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Synthesis and application of cellulose-based chiral stationary phases for HPLC

Bhavani Thadi Shetty
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SYNTHESIS AND APPLICATION OF CELLULOSE-BASED CHIRAL
STATIONARY PHASES FOR HPLC

A Thesis

Presented to

The Faculty of the Department of Chemistry

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Bhavani Thadi Shetty

December 2007

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ABSTRACT

SYNTHESIS AND APPLICATION OF CELLULOSE-BASED CHIRAL

STATIONARY PHASES FOR HPLC

by Bhavani Thadi Shetty

In this research stationary phases for liquid chromatography were prepared and evaluated. Acrylamidomethyl cellulose acetate propionate and acrylamidomethyl cellulose acetate butyrate chiral stationary phases (CSPs) were prepared using the silanization/hydrosilation process. The first step is the silanization process where most of the silanol groups of silica are converted to silica hydride followed by the second step hydrosilation, in which an organic moiety with a terminal olefinic (C=C) group is bonded to the silica hydride surface.

These newly synthesized cellulose-based CSPs were then characterized by the Neue test, elemental analysis, CP-MAS NMR spectroscopy, and DRIFT spectroscopy. Chromatographic measurements were made on a set of chiral aromatic small molecule drugs in the reverse phase and normal phase modes using various methanol/water and hexane/alcohol compositions respectively. The CSPs showed promise in the resolution of enantiomers when used in normal phase.

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Bhavani Thadishetty.

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1. INTRODUCTION

1.1 Chirality

The word *chiral* originated from the Greek word *cheir*, which refers to hand or handedness (1). Stereoisomers are the structures of the same substance but differ only in the spatial arrangement of their atoms. Stereoisomers that differ only in their capacity for rotating a plane of polarized light are called optically active or chiral compounds (2). An example of chiral compound is shown in Figure 1. The central carbon atom has four different groups attached to it and is considered as a chiral center (3). Molecules that can form non-superimposable mirror images are called enantiomers. Diastereomers are stereoisomers, which are not enantiomeric to each other. To describe the structure of an enantiomer the symbols R (rectus) and S (sinister) were proposed by R. S. Cahn, C. Ingold, and V. Prelog (2). Rules have been laid out to determine the R or S form of an enantiomer. The number of possible enantiomers of a chiral molecule can be calculated using the formula 2^n , where n is the number of chiral centers. There are two chiral centers in 2,3 dichloropentane, hence there are two pairs of enantiomers (1,2 and 3,4) as shown in Figure 2 and each enantiomer is a diastereoisomer of the other pair of enantiomers.

Not only the presence of chiral center but also a variety of other situations results in chirality. Consider molecules such as biphenyls in which two-rings are joined by a single bond and rotation about this single bond is hindered by bulky substituents. As a result the two rings are forced to occupy different planes. A simple example of this type of enantiomer is shown in Figure 3(3).

Figure 1. A pair of enantiomers.

Figure 2. Stereochemistry of 2,3 dichloropentane.

Figure 3. Chirality due to hindered rotation along a single bond.

Enantiomers have identical physical and chemical properties (e. g. melting point, boiling point, solubility etc.,) except in two important aspects: (a) They rotate a plane of polarized light in opposite directions, but in equal magnitudes. The enantiomer that rotates plane polarized light to the left (counterclockwise) is designated as laevorotatory L or (-), while the one that rotates plane polarized light to the right (clockwise) is termed as dextrorotatory D or (+). (b) They interact differently with other chiral compounds (4).

1.2 Significance of Chiral Separation

Biosynthesis and metabolism are controlled by chirality. Our body produces only D-glucose rather than a mixture of the sixteen possible hexoses. In the same way only L-amino acids are produced in our body, not a mixture of D- and L-isomers. Enzymes can recognize, bind and react with a specific isomer hence chiral products involved in biological reactions behave differently. Hence, accurate monitoring of the optical purity of pharmaceutical products is very essential in order to prevent undesirable toxic and physiological effects. Guidelines of the U.S. Food and Drug Administration in 1992 state that only one enantiomer can be brought to the market. It is very important to study the pharmacological effects and metabolic pathways of each enantiomer separately. This drives the necessity to separate the enantiomers, even though it has been recognized as one of the most difficult problems in chemical analysis (5).

1.3 History of Chiral Separation

Chiral resolution is very essential in the pharmaceutical, agricultural and food industries. Because enantiomers have similar physical and chemical properties, the

separation of chiral compounds is very hard to achieve and expensive. Enantiomer separations can be done indirectly by using derivatizing reagents to form diastereomeric derivatives. Because of the differences in the physical properties of the diastereomers, they can be separated by fractional distillation or by using a normal achiral chromatographic column. The separated compounds are then reacted with an appropriate reagent to get the enantiomers back. However, the indirect method approach has certain disadvantages, including multiple steps are involved, the chiral derivatization reagent has to be optically pure and one must be very careful that no racemization takes place during the reaction, thus failing to give a total separation of the enantiomers. Alternatively, a more efficient and widespread chromatographic method is to separate the enantiomers directly using chiral selectors, which can be incorporated either in the stationary phase or the mobile phase. Though a chiral selector in the mobile phase gives good results in many cases, it is cost-intensive when using expensive reagents. On the other hand, separations achieved by chiral stationary phases (CSPs), where the chiral selector is adsorbed or chemically bonded to a support material in the column are more elegant and convenient. The development of methods for chiral separation has attracted considerable attention during the past two decades.

Successful separations have been performed by various chromatographic methods such as gas chromatography (GC), high performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC), and thin layer chromatography (TLC). More recently, capillary electrophoresis (CE) and capillary electrochromatography (CEC) have been shown to be powerful alternatives to chromatographic methods but they are

presently limited to the analytical scale. GC and SFC require high temperature to operate and this may cause racemization or decomposition of chiral compounds. TLC is less reproducible. As a consequence, HPLC is a promising chromatographic technique for the separation of enantiomers on both preparative and analytical scales (6).

1.4 Chromatographic Chiral Separation by HPLC

1.4.1 High Performance Liquid Chromatography

High-performance liquid chromatography is the most popular chromatographic technique because of its sensitivity, adaptability to accurate quantitative determinations, reproducibility, its ability to separate nonvolatile species or thermally fragile ones, and above all its applicability to a wide range of materials such as amino acids, proteins, nucleic acids, hydrocarbons, carbohydrates, drugs, terpenoids, pesticides, antibiotics, and steroids. In all chromatographic methods, the components of a sample mixture are separated based on their distribution between a mobile phase and a stationary phase.

HPLC is classified into two types based on the relative polarities of the mobile phase and the stationary phase: (a) normal phase, where the mobile phase is non-polar and stationary phase is polar and (b) reverse phase, where the mobile phase is polar and the stationary phase is non-polar (7).

The simplest HPLC system is shown in Figure 4. It consists of a solvent reservoir, pump, injector, packed analytical column, detector, data recorder and waste reservoir. The mixture to be analyzed is dissolved in a suitable solvent, injected at one end of the column and carried through the column by a continuous flow of the mobile phase. The mobile phase is pumped through the column using a high pressure pump.

Figure 4. Diagram showing the components of HPLC instrument.

The separation takes place in the column, which contains a solid separating material also referred to as the stationary phase. Sample components reversibly interact with the stationary phase in a continuous manner. With the selection of the proper mobile phase and stationary phase, some components of the mixture will travel through the column more slowly than others. The separated components pass through the detector and the electrical response from the detector is sent to a recorder. The chromatogram is a record of the detector response as a function of time and indicates the presence of components as peaks (8).

1.4.2 Chiral Recognition Mechanism

The fundamental mechanism for chiral recognition is the “three-point rule” proposed by Dalglish in 1952, which postulates that three interactions have to take effect and at least one of them has to be stereoselective. The three-point contact model is shown in Figure 5 (6).

1.4.3 Chiral Stationary Phases

Chiral stationary phases (CSPs) are classified according to the type of solute-chiral stationary phase complexes formed and the interactions present between enantiomers and the CSP. The different types of stationary phases are as follows:

1.4.3.1 Polysaccharide-Based Chiral Stationary Phases

Polysaccharides are chiral and optically active. Among the various polymers of polysaccharides, cellulose and amylose are the best for chiral resolution. Cellulose is made up of polymeric chains of D- (+) glucose units with β -1, 4 linkages.

Figure 5. Three-point interaction model (Reprinted with permission from the copyright owner: Springer., 2004, Heidelberg, Germany (6)).

The glucose units have a chair conformation with the 2-OH, 3-OH, and the 5-CH₂OH groups all in equatorial positions. The structure of cellulose is shown in Figure 6. The degree of polymerization is not known exactly, but it ranges from 200 to 14,000 units of glucose.

At the molecular level, the chiral resolution mechanism is still unclear due to the difficulties associated with spectroscopic studies. Hasse and Hagel, and later Francotte et al., proposed an inclusion mechanism by which racemic compounds may be adsorbed in the chiral grooves of the cellulose matrix. It has been found that the main chiral sites of bonding are polar carbonyl groups of esters, which can interact with enantiomers through hydrogen bonding and dipole-dipole interactions for chiral discrimination. Recently, Aboul-enein and Ali observed that π - π interactions are crucial for the chiral resolution of aromatic racemates. Structures of polysaccharide-based derivatives like cellulose and amylose have chiral grooves on these chiral stationary phases. Electronegative atoms like nitrogen, oxygen and halogens of the enantiomers form hydrogen bonds. Also dipole-dipole interactions with the polysaccharide-based chiral stationary phase are responsible for enantioseparation. In addition, π - π interactions also exist between the phenyl rings of aromatic racemates and the CSP. Besides, steric effects also control chiral resolution on polysaccharide-based chiral stationary phases. In addition to these, weak achiral bonds like van der Waal forces and ionic bonds are also responsible for chiral resolution. The graphical representation of chiral resolution on a polysaccharide-based CSP, which have different interactions of the two enantiomers, is shown in Figure 7(9).

Figure 6. The chemical structure of cellulose.

Figure 7. Graphical representation of the mechanism of chiral resolution on a polysaccharide-based chiral stationary phase. (Reprinted with permission from the copyright owner: Marcel Dekker Inc., 2003, New York, U.S.A. (9)).

Figures 8 and 9 show the structure of chiral stationary phases that were selected for study in this research. The enantioselectivity of (acrylamidomethyl) cellulose acetate propionate and (acrylamidomethyl) cellulose acetate butyrate on various chiral compounds was investigated in this work.

1.4.3.2 Cyclodextrin-Based Chiral Stationary Phases

Schardinger identified three different naturally occurring forms of cyclodextrin (CD) and referred them as Schardinger's sugars, cyclohexamylose (α -CD), cycloheptamylose (β -CD), and cyclooctamylose (γ -CD). The three types of CD: α -, β -, γ - contain six, seven, and eight glucopyranose units, respectively. The interior of the cavity is hydrophobic and the exterior is hydrophilic. The chemical structure of CD is shown in Figure 10.

Chiral recognition is achieved due to the presence of stereospecific glucopyranose units and restricted conformational freedom of the secondary hydroxyl groups. Hence, diastereomeric inclusion complexes are formed by the two enantiomers. Inclusion complex stability is controlled by hydrogen bonding, interactions between dipoles and induced dipoles, and those of π - π forces. Not only these but other forces like van der Waals, ionic interaction, and solvation effect, also are responsible for chiral resolution. The chiral resolution strategies on CD phases may be predicted theoretically because of their well-known structures and sizes, which makes experiments inexpensive and less laborious. These phases have no serious limitation except that the exact chiral recognition mechanism should be evaluated (9).

Figure 8. Structure of (acrylamidomethyl) cellulose acetate propionate.

Figure 9. Structure of (acrylamidomethyl) cellulose acetate butyrate.

Figure 10. The chemical structure of cyclodextrin (Reprinted with permission from the copyright owner: Marcel Dekker Inc., 2003, New York, U.S.A. (9)).

1.4.3.3 Macrocyclic Glycopeptide Antibiotics-Based Chiral Stationary Phases

The concept of utilizing a macrocyclic glycopeptide as the CSP was introduced by Armstrong in 1994. The chemical structure of vancomycin is shown in Figure 11. Vancomycin is very effective for the enantiorecognition of anionic compounds containing carboxylic groups in their structure. The selectivity towards these compounds is due to the presence of amino groups in vancomycin. It has 3 cavities denoted as A, B, C and 18 chiral centers. Five aromatic rings and hydrogen donor and acceptor sites are available close to the ring structures.

Enantioselectivity of these antibiotics is possible because of functional groups like carboxylic, hydroxyl, amino, and amide, which are responsible for the ionization in buffer with various pK_a s. The possible interactions responsible for enantioselectivity may be π - π complexation, hydrogen bonding, inclusion complexation, dipole interactions, steric interactions, anionic and cationic bindings (9).

1.4.3.4 Pirkle-type and Related Chiral Stationary Phases

Mikes et al. in 1976, attached small chiral molecules to silica gel and the organic groups of the bonded moiety remained directed away from the silica gel. This chiral stationary phase looked like a brush; hence, it was called a brush-type phase. Pirkle and co-workers developed these types of CSP extensively and now these CSPs are called Pirkle-type CSPs. Depending on the nature of the group attached to the chiral molecule these CSPs are classified into three groups i.e., π -acidic (with electron-acceptor groups), π -basic (with electron-donor groups), π -acidic-basic (with electron-acceptor and -donor groups). A general structure of a Pirkle-type CSP is shown in Figure 12.

Figure 11. The chemical structure of vancomycin.

Figure 12. Graphical representation of a Pirkle-type CSP. (Reprinted with permission from the copyright owner: Marcel Dekker Inc., 2003, New York, U.S.A. (9)).

Pirkle type CSPs possess a chiral moiety having a phenyl ring and, hence, π - π charge-transfer diastereoisomeric complex formation of the enantiomers is very important. Therefore, it is necessary to choose π -acidic CSPs for the chiral resolution of π -donor solutes and vice versa. The newly developed CSPs having both π -acidic and π -basic groups are ideal for the chiral resolution of both types of solutes. Interactions responsible for chiral resolution on Pirkle-type CSPs are π - π interactions, hydrogen bonding, dipole-dipole forces, and steric effects. In these phases, the chiral moiety can be bonded selectively and, hence, these CSPs can be prepared as per the requirement of chiral resolution. However, chiral resolution on these CSPs require pre-derivatization of some racemic compounds, so enantiomeric resolution of biological samples is very limited (9).

1.4.3.5 Protein-based Chiral Stationary Phases

Enantioselectivity of a protein is possible because of different types of loops/grooves present in the protein molecule formed as a result of different intramolecular bonding. α_1 -Acid glycoprotein (AGP) or orosomucoid is a human plasma protein with 181 amino acids and is acidic in nature. Interactions involved in the chiral recognition on protein CSPs are non-polar, dipole interactions, hydrogen-bonding, and steric effects. Sometimes chiral loops on a protein are also responsible for enantiomeric resolution. Important structural features with interaction forces, identified by a qualitative structure relationship, for the binding of the basic drug (phinaramine) to the AGP protein, are shown in Figure 13. The use of a wide range of mobile phases makes them ideally suitable CSPs.

Figure 13. Structural features of the binding of a pheniramine molecule on AGP protein in the binding site. Hydrophobic moieties provide anchoring to the drug in the lipophilic region(s) of the site. (Reprinted with permission from the copyright owner: Marcel Dekker Inc., 2003, New York, U.S.A. (9)).

However, they are not suitable for preparative scale because of low loading capacity (9).

1.5 Synthesis of Chiral Stationary Phases

1.5.1 Silica Surface

The inorganic silica network (SiO_2) is the most commonly used base material because of its beneficial properties. Spherical particles of silica are available in a variety of particle diameters (typically 3, 5, or 10 μm) and pore sizes (typically 60-300 Å). In this study AstrosilTM silica was used. The mechanical strength of silica allows porous particles with large surface areas (typically 60-500 m^2/g), which can withstand high mobile phase pressures. The silica surface can be easily modified because of the presence of reactive SiOH sites.

In spite of its advantages there are several properties of silica that limit its usefulness. Silanol groups are weakly acidic and can interact with polar compounds through hydrogen-bonding and dipole-dipole interactions resulting in peak tailing and loss of chromatographic resolution. There are several types of surface silanols: isolated silanols, internally hydrogen-bonded vicinal silanols, and geminal silanols, which are shown in Figure 14 (10).

In chiral separations, the silanol groups may cause non-enantioselective interactions with the chiral solutes resulting in the reduction or loss of enantiomeric resolution.

Figure 14. The types of surface silanols present on silica gel. (Reprinted with permission from the copyright owner: Marcel Dekker Inc., 1998, New York, U.S.A. (10)).

1.5.2 Surface Modification

Chromatographers have tried different synthetic methods to chemically bond organic moieties onto the silica surface. The main goal of these developments is to decrease the polarity of the surface, which may overcome problems like peak tailing with the use of basic solutes, pH stability and irreproducibility.

The most primitive one is esterification of the silanol group by an alcohol as shown in Figure 15a. This reaction results in the formation of a silicon-oxygen-carbon (Si-O-C) linkage, which is hydrolytically unstable. This modified silica stationary phase cannot be used for aqueous mobile phases (11).

Organosilanization is another method used for silica surface modification, which is shown in Figure 15b. In this reaction an organosilane reagent is allowed to react with the silanols. This reaction yields a siloxane (Si-O-Si-C) linkage. An organosilane reagent with a single reactive group results in the formation of monomeric stationary phase, which is unstable at pHs lower than 2 or greater than 8. An organosilane reagent with three reactive groups results in the formation of a polymeric stationary phase, but it is hard to control the polymerization process. For this reason, stationary phase thickness is not reproducible and also column efficiency is reduced (12).

The third method involves chlorination of the silica surface with thionyl chloride followed by the attachment of the organic moiety onto the surface via a Grignard reagent or organolithium compound as shown in Figure 15c. This reaction yields direct Si-C linkage, which is hydrolytically stable. However, this is a two-step synthesis, which is more difficult to carry out when compared to the one-step organosilanization procedure.

This chlorination reaction method also produces undesirable side-products such as MgClBr or LiCl. These residual salts can often become entrapped in the silica matrix, which are difficult to remove (12).

Sandoval and Pesek introduced the silanization/hydrosilation process to overcome difficulties associated with the use of organosilane reagents. The first step, the silanization process, involves the reaction of the silica surface with triethoxysilane (TES) in the presence of water, an acid catalyst and an appropriate solvent to yield an Si-H monolayer. This step is followed by hydrosilation, which involves the catalytic addition of a terminal olefin to the surface silica hydride as shown in Figure 15d (12,13). The most commonly used catalyst is a solution of chloroplatinic acid hexahydrate ($\text{H}_2\text{PtCl}_6\cdot 6\text{H}_2\text{O}$) dissolved in 2-propanol (Speier's catalyst).

There are two disadvantages associated with Speier's catalyst: (1) the intermediate species can be of large size which may result in a low yield, and (2) the reaction between the hydride and the metal can result in the deposition of the Pt (0) on the surface.

One possible solution to both problems is the use of a free radical initiator compound such as tert-butyl peroxide (14,15). In this research the hydrosilation reaction is carried out using both Speier's catalyst and a free-radical catalyst as shown in Table 1. Therefore the chiral selectors chosen possess a terminal olefin as shown in Figures 8 and 9.

Figure 15a. Esterification of silanol group.

Figure 15b. Organosilanization (Y = Si or H, X=halogen)

Figure 15c. Modification by chlorination /organometallic reaction.

Figure 15d. TES silanization and hydrosilation.

Table 1. Chiral stationary phases synthesized via silanization/hydrosilation process.

Chiral Selector			Catalyst used
(Acrylamidomethyl) cellulose acetate butyrate			Speier's catalyst
(Acrylamidomethyl) cellulose acetate butyrate			tert-butyl peroxide
(Acrylamidomethyl) cellulose acetate propionate			Speier's catalyst
(Acrylamidomethyl) cellulose acetate propionate			tert-butyl peroxide

1.6 Aims of this work

The main goal of this project is to synthesize chiral stationary phases (CSPs) and to study their applications for enantiomeric separations by HPLC. This research is divided into three parts: (a) Synthesis of the chiral stationary phases via the silanization/hydrosilation process developed by Pesek et al. The chiral selectors that were chosen for study are (acrylamidomethyl) cellulose acetate butyrate (CAB) and (acrylamidomethyl) cellulose acetate propionate (CAP). The structures of the organic moieties chosen are shown in Figures 8 and 9. (b) Characterization of modified silica surface was done using the spectroscopic techniques cross polarization magic angle spinning nuclear magnetic resonance spectroscopy (^{13}C CP-MAS NMR) and diffuse reflectance infrared Fourier transform spectroscopy (DRIFT). Characterization based on the results of Neue test (18) and elemental analysis is also an important aspect of this work. (c) The final goal is the chromatographic evaluation of the newly synthesized CSPs for the separation of labetalol, terbutaline, 2,2,2- trifluoro-1-9-(9-anthryl) ethanol, 1,1'-bi-2-naphthol, tropicamide, quinine, DL-homatropine and 2-phenyl-1,2-propanediol.

2. EXPERIMENTAL

2.1 Chemicals

Table 2. List of chemicals used in this study.

Name	Source	CAS Number
Terbutaline	Sigma	[23031-32-5]
(R)-(-)-2,2,2-Trifluoro-1-(9-anthryl) ethanol	Aldrich Chemicals	[53531-34-3]
(S)-(+)-2,2,2-Trifluoro-1-(9-anthryl) ethanol	Aldrich Chemicals	[60646-30-2]
(S)-(-)-1,1'-Bi-2-naphthol	Aldrich Chemicals	[18531-99-2]
(R)-(+)-1,1'-Bi-2-naphthol	Aldrich Chemicals	[18531-94-7]
Tropicamide	Sigma	[1508-75-4]
Quinine	Aldrich Chemicals	[130-95-0]
DL-Homatropine	Sigma	[51-56-9]
2-phenyl-1,2-propanediol	Aldrich Chemicals	[4217-66-7]
Uracil	Matheson Coleman & Bell	[66-22-8]
Naphthalene	Sigma	[91-20-3]
Acenaphthene	Sigma	[83-32-9]
Amitriptyline	Sigma	[549-18-8]

2.2 Materials for Synthesis of Bonded Phases

All the stationary phases were prepared using Astrosil™ silica (Stellar Phases, Langhorne, Pennsylvania) having a pore size of 105 Å, a particle diameter of 4.2 µm and a surface area of 340 m²/gram. The silica was modified by 2.3 M HCl (Fisher Scientific) and triethoxy silane (Aldrich Chemical Co, Milwaukee, Wisconsin). One of the chiral selectors (acrylamidomethyl) cellulose acetate butyrate (Lot # 431060) was purchased from Aldrich Chemical Co. (Milwaukee, Wisconsin). The other chiral selector (acrylamidomethyl) cellulose acetate propionate (Lot # 431079) was also purchased from Aldrich Chemical Co. (St. Louis, Missouri). Both of the chiral selectors are relatively new products from Aldrich Chemical Co., which are not yet fully tested. Two catalysts, hexachloroplatinic acid and tert-butyl peroxide were supplied by Aldrich Chemical Co. (Milwaukee, Wisconsin). Deionized water was obtained from a Milli-Q™ purification system (Millipore Corp., Bedford, Massachusetts). The HPLC grade solvents methyl ethyl ketone, toluene, propanol and isopropanol were obtained from J.T. Baker Chemical Company (Phillipsburg, New Jersey). Other solvents used are tetrahydrofuran and chloroform from EM Science (Gibbstown, New Jersey), methanol and ethanol from Spectrum Chemical Co. (Gardena, California) and 1-butanol from Sigma Aldrich Chemical Co. (Milwaukee, Wisconsin) were all reagent grade.

2.3 Instrumentation

2.3.1 Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFT)

Diffuse reflectance infrared Fourier transformation (DRIFT) spectra of all the bonded phases and the silica hydride were run on a ATI Mattson Infinity Series FTIR™

spectrometer equipped with a deuterated triglycine sulfate (DTGS) detector and controlled by a digital Venturis Fx-2 series computer with WinFIRSTTM software. An EasiDiff diffuse-reflectance accessory with a 1.6 mm deep sampling cup was used for analysis. The DRIFT sample was prepared by adding 5 mg of KBr to 100 mg of the sample. This sample mixture was finely ground and transferred into the sample cup. The sample was pressed with a slide in order to obtain a smooth surface. The sample compartment was purged with nitrogen for 15 min to remove CO₂ and water. Spectra were recorded in the mid-infrared region of 4000-400 cm⁻¹ and averaged over 100 scans. The reference standard was KBr.

2.3.2 Cross-Polarization Magic Angle Spinning Nuclear Magnetic Resonance

Spectroscopy (CP-MAS NMR)

In solid-state NMR lines are much broader than in solution NMR due to dipolar coupling and chemical shift anisotropy. Dipolar coupling results from the number of interactions, which occur due to the presence of group of spins around a given spin. Chemical shift anisotropy arises from asymmetry in the electron density surrounding a given nucleus. The signal-to-noise ratio is also poor for ¹³C nuclei because of their low natural abundance. Line broadening caused by chemical shift anisotropy can be eliminated by a technique called magic-angle-spinning (MAS) where a solid sample is rotated rapidly (>2 KHz) in an air-driven rotor at an angle of 54.87 ° with respect to the applied field. Dipolar broadening can be avoided by the cross polarization (CP) technique where energy absorbed by ¹H nuclei is transferred to ¹³C nuclei. For the above

reasons solid state NMR was performed with a combination of cross polarization (CP) and magic angle spinning (MAS).

All ^{13}C CP-MAS NMR spectra were recorded on a Varian Inova 400 NMR Spectrometer at a spinning rate of 5000 Hz. Solid samples of 100-150 mg were placed in a ZrO_2 rotor and the spectra were obtained with a cross polarization contact time of 5 ms, a pulse interval of 5 sec and a pulse width of 6.5 μs . The chemical shifts in the ^{13}C spectra were referenced to tetramethylsilane using external glycine as the standard.

2.3.3 Elemental Analyzer

The bonded phase samples were sent to Desert Analytics (Tucson, Arizona) for the analysis. The carbon percentage of the synthesized bonded phases was determined using the micro-combustion method. The carbon percentage of the bonded phases provides quantitative information about the amount of organic moiety bonded on the silica surface.

2.3.4 Column Packing

In the column packing procedure, about 1.8 g of the bonded silica was mixed with isopropanol to form a uniform dispersion. This slurry was added slowly to the reservoir and the remaining volume of the reservoir was filled with HPLC grade methanol. The mixture in the reservoir was packed into the column in the presence of nitrogen under 6000 psi pressure. The column remained attached to the packing apparatus for about 30 minutes before removal.

2.3.5 High Performance Liquid Chromatography (HPLC)

Two HPLC systems were used to test the chiral resolution capability of the newly synthesized chiral stationary phases. All the normal phase analyses were done on a Hitachi (Tokyo, Japan) system which consists of a tertiary pumping system, a Hitachi L-6200, where the mobile phases are mixed and pumped through the column to the detector. The back pressure was maintained below 1700 psi. The samples were injected through a Rheodyne model 7125NS manual injector with a 20 μ L sample loop and were detected by a Hitachi model L-4000 UV detector. All the measurements were recorded on a Chromatojet integrator manufactured by Spectra-Physics (San Jose, California).

In the reverse phase mode, a Milton Roy (Riviera Beach, Florida) liquid chromatograph system equipped with a Milton Roy CM 4000 multiple solvent delivery system and a Milton Roy programmable SM 4000 UV detector was used. The samples were injected through a Rheodyne (Cotati, California) model 7125 manual injector with a 20 μ L sample loop. The backpressure was maintained below 1800 psi.

2.4 Synthetic Procedures

2.4.1 Preparation of Silica Hydride

The preparation of the silica hydride intermediate was carried out according to a method previously described by Pesek et al., (16). All the glassware was dried overnight at 110 °C. Prior to the synthesis, 15.6 g of AstrosilTM silica was weighed and dried overnight at 120 °C in a vacuum oven. The dried AstrosilTM silica was transferred to a 1000 mL three necked round bottom flask equipped with a thermometer, a condenser with a drying tube, an addition funnel with an equalizing tube, a stopper, a magnetic stir

bar, a magnetic stirrer and a heating mantle. Then 624 mL of dioxane was added followed by 7.64 mL of 2.3 M HCl solution and the reaction mixture was heated to 70 °C. Once the temperature was constant, 93.76 ml of dioxane was transferred into the addition funnel equipped with a stopper. Later, under argon, 21.68 mL of triethoxysilane (TES) was added slowly to the addition funnel containing dioxane. The prepared TES/dioxane mixture was then added dropwise into the RB flask over a period of 30-35 minutes with constant stirring. The temperature was then raised to 90 °C and reflux was continued for 90 minutes. After a cooling period, the reaction mixture was carefully transferred into a filter crucible attached to a filtering flask and the product was filtered using vacuum suction. The product was washed three times each with of dioxane, toluene and diethyl ether. The final product was dried in the hood at room temperature overnight to evaporate the ether and finally dried in a vacuum oven at 110 °C for 24 hours.

2.4.2 Solubility Test

Chiral stationary phases chosen for this study are newly synthesized and only limited information was available from the manufacturer. For the hydrosilation reaction to take place it was necessary to find the appropriate solvent where the chiral selectors dissolve completely. To test the solubility of the chiral selectors 1 mg of compound was placed in 1 ml of solvent. The organic compound solubility was tested in the following solvents: methylethyl ketone, toluene, chloroform, dioxane, tetrahydrofuran and methanol. Of all the solvents used to dissolve the chiral selectors, methylethyl ketone was the best. Solubility test results are shown in Table 3.

Table 3. Solubility Test Results.

Solvent Used	(Acrylamidomethyl) Cellulose acetate propionate	(Acrylamidomethyl) Cellulose acetate butyrate
Methyl Ethyl ketone	Soluble	Soluble
Toluene	Does not dissolve	Does not dissolve
Chloroform	Does not dissolve	Does not dissolve
Dioxane	Soluble	Does not dissolve
Tetra Hydro Furan	Soluble	Does not dissolve
Methanol	Soluble	Does not dissolve

2.4.3 Preparation of Chiral stationary phases

Hydrosilation either by Speier's catalyst or by free-radical addition is possible because the chiral selectors chosen for this research have a terminal double bond (14,17).

2.4.3.1 Synthesis of (Acrylamidomethyl) cellulose acetate propionate bonded phase using Speir's catalyst.

All the glassware was dried overnight at 110 °C in the oven and the Astrosil™ silica hydride was dried overnight in a vacuum oven at 120 °C. The experimental set up consisted of a 500 mL three necked round bottom flask equipped with a thermometer, a condenser with a drying tube, an addition funnel with an equalizing tube, a stopper, a magnetic stir bar, a magnetic stirrer and a heating mantle. In this synthesis, 4.5 g of (acrylamidomethyl) cellulose acetate propionate was dissolved in 130 mL of methyl ethyl ketone, and 1732 µL of 10 mM hexachloroplatinic acid in 2-propanol (Speier's catalyst) was added to the flask. The mixture was then heated to 60 °C for about an hour and 3 g of

dried AstrosilTM silica hydride was added to the flask. The system was then flushed with a slow stream of nitrogen gas. The temperature of the reaction mixture was then raised to 70 °C. The reaction was allowed to continue at the same temperature for 96 h. After the reaction was complete, the final product was filtered and washed four times with methyl ethyl ketone and four times with diethyl ether and dried in the hood at room temperature and in a vacuum oven at 110 °C for 24 hours.

2.4.3.2 Synthesis of (acrylamidomethyl) cellulose acetate propionate bonded phase using free radical tert-butyl peroxide.

A mixture of 162 mL of methyl ethyl ketone, 3.15 g of (acrylamidomethyl) cellulose acetate propionate and 391 µL of 10 mM hexachloroplatinic acid in 2-propanol were added to a 500 mL round bottom flask. The mixture in the round bottom flask was heated at a constant temperature of 60 °C for about an hour along with magnetic stirring. After the solution became clear, 3 g of dried AstrosilTM silica hydride was added slowly for 15-20 minutes through the open neck of the flask and the neck was then closed. The temperature of the reaction mixture was raised to 70 °C. The reaction was allowed to continue at the same temperature for 96 h. The final product was then filtered and washed 4 times with methyl ethyl ketone (30 ml each time) and one time with (60 mL) diethyl ether and dried at room temperature for at least five hours and overnight in the oven.

2.4.3.3 Synthesis of (acrylamidomethyl) cellulose acetate butyrate bonded phase using Speier's catalyst.

In the preparation, 4.5 g of (acrylamidomethyl) cellulose acetate butyrate was dissolved in 130 mL of methyl ethyl ketone, and 1732 μ L of 10 mM hexachloroplatinic acid in 2-propanol was added to the flask. The mixture was heated at a constant temperature of 60 °C for about an hour along with magnetic stirring. Three grams of dried AstrosilTM silica hydride was added slowly for 15-20 minutes through the open neck of the flask and then the neck was closed. The temperature of the reaction mixture was then raised to 70 °C. The reaction was allowed to continue at the same temperature for 96 h. After the reaction was complete, the final product was filtered and washed four times with methyl ethyl ketone and four times with diethyl ether and dried at room temperature for five hours and overnight in the vacuum oven.

2.4.3.4 Synthesis of (acrylamidomethyl) cellulose acetate butyrate bonded phase using the free radical tert-butyl peroxide.

A mixture of 162 mL of methyl ethyl ketone, 3.15 g of (acrylamidomethyl) cellulose acetate butyrate and 391 μ L of 10 mM hexachloroplatinic acid in 2-propanol were added to a 500 mL round bottom flask. The mixture in the round bottom flask was heated at a constant temperature of 60 °C for about an hour along with magnetic stirring. After the solution becomes clear 3 g of AstrosilTM silica hydride was added slowly for 15-20 minutes through the open neck of the flask and then the neck was closed. The temperature of the reaction mixture was raised to 70 °C. The reaction was allowed to continue at the same temperature for 96 h. The final product was then filtered and washed

4 times with methyl ethyl ketone (30 ml each time) and one time with (60 mL) diethyl ether and dried at room temperature for five hours and overnight in the oven.

2.4.3.5 Repetition of synthesis of (acrylamidomethyl) cellulose acetate propionate bonded phase using Speier's catalyst.

The procedure is same as described in the section 2.4.3.1 except that the chiral selector amount was decreased from 4.5 g to 3 g and the adjusted amount of Speier's catalyst was 1155 μ L (previously it was 1732 μ L).

All the glassware was dried overnight at 110 °C in the oven and the AstrosilTM silica hydride was dried overnight in a vacuum oven at 120 °C. The experimental set up consisted of a 500 mL three necked round bottom flask equipped with a thermometer, a condenser with a drying tube, an addition funnel with an equalizing tube, a stopper, a magnetic stir bar, a magnetic stirrer and a heating mantle. In this synthesis, 3 g of (acrylamidomethyl) cellulose acetate propionate was dissolved in 130 mL of methyl ethyl ketone, and 1155 μ L of 10 mM hexachloroplatinic acid in 2-propanol (Speier's catalyst) was added to the flask. The mixture was then heated to 60 °C for about an hour and 3 g of dried AstrosilTM silica hydride was added to the flask. The system was then flushed with a slow stream of nitrogen gas. The temperature of the reaction mixture was then raised to 70 °C. The reaction was allowed to continue at the same temperature for 96 h. After the reaction was complete, the final product was filtered and washed four times with methyl ethyl ketone and four times with diethyl ether and dried in a hood at room temperature and in a vacuum oven at 110 °C for 24 hours.

3. RESULTS AND DISCUSSION

In this research study, cellulose based chiral stationary phases were synthesized using the silanization/hydrosilation method. The hydrosilation reaction was carried out using both a platinum catalyst and a free radical catalyst. The success of the bonding process was evaluated by elemental analysis, DRIFT spectroscopy and CP-MAS NMR spectra. Hydrophobicity and silanophilic activity of the bonded phases were tested using Neue test. Chromatographic studies on the successfully synthesized chiral stationary phases were carried out in both the normal phase and reverse phase modes.

3.1 DRIFT Spectroscopic Evaluation

DRIFT Spectroscopy is used to confirm the formation of silica hydride and also to study the bonding of the organic group on the silica hydride surface.

3.1.1 AstrosilTM silica hydride

AstrosilTM silica hydride was prepared according to the silanization procedure developed by Pesek et al., where AstrosilTM silica was converted to AstrosilTM hydride (16). The DRIFT spectrum of silica hydride is shown in Figure 16. The strong band at 2250 cm^{-1} corresponds to the Si-H stretching vibration, which confirms the formation of AstrosilTM hydride. The broad band between $3800 - 3000\text{ cm}^{-1}$ is due to hydrogen-bonded silanols and adsorbed water. The other peaks in the spectrum are due to various fundamental vibrations of the silica matrix (16).

Figure 16. DRIFT spectrum of Astrosil™ silica hydride.

3.1.2 (Acrylamidomethyl) cellulose acetate butyrate bonded phase

(Acrylamidomethyl) cellulose acetate butyrate bonded phase was prepared by the hydrosilation process using both free radical catalyst and hexachloroplatinic acid catalyst. DRIFT spectra of (acrylamidomethyl) cellulose acetate butyrate bonded phase prepared by free radical and hexachloroplatinic acid catalyst are shown in Figures 17 and 18. The presence of a C-H stretching band at 3000 cm^{-1} indicates the success of bonding of the (acrylamidomethyl) cellulose acetate butyrate to the silica hydride surface. In addition to this the Si-H stretching band at 2250 cm^{-1} was reduced in intensity after the hydrosilation reaction. This decrease in intensity further indicates the success of bonding of the (acrylamidomethyl) cellulose acetate butyrate to the silica hydride surface.

3.1.3 (Acrylamidomethyl) cellulose acetate propionate bonded phase

The (acrylamidomethyl) cellulose acetate propionate bonded phase was prepared by the hydrosilation process using both free radical catalyst and hexachloroplatinic acid catalyst. DRIFT spectra of the (acrylamidomethyl) cellulose acetate propionate bonded phase prepared by free radical catalyst is shown in Figures 19. The presence of a C-H stretching vibration band at 3000 cm^{-1} indicates the success of bonding of the (acrylamidomethyl) cellulose acetate propionate to the silica hydride surface. In addition to this the Si-H stretching band at 2250 cm^{-1} was reduced in intensity after the hydrosilation reaction. This decrease in intensity further indicates the success of bonding of the (acrylamidomethyl) cellulose acetate propionate to the silica hydride surface.

Figure 17. DRIFT spectrum of (acrylamidomethyl) cellulose acetate butyrate bonded on AstrosilTM silica hydride using Speier's catalyst.

Figure 18. DRIFT spectrum of (acrylamidomethyl) cellulose acetate butyrate bonded on AstrosilTM silica hydride using free radical catalyst.

Figure 19. DRIFT spectrum of (acrylamidomethyl) cellulose acetate propionate bonded on Astrosil™ silica hydride using the free radical catalyst.

The (acrylamidomethyl) cellulose acetate propionate bonded phase synthesized using Speier's catalyst as described in Section 2.4.3.1 of the Experimental resulted in a paper like compound. This may be due to polymerization of the bonded phase. Thus, the experiment was repeated by changing the reaction conditions. The synthesis was repeated but the amount of the (acrylamidomethyl) cellulose acetate propionate and the amount of Speier's catalyst were decreased as described in Section 2.4.3.5 of the Experimental. This time a powder-like end product was obtained. A DRIFT spectrum of (acrylamidomethyl) cellulose acetate propionate bonded phase prepared by Speier's catalyst is shown in Figure 20. Though a C-H stretching band at 3000 cm^{-1} is present in the DRIFT spectrum of the (acrylamidomethyl) cellulose acetate propionate bonded phase prepared by Speier's catalyst (Fig. 19), it is less intense when compared to the C-H stretching peak in the DRIFT spectrum of the (acrylamidomethyl) cellulose acetate propionate bonded phase using the free radical catalyst (Fig. 20). This indicates that the bonding has not taken place as expected. This might be explained by the bulkiness of the (acrylamidomethyl) cellulose acetate propionate molecule, which might have formed very large platinum intermediate complex that hindered the reaction. For this reason, the (acrylamidomethyl) cellulose acetate propionate bonded phase prepared by Speier's catalyst was not used for further studies in this research work.

3.2 Elemental Analysis Results

Table 4 shows the carbon percentage values for all the bonded phases synthesized. These values indicate quantitatively how well the silica surface was modified by the chiral selectors.

Figure 20. DRIFT spectrum of (acrylamidomethyl) cellulose acetate propionate bonded on AstrosilTM silica hydride using Speier's catalyst.

A higher carbon percentage value means more of the organic moiety has been successfully bonded to the surface and thus better performance will be expected. The (acrylamidomethyl) cellulose acetate butyrate bonded phase prepared using Speier's catalyst has a higher carbon percentage value when compared to the other two bonded phases as seen in Table 4.

Table 4. Elemental analysis results.

Column	Catalyst	%C
CAB	Speier's	10.85
CAB	t-butyl peroxide	0.65
CAP	t-butyl peroxide	0.48

Columns:

CAB = (Acrylamidomethyl) cellulose acetate butyrate

CAP = (Acrylamidomethyl) cellulose acetate propionate

However, even this carbon percentage value is not unreasonable because the substituted cellulose molecule is a very large sugar molecule with a high molecular weight. Free radical hydrosilation using tert-butyl peroxide catalyst did not work well for the chosen chiral selectors. This might be explained by the bulkiness of the molecule near the olefin carbons, which may affect the efficiency of the hydrosilation reaction or formation of the free radical.

3.3 CP-MAS NMR Spectra

3.3.1 (Acrylamidomethyl) cellulose acetate butyrate bonded phase synthesized using Speier's catalyst

The ^{13}C CP-MAS NMR spectrum of the (acrylamidomethyl) cellulose acetate butyrate bonded phase synthesized using Speier's catalyst is shown in Figure 21. The chemical shifts at 11.108 and 15.905 ppm are due to aliphatic carbons. The peak at 33.335 ppm confirms the presence of amino groups. The peak at 60 ppm may be due to an alcoholic group and C-O-C linkages. The chemical shift at 70.015 ppm is due to the carbon near to oxygen in the pyranose ring. The peak at 98.679 ppm confirms the presence of O-C-O linkages. The chemical shift at 169.611 ppm confirms the presence of amide group. The peak at 218.437 ppm is due to aldehydes. All of the above characteristic peaks due to (acrylamidomethyl) cellulose acetate butyrate suggest that this particular chiral selector was bonded to the silica hydride surface.

3.3.2 (Acrylamidomethyl) cellulose acetate butyrate bonded phase synthesized using free radical catalyst

The ^{13}C CP-MAS NMR spectrum of the (acrylamidomethyl) cellulose acetate butyrate bonded phase synthesized using free radical catalyst is shown in Figure 22. The chemical shift at 43.051 ppm confirms the presence of amino group and saturated alkane group. As the spectrum is missing lot of other peaks, which are seen in Figure 21, we can conclude by saying that the bonding has taken place but the extent of reaction is not sufficient. The failure of free-radical hydrosilation is probably due to the bulkiness of the

molecule near the olefin carbons. The presence of bulky groups introduces steric hindrance and thus lowers the efficiency of free radical formation. The ^{13}C CP-MAS NMR spectrum results are consistent with the elemental analysis results.

3.3.3 (Acrylamidomethyl) cellulose acetate propionate bonded phase synthesized using free radical catalyst

The ^{13}C CP-MAS NMR spectrum of the (acrylamidomethyl) cellulose acetate propionate bonded phase synthesized using free radical catalyst is shown in Figure 23. The chemical shifts at -2, 5, 5.6, and 24.6 ppm confirm the presence of saturated hydrocarbons. The peak at 48.1 ppm is due to the presence of amino groups. The chemical shift at 57.9 ppm is due to an alcoholic group and C-O-C linkages. The peak at 70.3 ppm is due to the carbon near to oxygen in pyranose ring. The peak at 98.19 ppm is due to O-C-O linkages. The chemical shift at 172.4 ppm confirms the presence of an amide. As the spectrum is missing some peaks and also the peaks are less intense, when compared to Figure 22, it can be concluded that the bonding has taken place but the extent of the reaction is significantly less. The ^{13}C CP-MAS NMR spectrum results are consistent with the elemental analysis results. The failure of free-radical hydrosilation may be due to the bulkiness of the molecule near the olefin carbons which lowers the efficiency of free radical formation.

Figure 22. ^{13}C CP-MAS NMR spectrum of (acrylamidomethyl) cellulose acetate butyrate bonded on AstrosilTM silica hydride using free-radical catalyst.

3.4 Chromatographic Studies

3.4.1 Neue test

The Neue test is an industrial standard method used by column manufacturers to derive two important physico-chemical properties of packing materials. They are: (a) the hydrophobicity of the packing and (b) the silanophilic activity at pH 7. The solutes used in this test are uracil, naphthalene, acenaphthene, and amitriptyline. Uracil is used as the marker to determine the void volume for the column. Naphthalene and acenaphthene are hydrophobic compounds whereas amitriptyline is a basic compound. The structures of the solutes used for this test are shown in Figure 24.

The analysis was carried out in the isocratic mode at neutral pH using a 20 mM K_2HPO_4/KH_2PO_4 buffer:methanol (35:65). UV detection was set at 254 nm and the injection volume was 20 μ L. In order to extend column life, they were flushed with water for 30 minutes at a flow rate of 0.2 mL/min followed by methanol for 30 minutes at a flow rate of 0.1 mL/min at the end of the day. Table 5 shows the retention times of the solutes. (Acrylamidomethyl) cellulose acetate butyrate bonded phase synthesized using platinum catalyst showed good retention of naphthalene and acenaphthene. On the other hand, (acrylamidomethyl) cellulose acetate butyrate and (acrylamidomethyl) cellulose acetate propionate bonded phases synthesized using free radical catalyst the hydrophobic compounds are eluting before or at void volume with no retention at all as seen in Table 5 and Figures 25-27.

The hydrophobicity parameter (ψ) tells us how well the column will retain non-polar solutes.

Figure 24. Structures of the solutes used for Neue test. (A) uracil (B) amitriptyline (C) acenaphthene (D) naphthalene.

Figure 25. Retention time on the (acrylamidomethyl) cellulose acetate butyrate bonded phase synthesized using free radical catalyst. (A) uracil (B) naphthalene (C) acenaphthene (D) amitriptyline.

Figure 26. Retention time on the (acrylamidomethyl) cellulose acetate propionate bonded phase synthesized using free radical catalyst. (A) uracil (B) naphthalene (C) acenaphthene (D) amitriptyline.

Figure 27. Retention time on the (acrylamidomethyl) cellulose acetate butyrate bonded phase synthesized using platinum catalyst. (A) uracil (B) naphthalene (C) acenaphthene (D) amitriptyline.

The higher the value greater the retention of non-polar solutes. The hydrophobicity parameter (υ) can be calculated using the following equation:

$$\ln(k) = \upsilon \times V - 3.068$$

where V is the molar volume of the analyte (acenaphthene or naphthalene).

The silanophilic interaction parameter (S) measures strong polar/adsorptive interactions with the surface. A lower value indicates less surface adsorption, which results in better peak shape for polar analytes. The silanophilic interaction parameter (S) can be calculated using the following equation:

$$S = \ln(k_{\text{amitriptyline}}) - 0.7124 \times \ln(k_{\text{acenaphthene}}) + 1.9748$$

The hydrophobicity parameter (υ) for naphthalene and acenaphthene and the silanophilic interaction parameter (S) was calculated for three chiral bonded phases and the values are shown in Table 6.

The (acrylamidomethyl) cellulose acetate butyrate bonded phase synthesized using platinum catalyst showed higher hydrophobicity parameter values and lower silanophilic interaction parameter value, when compared to the other two chiral stationary phases prepared using free radical catalyst. As discussed above, the failure of free-radical hydrosilation is probably due to free radical formation. The same conclusion was reached by the results of the DRIFT spectra, elemental analyses and ^{13}C CP-MAS NMR spectra that a very small amount of organic moiety was attached to the silica hydride surface. For this reason, (acrylamidomethyl) cellulose acetate butyrate and (acrylamidomethyl) cellulose acetate propionate bonded phases synthesized using free

radical catalyst were not used in further chromatographic characterization in this research work.

Table 5. Neue test results showing retention times of the solutes.

Column	Catalyst	Solutes	Retention time
CAB	Speier's	Uracil	2.2
		Naphthalene	4.0
		Acenaphthene	5.2
		Amitriptyline	6.7
CAP	t-butyl peroxide	Uracil	1.44
		Naphthalene	1.39
		Acenaphthene	1.38
		Amitriptyline	6.0
CAB	t-butyl peroxide	Uracil	1.35
		Naphthalene	1.30
		Acenaphthene	1.42
		Amitriptyline	4.91

Stationary Phases: CAB = (Acrylamidomethyl) cellulose acetate butyrate

CAP = (Acrylamidomethyl) cellulose acetate propionate

Table 6. Neue test results.

Stationary phases	Catalyst used	Hydrophobicity Parameter (ψ)		Silanophilic Interaction Parameter (S)
		Naphthalene	Acenaphthene	
CAB	Speier's catalyst	0.0155	0.0144	2.602
CAB	t-butyl peroxide	0.0051	0.0042	4.3225
CAP	t-butyl peroxide	-0.0031	-0.001	5.232

Stationary Phases: CAB = (Acrylamidomethyl) cellulose acetate butyrate

CAP = (Acrylamidomethyl) cellulose acetate propionate

3.4.2 Structure of Samples

In order to explore the usefulness of the modified chiral stationary phase the chiral drugs, labetalol, terbutaline, 2,2,2- trifluoro-1-(9-anthryl) ethanol, 1,1'-bi-2-naphthol, tropicamide, quinine, DL-homatropine, and 2-phenyl-1, 2-propanediol were studied in this research. The structures of the drugs are shown in Figure 28.

For the UV detection of terbutaline, 2,2,2-trifluoro-1-(9-anthryl) ethanol (TFAE), and labetalol, the detection wavelength was set at 230 nm, 330 nm, and 308 nm respectively. The UV detection wavelength was set at 254 nm for 1,1'-bi-2-naphthol, tropicamide, quinine, DL-homatropine, and 2-phenyl-1, 2-propanediol. Equilibration of new columns involved flushing with methanol overnight at a flow rate of 0.1 mL/min. In order to extend the column life, in the reversed-phase mode they were flushed with methanol for 30 minutes at a flow rate of 0.1 mL/min and in the normal phase mode the column was flushed with the mobile phase for 30 minutes at a flow rate of 0.1 mL/min at the end of the day.

3.4.3 Chromatographic measurements

The capacity factor, k' , is a measure of how long a solute is retained on the column. The stronger the interaction between the solute and the stationary phase, the longer is the retention. The capacity factor can be calculated from the retention time (t_r) of the solute and the void volume as shown below.

$$k' = (t_r - t_0) / t_0$$

The separation factor (α) is a measure of selectivity of the column for any pair of solutes. In the case of a chiral separation, k_1' and k_2' are defined as the capacity factors for the first and second eluted enantiomers respectively. It can be calculated as follows:-

$$\alpha = k_1' / k_2'$$

3.4.4 Chiral separation of optical isomers in reverse phase mode

Ethanol-water mixtures may cause cellulose derivatives to swell up to 40% of its volume. Acetone, acetonitrile, dimethylformamide, chlorinated alkanes should be avoided as they dissolve cellulose derivatives. THF if used as the mobile phase results in gel formation. Cellulose derivatives swell strongly in pure 1,4-dioxane, or in a 50:50 v/v mixture of dimethoxyethane and toluene-1, 4-dioxane. For the above reasons, choices of a mobile phase in the reversed-phase mode are very limited (9). As lower alcohols are better when compared to higher alcohols methanol was selected for the mobile phase.

Chromatographic measurements were made in the reverse phase mode with various methanol/water compositions. All the chiral solutes were run using 100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20% and 10% methanol as well as with 100% water as the mobile phase. The void volume of the column was determined by injecting uracil, which was dissolved in methanol. The injection volume for all the runs was 20 μ L.

Based on the chromatographic analysis, there were no chiral separations of the drugs under investigation with the (acrylamidomethyl) cellulose acetate butyrate stationary phase synthesized using Speier's catalyst in the reverse phase mode over the range of concentrations of the organic modifier in the mobile phase.

Figure 28. Structures of chiral solutes. (A) terbutaline (B) 2,2,2-trifluoro-1-(9-anthryl) ethanol (C) 1,1'-bi-2-naphthol (D) tropicamide (E) quinine (F) DL-homatropine (G) labetalol (H) 2-phenyl-1, 2-propanediol.

The results of the retention of eight chiral drugs using 11 mobile phase combinations of methanol/water on the cellulose-based stationary phase are used to create retention maps. Retention maps of the chiral drugs are shown in Figures 29 and 30. It is not possible to characterize the behavior of the column based on these retention maps, as they do not follow any general trend. The maximum retention time for labetalol is 18 minutes (Figure 29) with 50% methanol mobile phase. The maximum retention time for quinine is 17 minutes with 70% methanol mobile phase (Figure 30).

Hence, even though an enantiomeric separation has not occurred, the retention times of some chiral drugs suggests a reasonable degree of interaction between the drug and the stationary phase. The chromatogram of R (+)-1,1'-bi-2-naphthol obtained using 40% methanol mobile phase is shown in Figure 31.

R (+)-1,1'-bi-2-naphthol is retained for 16.36 minutes which proves some degree of interaction between the solute and chiral stationary phase. Due to the polarity of the solvent used, successful separation of enantiomers in the reverse phase mode was not achieved. The organic modifier methanol in the mobile phase might have caused swelling of stationary phase, which causes distortion of the (acrylamidomethyl) cellulose acetate butyrate stationary phase three-dimensional structure. Hence, the inclusion mechanism by which racemic compounds adsorb in the chiral grooves of the cellulose matrix may not be possible. Overall, the three point interaction in the reversed-phase mode is not selective enough so, there is no resolution of the optical isomers. As indicated above, choices of a mobile phase in the reversed-phase mode are very limited. Thus, it was decided to continue the research in the normal phase mode.

Figure 29. Retention map of chiral solutes in the reverse phase mode.

Series 1 = labetalol, Series 2 = terbutaline, Series 3 = 2,2,2-trifluoro-1-(9-anthryl) ethanol, Series 4 = 1,1'-bi-2-naphthol.

Figure 30. Retention map of chiral solutes in the reverse phase mode.

Series 1 = tropicamide, Series 2 = quinine, Series 3 = DL-homatropine,

Series 4 = 2-phenyl-1, 2-propanediol.

Figure 31. Chromatogram of R (+)-1,1'-bi-2-naphthol on (acrylamidomethyl) cellulose acetate butyrate synthesized using Speier's catalyst; mobile phase: methanol/water (40:60);

3.4.5 Chiral separation of optical isomers in normal phase mode

The (acrylamidomethyl) cellulose acetate butyrate stationary phase synthesized using Speier's catalyst appears to exhibit better enantiomeric selectivity towards the chiral drugs in the normal phase when compared to that in the reversed phase. The column was conditioned before it was used for normal phase system. The column was washed for 1 hr each with methanol, ethyl acetate, ether, toluene, and hexane. The flow rate was 0.4 or 0.5 mL per minute depending on the backpressure of the column. In order to decrease the polarity of the mobile phase and increase the retention times of the enantiomers hexane was used as one of the main constituents of the mobile phase. The four mobile phases used were: hexane/propanol or butanol or ethanol or 2-propanol in the ratio of 90:10.

The effect of polarity of mobile phase i.e., 2-propanol, 1-butanol, 1-propanol and ethanol on the chromatographic separation of chiral drugs was studied. The polarities of 2-propanol, 1-butanol, 1-propanol and ethanol are 3.9, 3.91, 4.0 and 4.3 respectively. In general, it was expected that greater polarity of alcohol would lead to weaker interaction between enantiomers and the chiral stationary phase. In this study it was observed that greater polarity of the alcohol resulted in stronger interaction between enantiomers and the chiral stationary phase which is the opposite of typical normal phase retention. Hence, the chiral compounds will retain longer in a mobile phase with greater polarity. Possible separation of the optical isomers of terbutaline on the (acrylamidomethyl) cellulose acetate butyrate bonded phase is shown in Figure 32A (hexane/2-propanol) and Figure 32B (hexane/ethanol). As ethanol is more polar than 2-propanol the optical

isomers of terbutaline are retained slightly longer as seen in Figure 32B. The first peak in Figures 32A and 32B eluted before the void volume. This peak may be due to the first eluted enantiomer of terbutaline which indicates there is no interaction between this enantiomer and the chiral stationary phase. Separation of the optical isomers of terbutaline may be possible because it has a phenyl group, which is responsible for π - π interactions. Also, the three hydroxyl groups of terbutaline might be responsible for the hydrogen bonding with the acidic hydrogen on an amine group in the substituted cellulose molecule. In addition, the existence of an acidic hydrogen on an amine group of terbutaline further introduces hydrogen bonding with the carbonyl oxygen of the chiral stationary phase.

The normal phase separation of the optical isomers of quinine on (acrylamidomethyl) cellulose acetate butyrate synthesized using Speier's catalyst is shown in Figure 33A (hexane/ethanol) and Figure 33B (hexane/butanol). As ethanol is more polar than the butanol the second eluted isomer is retained for 22.5 minutes as seen in Figure 33A (11 minutes longer when compared to ethanol (Figure 33B)). The chiral separation of the quinine enantiomers is possible because it has two aromatic rings and a double bond, which are responsible for π - π interactions. Also, there is a hydroxyl group in quinine, which might be responsible for hydrogen bonding with the acidic hydrogen of the amine group in the chiral stationary phase. The ethyl bridge of quinine might possibly interact stereo specifically with the chiral stationary phase as well.

The chiral resolution of optical isomers of tropicamide is shown in Figure 34A (hexane/butanol) and Figure 34B (hexane/ethanol).

Figure 32. Normal phase separation optical isomers of terbutaline on (acrylamidomethyl) cellulose acetate butyrate synthesized using Speier's catalyst; mobile phases: (A) hexane/2-propanol (90:10), (B) hexane/ethanol (90:10).

Figure 33. Normal phase separation of the optical isomers of quinine on (acrylamidomethyl) cellulose acetate butyrate synthesized using Speier's catalyst; mobile phases: (A) hexane/ethanol (90:10), (B) hexane/butanol (90:10).

Figure 34. Normal phase separation of the optical isomers of tropicamide on (acrylamidomethyl) cellulose acetate butyrate synthesized using Speier's catalyst; mobile phases: (A) hexane/butanol (90:10), (B) hexane/ethanol (90:10).

Ethanol is more polar than butanol so the tropicamide enantiomers are retained somewhat longer as shown in Figure 34B. Chiral resolution of the optical isomers of tropicamide is possible because of the existence of two aromatic rings and two relatively bulky ethyl groups attached to the amine moiety which results in more extensive interaction between the solute and the chiral stationary phase. In addition to this, the hydroxyl group of tropicamide is responsible for hydrogen bonding with the carbonyl oxygen in the substituted cellulose molecule. Also, the existence of an acidic hydrogen on an amine group of the chiral stationary phase further introduces hydrogen bonding with the carbonyl oxygen of tropicamide.

The normal phase separation of the optical isomers of DL-homatropine can be seen in Figure 35A (hexane/butanol) and Figure 35B (hexane/ethanol). As expected DL-homatropine isomers are retained longer when ethanol is used as seen in Figure 35B (retained 10 more minutes when compared with the hexane/butanol mobile phase (Figure 35A)). Chiral resolution of the DL-homatropine enantiomers is possible because of the methyl substituted bridged nitrogen between a six member ring and a five member ring which introduces steric effects. The phenyl ring is responsible for π - π interactions. The hydroxyl group of DL-homatropine is responsible for the hydrogen bonding with the carbonyl oxygen of the chiral stationary phase. Also, the carbonyl oxygen of DL-homatropine can interact (can form a hydrogen bond) with the acidic hydrogen on an amine group of the bonded phase.

A single chiral stationary phase in combination with a single mobile phase cannot separate all racemic compounds.

Figure 35. Normal phase separation of the optical isomers of DL-homatropine on (acrylamidomethyl) cellulose acetate butyrate synthesized using Speier's catalyst; mobile phases: (A) hexane/butanol (90:10), (B) hexane/ethanol (90:10).

Therefore, in this research four different mobile phases were used for the attempted chiral resolution of different racemates. The chromatographic results (retention times, capacity factors and separation factors) for the study of the separation of eight chiral compounds using a hexane/ 2-propanol (90:10) mobile phase on the (acrylamidomethyl) cellulose acetate butyrate stationary phase are listed in Table 7. Based on separation factor (α) values it can be concluded that the hexane/ 2-propanol (90:10) mobile phase worked better for the chiral resolution of terbutaline, tropicamide, and 2-phenyl-1, 2-propanediol.

The chromatographic results for chiral compounds using hexane/ ethanol (90:10) mobile phase on the (acrylamidomethyl) cellulose acetate butyrate stationary phase are listed in Table 8. By evaluating the separation factor (α) values it appears that the hexane/ ethanol (90:10) mobile phase worked better for the chiral resolution of all compounds in the study except for 2,2,2-trifluoro-1- (9-anthryl) ethanol and 1,1'-bi-2-naphthol. The results for the study of the same enantiomeric compounds using a hexane/ butanol (90:10) mobile phase on chiral stationary phase are listed in Table 9. Based on the separation factor (α) values it appears that the hexane/ butanol (90:10) mobile phase worked better for the resolution of all the compounds except for 2,2,2-trifluoro-1- (9-anthryl) ethanol and labetalol. The chromatographic results for the compounds using a hexane/ 1-propanol (90:10) mobile phase on the (acrylamidomethyl) cellulose acetate butyrate stationary phase are listed in Table 10. By evaluating the separation factor (α) values it appears that the hexane/ 1-propanol (90:10) mobile phase worked better for the resolution of terbutaline, 2,2,2-trifluoro-1- (9-anthryl) ethanol, 1,1'-bi-2-

naphthol and tropicamide.

Table 11 shows the optimum conditions among the four mobile phases used for the separation of each chiral compound. Based on the separation factor (α) values, the hexane/ 2-propanol (90:10) mobile phase worked well for the chiral separation of terbutaline and 1,1'-bi-2-naphthol. The mobile phase hexane/ ethanol (90:10) was best for the chiral resolution of quinine, DL-homatropine and labetalol. For the chiral resolution of 2,2,2-trifluoro-1-(9-anthryl) ethanol, tropicamide, and 2-phenyl-1, 2-propanediol, the hexane/ 1-propanol (90:10) mobile phase worked better.

A chromatogram for the chiral separation of 2-phenyl-1, 2-propanediol using hexane/ 2-propanol (90:10) mobile phase is shown in Figure 36. The first enantiomer eluted at 7.6 minutes and the second one eluted at 26.4 min which indicates a very good chiral separation. The chiral selectivity is achieved by the two hydroxyl groups of 2-phenyl-1, 2-propanediol which are responsible for the hydrogen bonding with the carbonyl oxygen's of the stationary phase. In addition to this the phenyl ring of 2-phenyl-1, 2-propanediol is responsible for π - π interactions.

The typical chromatograms for the partial chiral separation of 1,1'-bi-2-naphthol using hexane/ butanol (90:10), and hexane/ propanol (90:10) mobile phases are shown in Figures 37B and 38B respectively. To determine which isomer eluted first, the pure enantiomer i.e., (R)-(+)-1,1'-bi-2-naphthol were injected separately (Figures 37A and 38A). By comparing the retention time values of Figures 37A with 37B and Figures 38A with 38B it was concluded that the first eluted enantiomers were of (R)-(+)-1,1'-bi-2-naphthol.

Table 7. Separation factors of chiral compounds using the (90:10) hexane/ 2-propanol mobile phase.

Solutes	t_r^1	t_r^2	t_0	k'_1	k'_2	α
Labetalol	7.392	7.392	3.4	1.174	1.174	1
Terbutaline	3.604	7.473	3.4	0.05	1.197	19.9
TFAE	5.621	5.621	3.4	0.65	0.65	1
Bi-2-naphthol	7.716	7.716	3.4	1.269	1.269	1
Tropicamide	5.856	7.280	3.4	0.722	1.141	1.583
Quinine	7.272	7.272	3.4	1.177	1.177	1
Homatropine	7.405	7.405	3.4	1.177	1.177	1
Phenyl-1, 2-propane diol	7.639	26.438	3.4	1.246	6.776	5.49

Column: (acrylamidomethyl) cellulose acetate butyrate

Flow rate: 0.5 mL/min

Table 8. Separation factors of chiral compounds using the (90:10) hexane/ ethanol mobile phase.

Solutes	t_r^1	t_r^2	t_0	k'_1	k'_2	α
Labetalol	8.292	23	3.6	1.303	5.38	4.13
Terbutaline	3.74	7.87	3.6	0.03	1.186	28.9
TFAE	6.339	6.339	3.6	0.76	0.76	1
Bi-2-naphthol	8.491	8.491	3.6	1.358	1.358	1
Tropicamide	6.425	8.241	3.6	0.784	1.289	1.64
Quinine	8.058	21.5	3.6	1.238	4.972	4.01
Homatropine	8.162	22.103	3.6	1.267	5.139	4.056
Phenyl-1, 2-propane diol	3.835	7.921	3.6	0.065	1.2002	18.4

Column: (acrylamidomethyl) cellulose acetate butyrate

Flow rate: 0.5 mL/min.

Table 9. Separation factors of chiral compounds using the (90:10) hexane/ butanol mobile phase.

Solutes	t_r^1	t_r^2	t_0	k'_1	k'_2	α
Labetalol	10.63	10.63	4.5	1.362	1.362	1
Terbutaline	4.529	10.748	4.5	0.0064	1.388	21.5
TFAE	6.456	6.456	4.5	0.434	0.434	1
Bi-2-naphthol	9.315	9.965	4.5	1.07	1.214	1.134
Tropicamide	6.392	10.39	4.5	0.42	1.308	3.113
Quinine	10.42	11.82	4.5	1.315	1.628	1.238
Homatropine	10.683	12.714	4.5	1.374	1.825	1.328
Phenyl-1, 2-propane diol	10.475	12.825	4.5	1.327	1.85	1.394

Column: (acrylamidomethyl) cellulose acetate butyrate

Flow rate: 0.4 mL/min.

Table 10. Separation factors of chiral compounds using the (90:10) hexane/propanol mobile phase.

Solutes	t_r^1	t_r^2	t_0	k'_1	k'_2	α
Labetalol	11.444	11.444	4.3	1.661	1.661	1
Terbutaline	4.478	11.185	4.3	0.041	1.601	39.05
TFAE	6.88	12.345	4.3	0.6	1.87	3.11
Bi-2-naphthol	8.881	9.946	4.3	1.065	1.313	1.232
Tropicamide	6.78	11.26	4.3	0.576	1.618	2.81
Quinine	12.499	12.499	4.3	1.906	1.906	1
Homatropine	11.899	11.899	4.3	1.767	1.767	1
Phenyl-1, 2-propane diol	11.816	11.816	4.3	1.747	1.747	1

Column: (acrylamidomethyl) cellulose acetate butyrate

Flow rate: 0.4 mL/min.

Table 11. Chromatographic data for chiral compounds by using the (acrylamidomethyl) cellulose acetate butyrate column.

Solutes	t_r^1	t_r^2	t_0	k'_1	k'_2	α	MP
Labetalol	8.292	23	3.6	1.303	5.38	4.13	B
Terbutaline	3.604	7.473	3.4	0.06	1.19	19.9	A
TFAE	6.88	12.345	4.3	0.6	1.870	3.11	C
Bi-2-naphthol	8.292	23	3.6	1.303	5.38	4.13	A
Tropicamide	6.78	11.26	4.3	0.576	1.618	2.806	C
Quinine	8.056	21.5	3.6	1.238	4.972	4.015	B
Homatropine	8.162	22.103	3.6	1.267	5.139	4.055	B
Phenyl-1, 2-propane diol	7.639	26.438	3.4	1.246	6.582	5.434	C

MP = Mobile Phase used:

A= (90:10) hexane/ 2- propanol

B = (90:10) hexane/ ethanol

C = (90:10) hexane/ 1- propanol

Figure 36. Normal phase separation of the optical isomers of 2-phenyl-1, 2-propanediol on (acrylamidomethyl) cellulose acetate butyrate synthesized using Speier's catalyst; mobile phase: hexane/ 2-propanol (90:10);

Figure 37. Normal phase separation of 1,1'-bi-2-naphthol on (acrylamidomethyl) cellulose acetate butyrate synthesized using Speier's catalyst; (A) (R)-(+)-1,1'-bi-2-naphthol (B) optical isomers of 1,1'-bi-2-naphthol; mobile phase: hexane/ butanol (90:10).

Figure 38. Normal phase separation of 1,1'-bi-2-naphthol on (acrylamidomethyl) cellulose acetate butyrate synthesized using Speier's catalyst; (A) (R)-(+)-1,1'-bi-2-naphthol (B) optical isomers of 1,1'-bi-2-naphthol; mobile phase: hexane/ propanol (90:10).

The chiral interactions may be possible because of the two naphthyl rings present in 1,1'-bi-2-naphthol, which are responsible for π - π interactions. 1,1'-bi-2-naphthol has two hydroxy groups which can form hydrogen bonds with the chiral stationary phase. Also, the two naphthyl rings are bulky enough to cause stereospecific interactions with the chiral groove of the derivatized cellulose bonded phase.

The normal phase separation of 2,2,2-trifluoro-1-(9-anthryl) ethanol (TFAE) on (acrylamidomethyl) cellulose acetate butyrate using the hexane/ propanol (90:10) mobile phase is shown in Figure 39B. The first enantiomer eluted at 6.8 minutes and the second one eluted at 12.3 minutes, which indicates good chiral separation. Injecting (R)-(-)-2,2,2-trifluoro-1-(9-anthryl) ethanol indicated that the first eluted enantiomer is of (R)-(-) enantiomer as seen in Figure 39A.

Francotte and Wolf investigated the effect of electron-withdrawing (halogen) groups on chiral resolution and found that substitution of halogens for hydrogens in phenyl-cycloalkane improved resolution (9). Chiral resolution of TFAE might be possible because of the three electron-withdrawing fluorine atoms. In addition to this the anthryl group is responsible for stereospecific and π - π interactions. Also, the hydroxyl group of TFAE can form a hydrogen bond with the carbonyl oxygen of the chiral stationary phase.

The chromatogram for the chiral separation of labetalol using the hexane/ ethanol (90:10) mobile phase is shown in Figure 40A. The first enantiomer eluted at 8 minutes and the second one eluted at approximately 23 minutes. Chiral selectivity is achieved by the two phenyl groups, which are responsible for π - π interactions.

Figure 39. Normal phase separation of 2,2,2-trifluoro-1-(9-anthryl) ethanol on (acrylamidomethyl) cellulose acetate butyrate (A)(R)-(-)-2,2,2-trifluoro-1-(9-anthryl) ethanol (B)optical isomers of 2,2,2-trifluoro-1-(9-anthryl) ethanol; mobile phase: hexane/ propanol (90:10).

In addition to this, the two hydroxyl groups, one carbonyl group, and the two amine groups of labetalol can form hydrogen bonds with the chiral stationary phase.

Finally, to check the performance of the (acrylamidomethyl) cellulose acetate butyrate bonded phase synthesized using Speier's catalyst the chromatograms of same chiral compound sample on both the chiral stationary phase and on C-18 column was compared. The chromatograms of labetalol using the hexane/ ethanol (90:10) mobile phase is shown in Figure 40A (derivatized cellulose chiral stationary phase) and 40B (C-18 column) was compared. It can be seen that a very good enantiomeric separation with the chiral stationary phase (Figure 40A) is obtained. On the other hand, the chromatogram is noisy and there is no chiral separation achieved when the same sample under same chromatographic conditions was run on the C-18 column (Figure 40B). By comparing these chromatograms it can be concluded that the derivatized cellulose-based chiral stationary phase is capable of successful separation of chiral compounds in the normal phase mode.

Figure 40. Comparison of the chromatograms of the optical isomers of labetalol on (A) (acrylamidomethyl) cellulose acetate butyrate synthesized using Speier's catalyst; (B) C18 column; mobile phase: hexane/ ethanol (90:10);

4. CONCLUSION

The primary goal of this study was to synthesize chiral stationary phases via the silanization/hydrosilation method. (Acrylamidomethyl) cellulose acetate butyrate and (acrylamidomethyl) cellulose acetate propionate were bonded to silica hydride surface using free-radical catalyst and Speier's catalyst. The success of bonding was evaluated quantitatively by elemental analysis, qualitatively by DRIFT spectroscopy and solid state NMR spectroscopy. Carbon analysis results, DRIFT spectra and solid state NMR spectroscopy showed that the (acrylamidomethyl) cellulose acetate butyrate bonded phase synthesized using Speier's catalyst had the highest bonding density when compared to the other bonded phases.

The Neue test showed similar results. The (acrylamidomethyl) cellulose acetate butyrate bonded phase synthesized using Speier's catalyst was more hydrophobic and had low silanophilic activity. This chiral stationary phase showed no enantiomeric resolution when operating in the reversed phase mode. This may be due to the shrinkage or swelling of the substituted cellulose when in contact with a polar solvent like methanol. On the other hand, in the normal phase mode several chiral compounds were very well separated using 90% hexane and 10% of a variety of alcohols as the mobile phase. Future work should focus on exploring more mobile phase conditions in the normal phase mode, as it is more promising. Enantioseparation of more chiral compounds using a greater variety of mobile phases would be beneficial for a better understanding of the chiral separation mechanism. Additional derivatives of cellulose and amylose chiral stationary phases should be synthesized and evaluated.

5. REFERENCES

1. Satinder, A. *Chiral Separations Applications and Technology*, Am. Chem. Soc, Washington, DC, **1997**, Ch-1.
2. Beesley, T. E.; Scott, R. P. W. *Chiral Chromatography*, John Wiley & Sons: New York, **1998**, Ch-1.
3. Lough, W. J.; *Chiral Liquid Chromatography*, Blackie and Son Ltd., New York, **1989**, Ch-1.
4. March, J.; *Advanced Organic Chemistry Reactions, Mechanisms and Structure*, John Wiley & Sons: Wiley Eastern Reprint, **1986**, Ch-4.
5. Subramanian, G.; *A practical Approach to Chiral Separations by Liquid Chromatography*, VCH Publishers: New York, **1994**, Ch-1.
6. Gerald, G.; Martin, G. S. *Chiral Separations Methods and Protocols*, Humana Press: New Jersey, **2004**, Ch-1.
7. Skoog, A. D.; Holler, J. F.; Niemann, A. T. *Principles of Instrumental Analysis*, 5th ed.; Saunders College Publishing: Florida, **1998**; Ch-26, 28.
8. Bidlingmeyer, A. B.; *Practical HPLC Methodology and Applications*, John Wiley & Sons: New York, **1992**, Ch-1.
9. Aboul-Enein, H. Y.; Ali, I. *Chiral Separations by Liquid Chromatography and Related Technologies*, Marcel Dekker, Inc: New York, **2003**.
10. Katz, E.; Eksteen, R.; Schoenmakers, P.; Miller, N. *Handbook of HPLC*, Marcel Dekker, Inc: New York, **1998**, Ch-8.

11. Pesek, J. J.; Matyska, M.T.; Sandoval, J.E.; Williamson, E.J. *J. Liq. Chrom. & Rel. Technol.* **1996**, *19*, 2843-3865.
12. Pesek, J. J.; Sandoval, J.E. *Anal. Chem.* **1991**, *63*, 2634-2641.
13. Pesek, J. J.; Matyska, M.T.; Yu, R.J.J. *Chromatogr. A.* **2002**, *947*, 195-203.
14. Pesek, J. J.; Matyska, M.T.; Williamsen, E.J.; Evanchic, M.; Hazari, V.; Konjuh, K.; Takhar, S.; Tranchina, R. *J. Chromatogr. A.* **1997**, *786*, 219-228.
15. Pesek, J. J.; Sandoval, J.E. *Anal. Chem.* **1989**, *61*, 2057-2075.
16. Montes, M.C.; Chu, C.-H.; Jonsson, E.; Auvinen, M.; Pesek, J.J.; Sandoval, J. E. *Anal Chem.* **1993**, *65*, 808-816.
17. Pesek, J. J.; Matyska, M.T.; Fu, P. F. *Chromatographia*, **2001**, *53*, 635-640.
18. Neue, U.D.; Tran, K.V.; Iraneta, P. C.; Alden, B. A. *J. Sep. Sci.* **2003**, *26*, 174-186.