the C=O group of AS bonded to the NH group of AS. The intensity enhancement (1.46 or 1.52) is smaller. The respective shift for the wavenumber of the benzoin OH groups with S2 are -192 cm\(^{-1}\), which is larger by 5% than the value of -183 cm\(^{-1}\) for B-B. This relative shift is exactly what was observed for the second OH peak (Figure 3.5). These results suggest that when benzoin enters the polymer cavities, the H-bonds between B and B break and stronger bonds form between the groups B-OH (or B-OD) and the groups C=O of the polymer. Indeed, the predicted energy of this S1-B H-bond is -35.03 kJ/mol, which is larger (in absolute value) than the value of -23.78 kcal/mol between B and B. For the simpler chain S1 the predicted H-bond energy is also large, -33.31 kcal/mol.

When the NH groups of S1 or S2 interact with the C=O groups of benzoin, the NH stretch wavenumber shifts by -107 cm\(^{-1}\) for S1, and by -90 cm\(^{-1}\) for S2 (R=9.20; Table 3). This number differs by only 20% from the value of -113 cm\(^{-1}\) predicted by DFT for the S2-S2 interaction (System 1, Table 3.3). The energies, in kJ/mol, are also comparable, at -23.03 for S2 with S2 vs. -18.39 for S2 with B and -24.20 for S1 with B. (The value for the energy for S1 with S1 is not available, because the computations would take too long time.) The predictions of \(\Delta\nu\) for NH-bending are, +30 and +22 cm\(^{-1}\),
which are higher than the value of 13 cm$^{-1}$ between S2 and S2. The C=O stretch band shifts by -34 cm$^{-1}$, or -33 cm$^{-1}$, for S1 or S2 with B, vs. -26 cm$^{-1}$ for S2 with S2. Overall, the DFT predictions are fairly accurate.

The interactions between NH groups of AS with the OH groups of benzoin are predicted to be weaker than those between the C=O groups of AS and the OH groups of benzoin. The H-bond energies, in kJ/mol, are -16.55 or -12.25 for the S1 or the S2 chains. The wavenumber shifts are also smaller, -81 cm$^{-1}$ or -90 cm$^{-1}$, respectively.

In summary, based on the DFT predictions and IR results, the following inferences are made:

1. In the pure polymer, most of the NH groups tend to form hydrogen bonds primarily with the C=O groups. At least three populations were identified by the relative strength of the H-bonds. The relative populations for NH are 1:2:3. NH may also form H-bonds with certain O-atoms on the polymer backbone. At least three populations of C=O groups were identified.

2. Pure solid benzoin is in the trans conformation. All OH groups are intermolecularly H-bonded with C=O groups, in two populations, (i) of strong H-bonds and (ii) of very strong H-bonds.

3. When benzoin is absorbed by AS, these H-bonds break, and benzoin OH (or OD) groups form two different kinds of H-bonds with C=O of AS, corresponding to two populations. One population is H-bonded with the same strength as B-B. The groups of
the other population are bonded more strongly than the groups with the strongest bonds between B and B.

4. When R- or S-benzoin molecules are absorbed by AS, their C=O groups may form H-bonds with the NH groups if it is sterically allowed. These H-bonds have slightly lower energies than those between NH (AS) and C=O (AS).

An important question is whether these IR results can be used to detect, directly or indirectly, any enantioselective interactions between AS and B. Taking the difference between spectra (AS+R-B) – (AS) and (AS+S-B) – (AS) in the 1760 to 1640 cm\(^{-1}\) region, we observe the following in the difference spectra (Figure 3.6):

(A). Between 1760 and 1700 cm\(^{-1}\), where the C=O bands of AS are expected, the absorbance differences are exactly the same for both enantiomers. This implies that the H-bonded state of the AS C=O groups changes upon absorption of benzoin, and that the changes are the same when either enantiomer is absorbed.

(B). Between 1700 and 1640 cm\(^{-1}\), where the C=O band of benzoin is expected to appear, there is a small and reproducible wavenumber difference of ca. 1 cm\(^{-1}\). This difference is higher than the estimated error of \(\pm 0.2\) cm\(^{-1}\). This result suggests that S-benzoin interacts less strongly with AS than R-benzoin. This inference is novel and is supported by MD results.
Figure 3.6 Difference spectra of the CO stretching bands of absorbed benzoin and of AS polymer upon interacting with R-benzoin (thick line) and S-benzoin (thin line). The effect on the polymer bands is the same for both enantiomers. The effect of AS on the benzoin band is small but significant; see text for details.
3.4 MM/MD/MC Results

3.4.1 Overview of Models Used

Although the DFT calculations are presumed to lead to fairly accurate predictions of electrostatic interactions and H-bonding interactions, they are limited to small scale systems. In order to understand the enantiomeric interactions involving polymers with chiral cavities, we had to use molecular simulations for structural predictions and for docking studies in an effort to understand plausibly the molecular basis for the observed enantioselectivity.

A 12-mer AS polymer with a 4-fold helix and three unit cells was built as the simplest model. This model is similar to the one used by Kasat et al. (2008), but smaller than the 36-mer, used for AD by Li et al (2010). Only monomers 5 through 8 at the center were examined for their possible cavities and the docking studies, to avoid possible end effects.

The CVFF force field was chosen for most of these simulations. The accuracy of this force field was evaluated, by comparison to DFT predictions of five types of H-bonds and of one π-π interactions (see Appendix B). The % differences vary from 7 to 130% for the energies, from 1 to 10% for distance, and from 1 to 7% for angles. Hence, the predictions should be considered as semiquantitative. These differences were significantly smaller than those determined using four other force fields available in Material Studio (PCFF, COMPASS, Dreiding, and Universal). Kasat et al. used the CVFF and PCFF force fields, Li et al. used the COMPASS force field. The accuracy of
the IR predictions based on these force fields is generally rather poor, probably because only classical mechanical effects are considered.

3.4.2 MD Simulations of Pure AS Polymer

The helical pitch of the AS backbone was fixed at 1.46 nm, as inferred from the XRD results. The predicted diameter of the AS rod was ca. 1.70 nm, which fits well the XRD result of 1.69 nm (Kasat et al. 2008). The agreement gives some additional confidence in the MD predictions.

The detailed binding states of the NH and the C=O groups of AS, as predicted are shown in Table 3.4. The data were averaged by randomly choosing 40 frames from the equilibrium states. The 2D representation of the AS binding structure is shown in Figure 3.7, as a 2D projection of the 3D representation. This representation allows pointing out more clearly the H-bonds than by showing images of the 3D structures. In this figure, we can identify three types of NH groups, which have either a strong (s) bond with C=O groups (Type i), or a medium (m) strength bond with O atoms (Type ii), or a weak bond or “free” (f) (Type iii), as judged from the bond distances and angles. The % relative populations of these groups in this MD model are 4:5:3 (f:m:s). The percentage of the “free” NH groups (about 30%) is slightly larger than the results (1:2:3 or about 17%) inferred from IR of the actual polymer and DFT. This suggests that there may be come additional H-bonds between adjacent molecules in the actual polymer material. More specifically, the type (i) bonds seem to occur between the C2 side chains of monomers 5-8 bonding with the C=O groups of the C3 side chain. The type (ii) bonds seem to occur
Table 3.4 Hydrogen Bonding Conditions of NH and CO Groups of the Central Unit Cell (5-8 Mer) for AS Model. 40 Frames were Randomly Chosen for Averaging, see Figure 3.7.

(a). H-binding status of NH groups

<table>
<thead>
<tr>
<th>mer</th>
<th>side chain</th>
<th>H-bond with</th>
<th>Length(Å)</th>
<th>Std</th>
<th>Angle(°)</th>
<th>Std</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>C2</td>
<td>5mer-C3-CO</td>
<td>2.89</td>
<td>0.12</td>
<td>140</td>
<td>4.62</td>
</tr>
<tr>
<td></td>
<td>C3</td>
<td>6mer-C2-O</td>
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<td>0.09</td>
<td>130</td>
<td>7.68</td>
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(b). H-binding status of CO groups

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<td>N/A</td>
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<td>5mer-C2-NH</td>
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<td>7mer-C2-NH</td>
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<tr>
<td></td>
<td>C3</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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</tr>
<tr>
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<td>C5</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Figure 3.7 2D representation of possible binding sites, NH, CO, and O, of a 12-mer AS polymer model in the central units (monomers 5 to 8), as predicted from MD simulations.

s≡ strong H-bond; m≡ medium-strength H-bond; f≡ free (or weakly bonded) group; see text and Table 3.4.
The simulation results in Table 4 suggest that some NH groups of AS tend to form intra-polymer H-bonds, leaving fewer NH groups available to bind with benzoin (or other solutes). The OH groups of benzoin are more likely to bind with the C=O and NH groups of AS, whereas the C=O groups of benzoin can only bind with the NH groups of AS, as shown in the docking simulations below.

3.4.3 MD and MC Docking Simulation Studies of Benzoin Enantiomers in AS Polymer

The MD docking simulation results were directly done first. Cavity A, which is the largest cavity among those observed, was chosen for these MD simulations. The results show that the R-benzoin could form two H-bonds simultaneously with the same side chain, the C2 chain of the 8th monomer, whereas the S-benzoin could only form the H-bond (SB) OH ⇄ O=C (AS). The predicted energy difference indicated that R-benzoin interacts more strongly with AS than S-benzoin by -56 ± 19 kJ/mol. The results appear to match the inferences from the IR spectral analysis. Since the MD docking simulation predictions can depend on the chosen initial orientation of the benzoin molecule in the cavity, as inferred from the IR results, the MD results could be biased, and may have less predictive value.

The MC docking simulation results were then used. Cavities A and B could be identified as having sufficient sizes and accessible binding sites for R-B and S-B. Inside cavity A, there is one NH group and two C=O groups which are exposed and free. The simulations showed that R-B could form two H-bonds, (AS) C=O ⇄ HO (RB) and (AS) NH ⇄ OC (R-B), and two π-π interactions, whereas S-B could form one H-bond, (AS)
C=O ↔ HO (S-B), and one \( \pi-\pi \) interaction. The complex docking configurations predicted by MC are different from those predicted by the MD simulations. The reason why S-B could not bind with the NH group of the cavity is as follows. As the OH group of S-B binds with the AS C=O group, its C=O group finds itself at an unfavorable orientation for binding with the NH group. In addition, the large steric hindrance effect caused by its two phenyl groups does not allow the rotation needed to obtain a favorable orientation. Evidently, the different R-B configuration allows it to form the second H-bond. Hence, cavity A allows a substantial enantioselectivity. Cavity B has two C=O groups for possible H-bonding sites with OH groups. The simulation results indicate that for this cavity, both R-B and S-B enantiomers have the same binding configuration, one H-bond, (AS) C=O ↔ HO (B), and two \( \pi-\pi \) interactions. Hence, no pronounced enantioselectivity is predicted for this cavity. The binding in cavity B may tend to decrease the predicted overall (or average) enantioselectivity, compared to the enantioselectivity in cavity A alone.

The energies show that although R-benzoin could form two H-bonds in cavity A, its energy is higher than that of the S-benzoin by +48 kJ/mol. The reason leading to the reverse enantioselectivity in energies is probably due to unrealistic predicted value of the H-bond length, which is \( d=1.66 \) Å, of the (AS) C=O ↔ HO (B) type of H-bond. The energies for this H-bond formed by S-benzoin with AS are overestimated.

To improve the MC simulation predictions and produce more accurate energy predictions with more realistic H-bond distances, the results from the MC simulations were used as the initial orientations and configurations for the subsequent MD
Figure 3.8 MC/MD simulation predictions of docking of R-benzoin and S-benzoin in cavity A. The initial orientations and configurations are from the results of MC simulations. This simulation predicts four interactions, two H-bonds and two π-π interactions, for R-B, and two interactions, one H-bond and one π-π interaction, for S-B.

This simulation predicts enantioselectivity for this cavity.
Figure 3.9 MC/MD simulation predictions of docking of R-benzoin and S-benzoin in cavity B. The initial orientations and configurations are from the results of MC simulations. This simulation predicts three interactions, one H-bonds and two π-π interactions, for R-B, and two interactions, one H-bond and one π-π interaction, for S-B. This simulation predicts no enantioselectivity for this cavity.
Table 3.5 Structural Information and the Most Important Specific Binding Sites with R-B and S-B for Cavity A and B by Using MD Simulations. The Initial Orientations and Configurations are from the Results of MC Simulations. Shown in Parentheses are the H-Bond Lengths and Angles.

**Cavity A**

<table>
<thead>
<tr>
<th>Number of side chains and accessible functional groups</th>
<th>4mer-C3: Ph</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>6mer-C5:Ph</td>
</tr>
<tr>
<td></td>
<td>7mer-C5: Ph, CO</td>
</tr>
<tr>
<td></td>
<td>8mer-C2:Ph, CO, NH</td>
</tr>
<tr>
<td>R-benzoin</td>
<td>7mer-C5-CO (3.41 ± 0.27Å; 125 ± 6.44°)</td>
</tr>
<tr>
<td></td>
<td>8mer-C2-NH (2.06 ± 0.07Å; 168 ±7.25°)</td>
</tr>
<tr>
<td></td>
<td>6mer-C5-Ph: T-shape</td>
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<tr>
<td></td>
<td>7mer-C5-Ph: parallel</td>
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<tr>
<td>S-benzoin</td>
<td>7mer-C5-CO (3.28 ± 0.10Å; 136 ± 5.85°)</td>
</tr>
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<td>N/A</td>
</tr>
<tr>
<td></td>
<td>4mer-C3-Ph: T-shape</td>
</tr>
<tr>
<td>ΔE&lt;sub&gt;R&lt;/sub&gt; ΔE&lt;sub&gt;S&lt;/sub&gt; (kJ/mol)</td>
<td>-62 ± 20</td>
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**Cavity B**

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
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<td>4mer-C2:Ph</td>
</tr>
<tr>
<td></td>
<td>6mer-C5: Ph, CO</td>
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<td>7mer-C2:Ph, CO</td>
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<td>R-benzoin</td>
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<td></td>
<td>3mer-C3-Ph: T-shape</td>
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<tr>
<td></td>
<td>3mer-C3-Ph: T-shape</td>
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<td>S-benzoin</td>
<td>6mer-C5-CO (2.13 ± 0.12Å; 149 ± 8.54°)</td>
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</tr>
<tr>
<td></td>
<td>3mer-C3-Ph: T-shape</td>
</tr>
<tr>
<td>ΔE&lt;sub&gt;R&lt;/sub&gt; ΔE&lt;sub&gt;S&lt;/sub&gt; (kJ/mol)</td>
<td>-26 ± 24</td>
</tr>
</tbody>
</table>
simulations. The results are shown in Figures 3.8 and 3.9, and Table 3.5. Since the atoms were allowed to move from the positions predicted from MC simulations, more plausible H-bond lengths were predicted, of $d=3.41 \pm 0.27$ Å. The bonding configurations remained the same, however, as in the MC simulations. The energy difference, $\Delta E= -62 \pm 20$ kJ/mol, would lead to a predicted enantioselectivity value of $\alpha \approx 7 \times 10^{10}$ for benzoin in cavity A, of $\Delta E \approx \Delta G$ and if all interactions involved cavity A. The cavity B also showed a small energy enantioselectivity, of $\Delta E= -26 \pm 24$ kJ/mol, in which the average value is close to the standard deviation. Hence, this cavity could be regarded as being nearly non-enantioselective.

In summary, the MC/MD docking simulations seem to be suitable for predicting or explaining some of the HPLC/IR data presented here. They suggest that there are two possible docking cavities which could have strong interactions with the benzoin molecule. Cavity A is quite enantioselective, and cavity B is nearly non-enantioselective. In all potential binding configurations, both the R-B and S-B enantiomers could form the (AS) C=O $\leftrightarrow$ HO (B) type of H-bond, which is also the strongest H-bond, as inferred from the DFT calculations. The AS structure described in Table 3.4 suggests that there are more accessible C=O groups than NH groups. The H-bond type (AS) C=O $\leftrightarrow$ HO (B) may be the easiest bond to form. The key enantioselective interaction would involve another H-bond (AS) NH $\leftrightarrow$ OC (RB) which may form simultaneously in the cavity A by R-B. The MC and MC/MD results are consistent with the IR, HPLC, and DFT results. R-benzoin interacts more strongly, and tends to form two H-bonds simultaneously, with AS. The MC results lead to the predictions that the OH groups of both R and S benzoin bind
similarly with the C=O groups of AS, as inferred from IR data. The key chiral distinction arises from the binding, or non-binding, of the NH group. This binding is controlled to some extent, by the steric effects of the phenyl rings. The π-π interactions are also somewhat different for the two enantiomers. Hence, the MC/MD method seems to be more suitable for elucidating realistically, and perhaps predicting, the basis for the observed enantioselectivity of benzoin, and for testing hypotheses derived from HPLC, IR, and DFT simulations.

3.5 Conclusions

HPLC results show that the retention factors of benzoin enantiomers with the sorbent AS increase with decreasing concentration of IPA in the IPA/hexane mobile solvent phase, as expected since the solvent becomes more hydrophobic. The retention factors are higher for R-benzoin. For pure n-hexane, the retention factors are the highest, and the enantioselectivity $S \equiv k_R/k_S$ is the highest, 2.13, compared to a range of 1.79-1.44 for IPA/ hexane, from 10 to 0.5 vol.% IPA. Detailed IR spectroscopy results and DFT simulations provide unique novel information on the state of hydrogen bonding of the NH and C=O groups in pure AS. There are three populations of NH-groups, as classified from the strength of their hydrogen bonding, weakly or non-H-bonded, medium H-bonded, and strongly H-bonded. DFT simulations reveal that upon H-bonding the NH stretch band shifts by up to 113 cm$^{-1}$, and that the band intensity increases by up to 12.5-fold. Based on these results, the relative populations are 1:2:3, or ca. 17-33-50%, even though the relative areas are 2-20-78%. Similarly, there are three populations of H-
bonded C=O groups. The NH groups bind mostly with the C=O groups in the surrounding side chains, and partly with the O-groups in the backbone.

The structure of the polymer cavities is determined with MD simulations, for monomers 5 through 8 of a 12-mer polymer model. These simulations are done using the CVFF force field, which seems to be more accurate than several other force fields tested in Material Studio, as found by comparison to DFT simulations. Such MD simulations are still rather inaccurate, but provide a comprehensive picture of the polymer structure, including the existence of chiral cavities capable of accommodating one benzoin molecule.

The enantioselective interactions of AS with benzoin enantiomers are studied via IR data and DFT simulations. By using pure n-hexane as the mobile phase, the key interactions of our system can be plausibly predicted with a two-component model system of sorbent and benzoin. DFT simulations are used for estimating H-bonding strengths. It is inferred that without steric hindrance benzoin may tend to form the type of H-bond (AS) C=O ↔ HO (B), which is the strongest H-bond for the side chain/B pairs. H-D exchange allowed the direct observation of the OH bands of benzoin. Both enantiomers form identical H-bonds with AS. Moreover, difference spectra suggest that the C=O groups of R-benzoin bind differently with AS than those groups of S-benzoin. These bonds are inferred to be the key difference for the mechanism of chiral recognition of B by AS.

Since, as suggested from the literature, the environment in the chiral cavities, plays a key role in the enantioselectivity, MD, MC, and hybrid MD/MC docking studies are used to model AS/B interactions. These molecular simulations suggest that for some
potentially enantioselective cavities, R-benzoin may form with AS two H-bonds simultaneously, and have two significant π-π interactions, whereas S-benzoin tends to form one type of H-bond (SB) OH ↔ O=C (AS), and have one significant π-π interaction. For some of those cavities, the differences in the numbers of H-bonds and π-π interactions between AS and the two benzoin enantiomers appear to be the main reason for the predicted and observed enantioselectivity.
CHAPTER 4. CHIRAL RECOGNITION MECHANISM OF ACYLOIN-CONTAINING CHIRAL SOLUTES BY AS SORBENT

4.1 Introduction

(Most material in this chapter was published in Tsui, Hung-Wei, Margaret Y. Hwang, Lei Ling, Elias I. Franses, and Nien-Hwa Linda Wang. 2013. *Journal of Chromatography A* 1279: 36–48)

Polysaccharide-based sorbents, especially derivatized amylose and cellulose, have been widely used for many enantioselective separations (Chankvetadze 2012; Lammerhofer 2010). The effectiveness and versatility of these sorbents are due to several of the following factors: (1) the structural chirality of the glucopyranose unit; (2) the conformational chirality of the backbone helical structure; (3) the side chains chirality if present; (4) the diverse supramolecular structures of the cavities formed inside polymer rods; (5) the diverse distributions of H-bonding and hydrophobic functional groups in the cavities; and (6) the supramolecular chirality in the regions between the adjacent polymer rods (Lammerhofer 2010). Although much has been done for studying the chiral recognition mechanisms of these sorbents, more work is needed to advance the fundamental understanding of such mechanisms (Chankvetadze 2012). In this chapter, the focus is on novel mechanistic studies for amylose tris (S-phenylethylcarbamate), or AS, sorbent and four structurally related solutes (see Figure 1).
Booth et al. compared the chromatographic behavior of three amylose-based sorbents, amylose tris (3,5-dimethylphenylcarbamate), or AD, the AS sorbent above, and amylose tris (R-phenylethylcarbamate), or AR, for a series of chiral amides (Booth et al. 1997). They inferred that the elution order depends on the chirality of the amylose backbone, while the enantioselectivities depend on the chirality of the polymer side chains. Aboul-Enein and Ali reported the enantioselectivities of econazole, miconazole, and sulconazole enantiomers on AD, AS, and AR sorbents (Aboul-Enein and Ali 2001). Their High Performance Liquid Chromatography, or HPLC, data showed the same elution orders for AS and AR, which have opposite chiralities in their side chains. The enantioselectivities of the enantiomers on the three sorbents were in the order AS>AD>AR. They concluded that the key interactions contributing to enantioselectivity were hydrogen bonds, or π-π, or dipole induced dipole interactions. Similar comparisons of the enantioselectivities on the side chain chiralities of AS and AR were reported by Okamoto and Kaida (Yoshio Okamoto and Kaida 1990). Five out of eight chiral solutes studied by them showed the same elution order. Most enantiomers were better resolved on AS than on AR. The above reports suggested that the recognition mechanism was due not only to interactions with the individual single side chains but with multiple side chains.

The radius of the helical twist is smaller for the cellulose than the amylose helical backbone (Lammerhofer 2010). Kasat et al. used X-ray diffraction (XRD) and infrared spectroscopy (IR) to study the helical structures of AD, AS, and OD (Kasat, Wang, and Franses 2008; Kasat, Wang, and Franses 2007). Upon adsorption of norephedrine, AS and AD were found to have the same helical pitch, 14.6 Å; OD has a larger helical pitch
of 16.2 Å. From the amide I band of the IR spectra, they inferred that the intra H-bonds between the side chains are stronger in AD than in OD. By using various methods including X-ray analysis, small angle neutron scattering, and optical rotatory dispersion (ORD), Zugenmeier and co-workers concluded that cellulose tri(phenylcarbamate) (CTPC) and cellulose tri-O-benzoyl (TBC) have a left-handed 3-fold helical structure with a pitch of 15 Å (Peter Zugenmaier and Vogt 1983; Vogt and Zugenmaier 1985; Steinmeier and Zugenmaier 1987). By combining computer modeling and NMR 2D NOESY, for AD in chloroform solution, Yamamoto et al. suggested a left-handed 4-fold helical structure with a pitch distance of 15.6 Å (Chiyo Yamamoto, Yashima, and Okamoto 2002). Recently, in studying AD and OD helical structures, Ma et al. used vibration circular dichroism (VCD), which provides direct and definitive evidence (Ma et al. 2009). They reported a left-handed helical structure for AD and a right-handed helical structure for OD. Hence, the helicity may depend on the type of side chain.

Okamoto et al. (Yoshio Okamoto et al. 1990) reported studies of AS, AR, cellulose tris (S-phenylethylcarbamate), or OS, and cellulose tris (R-phenylethylcarbamate), or OR, for ten enantiomers. They concluded that the chiral resolving ability depends significantly on the chirality of the side chains. For cellulose derivatives, the resolving ability of OR was better than that of OS. For amylose derivatives, AS showed a better resolving ability than AR. The amylose derivatives were generally more effective than the cellulose derivatives for enantiomer separations. The authors suggested that the chiralities of the polymer sorbent glucose units and the side chains affect the chiral recognition.
From the above studies, it is difficult to determine the key general features of the chiral recognition mechanisms, which seem to involve the helical backbone structure, the side chain structure, the H-bonding groups, and the hydrophobic groups. The elution order of benzoin, or B, with AS and AR sorbents, was S<R. These sorbents have the same helical amylose backbone but different chirality of the side chains (Yoshio Okamoto et al. 1990).

The idea that structurally complex chiral cavities are important for enantioselectivity was used for several molecular simulations studies (Kasat, Wang, and Franses 2008; C. Yamamoto, Yashima, and Okamoto 1999; Kasat et al. 2008; Kasat, Franses, and Wang 2010; Li et al. 2010; Tsui et al. 2011). Kasat et al. used attenuated total reflection infrared spectroscopy (ATR-IR), XRD, MAS solid-state nuclear magnetic resonance (NMR) spectroscopy, cross-polarization/magic-angle spinning (CP/MAS), density functional theory (DFT), and molecular dynamics (MD) simulations to study molecular environments in the AD, OD, and AS sorbents (Kasat, Wang, and Franses 2007). They inferred that the spatial distributions of the C=O, NH, and phenyl groups in the nanometer-sized cavities of the sorbents are the key for understanding the chiral recognition mechanism. These distributions depend on the structures of the backbone and the side chains, and the solvents used.

The mobile phase used for polysaccharide-based sorbents is often a hydrocarbon-alcohol solution. Many studies have focused on the effects of the solvent on the chiral recognition (Chankvetadze 2012; S. Ma et al. 2009; Tsui et al. 2013A; Wang and Chen 1999; Wang, Chen, and Vailaya 2000; Wang and Wenslow 2003; Wenslow and Wang 2001; Gyimesi-Forrás et al. 2009). The retention factors were found to depend
significantly on the type and the concentration of the alcohol. The elution order could be sometimes reversed when different types of alcohol modifiers were used. Ma and coworkers reported that the alcohol in the mobile phase may lead to conformational changes of the sorbent, and even to a reversal in the elution order (S. Ma et al. 2009).

Easson and Stedman postulated a three-point attachment (TPA) model for the general mechanism of chiral recognition (Easson and Stedman 1933). They stated that for achieving chiral discrimination, a minimum of three attractive interactions between a planar sorbent surface and a solute are required. Many recognition mechanisms, however, do not follow this TPA model strictly, since not all three interactions need to be attractive (Lammerhofer 2010; Feibush 1998). For example, one strong attractive interaction and two repulsive (or steric) interactions can lead to chiral recognition (Feibush 1998; Lammerhofer 2010; Davankov 1997). The repulsive interactions can be more important for non-flat binding surfaces than for flat binding surfaces. For polysaccharide-type stationary phases, Davankov argued that the sterical “fitting” of the chiral solutes to the sorbent cavities may result in at least three interactions (Davankov 1997). In most cases, however, there can be more than three possible interaction sites involving either attractive or repulsive interactions. The latter may depend on the binding configurations.

A more general idea, of chiral recognition involving two interactions, a leading and a secondary interaction, has been proposed (Feibush 1998; Lammerhofer 2010; S. Ma et al. 2009). When a leading long-range strong attractive interaction causes a solute to approach closely to a sorbent site, a weaker and shorter-range secondary interaction may result. Whereas the leading interaction is usually achieved through one or more bonds,
the secondary interaction can be either attractive or repulsive, and can lead to enantioselectivity, often when it is combined with a third interaction.

Although in most variants of the TPA model, one assumes that the functional groups of the sorbent and the solute are rigid, actual systems can have some molecular flexibility which may impact the enantioselectivity (Davankov 1997; Feibush 1998). Davankov argued that structurally rigid enantiomers can meet geometrical requirements and constraints for chiral recognition more easily than flexible molecules. This argument is more valid when steric hindrance is involved in the chiral recognition mechanism. In addition, upon binding, flexible molecules may lose their conformational degree of freedom, which leads to an unfavorable energy contribution. Nonetheless, the above statement is true when the cavity size and shape can match the dimension of rigid solute. Flexible molecules may be more likely than the rigid molecules to maximize the binding interactions by their conformational adaptation to the cavities (Lammerhofer 2010).

By using polarization modulation ATR-IR spectroscopy with hexane in a flow cell Wirz et al. studied the chiral recognition of ethyl lactate, or EL with AS (Wirz, Burgi, and Baiker 2003). They concluded that the C=O groups of the R-EL enantiomer form stronger hydrogen bonds with the polymer NH groups than those of the S-EL enantiomer. Such interactions lead to enantioselectivity. They also used a combination of HPLC and ATR-IR data to probe changes in the H-bonding states of AS with pantolactone, or PL, in 20 vol.% isopropanol, or IPA, in cyclohexane (Wirz, Ferri, and Baiker 2008). The observed enantioselectivity was attributed to stronger hydrogen bonding between the R-PL C=O groups and the AS NH groups than those of the S-pantolactone. Recently, a study on the chiral recognition of AS with benzoin (B) using IR and computational
techniques was reported (Tsui et al. 2011). The recognition mechanism was attributed for R-B to two H-bonds, of the kind (AS) CO ↔ HO (B) and (AS) NH ↔ OC (B), and for S-B one H-bond (AS) CO ↔ HO (B). The above acyloin-type solutes contain a hydroxyl group on the α-position of a carbonyl group O=C-C-OH (Figure 4.1).

The objective of this chapter is to establish the chiral recognition mechanism for four structurally related solutes with AS (Figure 4.1). These solutes have the same H-bonding functional groups, OH and C=O, form intra H-bonds with different strengths, and have different molecular rigidities; see Section 4.4. A novel feature of this study is the effect of the solute intramolecular and intermolecular H-bonds on the retention factors. Another objective is to determine the effects of possible leading and secondary attractive interactions on the enantioselectivity mechanism for the four solutes. To avoid potential complications in the interpretation of chromatographic results when 0.13 to 1.3 M (1 to 10 vol %) of 2-propanol is used in the mobile phase, data were also obtained for these solutes with only n-hexane as the mobile phase. In addition to simulations involving only polymer and solutes, we also did novel MD simulations, for probing possible effects of hexane in the structure of the polymer. The effect of the hexane on the H-bonding sites of AS was found to be negligible.

The results support the hypothesis that the general chiral recognition mechanism for these systems involves a non-enantioselective leading H-bonding interaction and an enantioselective secondary H-bonding interaction, which is affected by geometrical restrictions. There can be additional third interactions which may assist the secondary interactions and contribute further to the enantioselectivity. The third interaction may involve π-π interactions or an additional H-bond. Moreover, the results indicate that the
Figure 4.1 Molecular structures of the four chiral solutes; all may form an intra H-bond of the type OH ↔ O=C; EL and MM may form also a different H-bond of the type OH ↔ O.
enantioselectivities correlate with the molecular rigidity, being higher for the more rigid molecules.

4.2 HPLC Results: Effects of the Solvent Composition on the Retention Factors and Enantioselectivities

The retention factors of the four solutes decrease with increasing IPA concentration (see Figure 4.2 and Table 4.1). They are the lowest for ethyl lactate (EL) and the highest for pantolactone (PL). The enantioselectivity \( \alpha \) decreases with IPA concentration for EL and MM, and shows no clear trend for B and PL. Generally, the enantioselectivity is lower for EL and MM, and higher for B and PL.

For the PL enantiomers in pure hexane, no peaks were observed, apparently because the retention factors were very large (≥ 10 hours). A rough extrapolation from the HPLC data with IPA in hexane leads to the sequences \( k_{R,PL} > k_{R,B} \), or \( k_{S,PL} > k_{S,B} \), and \( \alpha_{PL} \geq \alpha_{B} > \alpha_{MM} \geq \alpha_{EL} \). The focus here is on the data of EL, MM, and B with pure hexane as the mobile phase. These data are affected almost completely by solute-sorbent interactions. The effects of any interactions with hexane are minor or negligible, as discussed in Section 4.5.2. Hence, the solute-sorbent molecular simulations can be used for interpreting the chromatographic results with hexane.
Figure 4.2 HPLC results for retention factors and enantioselectivities of the four chiral solutes
Table 4.1 HPLC Results for Retention Factors and Enantioselectivities of Four Chiral Solutes

<table>
<thead>
<tr>
<th>IPA (M)</th>
<th>EL-S</th>
<th>EL-R</th>
<th>MM-S</th>
<th>MM-R</th>
<th>B-S</th>
<th>B-R</th>
<th>PL-S</th>
<th>PL-R</th>
<th>EL</th>
<th>MM</th>
<th>B</th>
<th>PL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.9</td>
<td>10.7</td>
<td>47.42</td>
<td>76.54</td>
<td>49.6</td>
<td>106</td>
<td>N/A</td>
<td>N/A</td>
<td>1.35</td>
<td>1.61</td>
<td>2.14</td>
<td>N/A</td>
</tr>
<tr>
<td>0.065</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>13.62</td>
<td>19.6</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>1.44</td>
<td>N/A</td>
</tr>
<tr>
<td>0.13</td>
<td>2.9</td>
<td>3.61</td>
<td>11.74</td>
<td>15.34</td>
<td>10.87</td>
<td>16.4</td>
<td>35.4</td>
<td>53</td>
<td>1.24</td>
<td>1.31</td>
<td>1.51</td>
<td>1.50</td>
</tr>
<tr>
<td>0.325</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>7.69</td>
<td>12.3</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>1.60</td>
<td>N/A</td>
</tr>
<tr>
<td>0.65</td>
<td>1.49</td>
<td>1.81</td>
<td>4.33</td>
<td>5.67</td>
<td>4.13</td>
<td>6.35</td>
<td>10.32</td>
<td>18.82</td>
<td>1.21</td>
<td>1.31</td>
<td>1.54</td>
<td>1.82</td>
</tr>
<tr>
<td>1.3</td>
<td>1.08</td>
<td>1.3</td>
<td>2.63</td>
<td>3.18</td>
<td>2.58</td>
<td>4.62</td>
<td>6.26</td>
<td>10.76</td>
<td>1.20</td>
<td>1.21</td>
<td>1.79</td>
<td>1.72</td>
</tr>
</tbody>
</table>
Figure 4.3 OH Bands of IR spectra of 0.1 wt. % of EL, MM, B, and PL in pure n-hexane at 25 °C. For peak assignments and wavenumbers, see Table 4.2. For EL and MM, Peaks 1 and 2 overlap. For B and PL, Peak 2 was not observed. For PL, there is significant inter H-bonding.
Table 4.2 Comparison of Wavenumbers ($\nu_{\text{OH}}$) and Intensities (I) of IR OH Bands with DFT Predictions of Wavenumbers, Intensities, and Intra Hydrogen Bonding Energies for Four Chiral Solutes

<table>
<thead>
<tr>
<th>Band</th>
<th>Data</th>
<th>DFT-Predictions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\nu_{\text{OH}}$ (cm$^{-1}$)</td>
<td>$\Delta\nu_{\text{OH}}$ (cm$^{-1}$)</td>
</tr>
<tr>
<td>EL</td>
<td>3640</td>
<td>reference</td>
</tr>
<tr>
<td></td>
<td>3620</td>
<td>-20</td>
</tr>
<tr>
<td></td>
<td>3549</td>
<td>-91</td>
</tr>
<tr>
<td></td>
<td>3467</td>
<td>-173</td>
</tr>
<tr>
<td>MM</td>
<td>3643</td>
<td>reference</td>
</tr>
<tr>
<td></td>
<td>3615</td>
<td>-28</td>
</tr>
<tr>
<td></td>
<td>3536</td>
<td>-107</td>
</tr>
<tr>
<td></td>
<td>3463</td>
<td>-180</td>
</tr>
<tr>
<td>B</td>
<td>3635</td>
<td>reference</td>
</tr>
<tr>
<td></td>
<td>3478</td>
<td>-157</td>
</tr>
<tr>
<td></td>
<td>3355</td>
<td>-280</td>
</tr>
<tr>
<td>PL</td>
<td>3635</td>
<td>reference</td>
</tr>
<tr>
<td></td>
<td>3566</td>
<td>-69</td>
</tr>
<tr>
<td></td>
<td>3470</td>
<td>-165</td>
</tr>
</tbody>
</table>

$^a$The spectra are shown in Figure 4.3.

$^b$Relative Intensities, compared to that of free OH, are estimated from the relative peak areas.

$^c$Intensities are found from the predicted peak heights if all molecules form that intra H-bond.

$^d$No DFT simulation for inter H-bonding are available.
4.3 IR and DFT Results of Intra Hydrogen Bonding of Enantiomers and their Inter Hydrogen Bonding with AS Polymer

4.3.1 IR Spectra and DFT Simulations of the Intra Hydrogen Bonds of Enantiomers

For the OH band region of the IR spectrum of EL, four peaks are observed at 3640, 3620, 3549, and 3467 cm\(^{-1}\) (Figure 4.3 and Table 4.2). The wavenumber shifts of -20, -90, and -173 cm\(^{-1}\) relatively to peak 1 indicate that most of the OH groups are hydrogen-bonded. To help the assignments of these peaks, DFT simulations were done for each solute. For EL, they show a wavenumber of 3679 cm\(^{-1}\) for non-H-bonded OH groups. Hence, Peak 1 should be due to non-H-bonded OH. Peak 2 is assigned to the OH group forming an intra H-bond with the O atom of the C=O-C group, because DFT predicts a shift of -13 cm\(^{-1}\), which compares well with the -20 cm\(^{-1}\) shift in the data. With similar reasoning, Peak 3 is assigned to OH intra-H-bonded with the C=O group. Since DFT predicts no other peak, Peak 4 is assigned to the OH group forming one or more inter-H-bonds with the OH or the C=O group of another EL molecule.

The peak assignments for the other three solutes were made similarly. For the MM spectrum, four populations were observed. Peak 2 was not observed for B, which does not have a C-O-C group. Also, Peak 2 was not observed for the rigid PL molecule because such an H-bond is probably sterically inhibited. For the PL OH band, a significant and broad Peak 4 is observed, implying that there is a significant population of inter H-bonds of PL with other PL molecules. PL has a higher chance to form inter H-bonds than the other three solutes, because it forms weaker intra H-bonds. DFT energy predictions support this conjecture.
Figure 4.4 C=O Bands of IR spectra of 0.1 wt. % of EL, MM, B, and PL in pure n-hexane at 25 °C. For PL, there is significant inter H-bonding.
Table 4.3 Comparison of Wavenumbers ($\nu_{C=O}$) and Intensities ($I$) of IR C=O Bands\textsuperscript{a} with DFT Predictions of Wavenumbers, Intensities, and Intra Hydrogen Bonding Energies for Four Chiral Solutes

<table>
<thead>
<tr>
<th>Band</th>
<th>Data</th>
<th>DFT-Predictions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\nu_{C=O}$ (cm\textsuperscript{-1})</td>
<td>$\Delta\nu_{C=O}$ (cm\textsuperscript{-1})</td>
</tr>
<tr>
<td>EL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>1745</td>
<td>reference</td>
</tr>
<tr>
<td>b</td>
<td>1766</td>
<td>+21</td>
</tr>
<tr>
<td>c</td>
<td>1738</td>
<td>-7</td>
</tr>
<tr>
<td>d</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>MM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>1747</td>
<td>reference</td>
</tr>
<tr>
<td>b</td>
<td>1759</td>
<td>+13</td>
</tr>
<tr>
<td>c</td>
<td>1742</td>
<td>-5</td>
</tr>
<tr>
<td>d</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>N/A</td>
<td>reference</td>
</tr>
<tr>
<td>c</td>
<td>1687</td>
<td>N/A</td>
</tr>
<tr>
<td>d</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>PL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>1799</td>
<td>reference</td>
</tr>
<tr>
<td>c</td>
<td>1799</td>
<td>0</td>
</tr>
<tr>
<td>d\textsubscript{1}</td>
<td>1812</td>
<td>+13</td>
</tr>
<tr>
<td>d\textsubscript{2}</td>
<td>1787</td>
<td>-12</td>
</tr>
<tr>
<td>d\textsubscript{3}</td>
<td>1765</td>
<td>-34</td>
</tr>
</tbody>
</table>

\textsuperscript{a}The spectra are shown in Figure 4.4.

\textsuperscript{b}Relative Intensities, compared to that of free OH, are estimated from the relative peak areas.

\textsuperscript{c}Intensities are found from the predicted peak heights if all molecules form that intra H-bond.

\textsuperscript{d}No DFT simulation for inter H-bonding are available.
For the EL C=O band region of the spectrum, three partially overlapping peaks were detected with symbols, a, b, and c. Their relative intensities were established with spectral analysis (see Figure 4.4 and Table 4.3). One of these peaks is probably due to free EL, and two are due to intra H-bonded EL conformations. DFT predicts a wavenumber of 1707 cm$^{-1}$ for the C=O group of non-intra H-bonded EL. If there is an intra H-bond of the type of OH$\leftrightarrow$O H-bond, DFT predicts a upshift of +18 cm$^{-1}$ for the C=O group, which is therefore affected indirectly. Hence, Peak a is inferred to be due to the non-bonded EL, and Peak b is due to EL showing the effect of the OH$\leftrightarrow$O intra H-bond. DFT also predicts a shift of -7 cm$^{-1}$ when the C=O group is intra-H-bonded to the OH group. Hence, peak c is due to this type of H-bonded group. Since no fourth peak is detected, it is inferred that either C=O groups form no inter-H-bonds or that any signal of such conformation is too small to be detected.

Similar comparisons and assignments were done for the C=O bands of the MM, B, and PL spectra. For MM, three peaks were indicated by spectral analysis. Peak a, at 1755 cm$^{-1}$, is for the non-H-bonded conformation. Peak b, at 1759 cm$^{-1}$, is for the intra H-bonded O with HO affecting C=O. Peak c, at 1742 cm$^{-1}$, is for the intra H-bonded C=O with HO. For benzoin, only one peak was observed at 1687 cm$^{-1}$. and DFT predicts a wavenumber of 1656 cm$^{-1}$ for free C=O and 1684 cm$^{-1}$ for intra H-bonded C=O. It is not entirely clear which of these conformations represents the data. It is likely that what is observed corresponds to the intra H-bonded C=O, since the OH groups were found to be strongly intra H-bonded to C=O, in agreement with published results (Pawelka et al. 2003).
For the PL spectra, four populations are observed. Since the DFT simulations predict a small shift of +2 cm\(^{-1}\) for an intra H-bonded group, the peaks of the free and the intra H-bonded C=O groups are inferred to have overlapped, and to form the large peak at 1799 cm\(^{-1}\). Then, the unresolved peaks at 1765, 1787, and 1812 cm\(^{-1}\) (\(d_3\), \(d_2\), and \(d_1\). See Table 3), as found from spectral analysis, are assigned to the inter H-bonding configurations which were inferred from the OH group bands.

The peak areas for Peak 3 of the OH groups are larger for each of the four solutes than the areas for Peak 1 (Figure 4.3). By contrast, no Peak 2 is seen for B and PL. If one uses the relative peak areas to estimate the relative populations for the various conformations, one would estimate the following. For EL, the apparent mole fractions from Peaks 1, 2, 3, and 4 would be 0.09, 0.06, 0.8, and 0.05 (see Table 4.4). For MM, the mole fractions would be 0.11, 0.12, 0.76, and 0.01. For B Peaks 1, 3, and 4, they would be 0.41, 0.58, and 0.01. And, for PL, they would be 0.08, 0.17, and 0.76, respectively. Similarly, for the C=O bands, the mole fractions of peaks a, b, and c would be 0.58, 0.04, and 0.38 for EL, and 0.19, 0.08, and 0.73 for MM. For PL Peaks a and d, they would be 0.34 and 0.66.

Since DFT predicts that different structural configurations have different IR intensities, the above method of estimation based on the relative peak areas alone, may be quite inaccurate. To better estimate the relative populations of the conformations, the following method was used. In Table 4.2, the DFT predicted relative intensities represent the intensity enhancements relatively to the intensity of the free OH band, and are used as weighting factors in determining the observed IR intensities. The observed IR intensities
Table 4.4 Estimation of the Relative Populations, Based on the Peaks Areas Only (IR) and the Peaks Areas/DFT Intensities (IR-DFT), of the Conformations for EL, MM, and B

<table>
<thead>
<tr>
<th>Peak #a</th>
<th>Conformation</th>
<th>Mole fraction b</th>
<th>EL</th>
<th>MM</th>
<th>B</th>
<th>PL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IR-Areas</td>
<td>IR-DFT b</td>
<td>IR-Areas</td>
<td>IR-DFT b</td>
<td>IR-Areas</td>
</tr>
<tr>
<td>1</td>
<td>free (no H-bond)</td>
<td>1-x-y</td>
<td>0.09</td>
<td>0.25</td>
<td>0.11</td>
<td>0.28</td>
</tr>
<tr>
<td>2</td>
<td>intra-1 (OH↔O)</td>
<td>x</td>
<td>0.06</td>
<td>0.08</td>
<td>0.12</td>
<td>0.15</td>
</tr>
<tr>
<td>3</td>
<td>intra-2 (OH↔O=C)</td>
<td>y</td>
<td>0.80</td>
<td>0.67</td>
<td>0.76</td>
<td>0.57</td>
</tr>
<tr>
<td>4</td>
<td>inter H-bond</td>
<td>N/A</td>
<td>0.05</td>
<td>N/A</td>
<td>0.01</td>
<td>N/A</td>
</tr>
<tr>
<td>a</td>
<td>free (no H-bond)</td>
<td>1-x-y</td>
<td>0.58</td>
<td>0.58</td>
<td>0.19</td>
<td>0.19</td>
</tr>
<tr>
<td>b</td>
<td>intra-1 (OH↔O)</td>
<td>x</td>
<td>0.04</td>
<td>0.04</td>
<td>0.08</td>
<td>0.09</td>
</tr>
<tr>
<td>c</td>
<td>intra-2 (OH↔O=C)</td>
<td>y</td>
<td>0.38</td>
<td>0.38</td>
<td>0.73</td>
<td>0.72</td>
</tr>
<tr>
<td>d</td>
<td>inter H-bond</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

aPeaks 1, 2, 3, and 4 are for the OH bands; see Table 2. Peaks a, b, c, and d are for the C=O bands. It is believed that the OH-related data are more reliable; see text.
bPopulations of Inter H-bonds are not considered in the estimations.
cThe sum of the relative populations of the PL Peak a and Peak c is 0.34.
dSince most of the peak area is due to inter H-bond, no calculations of y are reported.
$I_{IR,x}$ and $I_{IR,y}$ depend on the DFT intensities $I_{DFT,x}$ and $I_{DFT,y}$ and the mole fractions of the hydrogen bonded states of 1, 2, 3 (or of a,b,c), 1-x-y, x, and y.

$$
\frac{I_{DFT,x} x}{1-(1-x-y)} = \frac{I_{IR,x}}{1}
$$

$$
\frac{I_{DFT,y} y}{1-(1-x-y)} = \frac{I_{IR,y}}{1}
$$

The inter H-bonded OH is ignored. With these equations, for the EL OH band, the predicted mole fractions are 0.25, 0.08, and 0.67 for Peaks 1, 2, and 3 (see Table 4.4). Overall, nearly 2/3 of the OH groups are H-bonded with C=O. The DFT predicted energies also show that the conformation of the intra H-bond (OH↔O=C) is more stable than that of free OH or intra H-bond (OH↔O).

For MM, the mole fractions are 0.28, 0.15, and 0.57 for free OH, intra H-bonded OH with O, and intra H-bonded OH with O=C. Since x=0 for B, the mole fractions for free OH and intra H-bonded OH are 0.57 and 0.43. The results for PL were not interpreted further, because the fraction of the groups with inter H-bonds is high.

Similarly, for the C=O bands, the mole fractions, for free C=O (a), intra H-bond (OH ↔ O) affecting C=O (b), and intra H-bonded C=O with HO (c), are 0.58, 0.039, and 0.38 for EL, and 0.19, 0.09, and 0.72 for MM (Table 4.4). The discrepancies of these mole fractions with the results for the OH bands may be due to errors in data or peak fitting. Nonetheless, the relative population estimates for the OH bands are more reliable than those for the C=O bands, because the peaks at the C=O region overlap. Applying the
new method makes some difference in the predicted mole fractions, but does not change
the quantitative interpretation significantly.

DFT results support the inferences from the IR data. From these results and the
DFT energy predictions, it is inferred that: (1) the trend in the intra (OH ↔ O=C) H-
bonding strength is: PL<B<MM<EL; (2) for PL, Peak 4 is quite broad and Peak d shows
three populations in the C=O band, indicating significant inter H-bonding between PL
molecules; (3) large Peaks 3 and c in the EL and MM spectra indicate a significant intra
H-bond of OH with O=C. The relative peak populations for each band correlate semi-
quantitatively with the DFT predictions of intra H-bonding strengths.

4.3.2 DFT-Based Ranking of Inter Hydrogen Bonding of Enantiomers with an AS Side
Chain

The mechanism of the chiral recognition in these systems is proposed here
primarily on the basis of IR data, to involve a strong leading interaction for each
enantiomer, a weaker secondary interaction for one of the enantiomers, in this case the R-
enantiomer, and a third interaction. The S enantiomer is prevented from having the
secondary interaction by a steric effect, which has to be clarified by further molecular
simulation studies. To help test this mechanism and establish which of the two possible
H-bonds would be the leading interaction, the energies of the H-bonds of the OH groups
and of the C=O groups of the four solutes were calculated with DFT.

The energies of the H-bonds of the OH groups for all four solutes are larger in
absolute value than those of the C=O groups; see Table 4.5. This postulated mechanism
Table 4.5 DFT Predictions of Inter Hydrogen Bonding Energies of Each Chiral Solute with the Model Side Chain S2 and Inferences on Interactions

<table>
<thead>
<tr>
<th>Pair</th>
<th>H-Bond Type</th>
<th>ΔE, (kJ/mol)</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>EL with S2</td>
<td>(EL) OH ↔ O=C (S2)</td>
<td>-24.4</td>
<td>Leading</td>
</tr>
<tr>
<td></td>
<td>(EL) C=O ↔ HN (S2)</td>
<td>-20.5</td>
<td>Secondary</td>
</tr>
<tr>
<td></td>
<td>(EL) O ↔ HN (S2)</td>
<td>-15.4</td>
<td>Possible Additional</td>
</tr>
<tr>
<td>MM with S2</td>
<td>(MM) OH ↔ O=C (S2)</td>
<td>-28.0</td>
<td>Leading</td>
</tr>
<tr>
<td></td>
<td>(MM) C=O ↔ HN (S2)</td>
<td>-20.2</td>
<td>Secondary</td>
</tr>
<tr>
<td></td>
<td>(MM) O ↔ HN (S2)</td>
<td>-20.0</td>
<td>Possible Additional</td>
</tr>
<tr>
<td>B with S2</td>
<td>(B) OH ↔ O=C (S2)</td>
<td>-33.3</td>
<td>Leading</td>
</tr>
<tr>
<td></td>
<td>(B) C=O ↔ HN (S2)</td>
<td>-18.4</td>
<td>Secondary</td>
</tr>
<tr>
<td>PL with S2</td>
<td>(PL) OH ↔ O=C (S2)</td>
<td>-34.0</td>
<td>Leading</td>
</tr>
<tr>
<td></td>
<td>(PL) C=O ↔ HN (S2)</td>
<td>-13.9</td>
<td>Secondary</td>
</tr>
<tr>
<td></td>
<td>(PL) O ↔ HN (S2)</td>
<td>-13.9</td>
<td>Possible Additional</td>
</tr>
</tbody>
</table>
for chiral recognition, involving a non-enantioselective leading interaction and an enantioselective secondary interaction, is consistent with certain literature reports (Tsui et al. 2011; Wirz, Ferri, and Baiker 2008; Wirz, Burgi, and Baiker 2003). Hence, for all four solutes, the leading interaction should be that of the type \( \text{OH} \leftrightarrow \text{O=C} \) bond, and the secondary interaction should be of the type \( \text{C=O} \leftrightarrow \text{HN} \) bond.

The energy of the leading interaction increases in the order \( \text{EL} < \text{MM} < \text{B} < \text{PL} \). The energy of the secondary interaction is in the opposite order, \( \text{EL} > \text{MM} > \text{B} > \text{PL} \). The sum of these energies, \(-44.9, -48.2, -51.7, \) and \(-47.9 \text{ kJ/mol} \), are in the order \( \text{EL} < \text{PL} < \text{MM} < \text{B} \), which does not correlate with the observed order of the retention factors. The total energy, including the energy of breaking the intra H-bond, are \(-23.7, -32.9, -37.0, \) and \(-39.5 \text{ kJ/mol} \), in the order \( \text{EL} < \text{MM} < \text{B} < \text{PL} \). This trend is consistent with the order of the retention factors, and suggests that intra H-bonding does play a significant role in the retention factors. In addition, for PL, the potential H-bond of the type \( \text{(PL) O} \leftrightarrow \text{HN (S)} \) has almost the same strength as that of the type \( \text{(PL) C=O} \leftrightarrow \text{HN (S)} \). The proximity of the C=O and the O groups of PL may lead to a simultaneous interaction with same side chain NH group. This effect may be one reason for the very high retention factors of PL. For more definitive inferences, one has to account for the effects of entropy differences, \( \pi-\pi \) interactions (for MM and B), and possible different binding capacities and sites. These effects are not considered here.
Figure 4.5 Molecular model of the O=C-C-O group for torsion angles for R-ethyl lactate:
(a) 0°; (b) 180°; (c) +120°; (d) -120°. For the other solutes, the torsion angles are defined similarly.
Figure 4.6 Torsion angle distributions and area percentages of R-EL, R-MM, R-B, and R-PL in vacuum, as determined from MD simulations without consideration of intra H-bonds. The torsion angles are defined in Figure 4.5. Conformations denoted by * tend to form intra H-bonds and to preclude enantioselective interactions because of molecular flexibility; see text.
4.4 MD Results of Molecular Rigidity or Flexibility

The distributions of the torsion angles of the acyloin O=C-C-O group were determined with MD simulations. These distributions provide an indicator of the molecular rigidity. The broad and bimodal distributions for EL and MM (see Figure 4.6) are due to their flexible chain-like molecular structures. For PL, the narrow and nearly unimodal distribution is probably due to its internal ring structure, which decreases the mobility of the functional groups. For B, the distribution may be narrow because of intra molecular π-π interactions. The area percentages of the second peaks in Figure 4.6 may provide a semi-quantitative measure of flexibility. Apparently, the enantiomers of EL and MM, unlike those of B and PL, have sufficient molecular flexibility to reduce the impact of the steric effects, and hence decrease the enantioselective interactions. For these reasons, the order of the enantioselectivity, EL ≤ MM < B ≤ PL (Table 4.1), correlates with increasing molecular rigidity, which may decreases strength of the intra H-bond.

4.5 Simulations of Polymer, Polymer with Hexane, and Polymer with Solutes

4.5.1 Structure of a Dodecamer Polymer Model with MD

To better visualize the available cavities, and their possible interactions with the solutes, it is important to produce accurate microstructures of the sorbent polymer. MD simulations were done for left-handed (LH) and for right-handed (RH) helical amylose backbone structure, to test for possible differences. For the LH structure the attachment sites of the C2 and C3 side chains are shown as dark spheres and medium dark spheres, and those of the C5 side chains are shown as light spheres (Figure 4.7a). The latter points
Figure 4.7 Molecular simulations of the molecular structure of an left-handed (LH) model polymer. (a) The dark and medium dark spheres represent the attachment points of the C2 and C3 side chains; the light balls represent the attachment points of the C5 side chains. (b) Structure of the polymer showing only the C2 and C3 side chains as dark and less dark balls; the space between these side chains define the empty helical space. (c) The insertion of the C5 side chains in the grooves produce an orderly array of cavities, some of which may be chiral; a few cavities are indicated by arrow.
form a helix with a smaller radius than that of the former points. When the C2 and C3 side chains are attached in the backbone (Figure 4.7b), one observes that the side chains define a helicoidal surface. After insertion of the C5 side chains, the empty space surrounded by this helicoidal surface is divided into a series of “grooves” or “cavities” (Figure 4.7c).

By contrast, a helix defined by the C5 attachment sites has a larger radius for the RH structure than for the LH structure (see Appendix C). For this reason, and since the chiralities of the stereogenic centers of the glucopyranose units are the same as for the LH structure, the cavities in the RH structure are not mirror images to those of the LH structure. Hence, reversing the orientation of the helical backbone may not necessarily lead to a reverse elution order. In a previous publication the RH structure was used for modeling and docking studies of B (Tsui et al. 2011). Since evidence points towards an LH structure for AD (Chiyo Yamamoto, Yashima, and Okamoto 2002; S. Ma et al. 2009), it will be assumed that the actual AS structure is LH, and we will only use an LH structure will be used in this study for reporting new docking studies for B.

One can detect several cavities in the polymer structure. In one example, shown later in Section 4.5.3, a cavity forms between the C3 side chain of Monomer #4, or M4, and the C5 side chain of monomer M5. Three other similar cavities, between M5 and M6, M6 and M7, and M7 and M8, form an ordered array and are the largest ones for the central polymer unit considered. These cavities can have somewhat fluctuating overall conformations. Several other smaller cavities can also be identified. The strategy is to use docking studies with specific solutes for finding chiral cavities, in which the solutes can fit and bind with a significant enantioselectivity. To obtain more comprehensive
distribution of all possible cavity conformations, one would have to run a much larger number of MD simulations than currently available. This is beyond the scope of this dissertation. Hence, docking simulations will be reported for a small number of polymer conformations and for the most effective cavities (see Section 4.5.3).

4.5.2 Structure of AS with Hexane with MD

MD simulations were done to determine whether there are any changes in the structure or the energy of the polymer model upon mixing it with 200 hexane molecules. A typical snapshot, in Figure 4.8a, shows little effect of hexane on the polymer structure. To better visualize any potential minor structural changes, two AS structures, one for pure AS and one for AS with hexane (without showing the hexane molecules) were superimposed in Figure 4.8b. Again, no significant changes were visually detected in the H-bonded regions.

The energies of the AS and its components before and after introduction of the hexane molecules were calculated (Table 4.6). The total energy increased slightly by about 136±143 kJ/mole, or about 3.4±3.5 %, apparently because of some structural changes induced by hexane. The electrostatic energy between the side chains showed no significant change, suggesting that the hexane molecules do not affect the H-bonding state. It has also been established from XRD and IR that for AD, hexane does not change the H-bonding state of the polymer (Kasat et al. 2006). A significant increase in the predicted vDW energies, +345±154 kJ/mol, or 9.6±4.3 %, indicates that the vDW interactions between the side chain phenyl groups become weaker. Such lower values may lead to an “energy relaxation” of the side chains, resulting in a slightly weakened
Figure 4.8 Molecular simulations of an AS 12-mer polymer model with 200 molecules of n-hexane. To help observe each hexane molecule an ellipsoid is drawn around it. (a) a random snapshot of the simulations in a periodic boundary cell. (b) two superimposed random snapshots of the simulations; light, AS in vacuum; dark, AS with hexane.
Table 4.6 Comparison of Predicted Energy Components of an AS Polymer Rod Model in Vacuum or with 200 Molecules of n-Hexane

<table>
<thead>
<tr>
<th></th>
<th>AS in vacuum (kJ/mol)</th>
<th>AS with n-hexane (kJ/mol)</th>
<th>Change (kJ/mol)</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS energy</td>
<td>4053 ±100</td>
<td>4189 ±102</td>
<td>136 ±143</td>
<td>3.4 ±3.5</td>
</tr>
<tr>
<td>valence (1)</td>
<td>5854 ±156</td>
<td>5662 ±119</td>
<td>-191 ±196</td>
<td>-3.3 ±3.4</td>
</tr>
<tr>
<td>non-bond (2)</td>
<td>-1801 ±120</td>
<td>-1473 ±91</td>
<td>327 ±151</td>
<td>18 ±8.4</td>
</tr>
<tr>
<td>vDW (2a)</td>
<td>3596 ±124</td>
<td>3941 ±92</td>
<td>345 ±154</td>
<td>9.6 ±4.3</td>
</tr>
<tr>
<td>electrostatic (2b)</td>
<td>-5397 ±23</td>
<td>-5415 ±28</td>
<td>-18 ±36</td>
<td>-0.33 ±0.67</td>
</tr>
</tbody>
</table>
valence energy (-191±196 kJ/mol). Since the hexane does not change much the H-bonding state of the polymer, subsequent docking studies were done without hexane. It is believed that these results are relevant to the chromatographic data with hexane.

4.5.3 Docking Studies

The MC docking results for PL showed that there are some achiral and some chiral binding configurations. The results for chiral configurations were used as the initial configurations and solute orientations for the subsequent MD simulations (Figure 4.9). In several achiral cavities the enantiomers form either one or two H-bonds simultaneously, and hence there is no energy difference for the two enantiomers. In a chiral cavity, the R-PL enantiomer is shown to form two strong H-bonds, of the type (R-PL) OH ↔ O=C (AS) and (R-PL) C=O ↔ HN (AS), with the C5 and C3 side chains (Figure 4.9a), with distances of 2.07 and 3.23 Å, respectively. By contrast, S-PL forms only one strong H-bond, of the type (S-PL) OH ↔ O=C (AS), with the C5 side chain and a distance of 1.85 Å. For these configurations there can be enantioselectivity. When the first H-bond forms, the location, conformation, and orientation of the solute in the cavity result in such an overall configuration that the distances and angles do not lead to the formation of a strong second H-bond. For the predicted distance of 3.75 Å, an H-bond would be weak. Moreover, the O group for both enantiomers may form an additional weak H-bond with the NH group. This bond may act synergistically with the C=O ↔ H-N bond, and may contribute to the large retention factors of PL.

MC simulations showed that the B enantiomers can be discriminated in the same cavity as the one for PL in Figure 4.9, that the R-B enantiomer forms two strong H-bonds,
Figure 4.9 Simulation predictions of docking of (R)- and (S)-pantolactone in a cavity formed by the C3 side chain of monomer M4 and the C5 side chain of monomer M5. The polymer structure is shown in the background. The numbers indicate the distances for potential H-bonds. The energy difference is $\Delta \Delta E = \Delta E_R - \Delta E_S = -13.1$ kJ/mol.
Figure 4.10 Same as Figure 9 but for (R)- and (S)-benzoin. $\Delta \Delta E = \Delta E_R - \Delta E_S = -50.6$ kJ/mol.

Some potential $\pi - \pi$ interactions are indicated by arrows.
and that the S-B enantiomer forms one strong H-bond, and a possible weaker H-bond (Figure 4.10). The third possible interaction for B may originate from several π-π interactions between its two phenyl groups with the phenyl groups of AS. The second H-bond “draws” the R enantiomer more deeply into the cavity than the S enantiomer. In the example shown in Figure 4.10, the phenyl groups of benzoin form three γ-shaped π-π interactions for R-B but only one for S-B. The distances of these interactions range from 3 to 5 Å. Since these π-π solute-sorbent interactions do not compete with IPA-sorbent interactions, their effect on the enantioselectivities is expected to be more significant for the higher IPA concentrations. These π-π interactions can contribute to both the high retention factors and the enantioselectivity (Table 4.1).

For MM (Figure 4.11), the mechanism seems to be quite similar as that of B, with the π-π interactions being weaker, because of the presence of only one phenyl group. The O group may also play a role, by analogy to PL and EL. For EL (Figure 4.12), the mechanism seems to be quite similar as that of PL, with two key differences. The O group is located in a flexible chain, instead of the five-member ring in PL, and at a larger distance from the NH group. This leads to a lower retention factor and a lower α-value.

The energy differences for the two enantiomers in the same cavity were found to be, in kJ/mol, -13 for PL, -51 for B, -30 for MM, and -32 for EL. They do not correlate with the observed enantioselectivities, for reasons which are unclear. It is presumed that the overall observed enantioselectivities are probably averages which include interactions with achiral cavities and other sites, as well as with chiral cavities.

Overall, the docking studies indicate that the mechanism for enantioselectivity involves one strong attractive interaction which is common for the two enantiomers, or
Figure 4.11 Same as Figure 10 but for (R)- and (S)-methyl mandelate. $\Delta \Delta E = \Delta E_R - \Delta E_S = -30.1 \text{ kJ/mol}$. 
Figure 4.12 Same as Figure 9 but for (R)- and (S)-ethyl lactate. $\Delta \Delta E = \Delta E_R - \Delta E_S = -32.2$ kJ/mol.
non-enantioselective, and one or more other enantioselective interactions. The differences in these interactions depend critically on the detailed microstructure of the polymer cavities and the different conformations produced by the two enantiomers. Moreover, for the solutes examined the molecular flexibility and the strength of intra H-bonds play significant roles.

4.6 Conclusions

The chiral recognition mechanism for the AS polymer was studied by using four structurally similar solutes, ethyl lactate (EL), methyl mandelate (MM), benzoin (B), and pantolactone (PL). Among these solutes, PL, EL, and MM have an O group adjacent to the acyloin group, and B and MM have two or one phenyl groups, respectively. These solutes were found to have quite different HPLC retention factors in the order EL< MM≤ B< PL, and enantioselectivities in the order EL≤ MM< B≤ PL. IR data and DFT simulations show evidence of an intra H-bond C=O ↔ HO for all solutes when dissolved in n-hexane mobile phase at 25 °C. The relative peak areas for the OH and C=O IR bands correlate semi-quantitatively with the intra H-bonding strengths between OH and C=O or O, which are in the order PL< B< MM< EL. The DFT energies of the inter H-bonds, of the solute OH groups and of the solute C=O groups, with the sorbent side chains were calculated. The results show that the strengths of the H-bonds of the solute OH groups are stronger than those of the solute C=O groups, and the strength of the leading interaction increases in the order EL< MM< B< PL. The strength of the secondary interaction is in the opposite order, EL> MM> B> PL. If an intra H-bond has to be broken before the formation of the inter H-bonds, the order of the energies is consistent with the order of
the retention factors. The O groups may also contribute synergistically to the retention factors of PL, and less on those of EL and MM.

The distributions of the torsion angles of the solute acyloin O=C-C-O group were determined with MD simulations. These distributions provide an indicator of the molecular flexibility or rigidity. The order of the enantioselectivity, EL ≤ MM < B ≤ PL, correlates with increasing molecular rigidity, which also affects the strength of the intra H-bond.

MD simulations were done for left-handed (LH) AS helical backbone 12-mer polymer structure. Simulations on the polymer structure and the energy components upon mixing one polymer molecule with 200 n-hexane molecules showed that the n-hexane does not change the H-bonding state of the polymer, and induces only a slight energy-related relaxation of the side-chain phenyl groups. Subsequent polymer-solute docking studies were done without hexane. MC and MD docking simulations were done for PL and B enantiomers, and MD docking simulations were done for EL and MM enantiomers. A certain cavity in which chiral recognition can be achieved was found. The results support the hypothesis that the general recognition mechanism involves a non-enantioselective “leading” strong H-bonding interaction and an enantioselective secondary H-bonding interaction, which is affected by geometrical and energetic restrictions and can lead to additional differences in interactions, either an H-bond by the O group of PL, EL, and MM or certain π-π interactions by the phenyl groups of B and MM with the phenyl groups of the sorbent polymer.
CHAPTER 5. RETENTION MODELS AND INTERACTION MECHANISMS OF ACETONE AND OTHER CARBONYL-CONTAINING MOLECULES WITH AS SORBENT

5.1 Introduction


In addition to solute-sorbent interactions, the H-bonding functional groups of the sorbents can bind with the polar modifier, or “solvent”, in the hydrocarbon mobile phase. The HPLC retention factors generally decrease with increasing concentration of the polar modifier in the hydrocarbon mobile phase (Kasat, Franses, and Wang 2010; Tsui et al. 2011). Many studies on the effects of the polar modifier in chiral separations and retention behavior in many other chiral sorbents have been reported (Wang, Chen, and Vailaya 2000; Wang and Wenslow 2003; Wenslow and Wang 2001; Wang and Chen 1999; Lammerhofer 2010).

Among the PS-based sorbents, AS (Figure 5.1), is widely used. Our previous studies of benzoin enantiomers with AS have shown that the S-benzoin forms one H-bond with AS and the R-benzoin forms two H-bonds with AS. This difference is considered to be the basis for the enantioselectivity (Tsui et al. 2011). The retention factors \( k \) depend on the concentration of the polar modifier, isopropanol, or IPA. When the \( k \) data of \( R \) and \( S \)-benzoins are plotted as a log-log plot vs. total IPA concentration \( C_i^0 \)
Figure 5.1 (A) Molecular structure of the polymer repeat unit of the AS polymer, with R being the side chain. (B) Molecular structure of benzoin. (C) Molecular structures of the five achiral solutes.
in \( n \)-hexane, a linear relationship, with \( R^2 > 0.9 \), of the retention behavior, \( \ln(k) = A - B \ln C^0 \), is generally observed (see Section 5.3). The slopes \( B \) are less than 1, namely 0.50 and 0.56, for the \( R \) and \( S \) enantiomers. Such slopes cannot be explained by the retention models from the literature.

In the early 1960’s, Snyder developed a retention model which has a form of \( \log\left(\frac{k_2}{k_1}\right) = \alpha' A_s (\varepsilon_1 - \varepsilon_2) \), where \( k_1 \) and \( k_2 \) are the solute retention factors in two solvent components (hexane and IPA here), \( \alpha' \) is the adsorbent activity parameter, \( A_s \) is the solute molecular area, and \( \varepsilon_1 \) and \( \varepsilon_2 \) are the solvent component strengths (L. R. Snyder 1974). In this model one assumes that the adsorbent surface is homogeneous and that there are no significant solute-solvent interactions. These assumptions may be valid for the adsorption on the entire surface (no specific adsorption sites) of an amorphous sorbent (such as alumina), on which the solute and solvent molecules can adsorb. For low modifier concentrations, the model can be reduced to the form of \( k = I/[D]^Z \), where \( I \) and \( Z \) are constants and \( [D] \) is the modifier concentration. The \( Z \)-value represents the ratio of the molecular areas of the solute and the solvent. A similar form of such a model, which is based on the concept of discrete adsorption, or binding sites, was reported by Soczewiński (Edward 1977). The mobile phase was assumed to be a mixture of a strong solvent, or modifier, and an inert solvent. In this “stoichiometric displacement” model, the \( Z \)-value represents the average number of modifier molecules displaced from the sorbent surface by a solute molecule. This \( Z \)-value can be an integer, 1, 2, etc, or an average of such integers, generally more than 1. In this model, as in Snyder’s model, the solute-solvent interactions were also ignored.
For the retention behavior of proteins in reverse phase chromatography, Geng and Regnier reported a stoichiometric displacement model, which is based on the application of the mass action law on the solute-solvent, solute-sorbent, and solvent-sorbent interactions (Geng and Regnier 1984). Although the form of the model is similar to Soczewiński’s model, the Z-value has a different physical meaning, namely the displacement of one or more solvent molecules on the solute and the sorbent surfaces upon the solute adsorption. Gyimesi-Forrás et al. applied Soczewiński’s model to describe the chiral separation of imidazo-quinazoline-dione derivatives on a quinine carbamate-based CSP (Gyimesi-Forrás et al. 2009). When their data were plotted in a log-log plot, the slope Z (we use the symbol B for our data below) ranged from 1.35 to 2.92. Based on such models, the B-values for benzoin would be between 1 and 4, which differ from our data, and therefore needs an explanation. To our knowledge, no slopes less than 1 have been reported previously for solutes with chiral sorbents, and none of the above models can help explain such small slopes.

Since benzoin has multiple functional groups, such as C=O, OH, and phenyls, it can form intra-molecular H-bonds, or multiple inter-molecular H-bonds with another benzoin molecule, or with the AS sorbent. To obtain insights into this mechanism for benzoin and other chiral molecules, we chose five simpler achiral solutes for a retention behavior study: acetone (AC), cyclo hexanone (CH), benzaldehyde (BA), phenylacetaldehyde (PA), and hydrocinnamaldehyde (HA) (see Figure 5.1 and Table 5.1). These solutes contain only one C=O group which may form one H-bond with the sorbent’s NH sites. The objective of this chapter is to understand the retention behavior
Table 5.1 Measured HPLC Retention Factors (k) for AS and for Different Isopropanol Concentrations ($C_I^0$) in n-Hexane and the Results of Linear Fits of ln k vs. ln $C_I^0$ Plots for Five Achiral Solutes

<table>
<thead>
<tr>
<th>Solutes</th>
<th>IPA concentration, $C_I^0$ (M)$^a$</th>
<th>ln k vs. ln $C_I^0$ plot$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.3</td>
<td>0.78</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Acetone</td>
<td>0.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Cyclohexanone</td>
<td>1.1</td>
<td>N/A</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>1.3</td>
<td>N/A</td>
</tr>
<tr>
<td>Phenylacetaldehyde</td>
<td>1.3</td>
<td>N/A</td>
</tr>
<tr>
<td>Hydrocinnamaldehyde</td>
<td>1.3</td>
<td>N/A</td>
</tr>
</tbody>
</table>

$^a$1.3 M corresponds to 10 vol.%.

$^b$The data were fitted to the equation \( \ln k = A - B \ln C_I^0 \)
qualitatively or quantitatively on the basis of plausible interaction mechanisms using a combination of thermodynamic theory, chromatography, IR experiments, molecular simulations, and dynamic chromatography simulations. In this study, we focus on new studies of the effects of the alcohol concentration on the retention factor of achiral molecules, and its detailed molecular interpretation, as a step to understand the mechanisms of binding of chiral molecules.

Three of the five molecules studied, BA, PA, and HA, have one phenyl group for possible π-π interactions with the sorbent. The IPA molecule can form at most one H-bond with the sorbent, either with the NH site or with C=O site. To understand the competitive H-bond formation with the sorbent, we present in Section 5.2 a new set of thermodynamic retention models. These models include IPA-IPA interactions via formation of H-bonded IPA aggregates, in addition to sorbent-solute, sorbent-IPA, and solute-IPA interactions. The conditions at which the new models can predict slopes \( B \) below 1 are described. Analysis of the equilibrium retention models indicates that only IPA aggregation can result in a slope less than 1 for a monovalent solute.

The idea of IPA-IPA aggregation in nonpolar solvents or in a gas phase is not new. Several papers have reported such effects (Fletcher and Heller 1967; S. L. Ma et al. 2008; O’Brien et al. 1997; Fujiwara and Ikenoue 1976), but there are no reports on their impact on the retention behavior. Ma et al. (2008) indicated that because isopropanol molecules have a lower tendency to form aggregates than that of linear alcohols, they tend to interact with the sorbent more efficiently. Stubbs and Siepmann used configurational-bias Monte Carlo (MC) simulations to study the aggregation of 1-hexanol in \( n \)-hexane (Stubbs
and Siepmann 2002). They concluded that in solutions of mole fractions from 0.01 to 0.05 at room temperature, tetramers and pentamers form. About half of these aggregates were inferred to be in cyclic conformations. By using “infrared cavity ring down laser absorption spectroscopy” and DFT, Provencal et al. reported that dimers, trimers, tetramers, and larger clusters of ethanol and butanol were present in the gas phase (Provencal et al. 2000). Førland et al. used IR to study the self-association of benzyl alcohol in carbon tetrachloride (Forland et al. 1997). For an alcohol molality of 0.2 m at 30°C, alcohol monomers were the dominant component (ca. 0.08 m), with the remainder being in aggregate form. They inferred that the average alcohol aggregation number was 4 for open chain (non-cyclic) conformations and 7 for less-abundant cyclic conformations.

This study focuses on the effects of IPA aggregation in n-hexane, for which no previous reports are available.

For acetone a more detailed study was done. IR data and DFT simulations were used to probe and further substantiate the existence of significant IPA-IPA aggregation and of AC-IPA complexation. The average number of molecules in IPA aggregates (or aggregate number), and the equilibrium constants for aggregation, complexation, and adsorption were estimated from the retention factors using the equilibrium retention models. The estimated aggregate number was consistent with those from the IR and DFT results. Chromatography simulations were also used to further test the retention models and the estimated model parameters. The simulations took into account competitive adsorption of AC and IPA, AC-IPA complexation, and IPA-IPA aggregation in a chromatography process. Both the equilibrium retention models and the dynamic
chromatography simulations indicated that IPA aggregation can reduce significantly IPA monomer concentration, resulting in a slope less than 1 for a monovalent solute in the log-log plot. Hence, complexation and aggregation of the polar modifier in the mobile phase must be accounted for in the retention models used for interpretation of the number of binding sites of a solute with a sorbent. Although similar, but more complex models, can be developed for benzoin and other chiral molecules, they were discussed in Chapter 6.

5.2 New Thermodynamic Retention Models

For modeling the retention factors, solvent-solvent (IPA-IPA) interactions, or IPA aggregation, are considered for the first time, in addition to solute-sorbent, solvent-sorbent, and solute-solvent interactions. Since the adsorption of a chiral solute onto a CSP may be heterogeneous, its retention factor would represent an average of various possible binding configurations. For this reason, a simple achiral solute, acetone (AC), is taken as an example for formulating the model. The AS polymer side chain contains equal amounts of NH and C=O groups. We expect that acetone may bind only to the NH groups, and that IPA may bind either to the NH or to the C=O groups. The possibility of having heterogeneous interactions and differences in the accessibility of the binding sites is expected to be small for both the solute (acetone) and the solvent (IPA). Homogeneous monovalent H-bonding to the sorbent is assumed for both acetone and IPA. The total capacities of NH groups and C=O groups in the column are assumed to be the same. One-to-one acetone (solute)-solvent complexation (AC-I) is assumed. Even though in reality
Figure 5.2 Schematic representations of the interactions considered in (a) Cases 1 and 2, (b) Cases 3-5, (c) Cases 6 and 7, and (d) Cases 8 and 9 in Table 2; see Section 3.1. In (d) we also show the possible binding of IPA to C=O. This binding does not affect the retention factor.
there may be an IPA-IPA aggregate size distribution (see Section 5.5), a one-step aggregation process with a single aggregate size $n$ is assumed for IPA (I)-IPA (I) H-bonding interactions. These interactions are treated thermodynamically as reversible reactions. The model is schematically represented in Figure 5.2d and is described in detail below.

The acetone (solute)-sorbent (NH groups) interaction,

$$AC + AS \rightleftharpoons \overline{AC},$$

the IPA (solvent)-sorbent (NH groups) interaction,

$$I + AS \rightleftharpoons \overline{I},$$

the IPA (solvent)-sorbent (C=O groups) interaction,

$$I + AS' \rightleftharpoons \overline{I'},$$

the acetone (solute)-IPA (solvent) interaction,

$$AC + I \rightleftharpoons AC - I,$$

and the IPA-IPA (solvent-solvent) interaction, or aggregation

$$nI \rightleftharpoons I_n,$$

are described by the following equations of “reaction” equilibria:

$$K_{AC} = \frac{c_{\overline{AC}}}{c_{AC}c_{AS}}$$  \hspace{1cm} (5.1)
\[ K_I = \frac{c_I}{c_I^* c_{AS}} \] (5.2)

\[ K_I' = \frac{c_I'^*}{c_I^* c_{AS'}} \] (5.3)

\[ K_{AC-I} = \frac{c_{AC-I}}{c_{AC} c_I} \] (5.4)

\[ K_n = \frac{c_{in}}{c_{in}^*} \] (5.5)

where

\( K_{AC} \) (in M\(^{-1}\)) is the equilibrium constant for acetone-AS (NH groups) interaction,

\( K_I \) (in M\(^{-1}\)) is the equilibrium constant for IPA-AS (NH groups) interaction,

\( K_I' \) (in M\(^{-1}\)) is the equilibrium constant for IPA-AS (C=O groups) interaction,

\( K_{AC-I} \) (in M\(^{-1}\)) is the equilibrium constant for acetone-IPA interaction,

\( K_n \) (in M\(^{1-n}\)) is the equilibrium constant for IPA aggregation,

\( c_{AS} \) (in mole per L of solid volume) is the concentration of free AS NH binding sites,

\( c_{AS'} \) (in mole per L of solid volume) is the concentration of free AS C=O binding sites,

\( c_{AC} \) (in mole per L of liquid volume, or M) is the acetone concentration in the mobile phase,

\( c_{AC-I} \) (in mole per L of solid volume) is the concentration of the adsorbed acetone on AS NH binding sites,

\( c_I \) (in M) is the free IPA monomer concentration in the mobile phase,

\( c_I^* \) (in mole per L of solid volume) is the concentration of the adsorbed IPA on AS NH binding sites,
\( C_I' \) (in mole per L of solid volume) is the concentration of the adsorbed IPA on AS C=O binding sites,

\( C_{AC-I} \) (in M) is the concentration of the acetone-IPA complex,

\( C_{n} \) (in M) is the concentration of the IPA aggregates,

and \( n \) is the IPA aggregation number in solution.

It is assumed that IPA aggregates and AC-IPA complexes do not adsorb to any significant extent.

By using the principles of local equilibrium of adsorption and reaction for linear systems, we derived the retention factor for acetone as follows.

\[
 k = \frac{\text{concentration of bound AC on sorbent}}{\text{total concentration of AC in mobile phase}} = \frac{c_{AC}^{\varphi}}{c_{AC} + C_{AC-I}} \tag{5.6}
\]

where \( \varphi \) is the ratio of the solid volume to the liquid volume in the column.

Then Eqs. (5.1)-(5.6) reduce to a relationship between \( k \) and \( C_I^0 \), as described “implicitly” (meaning we cannot solve Eq. (5.8) analytically for \( C_I \) as a function of \( C_I^0 \)) by the system of the following two equations with \( k \) and \( C_I^0 \) as unknowns:

\[
 \ln k = -\ln(C_I + K_{AC-I}C_I^2) + \ln \left[ \frac{K_{AC}C_I^0}{K_I} \right] \tag{5.7}
\]

\[
 C_I^0 = C_I + C_{AC-I} + nK_mC_I^n \tag{5.8}
\]

In a continuous chromatography process, the sorbent is first pre-equilibrated with the mobile phase. After the NH and C=O binding sites of the sorbent are equilibrated with the IPA, the total IPA concentration \( C_I^0 \) in the column, is given by Eq. (5.8), which does
not include the terms $C_T$ and $C_T'$. When an acetone pulse is introduced, it changes little
the concentrations $C_T$ and $C_T'$ of the bound IPA. Moreover, since during a pulse
experiment, the acetone concentration is expected to be much smaller than the IPA
concentration, Eq. (5.8) reduces to the equation.

$$C_T^0 \approx C_T + nK_nC_I^n$$

(5.9)

If the monomer IPA concentration were very high compared to the sorbent
capacity, or at the plateau region of the Langmuir isotherm, i.e. if $C_T \approx C_{AS}^0 K_I$, then the
adsorbed IPA concentration $C_T$ would be constant and equal to the total AS capacity $C_{AS}^0$, and $C_T$ would be independent of the value of $K_I$. Then the slope $B$ from Eq. (5.7) would be independent of $K_I$. However, in the presence of IPA-IPA aggregates, the effective monomer concentration may be such that $C_T < C_{AS}^0$. Then mass balances for the NH
binding sites lead to the equation

$$C_{AS}^0 = C_{AS} + C_T + C_{AC} \approx C_{AS} + C_T$$

(5.10)

Even if IPA-AC interactions are strong, they do not affect the IPA concentration in the
pulse experiments. The bound IPA concentration $C_T$ is related to $C_I$ with the Langmuir
isotherm.

$$C_T = \frac{C_{AS}^0 K_I C_I}{1 + K_I C_I}$$

(5.11)

This isotherm is not independent of Eqs. (5.1)-(5.10) but can be derived from Eqs. (5.2)
and (5.10). The isotherm for the other sorbent sites, C=O, is not considered, because the
adsorption on C=O sites is not expected to change upon the introduction of acetone. With the use of Eq. (5.11), Eqs. (5.7) and (5.9) are rearranged in the following form.

$$\ln k = -\ln(1 + (K_{AC-I} + K_I)C_l + K_{AC-I}K_IC_l^2) + \ln[K_{AC} \cdot C_{AS}^0 \cdot \phi]$$  \hspace{1em} (5.12)

$$C_l^0 = C_l + nK_nC_l^n$$  \hspace{1em} (5.13)

Hence, the dependence of $k$ on $C_l^0$, or the function $k(C_l^0)$, is predicted to depend on the four equilibrium constants defined above, and on the parameters $n$, $\alpha$, and $C_{AS}^0$. If it is assumed that $\ln k = A - B \ln C_l^0$, then the intercept $A$, which is the last term in Eq. (5.12), is affected only by the parameters $K_{AC}$, $C_{AS}^0$, and $\alpha$. The slope $B$ depends on the parameters $K_{AC-I}$, $K_I$, $C_{AS}^0$, $n$, and $K_n$, and it is not strictly constant. At the same mobile phase and the same modifier concentration range, the slope $B$ depends only on the value of $K_{AC-I}$. As seen in Eqs. (5.12) and (5.13), the model does not change when the parameters $K_{AC-I}$ and $K_I$ are interchanged. This means that the form of $\ln k$ vs. $\ln C_l^0$ does not change when an IPA molecule binds to the sorbent, as measured by $K_I$, or to the solute, as measured by $K_{AC-I}$.

There are two limiting cases when the total IPA concentration approaches infinity or zero. Then Eqs. (5.12) and (5.13) are reduced to either Eq. (5.14) or Eq. (5.15).

$$\lim_{C_l^0 \to \infty} (\ln k) = -\frac{2}{n} \ln(C_l^0) + \ln \left[\frac{K_{AC}^2(nK_n)^2/C_{AS}^0 \cdot \phi}{K_{AC-I}K_I}\right]$$  \hspace{1em} (5.14)

$$\lim_{C_l^0 \to 0} (k) = K_{AC}C_{AS}^0 \phi \approx K_{AC}C_{AS} \phi = \frac{c_{AC\phi}}{c_{AC}}$$  \hspace{1em} (5.15)
In the first case, the slope $B$ is $2/n$. In the second case, the slope $B$ is close to zero, and the model reduces to Eq. (5.6) with $C_{AC-I} \approx 0$.

To find out more generally this dependence, one would need to have an explicit solution for $C_I(C_I^0)$. Because this is not possible, it is convenient to use a dimensionless formulation by defining a dimensionless IPA concentration with a reference concentration equal to the concentration of the binding sites per volume of solution, $C_{AS}^0 \varphi$.

\begin{equation}
C_I^{0*} = \frac{\text{total concentration of IPA}}{\text{concentration of NH binding sites}} = \frac{C_I^0}{C_{AS}^0 \varphi} \quad (5.16)
\end{equation}

\begin{equation}
C_I^* = \frac{\text{concentration of monomer IPA}}{\text{concentration of NH binding sites}} = \frac{C_I}{C_{AS}^0 \varphi} \quad (5.17)
\end{equation}

Then several dimensionless numbers are defined below as follows.

\begin{equation}
N_1 \equiv K_{AC} C_{AS}^0 \varphi \quad (5.18)
\end{equation}

$N_1$ is a measure of solute-sorbent (NH groups) interactions.

\begin{equation}
N_2 \equiv K_I C_{AS}^0 \varphi \quad (5.19)
\end{equation}

$N_2$ is a measure of solvent-sorbent (NH groups) interactions.

\begin{equation}
N_3 \equiv K_{AC-I} C_{AS}^0 \varphi \quad (5.20)
\end{equation}

$N_3$ is a measure of solute-solvent interactions.

\begin{equation}
N_4 \equiv n K_n C_{AS}^0 n^{-1} \varphi^{n-1} \quad (5.21)
\end{equation}

$N_4$ is a measure of solvent-solvent interactions.
Equations (5.7) and (5.9) reduce to the following system of dimensionless equations for \( k \) and \( C_i^0 \).

\[
\ln k = -\ln\left(1 + (N_2 + N_3)C_i^* + N_2 N_3 C_i^{*2}\right) + \ln(N_1) \quad (5.22)
\]

\[
C_i^0 = C_i^* + N_4 C_i^{*n} \quad (5.23)
\]

From these two equations, three key combinations of the above dimensionless groups affecting the slope \( B \) were identified, \( N_2 C_i^* \), \( N_3 C_i^* \), and \( N_4 C_i^{*n-1} \). The value of the group \( N_2 C_i^* \) (which is equal to \( K_i C_i^* \)) is related to the fractional coverage of the NH sites by the IPA molecules. When \( N_2 C_i^* \gg 1 \) (see Eq. (5.11) for the Langmuir isotherm), where there is strong IPA adsorption or very high IPA concentration, the NH binding sites are almost completely occupied by IPA, which is at the plateau region of the Langmuir isotherm. This is one of the major assumptions often used in other retention models which do not consider IPA aggregation. When \( N_2 C_i^* \ll 1 \), where there is weak IPA adsorption or low IPA concentration, the system falls in the linear region of the Langmuir isotherm, and the NH sites fractional coverage is low.

From Eqs. (5.4), (5.5), and (5.9), and an acetone mole balance, one can derive Langmuir-isotherm-like equations for AC-I complexation and IPA aggregation.

\[
\frac{C_{AC-I}}{C_{AC-I} + C_{AC}} = \frac{K_{AC-I} C_i}{1 + K_{AC-I} C_i} \quad (5.24)
\]

\[
\frac{n C_{in}}{C_i^*} = \frac{nK_n C_i^{n-1}}{1 + nK_n C_i^{n-1}} \quad (5.25)
\]
For acetone molecules in the mobile phase, the value of the group $N_3 C_I^* (= K_{AC-I} C_I)$ is related to the fraction of acetone molecules which are bound to IPA. When there is strong AC-IPA interaction or a very high monomer IPA concentration, $N_3 C_I^* \gg 1$, or $K_{AC-I} C_I \gg 1$, based on Equation (5.24), nearly all the acetone molecules are bound to IPA in the mobile phase. When the value of the group $N_3 C_I^*$ is much lower than one, most acetone molecules are not bound to IPA. Similarly, the value of the group $N_4 C_I^{n-1} (= nK_n C_I^{n-1})$ is related to the fraction of IPA which are in aggregate form.

Several limiting cases are summarized in Table 5.2 and Figure 5.2. In the simplest Cases 1 and 2 (Figure 5.2a), only AC-sorbent and IPA-sorbent interactions are considered. In Cases 3-5 (Figure 5.2b), AC-I complexation is included. In Cases 6 and 7 (Figure 5.2c), there is aggregation without complexation. And in Cases 8 and 9 (Figure 5.2d), which are the most realistic, all interactions are considered. If IPA also binds to the C=O groups of AS, with equilibrium constant $K_I'$, this binding does not affect $k$, because acetone does not bind with C=O.

Case 1. When there is negligible aggregation, $N_4 C_I^{n-1} \ll 1$, negligible AC-I complexation, $N_3 C_I^* \ll 1$, and full coverage of NH binding sites by IPA, $N_2 C_I^* \gg 1$, Eqs. (22) and (23) reduce to Eq. (26) below.

$$\ln k \approx -\ln(C_I^{0*}) + \ln(N_1) - \ln(N_2)$$ (5.26)

The $B$ value is 1, which is in agreement with Soczewiński’s model. In this model, the slope represents the number of IPA molecules displaced by AC molecules. For such competitive monovalent binding, the displacement number is one.
Table 5.2 Summary of Limiting Cases for the Thermodynamic Retention Model; See Section 5.2 for the Symbols Meaning and Figure 5.2 for the Schematic Representations

<table>
<thead>
<tr>
<th>Case #</th>
<th>IPA aggregation</th>
<th>AC-I complexation</th>
<th>NH sites coverage</th>
<th>$B$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group $N_4C_i^{\pi n-1}$</td>
<td>Group $N_2C_i^*$</td>
<td>Group $N_2C_i^*$</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>No</td>
<td>No</td>
<td>Full</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>No</td>
<td>No</td>
<td>Moderate</td>
<td>from 0 to 1</td>
</tr>
<tr>
<td>3</td>
<td>No</td>
<td>Strong</td>
<td>Full</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>No</td>
<td>Moderate</td>
<td>Full</td>
<td>from 1 to 2</td>
</tr>
<tr>
<td>5</td>
<td>No</td>
<td>Moderate</td>
<td>Low</td>
<td>from 0 to 1</td>
</tr>
<tr>
<td>6</td>
<td>Strong</td>
<td>No</td>
<td>Full</td>
<td>1/n</td>
</tr>
<tr>
<td>7</td>
<td>Strong</td>
<td>No</td>
<td>Moderate</td>
<td>from 0 to 1/n</td>
</tr>
<tr>
<td>8</td>
<td>Strong</td>
<td>Moderate</td>
<td>Full</td>
<td>from 1/n to 2/n</td>
</tr>
<tr>
<td>9</td>
<td>Strong</td>
<td>Moderate</td>
<td>Low</td>
<td>from 0 to 1/n</td>
</tr>
</tbody>
</table>
Case 2. When there is negligible aggregation, $N_4 C_i^n \ll 1$, negligible AC-I complexation, $N_3 C_i^* \ll 1$, and moderate coverage of NH binding sites, $N_2 C_i^* = \mathcal{O}(1)$ (neither much smaller nor much larger than 1), Eqs. (5.22) and (5.23) reduce to Eq. (5.27) below.

$$\ln k \approx -\ln \left(1 + N_2 C_i^0 \right) + \ln(N_1) \quad (5.27)$$

The $B$ value ranges from 0 to 1, depending on the value of $N_2 C_i^*$. When this group is smaller than 1, indicating less competition between IPA and AC for adsorption, the $B$ value becomes smaller than 1. When there is very low coverage of NH binding sites, $N_2 C_i^* \ll 1$, the AC molecules can hardly “feel” the competition with IPA. The slope equals to zero, or near zero.

Case 3. When there is negligible aggregation, $N_4 C_i^n \ll 1$, strong AC-I complexation, $N_3 C_i^* \gg 1$, and full coverage of NH binding sites, $N_2 C_i^* \gg 1$, Eqs. (5.22) and (5.23) reduce to Eq. (5.28) below.

$$\ln k = -2 \ln \left( C_i^0 \right) + \ln(N_1) - \ln(N_2) - \ln(N_3) \quad (5.28)$$

The $B$ value is 2. This case is in agreement with Regnier’s model. Upon the adsorption of one AC, one molecule of adsorbed IPA is displaced from the sorbent and a second molecule of IPA is displaced from the AC-I complex. Hence, the overall displacement number of IPA is 2. This is the underlying physical reason why the slope increases from 1 to 2.
Case 4. When there is negligible aggregation, $N_4 C_i^{*n-1} \ll 1$, when moderate AC-I complexation, $N_3 C_i^* = O(1)$, and full coverage of NH binding sites, $N_2 C_i^* \gg 1$, Eqs. (5.22) and (5.23) reduce to Eq. (5.29) below.

$$\ln k = -\ln \left( C_i^{0*} + N_3 C_i^{0*2} \right)^{1/2} + \ln(N_1) - \ln(N_2)$$  \hspace{1cm} (5.29)$$

The slope $B$ is between 1 and 2, depending on the value of $N_3 C_i^*$. When the AC-I complexation is strong, or $N_3 C_i^* \gg 1$, Eq. (5.29) can be reduced to Eq. (5.28). When the AC-I complexation is weak, or $N_3 C_i^* \ll 1$, Eq. (5.29) can be reduced to Eq. (5.26). This case falls between Cases 1 and 3.

Case 5. When there is negligible aggregation, $N_4 C_i^{*n-1} \ll 1$, moderate AC-I complexation, $N_3 C_i^* = O(1)$, and low coverage of NH binding sites, $N_2 C_i^* \ll 1$, Eqs. (5.22) and (5.23) reduce to Eq. (5.30) below.

$$\ln k \cong -\ln \left(1 + N_3 C_i^{0*} \right) + \ln(N_1)$$  \hspace{1cm} (5.30)$$

The slope ranges from 0 to 1, depending on the value of $N_3 C_i^{0*}$. When the AC-I complexation is strong, or $N_3 C_i^* \gg 1$, the slope $B$ will be 1. Such a condition is different from Case 1 because the IPA molecule is displaced from AC-I complex.

Case 6. When there is strong aggregation, $N_4 C_i^{*n-1} \gg 1$, negligible AC-I complexation, $N_3 C_i^* \ll 1$, and full coverage of NH binding sites, $N_2 C_i^* \gg 1$, Eqs. (5.22) and (5.23) reduce to Eq. (5.31) below.

$$\ln k \cong -\frac{1}{n} \ln(C_i^{0*}) + \ln(N_1) - \ln(N_2) + \frac{1}{n} \ln(N_4)$$  \hspace{1cm} (5.31)$$
The $B$ value is $1/n$, because the effective IPA monomer concentration, $C_i^*$, is reduced to $C_i^{0+1/n}$

Case 7. When there is strong aggregation, $N_4C_i^{*\infty} \gg 1$, negligible AC-I complexation, $N_3C_i^* \ll 1$, and moderate coverage of NH binding sites, $N_2C_i^* = O(1)$, Eqs. (5.22) and (5.23) reduce to Eq. (5.32) below.

$$\ln k \approx -\ln \left(1 + N_2 \left(\frac{C_i^{0+1/n}}{N_4}\right) + \ln(N_1) \right) \quad (5.32)$$

The slope is between 0 and $1/n$, depending on the value of $N_2C_i^*$.

Case 8. When there is strong aggregation, $N_4C_i^{*\infty} \gg 1$, when there is moderate AC-I complexation, $N_3C_i^* = O(1)$, and when is full coverage of NH binding sites, $N_2C_i^* \gg 1$, Eqs. (5.22) and (5.23) reduce to Eq. (5.33) below.

$$\ln k = -\ln \left(\frac{C_i^{0+1/n}}{N_4}\right) + N_3 \left(\frac{C_i^{0+1/n}}{N_4}\right)^2 + \ln(N_1) - \ln(N_2) \quad (5.33)$$

The slope $B$ is between $1/n$ and $2/n$, depending on the value of $N_3C_i^*$. When there is strong AC-I complexation, or $N_3C_i^* \gg 1$, $B=2/n$. When $N_3C_i^* \ll 1$, $B=1/n$.

Case 9. When there is strong aggregation, $N_4C_i^{*\infty} \gg 1$, moderate AC-I complexation, $N_3C_i^* = O(1)$, and low coverage of NH binding sites, $N_2C_i^* \ll 1$, Eqs. (5.20) and (5.21) reduce to Eq. (5.34) below.

$$\ln k = -\ln \left(1 + N_3 \left(\frac{C_i^{0+1/n}}{N_4}\right)^{1/n} \right) + \ln(N_1) \quad (5.34)$$
Then the slope $B$ is between 0 and $1/n$, depending on the value of $N_3 C_i^*$. When there is strong AC-I complexation, or $N_3 C_i^* \gg 1$, the $B=1/n$. When $N_3 C_i^* \ll 1$, $B \approx 0$.

Hence, only when there is low or moderate coverage of NH binding sites (corresponding to the condition $C_i < C_{A5}^0$) or when there is IPA aggregation can the slope $B$ be smaller than 1. These two conditions are related. When there is no aggregation ($C_i^* = C_i^{0*}$), monomer IPA concentrations is generally high, and then $N_2 C_i^{0*} \gg 1$. The aggregation evidently reduces the monomer concentration $C_i^*$ of the solvent (IPA) and its thermodynamic activity, which controls the interactions with the sorbent and the solute.

The contributions of the groups $N_2 C_i^*$, $N_3 C_i^*$, and $N_4 C_i^{n-1}$ to $B$ are $B_{\text{adsorp.}}$, $B_{\text{complex.}}$, and $B_{\text{aggreg.}}$, respectively. The first one ranges from 0 to 1, the second one ranges from 0 to 1, and the third one ranges from 1 to $1/n$. The overall value of $B$ can vary from 0 to 2 and can be written as

$$B = B_{\text{aggreg.}}(B_{\text{adsorp.}} + B_{\text{complex.}})$$

(5.35)

In Section 5.4, the detailed application of these models to the HPLC data and the possible limiting cases for the data will be discussed.

5.3 **HPLC Results: Effects of the Solvent Composition on the Retention Factors**

The retention factors of the five achiral molecules, increase with decreasing molar concentration of IPA from 1.3 M to 0 M (or volume fraction $\phi = 0.10$ to 0.01), as generally expected (see Table 5.1 and Figure 5.3). The slopes $B$ of the curves $\ln k$ vs. $\ln C_i^0$ are smaller than one, ranging from 0.25 for HA to 0.45 for CH. As the solvent
Figure 5.3 Plots of relationships of ln k vs. ln $C_0^0$ at 25 °C for five achiral solutes; k is the retention factor and $C_0^0$ is the molarity of IPA in n-hexane. Δ, acetone; ◊, cyclo hexanone; ▽, benzaldehyde; □, phenylacetaldehyde; ○, hydrocinnamaldehyde. Inset: Data for acetone at higher IPA concentrations, from 2.6 to 6.5 M. In this range, the limiting slope is 0.65, or close to $2/n$, for n=3; see text.
becomes less polar, or as \( C_l^0 \) decreases, the solute C=O groups are expected to interact more with those of the polar sorbent and less with those of the polar solvent, thus providing less competition from the OH groups of IPA. For pure n-hexane as the mobile phase, the solutes bind much more strongly with AS when there is no competition from IPA. The straight line fit of the \( \ln k \) vs. \( \ln C_l^0 \) plot is good, although the slope \( B \) seems to increase slightly at higher IPA concentrations. Indeed, for very high IPA concentrations, from 2.6 to 6.5 M (see inset in Figure 5.3), the slope becomes 0.65; see Section 5.4 for a more detailed interpretation. \( B \)-values lower than 1.0 cannot be explained solely on the basis of solute-sorbent, solvent (IPA)-sorbent, and solute-solvent interactions, as inferred from the thermodynamic models (in Section 5.2), and as supported further by the IR results (Section 5.5) and the VERSE simulations (Section 5.6).

5.4 Estimation of the Parameters of the Thermodynamics Retention Model from the HPLC Data

Considering the nine limiting cases in Section 5.2, we infer that only Cases 8 and 9 for which \( N_4 C_l^{n-1} = O(1) \) or \( \gg 1 \) may apply to the data of the five achiral molecules considered here. If one applies the same models to all five molecules, one has to use the same values of \( n \), \( N_2 \), and \( N_4 \), but different values of the dimensionless numbers \( N_3 \) (different solute-solvent interaction, \( K_{AC-I} \)) and \( N_1 \) (different solute-sorbtent interaction, \( K_{AC} \)). If there is significant IPA-IPA aggregation with aggregate size \( n \), and there is no significant AC-I complexation, then \( B \) ranges from 0 to \( 1/n \); see Case 7 in Table 5.2. If there is significant aggregation with size \( n \), and if there is AC-I complexation, then \( B \) may
increase from the values of Case 7, ranging from 0 to 1/n (as in Case 9), or from 1/n to 2/n (as in Case 8).

The H-bond between the solute-C=O and IPA-OH is expected to be the dominant interaction between the solute and IPA. The $B$-values may also correlate with the strength of the H-bond, which may be linked to the charge of the oxygen atom O in the C=O groups of the solutes. DFT calculations support this conjecture. The predicted oxygen charges of the five achiral solutes are the following: -0.242 for HA, -0.245 for PA, -0.268 for BA, -0.273 for AC, and -0.294 for CH. The values of these charges seem to correlate with the increasing $B$-values.

To further test the validity of the retention model, Eqs. (5.12) and (5.13) were used to numerically fit the HPLC acetone data (from 0.13 to 1.3 M). The value of $\alpha=0.44$ was used (Xie et al. 2003). From Eq. (5.15) and the data from Table 5.1, at 0% IPA concentration, $k$ is equal to $N_1 = K_{AC}C_{AS}^0\varphi = 8.3$. The remaining four unknown parameters are $K_n$, $K_I$, $K_{AC-I}$, and $n$. The parameters are determined in two cases. In the first case, $n$ is an adjustable parameter. In the second and case, $n$ is fixed to be 3 and the data are fitted for the three remaining parameters. The best fit values are shown in Table 5.3 and Figure 5.4. The estimated parameters may not be quite accurate, because of the assumptions and data used. The resulted $B$ and $A$ values of these three fits are in fairly good agreement with the HPLC data. The values $K_{AC}$ and $K_{AC-I}$ are of the same order of magnitude, and are consistent with reported values when such H-bonds form (Vedernikova, Gafurov, and Ataev 2011; Abraham et al. 1987). The resulted values of the dimensionless groups, $N_2C_i^*=O(1)$, $N_3C_i^*=O(1)$ and $N_4C_i^{n-1}>>1$, indicate that there
Table 5.3 Parameters and Dimensionless Numbers Used in the Retention Model for the Acetone Solute; See Section 5.2

<table>
<thead>
<tr>
<th></th>
<th>Case 1</th>
<th>Case 2</th>
</tr>
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<tbody>
<tr>
<td>$n$</td>
<td>3.2 (best fit)</td>
<td>3 (fixed)</td>
</tr>
<tr>
<td>$K_I$ (M$^{-1}$)</td>
<td>258</td>
<td>277</td>
</tr>
<tr>
<td>$K_{AC-I}$ (M$^{-1}$)</td>
<td>258</td>
<td>277</td>
</tr>
<tr>
<td>$K_a$ (M$^{1-n}$)</td>
<td>1.1925 E+6</td>
<td>6.9754 E+5</td>
</tr>
<tr>
<td>$B$</td>
<td>0.39</td>
<td>0.41</td>
</tr>
<tr>
<td>$A$</td>
<td>-0.18</td>
<td>-0.18</td>
</tr>
<tr>
<td>$R^2$</td>
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<td>0.998</td>
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</tbody>
</table>
Figure 5.4 Fit of the thermodynamics retention model to the acetone $k (C_i^0)$ data, Cases 2 and 3 in Table 5.3. See also Table 5.4 for the values of other key parameters.
is moderate IPA adsorption, moderate AC-I complexation, and strong solvent (IPA) aggregation, as Cases 8 and 9 in Table 5.2. At the highest concentrations tested, 2.6 to 6.5 M (see inset of Figure 5.3), the observed slope increases and approaches 0.65, which is very close to the value of 2/3=0.67 predicted in Eq. (5.14) for the limiting case. The average aggregation number is inferred to be 3.

By applying these fitted parameters to the retention model, the mole fractions of the aggregated IPA molecules, AC-I complex, and the percent capacity coverage can be calculated (see Table 5.4). For the total IPA concentration of 0.13 to 1.3 M, the mole fractions of the aggregated IPA molecules range from 0.96 to 0.99 for \( n=3.2 \), and from 0.97 to 0.993 for \( n=3 \). These results indicate that most of the IPA molecules are in the aggregate form. If there were no aggregation, at highest concentration used, \( C_I^0 = 1.3 \) M, the IPA adsorption would be quite strong (\( N_2 C_I^* = 335 \gg 1 \) for \( n=3.2 \)), and \( C_I \) would be expected to be at the plateau region of the Langmuir isotherm and equal to \( C_{AS}^0 \). In the presence of aggregation, the IPA molecules occupied only 50%-70% of the sorbent NH binding sites. The low coverage of the NH sites is clearly due to the low concentrations of the IPA monomers. Most of the IPA molecules form aggregates instead of binding with the sorbent.

The inferences from the detailed study of acetone apply also to the other four achiral molecules. If one has 5 or more data points, one can use similar models for the other achiral molecules. For brevity, this is not done in this study.

If one applied the above model equations for the monovalent chiral enantiomers with the same assumptions, then one would have to assume that the \( R \) and \( S \) enantiomers
Table 5.4 Mole Fractions of Aggregated IPA Concentration, Acetone-IPA Complex Concentration and Occupied Binding Sites and the Dimensionless Groups by Using the Fitted Parameters from Table 5.3 for the Retention Model; See Section 3.1 for the Symbols Definitions

<table>
<thead>
<tr>
<th>$C_i^0$ (M)</th>
<th>1.30</th>
<th>0.78</th>
<th>0.65</th>
<th>0.52</th>
<th>0.26</th>
<th>0.13</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_T / C_i^0$</td>
<td>0.70</td>
<td>0.67</td>
<td>0.65</td>
<td>0.64</td>
<td>0.58</td>
<td>0.53</td>
</tr>
<tr>
<td>$C_{AC-I} / (C_{AC-I} + C_{AC})$</td>
<td>0.70</td>
<td>0.67</td>
<td>0.65</td>
<td>0.64</td>
<td>0.58</td>
<td>0.53</td>
</tr>
<tr>
<td>$nC_{ni} / C_i^0$</td>
<td>0.992</td>
<td>0.989</td>
<td>0.987</td>
<td>0.985</td>
<td>0.975</td>
<td>0.961</td>
</tr>
<tr>
<td>$N_2 C_i^*$</td>
<td>2.34</td>
<td>1.99</td>
<td>1.88</td>
<td>1.75</td>
<td>1.40</td>
<td>1.12</td>
</tr>
<tr>
<td>$N_3 C_i^*$</td>
<td>2.34</td>
<td>1.99</td>
<td>1.88</td>
<td>1.75</td>
<td>1.40</td>
<td>1.12</td>
</tr>
<tr>
<td>$N_4 C_i^{*n-1}$</td>
<td>123</td>
<td>86</td>
<td>76</td>
<td>65</td>
<td>40</td>
<td>24</td>
</tr>
</tbody>
</table>

$n=3.2$

<table>
<thead>
<tr>
<th>$C_T / C_i^0$</th>
<th>0.70</th>
<th>0.67</th>
<th>0.65</th>
<th>0.64</th>
<th>0.58</th>
<th>0.52</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{AC-I} / (C_{AC-I} + C_{AC})$</td>
<td>0.70</td>
<td>0.67</td>
<td>0.65</td>
<td>0.64</td>
<td>0.58</td>
<td>0.52</td>
</tr>
<tr>
<td>$nC_{ni} / C_i^0$</td>
<td>0.993</td>
<td>0.991</td>
<td>0.990</td>
<td>0.988</td>
<td>0.981</td>
<td>0.970</td>
</tr>
<tr>
<td>$N_2 C_i^*$</td>
<td>2.37</td>
<td>1.99</td>
<td>1.88</td>
<td>1.74</td>
<td>1.38</td>
<td>1.09</td>
</tr>
<tr>
<td>$N_3 C_i^*$</td>
<td>2.37</td>
<td>1.99</td>
<td>1.88</td>
<td>1.74</td>
<td>1.38</td>
<td>1.09</td>
</tr>
<tr>
<td>$N_4 C_i^{*n-1}$</td>
<td>152</td>
<td>108</td>
<td>95</td>
<td>82</td>
<td>51</td>
<td>32</td>
</tr>
</tbody>
</table>
have different equilibrium constants, $K_R$ and $K_S$ with AS. Then the enantioselectivity would be predicted to be equal to the ratio of these equilibrium constants,

$$ S \equiv \frac{k_R}{k_S} = \frac{K_R}{K_S}, \quad (5.36) $$

and independent of the IPA concentration.

The above model is based on the assumptions that there are monovalent interactions of solute-solvent, solute-sorbent, and solvent-sorbent, and that the binding sites are homogeneous for the adsorbates and for IPA. Most chiral solutes are expected to have non-monovalent and other more complex interactions with the sorbent, and possibly with IPA as well, by contrast to the five achiral molecules used here. Hence, the model and assumptions may not be valid for most chiral solutes. A more elaborate model is needed to realistically describe the behavior of chiral solutes.

5.5 Transmission IR and Density Functional Theory Results for IPA-IPA and Acetone-IPA Interactions

5.5.1 Probing of IPA-IPA Interactions

IR spectra of the IPA OH stretching band, with different concentrations of IPA in $n$-hexane, and pure IPA, are shown in Figure 5.5 (top). For all concentrations a peak ("Peak 1") centered around 3633 cm$^{-1}$ was observed. For pure IPA this peak was not fully resolved because the second peak was quite broad. A second broad OH-peak ("Peak 2") was observed, centered around 3342 to 3365 cm$^{-1}$. The peak around 3180 cm$^{-1}$ is mainly due to hexane.
The OH stretching band of isopropanol in the gas phase, where it should be non-H-bonded, was reported to be at 3648 or 3614 cm\(^{-1}\) [56]. The first peak is therefore assigned mainly to non-H-bonded OH stretch band. DFT calculations were done to test this inference. These calculations first revealed that there can be at least three major non-H-bonded IPA conformations, IPA1, IPA2, and IPA3, which have different torsion angles and energies (see Table 5.5). The first one was ignored in subsequent calculations, because it has the highest energy. For conformations IPA2 and IPA3, DFT predicts OH stretching wavenumbers of 3659 and 3678 cm\(^{-1}\). Hence, DFT supports the above OH assignment.

The second peak is quite broad and indicates a shift of ca. -50 to -500 cm\(^{-1}\) from the first peak. This shift is plainly due to IPA-IPA hydrogen bonding in IPA aggregates. The large shift range indicates a large distribution of H-bonding strengths, which may be linked to an aggregation size distribution. Thus, the IR data indicate that there are significant IPA-IPA H-bonding interactions in hexane. The question is what size or type of IPA aggregates can lead to such IR shifts.

To answer this question quantitatively, DFT simulations were done for different sizes and configurations of IPA aggregates, to help determine the likely types of aggregates in the mobile phase. The DFT simulations results for dimers, trimers, and tetramers are summarized in Table 5.6 and Figures 5.5 and 5.6. For dimers, four types of simulations were done, for pairs IPA2-IPA2, IPA2-IPA3, IPA3-IPA2, and IPA3-IPA3.
Table 5.5 DFT Predictions of Conformations, Relative Energies, and Wavenumbers, or Frequencies (ν), and Intensities (I) of the OH IR Stretching Band of Isopropanol

<table>
<thead>
<tr>
<th>IPA conformation</th>
<th>Torsion angle, (°) (H-O-C-H)</th>
<th>Relative stability, ΔE (kcal/mol)</th>
<th>ν, (cm⁻¹)</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPA1</td>
<td>0</td>
<td>reference</td>
<td>3724</td>
<td>44</td>
</tr>
<tr>
<td>IPA2</td>
<td>180</td>
<td>-0.998</td>
<td>3659</td>
<td>16</td>
</tr>
<tr>
<td>IPA3</td>
<td>64</td>
<td>-1.22</td>
<td>3678</td>
<td>19</td>
</tr>
</tbody>
</table>
Table 5.6 DFT Predictions of Wavenumbers of OH Stretching Frequencies ($\nu$) of IPA, IR Intensities Ratios ($R$), and Binding Energies $\Delta E$ for Different Configurations of Isopropanol Aggregates

<table>
<thead>
<tr>
<th>Aggregation type</th>
<th>IPAs configuration</th>
<th>Binding type</th>
<th>$\nu$, (cm$^{-1}$)</th>
<th>$R$</th>
<th>$\Delta E$, (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>dimer</td>
<td>(IPA2) OH$\leftrightarrow$OH (IPA2)</td>
<td>H$\leftrightarrow$O-H$^a$</td>
<td>3656</td>
<td>1.50</td>
<td>-5.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O-H$\leftrightarrow$O$^b$</td>
<td>3507</td>
<td>28.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(IPA2) OH$\leftrightarrow$OH (IPA3)</td>
<td>H$\leftrightarrow$O-H</td>
<td>3673</td>
<td>1.37</td>
<td>-5.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O-H$\leftrightarrow$O</td>
<td>3516</td>
<td>25.38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(IPA3) OH$\leftrightarrow$OH (IPA2)</td>
<td>H$\leftrightarrow$O-H</td>
<td>3656</td>
<td>1.50</td>
<td>-5.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O-H$\leftrightarrow$O</td>
<td>3519</td>
<td>28.53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(IPA3) OH$\leftrightarrow$OH (IPA3)</td>
<td>H$\leftrightarrow$O-H</td>
<td>3673</td>
<td>1.47</td>
<td>-5.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O-H$\leftrightarrow$O</td>
<td>3525</td>
<td>28.47</td>
<td></td>
</tr>
<tr>
<td>open chain trimer</td>
<td>three IPA2’s</td>
<td>H$\leftrightarrow$O-H</td>
<td>3652</td>
<td>1.63</td>
<td>-12.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O-H$\leftrightarrow$O</td>
<td>3468</td>
<td>32.19</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>O-H$\leftrightarrow$O</td>
<td>3432</td>
<td>42.25</td>
<td></td>
</tr>
<tr>
<td>cyclic trimer</td>
<td>three IPA2’s</td>
<td>O-H$\leftrightarrow$O</td>
<td>3484</td>
<td>35.06</td>
<td>-15.1</td>
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<tr>
<td></td>
<td></td>
<td>O-H$\leftrightarrow$O</td>
<td>3460</td>
<td>35.81</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>O-H$\leftrightarrow$O</td>
<td>3411</td>
<td>14.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>three IPA3’s</td>
<td>O-H$\leftrightarrow$O</td>
<td>3486</td>
<td>35.37</td>
<td>-15.4</td>
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<tr>
<td></td>
<td></td>
<td>O-H$\leftrightarrow$O</td>
<td>3466</td>
<td>40.89</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>O-H$\leftrightarrow$O</td>
<td>3420</td>
<td>7.58</td>
<td></td>
</tr>
<tr>
<td>open chain tetramer</td>
<td>four IPA3’s</td>
<td>H$\leftrightarrow$O-H</td>
<td>3673</td>
<td>1.42</td>
<td>-19.98</td>
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<tr>
<td></td>
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<td>O-H$\leftrightarrow$O</td>
<td>3445</td>
<td>20.89</td>
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<td>O-H$\leftrightarrow$O</td>
<td>3443</td>
<td>50.95</td>
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<tr>
<td></td>
<td></td>
<td>O-H$\leftrightarrow$O</td>
<td>3352</td>
<td>48.68</td>
<td></td>
</tr>
<tr>
<td>cyclic tetramer</td>
<td>four IPA3’s</td>
<td>O-H$\leftrightarrow$O</td>
<td>3365</td>
<td>16.95</td>
<td>-27.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O-H$\leftrightarrow$O</td>
<td>3344</td>
<td>80.32</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>O-H$\leftrightarrow$O</td>
<td>3323</td>
<td>90.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>O-H$\leftrightarrow$O</td>
<td>3265</td>
<td>0.26</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Refers to one H atom connected to only one O atom; similar to OH monomer.

$^b$Refers to one H atom connected to two O atoms.
Figure 5.5 Top: Transmission-IR spectra of IPA OH stretching bands for various molar IPA concentrations in n-hexane, 0.13 M, 0.65 M, and 1.3 M ($\phi$=0.01, 0.05, and 0.1). The cross-hatched regions indicate DFT-predicted wavenumbers of OH-stretch bands of IPA dimers (2), open chain trimers (3), cyclic trimers (3c), open chain tetramers (4), and cyclic tetramers (4c); see text and Table 5.6. Bottom: Effect of IPA on the C=O stretching band of acetone: (thin line) acetone alone in n-hexane, (thick line) acetone with 1.3 M IPA in n-hexane.
Figure 5.6 DFT predicted-molecular structures of H-bonded IPA aggregated: (A) dimer of IPA2 and IPA3; (B) open chain trimer of three IPA2 molecules; (C) cyclic trimer of three IPA2 molecules; (D) open chain tetramer of four IPA3 molecules; (E) cyclic tetramer of four IPA3 molecules; see Table 5.6.
Formation of trimers and tetramers with IPA2 or IPA3 conformers is considered, to reduce the number of the possible simulations. It is expected that this choice may not impact significantly the inferences, since IPA2 and IPA3 conformations have quite similar energies and IR wavenumbers.

Unlike the dimer and open chain trimers and tetramers (see molecular structures in Figure 5.6), the cyclic trimers and tetramers have every H-atom connected to two O-atoms. For this reason the cyclic aggregates do not have an OH-stretch band in the 3650 cm\(^{-1}\) range, i.e. they do not have an H atom bonded only to one O atom. For example, there is a 3652 cm\(^{-1}\) band for an open chain trimer but not for the cyclic trimer. Therefore, there must be some open chain aggregates, in addition to monomers at all IPA concentrations tested, and even in pure IPA.

The OH band shifts to lower values with each H-bond. For dimers the H-bond produces a shift of 149, 140, 159, and 153 cm\(^{-1}\), for the four conformations, or on average of ca. 150±10 cm\(^{-1}\). The predicted energies are about -5.1 to -5.6 kcal/mol, which are typical for such H-bonds. As the OH becomes H-bonded, the predicted intensities increase by about 20-fold. That is why the second observed peak intensities and areas are much larger than the first peak. If the second observed peak were narrow and were centered at about 3500 cm\(^{-1}\), one would infer that there were only monomers and dimers present. Since the second peak is centered at ca. 3350 cm\(^{-1}\), and since it is quite broad, one infers that there must be a wide distribution of aggregates with \(n\geq2\).

To probe this inference further, we did DFT simulations for trimers. For open chain trimers, the predicted \(\nu\)'s are 3468 or 3432 cm\(^{-1}\). For cyclic trimers, \(\nu\) can be as
small as 3411 cm$^{-1}$. Since the peak is centered around 3350 cm$^{-1}$, there must be aggregates with $n>3$. For open chain tetramers, $\nu$ is as small as 3352 cm$^{-1}$. And, for cyclic tetramers, $\nu$ ranges from 3365 to 3265 cm$^{-1}$. The $\nu$-ranges for the $n=2$, 3, and 4 are shown schematically in Figure 5.5. Thus, the second peak center wavenumber can be predicted by DFT if one assumes the presence of open chain or cyclic tetramers. Still lower values of $\nu$, as low as 3200 cm$^{-1}$ are observed, and they may be due to $n=5$ or higher. Hence, the data and DFT simulations clearly imply the presence of dimers, trimers, tetramers, and even some proportion of larger aggregates. If one uses a single size $\bar{n}$ to represent the average aggregate size, then $\bar{n}$ would be around 3, with both open-chain and cyclic trimers.

5.5.2 Probing of Acetone-IPA interactions

The IR spectra of the acetone-IPA interactions are shown in Figure 5 (bottom). The peak at 1722 cm$^{-1}$ is due to C=O of acetone in hexane, in which acetone is presumed to be non-aggregated or non-H-bonded. When acetone is in solution with IPA, the C=O peak shows a shift of ca. -5 cm$^{-1}$, which indicates that the C=O groups of acetone form H-bonds with the OH groups of IPA. DFT predictions support this inference. DFT predicts a value of 1706 cm$^{-1}$ for C=O. Upon the formation of an H-bond between an acetone molecule and an IPA molecule, DFT predicts a wavenumber shift of -11 cm$^{-1}$, and an intensity enhancement is 1.3 times. The energy of this H-bond is -7.45 kcal/mol, which implies that its formation is quite likely. Thus, IR spectra and DFT predictions indicate that the acetone forms an H-bond with IPA. It is, therefore, plausible that the acetone
retention factors are substantial, and could support the limiting Cases 8 and 9 of Section 5.3.

5.6 Chromatography Simulation Results

To further test the proposed hypotheses on the interaction mechanisms involving solvent-solvent and solute-solvent interactions, and understand better the chromatographic model basis for the observed HPLC data, we used dynamic simulations for certain retention behavior predictions. The simulation parameters and results for acetone as a solute are summarized in Table 5.7 and Figure 5.7. The column parameters, particle diameter, inter-particle voidage, and intra-particle voidage, were obtained from the parameters reported by Lee et al. [57] and Xie et al. [38] for Chiralpak AD. The AS parameters are assumed to be the same as those for AD. The mass transfer and dispersion parameters from Refs [46-49] were used. The numerical parameters were chosen for optimal accuracy. The parameters $K_I$, $K_{AC-I}$, $K_n$, and $C_{AS}^0 K_{AC}$ were from the fit parameters of the model (see Table 5.3). The aggregation number of the IPA molecules was fixed (not fitted) at $n=3$, based on guidance from the HPLC data, the thermodynamic models, and the IR results. The simulation calculations were done with dimensional numbers, and predictions were made of $k$ vs. $C_I^0$. The predictions fit well the data for $n=3$. The parameter values used represent well the dynamics of the HPLC experiments and predict $B$-values well below 1. Simulations without using aggregation effects (not shown here) predicted $B$-values of 1 or higher. Thus, the simulations provide firm support of the IPA aggregation hypothesis, and its effect on the values of the slopes $B<1.0$. 
Table 5.7 Parameters Used in the VERSE Simulations ($n=3$)

<table>
<thead>
<tr>
<th>Column Parameters</th>
<th>Value</th>
</tr>
</thead>
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<tr>
<td>Column length (cm)</td>
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<td>Internal diameter of column (cm)</td>
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</tr>
<tr>
<td>Particle diameter (μm)</td>
<td>20</td>
</tr>
<tr>
<td>Inter-particle voidage</td>
<td>0.32 (Xie et al. 2003)</td>
</tr>
<tr>
<td>Intra-particle voidage</td>
<td>0.55 (Xie et al. 2003)</td>
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<table>
<thead>
<tr>
<th>Mass-Transfer Parameters</th>
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<tbody>
<tr>
<td>Size exclusion factor</td>
<td>1 for all</td>
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<tr>
<td>Brownian diffusivity (cm$^2$ min$^{-1}$)</td>
<td>0.00186 for all molecules</td>
</tr>
<tr>
<td></td>
<td>(Castillo et al. 1994)</td>
</tr>
<tr>
<td>Intra-particle pore diffusivity (cm$^2$ min$^{-1}$)</td>
<td>0.0001743 for all molecules</td>
</tr>
<tr>
<td></td>
<td>(Lee et al. 2008)</td>
</tr>
<tr>
<td>Film mass transfer coefficient</td>
<td>Wilson and Geankoplis</td>
</tr>
<tr>
<td></td>
<td>correlation</td>
</tr>
<tr>
<td></td>
<td>(Wilson and Geankoplis 1966)</td>
</tr>
<tr>
<td>Axial dispersion coefficient</td>
<td>Gunn correlation</td>
</tr>
<tr>
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<td>(Gunn 1987)</td>
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<table>
<thead>
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<th>Forward and Reverse Reaction Rate Constants</th>
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<tr>
<td>$k_+$ for AC-I complexation (M$^{-1}$ min$^{-1}$)</td>
<td>1385</td>
</tr>
<tr>
<td>$k_-$ for AC-I complexation (min$^{-1}$)</td>
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</tr>
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<td>$k_+$ for IPA aggregation (M$^2$ min$^{-1}$)</td>
<td>3487700</td>
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<td>$k_-$ for IPA aggregation (min$^{-1}$)</td>
<td>5</td>
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<table>
<thead>
<tr>
<th>Langmuir Isotherm</th>
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</tr>
</thead>
<tbody>
<tr>
<td>$a=0.306, c_{AS}^0 K_{AC}$ (packing volume unit)</td>
<td>5.76</td>
</tr>
<tr>
<td>$b=K_{AC}$ (M$^{-1}$)</td>
<td>277$^a$</td>
</tr>
<tr>
<td>$a=0.306, c_{AS}^0 K_{I}$ (packing volume unit)</td>
<td>5.76$^a$</td>
</tr>
<tr>
<td>$b=K_{I}$ (M$^{-1}$)</td>
<td>277$^a$</td>
</tr>
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<table>
<thead>
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<th>Numerical Parameters</th>
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<tr>
<td>Axial elements per column</td>
<td>50</td>
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<tr>
<td>Collocation number for each element</td>
<td>4</td>
</tr>
<tr>
<td>Collocation number for the particle phase</td>
<td>2</td>
</tr>
<tr>
<td>Absolute tolerance</td>
<td>0.00001</td>
</tr>
<tr>
<td>Relative tolerance</td>
<td>0.0001</td>
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</tbody>
</table>

$^a$Independent parameters.
Figure 5.7 Fits of dynamic chromatographic VERSE simulations to the acetone $k (C_i^0)$ data for aggregation number of $n=3$ (top) and $n=2$ (bottom), slopes $B$ are 0.39 and 0.37, respectively, and comparison to the thermodynamic models. See also Tables 5.4 and 5.7.
5.7 Conclusions

The stoichiometric displacement models developed by Snyder (1974), Soczewiński (1977), and Regnier (1984) have been widely used for understanding the adsorption mechanisms of solutes in various chromatography systems. The models were used to explain the linear plots of the logarithms of solute retention factor versus the molar concentration of a competitive modifier in an inert solvent. The slope of the linear plot was inferred to be the total number of modifier molecules displaced from the sorbent and from the solute-modifier complex upon adsorption of a solute molecule. The slopes reported in the literature were generally greater than 1.

The retention factors of five simple achiral solutes, acetone (AC), cyclo hexanone (CH), benzaldehyde (BA), phenylacetaldehyde (PA), and hydrocinnamaldehyde (HA), in hexane-isopropanol mobile phase at 25°C for IPA concentration from 0.13 to 1.3 M were studied. These solutes have one C=O group, and are presumed to have a mono-valent H-bonding interaction with the NH groups of AS or the OH groups of IPA. The data fit well the equation \( \ln(k) = A - B \ln C_0 \). The slopes \( B \) range from 0.25 to 0.45, which cannot be explained by the previous literature models. According to the literature models which do not account for IPA aggregation, the slope \( B \) for a monovalent solute would be 1 or 2.

IR results, combined with DFT simulations, provide direct evidence of IPA aggregation and acetone-IPA complexation. IR data show a distribution of aggregates with \( n \)-values from 2 to about 5. A four-equilibrium-constant model for monovalent solutes, including solute-sorbent, solvent-sorbent, solute-solvent, and for the first time solvent-solvent aggregation equilibrium, was derived in dimensional and dimensionless forms. Three key dimensionless groups, which represent the fraction of sorbent binding
sites covered by IPA, the fraction of acetone molecules in complex form, and the fraction of IPA molecules in aggregate form, were found to control the value of $B$. These models suggest that only when there is significant IPA aggregation, the slope $B$ can be lower than 1 for large IPA concentrations.

For AC, the HPLC retention factor data were used to estimate an average aggregate number $n$ and the four equilibrium constants in the retention models. The best fit $n$ value was about 3, which was consistent with the IR and the DFT results. Chromatography dynamic simulations with $n=3$ and the same equilibrium constants from the retention models could fit well the acetone HPLC $k(C^0_I)$ data, and support the hypothesis of the IPA aggregation and acetone-IPA complexation in the solution. The results suggest strong IPA aggregation, significant AC-IPA complexation, about 50 to 70%, and incomplete coverage of NH sites by IPA, about 50 to 70%. The variations in the $B$-values of the five achiral solutes are probably due to different solute-IPA complexation strengths.

For a total IPA concentration $C^0_I$, IPA aggregation reduces significantly the monomer IPA concentration to $C_I$, which becomes proportional to $C^0_I^{1/n}$. At high IPA monomer concentrations, most of the acetone in the mobile phase is in complex form, most of the sorbent NH sites are bound to IPA, and most of the IPA is in aggregate form. When an acetone molecule (or a monovalent solute) is adsorbed, two IPA molecules are displaced, one from the sorbent and one from the complex. Therefore, the slope of $\ln k$ is 2 vs. $\ln C_I$ or $2/n$ vs. $\ln C^0_I$. In the absence of complexation, the slope is 1 vs. $\ln C_I$ or $1/n$ vs. $\ln C^0_I$ for a monovalent solute. By contrast, at very low IPA monomer
concentrations, most acetone molecules are not complexed and most sorbent NH binding sites are not bound by IPA. The value of $B$ is nearly zero because acetone adsorption does not displace IPA from the sorbent site or from the complex. Hence, the IPA-IPA aggregation and solute-IPA complexation in the mobile phase should be accounted for in the retention models used in interpretation of the retention factors and the adsorption mechanisms.
CHAPTER 6. EFFECT OF ALCOHOL MODIFIER ON THE RETENTION FACTORS OF CHIRAL SOLUTES WITH AS SORBENT: MODELING AND IMPLICATIONS FOR THE INTERACTION MECHANISM

6.1 Introduction

Polysaccharide (PS)-based chiral stationary phases (CSPs) have been used widely for most analytical, preparative, and production scale enantiomer chromatographic separations. For developing a successful chiral separation, it is often important to understand the interaction mechanisms of the solutes with the sorbent. Some important element in this understanding are the types and numbers of binding sites of the solute with the sorbent. Although many studies on this topic have been published (Davankov 1997; Yashima 2001; Lipkowitz 2001; Roussel et al. 2004; A. Rio et al. 2005; Piras and Roussel 2008; Rio 2009; Ma et al. 2009; Lammerhofer 2010; Kasat et al. 2008; Kasat, et al. 2010; Tsui et al. 2011; Chankvetadze 2012), more work is needed for understanding the binding involved in the chiral recognition mechanisms on these sorbents.

To study the molecular environments and the chiral recognition mechanisms of the polymers Chiralpak AD (amylose tris(3,5-dimethylphenylcarbamate), Chiralcel OD (cellulose tris(3,5-dimethylphenylcarbamate)), and Chiralpak AS (amylose tris(S)-α-methylbenzylcarbamate), or AS, Kasat et al. used cross-polarization/magic-angle spinning (CP/MAS), MAS solid-state nuclear magnetic resonance (NMR) spectroscopy, attenuated total reflection infrared spectroscopy (ATR-IR), X-ray diffraction (XRD),
density functional theory (DFT), and molecular dynamics (MD) (2006A; 2006B; 2007; 2008A; 2008B; 2010). From the results it was inferred that the sorbents contain many nanometer-sized cavities with intra-polymer hydrogen bonds. In these cavities, the configurations of the hydrogen bonding functional groups (C=O and NH) and the phenyl groups of polymer side chains are crucial for enantioselective interactions with the groups of the solutes. In some of the cavities, enantiomers either have a different number of binding sites (H-bond or π-π interactions) or different strength of the overall binding interactions, resulting in chiral recognition.

The mobile phase used for PS-based sorbents is often a solution of an alcohol in a hydrocarbon. The alcohol molecules can bind with the H-bonding functional groups of the sorbents, and possibly the solutes as well. The HPLC retention factors generally decrease with increasing concentration of the modifier in the mobile phase (Kasat et al. 2010; Tsui et al. 2011). Several studies on the effects of the alcohol on chiral separations and retention behavior have been reported (Chankvetadze 2012; Ma et al. 2009; Tsui et al. 2013A; Wang and Chen 1999; Wang et al. 2000; Wang and Wenslow 2003; Wenslow and Wang 2001; Gyimesi-Forrás et al. 2009). Several mathematical models have been used to describe the effects of the modifiers on the retention behavior (Gyimesi-Forrás et al. 2009; Tsui et al. 2013A; Lammerhofer 2010).

Snyder (1974) developed a model for the dependence of the retention factors on modifier concentrations. At low concentrations, the model is expressed in the form of

\[
\ln k = I - Z \ln [D]
\] (6.1)
where $I$ and $Z$ are constants and $[D]$ is the modifier concentration. The value of $Z$ represents the ratio of the molecular areas of the solute and the solvent. In this model one assumes that the adsorbent surface is homogeneous and that there are no significant solute-solvent interactions. These assumptions may be valid for adsorption on an amorphous sorbent with no specific adsorption sites for the solute and solvent molecules. Another model, called “stoichiometric displacement” model, which is based on the concept of discrete adsorption binding sites, was developed by Soczewiński (1977), and results in the same equation as above. The mobile phase was assumed to be a mixture of a modifier and an inert solvent. In Soczewiński’s model, the $Z$-parameter represents the average number of the modifier molecules displaced from the sorbent surface by one solute molecule. This $Z$-parameter is generally more than 1. As in Snyder’s model, the solute-solvent interactions were ignored.

For the retention behavior of proteins, Geng and Regnier (1984) developed a modified stoichiometric displacement model, which includes solute-solvent complexation interactions. Although the model again results in the same Eq. (6.1), the $Z$-parameter represents the total number of the modifier molecules displaced from the solute and the sorbent surface upon the solute adsorption. Gyimesi-Forrás et al. (2009) used Soczewiński’s model to describe the retention behavior of 10 imidazo-quinazoline-dione derivatives on a quinine carbamate-based CSP. The slope $Z$ was found to range from 1.35 to 2.92. The above retention models have been widely used for helping the understanding of the adsorption mechanisms of solutes in various chromatography systems. The slopes predicted by these models are greater than 1.
The further understand displacement of modifier molecules from the solute and the sorbent, Tsui et al. determined the retention factors $k$ of five monovalent solutes on an AS sorbent as a function of the concentration $C_1^0$ of isopropanol (IPA) in n-hexane (Tsui et al. 2013A).

$$\ln k = A - B \ln C_1^0$$

(6.2)

They used the symbol $C_1^0$ instead of $[D]$, and $A$ and $B$ instead of $I$ and $Z$, because the interpretation was different. The slopes $B$ of the log-log plots of $k$ vs. $C_1^0$ were found, however, to be smaller than 1 and to vary with concentration. Such values cannot be explained by the conventional displacement models. IR and DFT results showed clear evidence of IPA aggregation, which are not accounted in the previous models, and affect the values of $B$. For these reasons, a new thermodynamic retention model was developed, to take into account IPA aggregation, in addition to IPA-solute complexation and the competitive adsorption of solute and alcohol previously considered. They inferred that strong IPA aggregation with an average aggregation number $n=3$ (at 25 °C) can significantly reduce the IPA monomer concentration. Then, the slope of the log-log plot would approach $2/n$ at high IPA concentrations. It was concluded that the aggregation of the alcohol polar modifier in the mobile phase must be accounted for in the retention models used in the interpretation of the retention factors in terms of binding or displacement. Evidence of alcohol aggregation in nonpolar solvents has been known (Fletcher and Heller 1967; Ma et al. 2008; O’Brien et al. 1997; Fujiwara and Ikenoue 1976). Nonetheless, the impact of alcohol aggregation on the retention behavior has been covered only in Ref. (Tsui et al. 2013A).
The new retention model by Tsui et al. was developed and applied to simple achiral monovalent solutes, to help the fundamental understanding of the various phenomena for simpler molecules. Most chiral solutes are expected, however, to have multivalent and other more complicated interactions with the sorbent and the solvent. For such solutes, the interpretation of binding/complexation sites from the displacement models is more difficult than previously thought when (i) the solute may form intra-H-bonds or (ii) the polar modifier in the mobile phase, such as IPA or another alcohol, can have multivalent binding with the solutes. Hence, the model needs to be extended for describing the retention behavior of multivalent binding systems, and helping understand the mechanisms of binding of chiral solutes. This is the main objective of this study.

To obtain insights into the mechanism of chiral solutes with possible multivalent binding, we chose the same four chiral solutes for a retention behavior study with AS as those we used in a recent study of the chiral recognition mechanism (Tsui et al. 2013B): ethyl lactate (EL), methyl mandelate (MM), benzoin (B), and pantolactone (PL) (see Figure 6.1). These studies complement the previous mechanistic studies. Since these solutes have multiple functional groups, such as C=O and OH for all of them, O for EL and PL, and phenyl groups for MM and B, they can form intra H-bonds, and possibly multiple inter H-bonds with the AS sorbent and IPA. The MM and B solutes also have significant π–π interactions with AS. To understand the retention behavior of multivalent H-bonding formation with the sorbent and the solvent, a new thermodynamic retention model is presented in Section 6.2.
Figure 6.1(A) Molecular structure of the polymer repeat unit of the AS polymer, with R being the side chain. (B) Molecular structure of the acetone and cyclohexanone. (C) Molecular structures of the ethyl lactate and pantolactone.
The models include IPA aggregation and IPA-sorbent interactions, as previously (Tsui et al. 2013A), and also intramolecular H-bonding, multivalent solute-sorbent interactions, and multivalent solute-IPA interactions. The equilibrium constants and the average numbers of binding sites for complexation and adsorption of these solutes were estimated from the HPLC data by using the new model. The new models can describe the data well and provide more accurate and reliable parameters for the numbers of the complexation and the binding sites.

For EL and PL, a more detailed study of various interactions was done here for probing further the solute-IPA multivalent complexation. This study included IR, and DFT and MD simulations (Section 6.5). For testing its more general applicability, the new model was also used to describe and fit certain important literature HPLC data (Gyimesi-Forrás et al. 2009), and to estimate the number of sites for solute-IPA complexation and for solute adsorption. The results in this study may apply also to other alcohols used as polar modifiers, such as ethanol, propanol, etc., and other hydrocarbon solvents. These systems are known to also form aggregates. One would have to obtain the actual aggregation parameters for each new mobile phase as detailed in Ref. (Tsui et al. 2013A) for IPA in hexane, and apply the same methods as the ones used here.

6.2 New Multivalent Retention Models

The following model is a generalization of the model developed previously (see Chapter 5 and Tsui et al. 2013A). We consider a general solute (P), such as pantolactone, adsorbing on a sorbent (AS) from a mobile phase of an inert hydrocarbon (such as pure n-
hexane) with an IPA mobile phase modifier, called “solvent”. For modeling the retention factors, solvent-solvent (IPA-IPA) interactions, or IPA aggregation, and solute intra H-bonding interactions (Tsui et al. 2013A) are considered, in addition to multivalent solute-sorbent, monovalent solvent-sorbent, and multivalent solute-solvent interactions. Since the actual adsorption of a chiral solute onto a CSP may be heterogeneous, the measured retention factor would represent an average of various possible binding configurations. For simplicity, we assume that the NH and C=O groups of the AS polymer are equivalent sites, and bind similarly with P or IPA. It is also assumed that the sorbent capacity remains the same as the alcohol concentration increases. This assumption may not be valid when the alcohol changes the sorbent structure and its state of intra-H-bonding. There are reported cases of significant structure changes, even changes from right handed to left handed helical OD backbone from 1 to 20 vol % (Ma et al. 2009). Nonetheless, this assumption is used, to avoid unnecessary model complexity. Homogeneous monovalent H-bonding for the solvent (IPA) with the sorbent is assumed. For both the solute (P)-sorbent adsorption and the solute-solvent complexation, although different binding sites may have different equilibrium constants (see Section 6.5), one-step homogeneous multivalent H-bonding is still assumed, with x sites for the former and y sites for the latter. Even though in reality there may be an IPA aggregate size distribution, a one-step aggregation process with a single aggregate size n is assumed for IPA (I)-IPA (I) H-bonding interactions, as done previously (Tsui et al. 2013A). All interactions are treated thermodynamically as reversible reactions. The model is schematically represented in Figure 2 and is described in detail below.
Figure 6.2 Schematic representations of the interactions between the sorbent, solute, and IPA molecules.
The P (solute)-sorbent interaction,

\[ P + AS \rightleftharpoons P, \]

called the IPA (solvent)-sorbent interaction,

\[ I + AS \rightleftharpoons I, \]

called the P (solute)-IPA (solvent) interaction,

\[ P + yI \rightleftharpoons P - I_y, \]

called the P intra H-bonding interaction (established in Ref. [Tsui et al. 2013A]),

\[ P \rightleftharpoons P_{intra} \]

and the IPA-IPA (solvent-solvent) interaction, or aggregation

\[ nI \rightleftharpoons I_n, \]

are described by the following equilibrium equations:

\[ K_p = \frac{c_P}{c_p c_{AS}^x} \tag{6.3} \]

\[ K_I = \frac{c_I}{c_I c_{AS}} \tag{6.4} \]

\[ K_{PI} = \frac{c_{PI}}{c_p c_I y} \tag{6.5} \]

\[ K_{intra} = \frac{c_{intra}}{c_p} \tag{6.6} \]

\[ K_n = \frac{c_{in} c_I^n}{c_I} \tag{6.7} \]
where

\( C_{AS} \) (in mole per L of solid volume, or M') is the concentration of the free AS binding sites,

\( C_P \) (in mole per L of liquid volume, or M) is the P concentration in the mobile phase,

\( C_{\overline{F}} \) (in M') is the concentration of the adsorbed P on AS binding sites,

\( C_I \) (in M) is the free IPA monomer concentration in the mobile phase,

\( C_{\overline{T}} \) (in M') is the concentration of the adsorbed IPA on AS binding sites,

\( C_{\text{P}I} \) (in M) is the concentration of the P-IPA complex,

\( C_{\text{intra}} \) (in M) is the concentration of the intra H-bonded P,

\( C_{\text{ag}} \) (in M) is the concentration of the IPA aggregates,

\( K_P \) (in M\(^{-1}\)M\(^{1-x}\)) is the equilibrium constant for P-AS interaction,

\( K_I \) (in M\(^{-1}\)) is the equilibrium constant for IPA-AS interaction,

\( K_{\text{P}I} \) (in M\(^{2}\)) is the equilibrium constant for P-IPA interaction,

\( K_{\text{intra}} \) is the equilibrium constant for P intra H-bonding interaction,

\( K_n \) (in M\(^{1-n}\)) is the equilibrium constant for IPA aggregation,

\( x \) is the number of the binding sites of P adsorption,

\( y \) is the number of the binding sites of \( P - I_y \) complexation,

and \( n \) is the IPA aggregation number in solution.

It is assumed that the IPA aggregates and the P-IPA complexes do not adsorb to any significant extent. The above model is also based on the assumption that the Gibbs free energies of each binding site of the solute with the sorbent or the solvent (IPA) are equal, i.e., that all the sites are equivalent. Although this assumption is not necessarily valid, the
model represents the average Gibbs free energy of binding per site. Moreover, this model takes into account only H-bonding-type binding, and does not account for π-π interactions, which are known to affect binding by B or MM on the AS sorbent (Tsui et al. 2013).

By using the principles of local equilibrium of adsorption and reaction for linear systems, we derived the retention factor for a multivalent solute as follows.

\[ k = \frac{\text{concentration of bound AC on sorbent}}{\text{total concentration of AC in mobile phase}} = \frac{C_p \varphi}{C_p + C_pI + C_{\text{intra}}} \]  \hspace{1cm} (6.8)

where \( \varphi \) is the ratio of the solid volume to the liquid volume in the column (we had used the symbol \( \alpha \) for \( \varphi \) in Ref. (Tsui et al. 2013A)).

Then Eqs. (6.3)-(6.8) reduce to a relationship between \( k \) and \( C_{I}^{0} \), as described “implicitly” by the system of the following two equations with \( k \) and \( C_{I}^{0} \) as unknowns:

\[ \ln k = -\ln(1 + K_{\text{intra}} + K_{pI}C_{I}^{y}) - x \ln(K_{p}C_{I}^{x} \varphi) + \ln(K_{p}C_{I}^{x} \varphi) \]  \hspace{1cm} (6.9)

\[ C_{I}^{0} = C_{I} + C_{pI} + nK_{n}C_{I}^{n} \]  \hspace{1cm} (6.10)

Since during pulse experiments, which are used here for determining the retention factors, the solute concentration is expected to be much smaller than the IPA concentration, Eq. (12) reduces to the equation

\[ C_{I}^{0} \approx C_{I} + nK_{n}C_{I}^{n} \]  \hspace{1cm} (6.11)

If the monomer IPA concentration were very high compared to the sorbent capacity, or at the plateau region of the Langmuir isotherm, then the adsorbed IPA
concentration $C_T$ would be constant and equal to the total AS capacity $C_{AS}^0$. Moreover, $C_T$ would be independent of the value of $K_I$. Then the slope $B$ obtained from Eqs. (6.9) and (6.11) would be independent of $K_I$. However, in the presence of IPA-IPA aggregation, the effective monomer concentration may be such that $C_T < C_{AS}^0$ (Tsui et al. 2013A). Then mass balances for the binding sites lead to the equation

$$C_{AS}^0 = C_{AS} + C_T + C_{AC} \approx C_{AS} + C_T$$

(6.12)

This indicates that even if IPA-P interactions were strong, they would not affect the IPA concentration in the pulse experiments. The bound IPA concentration $C_T$ is related to $C_I$ by the Langmuir isotherm.

$$C_T = \frac{c_{AS}^0 K_P C_I}{1 + K_P C_I}$$

(6.13)

This equation is not independent of Eqs. (6.4) and (6.12), but can be derived from these equations. With the use of Eq. (6.13), Eqs. (6.9) and (6.11) are rearranged in the following form.

$$\ln k = -\ln(1 + K_{intra} + K_{PL} C_I^y) - x \ln(1 + K_I C_I) + \ln(K_P C_{AS}^0 x \varphi)$$

(6.14)

$$C_I^0 = C_I + nK_n C_I^n$$

(6.15)

The dependence of $k$ on $C_I^0$, or the function $k(C_I^0)$, is thus predicted to depend on the five equilibrium constants $K_{intra}$, $K_{PL}$, $K_I$, $K_P$, and $K_n$, and on the parameters $x$, $y$, $n$, $\varphi$, and $C_{AS}^0$. If it is assumed that the results fit the Eq. (6.2), then the intercept $A$, which is the last term in Eq. (6.14), is affected only by the parameters $K_P$, $C_{AS}^0$, $x$, and $\varphi$. The slope $B$ depends on the parameters $K_{intra}$, $K_{PL}$, $K_I$, $K_n$, $x$, $y$, and $n$, and on the concentration $C_I^0$. 
and it is not constant, although a “constant” value can be obtained by fitting a small concentration range of the data, as done in Section 6.3. At a given IPA concentration range, the slope $B$ depends on the values of $K_{\text{intra}}$, $K_{\text{PI}}$, $x$, and $y$.

In the limiting cases when the total IPA concentration approaches infinity or zero, Eqs. (6.14) and (6.15) are reduced to one equation, either Eq. (6.16) or Eq. (6.17).

\[
\lim_{c_I^0 \to \infty} (\ln k) = -\frac{x+y}{n} \ln(c_I^0) + \ln\left(\frac{K_P C_{\text{AS}}^0 \psi}{K_{\text{PI}} K_{\text{I}}} (n K_a)^{x+y/n}\right) \tag{6.16}
\]

\[
\lim_{c_I^0 \to 0} (k) = \frac{K_P C_{\text{AS}}^0 \psi}{1+K_{\text{intra}}} \approx \frac{K_P C_{\text{AS}} \psi}{1+K_{\text{intra}}} = \frac{C_{\text{PI}} \phi}{C_P + C_{\text{intra}}} \tag{6.17}
\]

In the first case, the limiting slope $LS$ is equal to $(x+y)/n$. In the second case, the slope $B$ is close to zero ($k$ is independent of $C_{I}^0$), and the model reduces to Eq. (6.8) with $C_{\text{PI}} \approx 0$.

To determine the $k(C_{I}^0)$ dependence more generally, one would need to have an explicit analytical solution for the monomer IPA concentration $C_{I}$ as a function of the total concentration, $C_{I}^0$. Because this is not possible, since the equations are nonlinear, it is convenient to use a dimensionless formulation by first defining the dimensionless IPA concentration with a reference concentration equal to the concentration $C_{\text{AS}}^0 \phi$ of the binding sites per volume of solution.

\[
C_{I}^0* \equiv \frac{\text{total concentration of IPA}}{\text{concentration of NH binding sites}} = \frac{C_{I}^0}{C_{\text{AS}}^0 \phi} \tag{6.18}
\]

\[
C_{I}^* \equiv \frac{\text{concentration of monomer IPA}}{\text{concentration of NH binding sites}} = \frac{C_{I}}{C_{\text{AS}}^0 \phi} \tag{6.19}
\]

Then various physically meaningful dimensionless groups are defined below as follows:
\[ N_P \equiv K_P C_A^0 \varphi \]  
(6.20)

\[ N_P \] is a measure of solute-sorbent interactions.

\[ N_I \equiv K_I C_A^0 \varphi \]  
(6.21)

\[ N_I \] is a measure of solvent-sorbent interactions.

\[ N_{PI} \equiv K_{PI}(C_A^0 \varphi)^y \]  
(6.22)

\[ N_{PI} \] is a measure of solute-solvent interactions.

\[ N_n \equiv nK_n C_A^{n-1} \varphi^{n-1} \]  
(6.23)

\[ N_n \] is a measure of solvent-solvent interactions.

Equations (6.14) and (6.15) reduce to the following system of dimensionless equations for \( k \) and \( C_i^{0*} \).

\[
\ln k = -\ln(1 + K_{intra} + N_{PI}C_i^{*y}) - x \ln(1 + N_I C_i^*) + \ln(N_P)
\]  
(6.24)

\[ C_i^{0*} = C_i^* + N_n C_i^{*n} \]  
(6.25)

From these two equations, three combinations of the above dimensionless groups affecting the slope \( B \) can be identified, \( N_I C_i^*, N_{PI} C_i^{*y} \), and \( N_n C_i^{*n-1} \). The value of the group \( N_I C_i^* \) (which is equal to \( K_I C_I \)) is related to the fractional coverage of the binding sites by the IPA molecules. When \( N_I C_i^* \gg 1 \) (see Eq. (6.13) for the Langmuir isotherm), where there is strong IPA adsorption or a very high IPA concentration, the binding sites are almost completely occupied by IPA. This condition defines the plateau region of the
Langmuir isotherm, and is one of the major assumptions often used in retention models which do not consider IPA aggregation. When there is weak IPA adsorption, or a low IPA concentration, or when $N_l C_l^* \ll 1$, the system of equations is in the linear region of the Langmuir isotherm. At this region the fractional coverage of the binding sites is low.

From Eqs. (6.5)-(6.7) and (6.11), and a solute mole balance, one can derive the equations for P-I complexation and IPA aggregation.

\[
\frac{C_{P-I}}{C_P + C_{intra} + C_{PI}} = \frac{K_{PI} C_I^y}{1 + K_{intra} + K_{PI} C_I^y}
\]

(6.26)

\[
\frac{n C_{intra}^y}{C_I^y} = \frac{n K_n C_I^{y-1}}{1 + n K_n C_I^{y-1}}
\]

(6.27)

The value of the group $N_{PI} C_I^{y-1}$ ($= K_{PI} C_I^{y}$) is related to the fraction of the solute P molecules which are bound to IPA. When there is strong P-IPA interaction or a very high monomer IPA concentration, it follows that $N_{PI} C_I^{y} \gg 1 + K_{intra}$, or $K_{PI} C_I \gg 1 + K_{intra}$, based on Equation (6.26). At these conditions, nearly all the solute molecules are bound to IPA in the mobile phase. When the value of the group $N_{PI} C_I^{y}$ is much lower than 1, most solute molecules are not bound to IPA. Similarly, the value of the group $N_n C_I^{y-1}$ ($= n K_n C_I^{y-1}$) is related to the fraction of IPA molecules in aggregate form.

The overall value of B can vary from 0 at very low concentrations to $(x+y)/n$ at an “infinite” concentrations, and can be written as a function of three individual contributions, $B_{adsorp}$, $B_{complex}$, and $B_{aggreg}$ of the dimensionless groups $N_l C_l^*$, $N_{PI} C_I^*$, and $N_n C_I^{y-1}$, respectively.
\[ B = B_{aggreg}(B_{adsorp} + B_{complex}) \] (6.28)

The derivation reveals that \( B_{adsorp} \) ranges from 0 to \( x \), \( B_{complex} \) ranges from 0 to \( y \), and \( B_{aggreg} \) ranges from \( 1/n \) to 1. The detailed application of these models to the HPLC data is discussed in Section 6.4.

If one applies the above model equations for an R-S chiral enantiomer pair, then one may have to assume that the \( R \) and \( S \) enantiomers have different equilibrium constants, \( K_R \) and \( K_S \), and different numbers of binding sites with AS, \( x_R \) and \( x_S \). Then the logarithm of the predicted enantioselectivity \( \alpha \) is found to be

\[
\ln \alpha = - (x_R - x_S) \ln (1 + K_I C_I) + \ln \left( \frac{K_{P,R}}{K_{P,S}} c_{AS}^{0} (x_R - x_S) \right) \] (6.29)

This result shows that the formation of intra H-bonds does not affect the predicted enantioselectivity. Moreover, in the case of \( x_R = x_S \), the enantioselectivity is predicted in this model to be independent of the IPA concentration and equal to the ratio of the solute-sorbent binding equilibrium constants.

\[
\alpha \equiv \frac{K_R}{K_S} \] (6.30)

6.3 HPLC Results: Effects of the Solvent Composition on the Retention Factors

The retention factors of the two achiral and four chiral solutes were found to decrease with increasing IPA concentration from 0 M to 7.8 M (see Table 6.1 and Figure 6.3), evidently because as the solvent becomes less polar, the solutes bind more strongly
with AS, because there is less competition from IPA, resulting in faster elution. For the PL enantiomers in pure hexane, no peaks were observed, probably because the retention times were very large (≥ 10 hours). For acetone, the slope $B$ increases from an average value of 0.38 at the low concentration range, from 0.065 to 1.3 M, to 0.66 at the high concentration range, from 2.6 to 7.8 M. Similarly, for cyclohexanone, the average slope increases from 0.45 to 0.63. For EL and PL, the slopes increase from 0.43 and 0.76, at $C_I^0 = 0.13$ to 1.3 M, to 1.0 and 1.25, at the higher concentration range. For benzoin, the slopes were found to increase slightly at the higher IPA concentration. Probably, because $\pi-\pi$ interactions do not compete with the sorbent-IPA H-bonding interactions, their effect on the retention behavior of benzoin is more significant at the higher IPA concentrations (Melander, Elrassi, and Horvath 1989). These interactions were not considered in our model, and are beyond the scope of this study. For EL, MM, and PL, the enantioselectivity $\alpha$ is mostly independent of $C_I^0$. (See Section 6.2). For benzoin, $\alpha$ was found to vary with $C_I^0$.

Such data are useful for determining estimates for the “limiting slope” (LS), which is defined as the slope at “infinite”, or practically at very high concentrations; see Eq. (19). No data were obtained beyond 7.8 M of IPA, which corresponds to a volume
Figure 6.3 Plots \( \ln k \) vs. \( \ln C_i^0 \) at 25 °C for two achiral and four chiral solutes; \( k \) is the retention factor and \( C_i^0 \) is the molarity of IPA in n-hexane. The filled circles and squares were used for determining the average limiting slope \( LS \) of the indicated concentration range; see Table 6.1.
Table 6.1 Data on HPLC Retention Factors (k) for Various Achiral and Chiral Solutes with AS Sorbent for Various Isopropanol Concentrations ($C_0^\text{P}$) in n-Hexane, and Results of Fits of ln k vs. ln $C_0^\text{P}$ Plots.

<table>
<thead>
<tr>
<th>Solutes</th>
<th>IPA concentration, $C_0^\text{P}$ (M)</th>
<th>ln k vs. ln $C_0^\text{P}$ plot</th>
<th>Slope (B)</th>
<th>Intercept</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7.8 6.5 5.2 3.9 2.6 1.3 0.78 0.65 0.52 0.325 0.26 0.13 0.065 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetone</td>
<td>0.26 0.30 0.34 0.41 0.54 0.70 0.90 1.0 1.1 N/A 1.4 N/A 8.3</td>
<td>0.66</td>
<td>0.0043</td>
<td>0.999</td>
<td></td>
</tr>
<tr>
<td>Cyclohexanone</td>
<td>0.40 0.43 0.50 0.57 0.80 1.1 N/A 1.6 N/A N/A 2.2 3.2 N/A 9.3</td>
<td>0.63</td>
<td>0.35</td>
<td>0.970</td>
<td></td>
</tr>
<tr>
<td>Ethyl Lactate-S</td>
<td>0.22 0.26 0.33 0.44 0.63 1.08 N/A 1.49 N/A N/A N/A 2.9 N/A 7.9</td>
<td>1.01</td>
<td>0.55</td>
<td>0.999</td>
<td></td>
</tr>
<tr>
<td>Ethyl Lactate-R</td>
<td>0.26 0.31 0.38 0.52 0.75 1.3 N/A 1.81 N/A N/A N/A 3.61 N/A 10.7</td>
<td>0.99</td>
<td>0.68</td>
<td>0.999</td>
<td></td>
</tr>
<tr>
<td>Methyl Mandelate-S</td>
<td>0.46 N/A N/A N/A N/A N/A 2.63 N/A 4.33 N/A N/A N/A 11.7 N/A 47.4</td>
<td>0.95</td>
<td>1.12</td>
<td>0.991</td>
<td></td>
</tr>
<tr>
<td>Methyl Mandelate-R</td>
<td>0.64 N/A N/A N/A N/A N/A 3.18 N/A 5.67 N/A N/A N/A 15.3 N/A 76.5</td>
<td>0.88</td>
<td>1.37</td>
<td>0.999</td>
<td></td>
</tr>
<tr>
<td>Benzoin-S</td>
<td>0.84 N/A N/A N/A N/A N/A 2.58 N/A 4.13 N/A 7.69 N/A 10.9 13.6 49.6</td>
<td>0.79</td>
<td>1.13</td>
<td>0.987</td>
<td></td>
</tr>
<tr>
<td>Benzoin-R</td>
<td>2.72 N/A N/A N/A N/A N/A 4.62 N/A 6.35 N/A 12.3 N/A 16.4 19.6 106</td>
<td>0.71</td>
<td>1.66</td>
<td>0.921</td>
<td></td>
</tr>
<tr>
<td>Pantolactone-S</td>
<td>0.72 0.89 1.17 1.71 2.83 6.26 N/A 10.32 N/A N/A N/A 35.4 N/A N/A</td>
<td>1.25</td>
<td>2.23</td>
<td>0.999</td>
<td></td>
</tr>
<tr>
<td>Pantolactone-R</td>
<td>1.31 1.62 2.11 3.03 4.84 10.8 N/A 18.82 N/A N/A N/A 53 N/A N/A</td>
<td>1.21</td>
<td>2.75</td>
<td>0.999</td>
<td></td>
</tr>
</tbody>
</table>

$^a$1.3 M corresponds to about 10 vol %.

$^b$The data at high concentrations were fitted to the equation ln $k$= A-B ln $C_0^\text{P}$, from 2.6 to 7.8 M for acetone and cyclohexanone, from 3.9 to 7.8 M for EL and PL, from 0.65 to 7.8 M for MM, and from 0.325 to 1.3 M for B.
fraction of ca. 0.60. Since the slope at high concentrations is related to the number $x$ of the solute-sorbent binding sites, the number $y$ of the solute-solvent (IPA) binding sites, the number $n$ of the IPA aggregates, the concentration, and other factors, it is important to use the data and the model for determining the actual values of $y$ and $x$.

6.4 Estimation of the Parameters of the Thermodynamics Retention Model from the HPLC Data

The value of 0.44 of the parameter $\phi$ was used for the sorbent at the conditions of the experiments, as detailed previously (Xie et al. 2003; Tsui et al. 2013A). To apply the model to the solutes with IPA, one has to use the same values of the binding constant $K_I$, the aggregation number $n$, and the aggregation equilibrium constant $K_n$, namely $K_I=290$, $n=3$, and $K_n=6.7\times10^5$, as those obtained by fitting the model to the acetone data (Tsui et al. 2013A). The four remaining unknown parameters for each solute are $K_{intra}$, $K_{Pl}$, $y$, and $x$. For the EL, MM, and B enantiomers, the $k_0$ values, $k_{0,R}$ and $k_{0,S}$, were obtained experimentally and were used in the fitting (Table 6.1). For R-PL and S-PL, the $k_0$ values were estimated from the fitting.

Because the values of $K_{intra}$, $K_{Pl}$, and $y$ are expected to be the same for each R- and S-enantiomer pair, the following strategy was used in the fitting procedures. For the EL and MM solutes, the data for the R-enantiomer were used to estimate the above three parameters and $x_R$. Then the same three parameters were used to determine the value of $x_S$ from the S-enantiomer data. For PL, the R-enantiomer data were used to determine $K_{intra}$, $K_{Pl}$, $y$, $x_R$, and $k_{0,R}$. Then the first three values were used with the S-enantiomer
data to estimate $x_S$ and $k_{0,S}$. For benzoin, it was preferable to fit first the S-enantiomer data, and then estimate $x_R$. Only the lower-concentration data were used to obtain the benzoin parameters, because the benzoin data may show significant effects of $\pi-\pi$ interactions, which are not accounted for in the present model. Such interactions may lead to an inflection point in the $\ln k$ vs. $\ln C_1^0$ plot, as observed in Figure 6.3E, and as discussed in Ref. (Tsui et al. 2013A).

The fitting routine involved the determination of the mean square error $E$ for each data point, and then minimizing it by varying the fitted parameters.

$$E = E(K_{p-1y}, K_{intra}, y, x_i, k_{0,i} \text{ for PL}) \equiv \left( \frac{\sum_{i=1}^{N} [\ln k_i(C_i^0)_{model} - \ln k_i(C_i^0)_{ exper}]}{N} \right)^2 \text{ min}$$  \hspace{1cm} (6.31)

where $N$ is the number of the experimental data used in the fitting. Further details of the fitting and some sensitivity analysis are given in the Supplementary Material. In certain cases the values of $y$ were first estimated as mentioned above, and were found to be fractional, between 2 and 3 (see Appendix D). The fitting procedure was repeated, with the values of $y$ assumed to be equal to an integer number of the respective H-bonding functional groups: 3, 3, 2, and 3 for EL, MM, B, and PL. The fitting error was little affected by this change (see Appendix D). For this reason, we report the set of $x$-values determined with this assumption. Complexation is also probed by IR and modeled with DFT. Additional insights on complexation can be obtained from the pair correlation functions estimated from MD simulations (see Section 6.5).
Table 6.2 Parameters Used in the Retention Model for the Enantiomers of EL, MM, B, and PL; See Section 6.4.

<table>
<thead>
<tr>
<th></th>
<th>EL-R</th>
<th>EL-S</th>
<th>MM-R</th>
<th>MM-S</th>
<th>B-R</th>
<th>B-S</th>
<th>PL-R&lt;sup&gt;a&lt;/sup&gt;</th>
<th>PL-S&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{PI}$ (M&lt;sup&gt;3&lt;/sup&gt;) $\times 10^b$</td>
<td>8.45</td>
<td>8.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.96</td>
<td>2.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.11</td>
<td>3.25</td>
<td>3.25&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>$K_{intra}$</td>
<td>10.7</td>
<td>10.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.2</td>
<td>6.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.7</td>
<td>4.8</td>
<td>4.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>$y$&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>2.0</td>
<td>2.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>X</td>
<td>1.4</td>
<td>1.3</td>
<td>2.2</td>
<td>2.1</td>
<td>1.9</td>
<td>1.6</td>
<td>2.8</td>
<td>3.0</td>
</tr>
<tr>
<td>$K_p C_{AS}^0$&lt;sup&gt;c&lt;/sup&gt;</td>
<td>284</td>
<td>210</td>
<td>1252</td>
<td>775</td>
<td>1374</td>
<td>642</td>
<td>5958</td>
<td>4455</td>
</tr>
</tbody>
</table>

<sup>a</sup> Because no HPLC were available, the $k_{0_{R}}$ and $k_{0_{S}}$ values were determined from the model, 338 for PL-S and 452 for PL-R.

<sup>b</sup> For EL, MM, and PL, the parameters obtained from fitting the R-enantiomer HPLC data were used for the S-enantiomer. For B, the S-B fitting parameters were used for the R-B.

<sup>c</sup> Parameters were calculated from Eq. (6.17).

<sup>d</sup> The $y$ values were fixed to 2 for B and 3 for EL, MM, and PL.
Figure 6.4 Fit of the thermodynamics retention model to the chiral solutes enantiomers $k$ ($C_i^0$) data. See also Table 6.2 for the values of other key parameters. For easier visualization of the fitting, the data are plotted in a normal scale, $k$ vs. $C_i^0$. 
The best fit results are shown in Table 6.2 and Figure 6.4. The fits look good for EL, MM, and PL. For benzoin, the fit is worse, probably because π-π interactions at high IPA concentrations were not considered in the model.

The values of the intra H-bond equilibrium constants $K_{\text{intra}}$ were found to increase in the order EL< MM< PL. This order is consistent with the DFT-predicted intra H-bonding energy values (Tsui et al. 2013B). The high $x$ value for PL indicates that the PL molecule forms simultaneously more H-bonds with AS than EL or MM. By using Eq. (6.16), the limiting slopes $LS$ were predicted to be 1.5 for EL, 1.7 for MM, 1.3 for B, and 2.0 for PL.

The values of $y$ indicate that two or three IPA molecules bind to the H-bonding functional groups of each solute. The values of the products of the binding constants $K_p$ and the sorbent capacity $C_{AS}^0$ can be determined from the values of $k_{0,i}$ and $K_{\text{intra}}$ (see Eq. (6.17)) They were found to increase in the order EL<MM<B<PL.

Perhaps the most important findings are the $x$-values. For all four solutes, the same values of the binding sites are found for the R- and S-enantiomers. In previous work on the molecular enantioselectivity mechanism (Tsui et al. 2013B), it was concluded from MD simulations and data that EL-R has two binding sites and EL-S has one. The thermodynamics data suggest, however, that the effective number of the binding sites is about 1.4, and the same for EL-R and EL-S. Moreover, the binding equilibrium constants are found to be significantly different. The discrepancies are attributed to the following factors:
1. The previous results refer only to the primary enantioselective interactions, and do not account for non-enantioselective interactions.

2. In the MD simulations, one ignores weaker interactions of the S-enantiomer, and designates them as non-H-bonded. By contrast, the thermodynamic model describes a weaker interaction as one with a lower equilibrium constant.

3. The MD simulations account for different energetic interactions, but not for differences in entropic interactions.

Similarly for MM and PL, the same values of $x$ and of $B$ were also observed. The inference is consistent with the findings reported in the literature (Wirz et al. 2003; Wirz et al. 2008). By using IR spectroscopy, Wirz et al. studied the chiral recognition mechanisms of EL and PL with AS. They concluded that the C=O groups of the R enantiomer form stronger hydrogen bonds with the polymer NH groups than those of the S enantiomer. Such interactions lead to enantioselectivity. By using MD simulations, Tsui et al. indicated that the enantioselective interactions of PL with AS are due to the different H-bonding strengths. For R-PL, the H-bonds of (PL) C=O $\leftrightarrow$ HN (AS) and (PL) O $\leftrightarrow$ HN (AS) are stronger than that of S-PL. Although the numbers $x$ of the binding sites are similar for the enantiomers, the strengths of the binding are different, resulting in different equilibrium constants. For benzoin, the values of $x$ are 1.6 for S-B and 1.9 for R-B. The difference may be due to the differences in $\pi-\pi$ interactions.

Hence, the effective number of the interaction sites can be obtained, with some confidence, from the $x$-values as estimated from the model. The $x$-values are about 2 for
MM and B, and 3 for PL. They seem to correlate well with the retention factors and are consistent with the results obtained from the mechanistic studies (Tsui et al. 2013B).

6.5 Transmission IR, Density Functional Theory, and Molecular Dynamic Simulations

Results for EL-IPA and PL-IPA Interactions

6.5.1 IR and DFT Results

The IR spectra of the solution of EL, EL-IPA, PL, and PL-IPA in hexane are shown in Figure 6.5. For EL, three partially overlapped peaks are detected for the C=O band region of the IR spectra. Spectral deconvolution was used to resolve these peaks (Tsui et al. 2013B). DFT was used to establish the peak assignments. One of these peaks is due to free EL at 1745 cm\(^{-1}\); two peaks are due to intra H-bonded conformations, one being OH↔O at 1766 cm\(^{-1}\), and the other being OH↔O=C H-bond at 1738 cm\(^{-1}\). The results indicate that a significant fraction of the solutes has an intra H-bond of OH with O=C. With EL at 0.65 or 1.3 M IPA, the intensities of the EL C=O bands at 1766 and 1738 cm\(^{-1}\) decrease, and the bands become broader. These results suggest that EL forms inter H-bonds with the OH groups of IPA, rather than intra H-bonds. To help understand the spectral changes upon the addition of IPA, DFT simulations were done for four possible H-bonding configurations between the EL and the IPA molecules (Table 6.3 and Figure 6.6). A significant shift of -24.7 cm\(^{-1}\) is predicted for the EL C=O group H-bonded with the IPA HO group. When the IPA OH group is H-bonded with an O atom of the OH group, or an O group of the EL, blue-shifts of +7.0 and +12.6 cm\(^{-1}\) are predicted, respectively. No significant shift is predicted for the C=O band when the EL OH group is
Figure 6.5 Transmission-IR spectra of EL and PL C=O stretching bands for various molar IPA concentrations in n-hexane, 0 M, 0.65 M, and 1.3 M.
H-bonded with the O atom of the IPA OH group. The energies of these H-bonds, range from -3.3 to -6.3 kcal/mol, and imply a high probability of binding. Since the spectral changes of the C=O bands cannot be attributed to any one of these H-bonding configurations, they may due in part to multivalent H-bonding of IPA with EL molecules.

For PL in pure hexane, four populations of C=O band can be detected. The band assignments were made from spectral deconvolution and DFT simulations (Tsui et al. 2013B). The large peak at 1799 cm\(^{-1}\) was inferred to be due to the overlapping bands of free C=O and of intra H-bonded C=O groups. The bands at 1765, 1787, and 1812 cm\(^{-1}\) were assigned to various inter H-bonding configurations. When PL is in solution with IPA, the intensity of the band at 1799 cm\(^{-1}\) decreases significantly, implying that there are fewer free PL molecules in IPA-hexane than in pure hexane. For the band at 1785 cm\(^{-1}\), a significant intensity increase was observed. It may have resulted directly from the interactions with IPA. From the DFT results, the shifts of -9.15, -5.71, and -4.44 cm\(^{-1}\) (red shifts) are predicted for the C=O band, for the H-bonding configurations of (PL) C=O ↔ HO (IPA), (PL) OH ↔ OH (IPA), and (PL) HO ↔ HO (IPA). A C=O band shift of +4.25 cm\(^{-1}\) is predicted for the PL O group H-bonded with the IPA OH group. Significant H-bonding energies, from -4.2 to -7.1 kcal/mol, indicate a high possibility of multivalent H-bonds with the IPA molecules. For testing this inference further, MD simulations were used for modeling solute-IPA interactions.
Table 6.3 DFT Predictions of Wavenumber Changes, IR Intensities Ratios ($R$), and Binding Energies $\Delta E$ for Different Dimer Configurations of EL-IPA and PL-IPA complexes

<table>
<thead>
<tr>
<th>Solute</th>
<th>Binding configuration</th>
<th>Band</th>
<th>$\Delta\nu$, cm$^{-1}$</th>
<th>$R$</th>
<th>$\Delta E$, kcal/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl lactate (EL)</td>
<td>(EL) C=O ↔ HO (IPA)</td>
<td>(EL) OH</td>
<td>2.43</td>
<td>1.14</td>
<td>-5.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(EL) C=O</td>
<td>-24.73</td>
<td>1.41</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(IPA) OH</td>
<td>-117.7</td>
<td>25.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(EL) OH ↔ OH (IPA)</td>
<td>(EL) OH</td>
<td>-185.9</td>
<td>26.8</td>
<td>-6.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(EL) C=O</td>
<td>-2.45</td>
<td>1.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(IPA) OH</td>
<td>-6.38</td>
<td>1.52</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(EL) HO ↔ HO (IPA)</td>
<td>(EL) OH</td>
<td>1.76</td>
<td>1.16</td>
<td>-5.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(EL) C=O</td>
<td>6.99</td>
<td>1.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(IPA) OH</td>
<td>-84.5</td>
<td>15.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(EL) O ↔ HO (IPA)</td>
<td>(EL) OH</td>
<td>-0.68</td>
<td>1.19</td>
<td>-3.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(EL) C=O</td>
<td>12.6</td>
<td>1.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(IPA) OH</td>
<td>-57.0</td>
<td>10.9</td>
<td></td>
</tr>
<tr>
<td>Pantolactone (PL)</td>
<td>(PL) C=O ↔ HO (IPA)</td>
<td>(PL) OH</td>
<td>3.51</td>
<td>1.11</td>
<td>-4.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(PL) C=O</td>
<td>-9.15</td>
<td>1.37</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>(IPA) OH</td>
<td>-41.5</td>
<td>21.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(PL) OH ↔ OH (IPA)</td>
<td>(PL) OH</td>
<td>-234</td>
<td>18.3</td>
<td>-7.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(PL) C=O</td>
<td>-5.71</td>
<td>1.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(IPA) OH</td>
<td>-0.94</td>
<td>1.39</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(PL) HO ↔ HO (IPA)</td>
<td>(PL) OH</td>
<td>-7.68</td>
<td>1.02</td>
<td>-4.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(PL) C=O</td>
<td>-4.44</td>
<td>0.97</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>(IPA) OH</td>
<td>-111.1</td>
<td>16.2</td>
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<tr>
<td></td>
<td>(PL) O ↔ HO (IPA)</td>
<td>(PL) OH</td>
<td>2.22</td>
<td>0.99</td>
<td>-4.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(PL) C=O</td>
<td>4.25</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(IPA) OH</td>
<td>-60.1</td>
<td>14.1</td>
<td></td>
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</table>
Figure 6.6 DFT predicted-molecular structures of four possible PL-IPA dimer complexes.
6.5.2 MD Simulations of Solute-IPA-Hexane Mixtures

When one EL or PL solute molecule interacts with 150 IPA molecules and 100 hexane molecules (at an IPA mole fraction of 0.6 or of an effective concentration $C_1^0$ of 6.0 M), IPA is predicted to adsorb, or concentrate preferentially, close to the solute. The pair correlation functions $g(r)$ for four different binding configurations are shown in Figure 6.7. The local densities ($\rho_{\text{average}}(g(r))$ for the type of H-bonds (solute) C=O ↔ HO (IPA) show similarly large peaks at short distances, indicating that this H-bond is the most likely between the solute and the IPA molecule. For types of H-bonds (solute) OH ↔ OH (IPA) and (solute) HO ↔ HO (IPA), the local densities are slightly higher for EL than for PL. The densities for the PL O group H-bonded with IPA HO group show much higher values at smaller distances than EL. It is inferred that the O atom of PL may be more accessible for binding with IPA molecules. The conformations (a), (c), and (d) seem to be the most likely, but they may not be allowed to occur simultaneously because of possible steric restrictions. Overall, the MD simulation provide additional insights on solute-IPA complexation in solution, and support the inferences made by IR.

6.6 Application of the Models to the Literature Data

The model was also used to fit certain important HPLC data for nine solutes reported by Gyimesi-Forrás et al. (2009) (Table 6.4, Figures 6.7 and 6.8, Appendix D). The parameters of $K_{PF}$, $x_i$, $y_i$, and $k_{0,i}$ were estimated. These data are for chemically attached molecular chains on a silica substrate (Figure 6.7), unlike the above data, which are for a continuous bulk polymer film of AS. These molecules and the sorbent molecules contain several potential solute-sorbent and solute-IPA binding sites. The estimated
Figure 6.7 Radial distribution functions $g(r)=\rho(r)/\rho_{av}$ for EL and PL molecules in mole fraction=0.6 IPA solution in hexane, for four different conformations a, b, c, and d.
number y of the complexation sites ranged from 3.0 to 4.3 (Table D 3). These values can be estimated roughly from an inspection of the molecular structures (Figure 6.7). The fitting procedure was repeated with the second method, with a fixed value of y equal to an integer number of the H-bonding functional groups (see Tables D 4 and 6.4). The values of the x-parameter changed slightly, but they ranged from about 2.6 to 4.5.

The values of x were found to be, respectively, as 3.2, 3.2, and 2.7, for solutes 7, 9, and 10, and 3.0, 3.5, 3.8, 3.7, 3.6, and 2.4 for solutes 1, 3, 4, 5, 6, and 8. On average, those for the first group were smaller, as expected, because they have one or two fewer functional groups. Moreover, they were quite larger than the reported slopes. The discrepancies between B and x indicate (i) that both the solute-IPA complexation and IPA aggregation should be accounted for obtaining reliable estimates of x; and (ii) that the experimental slopes B may not always reach the limiting slopes obtained from the model, although they were found to be close for solutes 3, 4, 5, and 10. Similarly with the results for the solutes with AS, the same values of x and of the slopes B were found for the R and the S enantiomers for all nine solutes, suggesting that for these solutes, the enantioselectivity is due mainly to different binding equilibrium constants.
Table 6.4 Results of Fitting the Multivalent Retention Model to Certain Data Reported by Gyimesi-Forrás et al. (2009).^a

<table>
<thead>
<tr>
<th>Solute^b</th>
<th>Slope</th>
<th>y^c</th>
<th>x</th>
<th>Limiting Slope (LS)^d</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.58</td>
<td>3.0</td>
<td>2.6</td>
<td>2.1</td>
</tr>
<tr>
<td>3</td>
<td>2.40</td>
<td>4.0</td>
<td>3.5</td>
<td>2.5</td>
</tr>
<tr>
<td>4</td>
<td>2.29</td>
<td>4.0</td>
<td>3.8</td>
<td>2.6</td>
</tr>
<tr>
<td>5</td>
<td>2.34</td>
<td>4.0</td>
<td>3.7</td>
<td>2.6</td>
</tr>
<tr>
<td>6</td>
<td>2.0</td>
<td>4.0</td>
<td>4.5</td>
<td>2.6</td>
</tr>
<tr>
<td>7</td>
<td>1.66</td>
<td>3.0</td>
<td>3.3</td>
<td>2.2</td>
</tr>
<tr>
<td>8</td>
<td>1.67</td>
<td>4.0</td>
<td>2.8</td>
<td>2.0</td>
</tr>
<tr>
<td>9</td>
<td>1.74</td>
<td>3.0</td>
<td>3.5</td>
<td>2.2</td>
</tr>
<tr>
<td>10</td>
<td>1.68</td>
<td>3.0</td>
<td>2.7</td>
<td>1.9</td>
</tr>
</tbody>
</table>

^a No intramolecular binding was considered ($K_{\text{intra}}=0$); the values of $K_{p-I}$ and $k_0$’s were determined, and are listed in the supplementary materials.

^b The structures of the solutes are given in Figure 6.5.

^c The $y$ values were fixed.

^d Limiting slopes, LS=(x+y)/3, determined from the model; see text.
Figure 6.8 Structures, of (A) quinine tert-butylcarbamate type sorbent and (B) nine solutes, which are quinazoline-1,5-dione derivatives, reported by Gyimesi-Forrás et al. (2009); data for solute 2 were not fitted because only four data were available.
Figure 6.9 Fit of the thermodynamics retention model to the reported selectand #1 enantiomers $k\left(C_i^0\right)$ data by Gyimesi-Forrás et al. (2009). See also Table 6.3 for the values of other key parameters.
6.7 Conclusions

Various literature stoichiometric displacement models have been widely used for understanding the competitive adsorption mechanisms of solutes and the polar modifiers (alcohols) of the mobile phase on the sorbent. The models were used for interpreting the plots of the logarithms of the retention factors versus the logarithms of the modifier concentrations $C_i^0$. The average slope of such a plot was generally inferred to be equal to the number of the modifier molecules which are displaced or desorbed from the sorbent upon adsorption of the solute on the sorbent and upon the solute-alcohol decomplexation. The slopes were generally found to be greater than 1.

In this study, the retention factors of enantiomer pairs of four structurally related solutes, ethyl lactate (EL), methyl mandelate (MM), benzoin (B), and pantolactone (PL), were measured for the amylose tris[(S)-α-methylbenzylcarbamate] sorbent, or AS, with isopropanol (IPA) in n-hexane as the mobile phase. The slopes ($B$) were found to increase with increasing IPA concentration $C_i^0$ and to range from less than 1, in the $C_i^0$ concentration range from 0.13 to 1.3 M, to slightly more than 1 at higher concentrations. The previous available literature models cannot account for the concentration dependence of $B$ or for such small slopes. In our previous results with simpler achiral monovalent binding molecules, the slopes were found to be even smaller than those here (0.25 to 0.43), and could not be accounted for by any previous model (Tsui et al. 2013A). When the aggregation of IPA was accounted for in the models, it was possible to explain such small slopes.

In this study, a new model is presented, in dimensional and dimensionless forms, accounting for multivalent solute adsorption ($x>1$), multivalent solute-alcohol
complexation \((y>1)\), and solute intra hydrogen-bonding, which is shown by IR to be quite pronounced. Three key dimensionless groups, which represent (i) the fraction of the sorbent binding sites covered by IPA, (ii) the fraction of the solute molecules in complex form, and (iii) the fraction of the IPA molecules in aggregate form, were found to determine the overall value of \(B\). The limiting slopes LS of \(\ln k\) vs. \(\ln C_1^0\), at the highest IPA concentrations approach the theoretically expected values of \(z/n\), where \(z=x+y\) is the number of the alcohol molecules displaced upon the adsorption of the solute and decomplexation of the solute-IPA complex; \(n\) is the average aggregation number of the alcohol aggregates.

The model was used to fit the HPLC data of the above four chiral solutes. The binding equilibrium constants, and the average numbers of the complexation binding sites \((y)\) and of the adsorption binding sites \((x)\), were estimated. The fits are good for EL, PL, and MM, and fair for B, which has significant \(\pi-\pi\) interactions not accounted for in the model. The values of the intra H-bond equilibrium constants \(K_{\text{intra}}\) were found to increase in the order EL< MM< PL, consistently with the DFT-predicted intra H-bonding energy values. At very high IPA concentrations, the limiting slopes LS were predicted to be 1.5 for EL, 1.7 for MM, 1.3 for B, and 2.0 for PL. For EL, MM, and PL, the HPLC data can be fitted with the same values of \(x\). Thus, the enantioselectivity is inferred to be due mainly to the difference of the enantiomer binding strengths, and not to differences in the number of the binding sites. For benzoin, the slightly different values of \(x\) may be due to differences in the \(\pi-\pi\) interactions.
The model was also tested with certain HPLC data reported in the literature (Gyimesi-Forrás et al. 2009). The same slopes $B$ were also reported for the pairs of the enantiomers. The parameters of $K_{pl}$, $x$, $y$, and $k_0$ were estimated. The $x$ values, ranging from 2.4 to 3.8, and the $y$-values, ranging from 3.0 to 4.3, were found to correlate semi-quantitatively with the numbers of the solute functional groups, from 4 to 5, determined by inspection of the solutes molecular structures. The reported slopes $B$ did not reach the limiting slopes $LS$ which were predicted by the model.

For EL and PL, IR results and DFT simulations indicated strong solute-IPA complexation, consistently with the fitting results. In IPA-hexane solution, EL forms inter H-bonds with the OH groups of IPA, instead of intra H-bonds. For PL, there are fewer free PL molecules in hexane-IPA than in pure n-hexane. MD simulations were used for modeling the multivalent solute-IPA complexation for EL and PL and determining the radial distribution functions, or local densities, for the four possible solute-IPA H-bonding configurations. Three types of the H-bonds, (solute) C=O ↔ HO (IPA), (solute) OH ↔ OH (IPA), and (solute) HO ↔ HO (IPA) were predicted for EL and PL. They may form simultaneously if sterically allowed. A significant additional H-bond, (solute) O ↔ HO (IPA), was predicted only for PL.

The new model has been shown to be capable of describing multivalent interactions, and fitting well several available retention factor data. Thus, the model is more reliable than previous models for estimating the numbers of the potential binding sites of the solute with the alcohol and of the solute with the sorbent.
CHAPTER 7. CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

In this thesis, the chiral recognition mechanisms for amylose tris(S)-α-methylbenzylcarbamate, or AS, sorbent were elucidated at the molecular and microscopic levels. One specific chiral solute, benzoin, which has a relatively high enantioselectivity, was chosen first for study. In Chapter 3, studies of the enantioselective interactions of AS with benzoin enantiomers are presented, via a combination of IR data and DFT simulations. By using pure n-hexane as the mobile phase, the key interactions of AS-benzoin-hexane systems can be plausibly predicted with a two-component model system of sorbent and benzoin. DFT simulations are used for estimating H-bonding strengths. It is inferred that without steric hindrance benzoin may tend to form the type of H-bond (AS) C=O ↔ HO (B), which is the strongest H-bond for the side chain/B pairs. Both enantiomers OH groups form identical H-bonds with AS. Moreover, difference spectra suggest that the C=O groups of R-benzoin bind differently with AS than those groups of S-benzoin. These bonds are inferred to provide the key difference for establishing chiral, or enantioselective, recognition of B by AS.

In Chapter 4, three chiral solutes, which are structurally similar to benzoin, were also chosen for study: ethyl lactate (EL), methyl mandelate (MM), and pantolactone (PL). These solutes were found to have quite different HPLC retention factors and
enantioselectivities. The DFT energies of the inter H-bonds, of the solute OH groups and of the solute C=O groups, with the sorbent side chains were calculated. The results show that the strengths of the H-bonds of the solute OH groups are stronger than those of the solute C=O groups. The distributions of the torsion angles of the solute acyloin O=C-C=O group were determined with MD simulations. These distributions provide an indicator of the molecular flexibility or rigidity. The order of the enantioselectivity correlates with increasing molecular rigidity. Molecular docking simulations were done for each of enantiomer of the four solutes. The results support the hypothesis that the general recognition mechanism involves a non-enantioselective “leading” strong H-bonding interaction and an enantioselective secondary H-bonding interaction, which is affected by geometrical and energetic restrictions and can lead to additional differences in interactions.

From the macroscopic level, when the retention factor $k$ data of $R$ or $S$-benzoin are plotted as a log-log plot, the slopes $B$ are less than 1, namely 0.50 and 0.56, for the $R$ and $S$ enantiomers. Such small slopes cannot be explained by any previously published retention model. Previous literature models have been widely used for understanding the competitive adsorption mechanisms of solutes and the polar modifiers of the mobile phase, provide useful complemental information to the mechanistic studies, and were used to explain the often-observed linear log-log plots. The slopes of the plots were inferred to be equal to the number of the displaced modifier molecules upon adsorption of one solute molecule, and were generally larger than one.

In Chapter 5, five achiral monovalent simple solutes were chosen and studied in detail. The slopes $B$ were even smaller. These are the first available values lower than
one in the literature. IR and DFT simulations provided indications that there is significant IPA aggregation with an average aggregation number of $n=3$. A new retention model for the monovalent solutes has been developed, which for the first time takes into account the effect of IPA aggregation on the retention factors. This is the first model which can explain small slopes and which can reliably and accurately probe the solute-sorbent and solute-alcohol binding.

More complex multivalent models were then developed, in Chapter 6, for chiral molecules, which also show unusually small slopes, which cannot be explained by previous models. The new model accounts for multivalent solute adsorption, as would be expected, multivalent solute-alcohol complexation, as also would be expected, and alcohol adsorption, and even for solute intra-H-bonding, which was discovered to be quite important. It also accounts, most importantly, for alcohol aggregation. The limiting slopes $LS$, at the highest IPA concentrations, are found to approach the newly theoretically predicted values of $z/n$, here $z=x+y$ is the number of the alcohol molecules displaced upon the adsorption of the solute and decomplexation of the solute-IPA complex; $x$ is the number of the adsorption binding sites; $y$ is the complexation binding sites; $n$ is the average aggregation number of the alcohol aggregates. Hence, if the value of $n$ can found from IR data and DFT simulations, then $k(C^0_I)$ data and the new model allow the reliable estimation of equilibrium constants of solute adsorption, alcohol adsorption, solute-alcohol complexation, and intra-H-bond formation. These constants cannot normally be estimated by independent experiments.

The model was used to fit the HPLC data of the above four chiral solutes and certain chiral solutes reported in the literature (Gyimesi-Forrás et al. 2009). Some
important parameters, the binding equilibrium constants, and the average numbers of the y- and x-values, were estimated. The x-values and the y-values correlate semi-quantitatively with the numbers of the solute functional groups, determined by inspection of the molecular structures. The HPLC data of the most enantiomers were fitted with the similar values of x, which inferred that the enantioselectivity is due mainly to the difference of the enantiomer binding strengths, and not to differences in the number of the binding sites. The new model has been shown to be capable of describing multivalent interactions, and fitting well many available retention factor data. Thus, the model is more accurate than previous models for estimating the numbers of potential binding sites of the solute with the sorbent and the solute with the alcohol. The aggregation of the polar modifier in the mobile phase must be accounted in the interpretation of the retention factors and the adsorption mechanisms. Overall, the data of the retention factor with alcohol allow one to obtain quantitative measures of solute-sorbent interactions, which affect the chiral recognition. These studies were shown to provide additional information for understanding chiral recognition mechanisms.

7.2 Recommendations

This dissertation focuses on the chiral recognition mechanisms for several acyloin-type chiral solutes with an important polysaccharide-based sorbent, amylose tris(S)-α-methylbenzylcarbamate, or AS. For exploring the applicability of the proposed general mechanisms, studies with other classes of solutes containing other functional groups, and with other of polysaccharide-based sorbents, are recommended.
More realistic, and more elaborate, MD simulations of polymer rods and polymer-solute systems may shed more light on the effects of interpolymer interactions. Using two or more polymer rods will allow the modeling of interpolymer interactions. For sorbent-solute-solvent systems, the effect of n-hexane may be examined further even if it is expected to be minor, as a prelude to examine the effect of the polar modifiers, IPA. The effect of alcohol may be quite strong, since it may change substantially the polymer structure. Moreover, the polymer-solute interactions may also be affected strongly by the alcohol. Using a combination of molecular simulations with new IR data, and possibly other data from solid-state NMR, VCD, and calorimetry, for sorbent-solute-hexane and alcohol, may help establish the key interactions which affect the retention factors and the enantioselectivities. By using VCD, as pioneered by Grinberg et al. (Ma et al. 2009), one may obtain how the helicity of the polymer sorbent may be affected by the alcohol, and in turn how the new structures may affect the solute-sorbent and the IPA-sorbent interactions.

The overall enantioselectivity may depend not only on the strong enantioselective interactions for the optimal cavities determined here but also on less enantioselective or non-enantioselective interactions with other cavities and other sites between the polymer rods. Hence, additional MC/MD simulations such as those used here, are needed to make quantitative predictions of relative energies and enantioselectivities. A more complex and realistic model will be needed.

The temperature dependence of the retention factors should be measured, to determine the enthalpic and entropic contributions to the enantiomer recognition. Further simulations on determining Gibbs free energies (and entropies), in addition to the
enthalpies would be helpful for a more comprehensive evaluation of the solute-sorbent interactions, with and without alcohol.

The retention models can also be applied for other types of the polar modifier molecules. The effect of the different alcohol molecules on the retention behavior can be elucidated. Moreover, for those solutes which have significant π-π interactions, mixed-model retention models are needed to include aromatic interactions, and develop new retention models which are more generally applicable.
REFERENCES


Appendix A  

**DFT Calculations of the Different H-bond Types of Pairs of S2-S2, S2-B (R-B), and S1 or S2 with R-B**

The following table contains detailed DFT simulation prediction of H-bonding for pairs of S2 with S2, B with B, and S1 or S2 with B; see Section 3.3 for the detailed discussion and interpretation.

Table A.1 H-Bond Energies, Lengths, and Angles of the Different H-Bond Types of Pairs of S2-S2, S2-B (R-B), and S1 or S2 with R-B from DFT Calculations (See also Section 3.3)

<table>
<thead>
<tr>
<th>System #</th>
<th>Pair</th>
<th>H-Bond Type</th>
<th>Energy, E (kJ/mol)</th>
<th>Length (Å)</th>
<th>Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S2 with S2</td>
<td>(S2) C=O ↔ H-N (S2)</td>
<td>-23.03</td>
<td>1.98</td>
<td>169</td>
</tr>
<tr>
<td>2</td>
<td>S2 with S2</td>
<td>(S2) C=O ↔ H-N (S2)</td>
<td>-17.35</td>
<td>2.50 (fixed)</td>
<td>170</td>
</tr>
<tr>
<td>3</td>
<td>S2 with S2</td>
<td>(S2) C=O ↔ O (S2)</td>
<td>-11.83</td>
<td>2.06</td>
<td>171</td>
</tr>
<tr>
<td>4</td>
<td>S2 with backbone</td>
<td>(S2) NH ↔ O (backbone)</td>
<td>-21.82</td>
<td>1.99</td>
<td>178</td>
</tr>
<tr>
<td>5</td>
<td>(b)</td>
<td>(cis-B) C=O ↔ HO</td>
<td>-14.76</td>
<td>1.97</td>
<td>119</td>
</tr>
<tr>
<td>6</td>
<td>R-B with R-B</td>
<td>(R-B) C=O ↔ HO (R-B)</td>
<td>-23.78</td>
<td>2.02</td>
<td>169</td>
</tr>
<tr>
<td>7</td>
<td>R-B with R-B</td>
<td>(R-B) OH ↔ OC (R-B)</td>
<td>-23.78</td>
<td>2.06</td>
<td>160</td>
</tr>
<tr>
<td>8</td>
<td>S1 with B</td>
<td>(S1) C=O ↔ H-O (B)</td>
<td>-35.03</td>
<td>1.83</td>
<td>168</td>
</tr>
<tr>
<td>9</td>
<td>S2 with B</td>
<td>(S2) C=O ↔ H-O (B)</td>
<td>-33.31</td>
<td>1.82</td>
<td>180</td>
</tr>
<tr>
<td>10</td>
<td>S1 with B</td>
<td>(S1) N-H ↔ O=C (B)</td>
<td>-24.20</td>
<td>2.02</td>
<td>162</td>
</tr>
<tr>
<td>11</td>
<td>S2 with B</td>
<td>(S2) N-H ↔ O=C (B)</td>
<td>-18.39</td>
<td>2.04</td>
<td>168</td>
</tr>
<tr>
<td>12</td>
<td>S1 with B</td>
<td>(S1) N-H ↔ O-H (B)</td>
<td>-16.55</td>
<td>2.11</td>
<td>161</td>
</tr>
<tr>
<td>13</td>
<td>S2 with B</td>
<td>(S2) N-H ↔ O-H (B)</td>
<td>-12.25</td>
<td>2.08</td>
<td>169</td>
</tr>
</tbody>
</table>

*a* Energy is half of what was calculated for two H-bonds between two benzoin molecules.

*b* This is for an intramolecular H-bond in cis-benzoin.
Appendix B  Predictions by MM Simulations with CVFF, and Comparisons to DFT

Predictions

The following table contains comparisons of DFT predictions of H-bond parameters with MM simulation predictions; see Section 3.4.1.

Table B.1 H-Bond Interaction Energies, Lengths, and Angles of Various Pairs Predicted by MM Simulations with CVFF, and Comparisons to DFT Predictions. Shown in Parentheses are the % Differences between the MM Simulations and the DFT Predictions

\( \% = \frac{X_{\text{MM}} - X_{\text{DFT}}}{X_{\text{DFT}}} \) (where X is E, d, or \( \theta \)) (See also Section 3.4)

<table>
<thead>
<tr>
<th>System #</th>
<th>Interaction Type</th>
<th>Energy, E (kJ/mol)</th>
<th>Length d (Å)</th>
<th>Angle ( \theta ) (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(S2) C=O ↔ H-N (S2)</td>
<td>-24.70 (7%)</td>
<td>1.97 (-0.5%)</td>
<td>166 (-1.8%)</td>
</tr>
<tr>
<td>2</td>
<td>(cis-B) C=O ↔ HO</td>
<td>-7.27 (-51%)</td>
<td>2.09 (6.1%)</td>
<td>116 (-2.5%)</td>
</tr>
<tr>
<td>3</td>
<td>(S2) C=O ↔ H-O (B)</td>
<td>-64.79 (95%)</td>
<td>1.64 (-9.9%)</td>
<td>168 (-6.7%)</td>
</tr>
<tr>
<td>4</td>
<td>(S2) N-H ↔ O=C (B)</td>
<td>-32.23 (75%)</td>
<td>1.96 (-3.9%)</td>
<td>170 (-1.2%)</td>
</tr>
<tr>
<td>5</td>
<td>(S2) N-H ↔ O-H (B)</td>
<td>-28.30 (131%)</td>
<td>1.95 (-6.3%)</td>
<td>175 (-3.6%)</td>
</tr>
<tr>
<td>6</td>
<td>(2 benzene) π - π</td>
<td>-21.36</td>
<td>3.79</td>
<td>N/A</td>
</tr>
</tbody>
</table>
The following figure contains 3D conformation of the molecular backbone structure of an RH model polymer with 12 monomer units; see Section 4.5.1.

Figure C.1 Molecular simulations of a 3D conformation of the molecular backbone structure of an RH model polymer structure with 12 monomer units. The dark and medium dark spheres represent the attachment points of the C2 and C3 side chains; the light balls represent the attachment points of the C5 side chains. The helix defined by the C5 attachment points has a larger radius for the RH structure model than for the LH structure model; see Section 4.5.1.
Appendix D  Estimation of Model Parameters by Fitting the Retention Factor Data for Each Enantiomer (See Sections 6.3 and 6.4)

The $k_i (C_i^0)$ data for the four acyloin-containing solutes in Figure 6.3 were fitted to the Eqs. (6.14), (6.15), and (6.17). The fitting strategy is described in Section 6.4. The number N of data points for each set is given in Table S1. The values of $K_{P\rightarrow L}$, $K_{intra}$, $y_R$ (or $y_S$), and $x_R$ (or $x_S$) were varied, and the average mean-square error E was calculated from Eq. (6.31). Then the error was minimized, and the optimal values of the parameters were determined. To avoid small inconsistencies in the values of $K_{P\rightarrow L}$, $K_{intra}$, and $y$ of each enantiomer, these values were determined first for the R-enantiomer of EL, MM, and PL, and were then used for the fitting of the S-enantiomer data. Thus, the comparison of the $x$-values should be more reliable. For PL the values of $k_{0,R}$ and $k_{0,S}$ were also determined from the fitting, since data were not available (see Section 6.3). For benzoin, the S-enantiomer data were fitted first, to obtain the values of $K_{P\rightarrow L}$, $K_{intra}$, $y_S$, and $x_S$. The value of $x_R$ was obtained from the fitting for $y_R=y_S$. Results of the fitting are given in Tables D1 and D2.

The values of the average error E in ln $k_i$ ranged from about 0.01 to 0.07. This means that the average error in $k_i$ was 1 to 7 %. The experimental error was estimated to be 1 to 4 %. This suggests that the data on benzoin fit the model less well than the data on the other three solutes. The values of E, and hence the values of the fitted parameters, are not as sensitive when the values of $K_{P\rightarrow L}$ and $K_{intra}$ are varied by 10 to 30 %. This is the estimated uncertainty in the
fitting of the these data to this model. Since our main goal is to obtain reliable estimates of $y_i$ and $x_i$, we did the fitting in a different way in Table D 2. We fixed the values of $y$ to 3, 3, 2, and 3, respectively, as the number of the H-bonding functional groups of EL, MM, B, and PL complexing with IPA. The respective error $E$ changed little for all sets of data, except for those of MM-S (0.062 vs. 0.0097). The estimated values of $K_{P-Ly}$, $K_{intra}$, and $x_i$ remained the same for EL-R and EL-S, changed little for B-R and B-S, and changed a lot for MM-R, MM-S, PL-R, and PL-S. The latter results appear to be closer to the values expected, based on our determined chiral recognition mechanism in Chapter 4. These results are presented in the main text of the article Section 6.4. The data seem to fit well the model with both estimation strategies (see Figure D 1 and Figure 6.4 in the article).

The values of $K_{intra}$ are in the order EL> MM> PL, as expected from the IR data. For benzoin, the value $K_{intra}=4.8$ is similar to that of PL, in contrast to what is expected from the IR data. The value for B, $K_{intra}=4.7$, are considered less reliable, because the data for B are expected to be affected by strong π-π interactions, which are not accounted for in the model. The values of the complexation constants $K_{P-Ly}$ for EL, MM, and PL are directly comparable, since the same value of $y=3$ was used. The results indicate that complexation is stronger for EL than for MM or PL.

The $x_R$ and $x_S$ values are about the same for each pair of enantiomers. The values are the smallest for EL (~1.4), and the largest for PL (~3). Previous chiral recognition studies suggested possible values of 2 or 1 for EL-R or EL-S and 3 or 2 for PL-R or PL-S. These issues, and the interpretation of the other parameters, are described in more detail in the text.
The following procedure was used to fit the nine sets of enantiomer data reported by Gyimesi-Forrás et al. (2009). Since it is unclear which were the R and S enantiomers, we use the symbol A for the first eluted enantiomer and B for the second one. Because the values of $k_{0,A}$ and $k_{0,B}$ were not reported, these values were also estimated from the data. The values of $K_{Pl}$, $y_i$, $x_i$, and $k_{0,i}$ for each enantiomer were estimated, with the same two methods. The results of the first method, with adjustable $y$, are shown in Table D 3. The value of $K_{intra}$ was taken as 0, since there is no evidence of intramolecular H-bonding in these solutes. Then the average values of $y$ and $x$ were determined. The average error in $E$ ranged from ca. 0.01 to 0.05, indicating an average fitting error in $k_i$'s of 1 to 5%. This suggests that the model fits the data fairly well for solutes 1, 3, 4, 5, 9, and 10. The fitting was worse for solutes 6, 7, and 8, resulting in significant discrepancies between $y_A$ and $y_B$, and $x_A$ and $x_B$.

From an inspection of the molecular structures, for solutes 7, 9, and 10, one would expect a maximum of four H-bonding groups, which are one N atom, two C=O groups, and one NH group. We found values of 3.3, 3.4, and 3.0. For solutes 1, 3, 4, 5, 6, and 8, one would expect a maximum value of 5 or 6. The values of 3.2, 4.0, 4.1, 4.0, 4.3, and 3.6 were found. For solute 1, the unexpected low $y$-value may be due to a very weak H-bond formed by the O atom. The N atom and O atom groups evidently form no complexes to any significant extent.

The $y$-values are slightly lower than the number of the expected functional groups, indicating a possible steric restriction that the possible H-bonding functional groups may not all bind with IPA molecules simultaneously, or that one of the functional group is not available to bind. Hence, $y$ can be estimated intuitively from the solute molecular
structure. The fitting results for the second method, with fixed y, are shown in Table D 4.

The errors were about the same, as those in Table D 3, indicating that they are not very sensitive to the small variations in y. The values of the x-parameters for the second method changed slightly, but they again ranged from about 3 to 4. These results are discussed in the main text.
Table D.1 Estimated Parameters of the Retention Model for the Enantiomers of EL, MM, B, and PL

<table>
<thead>
<tr>
<th>Solute</th>
<th>EL-R</th>
<th>EL-S</th>
<th>MM-R</th>
<th>MM-S</th>
<th>B-R</th>
<th>B-S</th>
<th>PL-R</th>
<th>PL-S</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{p-I_y}$ (M$^2$)</td>
<td>6.67</td>
<td>6.67$^b$</td>
<td>1.00</td>
<td>1.00$^b$</td>
<td>8.62$^b$</td>
<td>8.62</td>
<td>3.73</td>
<td>3.73$^b$</td>
</tr>
<tr>
<td>$K_{intra}$</td>
<td>13.4</td>
<td>13.4$^b$</td>
<td>10.7</td>
<td>10.7$^b$</td>
<td>1.46$^b$</td>
<td>1.46</td>
<td>4.4</td>
<td>4.4$^b$</td>
</tr>
<tr>
<td>$y_R$ or $y_S$</td>
<td>2.9</td>
<td>2.9$^b$</td>
<td>2.2</td>
<td>2.2$^b$</td>
<td>3.2$^b$</td>
<td>3.2</td>
<td>2.7</td>
<td>2.7$^b$</td>
</tr>
<tr>
<td>$x_R$ or $x_S$</td>
<td>1.4</td>
<td>1.3</td>
<td>1.5</td>
<td>1.4</td>
<td>2.2</td>
<td>1.9</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>$k_{0,R}$ or $k_{0,S}$</td>
<td>10.7</td>
<td>7.9</td>
<td>76.5</td>
<td>47.4</td>
<td>106</td>
<td>49.6</td>
<td>247$^a$</td>
<td>162$^a$</td>
</tr>
<tr>
<td>$K_Pc_{AS}^0$</td>
<td>350</td>
<td>259</td>
<td>2034</td>
<td>1260</td>
<td>593</td>
<td>277</td>
<td>3031</td>
<td>1988</td>
</tr>
<tr>
<td>N, for R or S</td>
<td>7</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>E, for R or S</td>
<td>0.0099</td>
<td>0.0091</td>
<td>0.042</td>
<td>0.0097</td>
<td>0.074</td>
<td>0.027</td>
<td>0.0088</td>
<td>0.019</td>
</tr>
</tbody>
</table>

$^a$ Because no HPLC were available, the $k_{0,R}$ and $k_{0,S}$ values were determined from the model.

$^b$ For EL, MM, and PL, the values of the parameters, $K_{p-I_y}$, $K_{intra}$, and $y$ were obtained from fitting the R-enantiomer HPLC data and were then used for the fitting of the S-enantiomer data, to estimate $x_S$. For the B solutes, the S-B fitting parameters were used for the R-B enantiomer.

$^c$ Parameters were calculated from Eq. (19).
Table D.2 Estimated Parameters of the Retention Model for the Enantiomers of EL, MM, B, and PL for Fixed \( y \) Values

<table>
<thead>
<tr>
<th>Solute</th>
<th>EL-R</th>
<th>EL-S</th>
<th>MM-R</th>
<th>MM-S</th>
<th>B-R</th>
<th>B-S</th>
<th>PL-R&lt;sup&gt;a&lt;/sup&gt;</th>
<th>PL-S&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_{P-l_y} (\text{M}^{-1}) \times 10^{-6} )</td>
<td>8.45</td>
<td>8.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.96</td>
<td>2.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.11</td>
<td>3.25</td>
<td>3.25&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>( K_{\text{intra}} )</td>
<td>10.7</td>
<td>10.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.2</td>
<td>6.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.7</td>
<td>4.8</td>
<td>4.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>( y_R ) or ( y_S )</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>2.0</td>
<td>2.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>( x_R ) or ( x_S )</td>
<td>1.4</td>
<td>1.3</td>
<td>2.2</td>
<td>2.1</td>
<td>1.9</td>
<td>1.6</td>
<td>2.8</td>
<td>3.0</td>
</tr>
<tr>
<td>( k_{0,R} ) or ( k_{0,S} )</td>
<td>10.7</td>
<td>7.9</td>
<td>76.5</td>
<td>47.4</td>
<td>106</td>
<td>49.6</td>
<td>452&lt;sup&gt;a&lt;/sup&gt;</td>
<td>338&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>( K_P C_{A_S}^{0,c} )</td>
<td>284</td>
<td>210</td>
<td>1252</td>
<td>775</td>
<td>1374</td>
<td>642</td>
<td>5958</td>
<td>4455</td>
</tr>
<tr>
<td>( N, ) for ( R ) or ( S )</td>
<td>7</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>( E, ) for ( R ) or ( S )</td>
<td>0.010</td>
<td>0.0098</td>
<td>0.048</td>
<td>0.062</td>
<td>0.072</td>
<td>0.030</td>
<td>0.014</td>
<td>0.020</td>
</tr>
</tbody>
</table>

<sup>a</sup> Because no HPLC were available, the \( k_{0,R} \) and \( k_{0,S} \) values were determined from the model.

<sup>b</sup> For EL, MM, and PL, the parameters obtained from fitting the R-enantiomer HPLC data were used for the S-enantiomer. For B, the S-B fitting parameters were used for the R-B.

<sup>c</sup> Parameters were calculated from Eq. (19).

<sup>d</sup> The \( y \) values were fixed to 3 for EL, MM, and PL, and to 2 for B.
Table D.3 Results of Fitting Certain Literature Data to the Multivalent Retention Model

<table>
<thead>
<tr>
<th>Solute</th>
<th>1-A</th>
<th>1-B</th>
<th>3-A</th>
<th>3-B</th>
<th>4-A</th>
<th>4-B</th>
<th>5-A</th>
<th>5-B</th>
<th>6-A</th>
<th>6-B</th>
<th>7-A</th>
<th>7-B</th>
<th>8-A</th>
<th>8-B</th>
<th>9-A</th>
<th>9-B</th>
<th>10-A</th>
<th>10-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{p-y} (\text{M}^2)$ $\times 10^{-6}$</td>
<td>4.98</td>
<td>4.99</td>
<td>502</td>
<td>502</td>
<td>502</td>
<td>502</td>
<td>502</td>
<td>502</td>
<td>817</td>
<td>502</td>
<td>7.10</td>
<td>5.00</td>
<td>117</td>
<td>404</td>
<td>5.40</td>
<td>5.57</td>
<td>6.08</td>
<td>5.64</td>
</tr>
<tr>
<td>$y_A$ or $y_B$</td>
<td>3.2</td>
<td>3.2</td>
<td>4.0</td>
<td>4.0</td>
<td>4.1</td>
<td>4.1</td>
<td>4.0</td>
<td>4.0</td>
<td>4.4</td>
<td>4.1</td>
<td>3.4</td>
<td>3.2</td>
<td>3.0</td>
<td>4.2</td>
<td>3.4</td>
<td>3.3</td>
<td>2.9</td>
<td>3.1</td>
</tr>
<tr>
<td>$x_A$ or $x_B$</td>
<td>3.0</td>
<td>2.9</td>
<td>3.5</td>
<td>3.4</td>
<td>3.9</td>
<td>3.6</td>
<td>3.7</td>
<td>3.6</td>
<td>3.9</td>
<td>3.3</td>
<td>3.4</td>
<td>3.0</td>
<td>2.0</td>
<td>2.7</td>
<td>3.2</td>
<td>3.1</td>
<td>2.6</td>
<td>2.8</td>
</tr>
<tr>
<td>$k_{0,A}$ or $k_{0,B}$</td>
<td>1855</td>
<td>1962</td>
<td>35016</td>
<td>35618</td>
<td>31538</td>
<td>29843</td>
<td>29286</td>
<td>32057</td>
<td>12012</td>
<td>8550</td>
<td>2248</td>
<td>1941</td>
<td>24498</td>
<td>1706</td>
<td>1926</td>
<td>1979</td>
<td>2919</td>
<td>2333</td>
</tr>
<tr>
<td>$N$, for A or B</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>$E$, for A or B</td>
<td>0.014</td>
<td>0.015</td>
<td>0.034</td>
<td>0.039</td>
<td>0.051</td>
<td>0.054</td>
<td>0.043</td>
<td>0.046</td>
<td>0.034</td>
<td>0.035</td>
<td>0.024</td>
<td>0.025</td>
<td>0.037</td>
<td>0.035</td>
<td>0.032</td>
<td>0.031</td>
<td>0.025</td>
<td>0.024</td>
</tr>
</tbody>
</table>

\(^a\) All $k_0$-values were determined from fitting the data, since no HPLC data are available for 0 % IPA.

\(^b\) The $y$-values were fitted to the model.
Table D.4 Results of Fitting Certain Literature Data to the Multivalent Retention Model for Fixed y values

<table>
<thead>
<tr>
<th>Solute</th>
<th>1-A</th>
<th>1-B</th>
<th>3-A</th>
<th>3-B</th>
<th>4-A</th>
<th>4-B</th>
<th>5-A</th>
<th>5-B</th>
<th>6-A&lt;sup&gt;a&lt;/sup&gt;</th>
<th>6-B</th>
<th>7-A&lt;sup&gt;b&lt;/sup&gt;</th>
<th>7-B</th>
<th>8-A&lt;sup&gt;c&lt;/sup&gt;</th>
<th>8-B</th>
<th>9-A</th>
<th>9-B</th>
<th>10-A</th>
<th>10-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>(K_{P-I, y} (M^3) \times 10^{-6})</td>
<td>3.99</td>
<td>4.30</td>
<td>502</td>
<td>502</td>
<td>464</td>
<td>504</td>
<td>502</td>
<td>502</td>
<td>151</td>
<td>155</td>
<td>2.02</td>
<td>2.34</td>
<td>206</td>
<td>190</td>
<td>1.22</td>
<td>1.09</td>
<td>5.02</td>
<td>5.20</td>
</tr>
<tr>
<td>(y_A) or (y_B)</td>
<td>3.0</td>
<td>3.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
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<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>(x_A) or (x_B)</td>
<td>2.6</td>
<td>2.5</td>
<td>3.5</td>
<td>3.4</td>
<td>3.9</td>
<td>3.7</td>
<td>3.7</td>
<td>3.6</td>
<td>4.4</td>
<td>4.5</td>
<td>3.4</td>
<td>3.1</td>
<td>2.7</td>
<td>2.8</td>
<td>3.4</td>
<td>3.6</td>
<td>2.7</td>
<td>2.6</td>
</tr>
<tr>
<td>(x_{av.})</td>
<td>2.6</td>
<td>3.5</td>
<td>3.8</td>
<td>3.7</td>
<td>4.5</td>
<td>3.3</td>
<td>2.8</td>
<td>3.5</td>
<td>2.7</td>
<td>2.7</td>
<td>2.7</td>
<td>2.7</td>
<td>2.7</td>
<td>2.7</td>
<td>2.7</td>
<td>2.7</td>
<td>2.7</td>
<td>2.7</td>
</tr>
<tr>
<td>(k_{0,A}) or (k_{0,B})</td>
<td>1742</td>
<td>2109</td>
<td>3516</td>
<td>35618</td>
<td>36141</td>
<td>38162</td>
<td>29286</td>
<td>32057</td>
<td>21218</td>
<td>27215</td>
<td>2466</td>
<td>2406</td>
<td>1588</td>
<td>2011</td>
<td>2435</td>
<td>3542</td>
<td>1977</td>
<td>2056</td>
</tr>
<tr>
<td>(N_0) for A or B</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
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<td>5</td>
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<td>5</td>
<td>5</td>
</tr>
<tr>
<td>(E_0) for A or B</td>
<td>0.015</td>
<td>0.151</td>
<td>0.034</td>
<td>0.039</td>
<td>0.051</td>
<td>0.052</td>
<td>0.043</td>
<td>0.046</td>
<td>0.035</td>
<td>0.034</td>
<td>0.024</td>
<td>0.025</td>
<td>0.035</td>
<td>0.035</td>
<td>0.032</td>
<td>0.032</td>
<td>0.026</td>
<td>0.026</td>
</tr>
</tbody>
</table>

<sup>a</sup> All \(k_0\)-values were determined from fitting the data, since no HPLC data are available for 0 % IPA.

<sup>b</sup> The \(y\)-values were fitted to the model.
Figure D.1 Fit of the thermodynamic retention model to the chiral solutes $k \left( C^0_t \right)$ data.

See also Tables D 1 and D 2 for the values of the parameters.
VITA
VITA

Hung-Wei Tsui
Graduate School, Purdue University

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PUBLICATIONS

Publications from M.S. Thesis Research


Publications from Ph.D. Thesis Research, to Date


