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Optimization of Chiral Separation of Nadolol by Simulated Moving Bed Technology

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OPTIMIZATION OF CHIRAL SEPARATION OF NADOLOL BY SIMULATED MOVING BED TECHNOLOGY

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Abstract

Simulated Moving Bed (SMB) technology has gained increasing attention as one of the most powerful techniques for chromatographic separations due to its cost-effectiveness and efficiency. Application of SMB technology is especially important in the pharmaceutical industry for production of enantiopure drugs, as required under strict FDA regulations, to avoid possible adverse effects of racemic drugs. In this study, the performance of the SMB process in separation of racemic nadolol on a perphenyl carbamoylated beta cyclodextrin (β-CD) stationary phase was investigated. The equilibrium dispersive model coupled with bi-Langmuir adsorption isotherm and lumped kinetic approximation, constitute the mathematical model used to simulate the dynamic behavior of SMB. Multi-objective optimization was carried out using a robust state-of-the-art optimization technique, non-dominated sorting genetic algorithm (NSGA). Two optimization problems were solved to simultaneously maximize productivity and purity of the product and minimize consumption of desorbent. The generated Pareto optimal solutions showed that selection of operating conditions can significantly affects the performance of SMB to meet the desired objectives.

Keywords:

Nadolol, racemic compound, chirality, simulated moving bed chromatography, chiral stationary phase, Pareto optimal, competitive adsorption isotherm, enantiomer separation, Nadolol, β- cyclodextrin, genetic algorithm, multi-objective optimization

Dedication

First and foremost, all praise is due to the most graceful and most compassionate the almighty God for providing me with uncountable blessings, one of which is granting me the capability to proceed successfully with this project.

This thesis appears in its current form due to the assistance and guidance of several people who always believed in me and to whom I would like to offer my sincere thanks and gratitude.

To my mother Iman El-Adawi whose unconditional love and continuous support have sustained me throughout my life, and whose words of inspiration and encouragement in pursuit of excellence keep moving me forward. I am truly indebted to you for who I am today and words *can never express the deepest gratitude I have for you.*

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Nomenclature

a_i	Intrinsic affinity coefficients (dimensionless)
A	Cross section area of chromatographic column (cm^2)
b_i	Langmuir competitive interference coefficient (ml/mg)
c_i	Mobile phase concentration based on fluid volume (mg/ml)
c_i^f	Feed concentration based on fluid volume (mg/ml)
c_i^j	Mobile phase concentration of component i in section *j* of SMB (mg/ml)
$C_{S,i}$	Concentration in stationary phase (mg/ml)
C_0	Pulse injection concentration (mg/ml)
D_e	Effective diffusion coefficient (cm^2/s)
D_L	Axial dispersion coefficient (cm^2/s)
D_m	Solute molecular diffusivity (cm^2/s)
d	Diameter of the column (cm)
d_P	Particle diameter (μm)
F	Phase ratio, equal to (1-ε)/ε
H	Equilibrium constant (dimensionless)
HETP	Height equivalent to a theoretical plate (cm)
k_m	Lumped mass transfer coefficient (s^{-1} or min^{-1})
k'	Elution capacity (retention) factor of the solute at infinite dilution (dimensionless)
k'_w	Capacity (retention) factor obtained in water

k_f External film mass transfer coefficient (cm/s)

K'_i Frontal capacity factor (dimensionless)

K Equilibrium constant (dimensionless)

L Column length (cm)

m_j Fluid phase flow rate over solid phase flow rate in *j* section (dimensionless)

m'_j Net fluid phase flow rate over solid phase flow rate in *j* section (dimensionless)

N Theoretical number of switching period of columns

q_i Concentration of component i on stationary phase (mg/ml)

q^*_i Equilibrium concentration of component i on stationary phase (mg/ml)

q^j_i Concentration of component i on stationary phase in section *j* of SMB (mg/ml)

Q_D Desorbent flow-rate fed to SMB process (ml/s)

Q_E Extract flow-rate out of SMB process (ml/s)

Q_F Feed flow-rate fed to SMB process (ml/s)

Q_R Raffinate flow-rate out of SMB process (ml/s)

Q_s Solid phase flow rate in both TCC and SMB process (ml/s)

t^* Switching time in SMB process (s)

T_0 Column hold up time (s)

t_R Mean retention time of an adsorbed component (s)

t_0 Mean retention time for an unretained compound (s)

t_{0R} Mean retention time for an unretained compound (s)

u Superficial velocity (cm/s)

v Interstitial fluid velocity of the mobile phase (cm/s)

V Column volume (cm^3)

V Volumetric flow rate of the mobile phase (ml/s)

$W_{0.1}$ Width of the peak at the position of 10% peak height

$W_{0.5}$ Width of the peak at half peak height

Subscripts

A More retained component of racemic compound ((RSR)-Nadolol)

B Less retained component of racemic compound ((RRS)-Nadolol)

D Desorbent port

E Extract port

F Feed

i,j Component i and j

R Raffinate

T Total

z Space coordinate

Greek symbols

ε Bed voidage, equal to external bed porosity ε_{ext} (dimensionless)

ε_i Internal porosity (dimensionless)

ε_T Total porosity (dimensionless)

λ Flow-geometry dependent constant in equation

μ Dynamic viscosity (g/(s·cm))

ρ Density of mobile phase (g/cm3)

Chapter 1: Introduction

1.1 General Background

Isomers are compounds with the same molecular formula that can be further classified into: stereoisomers if they have the same atom connectivity but different arrangement of the atoms in space, or constitutional (structural) isomers if the order in which atoms are joined together differs. Two kinds of stereoisomers are diastereomers and enantiomers. Diastereomer molecules are not related as mirror images and have different physical and chemical properties, while enantiomers are a pair of non-super imposable mirror images. Diastereomerism is more commonly exhibited by molecules in which more than one stereogenic centre is present. Diastereomers of a chiral molecule with multiple stereocentres can be converted to enantiomers by inversion of all stereogenic centres, and to an epimer of the original structure by inversion of only one stereogenic centre. However, diastereomers can be formed by inverting the configuration at only some stereocentres (Lin, Zhang, & Cheng, 2011; He, 2010). The flowchart below (Fig. 1) summarizes the different types of isomerism:

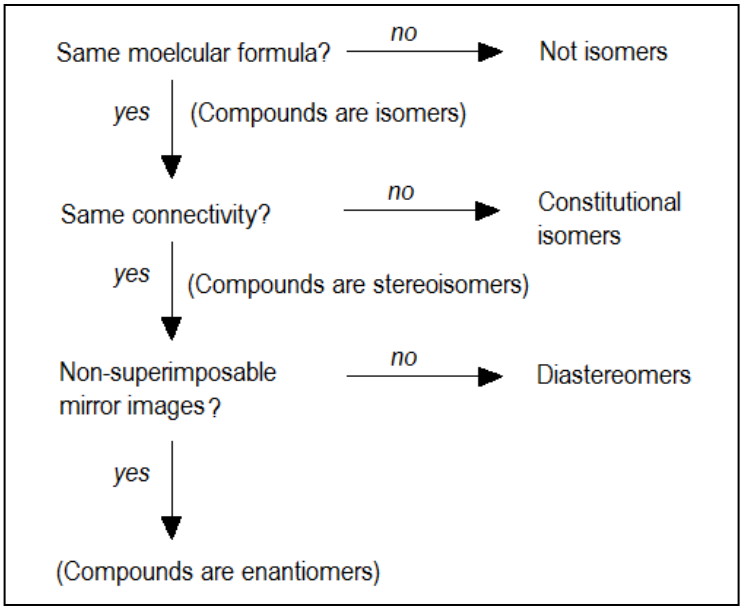

Figure 1: Types of Isomerism

1.1.1 Chiral Molecules

A chiral molecule has a stereogenic center, also called the chiral center, attached to four different substituents making it asymmetric. Such a molecule is not super imposable on its mirror image; hence the two mirror image stereoisomers are called enantiomers. Although these enantiomers possess identical physical and chemical properties, their biological profiles may be quite distinct. Unlike achiral molecules which are not optically active, each enantiomer of a chiral molecule has an equal but opposite optical rotation; i.e. one enantiomer is (+) dextrorotary (right handed) so it rotates polarized light in a clockwise direction while the other is (-) levorotary (left handed) and rotates polarized light in a counter-clockwise direction. These enantiomers are commonly referred to as d- or r-enantiomer and l-or s- enantiomer, respectively. The R- or S- of isomer based on Cahn-Ingold-Prelog (CIP) system, whereas D- or L- are based on Fisher projection. An equimolar

mixture of these two enantiomers is known as a racemic mixture and has no optical rotation since they cancel each other out (Xin, 2004). Lactic acid is an example of a chiral molecule whose two forms (Fig. 2), the levorotary enantiomer (S)-lactic acid and the dextrorotary (R)-lactic acid, rotate plane polarized light in opposite direction.

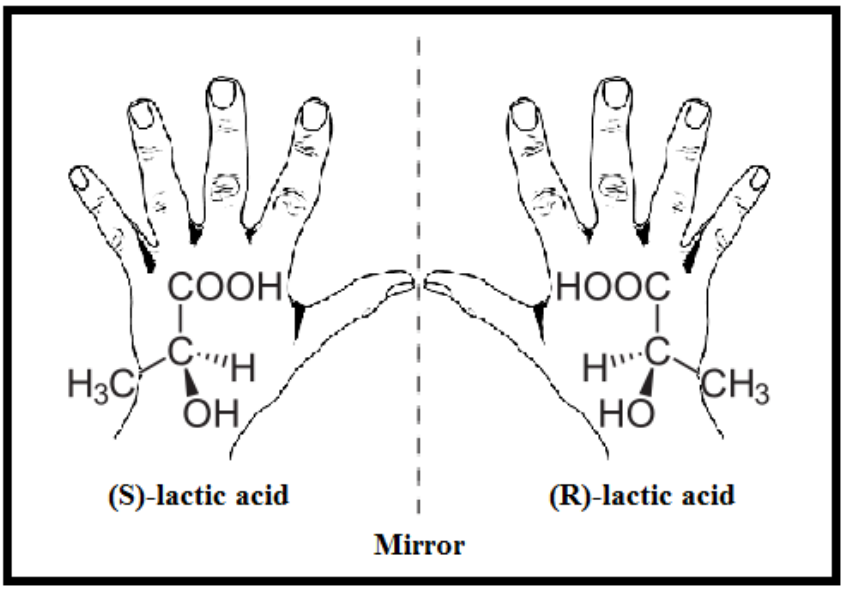

Figure 2: Lactic Acid Chiral Molecule

Louis Pasteur made the first discovery of the concept of chirality and optical activity in 1848 in the midst of his attempts to make crystals of sodium tartrate, which is not optically active. Upon crystals formation in the solution, Pasteur noticed that some crystals were mirror images of each other, and when separated, solutions of each batch rotated light in a different direction due to the molecule's structure (He, 2010; Xin, 2004).

1.1.2 Background on chiral separation

Chiral or optically active molecules are naturally prominent in living systems. Most of the basic building blocks that make up plants, animals and human body, such as proteins,

nucleic acids and polysaccharides are able to perform their functions because of their chiral characteristic structures. Interestingly, usually only one of the two possible enantiomeric forms of the chiral molecule occurs naturally in a given species, for e.g. proteins consist exclusively of L-amino acids and nucleic acids of D-sugars. This phenomenon of homo-chirality of biological molecules in the human body is responsible for its highly stereospecific environment. Although chiral molecules have the same chemical formula and identical physiochemical properties they are different in three dimensions giving rise to very different biological properties. Therefore, different enantiomers in drugs, food, proteins and other compounds bind selectively to certain molecules and compounds that are also enantiomers resulting in different biological responses to each enantiomer. Amongst many examples, this phenomenon explains the lock and key model for enzyme-receptor interaction. This enantioselective feature in living things generally and humans specially makes chirality an integral part of drug research and development due to the significance of chiral drug applications in the pharmaceutical industries, and the profound impact it has on people's well-being and lives (Welch, 2004; Hutt & Valentová, 2003; Collins, 1992).

Very often in a racemic drug, one enantiomer will serve the therapeutic purpose while the other enantiomer's role can vary from being inactive to posing harmful pharmacodynamic side effects and toxic effects. For instance, the Thalidomide case which took place in the 1960s stirred awareness of stereo selectivity of chiral drugs when a sedating drug called thalidomide was prescribed for pregnant women to treat morning sickness. This chiral drug however resulted in the birth of more than 10,000 deformed babies because while

one enantiomeric form of thalidomide was sleep inducing and anti-nausea (the R-Isomer), the other (S-Isomer) was later discovered to be teratogenic (i.e. can cause malformations of an embryo or fetus). The two enantiomers of Thalidomide are shown in figure 3. Investigations indicated that (-)(S)-thalidomide inhibits the growth of new blood vessels hindering proper development of a fetus. Moreover, even if the racemic mixture is purified, the human liver is capable of converting enantiomerically pure (+)(R)-thalidomide to the unwanted enantiomer resulting in a racemic mixture (McConathy & Owens, 2003). Thalidomide was banned in 1962 by World Health Organization (WHO), until The Food and Drug Administration (FDA, U.S.A) approved it again in 1998, but under a special restricted distribution program, when it was proven to be effective for treatment of some cancers and inflammatory diseases (FDA, 1992). This shows that there is no general rule for all chiral drugs, but instead they must be investigated each individually, very carefully (Davies & Teng, 2003).

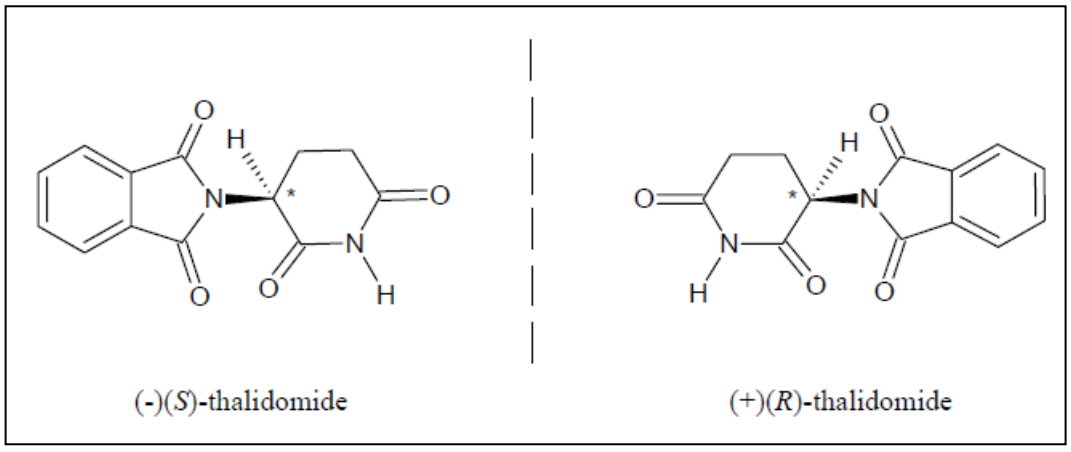

Figure 3: Thalidomide Enantiomers

Previous high-profile cases verifying the potential risks associated with racemic drugs led to the release of strict regulations on the production of chiral bioactive molecules by FDA in 1992. This regulatory authority provided guidelines indicating that enantiomers of drugs, new or improved, with a chiral centre have to be separated and their pharmacological and toxicological profiles studied, and new chiral drugs be brought to the market in the form of pure enantiomer i.e. only the therapeutically active enantiomer of the chiral molecule can be present (FDA, 1992; Davies & Teng, 2003).

1.2 Implications in Pharmaceutical drugs

Currently, approximately 40% of marketed drugs have chiral active ingredients and almost half of these drugs are marketed as racemic mixture, contributing an estimated world market of $320 billion in 2011. The top three drug categories being antibiotics, cardiovascular, and hormones. However, with the new regulatory requirements there has been a lot of collaboration world-wide between pharmaceutical companies and research institutes and accelerated efforts to find new methods or improve the efficiency of existing methods of enantioseparation, also known as racemic or chiral switching, on industrial scale (Stinson, 2000; Li & Haynie, 2006).

Pharmaceutical companies are actually benefiting from FDA's new regulations as it gives them a chance to use racemic switching as a marketing strategy to make more profit out of enantiopure drug formulas. It is also advantageous as a defense strategy against generic competition, since the new single-isomer drug is subject to extended patent lifetime if it has distinctly different pharmacological effects. The market for chiral technologies is

speculated to increase to hit $2.0 billion in 2011, in the United States alone, suggesting that commercialization of chiral technologies will be a profitable and rewarding field for biopharmaceutical research in academic institutions. Despite the economic benefits to both pharmaceutical and technology industries, the most important driving force for the effort and money put towards chiral switching should ideally be better functioning drugs to benefit patients (Van Arnum, 2006; Bojarski, Aboul-Enein, & Ghanem, 2005). Several advantages of the use of single enantiomer considered to be key market drivers are:

- Improved safety margin through greater selectivity and potency, while eliminating the potential of enantiomer-enantiomer interaction
- More selective pharmacological profiles leading to improved efficacy
- Absence of unwanted pharmacodynamic side effects and toxic effects
- Lower dose exposure while maintaining or improving therapeutic effect
- Eliminates risks associated with possibility of bio-inversion
- Easier and more accurate assessment of drug co-administration effects preventing additive effects
- Simpler pharmacokinetics and reduced drug interactions
- Easier to monitor efficacy and relationship between plasma concentration and effect

Racemic drugs typically fall under one of the following four groups from a therapeutic point of view (Mehvar, 2001; Crosby, 1997):

1. Racemates with equipotent enantiomers such as the anti-malarial agent, primaquine, whose enantiomers are both equally effective and safe to use for its purpose whether in the form of a racemic mixture or single-enantiomer.
2. Racemates with one dominating enantiomer in terms of beneficial properties. The majority of racemic drugs belong to this category in which the inactive enantiomer, or one with lower pharmacological activity and/or sometimes adverse effects, is called the distomer. Whereas, the eutomer is the enantiomer possessing majority or all the beneficial properties.
3. Racemates superior to enantiomers, such as the diuretic drug named indocrinone, show that optically pure compounds are not necessarily always better than racemates. Each chiral drug is a special case with different therapeutic, pharmacological, and toxicological profiles corresponding to its different chiral states.
4. Racemates with enantiomers possessing different pharmacological effects, for e.g. separation of enantiomers of Propoxyphene results in two different drugs, the (-)-enantiomer being an analgesic and (+)-enantiomer an anti-tussive.

Examples of drugs that undergo chiral switching and the effect of each enantiomer are listed in table 1.

Table 1: Example of Chiral Drugs and their Bioactivity

Drug	Stereoisomer	Bioactivity
Penicillamine	D-Isomer	Anti-arthritic
	L-Isomer	Toxic
Chloramphenicol	(+)-Isomer	High antibacterial activity
	(-)-Isomer	No known effect
Citalopram	S-Isomer	Serotonin reuptake inhibitor
	R-Isomer	30-fold less potent
Fluoxetine	S-Isomer	Racemate is superior to isomer
	R-Isomer	Racemate is superior to isomer

1.3 Scope of this work

Nadolol is a beta-blocker drug or beta-adrenergic blocking agent that contains more than one chiral center, hence it exists as a mixture of enantiomers and diastereosiomers. It is widely used to treat high blood pressure, prevent migraine headaches as well as symptoms of angina pectoris, which is chest pain due to restriction in blood supply to the heart muscle. Nadolol's therapeutic effects are attributed to its ability to competitively bind to beta-adrenergic receptor sites on the heart and by doing so preventing the production of hormones that signal the heart to beat faster. Hence, nadolol reduces the demands on the heart by causing it to beat more slowly and with less force which in turn imporves blood flow and decreases the amount of oxygen the heart needs to pump blood around the body. Although the therapeutic β-blocking properties of nadolol are found only in the

levorotatory enantiomers, it is currently marketed as an equal mixture of four stereoisomers. Separation and production of the most potent enantiomer (RSR)-nadolol however is desirable for safer, and more effective use as well as from an economic point of view. In this study the preperative enantioseparation of racemic nadolol by simulated moving bed (SMB) chromatography, on beta cyclodextrin chiral stationary phase (CSP), is investigated theoretically with the aim of a complete separation of the most potent enantiomer (RSR)-nadolol.

A literature survey on chiral separation which reviews the methodological approaches of chiral separation methods is given in Chapter 2 to explain the choice of chromatographic technique. In addition, various factors that affect the enantioseparation of racemic nadolol, which include the mobile phase composition, flow rate, pH, and temperature are discussed in Chapter 3 to identify the optimum separation conditions for the mobile phase. Furthermore, the kinetics of mass transfer, model parameters, and adsorption isotherms on the columns used for the chiral separation of racemic nadolol are examined in Chapter 4 characterized by the parameters of bed voidage and axial dispersion coefficient. In Chapter 5, the concept of continuous countercurrent separation is introduced and the difference between true moving bed (TMB) and simulated moving bed (SMB) processes is addressed.

The triangle theory which will be applied to the design and complete separation of nadolol, in Chapter 7, and the performance parameters used to evaluate the process are covered in Chapter 6 of this dissertation. The actual modeling, simulation and validation of the pseudo-binary separation of nadolol by SMB chromatography are presented in Chapter 7,

and the effects of switching time, feed flow rate, raffinate flow rate, and extract flow rate on the separation performance is discussed. In Chapter 8, multi-objective optimization with diverse objectives was carried out using a non-traditional optimization technique, non-dominated sorting genetic algorithm, to generate Pareto optimal solutions. Finally in Chapter 9, the overall conclusions are stated and recommendations for future work are listed.

Chapter 2: Literature Review on Chiral Separation

2.1 Chiral Separation Methods

As mentioned earlier, the majority of therapeutics and drugs derived from natural products are usually in the optically active or pure one enantiomeric form. However, in cases where drugs are chemically synthesized in an achiral environment, a racemic mixture is produced containing an equal proportion of both enantiomers. The similarity in physiochemical properties of enantiomers, for e.g. melting point, solubility, and IR (infrared) spectra, makes chiral separation a difficult task, however, critical to make use of each enantiomer's different biological activities. Thus, increased interest of the pharmaceutical industry in the topic "chiral switch" is behind the proposal and invention of new enantioselective technologies that utilize various methods presented in this dissertation (Caner, 2004; Rouhi, 2003).

2.1.1 Chiral Synthetic Approach

(1) Asymmetric synthesis

This method uses a chiral catalyst, auxiliary or substrates which introduces a temporary stereocentre to bind the molecule in a way that influences the reaction by using steric hindrance as shown in figure 4, forcing the production of predominantly one enantiomer over the other (enantio-selective), or only one enantiomer (enantio-specific). Even though the catalyst can be recycled in a separate step for re-use, this method is highly complex and too expensive for industrial scale productions (Sheldon, 1993).

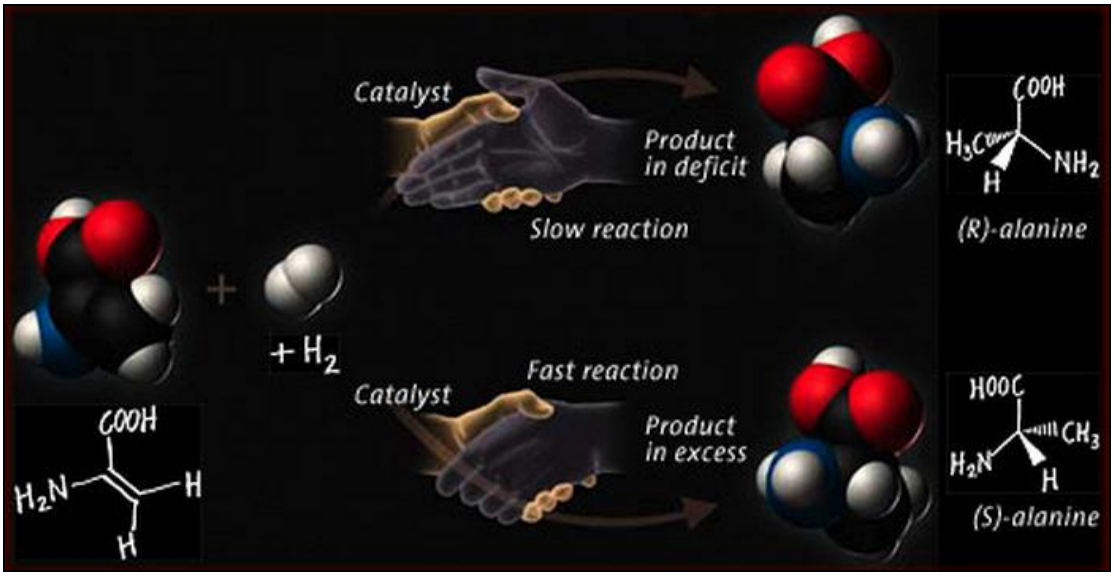

Figure 4: Mechanism of Action of Chiral Catalysts (Nobel Media AB 2012)

(2) Biological methods

This method uses enzymatic catalysis, in which an enzyme acting as chiral catalysts is combined with a racemic mixture. The enzyme will either preferentially bind with one of the enantiomers, making resolution of the desired enantiomer possible, or the enzyme will react with a pro-chiral molecule producing the desired pure enantiomer of the chiral molecule. These two routes are known as enzymatic kinetic resolution and enzymatic asymmetric synthesis, respectively. Pro-chiral molecules are those that can be converted from achiral to chiral, i.e. with a new stereocentre, through the addition or exchange of one group on the molecule in a single step. In figure 5 prochiral $NADH_2$ molecule conversion to chiral NAD is achieved via enzymatic catalysis by dehydrogenase. The main problem here lies in the gradual decrease of catalytic activity with time, in addition to the time-consuming and costly enzyme-screening process (Rasor, 2001; Ölceroglu, 2006).

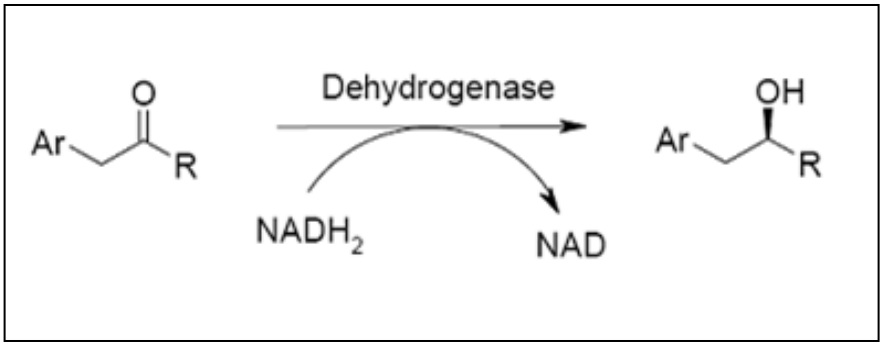

Figure 5: Prochiral $NADH_2$ molecule conversion to chiral NAD via enzymatic catalysis by dehydrogenase

2.1.2 Racemic Approach

This approach relies on separation or resolution of racemates, which is done mostly by crystallization and chromatographic techniques.

2.1.2.1 Crystallization techniques

Chiral resolution of racemates by the traditional optical resolution techniques, based on crystallization, is the most widely employed method to obtain pure enantiomers in the pharmaceutical industries. This is so because the low cost of operation and high efficiency of crystallization are added advantages to the simplicity and practicality of the process for industrial-scale production and purification of enantiomers. Most importantly, the efficiency of crystallization methods have been greatly improved as a result of extensive employment for a few decades which led to better understanding of the underlying principles of crystallization and the properties upon which its application in separation are based. Identification of racemate types can help to determine which crystallization technique would be best to apply, time- and cost-wise, to maximize efficiency. The

crystalline racemate can be classified into three fundamental types which are conglomerate, racemic compound and pseudo-racemate (solid solution). The categorization of enantiomer mixture is based on their melting point phase diagram, also known as binary diagram. It may also be defined by solubility diagram, also known as ternary diagram, as well as other thermodynamic and structural studies. A racemic conglomerate, as illustrated in figure 6, is an equimolar physical mixture of crystals each containing only one of the two forms of pure enantiomer. Molecules in the crystal lattice have a greater affinity for the same enantiomer than for the opposite enantiomer, and the melting point of the pure enantiomer is higher than that of the racemic conglomerate (He, 2010; Li & Haynie, 2006; Lorenz et al., 2007).

A racemic compound, also known as a true racemate, refers to a single crystalline phase comprised of the two enantiomers coexisting in an ordered 1:1 ratio in one unit cell. Molecules in a unit cell have a lower affinity for the same enantiomer than for the opposite enantiomer, and the melting point may be decreased by adding a small amount of one enantiomer to the racemic compound. A pseudo-racemate or racemic solid solution is a homogenous mixture of equal proportions of two enantiomers existing in an unordered manner in the crystal lattice. Unlike the first two types, there is no significant difference in affinity between the same and opposite enantiomers in a racemic solid solution, and the melting point is also either affected very slightly or not at all by adding a small amount of one enantiomer (Wang, Liu, Yu, & Ching, 2006).

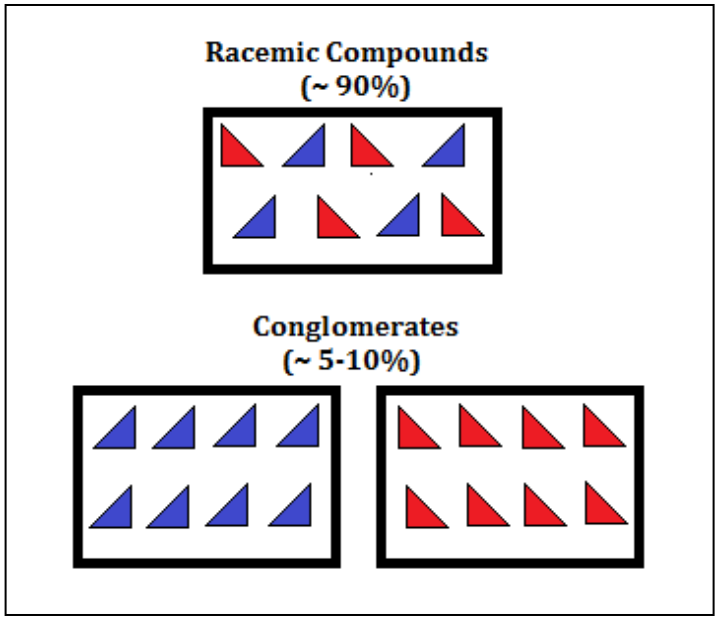

Figure 6: Racemates crystallize as racemic compounds or as conglomerates

The type of racemate, which is strongly dependent on the solid-state nature of the racemate, determines what type of crystallization resolution, direct crystallization or diastereomeric crystallization, would be the most efficient method to utilize for the enantioseparation of racemic species.

2.1.2.1.1 Direct crystallization

(1) Spontaneous resolution:

Optical resolution by direct crystallization makes the two enantiomers of chiral compound crystallize out directly. This technique is known as spontaneous resolution, and is only possible with a racemic conglomerate due to its characteristic aggregate-forming properties, which also makes it possible to separate different enantiomorphous crystals. However, this kind of separation is rarely used because it is extremely time-consuming and

laborious, besides only 5-10% of all racemates are known to crystallize as mixtures of pure enantiomer crystals or conglomerates (Xin, 2004; Collet, 1999).

(2) Preferential crystallization

This method, also called resolution by entrainment, manages to promote the preferential crystallization of one of the enantiomers, while keeping the other in supersaturated state, in substances with identical physiochemical properties but different metabolic effects. Resolution by preferential crystallization is performed in batch, where both enantiomers are initially dissolved, and the solution's temperature is maintained in a range where secondary nucleation is much more important than primary nucleation. Understanding and proper use of control of the ternary phase diagram facilitates the crystallization process by controlling the crystallization rates of the two enantiomers. Very briefly, preferential crystallization relies on the addition of a pure enantiomer seed to a supersaturated solution to break the solution equilibrium making precipitation of this type of enantiomers favorable. The pre-requisites for this resolution method are that the solution does not reach equilibrium, and the racemic mixture does not crystallize during the operation. Preferential crystallization is one of the most straightforward, efficient, and economic separation processes for industrial-scale resolution of a racemic conglomerate; products are so pure that they can be directly used in the production of pharmaceuticals, food ingredients and fine chemicals (Stinson, 1998; Coquerel, 2007).

2.1.2.1.2 Diastereomeric crystallization

Diastereomeric crystallization, also known as indirect crystallization, is a predominant two-step method employed in chiral resolution, especially for acids and bases. First of all, enantiomers of chiral compounds are converted into diastereomers by way of chemical reaction, or by interaction of a racemic mixture with a resolving agent (single enantiomer) to form two diastereomeric salts of optically pure compound. Since diastereomers have different properties, for e.g. solubility and boiling points, like any two different molecules, the salts formed can be easily separated by physical means such as recrystallization. In most cases, the chiral material that functioned as a resolving agent can be recovered and re-used to repeatedly re-racemize the undesired enantiomer, after resolution of desired enantiomer from the diastereomeric salt, for a higher yield (Lorenz, 2006). Despite its image as a "low-tech method" and its low theoretical yield of 50%, diastereomeric crystallization plays a key role in separation of racemic mixtures accounting for 65% of major bulk drug production. It has the advantage of simplicity and convenience as it is well suited to batch production which means it can be accomplished with standard equipment used in pharmaceutical industry. The difference between this technique and asymmetric synthesis is that the latter produces a single enantiomer versus separating two enantiomers mixed in a solution (Kozma, 2001; Lorenz, 2007; Xin, 2004). As shown in Figure 7, efficient resolution of 1,1'-Bi-2-naphthol (BINOL) into its enantiomeric form can be achieved by diastereomeric crystallization using N-benzylcinchonidinium chloride (Wang, Sun, & Ding, 2000; Wongso, Hidajat, & Ray, 2005).

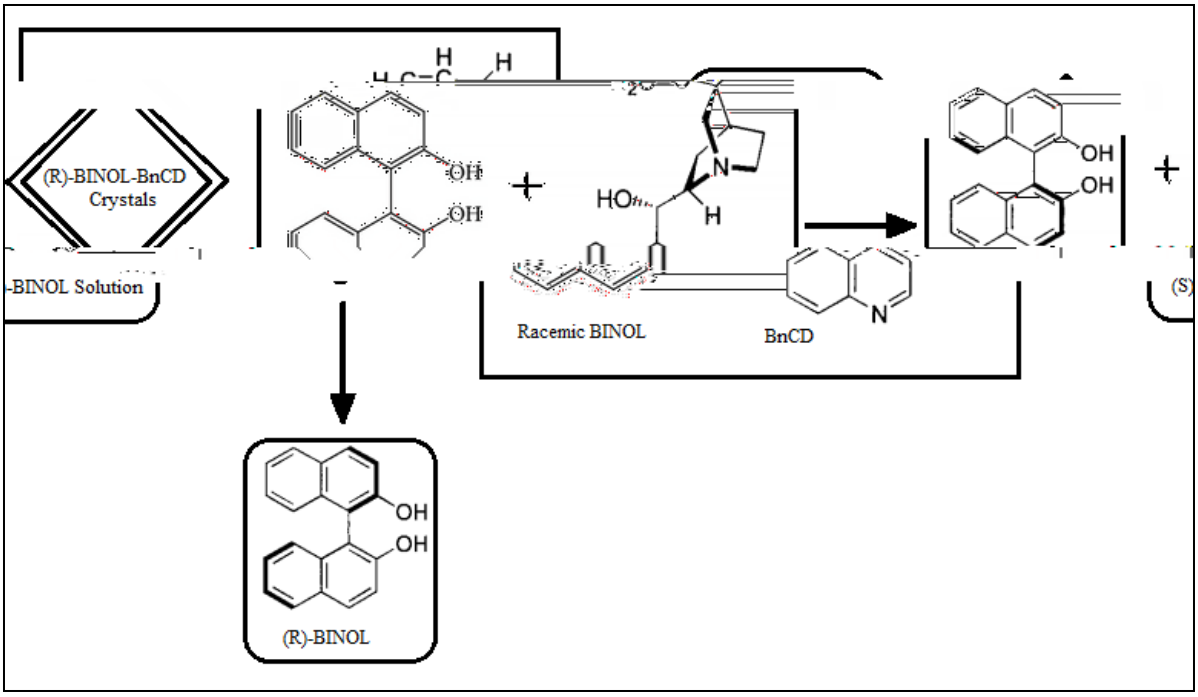

Figure 7: Separation of (R)-BINOL from racemate by diastereomeric crystallization using N-benzylcinchonidinium (BnCD)

2.1.2.2 Chromatographic techniques

In the past few years, chiral liquid chromatography or chromatographic resolution has entered into a new era of development and improvement in techniques that it has become the main and most efficient way, on analytical scale, for purification and production of pure enantiomers by resolution of racemic mixtures. Large-scale chiral chromatography has attracted increasing interest in the pharmaceutical industry due to ease of operation with fully automated equipment enabling cost-effective multi-ton scale production, as well as significantly reduced time to market, of active pharmaceutical ingredients (APIs) using cost efficient commercial chromatography processes. Moreover, chiral

chromatographic separation may be used in conjunction with traditional synthesis methods to obtain the best yield of purest possible product.

The difference between 'conventional chromatography' and 'chiral column chromatography' is that the latter technique depends upon the differential adsorption of enantiomers; i.e. introduction of an asymmetric or chiral environment allowing diastereomeric interactions. This is achieved by using either a chiral mobile phase, which can be gas or liquid giving rise to chiral gas chromatography and chiral liquid chromatography, or special selective columns that result in increased retention of one enantiomer over the other. A third method is by forming a diastereoisomeric derivative using standard achiral stationary phases, however, its disadvantages in terms of selectivity and purity of the derivatization agent makes it the least favorable option (Bojarski, Aboul-Enein, & Ghanem, 2005; Saari, 2011; Mack, 2012).

2.1.2.2.1 Chiral mobile phase additives

Resolution of enantiomeric compounds has been alternatively accomplished through chiral mobile phase additives (CMPAs) as a means to forming diastereomeric complexes with different stabilities, solvation in the mobile phase and the complexes binding to the solid support. The main advantages of CMPAs over chiral stationary phases (CSPs) is that the former uses standard less expensive columns with high loading capacities, as well as the availability of a wide range of additives and possibility to modify solute character, for e.g. ion pairing. The removal of the chiral selector after chromatography, expenses associated with large scale production with lack of recycling additives, in addition to difficulty to

develop separation are the major disadvantages of using CMPAs. There are three main types of CMPAs: ion pairing, ligand exchange, and inclusion complexes (Xin, 2004; Aboul-Enein et al., 2003).

(1) Ion pairing

Chiral ion pairing agents rely on the use of a charged solute and a counter-ion of opposite charge, both of which are optically active, to form diastereomeric ion pairs that are usually separated either by differences in their solubility in the mobile phase or in their binding to the stationary phase. Propanolol, naproxen, quinine (an amino alcohol), and amino acids such as tryptophan are examples of some solutes that have been resolved by chiral ion pairing chromatography. Unfortunately application of this method is difficult because it has low capacity and stability as it can be affected by several factors such as temperature and water content in the mobile phase, among others.

(2) Inclusion

The mobile phase additive used for forming inclusion complexes is beta-cyclodextrin (β-CD). The separation mechanism, however, is more difficult than if the β-CD was loaded onto a stationary phase because both inclusion complexion and adsorption affect elution order and speed. Removal of cyclodextrins (a seven sugar ring molecule) after chromatography can be achieved by one of two ways, firstly by using acid/base extractions and secondly by solid phase extraction. Separation of a few milligrams of mephenytoin and hexobarbital has been achieved using cyclodextrins.

(3) Ligand Exchange

This method uses a chiral selector and a transition metal ion for the formation of diastereomeric complexes with different stabilities between the mobile and the stationary phases, which forms the basis of chiral selectivity. It is best used for resolution of molecules able to form coordination complexes with transition metal ions such amino acids and amino acid like compounds.

2.1.2.2.2 Direct method using chiral stationary phase (CSP)

The chiral selectivity of the chiral stationary phase column is usually attained by coating an achiral support, such as silica gel, with a suitable chiral adsorbent enriched with single enantiomer of a chiral compound chosen so that the spatial arrangement of its composite atoms significantly favors and increases the likelihood or proximity of interaction with only one of the two enantiomers. Since enantiomers of the same analyte compound have differing affinities to other single enantiomers of a chiral compound, when passed through the column packed with enantiomer-enriched adsorbent material, called the chiral stationary phase (CSP), the different enantiomers will exit at different times; hence, chiral separation is successfully achieved as illustrated in figure 8.

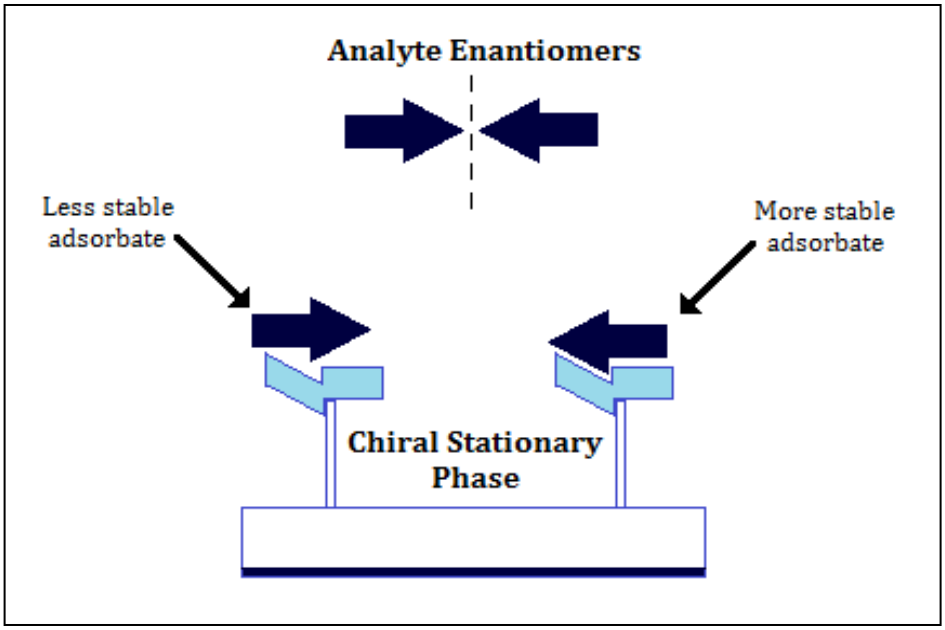

Figure 8: Chromatographic resolution of enantiomers depends on differential adsorption of enantiomers on the chiral stationary phase (CSP)

Chiral recognition leading to chiral separation depends on the formation of a minimum of three simultaneous interactions, between the analyte enantiomer and chiral selector, on the CSP resulting in the formation of temporary diastereomeric complexes with different energies. One of the interactions in the 3-point-interaction model of Dalgliesh must be stereo-chemically dependent. The compounds in the diastereomeric complexes are bound in a certain three-dimensional structure by forces of interactions giving one enantiomer selective advantage over the inactive enantiomer. The forces of interaction may be: H-bonding, n-n interaction, dipole stacking, steric bulk and inclusion complexing (Valle, 2005).

Different stationary phases are better suited to specific types of analytes. There are five general types of chiral stationary phases (CSPs) used in chromatography:

(1) Polymer-based carbohydrates

Among the various CSPs available that are based on polysaccharides, the most commonly used are the Yoshio Okamoto's invented amylose and cellulose CSPs which Daicel Chemical Industries, Ltd. commercialized. It is basically a chiral polysaccharide derivative, i.e. amylose and cellulose, coated on a macroporous silica gel support. Steric restriction at the polysaccharide backbone, preventing one of the enantiomers from forming H-bonds with carbamate, links between side chains and polysaccharide backbone is the mechanism of chiral recognition adopted by this CSP. Several beta-blockers have been successfully separated using polymer-based carbohydrates CSPs. Polymer-based CSPs can be used with normal phase high-pressure liquid chromatography (HPLC), supercritical fluid chromatography (SFC), reversed-phase high-pressure liquid chromatography (RP-HPLC) and the only limitation is its incompatibility with a wide range of solvents other than alcohols (Aboul-Enein, 2001; Chen, 2007). Examples of available columns are: Chiralpak AD, AS-RH, and Chiralcel OD, OD-RH from Chiral Technologies, Inc.

(2) Pirkle or brush-type phases

Columns within this group are designed to give a strong three-point interaction with one of an enantiomer pair through 3 classes: π-donor phases, π-acceptor phases, and mixed donor-acceptor phases. The first CSP of this type was synthesized by Bill Pirkle and are now readily available commercially at reasonable cost for use mostly with normal phase HPLC and SFC. Despite its high efficiency and selectivity, the requirement of derivatization for resolution of many chiral solutes and the fact that it only works with aromatic compounds are the main limitations of using this column. Phenomenex Chirex phases,

Whelk-O 1, DACH-DNB (mixed phases), β-Gem 1 (π-acceptor phases), and Naphthylleucine (π-donor phases), from Regis Technologies, Inc. are some pirkle type CSP columns (Aboul-Enein, 2001; Xin, 2004).

(3) Cyclodextrins

Alpha, beta and gamma-cyclodextrins (α, β, γ-CD), the three most common forms of the cyclic oligosaccharides containing 6, 7 and 8 glucose units respectively, bond to silica and form chiral cavities that contain hydroxyls for H-bonding with polar groups of analyte and non-polar cavities in which the hydrophobic portion of analyte fits. Only one enantiomer will fit better in the cavity resulting in the desired chiral resolution. This type of CSP is used in RP-HPLC and polar organic mode, given that the analyte has a hydrophobic group to fit into cavity (Martin Del Valle, 2004). Some commercial columns of this type are Cyclobond (α-, β-, and γ-cyclodextrins) and ORpak CDA (α), ORpak CDB (β), ORpak CDC (γ).

(4) Protein-based

Natural proteins which are high-molecular-weight polymers composed of chiral subunits were bonded to a silica matrix for the first time by Hemansson who introduced the first protein-based CSP which was α_1-acid glycoprotein (AGP). Since proteins contain large numbers of chiral centers, they form strong bonds with small chiral analytes through hydrophobic and electrostatic interactions, H-bonding. This CSP requires aqueous based conditions in RP-HPLC and the other condition is that the analyte must have ionizable groups such as amine. This type of CSP has the limitation that all protein columns have a

low sample capacity, which makes them best suited for analytical scale work (Ahuja, 1997; Aboul-Enein and Wainer**,** 1997). Chiral AGP (α-glycoprotein), HSA (human serum albumin), and BSA (bovine serum albumin) are protein-based columns available.

Chromatographic resolution of enantiomers can be and has been achieved by application of chiral stationary column in chiral high pressure liquid chromatography (HPLC), supercritical fluid chromatography (SFC), and chiral gas chromatography (GC). The advantage of using chiral chromatography is that it combines simplicity, speed, robustness *to* meet the most stringent regulatory standards in cGMP (current good manufacturing practices) compliant systems, cost-efficiency and manufacturing versatility. From an environmental point of view chiral chromatography has a reduced impact compared to batch chemistry due to automated solvent recycling which recycles more than 99% of organic solvents. Low solvent consumption and the speed of the process are major contributors to cost-effectiveness and help leading to a rapid return on investment. Moreover, the adaptability of the equipment allows changing scale of production to suit market demands (Bojarski, Aboul-Enein, & Ghanem, 2005; Davies & Teng, 2003; Sekhon, 2010). Several active pharmaceutical ingredients (APIs) have been fully developed and produced as pure enantiomers using large-scale chromatography, some of which are shown in Table 2.

Table 2: Examples of drugs produced as pure enantiomers

Drug Name	Indication	Company
Armodafinil Nuvigil®	Narcolepsy, Jet lag disorder, obstructive sleep apnea	Cephalon
Levitiracetam Keppra®	Epilepsy	UCB
Escitalopram Lexapro®	Depression, generalized anxiety disorder	Lundbeck

Chapter 3: Liquid Chromatographic Retention Behavior and Enantioseparation of Nadolol

3.1 Introduction and elution order of the enantiomers of Nadolol

Nadolol is a non-selective beta blocker (β-blocker), also known as beta-adrenergic receptor blocking agent, that blocks both β-1 receptors predominantly located in the heart and β-2 receptors located in the lungs, but has a preference for β-1 receptors. This white crystalline powder is polar, hydrophilic at pH 2, soluble in alcohol and methanol, has low lipid solubility and is slightly soluble in chloroform. Nadolol and beta-blockers in general, displays a high degree of stereoselectivity in binding β-receptors. It is approved by the U.S. Food and Drug Administration (FDA) for treatment of hypertension (high blood pressure), migraine headaches and long-term treatment of angina pectoris (chest pain due to insufficient blood supply to the heart). Nadolol's mechanism of action lies in its ability to compete with adrenergic neurotransmitters for binding at sympathetic receptor sites. Its inhibition of β-1 receptors in the heart and vascular smooth muscle prevents binding of catecholamines epinephrine and norepinephrine, hence leading to decreased heart rate, reduction in the force of the heart muscle contraction and lowering of blood pressure. Similarly, by binding β-2 adrenergic located in the bronchiole smooth muscle, nadolol inhibits vasoconstriction and water retention, which are the effects that would be otherwise caused by production of rennin. The chemical structure of nadolol has three chiral centers and although β-blocking properties are found only in the levorotatory enantiomers, this drug is synthesized and marketed as an equal mixture of only four stereoisomers because of the cis-orientation of the two adjacent hydroxyls on the

cyclohexane ring. The four possible stereoisomers are labeled “racemate A”, which includes an equimolar mixture of the most potent stereoisomer RSR-nadolol and its enantiomer SRS-nadolol, and “racemate B”, which is has a 1:1 molar ratio of the stereoisomer RRS-nadolol and its enantiomer SSR-nadolol. The stereochemical structures of the four enantiomers of nadolol are shown in figure 9. Separation of the most active stereoisomer RSR-nadolol and its production in a pure enantiomer form is highly desirable for safer and more effective use, and to abide by the FDA’s regulations favoring marketing drugs as pure enantiomers rather than racemic mixtures (Xin, 2004; Aboul-Enein, 2001; Wang & Ching, 2003).

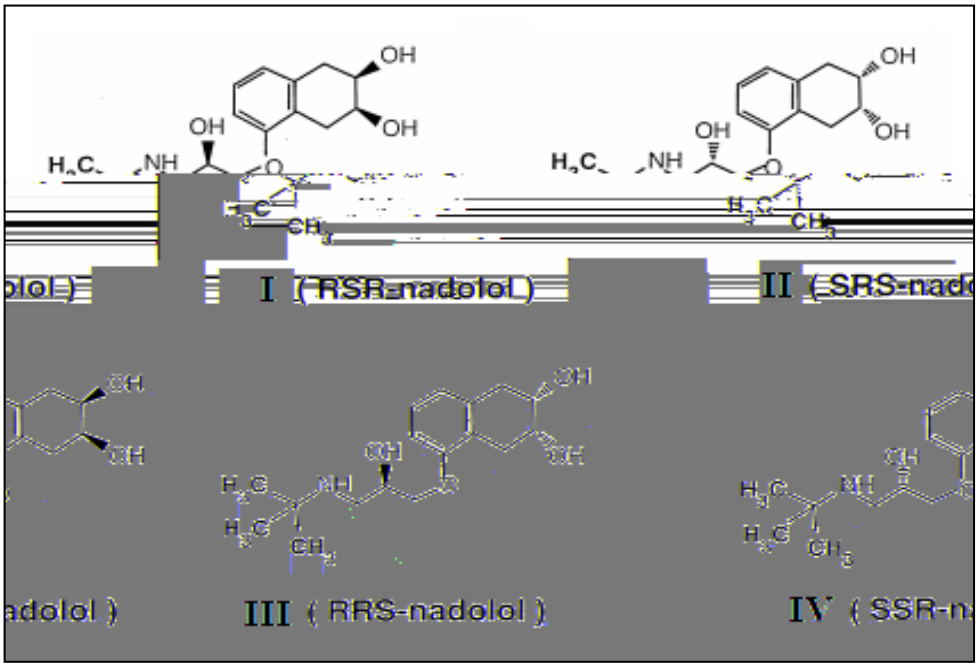

Figure 9: Stereochemical structure of the four nadolol enantiomers. I and II constitute nadolol racemate A; III and IV constitute nadolol racemate B.

Separation of multi chiral center drugs like nadolol, using direct crystallization is very difficult and complicated unless the racemic mixture belongs to conglomerate, hence separation has been attempted via different chromatography methods such as HPLC using CD column and SFC using α1-acid glycoprotein column. A successful approach is to separate these analytes, which is very challenging due to their complex structure, in a continuous mode using 4-zone non-linear SMB chromatography to get the most potent (RSR)-nadolol from its racemic mixture. The elution order of the enantiomers of nadolol in the chosen CSP, beta-cyclodextrin, was established as (SRS)-nadolol and (SSR)-nadolol eluting together and overlapped in the first peak, followed by (RRS)-nadolol in the second peak, and the most potent (RSR)-nadolol is the third eluted component (Wang & Ching, 2002; Xin, 2004).

3.2 Beta-Cyclodextrin Chiral Stationary Phase

The choice to use a chromatographic method over a classical resolution process using crystallization is not only because of its limited performance in separating racemic compounds that are not conglomerates but also because of the advantages chromatographic processes offer summarized into: easy automation of operation in a continuous way, eliminate loss of valuable optically active material, and availability of highly selective chiral stationary phases making separation of more than one chiral center racemic compounds possible. Cyclodextrins, also referred to as cycloamyloses, are a group of cyclic oligosaccharides naturally produced by partial degradation or bacterial digestion of starch, and enzymatic coupling of 6-8 glucopyranose units into crystalline toroidal

structures. As show in figure 10, the three well-characterized cyclodextrins are alpha (α), beta (β) and gamma (γ), containing 6, 7 and 8 glucose units, respectively (Xin, 2004; Martin Del Valle, 2004; Huthmann & Juza, 2001).

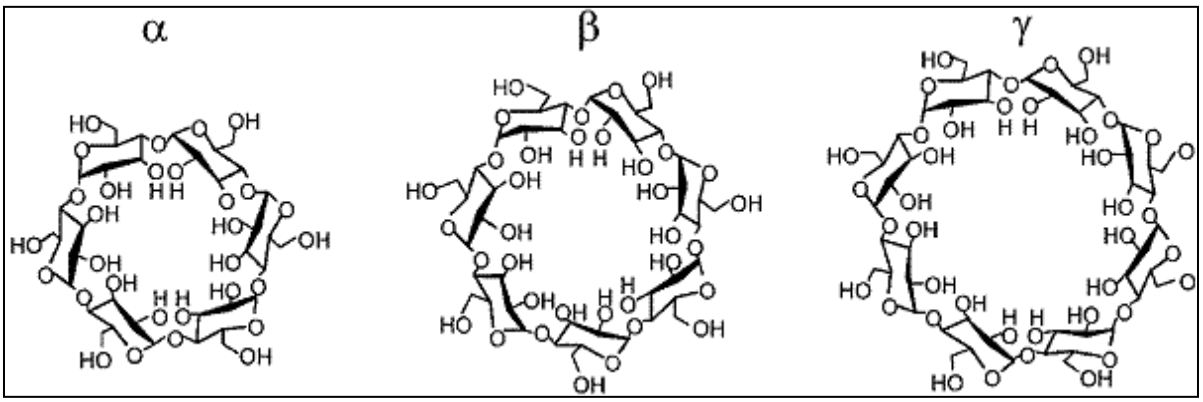

Figure 10: Molecular structure of alpha (α), beta (β), and gamma (γ) cyclodextrin

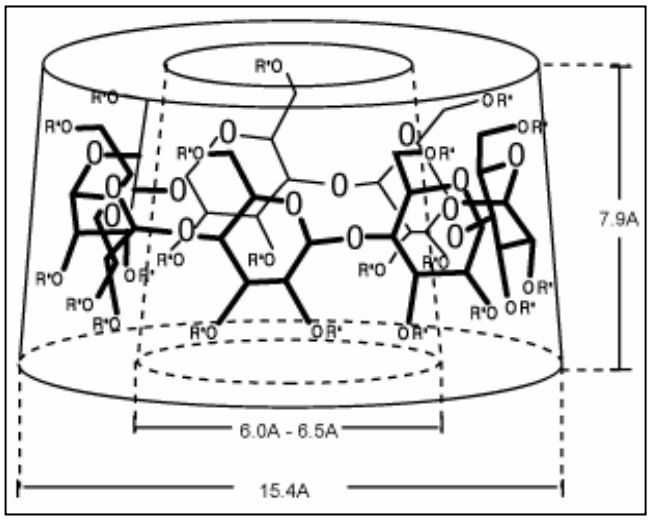

Figure 11: Toroidal structure of β-cyclodextrin

Among the important selectors available, beta-cyclodextrin (β -CD) has been one of the most widely used to produce chiral stationary phases, especially for efficient chiral separation of various drugs and pharmaceutical applications because of its attractive

properties: low UV absorbance, low cost, and water solubility. This truncated cone-shaped chiral structure (Fig. 11) with two openings of different size provides a lipophilic central cavity and a hydrophilic exterior resulting from hydroxyl groups making them water soluble; hence the ability to form inclusion complexes in aqueous solution. The hydrophobic character of the cavity is due to its composition of glucoside oxygen and methylene hydrogen, which gives cyclodextrin the capacity to include bulky hydrophobic groups of the analyte while binding through interaction with hydroxyl groups using dipole-dipole interactions, hydrogen bonding or London dispersion forces. The enantioselectivity of the CD-CSPs can be altered by substituting hydroxyl groups in β-CD with other functional groups which may give different properties to these derivatized β-CD based chiral stationary phases. In most cases reported in literature, the native β-CD is chemically bonded to the support material, which is a porous silica gel, and then it is synthetically modified in what is known as a post-immobilization derivatization procedure. However, studies have shown that preparation of pre-immobilized β-CD-CSPs leads to more complete functionalization of CD-moieties and hence better reproducibility of results (Sigma-Aldrich, 2000; Wang & Ching, 2002; Huthmann & Juza, 2001).

Wang et al. (2004) successfully achieved enantiomeric separation of the stereoisomers of nadolol with complete resolution of the most potent enantiomer (RSR)-nadolol, using HPLC, on a column packed with heptakis (6-azido-6-deoxy-2, 3-di-O-phenylcarbamolyted)-β-CD-CSP, in which all 2- and 3-secondary hydroxyl groups are substituted by phenylcarbamate groups as the derivative group, and the seven primary hydroxyl groups are replaced by the azido group to link the β-CD derivative to the silica gel. Besides its

success in the chiral resolution of the active ingredient of interest for this dissertation study, the improved stability, reproducibility and effectiveness with a versatile mobile phase systems support the decision to use perphenyl carbamoylated β-CD based stationary phase in simulating the SMB separation performance.

Retention factor, k, is the measure of the time the analyte spends retarded by the CSP relative to the time spent in the mobile phase. In essence, this is a measure of the degree of adsorption of each enantiomer and therefore k is proportional to the equilibrium adsorption constant. On the other hand, retention time is the time between sample injection and a solute reaching a detector at the end of the column; the most soluble or least adsorbed solute travels faster with the mobile phase (Snyder et al., 1997). Since each solute has its own equilibrium between adsorption on the surface of a given solid and solubility in the solvent also known as an eluent, the retention time for different solutes and in this case different enantiomers varies resulting in separation into bands containing different solutes as shown in Figure 12. Mathematically, retention factor is given by the following equation:

$$k = \frac{amount\ of\ component\ in\ stationary\ phase}{amount\ of\ component\ in\ mobile\ phase} \quad (1)$$

$$k_1 = \frac{t_1 - t_0}{t_0} \quad (2)$$

$$k_2 = \frac{t_2 - t_0}{t_0} \quad (3)$$

Where t_j (j=1, 2) is the retention time of the enantiomer and t_0 is the time taken by an un-retained analyte or mobile phase to pass through the column.

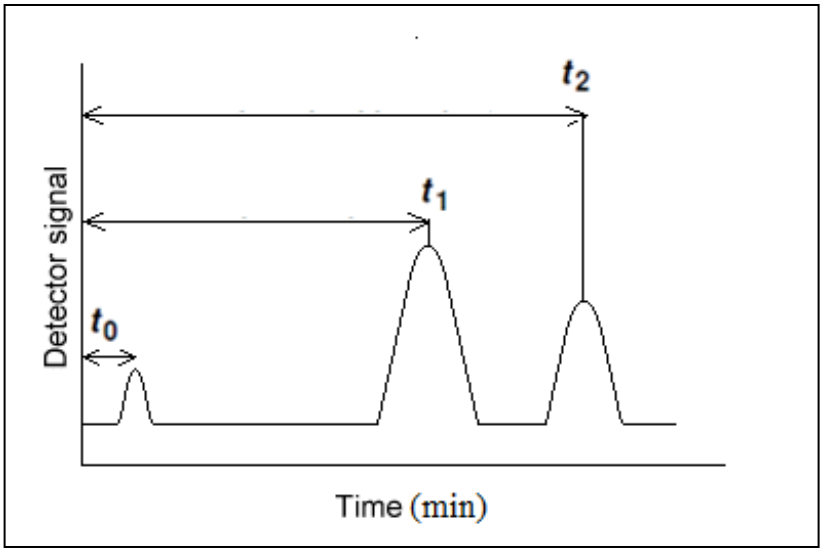

Figure 12: Illustration of chromatographic elution profile

The extent of resolution of enantiomers is generally described by α, the separation factor, sometimes referred to as selectivity factor and given by:

$$\alpha = \frac{k_2}{k_1} \tag{4}$$

Where species two elutes slower than species one, the selectivity factor must be greater than 1 otherwise there would absolutely no separation of the two enantiomers; typically a separation factor of at least 1.2 or larger is desirable for efficient separation (Altenhoner, 1997).

3.3 Operating Conditions

3.3.1 Mobile Phase Composition

Previous studies have shown that using methanol as an organic modifier in mobile phases for chromatographic separation of chiral molecules on β-CD yielded the highest resolution of enantiomers. Experimental work done by Xin et al. (2004) established that there exists an inversely proportional relationship between the concentration of methanol and

retention time on the CSP. Considering both separation of nadolol and a reasonable retention time, a ratio of 80:20 aqueous-organic mobile phase composed of methanol as organic modifier and the aqueous buffer solution of triethylamine acetate (TEAA) at a 1% (w/v) concentration, which helps control ionization of solutes, would make the optimum mobile phase composition.

3.3.2 Effects of pH

An increase of mobile phase pH is shown to enhance the hydrophobic interaction between the apolar segment of nadolol and the hydrophobic cavity of the CSP, therefore increasing pH results in a longer retention time. It is reported in literature that the enantiomeric resolution of nadolol increases with pH in the range of 4-6.5, the optimum pH being 5.5 for a reasonable retention time without compromising a high enough resolution of nadolol.

3.3.3 Effects of Flow Rate

The optimal mobile phase flow rate was fixed at 0.3ml/min based on results of investigations carried out Xin et al. (2004) on the effect of flow rate on the retention and resolution of enantiomeric separation. It has been reported that increasing the flow rate from 0.2 ml/min to 0.6 ml/min, at room temperature, causes a significant decrease in retention time and resolution of nadolol due to insufficient interaction time between the solute and the cavity of β-CD. Fixing the flow rate at 0.3 ml/min achieved maximum resolution at a reasonable retention time.

3.3.4 Effects of Temperature

The effect of temperature on the retention and resolution of enantiomeric separation of nadolol studied by Xin et al. (2004) confirmed previous studies which suggest that

retention time of nadolol's enantiomers as well as resolution decrease with temperature increase. Studies indicated that this is due to the decrease in binding constants of individual enantiomers of nadolol to the β- CD with increasing temperature, and that operating at a column temperature of 20 ^{o}C gave the best elution chromatogram showing full resolution of all components of Nadolol.

Chapter 4: Kinetic and Equilibrium Study of the Enantioseparation of Nadolol

In order to achieve preparative separation of nadolol by chromatographic method, it is important to study the kinetics and equilibrium of enantioseparation of nadolol on the β-CD columns along with other system properties such as bed voidage and axial dispersion coefficient which are required for modeling and simulation of the process.

4.1 Theories for Liquid Chromatography

Researchers have contributed various theories to the mathematical modeling of isothermal adsorption of liquid chromatography. Among the most famous and useful models are those developed by Ruthven (1984), Zhong et al. (1996) which can be classified into four general categories: equilibrium theory, plate models, rate models, and equilibrium-dispersive model.

4.1.1 Equilibrium Theory

Equilibrium theory, also known as the 'ideal model', assumes that both phases, mobile phase and stationary phase, are in thermodynamic equilibrium, and that dispersive effects and mass transfer resistance are negligible which leads to effective prediction of retention times of elution peaks for chromatographic columns but fails to describe peak shapes accurately if mass transfer effects are significant. This framework provides a more fundamental approach for deriving analytical solution and basic design of conventional fixed-bed processes, countercurrent schemes as well as reactive chromatography equally (Langel, 2010; Weintraub, 2010).

4.1.2 Plate Models

This model originated from fractional distillation columns and supposes that the chromatographic column contains a number of separate layers, called 'theoretical plates', in which separate equilibrations of the sample between the stationary and mobile phase occur. The analyte moves down the column by transfer of equilibrated mobile phase from one plate to the next for a number of theoretical plates that is directly related to equilibrium rate. Two ways of measuring efficiency of the column are either using the number of theoretical plates in a column, *N*, where the more plates the better, or by using the "Height Equivalent to a Theoretical Plate", *HETP*, which is better if smaller because *N* would be large (Ruthven, 1984; Xin, 2004). If the column has a length *L* and *N* theoretical plates, then the *HETP* is defined by:

$$H = \frac{L}{N} \tag{5}$$

The number of theoretical plates, *N*, can be determined by using information from a chromatographic peak after elution

$$N = \frac{5.55\ t_R^2}{w_{1/2}^2} \tag{6}$$

Where t_R is the retention time and $w_{1/2}$ is the peak width of the eluting chromatographic peak at half-height.

Two kinds of plate models exist, of which one model is directly analogous to the tanks in series model for no ideal flow systems in which a column is divided into a series of small artificial cells, each with complete mixing giving a set of first order ordinary differential equations (ODEs) that describe the adsorption and interfacial mass transfer. This model

however, is not suitable for multi-component chromatography because the equilibrium stages may not be the same for different components (Ruthven, 1984). The other model is formulated based on distribution factors, the most popular being Craig distribution models, which determine the equilibrium of each component in each artificial stage, using recursive iterations rather than solving ODE systems. Considering the blockage effect, the Craig models are applicable to multicomponent systems and have been used by Lazo et al. (2000) to simulate elutions and frontal adsorptions.

4.1.3 Equilibrium-dispersive Model

The equilibrium-dispersive dynamic model originally proposed by Teoh et al. (2001) assumes the concentrations in the mobile and stationary phases are in equilibrium throughout the whole column. It also assumes that there is no temperature gradient in the column, and that the contributions of axial dispersion and non-equilibrium effects, such as mass transfer resistance, can be lumped together and described by an apparent dispersion coefficient, D_a, which Guiochon (2000) derived, in his work on frontal analysis, as the kinetic parameter providing the best agreement between the experimental breakthrough curve and the theoretical one calculated using the equilibrium-dispersive model.

4.1.4 Rate Models

A rate model usually consists of two sets of differential mass balance equations, one for the bulk-fluid phase, the other for the particle phase, and if considers axial dispersion, external mass transfer, intraparticle diffusion and nonlinear isotherms then it is considered a general multicomponent rate model. This general rate model of chromatography offers the most detailed description of the adsorption and mass transfer processes in

multicomponent chromatography. Several researchers have solved a variety of general multicomponent rate models using different numerical approaches and considered it to be more exact than equilibrium-dispersive models (Lorenz et al., 2006; Zhang et al., 2007).

The relationship between the local concentration of a compound in the mobile and stationary phases should be given by a rate or kinetic equation that relates $\partial C_{S,i} / \partial t$ to the compositions of both phases so that the diffusion coefficient accounts for axial dispersion, including axial and eddy diffusion:

$$\frac{\partial C_{S,i}}{\partial \mathrm{t}} = g_i(C_1, C_2, \dots, C_i, \dots C_n, C_{S,i}, \dots, C_{S,n}) \tag{7}$$

In the context of chromatography, the differential mass balance equation for a component i over a slice of the column is written as:

$$\frac{\partial C_i}{\partial \mathrm{t}} + F\frac{\partial C_{S,i}}{\partial \mathrm{t}} + \nu\frac{\partial C_i}{\partial \mathrm{z}} = D_L\frac{\partial^2 C_i}{\partial z^2}, \quad (i = 1, 2) \tag{8}$$

$$F = \frac{(1-\varepsilon)}{\varepsilon} \tag{9}$$

where C_i and $C_{S,i}$ is the concentration of component *i* in mobile phase and stationary phase, respectively, *F* is the phase ratio and *ε* is the bed voidage of the column, *v* is the interstitial velocity, D_L is the axial dispersion coefficient, while *z* and *t* are the space and time coordinates. This equation was derived first by Lapidus and Amundson based on a set of assumptions, the most important being:

- Homogeneous packing, constant axial dispersaion, and isothermal conditions
- Negligibility of the compressibility of mobile phase so that mobile phase flows at a constant velocity along the column

- The same partial molar volumes of the components is present in both phases
- The mobile phase is completely inert and none of it adsorbs to the column which is radially homogeneous

In the chromatographic process there are two mobile phase fractions, interparticle which flows between the paticles and occupies the equivalent volume of bed voidage in the column, and the othe fraction is the intraparticle which is stagnant and fills the inner porosity of the packing. In order to achieve enantioseparation, the analyte must leave the bulk of the fluid phase and move toward the intraparticle wall surface of the porous packing where it becomes adsrobed to the column. During this path, the fluid meets various resistances including that in the fluid outside the particles (external film resistance), resistance inside the particles due to diffusion in the pore volume, and surface diffusion resistance on the pore wall surface. Moreover, resistance due to adsorption and desorption at the surface also exists. One or more of these resistances are likely to be limiting the overall reaction rate and therefore contribute the rate-determining step may be attributed to them (Ruthven et al., 1989; Xin, 2004).

There are four commonly used rate models for chromatography with physical interpretation and mathematical description for each covered by Ruthven (1984). Out of these models, the model of external film resistance plus two intraparticle diffusional resistances provides a realistic description of almost all practical systems and therefore it is very widely used. In 1949, Glueckauf proposed the solid film resistance hypothesis which uses a solid film linear driving force model to describe the adsorption rate. This model assumes that the solid matrix is homogeneous in its structure as well as in concentration,

represented by the average concentration within the particle, and thus the rate equation thus becomes a simple function of time and position only. Using the solid film linear driving force model we can assume that both the kinetics of adsorption-desorption is infinitely fast and the mass transfer kinetics are fast enough to satisfy the uniformity of concentration condition throughout the bulk phase (Hsuen, 2000; Duan, Ching, & Swarup, 1998).

Lagergren (1898) presented the earliest known first-order adsorption rate equation that describes the kinetic process of liquid-solid phase adsorption based on the adsorption capacity. Figure 13 shows the proportionality of the mass transfer rate from the bulk phase to solid particles, to the difference between the bulk average concentration within the particles, $C_{S,i}$, and the surface concentration, q_i^*, which is assumed to be in equilibrium with the fluid phase outside the particle (Xin, 2004).

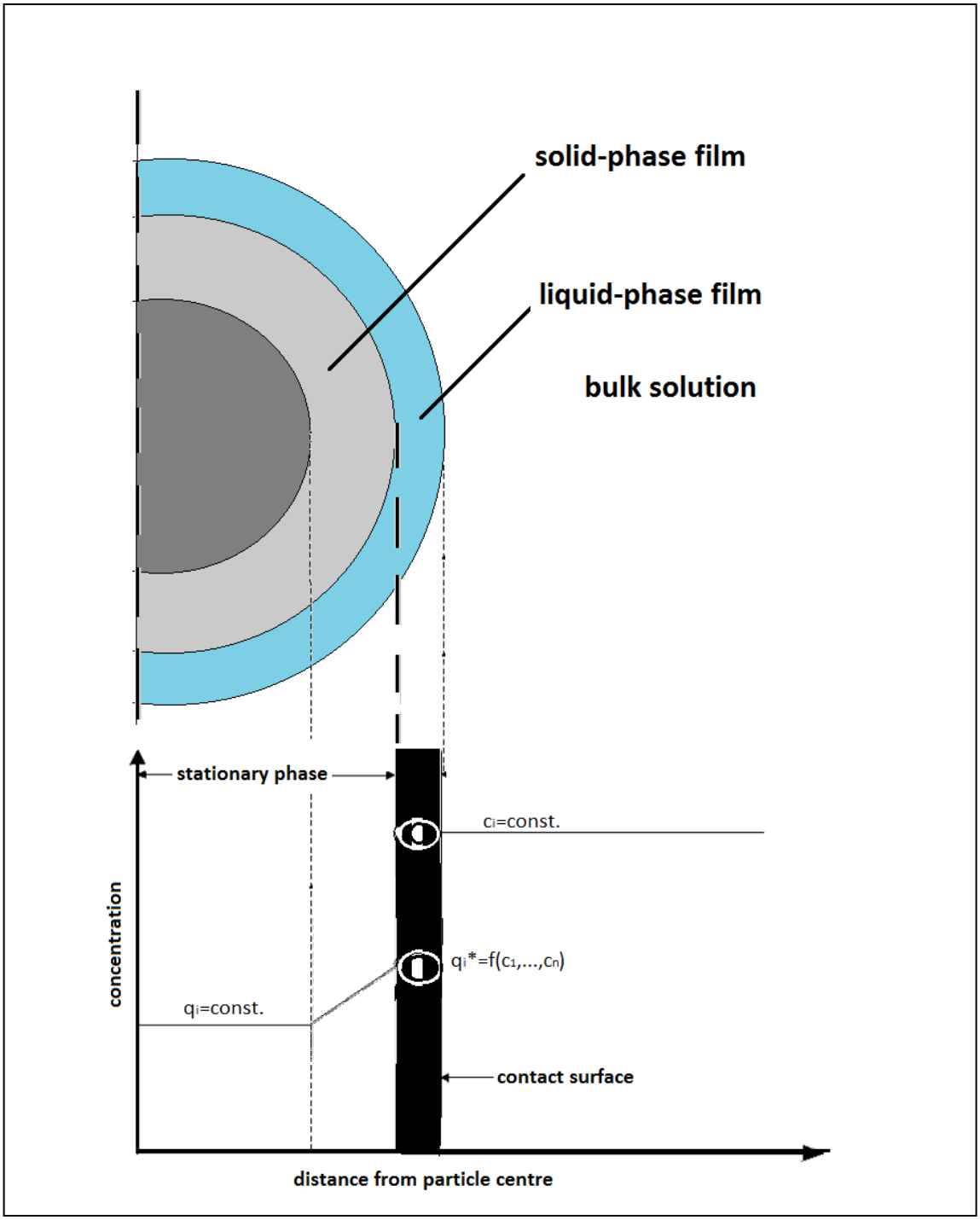

Figure 13: Solid film linear driving force model

Hence, the dominating mass transfer resistance in this model lies in the particle diffusion step represented by the thin solid film in which the CSP concentration changes from the equilibrium concentration, q_i^*, at the contact surface to $C_{S,i}$, the bulk avergae concentration. This resistance is described in the rate equation given below by an overall mass transfer coefficient, k_i.

$$\frac{\partial C_{S,i}}{\partial \mathrm{t}} = k_i(q_i^* - C_{S,i}) \tag{10}$$

The linear isotherm q_i^* varies based on the type of adsorption isotherm being used, but in a diluted region the linear isotherm, where K_i is the equilibrium constant, is given as follows:

$$q_i^* = K_i . C_i \tag{11}$$

The initial and boundary conditions (Danckwerts conditions) for this problem are:

$$c_i(z, t = 0) = 0 \qquad C_{S,i}(z, t = 0) = 0 \tag{12}$$

$$c_i(z = 0, t) = \begin{cases} c_{F,i} & 0 < t < t_F \\ 0 & t > t_F \end{cases} \qquad C_{S,i}(z = 0, t) = f_i\left(c_{F,1}, c_{F,2}\right) \tag{13}$$

$$D_L \frac{\partial c_i}{\partial \mathrm{z}}\Big|_{z=L} = v[c_i(t, 0^+) - c_i(t, 0^-)] \tag{14}$$

$$\frac{\partial c_i}{\partial \mathrm{z}}\Big|_{z=L} = 0 \tag{15}$$

Where $c_{F,i}$ is the sample feed concentration, t_F is the injection time, and f_i is the function of the adsorption isotherm.

4.2 Competitive adsorption isotherm models

The competitive adsorption isotherm is the equilibrium isotherm which gives the relationship between the concentrations of a component i in the stationary phase q_i^* and

the mobile phases. Several adsorption isotherm models have been introduced to describe the adsorption behaviour on chromatography columns. Among these models, the most popular ones are the Langmuir and the bi-Langmuir models due to their simplicity, especially in the theoretical development of multi-component adsorption process (Xin, 2004).

4.2.1 Competitive Langmuir Isotherm

The competitive Langmuir isotherm model assumes monolayer coverage of the adsorbate molecules over a homogeneous adsorbent surface (Lorenz et al. 2006; Row, Choi, Han, & Chung, 2007). In the case of equilibrium, the rate of adsorption and desorption are equal for each component. This model assumes the number of adsorption sites is limited for solute molecules, hence the total concentration in the stationary phase can not exceed a limiting concentration q_{max}. Amongst other researchers, Choi et al. (1998) applied this model to determine the adsorption behaviour of racemic bupivacaine on Kromasil® CHI-TBB CSP column as part of his work on enantioseparation of bupivacaine using SMB, while Asnin et al. (2010) used it to study the adsorption of Naproxen enantiomers on the CSP Whelk-O1. The Langmuir model is most commonly used to describe nonlinear adsorption and for each component i it is given as:

$$q_i^* = \frac{q_s b_i c_i}{1+b_1 c_1+b_2 c_2} = \frac{H_i c_i}{1+b_1 c_1+b_2 c_2} \tag{16}$$

Where q_s is the monolayer capacity of the CSP, q_i^* is adsorbed amount of each component in the stationary phase at equilibrium of the mobile phase concentration c_i, and $b_i$is the equilibrium constant of adsorption. The subscripts on the equilibrium constants, 1 and 2,

refer to the less retained component (RRS)-nadolol and the more retained component (RSR)-nadolol respectively.

4.2.2 Competitive bi-Langmuir Isotherm

The competitive bi-Langmuir adsorption isotherm model assumes two different types of binding sites exist on the surface of the CSP with either one being homogeneous. This model is widely applied to describe the competitive retention mechanism of chiral molecules on various CSPs and was used to study the isotherm behaviour of the pindolol on α1-acid glycoprotein (AGP) chiral stationary phase (Zhang, 2007; Santos, Veredas, Silva Jr, Correia, T, & Santana, 2004). The competitive bi-Langmuir isotherm model is written as:

$$q_i^* = \frac{q_{1s} b_{1,i} c_i}{1+\sum_{i=1}^{2} b_{1,i} c_i} + \frac{q_{2s} b_{2,i} c_i}{1+\sum_{i=1}^{2} b_{2,i} c_i} \quad (17)$$

where q_{1s} and q_{2s} are the saturation capacities of the two sites; $b_{1,i}$ and $b_{2,i}$ are the equilibrium constants of the two sites for component i.

The inverse method was used in determining the isotherm parameters or coefficients for the selected adsorption isotherm model. The optimization is carried out by minimizing the error function $F(p)$, where p is the total number of experimental data, defined as the sum of square of difference between the experimental and calculated concentration profiles using the isotherm and column models (Francotte, Richert, Mazzotti, & Morbidelli, 1998; Mao, 2012).

$$F(p) = min_\theta \sum_{j=1}^{p} (c_j^{exp} - c_j^{cal})^2 \ , \theta = q_s, b_1, b_2, k_v, etc \quad (18)$$

The Non-dominated sorting genetic algorithm (NSGA-II-JG) was used in this study to obtain the best-fit values of the isotherm parameters, including the bi-Langmuir coefficients of

the stereoisomers of nadolol summarized in Table 3 in Chapter 6 whereas the NSGA is explained in more detail in Section 7.1 of this dissertation.

4.3 Model Parameters

4.3.1 Bed Voidage

There are three types of porosities into which the column volume can be divided. These are internal porosity ε_{int}, external porosity (also known as the bed voidage ε) ε_{ext}, and the total porosity ε_T and are related by:

$$\varepsilon_T = \varepsilon_{ext} + (1 - \varepsilon_{ext})\varepsilon_{int} \tag{19}$$

The bed voidage can generally be evaluated from the zero retention time which is defined as the time that marks the first significant disturbance to the baseline. This disturbance is caused by column detection of difference in compositions of sample solution and the mobile phase or time at which a non-adsorbed component, such as a buffer or strong mobile, leaves the CSP column as an unresolved plug (Cavalcantre Jr., 2000; Xin, 2004).

For a component that enters the pore system but does not adsorb to the surface of the CSP, the retention time is given by:

$$t_{OR} = \frac{V\varepsilon_T}{V} = \frac{AL\varepsilon_T}{Au} = \frac{L\varepsilon_T}{u} \tag{20}$$

Therefore from this equation, where u is the superficial velocity and V the volumetric flow rate of the mobile phase respectively, it becomes clear that the bed voidage ε can be calculated using knowledge of total porosity and internal particle porosity.

Wang et al. (2006) determined the total porosity ε_T to be 0.77 by plotting the mean retention time against the inverse flow rate for a non-adsorbed component of 1,3,5 tri-

tert-butyl benzene (TTBB) which can enter the pores of the chiral stationary phase (CSP). The following correlation suggested by Ruthven (1984) was then used to evaluate the bed voidage which was found to be 0.58 for the perphenyl carbamoylated β-CD column.

$$\varepsilon_T = 0.45 + 0.55\varepsilon \tag{21}$$

4.3.2 Axial Dispersion Coefficient

There is a tendency for axial mixing to occur when a fluid flows through a packed bed, and reduces the efficiency of separation. All the phenomena that contribute to axial mixing, except that of mass transfer resistance, are lumped into an axial dispersion coefficient, D_L. Since it is caused by the twin effects of molecular diffusion and eddy diffusion occurring through the interparticle space, axial dispersion coefficient is given by:

$$D_L = \gamma_1 D_m + \gamma_2 d_p v \tag{22}$$

where d_p is the diameter of the particle size, γ_1 and $\gamma_2$are geometrical constants, and D_m is the molecular diffusion coefficient of the solute in the mobile phase (Ruthven, 1984). For a pair of enantiomers that exhibit identical chemical and physical properties, their diffusion coefficients D_m and therefore their dispersion coefficients D_L are equal. This dispersion coefficient is also assumed as constant in all the columns of the SMB process for the sake of the simplicity (Cherrak, Khattabi, & Guiochon, 2000; Lorenz et al., 2007).

For convenience, the dispersion coefficients $D_L$can also be expressed as follows, where η is the tortuosity factor for a packed column, and λ is the flow-geometry dependent constant.

$$D_L = \eta D_m + \lambda v \tag{23}$$

Since the molecular diffusivities D_m of liquids is too small to contribute significantly to axial dispersion the expression for dispersion coefficient can be further simplified to:

$$D_L = \lambda v \tag{24}$$

From the moment analysis of the solution of the general rate model in the Laplace domain, the expression for (HETP) for a solid film linear driving force model is given by:

$$HETP = \frac{L}{N} = \frac{\sigma^2 L}{\mu_1^2} = \frac{2D_L}{v} + 2v\left[\frac{\varepsilon}{1-\varepsilon}\right]\frac{1}{kK}\left[1 + \frac{\varepsilon}{(1-\varepsilon)K}\right]^{-2} \tag{25}$$

Where

$$K = \varepsilon_p + (1 - \varepsilon_p)H \tag{26}$$

$$N = 5.545\left[\frac{t_R}{w_{1/2}}\right]^2 \tag{27}$$

Where *L* is the column length, *N* the number of plates, $w_{1/2}$ the peak width at half height, D_L the axial dispersion coefficient, *k* the overall mass transfer coefficient, *K* is the equilibrium constant, and *H* is the Henry constant.

Using equations (24-27) the axial dispersion and overall mass transfer coefficient were evaluated and reported to be 0.001364*v* and 86.8 min^{-1}.

Chapter 5: Continuous Counter-Current Separation of Nadolol Enantiomers

5.1 Background of Chromatography

Chromatography is a powerful and efficient separation technology that was applied for the first time in 1906 by Mikhail Tswett for the separation of plant pigments with calcium carbonate, and ever since has proven its value and importance in various fields requiring separation of binary or even multi-component mixtures. The pharmaceutical industry specifically owes some of its major advancements to preparative chromatography, especially in the development of new drugs from chiral drugs whose components are made available for evaluation by this technology. The separation is based on the difference in adsorption affinities of different components in the flowing mobile phase to a solid stationary phase (Xin, 2004).

Two approaches to operate and scale up chromatographic processes, are batch and continuous chromatography. The batch-wise route however has major drawbacks, the two main ones being the finite recovery yield of the components of interest due to low production rates and difficulty recycling mixed fraction that have not been separated completely. Therefore, optimising the batch chromatography process is complex. Continuous chromatography, which refers to a process configuration that allows continuous introduction of feed and continuous withdrawal of products, results in higher throughputs and usually recyling is already built into the process. The consistent product quality obtained from this continuous approach as well as minimum capital operating costs are added advantages that make this process preferred for industrial applications. These continuous systems can be further divided into cross-current and counter-current

configurations based on the relative motions between the fluid and sorbent phases. In the latter the two phases move in opposite directions while in cross-current systems the motions of fluid and sorbent phases “cross” each other at 90 degrees (Ganetsos and Barker, 1993). Only the counter-current process will be discussed in more details since it has proved very successful for large-scale operations and therefore used in SMB technology.

5.2 Continuous counter-current chromatography

Counter-current processes can be classified into two categories, namely true counter-current processes in which the solid phase is mobile, and the simulated counter-current processes in which the solid phase is actually stationary but its motion is simulated by mechanical shifting of inlets and outlets as will be discussed later in this chapter. Figure 14 illustrates an analogy for countercurrent chromatography where the snail and rabbit are analogous to a strongly adsorbed component and less adsorbed component respectively (Mazzotti et al., 2009). Both are pushed by the conveyer belt in the opposite direction but since the rabbit runs at a greater velocity than the snail, the rabbit will separate from the snail and reach the end of the conveyer belt faster. If the velocity of the conveyer belt is intermediate to that of the two animals, the rabbit will have a net velocity to the right and the snail to the left resulting in complete separation and collection of the each. If the speed of the conveyer belt, however, is less than the snail or greater than that of the rabbit then either both will be conveyed to the right or pushed back to the left. Therefore, operating the counter-current process at the intermediate velocity is key to efficient

separation achieved by maximizing the separation driving force between the liquid phase and solid phase (Hsuen, 2000; Grosfils, 2009; Xin, 2004).

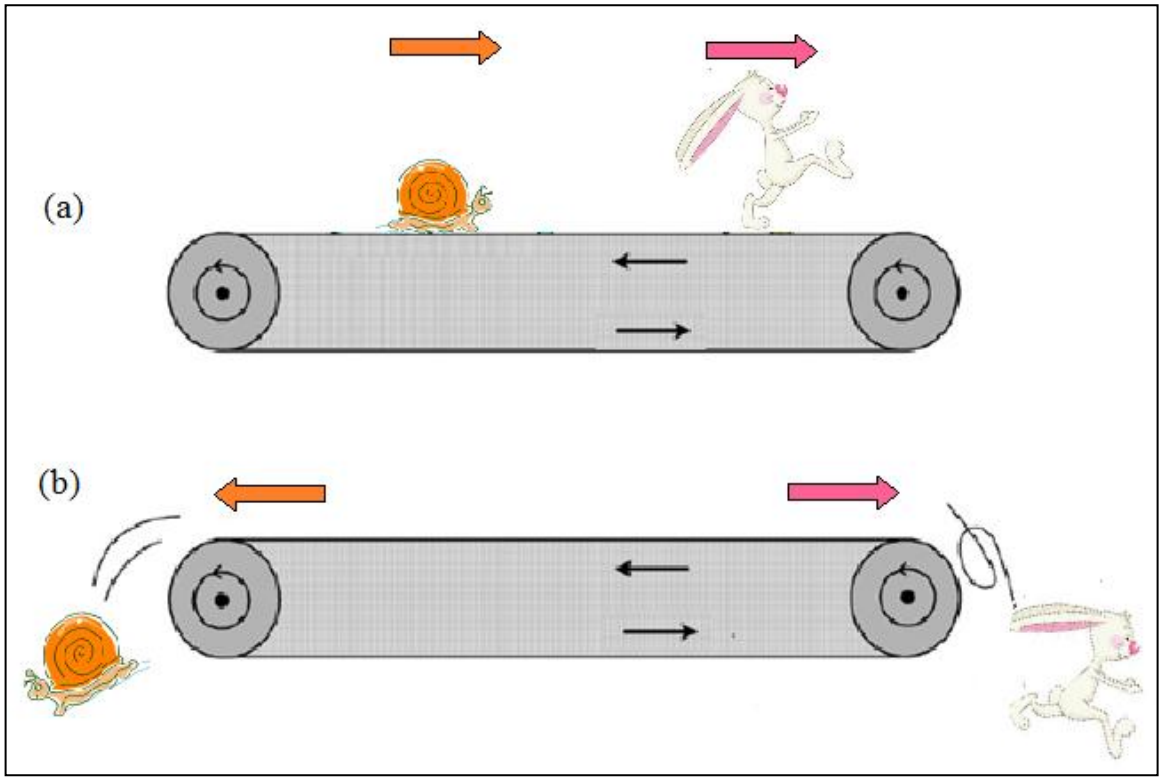

Figure 14: The snail-rabbit separator analogy in a chromatographic column where the more-retained species A is represented by a Snail and less retained species B by a Rabbit. (a) Analogy for countercurrent chromatography when the belt velocity has no effect . (b) Analogy with efficient belt velocity for separation

5.2.1 True Moving Bed (TMB)

In a true moving bed (TMB), the solid phase (adsorbent) physically moves opposite to the liquid phase (desorbent) flow, contacting each other in a pattern of countercurrent flow as illustrated in Figure 15 for the binary separation of more retained species A and less retained species B. The column consists of four sections as shown and the sample with species A and B is fed continuously in the middle of the column between sections 2 and 3. Meanwhile, the desorbent mobile phase flows continuously into Section 1 and the adsorbent solid phase is introduced into Section 4. The adsorbent will retain species A in

Sections 2 and 3 so it can be collected in the extract port by washing the solid phase with flowing mobile phase, while the flowing desorbent will wash component B in Sections 3 and 4 for collection in the raffinate port. The solvent that exits Section 4, which should contain none of the two species A and B, can then be recycled to Section 1 and similarly, the solid phase after regeneration is recycled from Section 1 to Section 4.

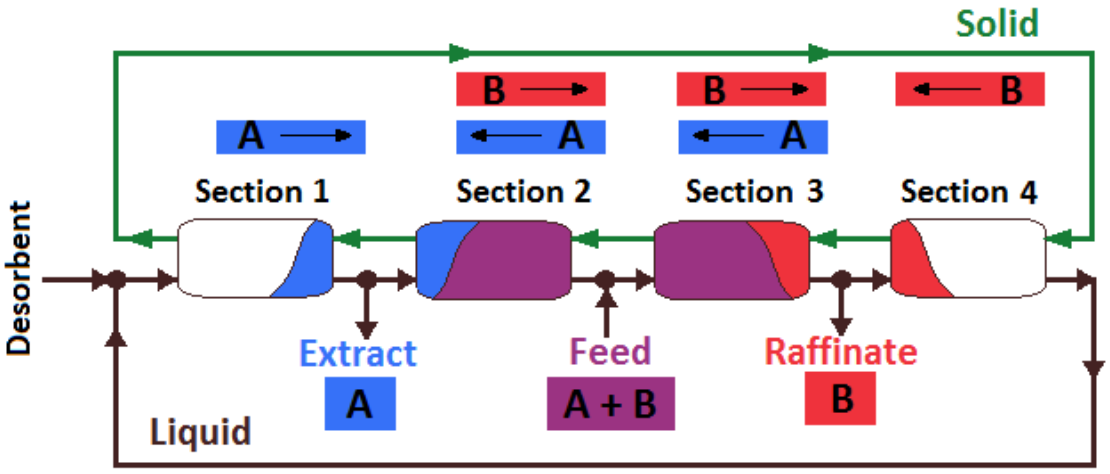

Figure 15: Schematic of four-section true moving bed (TMB)

The hypersorption process developed by Union Oil Company in the early 1950s is the earliest known example of large-scale moving bed process (Xin, 2004; Berg, 1946). Besides that it allows for continuous processing of the feed leading to an increase in productivity, another advantage of the TMB process compared to the elution chromatography is reduced solvent consumption by recycling. In addition it is still possible to obtain pure products without necessarily achieving complete separation which makes effective resolution of mixtures whose components have similar retention properties easier and more efficient with a TMB rather than conventional elution chromatography. Much work had been carried out on true moving bed systems and some large scale systems were

used. However, TMB process has disadvantages associated with the physical movement of the solid phase which include solid particle attrition, loss of efficiency due to back-mixing and limited liquid velocity to avoid fluidization of the solid particles. Obtaining uniform flow of both solid phase and liquid phase in chromatography columns of large diameter is also difficult which can impair the performance of the TMB, thus hindering separation (Broughton, 1978; Ruthven & Ching, 1989; Mao, 2012).

5.2.2 Simulated Moving Bed (SMB)

The first implementation of the concept of the simulated moving bed (SMB) was the Sorbex technology, which was designed for p-xylene recovery from mixed xylenes, by UOP in 1961. This continuous countercurrent multi-column chromatographic separation technique was invented by Broughton and Gerhold (1961) to overcome those aforementioned drawbacks of the true moving bed (TMB). The counter-current contact in the SMB is simulated by means of a rotary valve that periodically shifted the feed, eluent, raffinate, and extract lines along the fixed-bed chromatographic columns at fixed time intervals in the direction of fluid flow. Since its early stages of development this technology has also been employed on a commercial scale in so many industries ranging from the food and pharmaceutical industries to the fine chemical and petro-chemical industries (Erdem, 2004; Rathore & Velayudhan, 2003). Ironically, even though SMB technology was first developed for the hydrocarbon separations, the number of hydrocarbon separations where SMB technology is economically competitive is very limited. Instead, due to increasing demand for the pure enantiomers by the regulation of FDA, SMB has been increasingly gaining special attention as the efficient and effective method of chiral

separation in the pharmaceutical industry. The first successful chiral resolution by SMB was performed by Negawa and Shoji who separated 1-phenylethanol on Chiralcel OD in 1992. Following that a large number of chiral SMB separations were reported in the literature and with advancements in chiral stationary phase (CSP) development in recent years the number of SMB chiral separation candidates is virtually unlimited with access to sufficient amounts of stable and selective CSPs (Broughton, 1978; FDA, 1992; Gomes, 2009). A typical configuration of the SMB is shown in Figure 16.

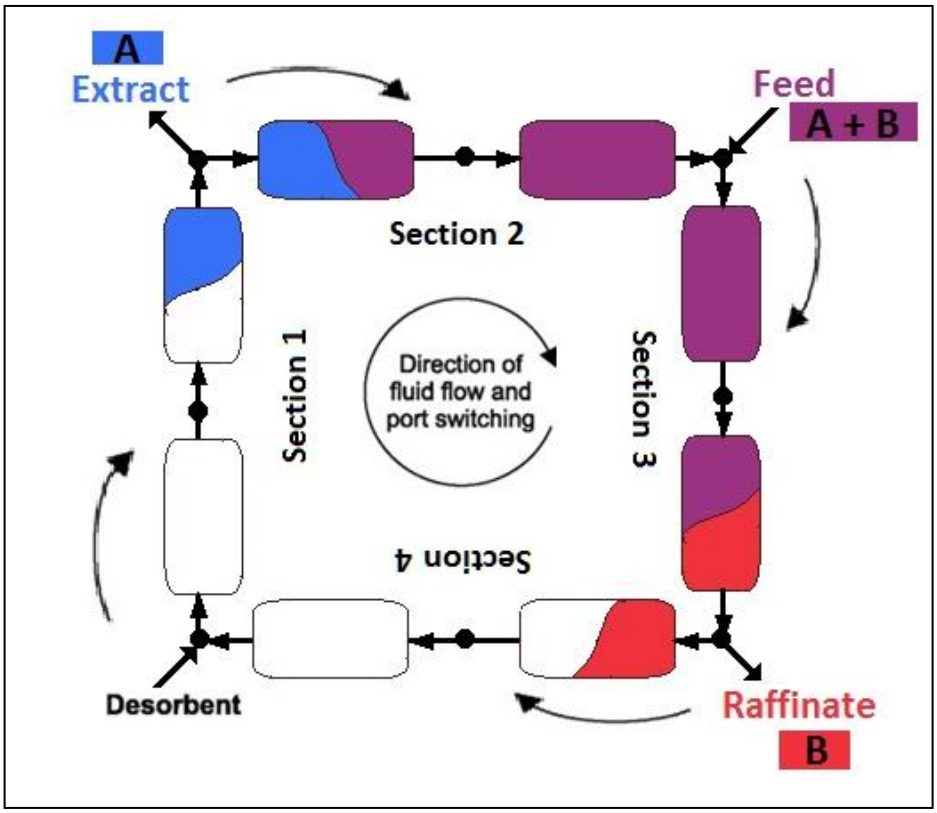

Figure 16: Schematic of four-section simulated moving bed (SMB)

In a standard SMB set-up, the CSP-packed columns are divided into four sections which can be further divided into several subsections. All inlets and outlets are constantly switched

into the next valve positions at the same time in the direction of fluid flow, simulating the flow of solid adsorbent in the direction opposite to that of the mobile phase. Due to this continuous nature of operation of SMB and an efficient use of the stationary and mobile phases, the desorbent requirement is much less than that of classical elution chromatography, whereas the productivity per unit time and unit mass of stationary phase is much higher. Moreover, high purity is achievable without sacrificing yield and the reduced footprint, equipment size, and manpower make using SMB for large scale chiral separations more economical than batch preparative process (Mazzotti, Rajendran, & Paredes, 2009; Abel, 2004).

In order to achieve complete separation of the two species A and B, the fluid to solid flow ratio m_1 must be large enough to completely regenerate the solid stream before it is recycled and the flow ratio m_4 must be low enough to adsorb all raffinate components from the recycled desorbent. On the other hand, the flow ratios m_2 and m_3 must also be limited in a certain operating parameter space as will be explained in SMB design section (Abel, Mazzotti, & Morbidelli, 2004). Research has shown that even though the greater the number of ports of a SMB unit the more it resembles the performance of the TMB, a small subdivision of the bed (usually 2 columns per section) is sufficient to ensure that an SMB unit performs close to the ideal TMB countercurrent operation. In fact, an SMB system with infinite number of ports or adsorbent fixed-bed columns and infinitesimal switch times is considered to be theoretically identical to the equivalent TMB process (Pais, Loureiro, & Rodrigues, 1998).

Chapter 6: Simulated Moving Bed (SMB) Technology in Chiral Separation

6.1 Simulated Moving Bed Model

Chromatographic columns can be modeled in many different ways corresponding to different levels of simplification (Guiochon et al., 2006). A mathematical model of the SMB process can be obtained using the model of a single column as a building block while considering the cyclic port switching by implementing material balance equations at the nodes of the unit connecting the dynamic models of the columns. The following basic assumptions and considerations are generally followed in establishing a model for the SMB process, however if the practical situation warrants then one or more of these can be relaxed (Lu, Yang, & Wu, 2006; Yan, 2006). The assumptions of the model are:

1. No radial variation occurs in the column so concentration gradients in the radial direction of the bed are negligible
2. Thermal effects are negligible since operation of SMB process is under isothermal conditions
3. The axial dispersion and mass transfer coefficients are considered constant
4. The flow rates in the column are constant and all columns have identical void fraction and porosity
5. The pressure drop in the column is disregarded and compressibility of the mobile phase is neglected.
6. The solvent or mobile phase does adsorb to the stationary phase particles
7. The intraparticle mass transfer is described by a linear driving force (LDF)

6.1.1 Node Model

The following mass balances at the inlet and outlet nodes in the SMB configuration relate the internal flow rates Q_j, (j = 1, . . . , 4) to the external flow rates Q_D, Q_E, Q_F, Q_R, where the subscripts D, E, F and R stand for desorbent, extract, feed and the raffinate streams, respectively and superscripts "in" and "out" refer to the inlet and outlet streams, respectively (Abel, Mazzotti, & Morbidelli, 2004):

Desorbent inlet node (Eluent)

$$Q_D = Q_1 - Q_4 \tag{28}$$

$$c_{i,D} = \frac{Q_1 c_{i,1}^{in} - Q_4 c_{i,4}^{out}}{Q_D} \tag{29}$$

Extract withdrawal node

$$Q_E = Q_1 - Q_2 \tag{30}$$

$$c_{i,E} = c_{i,1}^{out} = c_{i,2}^{in} \tag{31}$$

Feed inlet node

$$Q_F = Q_3 - Q_2 \tag{32}$$

$$c_{i,F} = \frac{Q_3 c_{i,3}^{in} - Q_2 c_{i,2}^{out}}{Q_F} \tag{33}$$

Raffinate withdrawal node

$$Q_R = Q_3 - Q_4 \tag{34}$$

$$c_{i,R} = c_{i,3}^{out} = c_{i,4}^{in} \tag{35}$$

6.1.2 Triangle theory method

The equivalence between TMB and SMB processes can be quite useful in guiding the design and selection of operation criteria of an SMB unit for complete separation of a binary mixture of more retained component A and less retained component B. The "Triangle Theory" is a design method that facilitates the preliminary and rather accurate estimation of the optimum operating conditions for the desired separation and provides a precise explanation of the main features of SMB operations in both linear and nonlinear ranges (Gomes, 2009). This strategy developed by Storti et al. (1989) works in the framework of equilibrium theory model which neglects mass transfer resistance and axial dispersion and describes the transport of a solute i along the axial coordinate z of a chromatographic column in section j of the SMB unit by the following material balance:

$$\nu_j \frac{\partial c_{i,j}}{\partial \mathrm{z}} + \frac{\partial}{\partial \mathrm{t}}\left[c_{i,j} + \frac{1-\varepsilon}{\varepsilon} q^*_{i,j}\right] = 0 \tag{36}$$

Under the assumption of linear adsorption isotherms it is shown that both SMB and TMB models are equivalent, therefore leading to identical design criteria that satisfy the following equivalence relationships:

$$Q_j^{SMB} = Q_j^{TMB} + \left(\frac{\varepsilon}{1-\varepsilon}\right) Q_s \tag{37}$$

$$\frac{V}{t^*} = \frac{Q_s}{1-\varepsilon} \tag{38}$$

$$Q_s = u_s A(1-\varepsilon) \tag{39}$$

where Q_j is the volumetric flow rate of the fluid in section *j*, Q_s the volumetric flowrate of the solid, *V* the volume of the chromatographic column, ε is the void fraction of the column and t^* is the switch time of the SMB unit.

These relationships enforce the condition that the ratio of fluid phase velocity to solid phase velocity, also known as net flow rate ratio m_j, must be the same in the SMB and in the TMB columns. This dimensionless net flow rate ration is defined as:

$$m_j = \frac{Q_j t^* - V\varepsilon}{V(1-\varepsilon)} = \frac{net\ fluid\ flow\ rate}{net\ solid\ flow\ rate} \tag{40}$$

where Q_j^{SMB} and Q_j^{TMB} are the flow rates of the fluid phase in section j of a SMB unit and in the equivalent TMB unit, respectively.

In the linear case the adsorption isotherm is described as follows where H_i is the Henry constant of the component i, and $H_A > H_B$:

$$q_i^* = H_i c_i \tag{41}$$

The Henry constants, is given by Pedeferri et al. (1999) as:

$$H_i = \frac{t_{i,j}^R - t_{0,j}}{t_{0,j}} \left(\frac{\varepsilon}{1-\varepsilon}\right) \tag{42}$$

$$t_{0,j} = \frac{V\varepsilon}{Q_j} \tag{43}$$

$$t_{i,j}^R = \frac{V\varepsilon}{Q_j^{SMB}} \left(1 + \frac{1-\varepsilon}{\varepsilon} H_i\right) \tag{44}$$

where $t_{i,j}^R$ is the retention time and t_{0j} is the column dead time or residence time of the mobile phase in section j, respectively.

The triangle theory specifies a triangle region formed in the plot of the flow ratios of Sections 2 and 3 where separation is perfomed, which identifies the region of complete

separation based on a number of constraints considering the specific role of the different sections in the SMB unit. Recalling the the function of each section of the the four-zone SMB where the binary mixture is fed between Sections 2 and 3 , the constraints and criteria for achieving complete separation of the more retained component A (in extract between Sections 1 and 2) and less retained component B (in raffinate between Sections 3 and 4) can be deduced. Operation of Sections 2 and 3 should be designed in such a way that the switch time is larger than retention time of component B and smaller than that of component A. This allows component B to reach the raffinate port without any contamination from component A, and ensures that component A reaches the extract port alone since component B will have been completely removed from the column. Since the role of Section 1 is to ensure elution of component A and total regeneration of the adsorbent or solid stationary phase, the switch time in Section 1 should be larger than the retention time of component A. Likewise, to ensure complete regeneration of a pure desorbent or mobile phase, which is the role of Section 4, the switch time in this section should be smaller that the retention time of component B. The conditions described above can be expressed as the following constraints (Mazzotti, Rajendran, & Paredes, 2009):

$$t_{A,1}^{R} \leq t^{*} \tag{45}$$

$$t_{B,2}^{R} \leq t^{*} \leq t_{A,2}^{R} \tag{46}$$

$$t_{B,3}^{R} \leq t^{*} \leq t_{A,3}^{R} \tag{47}$$

$$t^{*} \leq t_{B,4}^{R} \tag{48}$$

Substituting Eq. (44) into these inequalities yields the following constraints on the net flow rate ratios that must be satisfied for complete separation of the binary mixture:

$$H_A \leq m_1 \tag{49}$$

$$H_B < m_2 \leq H_A \tag{50}$$

$$H_B \leq m_3 \leq H_A \tag{51}$$

$$\frac{-\varepsilon}{1-\varepsilon} < m_4 \leq H_B \tag{52}$$

The constraints on m_2 and m_3 can be combined as follows to satisfy the positive feed flow rate constraint that m_3 be greater than m_2:

$$H_B < m_2 < m_3 < H_A \tag{53}$$

This inequality defines the projection of triangle region in the (m_2, m_3) plane and (m_1, m_4,) plane, as shown in Figures 17 and 18, which provides a number of feasible operating conditions to ensure that the extract and raffinate collected at their respective ports are 100% pure. Regions for a pure raffinate product or extract product are also specified as the lower-left square ($m_2 < H_B$) and upper-right square ($m_3 > H_A$), respectively. Whereas, neither pure extract nor pure raffinate can be obtained by operating the SMB unit in the upper-left square conditions where $m_2 < H_B$ and $m_3 > H_A$, and ideally the vertex in the triangle region provides the optimal operating conditions.

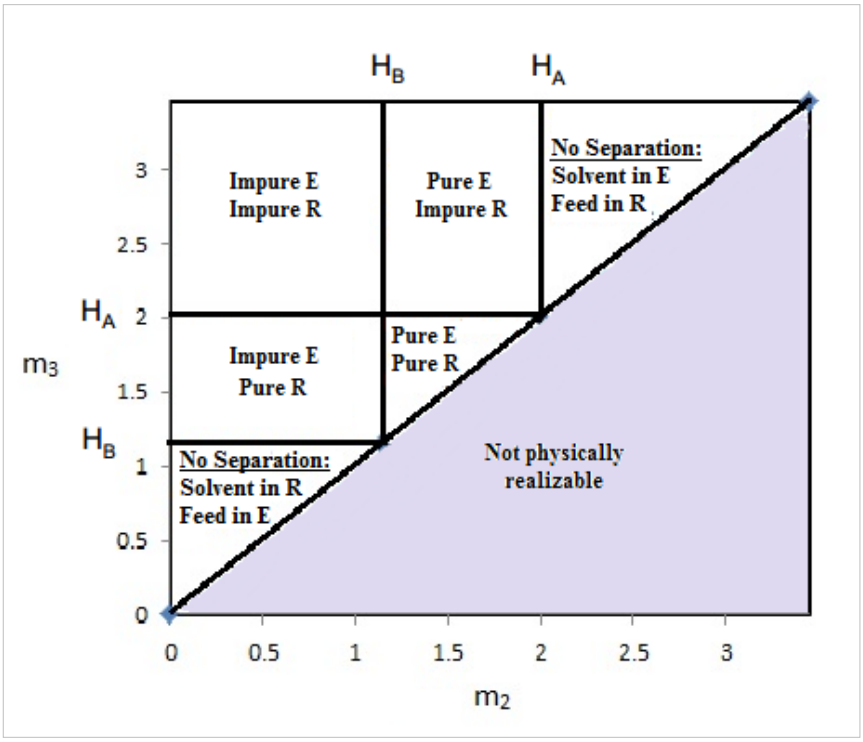

Figure 17: Separation zones defined by the triangle theory for binary separation with linear adsorption isotherm, where R is for raffinate and E for extract

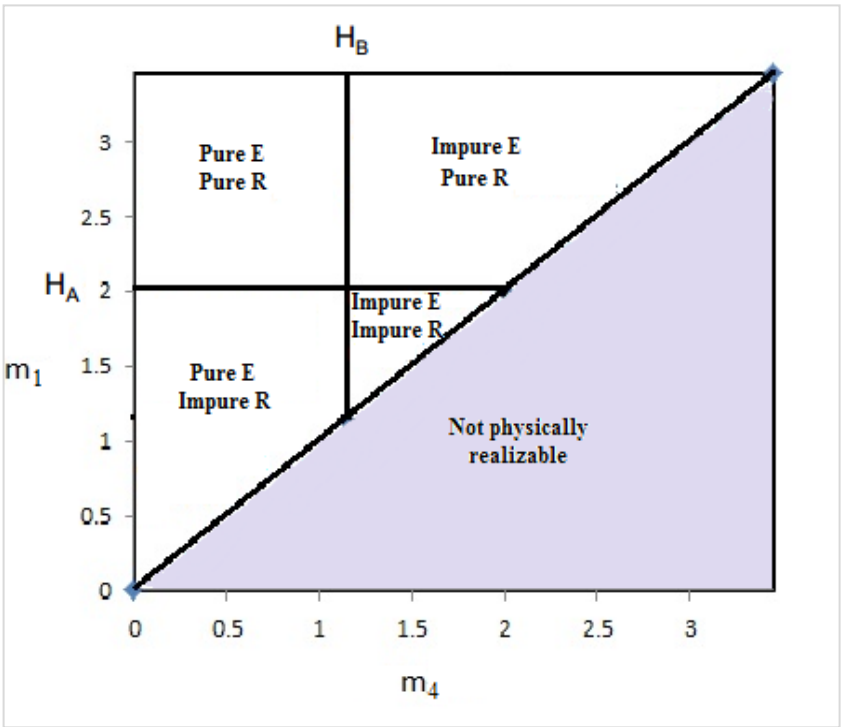

Figure 18: Triangle theory: the different separation on the (m_1, m_4) plane for binary separation with linear isotherm

6.1.3 Triangle theory extended (non-linear isotherm)

Knowledge, understanding and application of the triangle theory to obtain design criteria for SMB separation based on a linear isotherm is very importnant and practically useful. However, the need developed to extend this theory to deal with the nonlinear case where the equilibrium theory for binary separations is subject to nonlinear adsorption isotherms like the competitive Langmuir (Storti et al., 1993; Mazzotti, 2006), the modified Langmuir (Charton and Nicoud, 1995; Sa Gomes, 2006) and bi-Langmuir isotherms that apply to most cases of interest (Gentilini et al., 1998; Xin, 2004). Taking the competitive Langmuir isotherm as an example, the triangle for complete separation is given in Figure 19 and the constraints on the net flow ratios based on the equilibrium theory model differ from the linear case as follows:

Langmuir isotherm:

$$q_i^* = \frac{q_s b_i c_i}{1+b_1 c_1+b_2 c_2} = \frac{H_i c_i}{1+b_1 c_1+b_2 c_2} \tag{54}$$

$$m_{1,min} \leq m_1 \tag{55}$$

$$m_{2,min} \leq m_2 \leq m_{2,max} \tag{56}$$

$$m_{3,min} \leq m_3 \leq m_{3,max} \tag{57}$$

$$m_4 \leq m_{4,max} \tag{58}$$

These constraints on the net flow rate ratios can be summarized into:

$$H_A = m_{1,min} \leq m_1 < \infty \tag{59}$$

$$m_{2,min} \leq m_2 < m_3 \leq m_{3,max} \tag{60}$$

$$\frac{-\varepsilon}{1-\varepsilon} < m_{4,max}(m_2, m_3) < H_B$$

$$= \frac{1}{2}\left\{H_B + m_3 + b_B c_{B,f}(m_3 - m_2) - \sqrt{\left[H_B + m_3 + b_B c_{B,f}(m_3 - m_2)\right]^2 - 4H_B m_3}\right\} \quad (61)$$

Similar to the linear case, the implicit constraints on m_2 and m_3 still define the complete separation region in the (m_2, m_3) plane for the case of a non-linear isotherm. However, the lower bound on m_1 and upper bound on m_4 were found to be explicit and m_4 was found to be dependent on the flow rate ratios m_2 and m_3. Although the region for the complete separation in the (m2, m3) plane is no longer strictly triangle-shaped this approach is still referred to as the triangle theory (Pedeferri, Zenoni, Mazzotti, & Morbidelli, 1999). This theory is now widely used for SMB process design as it has been demonstrated to represent precisely the real SMB behavior providing a sound basis for simulation and optimization based on the detailed SMB model discussed.

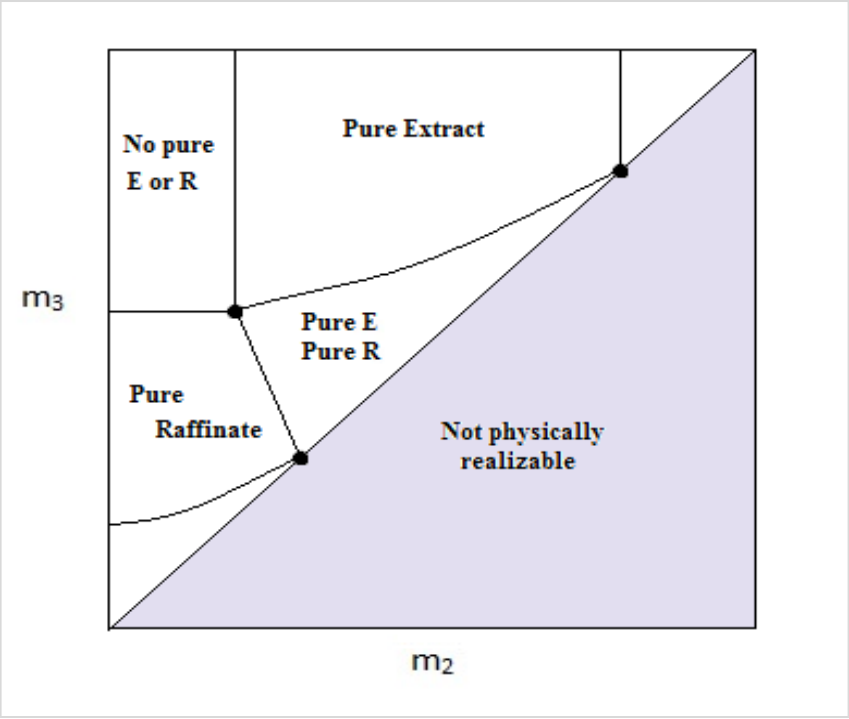

Figure 19: Separation zones in the (m, m) plane defined by the triangle theory for binary separation with Langmuir isotherm

6.1.4 Performance indicators

The performance and optimization of the operation of an SMB unit with the objective of achieving a complete separation are commonly evaluated using the the following performance indicators: purity, recovery, productivity, and desorbent requirement. These performance parameters are defined for the case of a binary mixture as given below (Mazzotti, Rajendran, & Paredes, 2009):

(1) Purity

The purity Pu of the raffinate and extract streams at cyclic steady state, in the case of a binary separation, is defined as the ratio of target component collected to sum of both components collected within a switching period.

$$Pu_R = \int_t^{t+t^*} \frac{c_{B,R}}{c_{A,R}+c_{B,R}} dt \tag{62}$$

$$Pu_E = \int_t^{t+t^*} \frac{c_{A,E}}{c_{A,E}+c_{B,E}} dt \tag{63}$$

where $c_{i,R}$ is concentration of the solute in the raffinate and $c_{i,E}$ that of the solute in the raffinate and extract stream, and subscripts R and E stand for raffinate and extract eluent, respectively.

(2) Recovery

The recovery or yield of the target component from mixture to be separated by the SMB process is a very important performance indicator from an economical point of view since a low yield of a pure product may not be enough to cover the costs of production. Recovery is essentially determined by the ratio of mass flow rate or amount of a particular

species to the total feed flow or amount of that species fed into the system within a switching period (Wang L. , 2009). The recoveries of more retained species A in extract, Re_A, and the less retained species B, Re_A, in raffinate stream over a complete cycle are defined as:

$$Re_A = \frac{Q_E}{Q_F}\int_t^{t+t^*} \frac{c_{A,E}}{c_{A,F}} dt \tag{64}$$

$$Re_B = \frac{Q_R}{Q_F}\int_t^{t+t^*} \frac{c_{B,R}}{c_{B,F}} dt \tag{65}$$

where $c_{i,R}$ and $c_{i,E}$ are the concentrations of the solute in the raffinate and extract streams, respectively.

(3) Productivity

The productivity, PR, is typically defined as the ratio of the mass of feed being processed to the mass of chiral stationary phase (CSP) per unit time deduced mathematically as follows:

$$PR = \frac{Q_F c_{T,F}}{(1-\varepsilon)\rho_S V_T} = \frac{c_{T,F}(m_3 - m_2)}{\rho_S t^* \sum_{j=1}^{4} N_j} \tag{66}$$

where $c_{T,F}$ is the overall concentration of feed mixture, ρ_s is the density of the adsorbent or solid phase, V_T is the total column volume, ε is the void fraction of the column, t^* is the switching time, N_j is the number of columns in section j, while m_3 and m_2 are the flow-rate ratios of Sections 3 and 2 respectively.

It would appear from this definition that the maximum productivity can be obtained by maximizing the difference between m_3 and m_2 which can be achieved by operating the SMB process on the vertex of the triangular complete separation region discussed earlier. However, operating at these conditions decreases robustness hence leading to

compromising the purity of the products. Another possibility that can be construed from the definition of productivity for maximizing the output of the process is by decreasing the switching time which is feasible theoretically but practically limited. Increase in the internal flow rates, Q_j, as a result of reducing the switching time is the limitation on how much the switching time can be reduced. The reason behind this being that large flow rates are accompanied with high pressure drops which can completely ruin the column or in the best-case scenario reduce the column efficiency. The typical minimum recommended for switch time to ensure robust and stabilized operation is reported as one minute (Dunnebier, Fricke, & Klatt, 2000; Lee, 2005).

(4) Desorbent requirement

Another important parameter used to assess process performance is the desorbent requirement, *DR*, defined as the ratio of the mass of desorbent to that of feed.

$$DR = \frac{(Q_D+Q_F)\rho_D}{Q_F c_{T,F}} = \frac{\rho_D}{c_{T,F}}\left(1+\frac{m_1-m_4}{m_3-m_2}\right) \quad (67)$$

Where Q_D is the amount of desorbent and ρ_D is the desorbent density.

The importance of this parameter lies in that one of the major advantages of SMB that distinguish it from conventional chromatography is that the consumption of desorbent is smaller. Operating with the minimum amount of desorbent that still yields pure products is one of the most studied optimization problems since it reflects on the costs involved in the complete separation of the binary mixture. In the above formulation, it is worth noting that minimizing the difference between m_1 and m_4 minimizes the desorbent requirement , however operating the SMB unit in the region of the triangle theory diagram

correspondant to this condition renders the process to be less robust due to pollution to the product streams which may occur due to minor deviations (Lee, 2005; Mazzotti, Rajendran, & Paredes, 2009).

To summarize, the operating conditions chosen within the region of complete separation must take into consideration the requirement to minimize the desorbent requirement, *DR*, and at the same time maximize productivity, *PR*, and purity, *Pu*, of the products.

Chapter 7: Modeling and Simulation of SMB Separation of Nadolol

7.1 Introduction to Non-dominated Sorting Genetic Algorithm

Sorting Genetic Algorithm (SGA or GA), invented in 1975 by John Holland, is a form of evolution that mimics the process of natural selection on a computer to solve optimization problems through exploitation of historical information to direct the search into the region of better performance within the search space. The basic techniques of the GA's simulate the principle of "survival of the fittest" laid down by Charles Darwin among individuals, which are analogous to chromosomes in our DNA, in the population (gene pool) over consecutive generations for solving a problem. Similar to the process mother nature uses, the GA evolves to an improved generation of chromosomes through three main operators: selection, crossover or recombination, and mutation (D'Souza, Sekaran, & Kandasamy, 2010).

Selection implements the "survival of the fittest" theory by generating the initial population comprised of copies of chromosomes which are evaluated and assigned fitness values based on an objective function or subjective judgment specific to the optimization problem. These selected individuals are deemed fittest to pass on their genes. The crossover operator recombines two random parent chromosomes resulting in a new pair of daughter chromosomes which create the next generation of population that will hopefully create even better individuals given they actually turn out to be better than the parent chromosomes. Following recombination, the mutation operator will flip some bits in some of the new individuals. This happens with a very small probability but is important to maintain diversity in the population and improve the ability for reaching the Pareto

optimal solution by inhibiting premature convergence. Pareto optimal solutions are those solutions in the set which are not dominant or superior to any other in all the objective function evaluations and therefore any one of the these solutions in the Pareto set is an acceptable solution. This entire process of selection, crossover and mutation is repeated again and again until the termination criteria such as a set maximum number of generations or a specific function tolerance are satisfied (Srinivas & Deb, 1994; Tarafder, Ray, & Gupta, 2004).

The difference between the SGA and Non-dominated Sorting Genetic Algorithm (NSGA) is in the way the selector operator works. It removes fitness-proportionate selection problems by basing selection on relative rather than absolute fitness which can lead to premature convergence due to one or two highly fit members gaining dominance early on at the expense of others that are less fit. Hence, NSGA's ranking selection method preserves diversity in the population promoting the possibility to find multiple non-dominated solutions to various multi-objective optimization problems. NSGA uses a ranking selection method which identifies non-dominated individuals in a current population and assigns them a large dummy fitness value arbitrarily. The niche method is the sharing procedure that is then used to maintain a stable and diverse population by dividing the original dummy fitness value by the niche count, a quantity proportional to the number of neighbors around it, representing how crowded the individuals are in the decision variable space.

The process of identifying non-dominated individuals for the second and third front and assigning them new dummy fitness values smaller than the minimum shared dummy

fitness value of previous fronts is repeated until all the chromosomes in the gene pool are assigned shared fitness values. The population then undergoes reproduction, crossover and mutation with non-dominated members of the 1st front having higher representation in the mating pool while dominated ones of later fronts get fewer copies than the rest of the population. This division causes multiple optimal points to co-exist in the population and therefore the population converges rapidly (D'Souza, Sekaran, & Kandasamy, 2010). This processing of populations in NSGA-II is illustrated in Figure 20.

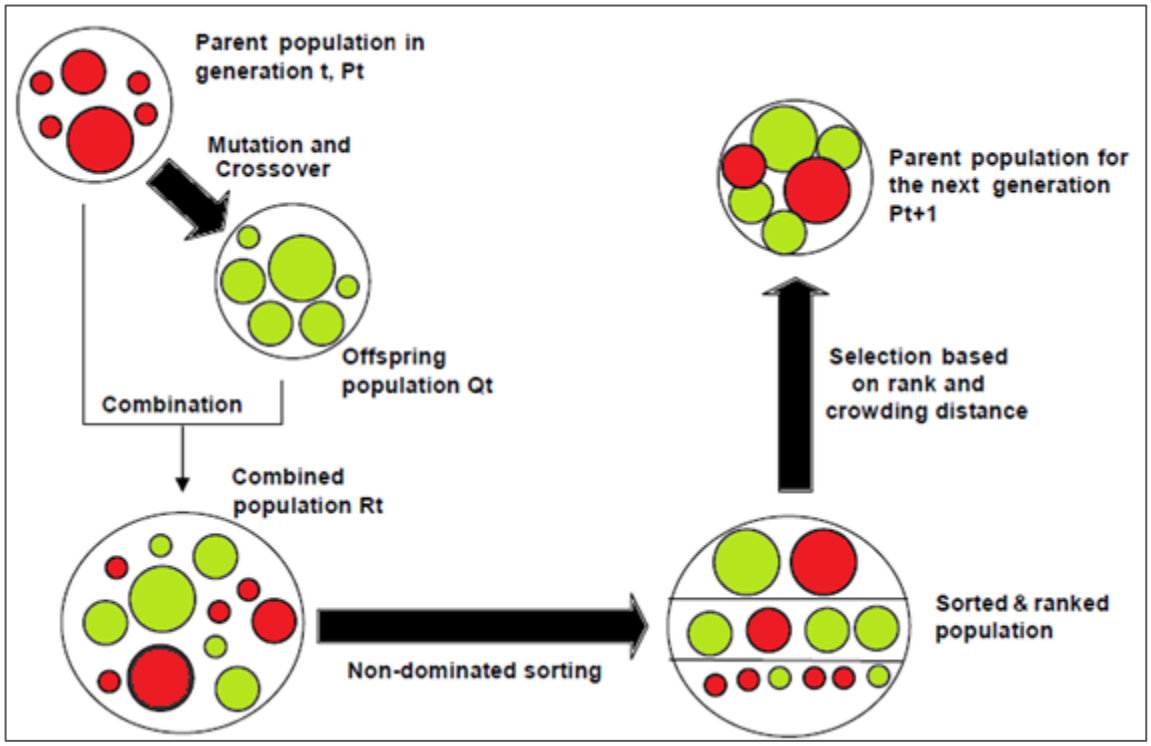

Figure 20: Illustration of processing of populations in NSGA-II over one generation

Some of the advantages of using GA's instead of other artificial intelligence (AI) methods are:

- More robust, works very well even in reasonably "noisy" environments
- Solves problems with multiple solutions and all solutions are good but they can get better with time
- Handles a wide range of constraints and objectives with ease
- Requires no linearization of problem and computation of partial derivatives

7.2 SMB Modeling

In the last few decades, design, control and optimization of chromatographic separation processes, including SMB, has been one of the most researched subject. This led to the development of several modelling methods, strategies and approaches that are now available to solve the same problem but in many different ways. The column model, mass balance of nodes and the SMB performance equations constitute the detailed mathematical model of the SMB process, which is essential to have to successfully design and operate an SMB unit. Moreover, the competitive adsorption isotherm is taken into account in the SMB design since it describes the retention behaviour of the enantiomers to be separated and their dependence on their concentration in the CSP (Santos, Veredas, Silva Jr, Correia, T, & Santana, 2004). For modeling and simulation of the pseudo-binary separation in our case, the ternary mixture of nadolol can be reduced to an equivalent pseudo-binary mixture because the desired component is the most or least retained, so the other components do not have to be separated. This mixture is constituted only of the

weak-key (component 2) and the strong key (component 3) components which are (RRS)-nadolol and (RSR)-nadolol, respectively (Juza, Mazzotti, & Morbidelli, 2000; Wang & Ching, 2004 Yu, Hidajat, & Ray, 2003)). In this study a competitive bi-Langmuir isotherm model, which assumes two different types of adsorption sites exist on the surface of the CSP each subject to an independent Langmuir isotherm and either one being homogeneous, has been used to describe the phase equilibrium of (RRS)-nadolol and (RSR)-nadolol on perphenyl-carbamoylated beta cyclodextrin (β-CD) chiral stationary phase (CSP).

The competitive bi-Langmuir isotherm model is given as:

$$q_i^* = \frac{q_{1s} b_{1,i} c_i}{1+\sum_{i=1}^{2} b_{1,i} c_i} + \frac{q_{2s} b_{2,i} c_i}{1+\sum_{i=1}^{2} b_{2,i} c_i} \quad (68)$$

where c_i is the mobile phase concentration, q_{1s} and q_{2s} are the saturation capacities of the two sites, while $b_{1,i}$ and $b_{2,i}$ are the equilibrium constants of the two sites for component i= A or B. The components A represents the more retained component (RSR)-nadolol, whereas B represents the less retained component of (RRS)-nadolol.

The equilibrium dispersive model with solid linear driving force is the column model used to describe the intra-particle adsorption process of the mixture components in the stationary phase column. This model is developed assuming axially dispersed plug flow for the fluid and a linear mass transfer rate expression, in addition to the following assumptions listed as follows:

- Thermal effects, pressure and velocity variations are negligible
- Complete radial mixing eliminates possibility of radial concentration gradient
- Mass transfer coefficients and physiochemical parameters independent of mixture composition

- Constant voidage and radius of the column, as well as flow rates in each section

A summary of the model equations, including mass balance, kinetic models as well as initial and boundary conditions for each column in the SMB unit is as follows:

Mass balance of component i in the mobile phase:

$$\frac{\partial c_{i,j}^{N}}{\partial \mathrm{t}} + \frac{1-\varepsilon}{\varepsilon}.\frac{\partial q_{i,j}^{N}}{\partial \mathrm{t}} + \nu_j \frac{\partial c_{i,j}^{N}}{\partial \mathrm{z}} = D_L \frac{\partial^2 c_{i,j}^{N}}{\partial z^2}, \quad (i = 1, 2) \tag{69}$$

Lumped kinetic model or mass balance of component i in the stationary phase:

$$\frac{\partial q_{i,j}^{N}}{\partial \mathrm{t}} = k_m (q_{i,j}^{*(N)} - q_{i,j}^{(N)}) \tag{70}$$

Initial conditions:

$$\text{When } N = 0, \;\; c_{i,j}^{0} = 0 \quad and \quad q_{i,j}^{0} = 0 \tag{71}$$

$$\text{When } N \geq 1, \;\; c_{i,j}^{N} = c_{i,j+1}^{(N-1)}, \quad for \;\; j = 1, (N_{col} - 1) \tag{72}$$

$$\text{and } c_{i,j}^{N} = c_{i,1}^{(N-1)}, \quad for \;\; j = N_{col} \tag{73}$$

Boundary conditions:

Feed node, $$c_{i,N_1+N_2+1}^{N}\Big|_{z=0} = \frac{Q_2}{Q_3} c_{i,N_1+N_2}^{N}\Big|_{z=L} + \frac{Q_F}{Q_3} c_{F,i} \tag{74}$$

Raffinate node, $$c_{i,N_1+N_2+N_3+1}^{N}\Big|_{z=0} = c_{i,N_1+N_2+N_3}^{N}\Big|_{z=L} \tag{75}$$

Eluent node, $$c_{i,1}^{N}\Big|_{z=0} = c_{i,N_{col}}^{(N-1)}\Big|_{z=L} \tag{76}$$

Extract node, $$c_{i,N_1+1}^{N}\Big|_{z=0} = c_{i,N_1}^{N}\Big|_{z=L} \tag{77}$$

where c_i and q_i is the concentration of component i in mobile phase and stationary phase, respectively, ε is the bed voidage of the column, v is the interstitial velocity, D_L is the axial dispersion coefficient, N is the number of switching period, q_i^* is the linear isotherm, k_v is the lumped mass transport coefficient, while z and t are the space and time coordinates.

In this study, the purity of extract Pu_{Ex}, productivity PR, and desorbent requirement DR are the paramaters used to evaluate the perfomance and efficiency of the SMB process. These performance indicators are defined as:

$$Pu_E = \int_t^{t+t^*} \frac{c_{A,E}}{c_{A,E}+c_{B,E}} dt \tag{78}$$

$$PR = \frac{Q_F c_{T,F}}{(1-\varepsilon)\rho_S V_T} \tag{79}$$

$$DR = \frac{(Q_D+Q_F)\rho_D}{Q_F c_{T,F}} = \frac{\rho_D}{c_{T,F}}\left(1+\frac{m_1-m_4}{m_3-m_2}\right) \tag{80}$$

7.3 Simulation and Validation of Adsorption Isotherms of Nadolol

Chromatography is a complex phenomenon because of all the factors involved in this process. These factors include fluid dynamics, mass transfer phenomena and equilibrium thermodynamics that play an important role in predicting the overall SMB performance for chiral separation. The bed voidage ε, axial dispersion coefficient D_L, equilibrium constant K_i and mass transfer coefficient k_v obtained from single column experiments in previous work were used as the basis to simulate the elution profiles of racemate nadolol (Wang & Ching, 2002; Xin, 2004; Wang & Ching, 2004).

The SMB model with the initial and boundary conditions were solved using the Method of Lines by commercial software a FORTRAN and the simulated results were compared with

the experimental results from previous work as shown in Figure 20. The method of lines is a technique for solving partial differential equations by converting them to initial value ordinary differential equations. The best-fit isotherm parameters for the competitive bi-Langmuir isotherm model were then determined by minimizing the square difference between experimental and model predicated band profiles through Non-dominated Sorting Genetic Algorithm (NSGA) optimization. The isotherm and model parameters for the design and simulation of chiral separation of (RSR)-nadolol in the SMB chromatography are listed in Table 3.

Table 3: Isotherm parameters and rate coefficients of (RRS)- and (RSR)-Nadolol in SMB colum

Competitive bi-Langmuir isotherm parameters					
q_{1s} (g/L)	**b_{11} (L/g)**	**b_{12} (L/g)**	**q_{2s} (g/L)**	**b_{21} (L/g)**	**b_{22} (L/g)**
200.15	0.0057	0.0066	2.15	0.096	0.932
Model parameters at Q = 0.3 mL/min					
k_v (s^{-1})	**D_L ($x10^{-3}$ cm^3/min)**				
86.8	1.36				

The equilibrium data were fitted to the competitive bi-Langmuir model and are given by the following expressions where the subscripts 1 and 2 refer to the order of breakthrough of components (RRS)-nadolol and (RSR)-nadolol from the column, respectively:

$$q_1^* = \frac{1.14c_1}{1+0.0057c_1+0.0066c_2} + \frac{0.2c_1}{1+0.096c_1+0.932c_2} \quad (81)$$

$$q_2^* = \frac{1.32c_1}{1+0.0057c_1+0.0066c_2} + \frac{2c_1}{1+0.096c_1+0.932c_2} \quad (82)$$

In modeling a chromatographic process, a model is only valid if it is very well able to predict band profiles for the separation of mixtures. Hence, the validity of the determined parameters was verified by comparing the model predictions with experimental elution profiles from previous studies done by Wang et al. (2004) which were in good agreement in terms of elution times and peak shapes of the two components. The PDE systems with the initial and boundary conditions were solved and the steady state concentration profiles were calculated by using the IMSL integration routine IVPAG in a FORTRAN program.

Figure 20 gives the comparison between experimental and simulated profiles for non-linear elution chromatography at concentration of 0.135 mg/ml and mobile phase flow rate of 0.3 ml/min. Since the experimental and simulated results match well, the validity of the obtained adsorption isotherms is confirmed.

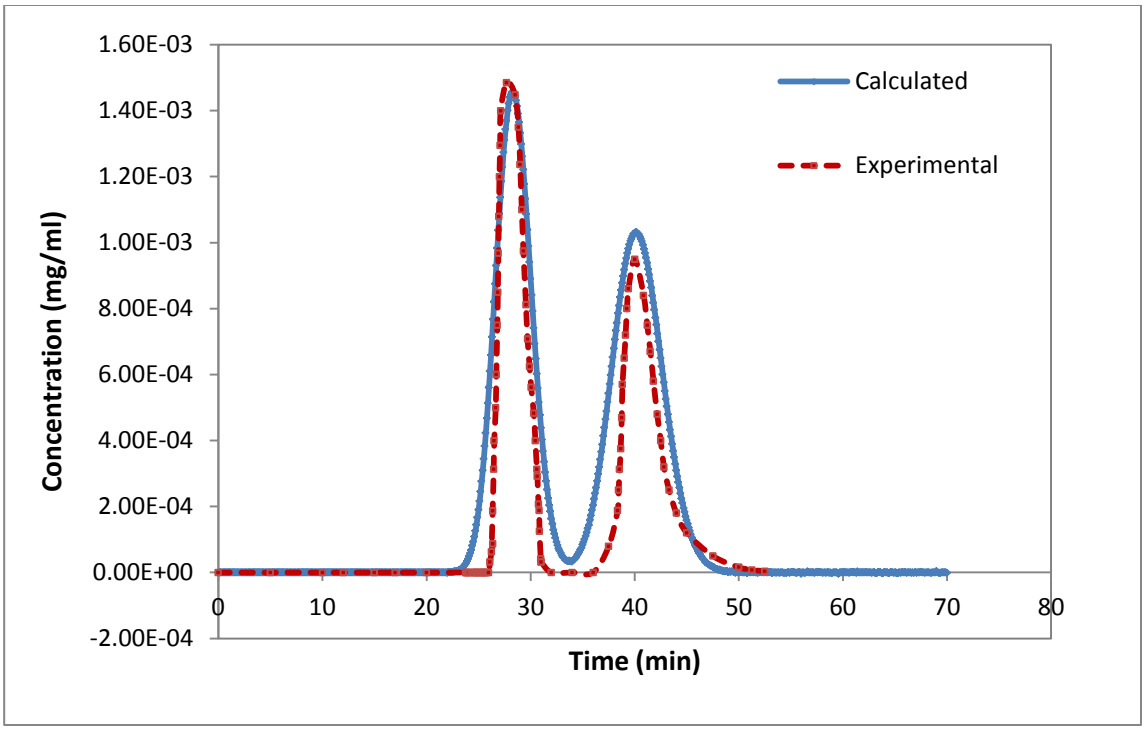

Figure 21: Comparison of the simulated and experimental (Wang, 2004) band profiles of racemate nadolol. Condition: Flow rate = 0.3 mL/min , Feed concentration = 0.135 mg/mL (non-linear region)

Although simulation of enantioseparation of all the four stereoisomers of nadolol was not accomplished in the perphenyl carbamated β-CD covalently bonded CSP, successful separation of two components including that of the most active enantiomer, (RSR)-nadolol, was achieved in simulation. The optimum separation conditions and model parameters obtained are very important and useful for design of the 4-zone simulated moving bed (SMB) so that it would carry out the continuous counter-current chromatographic separation of racemate nadolol.

7.4 Design of SMB for Separation of Nadolol

A four-unit simulated moving bed (SMB) unit in this study is simulated to be composed of six perphenyl carbamoylated beta cyclodextrin (β-CD) chiral stationary phase (CSP)

columns arranged in a 1-2-2-1 configuration. Choosing the right operating conditions, which can be determined by the flow rate ratio between the net mobile and solid phase mass flow in each section, m_j, is crucial for optimal and robust operation of SMB process to successfully achieve complete separation of nadolol by the SMB unit (Kaspereit, Jandera, Skavrada, & Seidel-Morgenstern, 2002).

$$m_j = \frac{Q_j t^* - V\varepsilon}{V(1-\varepsilon)} = \frac{net\ fluid\ flow\ rate}{net\ solid\ flow\ rate} \tag{83}$$

$$\varepsilon = \frac{Q_j t_{0,j}}{V} \tag{84}$$

where Q_j is the volumetric flow rate of the fluid in section *j*, *V* the volume of the chromatographic column, ε is the void fraction of the column, t_{0j} is the column dead time or residence time of the mobile phase in section j, and t^* is the switch time of the SMB unit.

Since component A of stereoisomer (RSR)-nadolol is the most potent and desirable enantiomer, as well as the most retained on the CSP column, the purpose of the SMB separation is to produce an extract stream with the highest possible purity of this component, highest productivity, and least desorbent requirememnt possible.

The competitive isotherm parameters of nadolol enantiomers (Table 3) and the feed concentration were used to obtain the operating conditions of SMB based on triangle theory. Provided that the adsorbent and eluent are to be completely regenerated in Sections 1 and 4, respectively, the triangle theory can be applied to determine the pseudo-binary separation performance of the SMB unit from the (m_2, m_3) plane where each of the four different regions correspond to different operating conditions. The projection of the

four-dimensional space m_j (j=1,…4), onto (m_2, m_3) plane is of paramount importance because this is the plane in the operating parameter space spanned by the net flow rate ratios of the two fundamental sections for separation of the racemic mixture in the SMB unit. Graphical representations of the complete separation regions for nadolol is contructed in the (m_2, m_3) plane, as shown in Figure 17 for a system described by the linear isotherm. However, for nonlinear system determination of the complete separation region is not straightforward and since constraints for m_j values are not explicit, numerical simulation has to be applied to determine the boundaries of complete separation region.

For design and operation of SMB process values of m_1 to m_4 are selected within constraints introduced earlier based on equilibrium theory and the equivalence between SMB and TCC process. Attempts should be made to select these net flow ratios such that their correspondant section flow rates Q_j *(j=1,…,4)* result in the maximum production rate and purity at minimum desorbent consumption while maintaining the robustness of operation. First, the switch time t^*, which is the time interval between two successive port switches, is selected taking into account the compromise between high production rate and high flow rates if the switching time is short. This means that there is a lower limit for t^*, set according to the maximum flow-rate or maximum permissible pressure drop allowed in the SMB system. Alternatively, Q_1, which is the largest flowrate in the SMB unit, can be estimated based on the aforementioned limitation of the SMB, and the switching time can be determined by the following expression where V_1^D is the dead volume in section 1:

$$t^* = \frac{V[(m_1(1-\varepsilon)+\varepsilon)]+V_1^D}{Q_1} \tag{85}$$

The explicit constraints of *m1* and *m4* for the complete regeneration of adsorbent and desorbent in sections 1 and 4, as well as complete separation conditions expressed in terms of m_2 and m_3 were obtained using the approach proposed by Storti et al. as given below:

$$Section\ 1: \quad m_1 > \frac{q^*_{A,1}}{c_{A,1}}, m_1 > \frac{q^*_{B,1}}{c_{B,1}} \quad where\ Q_1 = Q_D \tag{86}$$

$$Section\ 2: \quad \frac{q^*_{B,2}}{c_{B,2}} < m_2 < \frac{q^*_{A,2}}{c_{A,2}} \qquad where\ Q_2 = Q_D - Q_E \tag{87}$$

$$Section\ 3: \quad \frac{q^*_{B,3}}{c_{B,3}} < m_3 < \frac{q^*_{A,3}}{c_{A,3}} \qquad where\ Q_3 = Q_D - Q_E + Q_F \tag{88}$$

$$Section\ 4: \quad m_4 < \frac{q^*_{B,3}}{c_{B,3}}, m_4 < \frac{q^*_{A,4}}{c_{A,4}} \quad where\ Q_4 = Q_D - Q_E - Q_R + Q_F \tag{89}$$

Finally, having decided m_j (j=1,…5) and t^*, the liquid flow rate in the four sections of the SMB unit and hence the flow rates of the inlet and outlet streams are determined by solving the following expression for Q_j:

$$m_j = \frac{Q_j t^* - V\varepsilon}{V(1-\varepsilon)} = \frac{net\ fluid\ flow\ rate}{net\ solid\ flow\ rate} \tag{90}$$

The advantage of this method is that it can be applied in both linear and non-linear systems regardless of sepecifications such as configuration or size and productivity of an SMB unit. Credit for this goes to the dimensionless flow rate ratios which bring together information about column volume, *V*, unit flow rates, *Q*, and switch time, t^* to ease the design process (Xie, Koo, & Wang, 2001; Weifang, 2003).

7.4.1 Complete separation region

Complete separation region for (RRS)- and (RSR)-nadolol, with a feed concentration of 0.135mg/mL and extract outlet stream that is over 99% pure can be achieved if the m_1 value is higher than 3.76 and m_4 value is lower than 2.26 calculated based on the SMB design method discussed. Operating parameters such as flow rates in Sections 2, 3, and 4 as well as switching time can be derived from any combination of m_2 and m_3 within the complete separation region based on extract purity criteria. The operating conditions and separation results are listed in Table 4.

Table 4: Operation conditions and parameters for the SMB experiments

Run	Operation parameters					Operation Conditions				Performance Indicators (%)	
	Flow rate ratio				t^*	Flow rate (mL/min)				Extract Purity	Extract Recovery
	m_1	m_2	m_3	m_4	(min)	Q_1	Q_F	Q_R	Q_E	Pu_{Ex}	Re_A
1	5.08	1.46	1.77	0.07	2.0	10.67	0.30	2.81	5.97	79.77	93.95
2	6.70	2.18	2.56	0.43	2.5	10.67	0.30	2.81	5.97	96.06	87.57
3	11.55	4.32	4.93	1.52	4.0	10.67	0.30	2.81	5.97	76.88	9.75
4	8.32	2.89	3.35	0.79	3.0	10.67	0.30	2.81	5.97	99.98	90.55
5	8.32	2.89	3.35	0.79	3.0	10.67	0.20	2.81	5.97	96.81	52.10
6	8.32	2.89	3.35	0.79	3.0	10.67	0.80	2.81	5.97	88.71	76.79
7	8.32	2.89	3.35	0.79	3.0	10.67	0.50	2.81	5.97	89.23	77.69
8	8.32	2.70	3.35	0.79	3.0	10.67	0.30	2.81	6.18	89.59	76.98
9	8.32	2.50	3.35	0.79	3.0	10.67	0.30	2.81	6.4	80.61	89.56
10	8.323	3.10	3.35	0.79	3.0	10.67	0.30	2.81	5.74	99.99	78.15
11	8.323	2.89	3.35	0.79	3.0	10.67	0.30	2.81	5.97	99.98	90.55
12	8.323	2.89	3.15	0.79	3.0	10.67	0.30	2.58	5.97	99.98	14.67
13	8.323	2.89	2.80	0.79	3.0	10.67	0.30	2.20	5.97	99.99	7.05
14	8.323	2.89	3.50	0.79	3.0	10.67	0.30	2.98	5.97	99.30	23.08

7.4.2 Effects of operating conditions

7.4.2.1 Effect of Switching Time, t^*

As can be seen from results of varying switching time t^* in Runs 1 to 4 in Table 4, upon increasing t^* from 2 to 2.5 minutes the purity of extract increases from 79.77% to 96.06%. However, further increase of t^* from 2.5 to 4 minutes decreases extract purity to 9.75%. At the same time, it appears to be that increase in t^* is associated with a decrease in recovery of the more retained component (RSR)-nadolol in the extract stream. The reason behind this behaviour is that increasing swtiching time reduces the solid phase pseudo-velocity since it leads to a relatively higher fluid to solid flow rate ratio which translates into longer residence time of components in each section. This allows for improved separation especially of the strongly adsorbed component which benefits from the increased desorbing time leading to high extract purity. But, it also means that components will travel more in the fluid or mobile phase hence more of both components will elute from the raffinate stream versus the extract stream, explaining the decreased recovery of (RSR)-nadolol. However, if the switching time is too high both purity and recovery decrease due to pollutions of both outlet streams as a result of improper regeneration of the adsorbent and desorbent. It is worth noting that the feed concentration and flow rate are kept constant to ensure the changes in results are solely due to change in switchinig time.

7.4.2.2 Effect of Feed Flow Rate, Q_F

If the switch time is kept constant, increasing the feed flow rate Q_F from 0.2 to 0.8 will result in a decrease in extract purity from 96.81% to 88.71%, and recovery of (RSR)-nadolol

increases from 52.10% to 76.79%. This is mainly due to an increase in m_4 which may cause some of the less adsorbed component to contaminate the extract stream. The advantage of increasing flow rate is that it improves productivity of the desired component, however if the feed flow rate is too high it can cause unstable operation of the SMB unit.

7.4.2.3 Effect of Extract Flow Rate, Q_{EX}

The effect of the flow rate of extract on the performance of the SMB process was studied by varying Q_{EX} in Runs 7 to 10, while keeping the feed flow rate, feed concentration, switch time and all other flow rates constant as shown in Table 4. When the extract flow rate increases from 5.97 mL/min to 6.4 mL/min the extract purity decreases from 89.23% to 80.61%. The reason is that the decrease in m_2 value as a result of extract flow rate increase is reflected in the flow rates in Section 2 also decreases due to poor desorption as a result of the decreased fluid-phase velocity. Recovery of the the more retained component (RSR)-nadolol however increased slightly with the increase in extract flow rate.

7.4.2.4 Effect of Raffinate Flow Rate, Q_R

When Q_R increases from 2.20 mL/min to 2.98 mL/min as revealed in Table 4, while all other values are kept fixed, flow rate through desorption and regeneration zones decreases so complete regeneration of the adsorbent is hindered. Hence the purity decreases from 99.99% to 99.30% while recovery of the more potent component (RSR)-nadolol slightly increases from 7.05% to 23.08%.

7.5 Conclusion

The results of this theoretical investigation show that racemic nadolol can be successfully separated on the perphenylcarbamoylated β-Cyclodextrin column using a four-section SMB unit in which the columns are arranged in a 1/2/2/1 configuration. The triangle theory was used to design the simulated moving bed (SMB) unit then its dynamic behaviour was simulated by the equilibrium dispersive column model and using a competitive bi-Langmuir adsorption isotherm. The results of 14 different runs which were simulated with variation in design parameters provide information about the effect of the different operating conditions on the SMB unit performance. Since the extract is the most potent enantiomer of nadolol, this study focuses on the extract purity as the main objective. The recovery of the more potent component from the extract stream is the reason why raffinate purity is taken into consideration as it affects that yield as shown later on in the optimization problems in Chapter 8.

As observed, the recovery of (RSR)-nadolol in the extract stream declines with increasing switching time, while the purity of extract increases until the switching time reaches 3 minutes then it starts declining. Increasing the feed flow rate, however, decreases the purity and recovery of extract although the productivity is higher at higher feed flow rates. Similarly, increase in extract flow rate results in a decline in purity of extract and recovery of storngly-adsrobed component A which in this study is (RSR)-nadolol. These results support Mazzotti's (2009) observations, as summarized in Figure 21, on how the different parameters affect the separation process in an SMB unit (Yun, Zhong, & Guiochon, 1997; Xie, Koo, & Wang, 2001).

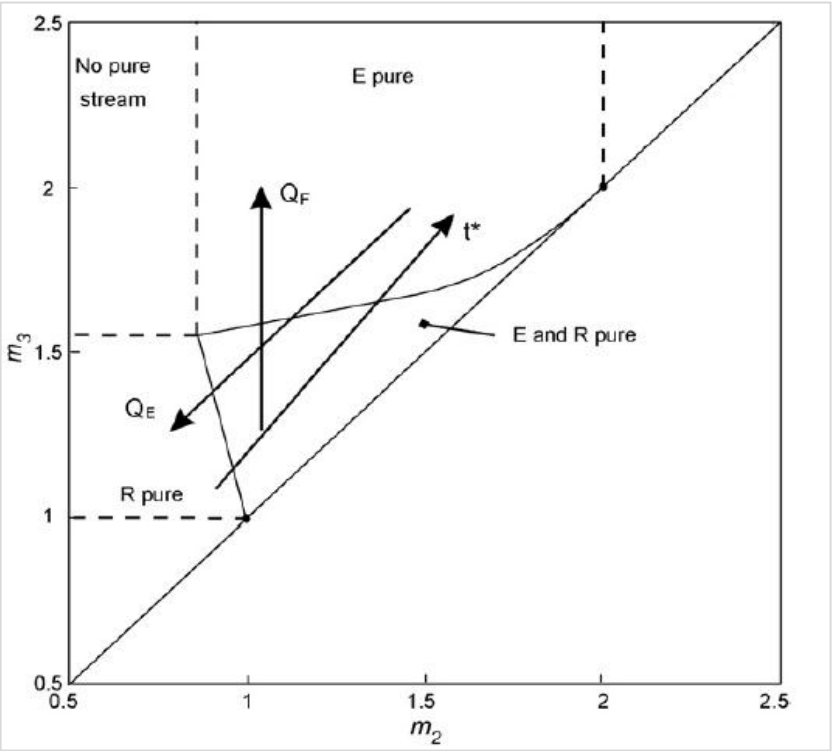

Figure 22: Effect of operating conditions on the position of the operating point on the (m_2, m_3) plane (Mazzotti, 2009)

Chapter 8: Multi-objective Optimization of SMB for Chiral Separation of Nadolol

8.1 Multi-objective optimization

The main goal of single-objective optimization is to find the best solution which is usually the global minimum or maximum by transforming all but one single objective into constraints. This type of optimization can be a useful tool in some cases, however in most real world design problems, such as that of the simulated moving bed (SMB) where operating variables have conflicting effects on performance, simultaneous optimization of multiple objective functions is extremely important (Savic, 2002; Narzisi, 2008; Rathore & Velayudhan, 2003; Srinivas & Deb, 1994). The principle of multi-objective optimization with conflicting objectives is different from that of single objective optimization in that the former's goal is to find a set of equally good solutions rather than a single solution that is the best or global optimum. These optimal solutions are known as non-dominated or Pareto-optimal solutions. As mentioned, these are all acceptable solutions and no solution can be considered a superior or dominant solution, with respect to all objective functions, because at any point chosen at least one objective function improves while the other deteriorates. Thus, the choice of one optimal solution over the many other solutions provided by the Pareto set often depends on non-quantifiable information of the process at hand and its purpose (Yu, Hidajat, & Ray, 2003). In this study, the multi-objective optimization for the separation of nadolol in a SMB was performed using the Non-dominated Sorting Genetic Algorithm (NSGA) which is an optimization method based on principles of natural selection in genetics. The intention of performing such optimizations is to develop a better understanding of the SMB process as well as generate a wider range

of meaningful and useful optimal operating conditions to aid decision makers in choosing the right parameters at which to operate the SMB unit in order to achieve the intended goals behind the separation process (Lee, 2005; Narzisi, 2008).

Two multi-objective cases that are typically encountered when considering chiral separation by simulated moving bed (SMB) are considered in this chapter. These two multi-objective cases which consider the three most important objectives for SMB performance namely, maximization of productivity and purity of extract stream using minimum desorbent are formulated as presented in Table 5.

Table 5: Multi-objective optimization problems solved in this study

Problem	Objective Function	Constraints	Decision Variables	Fixed Variables
Case 1	Max Pu_E Max PR	$Pu_E > 90.0\%$ $Pu_R > 90.0\%$	$2 < Q_D < 25$ mL/min $1.5 < Q_R < 3$ mL/min $2 < t^* < 4$ min	$Q_1 = 10.67$ mL/min $Q_F = 0.3$ mL/min L= 10 cm N = 6
Case 2	Max Q_F Min Q_D	$Pu_E > 99.9\%$ $Pu_E > 99.0\%$ $Pu_E > 95.0\%$ $Pu_R > 90.0\%$	$0.2 < Q_F < 1.4$ mL/min $1 < Q_D < 25$ mL/min $1.5 < Q_R < 3$ mL/min $2 < t^* < 4$ min	$Q_1 = 10.67$ mL/min L= 10 cm N = 6

8.1.1 Case 1: Maximization of Purity and Productivity of Extract Stream

The objective functions in this case are maximization of productivity and purity of the extract stream, which are two aspects that can be achieved by simulated moving bed (SMB) making it a promising technology for fine chemical and pharmaceutical industries. The conflicting influence of the different operating and design variables on these two objectives makes it necessary to use multi-objective optimization to determine the optimal

operating conditions that lead to maximization of both functions. The raffinate purity Pu_R constraint set to be greater than 90% in this case serves to maintain the recovery of the more retained component in the extract stream, whereas the extract purity Pu_E is set to be greater than 90%. The raffinate flow rate, desorbent flow rate, and switching time are used as the decision variables, while the flow rate in Section 1, Q_1, as well as the feed flow rate Q_F, number of columns N and length of column L are fixed. Figure 22 shows the corresponding plots for the decision variables. As is expected the productivity of extract corresponding to certain purity is lower for a higher feed concentration than the productivity of extract at that same purity but at a lower feed concentration. This is because with increasing feed the loading increases which causes the extract purity and recovery to decrease while the productivity increases.

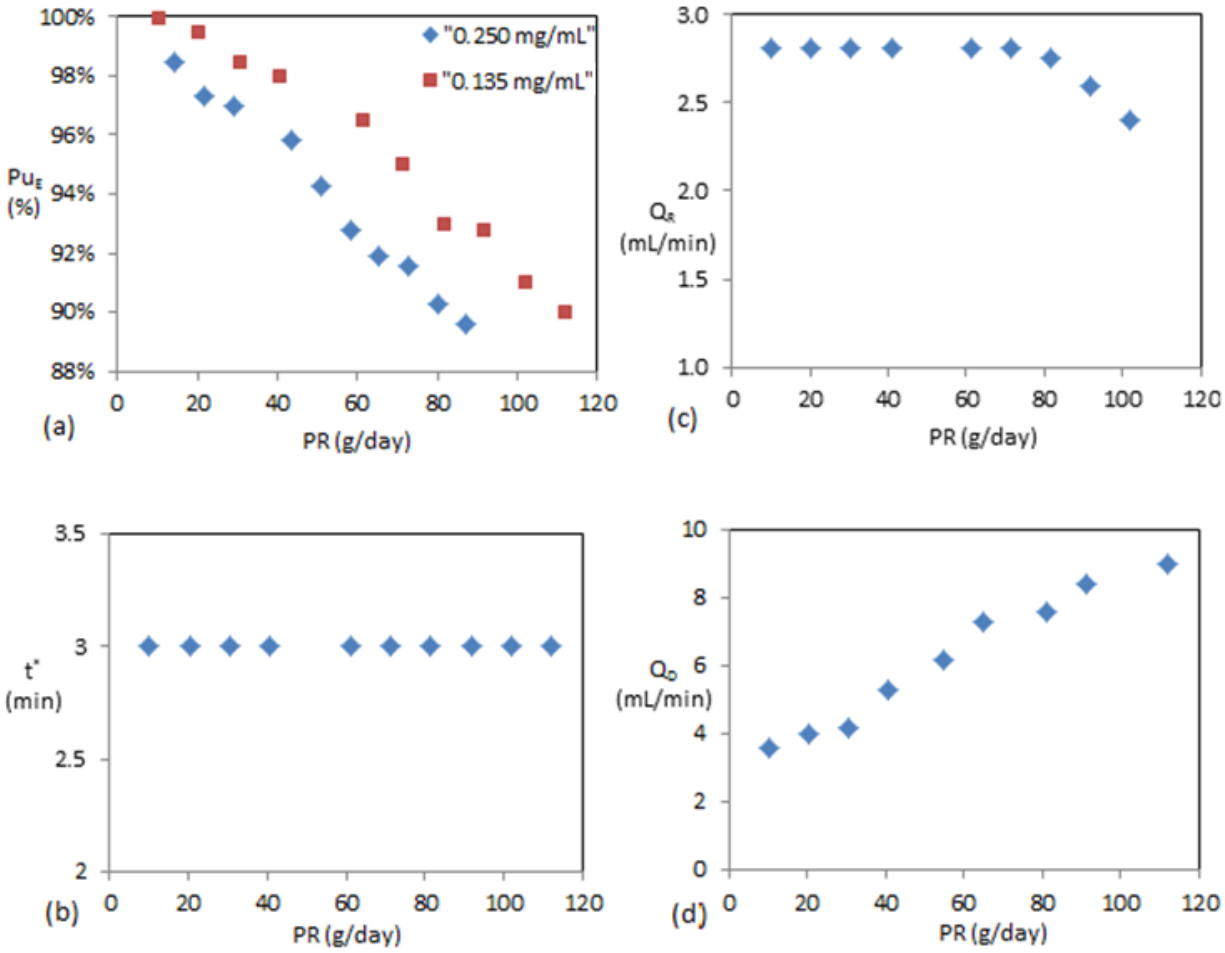

Figure 23 (a-d): Pareto optimal solutions and corresponding decision variables (Case 1) for SMB

As shown by Figure 22b switching time t^* is a key parameter since it restricts separation and lowers purity if operated under a minimum value that provides sufficient residence time for the more retained component to be adsorbed and then desorbed for collection in the extract stream. On the other hand, the desorbent flow rate increases slightly while the raffinate flow rate is found to be less sensitive towards the objective functions.

8.1.2 Case 2: Maximization of Feed Flow Rate and Minimization of Desorbent Consumption

Simultaneous maximization of feed flow rate Q_F (throughput) and minimization of desorbent consumption are economically important factors in the determination of SMB chiral separation process costs. Since the desorbent consumption generally increases with increasing feed load, multi-objective optimization that takes into account other variables is important to carry out in order to find the optimal design conditions of the SMB process that satisfy the objective functions of maximum feed flow rate and minimum desorbent consumption. Like the previous case, all variables except desorbent flow rate Q_D, raffinate flow rate Q_R and switching time t^* are fixed, whereas the extract purity Pu_E was varied at three different values 99.9%, 99.0% and 95.0% to observe how the solution changes and accommodate commercial market requirements. The raffinate purity Pu_R constraint is again set at a minimum of 95% to ensure recovery of the more retained component in the extract stream. The detailed formulation of the multi-objective optimization problem is given in Table 5. The optimization result is shown in Figure 23 of the Pareto optimal solution and the corresponding decision variable plots.

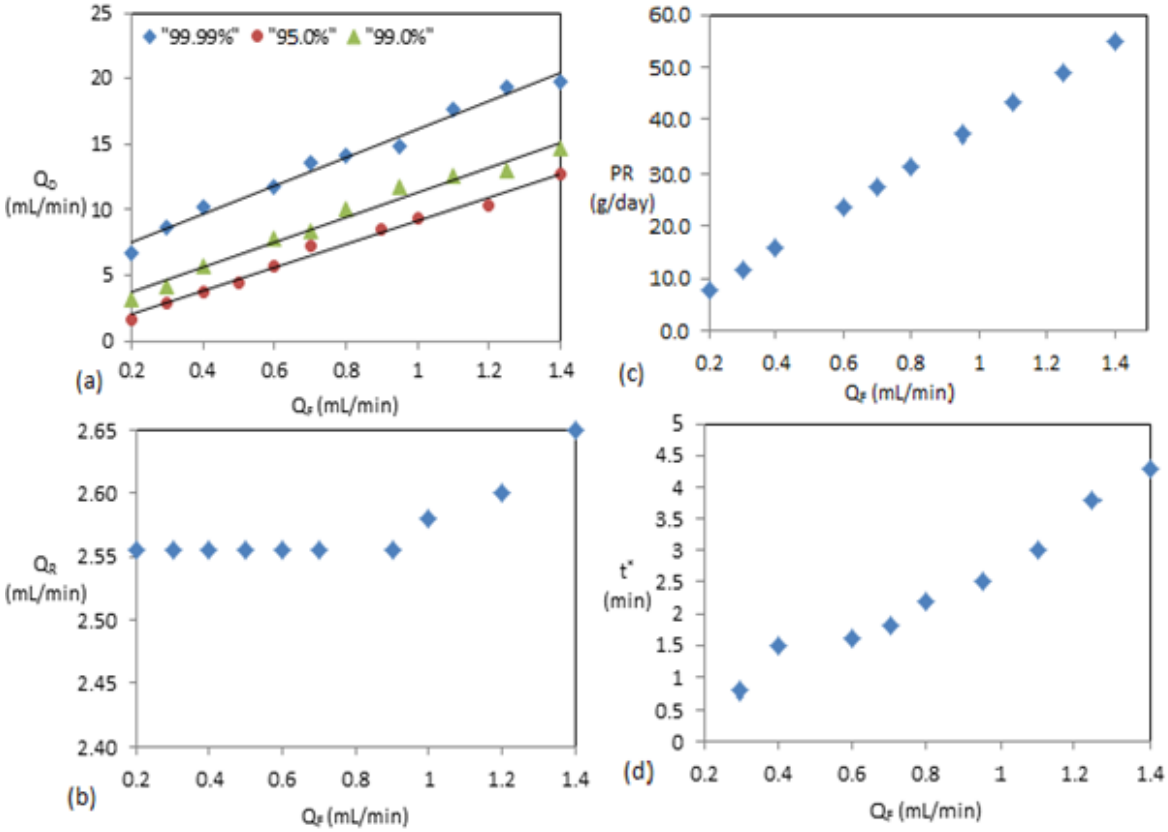

Figure 24 (a-d): Pareto optimal solutions and corresponding decision variables (Case 2) for SMB

The Pareto optimal solution shows that the higher the feed flow rate, the higher the productivity and the more desorbent is required. A longer switching time is also needed as the feed flow rate increases in order to achieve the desired extract purity and productivity since the separation task becomes more difficult. The raffinate flow rate also increases slightly at higher feed loading in anticipation of the increasing task of separation in the SMB unit.

8.2 Conclusion

In summary, the two cases presented each of two objective functions optimization problems, show that the productivity increases with decreasing purity. On the other hand

the desorbent consumption increases to achieve near 100% pure extract product. Using multi-objective optimization has demonstrated its ability to provide valuable information that can be used to implement a realistic, effective and efficient design of SMB unit.

Chapter 9: Conclusions and Recommendations

9.1 Conclusions

In this thesis, a systematic study theoretically investigating the chiral separation of racemic nadolol on perphenyl carbamoylated β-cyclodextrin (β-CD) immobilized chiral stationary phase (CSP) by simulated moving bed (SMB) is presented. Firstly, the optimal mobile phase was determined to be 80:20 triethylamine acetate (TEAA) buffer solution (1% w/v, pH=5.5) and methanol, at a flow rate 0.3 mL/min and temperature of 20°C. The competitive adsorption isotherm parameters summarized in Section 7.3 , dispersion coefficient (1.36 $x10^{-3}$ cm^3/min) and mass transfer coefficient (86.8 s^{-1}) were determined for the liquid chromatography resolution of racemic nadolol, and their accuracy and reliability were verified by comparing the simulated elution profile predicted with the experimental slution profile from another study which matched quite well (Xin, 2004).

A rigorous mathematical model describing the dynamic behavior and separation performance of the SMB was then developed based on equilibrium-dispersive model combined with competitive bi-Langmuir adsorption isotherm. The triangle theory is applied to find the complete separation region of racemic nadolol for this non-linear system using SMB. It was estimated that complete separation region for (RRS)- and (RSR)-nadolol, with a feed concentration of 0.135mg/mL and 99% pure extract outlet stream, can be achieved if the m_1 value is higher than 3.76 and m_4 value is lower than 2.26

By studying the effect of parameters such as feed flow rate, switching time, extract flow rate, and flow rate of raffinate on the separation performance, it becomes possible to choose design and operating parameters of SMB that lead to successfully achieving the

complete separation desired. It is observed that a reasonable increase in switching time causes the recovery of (RSR)-nadolol in the extract stream to decline and purity of extract to increase. Whereas increasing the feed flow rate, causes the purity and recovery of extract to decrease but increases the productivity. Increase in extract flow rate has the same effect as feed flow rate on the purity of extract and recovery of strongly-adsrobed (RSR)-nadolol. However, increase in raffinate flow rate has insignificant effect or decreases extract purity and recovery to decreases very slightly.

Finally, the multi-objective optimization, which provides a more sensible and rigorous approach towards a realistic design of SMB study, was performed for the separation of the most potent (RSR)-nadolol enantiomer in simulated moving bed reactor (SMB). Two-objective functions optimization were carried out, using the state-of-the-art non-dominated sorting genetic algorithm (NSGA) technique, to obtain a series of non-dominated (Pareto optimal) solutions rather than one global optimum solution as would be the case in a single-objective optimization. It is observed that with the same recovery requirement, the productivity is higher when the purity constraint is lower, and that for the same feed flow rate or throughput, desorbent consumption is higher when the purity constraint is lower. This study shows that an industry may operate the SMB unit at different conditions that enable achievement of desired purity versus productivity standardly required in that particular industry according to regulations, in this case the pharmaceutical industry, based on the Pareto optimal sets obtained from the multi-objective optimization.

9.2 Recommendations for Future Work

- Provide further verification of the model developed in this study by running experiments in the laboratory to determine appropriate design and successful implementation of SMB on an industrial scale. Due to limited time and availability of SMB unit in the laboratory, experimental work was not feasible to perform, however, simulation of the theoretical work and validation using experimental work provides a strong indication of the success of separation process.
- Use different column configurations in the four-zone SMB unit, and try five-zone SMB units with multiple raffinate or extract outlets to observe changes in performance and evaluate efficiency and quality of separation (Khan & Younas, 2011).
- Also, coupling SMB technology with other separation processes such as crystallization and membrane separation methods deserve attention, as they can provide efficent methods for chiral separation. Employing two methods in a well designed coupled separation system is very likely to allow production of lower purity product from SMB, as only partial separation may be necessary using this technique, hence lowering the overall cost by reducing desorbent consumption as well as increasing productivity (Mao, 2012; Wang, Gao & Lee, 2002).

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Curriculum Vitae

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EDUCATION

Master of Engineering Science, Chemical and Biochemical Engineering **2012**
Western University, London, Ontario
- Research topic:
- Project Supervisors: Dr. Ajay Ray and Dr. Hassan Gomaa

Bachelor of Engineering Science, Chemical Engineering **2010**
Western University, London, Ontario

RELEVANT EXPERIENCE

Graduate Research Assistant **2011-2012**
Western University*, London, Ontario*

Graduate Teaching Assistant **2008-2011**
Western University*, London, Ontario*

WORK EXPERIENCE

Research and Design Engineer **2009**
ICFAR, London, Ontario
- Conducted literature review on biomass pyrolysis and upgrading of bio-oil
- Designed a laboratory-scale system of vaporization and fractional condensation
- Presented a poster on "Fractional Condensation of Bio-oil" at the opening of ICFAR
- Carried out experiments and developed skills in data collection and manipulation

Research Engineer and Assistant in Wastewater Laboratory **2008**
Western University, London, Ontario
- Assisted in pretreatment of anaerobic sludge for methanol production using ultrasonication and chemical methods
- Conducted literature review on wastewater treatment methods
- Analyzed data collected and summarized results for weekly meetings with supervisor

AWARDS AND ACHIEVEMENTS

- World Petroleum Council Millennium Scholarship ***2009***
- Faculty of Engineering Dean's Honor List –Western University ***2009***
- Western Scholarship of Distinction ***2005***

PROFESSIONAL DEVELOPMENT

- The Teaching Assistant Training Program (TATP) – Western University ***2011***
  - Certificate of completion of an interdisciplinary course for graduate teaching assistants on the strategies and practice of university teaching

- Leadership Education Program – Western University ***2010***
  - Letter of Accomplishment in Individual Leadership
  - Letter of Accomplishment in Advanced Student Leadership

- Certificate of Achievement for "*Young Leaders of Tomorrow*" -United Way ***2009***

VOLUNTEER EXPERIENCES

- Student coordinator for SDC's 'Volunteer in Progress' Program at UWO ***2011***
- Host Program Volunteer at Cross Cultural Learner Centre ***2010***
- Mentor for High School students at the MAC Youth Centre ***2010***
- Co-editor of a newsletter published by a University student body ***2008***

PUBLICATIONS

- Gomaa, H. G., **Hashem, N.**, Al-Taweel, A. M. (2012). Dispersion Characteristics and Mass Transfer in a Pilot-Scale Gas-Liquid Oscillatory-Plate Column. *Chemical Engineering and Technology 35(7),* 1300-1311