1.1. Importance of Chiral Separation

The separation of chiral compounds has been of great interest because the majority of bioorganic molecules are chiral. Living organisms, for example, are composed of chiral biomolecules such as amino acids, sugars, proteins and nucleic acids. In nature these biomolecules exist in only one of the two possible enantiomeric forms, e.g., amino acids in the L-form and sugars in the D-form. Because of chirality, living organisms show different biological responses to one of a pair of enantiomers in drugs, pesticides, or waste compounds, etc.

Chirality is a major concern in the modern pharmaceutical industry. This interest can be attributed largely to a heightened awareness that enantiomers of a racemic drug may have different pharmacological activities, as well as different pharmacokinetic and pharmacodynamic effects. The body being amazingly chiral selective, will interact with each racemic drug differently and metabolize each enantiomer by a separate pathway to produce different pharmacological activity. Thus, one isomer may produce the desired therapeutic activities, while the other may be inactive or, in worst cases, produce unwanted effects. Consider the tragic case of the racemic drug of n-phthalyl-glutamic acid imide that was marketed in the 1960’s as the sedative Thalidomide. Its therapeutic activity resided exclusively in the R-(+)-enantiomer. It was discovered only after several hundred births of malformed infants that the S-(+)-enantiomer was teratogenic.

The U.S. Food and Drug Administration, in 1992, issued a guideline that for chiral drugs only its therapeutically active isomer be brought to market, and that each enantiomer of the drug should be studied separately for its pharmacological and metabolic pathways. In addition, a rigorous justification is required for market approval of a racemate of chiral drugs. Presently, a majority of commercially available drugs are both synthetic and chiral. However, a large number of chiral drugs are still marketed as
racemic mixtures\textsuperscript{9-10}. Nevertheless, to avoid the possible undesirable effects of a chiral drug, it is imperative that only the pure, therapeutically active form be prepared and marketed. Hence there is a great need to develop the technology for analysis and separation of racemic drugs.

Chiral compounds are also utilized for asymmetric synthesis\textsuperscript{11}, i.e., for the preparation of pure optically active compounds. They are also used in studies for determining reaction mechanisms, as well as reaction pathways. Chiral compounds are also important in the agrochemical industries\textsuperscript{12-13}.

Current methods of enantiomeric analysis include such non-chromatographic techniques as polarimetry, nuclear magnetic resonance, isotopic dilution, calorimetry, and enzyme techniques. The disadvantages of these techniques are the need for pure samples, and no separation of enantiomers are involved. Quantitation, which does not require pure samples, and separation of enantiomers can be done simultaneously by either gas chromatography (GC) or high performance liquid chromatography (HPLC).\textsuperscript{14}

Chiral HPLC has proven to be one of the best methods for the direct separation and analysis of enantiomers\textsuperscript{15-16}. It is more versatile than chiral GC because it can separate a wide variety of nonvolatile compounds. It provides fast and accurate methods for chiral separation, and allows on-line detection and quantitation of both mass and optical rotation of enantiomers if appropriate detection devices are used\textsuperscript{17-19}. Current chiral HPLC methods are either direct, which utilizes chiral stationary phases (CSPs) and chiral additives in the mobile phase, or indirect, which involves derivatization of samples.\textsuperscript{20-22} Direct chiral separations using CSPs are more widely used and are more predictable, in mechanistic terms, than those using chiral additives in the mobile phase\textsuperscript{23}.

To date nearly a hundred HPLC CSPs have been developed and are commercially available.\textsuperscript{21} However, there is no single CSP that can be considered universal, i.e., has the ability to separate all classes of racemic compounds. Choosing the right CSP for the enantioseparation of a chiral compound is difficult. The decision relies mostly on empirical data\textsuperscript{22-23}. Most chiral separations achieved on CSPs, however, were obtained based upon the accumulated trial-and-error knowledge of the analyst, intuition, and often simply by chance. An alternative way of choosing a CSP is by using predictive empirical
rules that have been developed based on empirical structures\textsuperscript{24-26}. Neither scheme of choosing a right CSP offers a guarantee for a successful enantiomeric separation. Although enantioseparation is hoped to be achieved by knowing the chemistry of the racemic analytes and the CSP sometimes, however, it does not work because the interactions of the mobile phase with both the racemic analyte and CSP have to be considered. All three components, analyte, CSP, and mobile phase, must be taken into consideration when developing a chiral separation method. The key, therefore, to a successful enantioseparation of a particular class of racemates on a given CSP is the understanding of the possible chiral recognition mechanisms\textsuperscript{27-28}.

1.2 Nonsteroidal Anti-inflammatory Drugs of 2-Methylarylpropionic Acids (Profens)

A variety of 2-arylmethylpropionic acids (profens) (Fig. 1) have been widely used as nonsteroidal anti-inflammatory drugs for the relief of acute and chronic rheumatoid arthritis and osteoarthritis, as well as for other connective tissue disorders and pains\textsuperscript{29-30}. Examples are fenoprofen, ibuprofen, ketoprofen, flurbiprofen, and naproxen. Another profen (carprofen) has been studied here but is not yet commercially available. All are chiral and, except for naproxen, are marketed in racemic form. The chirality of these molecules arise from the $sp^3$-alpha carbon. Direct enantioseparations of profens have been of considerable interest because their anti-inflammatory and analgesic effects have been attributed almost exclusively to their S-enantiomers.\textsuperscript{31} To avoid the unwanted effects of the R-enantiomer, the use of pure S-enantiomer of the profens is desirable. Hence development of a preparative scale separation direct enantiomeric analysis is important. Furthermore, some of the profens generally undergo a unidirectional in vivo chiral inversion\textsuperscript{32-36} from the inactive R-enantiomer to the active S-form, as well as bi-directional chiral inversion\textsuperscript{37-39}. The metabolic and pharmacokinetic studies of both isomers require direct enantioseparations.

Enantioseparation of profens by HPLC has been studied extensively, using both indirect and indirect methods. In the indirect method, a racemic profen is derivatized to form diastereomers and then separated using a chiral column\textsuperscript{40-56}. Whereas in the direct
Figure 1. Structures of racemic racemic 2-aryl methyl propionic acids (profens) analyzed.
method, there is no derivatization of profens, and CSPs are used to separate the isomers. At present numerous direct chiral separations of profens on HPLC chiral stationary phases have been reported. To mention some of the CSPs used for the enantioseparation of profens with aqueous buffer solutions as eluents are the $\alpha_1$-acid glycoprotein$^{57-61}$, ovomucoid$^{62-65}$, bovine serum albumin$^{66}$, cyclodextrin$^{67-68}$, and serum albumin$^{69}$, and avidine$^{70-71}$. Examples of the CSPs used for the separations of profens with normal phase eluents are the derivatized polysaccharides$^{72-81}$ adsorbed on macroporous silica gel such as Chiralcel OD, Chiralcel OJ, and Chiralpak AD.

The enantiomeric separation of profens using tris(3,5-dimethylphenylcarbamate)s of amylose and cellulose coated on macroporous silica (ADMPC and CDMPC, respectively, which was later commercialized as Chiralpak AD and Chiralcel OD) (Fig. 2) was first studied by Okamoto et al.$^{82}$ Enantioseparations of ibuprofen, ketoprofen, flurbiprofen, and tiaprofenic acid were performed at varying compositions of hexane/2-propanol, but with 1% trifluoroacetic acid (TFA) of the total volume of mobile phase. In their work, all racemic profens were incompletely separated on CDMPC. On ADMPC, only flurbiprofen and tiaprofenic acids were completely enantioseparated. Ibuprofen was also completely enantioseparated on ADMPC, but only in its derivatized form. Chiral separations were completed in more than 15 minutes. Based on the racemic profens separated, ADMPC appears to have a superior enantioseparating ability.

Wainer et al.$^{83}$ studied on the enantioseparation of 2-alkylarylpropionic acids on Chiralpak AD, including the 2-aryl methylpropionic acids. They investigated the chiral recognition mechanism for the enantiomeric separations of these acids using quantitative structure-enantioselective retention relationship (QSERR). The retention data collected from the enantioseparation of racemic acids, with 95/5/1% hexane/2-propanol/TFA as eluents, were correlated to a series of molecular descriptors including the hydrogen bonding ability and aromaticity of the analytes. In this QSERR analysis, the influence of the nature of mobile phase on the structure of CSP and analyte is not considered. Based on their results the chiral recognition mechanism on Chiralpak AD mainly involves attractive interactions, primarily hydrogen bonding, and is conformationally driven.
Figure 2. Structure of derivatized polysaccharide CSPs: (a) cellulose tris(3,5-dimethylphenylcarbamate) and (b) amylose tris(3,5-dimethylphenylcarbamate).
1.3 Research Objectives

There were several interrelated objectives in this dissertation. The first objective was to develop a systematic method for optimized separation of racemic profens geared for the analysis of both enantiomers. The chiral separation method should take only a short time and preferably use only inexpensive solvents. This study explored the enantioseparation of profens on Chiralpak AD and Chiralcel OD using a normal phase eluent of hexane, as the apolar solvent, and alcohol as the polar modifier. Carboxylic acids were studied as the acidic mobile phase modifiers. Variation of column temperatures are also investigated for optimization of enantioseparation of profens on both CSPs.

Chiralpak AD and Chiralcel OD were the CSPs chosen in the study because, by virtue of the tris(3,5-dimethylphenylcarbamate)-D-glucose units as the chiral adsorbing sites, they are capable of interacting with the small racemic profens leading to chiral separation. In addition, since Chiralpak AD and Chiralcel OD have the same chiral adsorbing sites but are of different structures their enantioseparating abilities for profens are expected to be different, but complementary. That is, a profen that could be only partially enantioseparated in one CSP, hopefully could be well resolved in the other. Lastly, Chiralpak AD and Chiralcel OD both require a normal phase eluent that is well suited for the analysis of the pure S-enantiomer of profens.

The second objective of this study was to investigate the influence of temperature on retention and enantioselectivity of profens on Chiralpak AD and Chiralcel OD. It is well known that a change in temperature alters retention and enantioselectivity, and thus enantiomer resolution\(^{84-86}\). From the retention behavior and the corresponding enantioselectivity, inferences can be drawn for the possible chiral recognition mechanism of profens on both CSPs. To achieve this result, the thermodynamic parameters - differences in enthalpy, entropy, and Gibbs free energies for the association of enantiomers and CSP were measured.

The third goal of this research was to explore the influence of mobile phase, both acidic and alcoholic modifiers, on the enantioseparation of profens and profen methyl esters on Chiralpak AD and Chiralcel OD. The general mechanisms for chiral recognition
on both CSPs are attractive interactions and inclusion or steric fit of analytes to the chiral cavities. Nevertheless, it is obvious that any competing interaction involving the mobile phase may alter enantioselectivity and, thus, enantiomeric resolution. In addition, this study aimed to obtain a glimpse of the mechanism involved for chiral discrimination of profens on both CSPs.

Lastly, this study aimed to elucidate the chiral recognition of mechanisms for the separation of racemic profens on Chiralpak AD and Chiralcel OD. The retention behavior and the corresponding enantioselectivity from the optimization studies and the studies of the influence of temperature and acidic and alcoholic mobile phase modifiers were used in depth to evaluate the mechanism for chiral discrimination of profens on both CSPs. This involves structural correlation of analyte and CSP to the observed retention behavior and corresponding enantioselectivity from the different studies. In this study, methyl esters of fenoprofen, ibuprofen, and ketoprofen, and the aromatic hydrocarbons of benzene, naphthalene, and anthracene, were included as probe analytes aside from the six profen molecules (Fig. 1).

As noted in Section 1.2, Wainer et al. investigated the chiral recognition for the enantioseparation of 2-arylmethyl propionic acids on Chiralpak AD by QSERR analysis. The study of the chiral recognition of profens on Chiralpak AD, in this dissertation, was already completed before the work of Wainer et al. was reported.