6-15-2011

Determination of Ibuprofen Isotherm Using Supercritical Fluid Chromatography

Loi Ho
University of South Florida, ldho@mail.usf.edu

Determination of Ibuprofen Isotherm Using Supercritical Fluid Chromatography

by

Loi Ho

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Chemical Engineering
Department of Chemical and Biomedical Engineering
College of Engineering
University of South Florida

Major Professor: Aydin Sunol, Ph.D.
Sermin Sunol, Ph.D.
Timothy Fawcett, Ph.D.
Babu Joseph, Ph.D.

Date of Approval:
June 15, 2011

Keywords: computation, chiral, adsorption, model, comsol

Acknowledgments

I would like to thank my major professor Dr. Aydin Sunol and Dr. Sermin Sunol for their many years of help with the thesis, financial support, and patience. I like to thank my committee member Dr. Timothy Fawcett and Dr. Babu Joseph for their suggestions on improving the thesis. I also like to thank my colleague Wade Mack for allowing me to use his valuable data after years of research and trial in the laboratory.

Table of Contents

List of Tables

List of Figures

Abstract

Chromatography is widely used to determine physiochemical properties data including adsorption isotherm. In the separation of enantiomers through sorptive processes, supercritical fluids allow efficient and green alternatives to liquid solvent based systems. The isotherm information is vital in the development of operating policies and design of preparative chromatographic or moving bed separation schemes. Determination of sorption isotherms from experimental chromatographic elution data is automated and compared with different chromatogram model. Procedures were developed and validated for separation of R- and S- ibuprofen with supercritical CO_2 and ethanol mixture as the mobile phase over a chiral stationary phase. The isotherms for the Langmuir, Freundlich, and Toth gave the similar results with a small residual for S-Ibuprofen at 150 bars and 40℃. Relative small amount of sample can be use from experiments to determine isotherms data that later be use for scale-up of the sorptive processes in industry to reduce time and cost.

Chapter 1

Introduction and Background

The objective of this research is to find the isotherm data using small quantity of sample to predict the larger sample for industrial use and lower cost.

In this chapter, we will first discuss what chirality is and the importance of it in daily life. Next, Supercritical Fluid Chromatography (SFC) methods of isolating compounds with similar physical and chemical properties will be discussed. Finally, the methods of determining key parameters for the isotherm will be defined for design of separation processes and modeling of chiral systems. R and S-Ibuprofen (iso-butyl-propanoic-phenolic acid), will be used to demonstrate principles. The data for the isotherms and chromatograms were obtained from Mack (2012).

1.1 What is Chirality?

Louis Pasteur first discovered chirality in 1848, when he was working with tartaric acid; he realized that this molecule is asymmetrical (Subramanian and Brown, 1994). A molecule is chiral when it has a mirror image that is not superimposable, such as protein, polysaccharide, and nucleic acid. Van't Hoff and Le Bel independently postulated that a molecule can be moved around its center atom to make a mirror image (Subramanian and Brown, 1994). Figure 1.1 depicts the chiral structure of Ibuprofen.

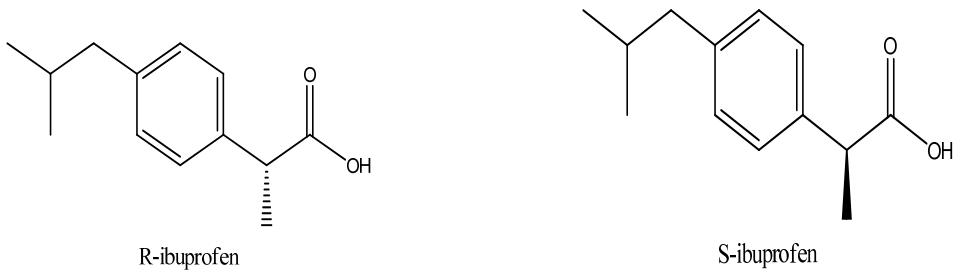


Figure 1.1 R and S-Ibuprofen

Many of the compounds that exist on the earth and in our bodies have many chemical configurations. For example, glucose has sixteen distinct configurations, but only one, D-glucose, can be used in the body for energy (Subramanian and Brown, 1994). Some compounds can change from one configuration to another when they enter the body. Compounds with some of these distinct configurations can have undesirable effects (Subramanian and Brown, 1994).

1.1.1 Applications of Chiral Compounds

The impact of the chiral technology in pharmaceutical and food industries is very significant. The medicines we take for fever or headache may have chiral compounds and/or may have been separated using a chiral stationary phase. Similarly, orange or lemon flavoring we taste or smell in our food may be due different chiral structures of limonene (Subramanian and Brown, 1994).

1.1.1.1 The Pharmaceutical Industries Use of Chiral Compounds

Racemic is a mixture of a molecule that has both the right and left side configuration. Thalidomide was thought to be a sedative and anti-nausea drug in the 1960s. In 1979, the R-isomer of Thalidomide was the effective medicine while the S-Isomer was a teratogenic, which causes pregnant women to have

malformation in the fetus (Ahuja, 2000).The S-Isomer of barbiturates were used as a depressant while the R-isomer as a convulsant (Hassan and Ali, 2003). Table 1.1 shows examples of chiral drugs (Subramanian, 1994), (Ahuja, 2000), and (Ager, 1999).

Table 1.1 Pharmaceuticals for Which Chiral Compounds are the Medically Active Components. Reproduced with permission.

Compounds	Isomer	Properties
Thalidomide	S-Isomer R-Isomer	Teratogenic Sleeping inducing, anti-nausea
Barbiturates	S-Isomer R-Isomer	Depressant Convulsant
Ibuprofen	S-Ibuprofen R-Ibuprofen	Reduced fever, headache Non-active
Asparagine	D-Asparagine L-Asparagine	Sweet Taste Bitter Taste
Opiates	S-Isomer R-Isomer	Narcotics Non-addictive cough mixture
Labetalol	S-Isomer R-Isomer	Alpha-blocker Beta-blocker
Amphetamine	D-Isomer L-Isomer	Central Nervous System Stimulant Little effect
Ascorbic Acid	S-Isomer R-Isomer	Antiascorbutic No properties
Enalapril	R- Isomer	Angiotensin-Converting Enzyme Inhibitor
Simvastatin	R-Isomer	Reductase Inhibitor
Pravastatin	S- Isomer	Inhibition of Cholesterol Biosynthesis
Amoxicillin	R-Isomer	Semisynthetic Penicillin Antibiotic
Lisinopril	S-Isomer	Angiotensin-Converting Enzyme Inhibitor
Diltiazem	S-Isomer	Antihypertensive
Sertraline	S-Isomer	Antidepressant

1.1.1.2 The Food Industries Use of Chiral Compounds

In the food industry, using natural ingredients in the products can be expensive due to significant development time and costly standardization issues. Therefore, in many cases, the food industry opted to go with a mixture of chiral as opposed to a specific enantiomer. This is possible because most natural compounds of utility exists as a mixture of chiral forms, side effect issue that exists in pharmaceuticals is usually not applicable, and the flavors are not compromised in the products. The consumption of chiral compounds in the food industry makes only 25% of the chiral products industry and only about 8% is used as single isomers. For example, limonene exists as a racemic mixture where R-Isomer gives an orange odor while the S-Isomer provides a lemon odor (Subramanian, 1994, Ahuja, 2000). Many diet drinks do not use sugar in the product, but instead use Aspartame, a chiral compound, as a sweetener. The aspartame R, R-Isomer has a sweet taste while the S, R-Isomer has a bitter taste (Subramanian and White, 1994) (Ahuja, 2000). Table 1.2, reproduced with permission, shows examples of chiral applications for flavors in the food industry (Subramanian, 1994) (Ahuja, 2000).

Table 1.2 Food for Which Chiral Compounds are the Primary Components. Reproduced with permission.

Carvone	R-Isomer S-Isomer	Spearmint odor Caraway odor
Limonene	R-Isomer S-Isomer	Orange odor Lemon odor
Aspartame	R-Isomer S-Isomer	Sweet taste Bitter taste

1.1.1.3 Use of Ibuprofen Enantiomers in Industry

Ibuprofen is a nonsteroidal anti-inflammatory drug that is produced industrially as a racemate, because it hard to separate the enantiomer due to having the same physical properties and cost associate with it. Two enantiomers of ibuprofen occur, with the potential for different biological effects and metabolism for each enantiomer. Indeed, the (S)-(+)-ibuprofen (dexibuprofen) was found to be the active form both in vitro and in vivo, while the (R)-(+)-ibuprofen is non active. The (S)-(+)-ibuprofen cost more than the (R)-(+)-ibuprofen because of it properties to help relieve pain and fever (Subramanian, 1994).

It is therefore, then to reconsider the potential for improving the selectivity and potentcy of ibuprofen formulations by marketing ibuprofen as a single-enantiomer product (as occurs with naproxen, another NSAID). Such as endeavor involves fractionating the racemate.

1.2 Methods of Fractionating Chiral Mixtures

There are many different methods of fractionation that can separate compounds that have similar physical and chemical properties (Guiochon, et al., 2006). The Simulated Moving Bed (SMB) and the High Performance Liquid Chromatography (HPLC) are two popular methods that will be discussed (Guiochon, et al., 2006).

1.2.1 Simulated Moving Bed (SMB)

Simulated Moving Bed, popular the oil industry in the 60's, is a cyclic process, where pseudo continuous performance flow for a stationary solid sorbent phase is achieved by a portfolio of mechanisms such as inlet valve switching for the fluid phase. It is assumed that the solvent is in contact with the column by a continuous countercurrent flow. The process for the SMB breaks down into four different zones. The feed first enters zone II and goes to zone III as raffinate. The raffinate (the liquid stream which remains after solutes from the original liquid are removed) travels along to zone IV and mix with the effluent. After the mixing, the feed travels to zone I to be extracted. The feed goes around the 4 zones 50 to 100 cycles before it can be extracted for further study. The assumptions for the SMB model are that the mass transfer is in equilibrium between feed and the adsorbent and the driving force is linear (Guiochon and Zhong, 1997).

The SMB operates at isothermal conditions and its application may be based on elution or displacement chromatography. The advantages of SMB include small amount of solvent needed per run, higher purity extraction, and stable product quality. The SMB has advantages over batch chromatography such as low consumption of solvent, high concentration, and purity of product. On the other hand, although batch chromatography has high consumption of solvent, they are easier to operate (Guiochon and Zhong, 1997). Figure 1.2, shows the stages of the simulated moving bed as different stages such as extract, effluent, feed, and raffinate (Gouichon and Zhong, 1997).

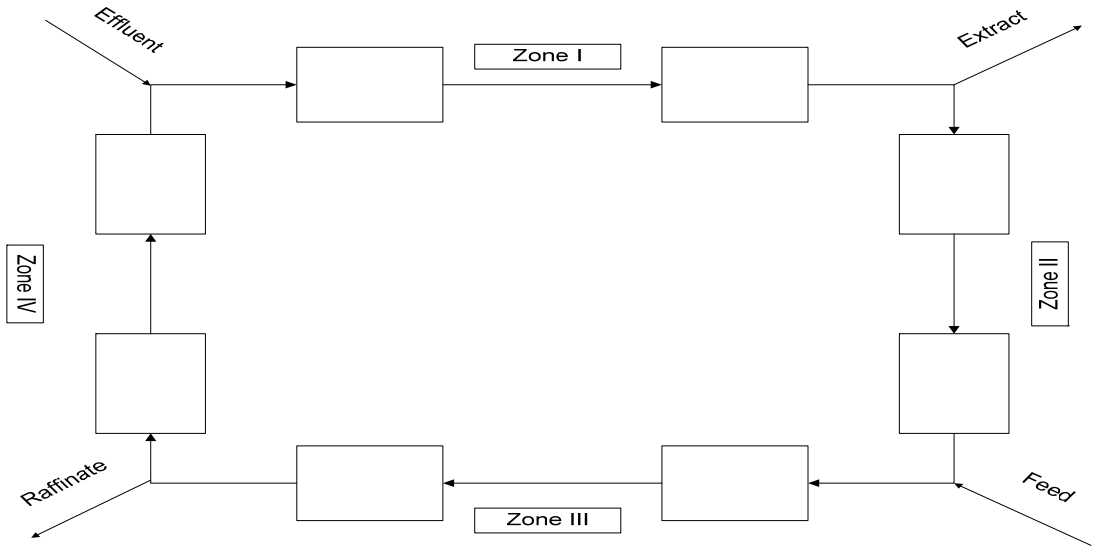


Figure 1.2 Simulated Moving Beds. Reproduced with permission.

1.2.2 High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography is well established method to separate compounds with similar physical characteristics. It is an analytical technique used in identification and purification of similar compounds. HPLC has normal and reversed phase modes of operation. In the normal mode of HPLC operation, silica gel packing with polar constituents facilitates attraction of polar components of the mobile phase while the non-polar solvent moves through the column faster. In the reversed phase HPLC, the stationary phase is non-polar and has affinity for the non-polar compounds, thus, the polar solvent moves through the column faster (Meyer, 1994).

High Performance Liquid Chromatography (HPLC) was first used in 1848 for separation of sodium ammonium tartrate crystals (Subramanian, 2000) while Gas Chromatography was used in 1966 to separate acylated amino acids. With the development of new columns, manufacturers started to produce equipment

that could separate chiral compounds. The first use of silica gel to separate chiral compounds was in 1979. In 1981, the first commercial HPLC chiral column became available. The first chiral screening kit was developed in 1991 (Subramanian, 2000).

1.3 Supercritical Fluid Chromatography

Supercritical Fluid Chromatography (SFC) has been commercially available since approximately 1982. It is a technique derived from High Performance Liquid Chromatography and Gas Chromatography. The main difference between SFC and HPLC is that SFC uses a supercritical mobile phase as opposed to a liquid mobile phase. Therefore, SFC doesn't require an organic solvent as the mobile phase; instead it uses compounds such as carbon dioxide that are safe, cost effective, and environmentally friendly. Supercritical fluids have physical properties that are between liquid and gas states resulting in higher mass transfer rates. Supercritical fluids have lower viscosities and higher diffusivities compared to liquids.

The critical temperature and pressure of CO_2 is 31 ℃ and 79 bars, respectively. SFC can separate chiral compounds much faster than HPLC because of less resistance within the stationary phase in the column. SFC requires smaller column dimensions, lower modifier concentrations, and provide faster separation times. The most popular mobile phase in SFC is CO_2 which is non-toxic and non-corrosive.

The limitations for SFC are encountered when working with proteins and polypeptides, which are chain of amino acids that are not soluble in CO_2. Another disadvantage of SFC is the relatively larger capital investment required for the equipment (Charpentier and Marentis, 1998).

1.3.1 Properties of Supercritical Fluids

Supercritical is a physical state where properties of a molecule are intermediate between gas and liquid properties of that substance. SCFs have lower density and kinematic viscosity than a liquid, but have higher diffusivity and diffusion coefficient than a liquid (Sivasankar, 2005). The physical and chemical properties of SCFs are given in Table 1.3 (Székely) while critical pressures and temperatures of some SCFs used as mobile phase are given in Table 1.4.

Table 1.3 Properties of Liquid, Gas, and Supercritical Fluids (SCF). Reproduced with permission.

Property	Liquid	Gas	SCF
Density(kg/m^3)	1000	1	100-1000
Viscosity ($\mu Pa * s$)	500-1000	10	50-100
Diffusion Coefficient (mm^2/s)	0.001	1-10	0.01-0.1

Table 1.4 Popular Mobile Phase Solvents and their Critical Properties

Solvent	Critical Temperature (K)	Critical Pressure (bars)
Carbon Dioxide (CO2)	304	73.8
Methane	190	46.0
Methanol	513	81.0
Ethane	305	48.7
Ethanol	514	61.4
Isopropanol	508	47.6
Isobutanol	547	43.0

Table 1.4 Continued

Propane	349	42.5
1-Hexanol	611	35.1
Ethylene	282	50.3

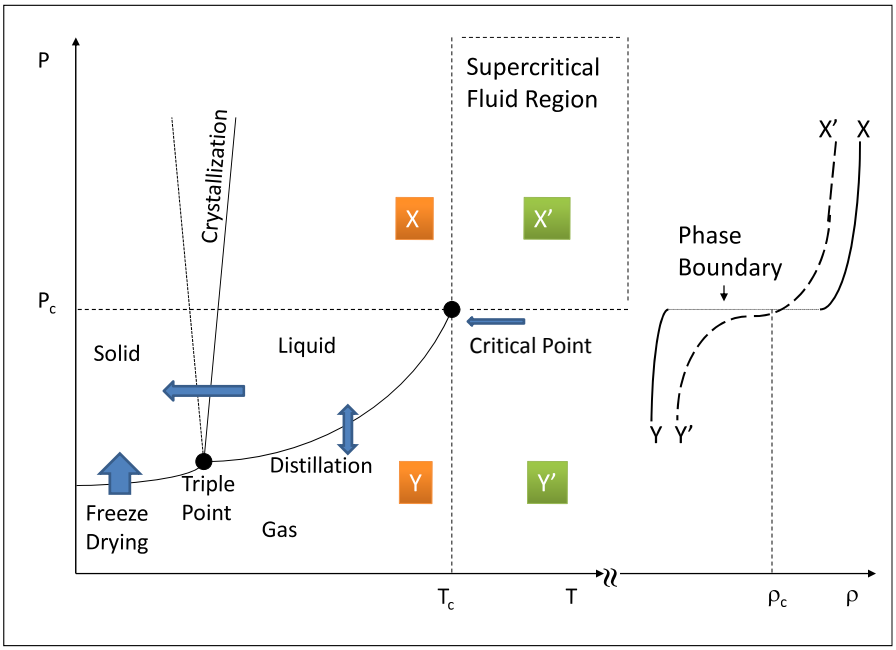


Figure 1.3 Pressure Temperature Diagram of a Pure Compound Including Supercritical Region. Reproduced with permission.

Figure 1.3 is a sketch showing the gas, liquid, solid, and SFC states using a temperature and pressure diagram. The boundaries of regions and the phase diagrams are slightly different for different compounds. The phase transition continuums are illustrated using pressure –density diagrams below and above the critical point. For Carbon Dioxide the supercritical region is above 31 ℃ and 79 bars.

Hannay and Hogart (1879) studied the solubility of solids in carbon dioxide and found that CO_2 has unexpectedly high dissolving power that is pressure dependent. $SCCO_2$ is cost effective, environmental friendly, has low viscosity, low density, high diffusivity, and low critical temperature and pressure (Charpentier and Marentis, 1998) and(Bracey, 1989). The critical volume is 93.9 cm^3/mol, the acentric factor is 0.239, and the triple point temperature is 217 K. The heat of fusion for supercritical carbon dioxide is 940 $J/mol * K$, a solid heat capacity of 59.4 $J/mol * K$, and a liquid heat capacity of 78 J/mol.

$SCCO_2$ has been used in many chemical processes such as pharmaceutical coating of tablets, food purification, cleanings of parts, extraction of soil, flavor extraction, waste water extraction, purification of essentials oil such as citrus peel oil and terpenes, fractionation of fatty acids and glycerides, regeneration of catalyst and absorbents, chromatographic fractionation of vitamins and optical isomers, and removal of trace compounds (Sunol and Sunol, 2001). The American Society of Heating, Refrigerating and Air-Conditioning Engineering show that at high temperature, the density of carbon dioxide will decrease linearly and at low temperature, the density behaves exponentially (ASHRAE, 2010). The density of carbon dioxide at supercritical fluid condition does not behave ideally. When the reduced pressures increase, reduced viscosity increases. For carbon dioxide at 50 ℃, both the dielectric constant and density increases as pressure increases in the system (Charpentier and Marentis, 1998).

1.3.2 Separation of Chiral Compounds using Supercritical Fluid Chromatography

The pharmaceutical study with Ibuprofen in human plasma with high performance liquid chromatography shows us that the S-Ibuprofen has a better mean plasma concentration than R-Ibuprofen. The retention time for S-Ibuprofen is 1.27 hours and the R-Ibuprofen is 1.31 hours (Canaparo et.al., 1999).The solubility of ibuprofen in supercritical carbon dioxide increases as a function of temperatures between 35 to 50℃ at a pressure range of 100 to 250 bars (Suleiman, et al., 2005). The dissolution of Ibuprofen at 25 megapascal (250 bars) and 313 K (39.9 ℃) in Supercritical CO_2 shows that when Ibuprofen is being treated with rapid expansion of supercritical solution (RESS), it took 20-25 minutes for complete dissolution, while it took 40 minutes for not treated with rapid expansion of supercritical solution RESS (Wischumerski, et. al., 2007). When ibuprofen is inside plasma they found that it can stay in the system between five and eight hours (Plakogiannis, et al., 1980).

1.4 Thermophysical Property Measurement using Supercritical Chromatography

Chromatographic techniques have been used not only for analysis and separation. They can be used for measurement of thermo-physical and transport properties. These methods are quite popular since they are quick, use off the shelf equipment and are reliable. The extension of gas and liquid chromatography techniques for measurement of these properties to supercritical conditions has been subject of intense work in the past twenty years (S. G. Sunol et.al., 1997).

The relevant methods for isotherm determination for chiral separations include Frontal Analysis (FA), Frontal Analysis by Characteristic Points (FACP), Elution by Characteristic Points (ECP), Pulse Methods, and the Retention Time Method (Guiochon, et al., 2006).

This following section will be focus on the separation factor, peak resolution, and the number of theoretical plates that are applicable to all SFC based separations and property measurement methods.

1.4.1 Separation Factor

The equation below shows the separation factor for any mixture with the retention time of the mixture (Meyer, 1994).

$$\alpha = \frac{t_{R2} - t_0}{t_{R1} - t_0} \tag{1.1}$$

Johannsen (2001) showed that at $40℃$ and 160 bars, with Ethanol as the modifier; the separation factor is 1.2 for Ibuprofen. The separation factor increased as the retention factor increased in a Kromasil CHI-TBB analytical column (Johannsen, 2001). Whelan (2003) showed that when Isopropanol was used as a modifier, the separation factor decreased as the modifier concentration increased. He also showed that at 5 % Ethanol and 5% Isopropanol as a modifier, the separation factor was high, when the temperature of the system increased, the separation factor decreased slightly (Whelan, 2003). Johannsen showed that, for Ibuprofen at $40℃$ and 160 bars using Isopropanol as the modifier, the modifiers concentration increased as the separation factor for

Ibuprofen decreased for Kromasil CHI-TBB, Kromasil CHI-DMB, Chirobiotik T, and Chiralcel OBH columns (Johannsen, 2001).

1.4.2 Peak Resolution

The peak resolution equation given below (Meyer, 1994) takes into account the width base of the two peaks.

$$R_s = \Delta t \left(\frac{2}{W_{b1} + W_{b2}}\right) \quad (1.2)$$

It shows that at 40 ℃ and 160 bars with Isopropanol as the modifier in supercritical carbon dioxide that as the modifier concentration increases the peak resolution of Ibuprofen decreases in Kromasil CHI-TBB, Kromasil CHI-DMB, Chirobiotik T, and Chiralcel OBH columns (Johannsen, 2001). Whelan (2003) showed that when Isopropanol is used as a modifier, the peak resolution decreases as the modifier concentration increases. The results from Whelan and Johannsen show that the best peak resolution observed was obtained at 5% modifier concentration (Johannsen, 2001), while Whelan (Whelan, 2003) showed that 10 % Isopropanol and Ethanol, respectively, mixed at 35 ℃ , peak resolution decreased as a function of increasing pressure in the system. Whelan (2003) showed that using 5% Ethanol and 5% Isopropanol as a modifier, as the temperature increases the peak resolution decreases. Wilson (1994) found that a Chiralpak AD column can separate Ibuprofen enantiomer in about 7 minutes with 5 % methanol and the flow rate of 2 ml/ min at 100 bars and 35 ℃ . Wilson also found that as the flow rate, temperature, and pressure increase, the peak resolution decreases.

1.4.3 The Number of Theoretical Plates

The higher number of theoretical plates in a column permits a better separation of substance. At higher theoretical plates, the relative retention factor goes to one (Meyer, 1994). The equation below shows the equation for calculating the number of theoretical plates with the retention time and width of the peak (Meyer, 1994).

$$\mathrm{N} = \mathrm{a}\,(\frac{\mathrm{t_R}}{\mathrm{w}})^2 \tag{1.3}$$

where a =16

The number of theoretical plates is related to the resolution (Meyer, 1994)

$$R = \frac{1}{4} * \sqrt{N} * \left(\frac{\alpha - 1}{\alpha}\right) * \frac{k'_2}{1 + \overline{k'}} \tag{1.4}$$

where k

$$\overline{k'} = \frac{k'_1 + k'_2}{2}$$

Table 1.5, shows the relationship between the separation factor and the resolution as it impacts the theoretical plate numbers (Meyer, 1994).

Table 1.5 Theoretical Plates as a Function with Relative Retention and Resolution. Reproduced with permission.

Relative retention, α	R=1.0	R=1.5
1.005	650000	1450000
1.010	163000	367000
1.050	7100	16000
1.100	3700	8400
1.250	400	900
1.500	140	320
2.000	65	145

1.5 Objectives and Outline of the Thesis

The objective of this thesis is to evaluate the merits of isotherm determination for chiral compounds using SFC. Elution chromatographic data (ECD) will be used to determine isotherms and the results will be consolidated into known models such a Lagmuir as constants. Mechanistic first principle chromatographic models will also be used along with chromagrams to compare the results with ECD method.

Chapter 2

Determination of Adsorption Isotherm by Elution Chromatography

Chromatographic data can generally be obtained by four procedures: elution chromatography, frontal analysis, the combined frontal-elution method and the displacement technique (Guiochon, et al., 2006). The technique used in the present study is elution chromatography and therefore procedures related to this particular technique are discussed in this chapter.

In the elution technique, a discrete sample (pulse) of material is introduced into the carrier fluid stream. During passage through the column, a given component is distributed in a constant ratio between the mobile and the stationary phase. This ratio is governed by a fundamental physical quantity, the partition coefficient. If the partition coefficients for each component of mobile phase differ sufficiently, they emerge from the column as separate peaks. At fixed temperature, pressure, and flow rate, the time till emergence of a peak is characteristic of each component of a mobile phase. The repeated distribution of material between phases leads to a concentration profile along the column for each component, and this gives rise to the familiar elution chromatogram comprising a set of peaks with diffuse rear end (Guiochon, et al., 2006).

These peaks are characterized by two sets of parameters, namely the retention time and retention volume and the shape and behavior of the chromatographic peak (Guiochon, et al., 2006).

2.1 The Mechanism for Sorptive Separation of Chiral Compound

The isolation of chiral compounds can be achieved using chromatographic, HPLC or Supercritical, or membrane based techniques. Our focus in this thesis is sorptive and as such, the mechanism of chiral columns will be discussed (Subramanian, 1994).

The separation for chirality occurs on the column as the solvent binds to the active site and then moves down the column to the detector. Many different companies make different columns based on the material, size, cost, and efficiency (Meyer, 1998). The column diameter is important, because it determines the flow rate through the column at a given time (Meyer, 1998). The column material has to withstand the pressure and temperature range of the experiment (Meyer, 1994).

2.1.1 The Transport of Single Solute through a Chiral Column

The Figure 2.1, below shows what happens inside the Whelk-01 Column with a 10 cm long tube made of stainless steel (Regis, 2000) and (Meyer, 1998). The carbon dioxide, Ethanol, and S-Ibuprofen enter the column at the same time. The carbon dioxide and the Ethanol (which act as a modifier) move through the column at a constant rate of 3 mL/ min. The S-Ibuprofen is absorbed and desorbed on the active site as it moves along the column. The carbon dioxide and Ethanol will leave the column first, to go into the vent. The S-Ibuprofen then

reaches the detector and the signals are recorded on a computer for later analysis. There is only one peak because there is only one solute in the detector. As higher concentrations are injected into the column, there are more chances that the molecule will get to the active site and a higher chance for the detector to pick up the signal and that will give a big absorbance (Guiochon, et al., 2006) and (Meyer, 1998).

The Figure 2.1 below shows how the solute moves through the column and how it interacts with the stationary phase (Meyer, 1998). Figure 1.2 (a) shows the initial condition, (b) shows the later time as the solute moves inside the column and (c) shows what happens after the carrier leaves the column (Meyer, 1994).

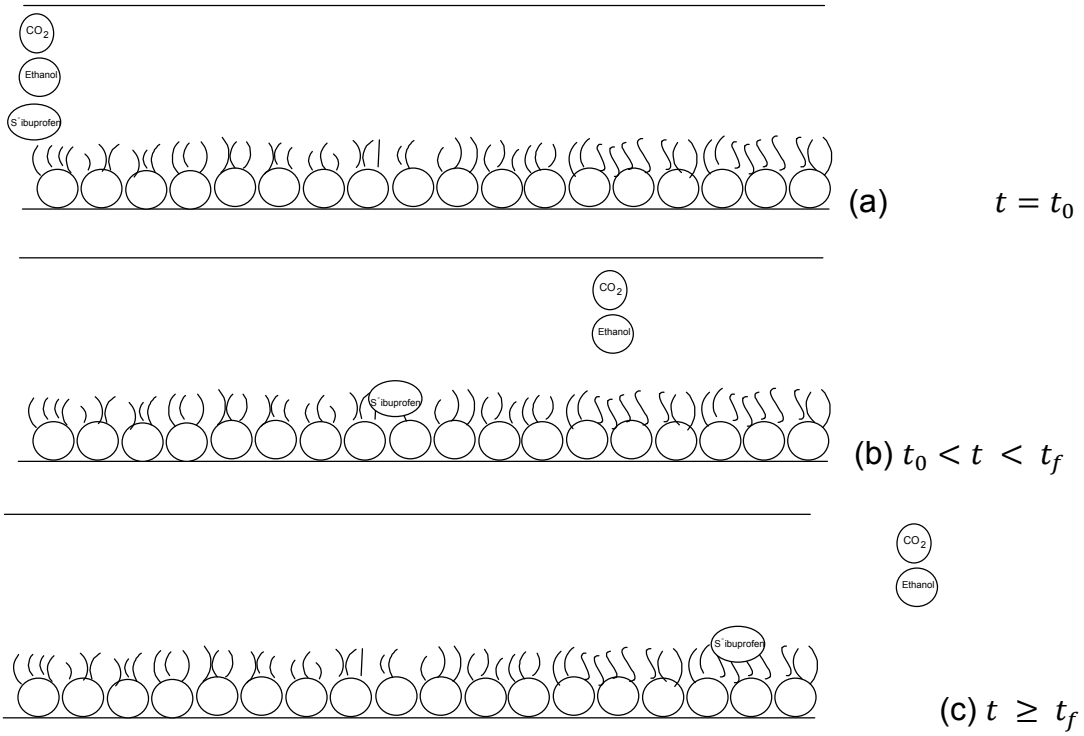


Figure 2.1 Sorption of a Single Adsorbate within a Chromatographic Column

2.1.2 The Separation of Multiple Solutes through a Chiral Column

The Figure 2.2 below shows two molecules competing for the active site. What we see here is Carbon Dioxide, Ethanol, S-Ibuprofen, and R-Ibuprofen. Both the S and R have the almost identical chemical properties and structure, the only difference being that they are a reflection of each other (Subramanian, 1994). The reasons why there are different times for the solute mixture is because of the type of column that is used. The same process occurs in the case of the single solute, but this time there is a competition to get to the active site. The Carbon Dioxide and Ethanol move out of the column first. The S-Ibuprofen will always go out after the solvent and is followed by the R-Ibuprofen (Meyer, 1998). The processed data show that there are two peaks corresponding to the solute that was injected into the auto sampler. We see that the first peak in the chromatogram is the S-Ibuprofen and the second one is R-Ibuprofen.

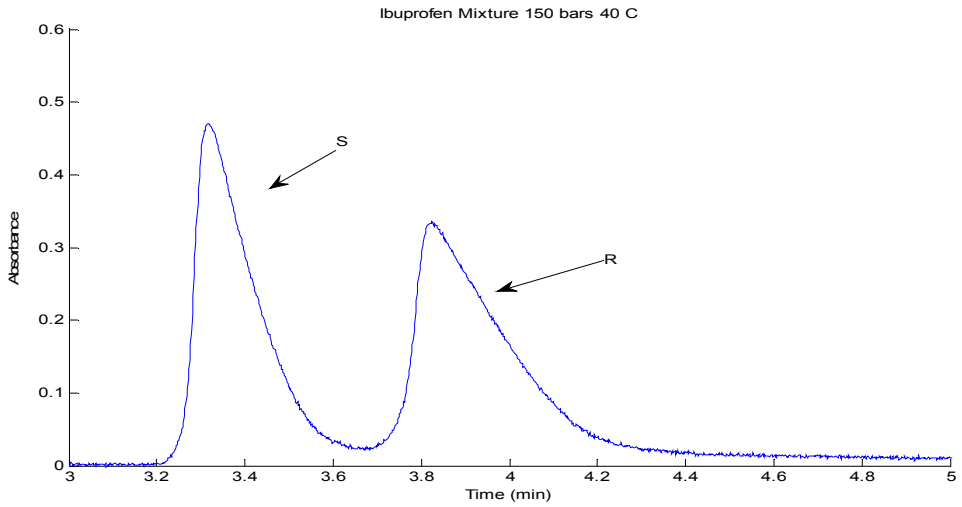


Figure 2.2 A Chromatogram for Mixture of Ibuprofen Enantiomers at 150 bars 40 ℃

There are different columns that can be selected to enhance the R-Ibuprofen come out first instead of S-Ibuprofen (Meyer, 1994). Figure 1.4 (a)-(c) shows what happens in the column when there is a mixture of Ibuprofen involved. The figure shows that the mixture sample is injected into the column and the solute will go attach itself to the stationary phase as it moves along the column (Meyer, 1994). Figure 2.3 (a) shows the initial condition for (R&S)-Ibuprofen, (b) shows a later time as the S-Ibuprofen moves first followed by the R-Ibuprofen, and (c) shows what happens as ibuprofen moves along the column (Meyer, 1994).

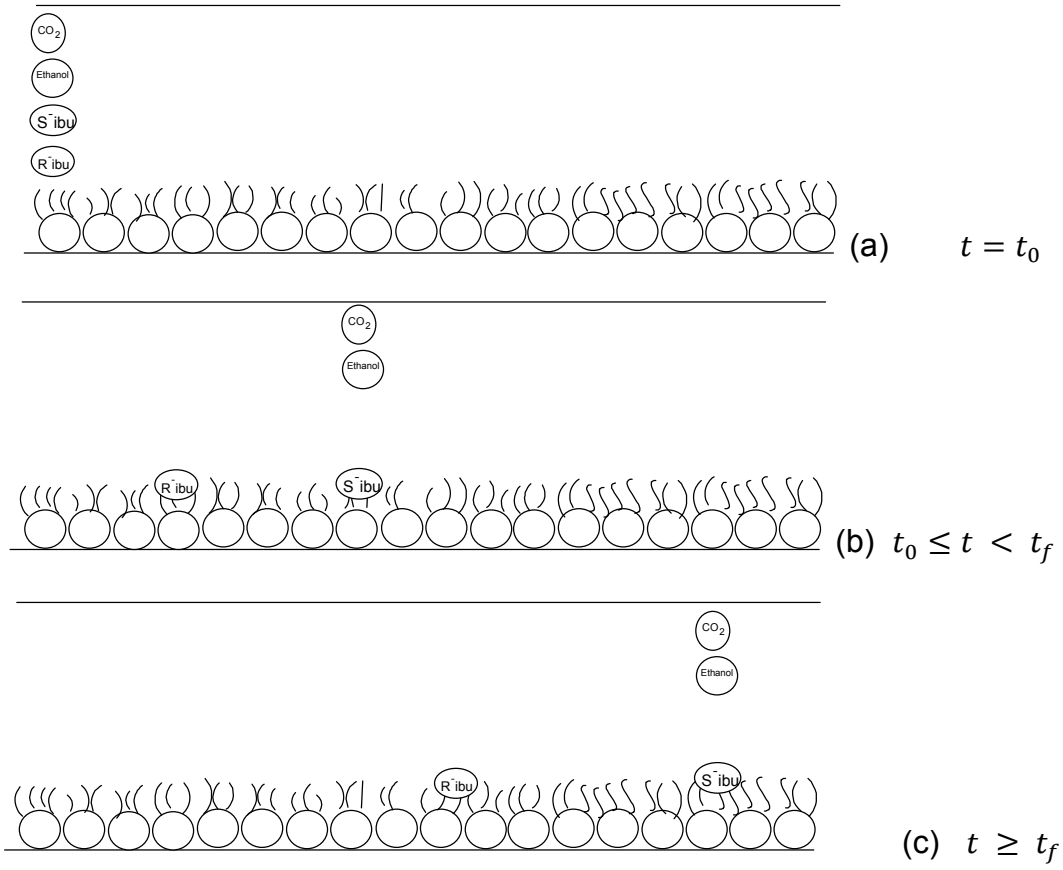


Figure 2.3 Separation of Multiple Adsorbates within a Chromatographic Column

2.2 The Components of Supercritical Fluid Chromatography Columns

In Supercritical Fluid Chromatography the important factors that made the result better or worse are the mobile and stationary phase that we choose in our experiment. There are no single mobile or stationary phase that will gave us the best results for every experiment, so there will be a compromise (Meyer, 1994).

2.2.1 The Mobile Phase

The choice of the mobile phase depends on some important properties. The mobile phase needs to be inert and inexpensive. The solvent must be transparent over the spectral sensitivity range of the UV detector, and if not, an entrainer must be added to the solvent to help the detector to pick up the compound more easily (Myer, 1994). The solvent also has to be inert to the column and non-toxic if leaked to the environment. The viscosity of the mobile phase determines flow rates through the column. Veronika Meyer shows graphically how to find the number of theoretical plates for low and high viscosity mobile phases based on known parameter such as length of the column [cm], the optimum flow rate [mm/s], time [sec], and pressure [bar] (Meyers, 1994).

2.2.2 The Stationary Phase

The analytes in the mobile phase gets absorbed to active sites on the stationary phase and held there for some time and then moves along the length of the column to the detector (Meyer, 1994). The stationary phase that is used to separate the mixture is a (R, R) Whelk 01 column (Regis, 2000).

2.3 Other Stationary Phases

The column is packed with silica gel, with a density of 2.2 $\frac{g}{cm^3}$, and a narrow distribution of the pore size for better separation (Meyer, 1994). The pore diameter is below 5 nm. The surface of the gel can react with either a base, acid, or neutral mixture. The silica gel has to be coated to separate the analyte mixture as it being contact with the mobile phase.

The silica gel has to be small so that the coating will be uniform and result in good separation and if the pore size is less than 5 nm it would be hard coat all of the silica gel particles, yet it would give great separation of the mixture because there is more contact by the solvent. The smaller the silica gel in the column, the better it is for the molecules to be in contact with the active site and results in better separation (Meyer, 1994). When the silica gel is too large, the silica gel has a large void in the column and the solvent can pass through the column as if nothing is there. If the silica gel is too small, the solvent would have a harder time traveling through the column (Meyer, 1994). Figure 2.4, reproduced with permission, show the functional group that is attached to the silica gel (Regis, 2000).

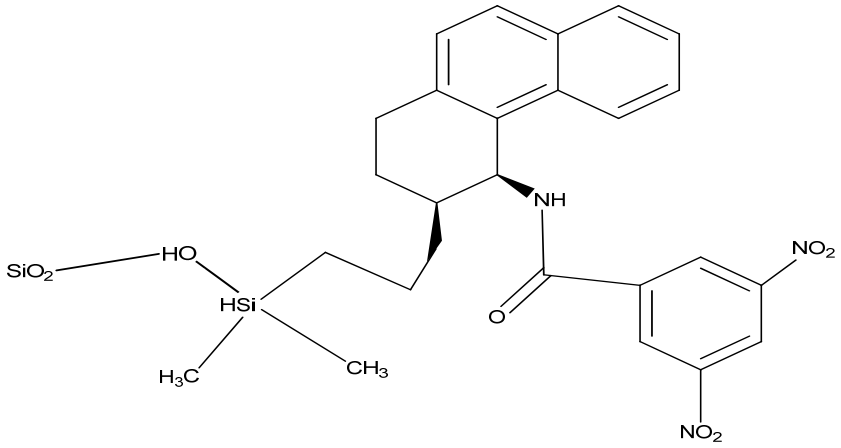


Figure 2.4 Functional Group of Whelk-0 1 Column

In Chapter 3, we will discuss the modeling aspect of chromatography as the concentrations of ibuprofen enter the column and what factors are important in determine the best isotherm.

Chapter 3

Modeling and Analytical

This chapter is based on the continuity equation for chromatography material balance and the analytical solution. Section 3.1 discusses the retention time and volume while section 3.2 focuses on adsorption isotherms. Section 3.3 discusses the determination of isotherm from the diffuse rear boundary of the elution peak while section 3.4 focuses on the determination of isotherms using elution characteristic point techniques. Section 3.5 discusses the determination of isotherm constants of the Langmuir while section 3.6 on the adsorption of the analyte inside the column. Section 3.7 discusses the desorption of analyte on the column while section 3.8 discusses the analytical solution of the analyte in chromatography. Next, section 3.9 discusses the analyte concentration in the column and section 3.10 focuses on the liquid chromatography model in the column. Section 3.11 discusses the Freundlich Isotherm and section 3.12 is on the Toth Isotherm.

3.1 Retention Time and Retention Volume

In chromatographic systems, all measurements are taken as time value or they may be associated with a time value. The equipment used to pick up the signal is a Hewlett Packard 1050 Series Variable Wavelength Detector (Agilent Tech, 2001). The wavelength was held constant before each experimental run and the amount of detection the equipment can do is 1, 2, and 4 AU.

The detector has to warm up for about 30 minutes before it fully functional. The detector has a noise level of 0.05 AU. The range of the UV spectrum is 240 to 260 nm (Regis, 2000). The signal is converted from analog to digital and is recorded (Agilent Tech, 2001).Retention time values are defines as shown in Figure 3.1 (Meyer, 1994).

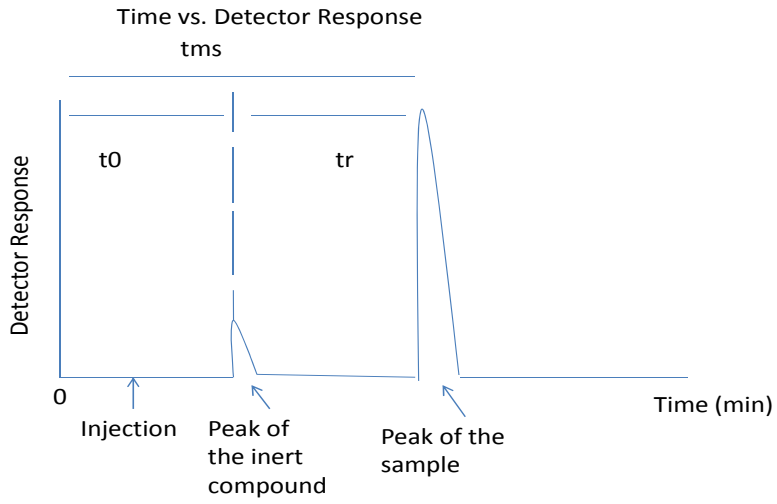


Figure 3.1 Schematic Presentation of a Sample Chromatogram

$$t_{MS} = t_0 + t_R \quad (3.1)$$

The retention time equation above is the time it takes a substance to go through the column. It consists of the holdup time plus the adjusted retention time (Martin, 2004).

where,

t_0 = the holdup time

t_R = the adjusted retention time

The time spent in the mobile phase as the sample travels the column from one end to the other, t_0, and the time spend on the surface of the stationary phase, t_R. Here, t_0 is invariant with any sample, if the column conditions are not altered, but t_R is specific for each sample (Meyer, 1994).

If the carrier flow rate is held constant, a similar relation for the retention volume can be derived (Martin, 2004):

$$F_C * t_R = F_c * (t_{MS} - t_0) \tag{3.2}$$

where F_C is the mobile phase flow rate in the column (Martin, 2004).

$$V_R = V_{MS} - V_0 \tag{3.3}$$

The retention volume V_R may be defined as the volume of mobile phase and entrainer required to carry a zone of given concentration on the boundary from inlet to outlet of the column (Martin, 2004).

3.2 Adsorption Isotherms

The adsorption isotherm is the relation between the concentration of the adsorbent in the fluid and the concentration of the adsorbent on the solid surface at equilibrium conditions and constant temperature (Hubber and Shafei, 2002). Adsorption isotherms may be obtained from the shape of the diffuse rear boundary of an elution peak (Cremer and Huber, 1961). An expression relating the concentration of the sample in the effluent to the volume of carrier fluid, which has passed through the column since the injection of the sample, was derived under the following simplifying assumptions. First, the temperature of the column is constant and uniform along its length. Second, the volume velocity of the

carrier fluid, averaged over any cross section of the column is constant. Third, the axial transport inside the column is due to convection. Fourth, the equilibrium is maintained at all time within any cross section (Guiochon, et al., 2006).

The retention volume at a given concentration is related to the derivative of the adsorption isotherm at that concentration. Figure 3.2 shows the stationary phase in the column (Guiochon, et al., 2006). At finite concentration, the material balance equation can be written for the solute as follows (Guiochon, et al., 2006):

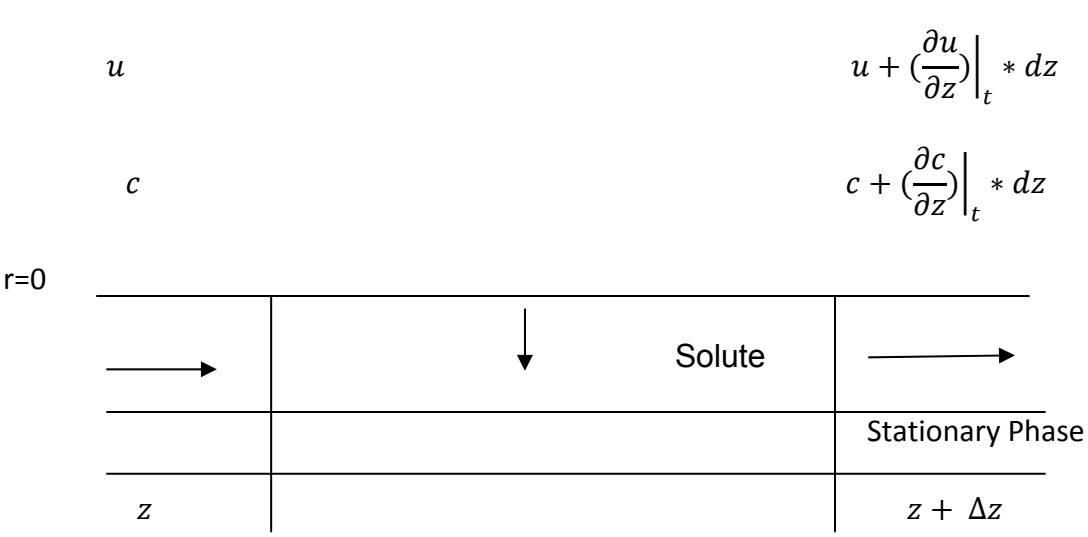


Figure 3.2 Stationary Phase

$$In = c * u * dt \tag{3.4}$$

$$Out = \left[c + (\frac{\partial c}{\partial z})\Big|_t dz\right]\left[u + (\frac{\partial u}{\partial z})\Big|_t dz\right] dt$$

$$= \left[cu + c\,\frac{\partial u}{\partial z}dz + u\frac{\partial c}{\partial z}dz + \frac{\partial u \partial c}{\partial z^2}\,dz^2\right] dt$$

where $\left(\frac{\partial u \partial c}{\partial z^2}\right) dz^2$ is neglected. We neglected the term because we assume that the dispersion in the column is zero (Guiochon, et al., 2006).

$$= \left[cu + c\ \frac{\partial u}{\partial z}dz + u\frac{\partial c}{\partial z}dz\right] \tag{3.5}$$

$$Accumulation\ in\ the\ fluid\ phase\ = \frac{\partial c}{\partial t}\ dz\ dt \tag{3.6}$$

$$Accumulation\ in\ the\ solid\ phase = \frac{1-\varepsilon}{\varepsilon}\frac{\partial q}{\partial t}\ dz\ dt \tag{3.7}$$

This equation relates the sorption of the silica gel in the column and how porous it is (Guiochon, et al., 2006).

$$In - out = accumulation$$

$$\left[-c\ \frac{\partial u}{\partial z} -\ u\ \frac{\partial c}{\partial z}\right] = \frac{\partial c}{\partial t} + \frac{1-\varepsilon}{\varepsilon}\frac{\partial q}{\partial t} \tag{3.8}$$

where

t= time spent in the column [min]

c= concentration of the solute in the mobile phase [mmol/mL]

u= velocity of the mobile phase [mm/s]

z= axial dimension of the column [mm]

q= concentration of the solute on the stationary phase [mmol/mL]

ε = void fraction of the column

The balance equation on total material that includes both the solute and the mobile phase can be written as follows (Gonenc, 1985) and (Conder and Young, 1979).

The axial dispersion coefficient is assumed to be zero (Guiochon, et al., 2006)

c_t = total concentration (solute + carrier)

$$\text{Inflow } = c_t * u * dt \tag{3.9}$$

$$\text{Outflow } = [c_t + \frac{\partial c_t}{\partial z}\, dz] * \left[u + \left(\frac{\partial u}{\partial z}\right) dz\right] dt$$

$$= c_t * u * dt + c * \frac{\partial u}{\partial z} * dzdt + u\frac{\partial c_t}{\partial z} * dzdt$$

$$= \left[c_t u + c_t \frac{\partial u}{\partial z} dz + u\frac{\partial c_t}{\partial z} dz\right] dt \tag{3.10}$$

$$accumulation\ in\ the\ gas\ phase = \left(\frac{\partial c_t}{\partial t}\right) * dzdt \tag{3.11}$$

$$accumulation\ in\ the\ solid\ phase = \frac{1-\varepsilon}{\varepsilon} * \frac{\partial q}{\partial t}\, dz\, dt \tag{3.12}$$

$$In - out = accumulation$$

$$\left[-c_t \frac{\partial u}{\partial z} - u\, \frac{\partial c_t}{\partial z}\right] = \frac{\partial c_t}{\partial t} + \frac{1-\varepsilon}{\varepsilon} * \frac{\partial q}{\partial t} \tag{3.13}$$

The concentration along the column assumed to be zero. Total concentration c_t changes with neither z nor t

$$-c_t * \frac{\partial u}{\partial z} = \frac{1-\varepsilon}{\varepsilon} * \frac{\partial q}{\partial t} \tag{3.14}$$

Define y, mole fraction of the solute in the mobile phase, as

$$y = \frac{c}{c_t} \tag{3.15}$$

Multiply Equation 3.14 by y

$$y - c\, \frac{\partial u}{\partial z} = \frac{c}{c_t} * \frac{1-\varepsilon}{\varepsilon} \frac{\partial q}{\partial t} \tag{3.16}$$

Subtract Equation 3.16 from Equation 3.8

$$-u * \frac{\partial c}{\partial z} = \frac{\partial c}{\partial t} + (1 - y) * \frac{1 - \varepsilon}{\varepsilon} * \frac{\partial q}{\partial t} \tag{3.17}$$

Equation 3.17 can be written in terms of $\frac{\partial \mathrm{t}}{\partial \mathrm{z}}$ using chain rule

$$\frac{\partial t}{\partial z} = -\left(\frac{\partial t}{\partial c}\right) * \left(\frac{\partial c}{\partial z}\right) \tag{3.18}$$

Using Equation 3.18 one can obtain from the following equation 3.17

$$u\frac{\partial t}{\partial z} = 1 + (1 - y) * \frac{1 - \varepsilon}{\varepsilon} * \frac{\partial q}{\partial c} \tag{3.19}$$

Integrating the time in Equation 3.19 from 0 to t_R and the length from 0 to L gives (Guiochon, et al., 2006).

$$t_R = \frac{L}{u}(1 + \frac{1 - \varepsilon}{\varepsilon} * (1 - y) * \frac{dq}{dc}) \tag{3.20}$$

The volumetric flow rate is given as the product of the cross sectional area and velocity.

$$F = S_1 * u \tag{3.21}$$

where F is the volumetric flow rate, S_1 is the cross sectional area, and u is the velocity.

The retention volume is defined as

$$V_R = t_R * F \tag{3.22}$$

The gas holdup is given by

$$V_M = L * S_1 \tag{3.23}$$

where L is the column length

Combining Equation 3.20 and with 3.21- 3.23 gives

$$V_R = V_M + V_s(1-y) * \frac{dq}{dc} \tag{3.24}$$

where V_s is the volume of the stationary phase. The equation 3.23 shows that the derivative of the adsorption isotherm $\frac{\partial q}{\partial c}$ is proportional to the retention volume (V_R).

3.3 Determination of Isotherm (for a Single Solute) from the Diffuse Rear Boundary of the Elution Peak

A pulse of a single solute is introduced into the carrier fluid stream and the elution curve at the outlet is recorded. A pulse of inert component is also introduced into the column under the same experimental conditions and its retention time recorded so that the retention of the enantiomer on the chiral column due to adsorption may be calculated. As stated by Beer Lambert Law, the absorbance is proportional to the concentration, the path length, and the molar absorbtivity (Meyer, 1998)

$$A_1 = e * b_1 * c \tag{3.25}$$

A_1= absorbance

e= the molar absorbtivity $[\frac{L}{mol*cm}]$

b_1= path length of the sample [cm]

c= concentration of the compound $[\frac{mol}{L}]$

A typical chromatogram is shown below as Figure 3.3.

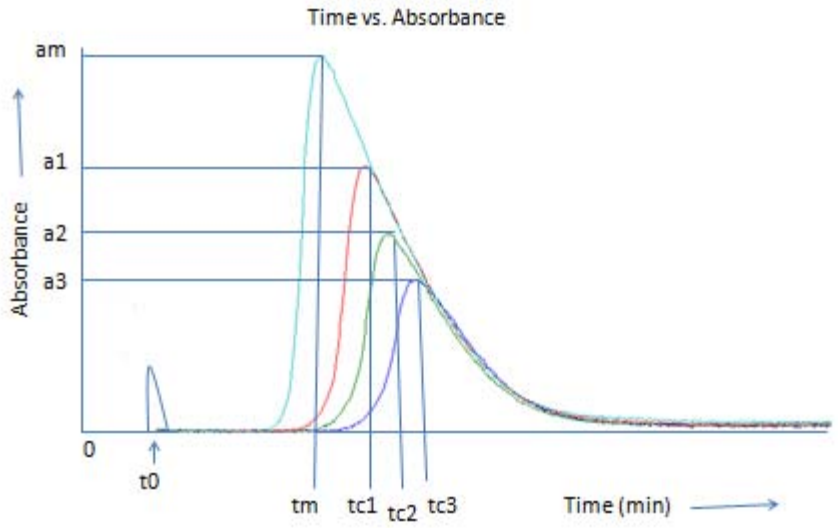


Figure 3.3 Absorbance versus Time

3.3.1 Determination of Retention Volumes

The retention volume corresponding to each concentration on the diffuse end of the peak can be calculated as follows (Martin, 2004):

$$V_R = F_C t_R = F_C * \frac{t_C - t_0}{w} \tag{3.26}$$

where F_C and t_0 are as defined earlier. t_C is the retention time on the rear boundary of the peak and w is the weight of the adsorbent in the column which is 3.5 grams (Regis, 2000).

The corresponding concentrations can be calculated from the absorbance values in two different ways (Martin, 2004): Either, the concentration of the solute in the mobile phase can be calculated from the absorbance, correcting it using the peak area under the rear boundary of the peak. The concentration of the solute in the mobile phase is varied in separate experiments and the absorbance

corresponding to those concentrations is measured and the calibration is made using these values (Martin, 2004). These two methods are described Sections 3.3.2 and 3.3.3.

3.3.2 Determination of the Concentration of Solutes in the Mobile Phase Using Peak Areas

The concentration of the solute in the mobile phase is determined from

$$c = \frac{a * N}{A * F_c} \tag{3.27}$$

$$c = \frac{AU * mmol}{(AU * min) * \frac{mL}{min}} = \frac{mmol}{mL}$$

where c is the concentration of the solute in the mobile phase [mmol/mL], a is the absorbance, N[mmol] is the number of moles of the solute injected, A is the area of the diffuse end of the peak, and F_c [mL/min] is the mobile phase flow rate in the column (Martin, 2004). Absorbance, a, is measured in Absorbance Units, which is unitless, which is related to transmission. For example, ~ 1.0 Au is equal to 10 % transmittance, ~ 2.0 Au is equal to 1% transmittance, and so on in a logarithmic trend.

3.3.3 Determination of the Concentration of Solutes from Concentration Calibrations

In order to determine the relation between the absorbance values and the concentration of the solute in the mobile phase, a calibration curve is first created. To do this, a known amount of the solute is mixed with the carrier in the pump, and the carrier with the constant amount of solute is passed through the column and the corresponding absorbance is recorded. The procedure is carried out at different concentrations of solute and an absorbance versus concentration

curve is plotted (Gonenc, 1985). The graph in Figure 3.4 is on the absorbance versus concentration

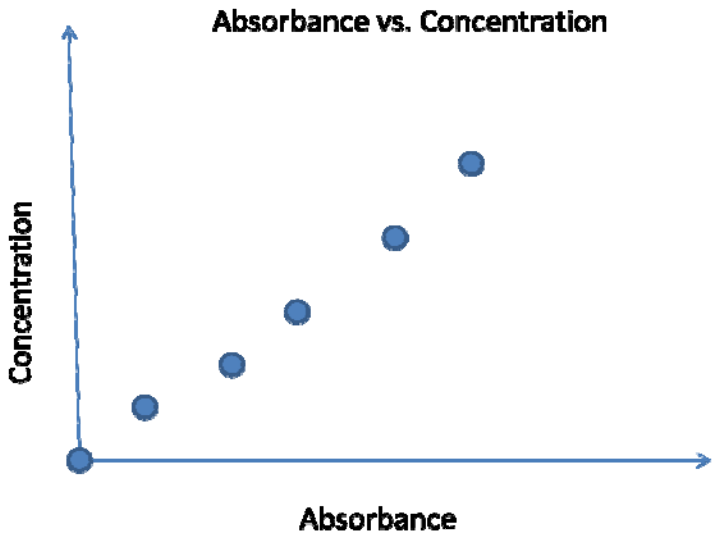


Figure 3.4 Absorbance versus Concentration of Solute in the Mobile Phase

The points in Figure 3.4 are fit into polynomial as (Gonenc, 1985):

$$c = A + B * a + C * a^2 + D * a^3 \quad (3.28)$$

for each absorbance value on the chromatogram, concentration values c_m, c_1, c_2 are then calculated from the above polynomial equation (Gonenc, 1985).

3.3.4 Determination of Isotherms

The retention volume vs. the concentration in the mobile phase is shown in Figure 3.5.

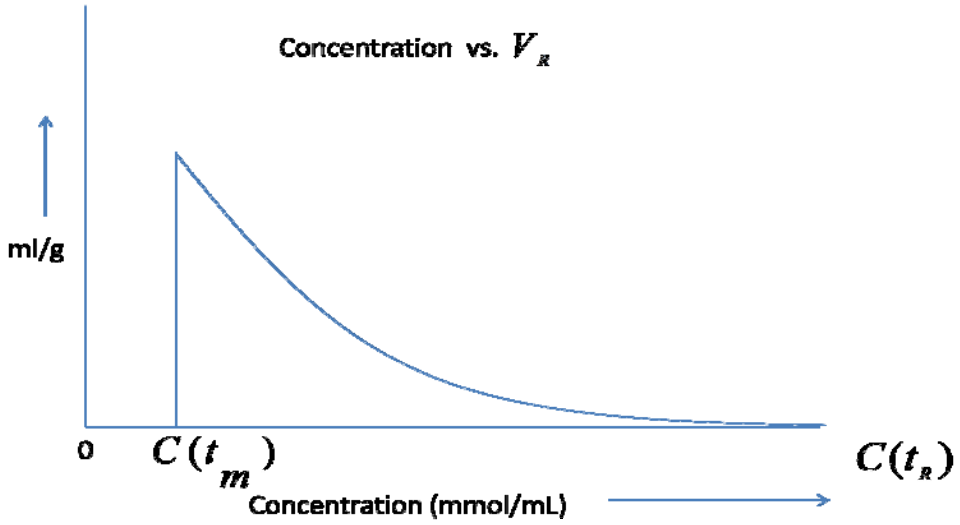


Figure 3.5 Concentrations vs. Retention Volume (V_R)

Integrating the V_R versus concentration curve, which is from the peak maximum to the base line, yield to the adsorption isotherm for Figure 3.6. The equation below is for the amount of concentration of the stationary phase for the rear end elution profile (Guiochon, et al., 2006).V is the volume of the column.

$$q = \frac{1}{V} \int_{C(t_M)}^{C(t_R)} V_R * dc \tag{3.29}$$

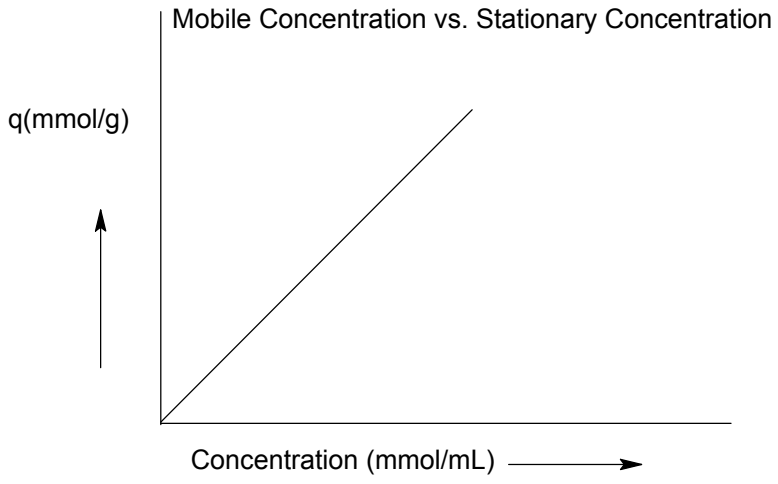


Figure 3.6 Adsorption Isotherm

The procedure for determining the adsorption isotherm using the peak area for concentration calibration and using the calibration experiments for determination of concentration of solute in the mobile phase are summarized in Figures 3.7 and 3.8, respectively.

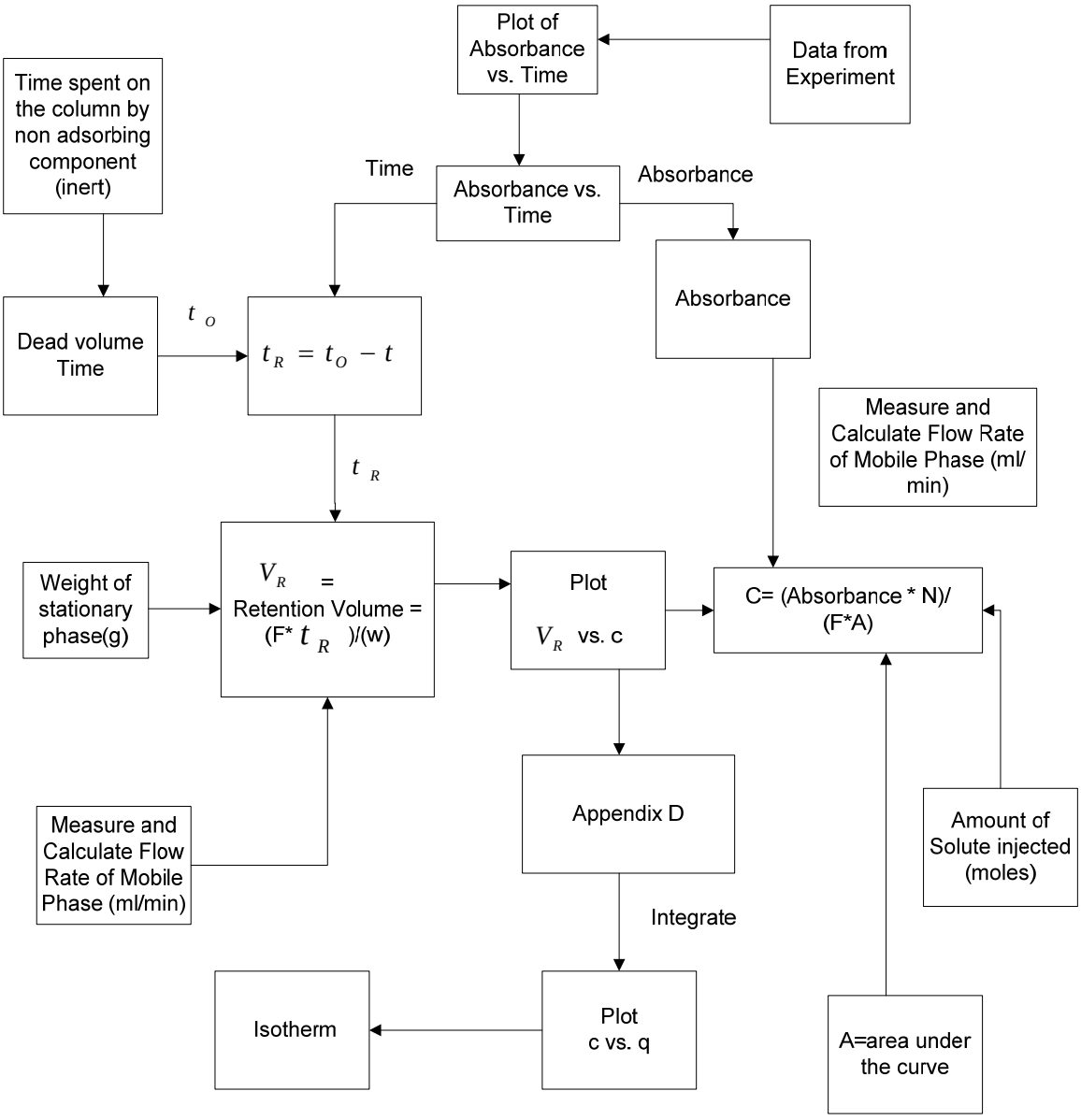


Figure 3.7 Determination of the Isotherm Using Peak Area for Concentration Calculation

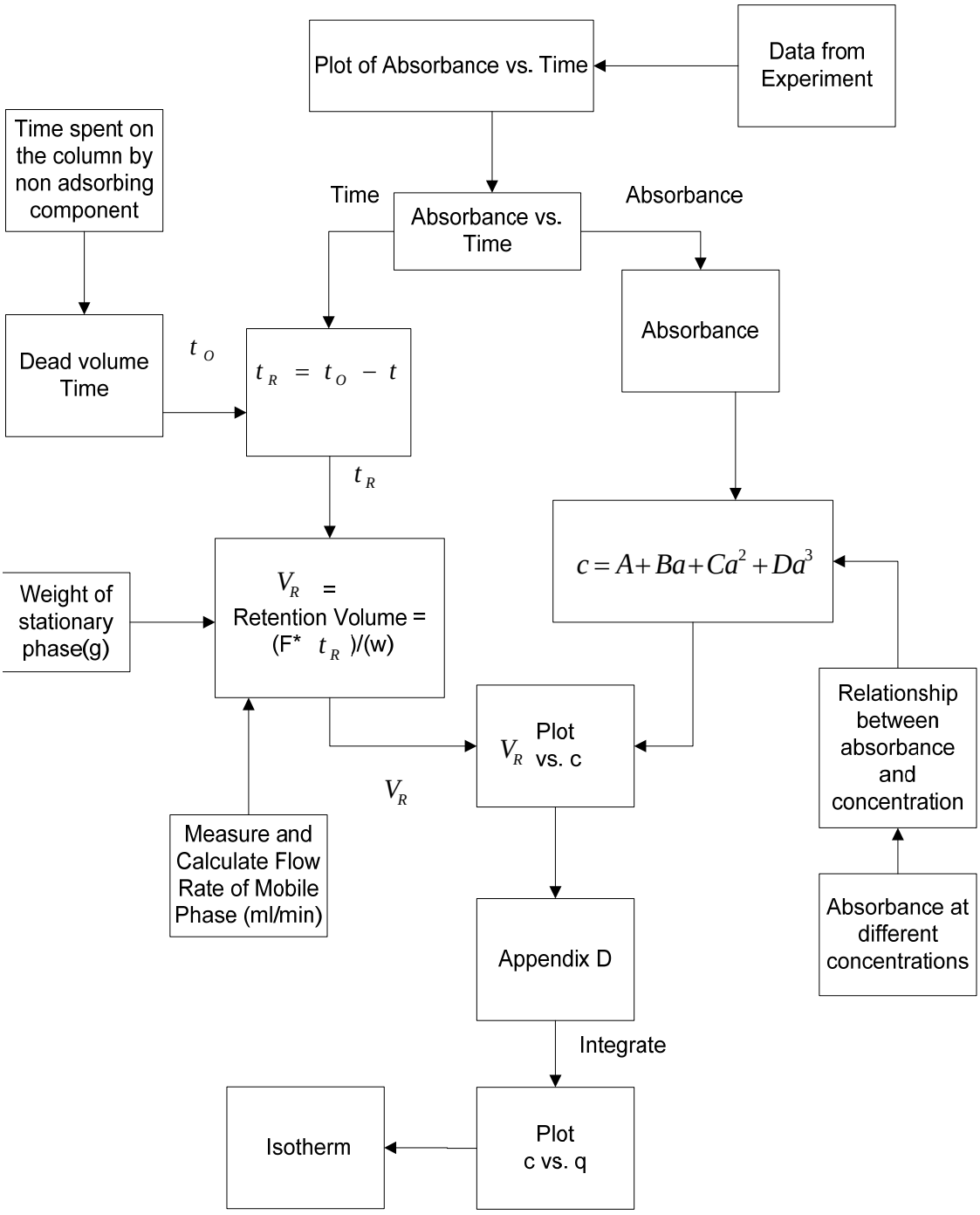


Figure 3.8 Determination of Isotherm Using Absorbance Measurement at Different Concentration

The flow rate of the mobile phase in the column is used for calculating the retention volume from the retention time and for converting the absorbance to concentration (Gonenc, 1985). Carbon dioxide and the entrainer are mixed in the column at a fixed composition in the liquid state. The mobile phase flows through the column can be measured with a flow meter (Whelan, 2003).

3.4 Determination of Isotherms using Elution Characteristic Point (ECP) Techniques

The material balance equation for the solute in the chromatographic column can be derived for ideal (equilibrium) chromatography where the assumptions are the instantaneous mass transfer, no longitudinal diffusion, uniform mobile phase velocity along the column cross section, and constant mobile phase velocity along the column (Guiochon, et al., 2006).

The material balance equation is written for the column shown in Figure 3.9 (Guiochon, et al., 2006).

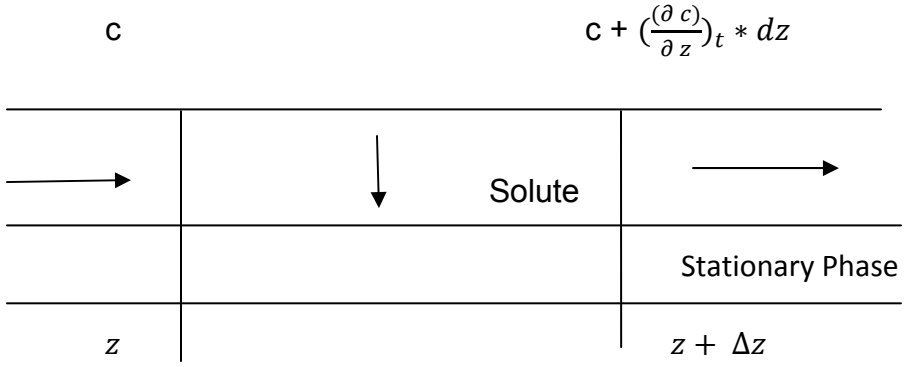


Figure 3.9 Stationary Phase Material Balance

$$in = c * u * dt \tag{3.30}$$

$$out = \left[c + \frac{\partial c}{\partial z}\, dz \right] u * dt = c * u * dt + u \frac{\partial c}{\partial z}\, dz * dt \tag{3.31}$$

$$accumulation\ in\ the\ gas\ phase = \frac{\partial c}{\partial t} * dz * dt \tag{3.32}$$

$$accumulation\ in\ the\ solid\ phase = \frac{1-\varepsilon}{\varepsilon} * \frac{\partial q}{\partial t} * dz * dt \tag{3.33}$$

$$out - in + accumulation = 0$$

$$c * u * dt + u \frac{\partial c}{\partial z} * dz * dt - c * u * dt + \frac{\partial c}{\partial t} * dz * dt + \frac{1-\varepsilon}{\varepsilon} * \frac{\partial q}{\partial t} * dz * dt = 0$$

$$u \frac{\partial c}{\partial z} + \frac{\partial c}{\partial t} + \frac{1-\varepsilon}{\varepsilon} * \frac{\partial q}{\partial t} = 0 \tag{3.34}$$

c= concentration of the solute in the mobile phase [mmol/ml]

u= velocity of the mobile phase [mm/s]

z= axial dimension of the column [mm]

q= concentration of the solute on the stationary phase [mmol/mL]

t= time

$\varepsilon =$ porosity of the silica gel

Equation 3.32 is solved with the following initial and boundary conditions (Choi, et al., 2004):

Initial conditions	$t = 0$	$c(i,z) = 0$
	$t = 0$	$q(i,z) = 0$
Boundary conditions	$z = 0$	$c = c_{in}$
	$z = L$	$\frac{\partial c}{\partial z} = 0$

Equation 3.35 allows us to calculate the retention time of the peak (Guiochon, et al., 2006).

$$t_R = t_o\left(1 + \varphi\frac{dq}{dc}\right) \tag{3.35}$$

where $\varphi = \frac{1-\epsilon}{\epsilon}$

The relationship between q and c is given by the Langmuir isotherm.

The assumptions for the Langmuir isotherm are the solution is ideal, the solute have monolayer coverage, the adsorption layer is ideal, there are no solute-solute interactions in the monolayer, and there are no solvent-solute interactions (Guiochon, et al., 2006).

The equations below show the concentration in the stationary phase with "a" and "b" as parameter (Guiochon, et al., 2006).

$$q = \frac{ac}{1 + bc} \tag{3.36}$$

$$c = \frac{q}{a - bq} \tag{3.37}$$

$$t_R = to\left(1 + \varphi\frac{dq}{dc}\right) = t_0 * (1 + \varphi\frac{dq}{dc}) \tag{3.38}$$

The equation 3.39-3.48 and 3.52 are derived from Gonenc (Gonenc, 1985).

Taking the derivative of c with respect to q in Equation 3.37 yields

$$\frac{dc}{dq} = \frac{a}{(a - bq)^2} \tag{3.39}$$

Substituting Equation 3.39 into Equation 3.38 gives

$$t_r = t_0\left(1 + \varphi\ \frac{(a - bq)^2}{a}\right) \tag{3.40}$$

Rearranging Equation 3.40 gives

$$t_R = t_0(1 + \frac{\varphi a}{(1 + bc)^2}) \tag{3.41}$$

Material balance for the solute injected is

$$N = F * \int_{t_R}^{t_R o} c(t) dt_R \tag{3.42}$$

F= volumetric flow rate in the column

N= total number of moles injected

Substituting Equation 3.37 into Equation 3.42 gives

$$N = F \int_{t_R}^{t_{R0}} \frac{q}{a - bq} dt_R \tag{3.43}$$

Taking the derivative of t_R with respect to q in Equation 3.40 gives

$$\frac{dt_R}{dq} = -2t_0\varphi\ b\ \frac{(a - bq)}{a} \tag{3.44}$$

Multiply both sides with dq and rearrange

$$dt_R = \frac{-2\ t_0\ \varphi\ b\ (a - bq)}{a}\ dq \tag{3.45}$$

Substituting dt_Rinto Equation 3.42 gives

$$N = F\frac{2t_0\ \varphi\ b}{a}\int_{q_m}^{0} q dq \tag{3.46}$$

Integrating yields

$$N = F\frac{t_0\ \varphi\ b}{a}{q_m}^2 \tag{3.47}$$

Solving for q_m, we get

$$q_m = \sqrt{\frac{Na}{F\ t_0\ \varphi\ b}} \tag{3.48}$$

where Langmuir constant can be estimated using retention time (Guiochon,et al., 2006).

$$a = \frac{t_R\ - t_0}{v * t_0} \tag{3.49}$$

$$b = \frac{Lf * v * (t_R\ - t_0)}{N_m} \tag{3.50}$$

$$Lf = (\left(1 - \left(\frac{t_f\ - t_0\ - t_p}{t_R\ - t_0}\right)^{.5}\right)^2 \tag{3.51}$$

where v the linear velocity inside the column is, t_f is the final time, and t_p is the width of the peak (Guiochon, et al., 2006).

The isotherm constants are also calculated by regressing the equation for the retention time (Gonenc, 1985)

$$t_R\ =\ t_0\ (1 + \varphi \frac{(a - bq)^2}{a})$$

or in terms of c

$$t_R\ =\ t_0(1 + \varphi \frac{\left(a - \frac{bac}{1+bc}\right)^2}{a}) \tag{3.52}$$

The concentration for each maximum t_r in Figure 3.6 is calculated using the calibration for absorbance with equation 3.28 (Gonenc, 1985). Equation 3.52 is linearly regressed in order to calculate constants of the Langmuir equation, “a” and “b” (Guiochon, et al., 2006).

Figure 3.10 shows the procedure for calculating the isotherm using the ECP method. In this method, different amounts of the solute are injected into the column and the retention times for peak maxima are recorded for each of the pulse. The amount of the solute adsorbed is calculated using equation 3.36 with the initial values of the isotherm constant calculated using equation 3.48-3.51. Mobile phase concentration is calculated using the Langmuir model and the isotherm obtained is compared with the isotherm obtained using the other methods described in Sections 3.2 and 3.3.

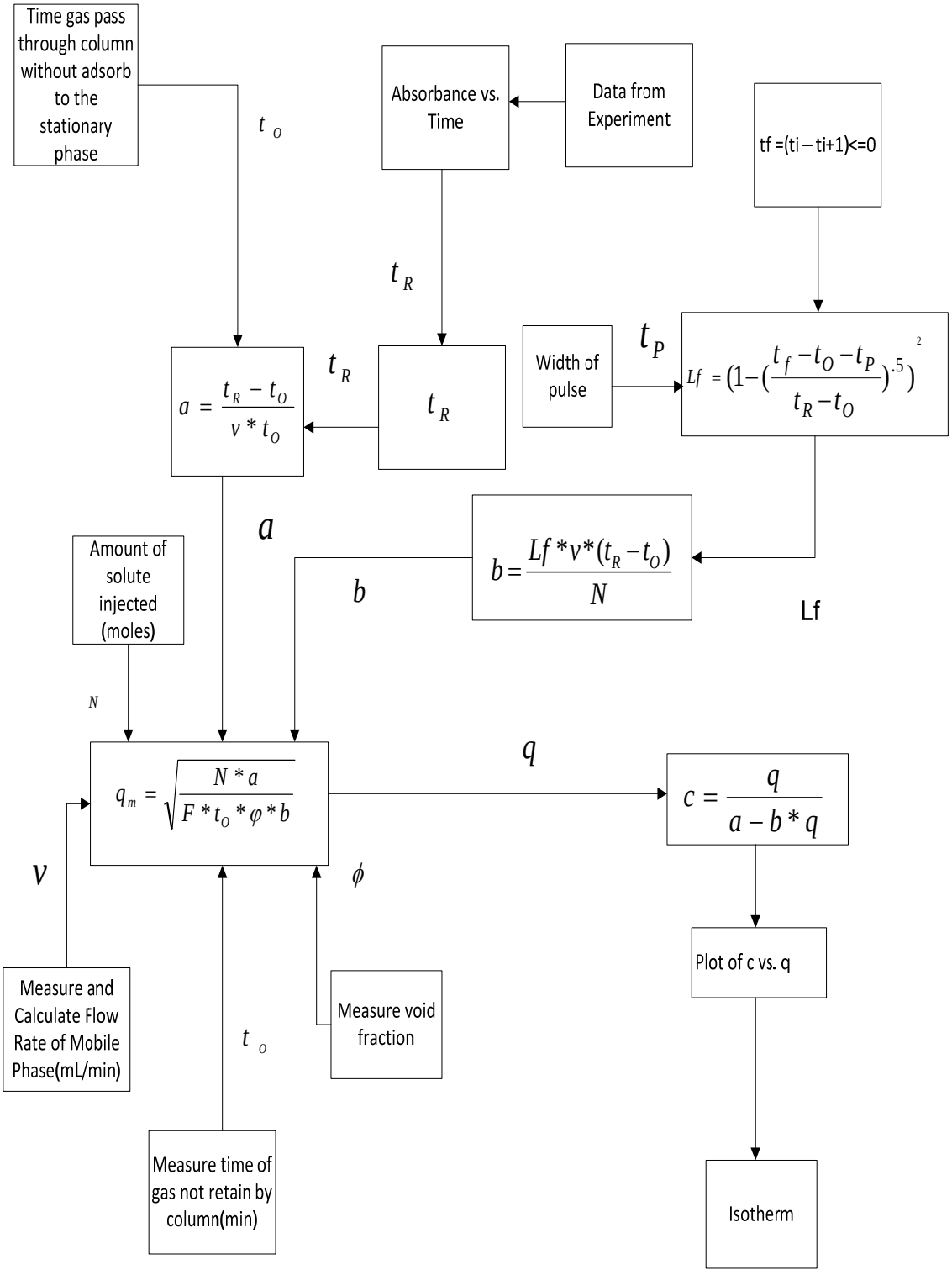


Figure 3.10 Procedure for Determination of an Isotherm from Elution Characteristic Point Method

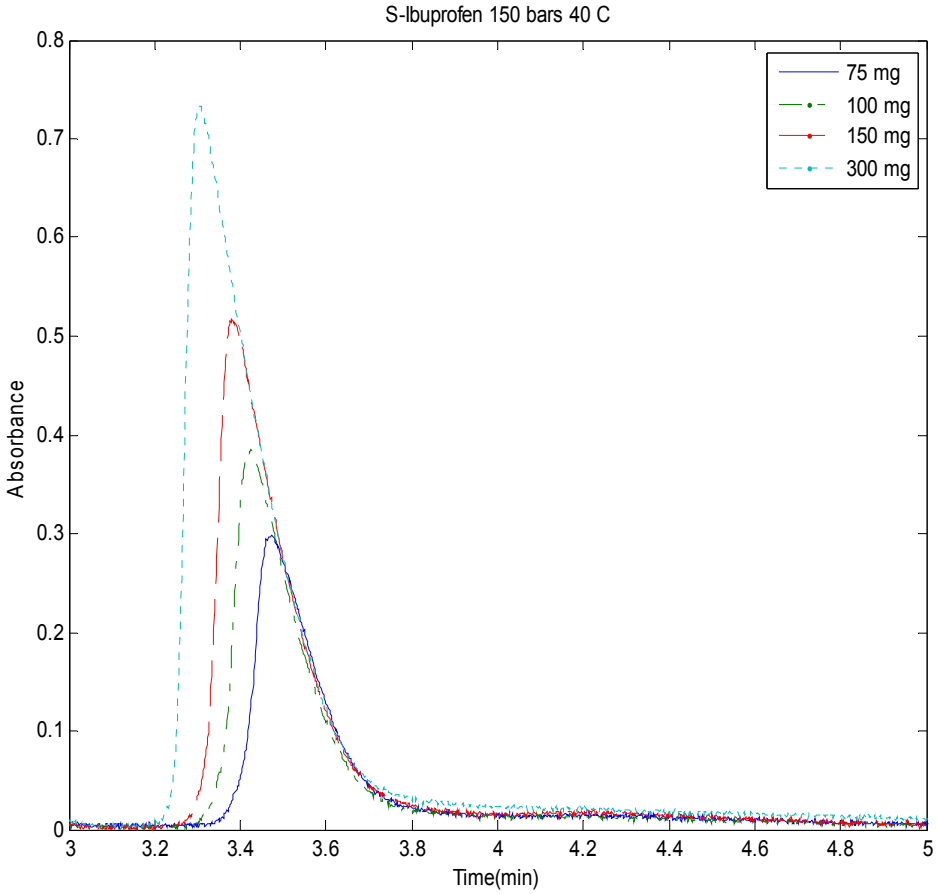


Figure 3.11 Chromatogram of S-Ibuprofen at Different Concentrations

3.5 Determination of Isotherm Constants of the Langmuir Equation

The chromatograms in Figure 3.11 are taken at each concentration from the maximum peak to the diffuse rear to get the isotherm. The Langmuir equation is in the following form (Guiochon, et al., 2006):

$$q = \frac{a * c}{1 + b * c} \tag{3.53}$$

Linearizing the Langmuir equation gives (Guiochon, et al., 2006)

$$\frac{c}{q} = \frac{b * c}{a} + \frac{1}{a} \tag{3.54}$$

A plot of $^{c}/_{q}$ versus c gives a straight line with slope equal to $^{b}/_{a}$ and the intercept equal to $^{1}/_{a}$. With the initial estimates obtained, a linear regression of q vs. c data yields values of “a” and “b” (Guiochon, et al., 2006).

The equations in this section are derived from Guiochon (Guiochon, et al., 2006). We will start with the mass balance equation

$$\frac{\partial c}{\partial t} + F\frac{\partial q}{\partial t} + u\frac{\partial c}{\partial z} = 0 \tag{3.55}$$

where

F = the phase ratio

u = the flow rate

q = the stationary phase concentration in equilibrium

Here, we assume that there is no axial dispersion, and there is instant equilibrium between the two phases (Guiochon, et al., 2006).

The following differential equation models the SMB in the chromatography column with single solute (Guiochon, et al., 2006):

We will take flux, $N_{i,z}$, that would enter a differential cylindrical control volume as a small slice in the column

$$N_{i,z} = \varepsilon * S * (uc_i - D_{L,i} * \frac{\partial c_i}{\partial z})_{z,t} \tag{3.55.1}$$

where

ε= the porosity in the column

S= the cross sectional area of the column

$D_{L,i}$= the axial dispersion coefficient

z= distance along the column

The flux at the exit of the control volume

$$N_{i,z} = \varepsilon * S * \left(uc_i - D_{L,i} * \frac{\partial c_i}{\partial z}\right)_{z+\Delta z,t} \tag{3.55.2}$$

The rate of accumulation

$$S\Delta z\left(\varepsilon\frac{\partial c_i}{\partial t} + (1-\varepsilon)\frac{\partial c_{s,i}}{\partial t}\right)_{z,t} \tag{3.55.3}$$

Combining the equation into a differential mass balance

$$\varepsilon * S * (uc_i - D_{L,i} * \frac{\partial c_i}{\partial z})_{z,t} - \varepsilon * S * \left(uc_i - D_{L,i} * \frac{\partial c_i}{\partial z}\right)_{z+\Delta z,t} = S\Delta z\left(\varepsilon\frac{\partial c_i}{\partial t} + (1-\varepsilon)\frac{\partial c_{s,i}}{\partial t}\right)_{z,t} \tag{3.55.4}$$

Taking the Δz as it approach 0, we obtain

$$\frac{\partial c_i}{\partial t} + \frac{1-\varepsilon}{\varepsilon}\frac{\partial q_i}{\partial t} + u\ \frac{\partial c_i}{\partial z} = D_i\frac{\partial^2 c_i}{\partial z^2} \tag{3.55.5}$$

$$\frac{\partial q_i}{\partial t} = k_i(q_i^* - q_i) \tag{3.55.6}$$

Assuming the isotherm is linear.

The boundary conditions are (Choi, et al., 2004)

$$\text{at z} = 0 \qquad \text{c(i, 0)} = \text{c}_{\text{in}}\text{(i)}$$

$$\text{at z} = \text{L} \qquad \frac{\partial \text{c(i, L)}}{\partial \text{z}} = 0$$

The initial conditions are (Choi, et al., 2004)

$$\text{at t} = 0 \qquad \begin{cases} \text{c(i, z)} = 0 \\ \text{q(i, z)} = 0 \end{cases}$$

3.6 The Adsorption of Analyte Inside the Column

We will use equation 3.55 as the basic for the development of the adsorption that occurs on the column. The equations in section 3.1 and 3.2 are derived from Bracey (Bracey, 1989). We will assume that the concentration in the stationary phase is linear with the equation

$$\frac{1-\varepsilon}{\varepsilon}\left(\frac{\partial q}{\partial t}\right) = k_i * (c_i - c_i{}^*) \tag{3.56}$$

where

q= the concentration in the stationary phase

ε = the porosity of the silica gel

k= the rate constant

c_i= equilibrium concentration

$c_i{}^*$= local concentration in the stationary phase

The initial conditions for the equation 3.55 are

$$c_i = c_{i0} \quad x = 0$$

$$c_i = 0 \quad t = 0$$

By applying the Laplace transform, we get

$$c_i(x,t) \xrightarrow{L} \tau_i(x,s) = \tau_i$$

$$q_i(x,t) \xrightarrow{L} q_i(x,s) = q_i$$

We will then substitute in the Laplace transform in equation 3.55, to obtain

$$sq_i * \left(\frac{1-\varepsilon}{\varepsilon}\right) + u\frac{\partial c}{\partial z} + s\tau_i = 0 \tag{3.57}$$

$$sq_i * \left(\frac{1-\varepsilon}{\varepsilon}\right) = k_i * \left(\tau_i - \frac{q_i}{B_i}\right) \tag{3.58}$$

$$\tau_i = \frac{c_{i0}}{s} \qquad x = 0 \tag{3.59}$$

Solving equation for q_i in equation 3.58, we get

$$q_i = \frac{k_i * B_i * \tau_i * \left(\frac{\varepsilon}{1-\varepsilon}\right)}{B_i * s + k_i * \left(\frac{\varepsilon}{1-\varepsilon}\right)} \tag{3.60}$$

Then, upon substitution of q_i into equation 3.57 to obtain

$$u * \frac{\partial \tau_i}{\partial z} + \left(s + \frac{k_i * B_i * s}{B_i * s + k_i * \left(\frac{\varepsilon}{1-\varepsilon}\right)}\right) * \tau_i = 0 \tag{3.61}$$

Solving for τ_i

$$\tau_i = \frac{c_{io}}{s} * exp^{\left(-\frac{x}{u}\right)*s} * exp^{\left(-k_i*\left(\frac{x}{u}\right)*\left(\frac{s}{s+k_i*\left(\frac{\varepsilon}{1-\varepsilon}\right)*B_i}\right)\right.} \tag{3.62}$$

and taking the inverse Laplace transforms

$$c_i = c_{i0} * e^{-\xi_i} \int_0^{\tau_i} e^{-u} * I_0 * \left(2 * \sqrt{\xi_i * u}\right) * du + e^{-(\xi_i+\tau_i)} * I_0 * \left(2 * \sqrt{\xi_i * \tau_i}\right) \tag{3.63}$$

where

$$\tau_i = k_i * \left(t - \frac{L}{u}\right)$$

$$\xi_i = \frac{k_i * B_i}{u} * \left(\frac{1-\varepsilon}{\varepsilon}\right)$$

I_0= Bessel function

The analytical solution of the equation is

$$c_i = \frac{1}{2} * c_{i0} * ERFC\left(\sqrt{\xi_i} - \sqrt{\tau_i} - \frac{1}{8} * \sqrt{\xi_i} - \frac{1}{8} * \sqrt{\tau_i}\right) \quad (3.64)$$

With ERFC the complimentary Error Function

$$ERFC\ (x) = 1 - ERF(x) = \frac{2}{\sqrt{\pi}} \int_x^{\infty} e^{-t^2} dt$$

3.7 The Desorption of Analyte Inside the Column

We will use the same method in the adsorption model to transform the entire variable into new one

$$\Theta = c_{i0} - c_i$$

$$\psi = q_{i0} - q_i$$

we also have

$$-\frac{\partial c_i}{\partial z} = \frac{\partial \Theta}{\partial z}$$

$$-\frac{\partial c_i}{\partial t} = \frac{\partial \Theta}{\partial t}$$

$$-\frac{\partial q_i}{\partial t} = \frac{\partial \psi}{\partial z}$$

The boundary conditions are

$$c_i = c_{i0} \qquad x = 0 \ \rightarrow \ \Theta = \mathrm{c_{i0}}$$

$$c_i = 0 \qquad t = 0 \ \rightarrow \ \Theta = 0$$

$$q_i = q_{i0} \qquad t = 0 \ \rightarrow \psi = 0$$

The same procedure will be followed to solve for the analytical solution

$$c_i = c_{i0}(1 - e^{-\xi_i} \int_0^{\tau_i} e^{-u} * I_0\left(2 * \sqrt{\xi_i u}\right) * du + \ e^{-(\xi_i + \tau_i)} * I_0 * \left(2 * \sqrt{\xi_i * \tau_i}\right) \tag{3.65}$$

The equation becomes

$$c_i = \frac{1}{2} * c_{i0} * ERFC\ \left(\sqrt{\xi_i} - \sqrt{\tau_i} - \frac{1}{8} * \sqrt{\xi_i} - \frac{1}{8} * \sqrt{\tau_i}\right) \tag{3.66}$$

3.8 Analytical Solution of the Analyte in Chromatography

The analytical solution of the chromatography mass balance equation will be derived using equation 3.55 (Guiochon, et al., 2006). The equations in section 3.3 and 3.4 are derived from Guiochon (Guiochon, et al., 2006). When the solute is injected into the column, equation 3.55 can be written using the theory of characteristic lines

$$\frac{\partial c}{\partial t} + \frac{u}{1 + F\frac{\partial q}{\partial c}}\left(\frac{\partial c}{\partial z}\right) = 0 \tag{3.67}$$

We can write the second term as

$$u_z = \frac{u}{1 + F\frac{\partial q}{\partial c}} \tag{3.68}$$

When the concentration is continuous, we can write the equation 3.68 as (Guiochon, et al., 2006)

$$t(c) = \ t_p + \frac{L}{u_z} = t_p + \frac{L}{u} * \left(1 + F\frac{\partial q}{\partial c}\right) \tag{3.69}$$

where t_p is the width of the injection profile.

The concentration shock that occurs in the column can be written as (Guiochon, et al., 2006)

$$u_s = \frac{u}{1 + F\frac{\partial q}{\partial c}} \tag{3.70}$$

By applying the Langmuir Isotherm for equation 3.68 and 3.70

$$u_z = \frac{u}{1 + \frac{a*F}{(1+b*c)^2}} \tag{3.71}$$

$$u_s = \frac{u}{1 + \frac{a*F}{1+b*c}} \tag{3.72}$$

Plugging equation 3.71 backed into equation 3.69 we get

$$t(c) = t_p + t_0 * \left(1 + \frac{a * F}{(1 + b * c)^2}\right) \tag{3.73}$$

with c equal to

$$c = \frac{1}{b} * \left[\sqrt{\frac{t_{R,0} - t_0}{t - t_p - t_0}} - 1\right] \tag{3.74}$$

So, for the band maximum and the front shock to find the retention time

$$t_R = t_p + t_0 + \left(t_{R,0} - t_0\right) * (1 - \sqrt{L_f})^2 \tag{3.75}$$

where

$$L_f = \frac{n * b}{\varepsilon * S * L * k_0'} \tag{3.76}$$

n= number of mole

k_0'= a*F^2

The maximum concentration can be calculated by combining equation 3.73 and 3.75

$$c_M = \frac{\sqrt{L_f}}{b * (1 - \sqrt{L_f})} \tag{3.77}$$

3.9 The Analyte Concentration in the Column

We want to model the concentration as the solute moves along the column at a certain time and replace L with z in equation 3.69

$$t = t_p + \frac{L}{u} * \left(1 + F\frac{\partial q}{\partial c}\right) = \ t_p + \frac{z}{u} * [1 + \frac{k_0'}{(1 + b * c)^2}] \tag{3.78}$$

Solving for the concentration in the column

$$z(c) = \frac{u(t - t_p)}{1 + \frac{k_0'}{(1+b*c)^2}} \tag{3.79}$$

Solving for c in equation 3.79, we get

$$c = \frac{1}{b} * \left[\sqrt{\frac{k_0' * z/u}{t - t_p - z/u}} - 1\right] \tag{3.80}$$

When the shock passes through the column at a certain point in time defined as

$$t = t_p + \frac{z}{u} + \frac{k_0' * z}{u} * [1 - \sqrt{\frac{c_0 * t_p * b * u}{k_0' * u}}]^2 \tag{3.81}$$

The maximum concentration along the column is achieved

$$c_M = \frac{1}{b} * \left[\frac{\sqrt{\frac{c_0 * t_p * b * u}{k_0' * z}}}{1 - \sqrt{\frac{c_0 * t_p * b * u}{k_0' * z}}}\right] \tag{3.82}$$

3.10 The Liquid Chromatography Model in the Column

The model for the Liquid Chromatography is based on the separation of mixture that has closely related properties such as temperature and density. The equations in this section are derived from COMSOL (COMSOL, 2010) using the convection and diffusion module. The model is based on the amount of concentration in the mobile phase entering the column and adsorbed onto the stationary phase. Non-linear chromatography with 1-D geometry is assumed in the COMSOL (COMSOL, 2010) model.

The analytes enter the column would follow a mass transport equation given by

$$S\frac{\rho(1-\varepsilon)}{\varepsilon} * \frac{\partial n_i}{\partial t} + \frac{\partial c_i}{\partial t} = -\frac{\mathrm{v}}{\varepsilon A} * \frac{\partial c_i}{\partial x} \tag{3.83}$$

S= Surface area of particles in column $[\frac{m^2}{kg}]$

ρ= Density of the solid particles $[\frac{kg}{m^3}]$

ε= column porosity

A= Inner area of the column tube

n_i= Analyte concentration in the stationary phase of component i $[\frac{mol}{m^3}]$

v= Mobile phase flow rate $[\frac{m^3}{s}]$

c_i= Analyte concentration in the mobile phase of component i $[\frac{mol}{m^3}]$

Assuming that the analyte between the mobile and stationary phase is in equilibrium

$$\frac{\partial n}{\partial t} = \left(\frac{dn}{dc}\right) * \frac{\partial c_i}{\partial t} \tag{3.84}$$

The chromatography mass transport equation becomes

$$\left(1 + S\frac{\rho(1-\varepsilon)}{\varepsilon} * \frac{dn}{dc}\right) * \frac{\partial c_i}{\partial t} = -\frac{\mathrm{v}}{\varepsilon A} * \frac{\partial c_i}{\partial x} \tag{3.85}$$

There will be a dispersion of the analyte as it enters the column and each of the molecules will randomly moves along the column. The dispersion can be expressed as the effective diffusion constant

$$D_{eff} = \frac{H v_{zi}}{2} \tag{3.86}$$

D_{eff}= Diffusion effective constant [$\frac{m^2}{s}$]

H= Height equivalent of a theoretical plate

v_{zi}= Migration velocity of the analyte zone through the column

The mass balance equation for the chromatography

$$\left(1 + \Phi * \frac{dn}{dc}\right) * \frac{\partial c_i}{\partial t} = -\mathrm{v}_i \frac{\partial c_i}{\partial x} + D_{eff} \frac{\partial^2 c_i}{\partial x^2} \tag{3.87}$$

where

$\Phi = S\frac{\rho(1-\varepsilon)}{\varepsilon}$[$\frac{m^2}{m^3}$]

v_i= Linear velocity of the mobile phase in the column [$\frac{m}{s}$]

The adsorption isotherm follows a Langmuir model for both the mobile and stationary phase

$$n_i = \frac{n_{0i} K_i c_i}{1 + K_i c_i} \tag{3.88}$$

then take the differential with respect to concentration

$$\frac{dn_i}{dc_i} = \frac{n_{0i} K_i}{(1 + K_i c_i)^2} \tag{3.89}$$

K_i= Adsorption constant for the component i $[\frac{m^3}{mol}]$

n_{0i}= monolayer coverage capacity of the stationary phase for component i $[\frac{mol}{m^2}]$

3.11 Freundlich Isotherm

The Freundlich isotherm is a model that was developed by Boedeker, but made popular by Freundlich by taking into account the presence of strong polar compounds in a polar solvent (Guiochon, et al., 2006). The equation used to model this is given by

$$q = a * c^{1/n} \tag{3.90}$$

If n=1, then the isotherm is linear. The Freundlich isotherm had two flaws: 1) it was not thermodynamically consistent, and 2) it violates the Gibbs-Duhem equation (Guiochon, et al., 2006).

3.12 Toth Isotherm

The Toth isotherm was the first used for gas-solid equilibrium, but also can be used for the liquid-solid equilibrium. The Toth isotherm is described by the equation

$$\theta = \frac{c}{(b + c^t)^{1/t}} \tag{3.91}$$

The parameters for the equation above are b and t, which is the initial slope and curvature of the isotherm, respectively, and c is the concentration. When t=1 the equation reduces to the Langmuir model (Guiochon, et al., 2006).

In Chapter 4, we will discuss the way of obtaining the isotherm for the single and multiple solutes.

Chapter 4

Derivation of Isotherms from Experimental Elution Chromatograms

In this chapter, calculation procedures for obtaining isotherms from the chromatogram are discussed and described. The figures in this chapter are generated using Matlab (Matlab, 2010).

4.1 Evaluation of Data

The data were collected in real time using Labview of the time it took for the analyte to pass through the column and the absorbance level.

4.1.1 Evaluation of Data for Adsorption Isotherms Using Calibration Through Peak Area

The adsorption isotherms were obtained from single elution chromatograms. Specific retention volume,V_R, and concentration of the adsorbate in the mobile phase, c, are calculated using Equation (3.26) and (3.27) respectively. As discussed in Chapter 3, the adsorption isotherm was obtained by integrating the $V_R \; vs. \; c$ curve. A sample calculation is given below for the isotherm of S-Ibuprofen adsorption at 150 bars and $40\,℃$. The sample to be injected was prepared by dissolving 300 mg of Ibuprofen in 1 ml of Ethanol. 10 μl of this sample is injected into the column. Retention volumes and corresponding concentrations of solute in the mobile phase are calculated for any retention time on the abscissa of the chromatographic peak, which is equal or greater than the

retention time corresponding to the maximum of the sample peak (Martin, 2004). Time versus absorbance data are given in Appendix A.

Retention volume is described by (Martin, 2004)

$$V_R = \frac{[F_C * (t_c - t_0)]}{w} \tag{4.1}$$

For the given conditions of temperature and pressure, t_0, the hold-up time was measured as being equal to 1.28 min. The weight of the adsorbate (column material) was 3.5 g (Regis, 2000). The volumetric flow rate of the mobile phase (2.85$\frac{ml}{min}$ of CO_2 and 0.15 $\frac{ml}{min}$ of Ethanol mixture at pump conditions $4℃$ and 100 bars) at 150 bars and 40 ℃ is calculated from the feed rates as described in Chapter 2. The compressibility factor was calculated using the Peng Robinson Equation of State with Wong-Sandler mixing rules (Patel, 2008) .The compressibility is equal to 0.3 at 150 bars and 40 ℃ (Patel, 2008). The calculation of the flow rate is given below (Martin, 2004). The corrected retention times $(t_c - t_0)$ are also given in Appendix A. The V_R values as calculated from the above equation are also given in Appendix A. The equations below are used to calculate the compressibility at the supercritical conditions used in the experiment (Martin, 2004).

$$F = n * Z \tag{4.2}$$

$$F = 3.3\ \frac{mL}{min}$$

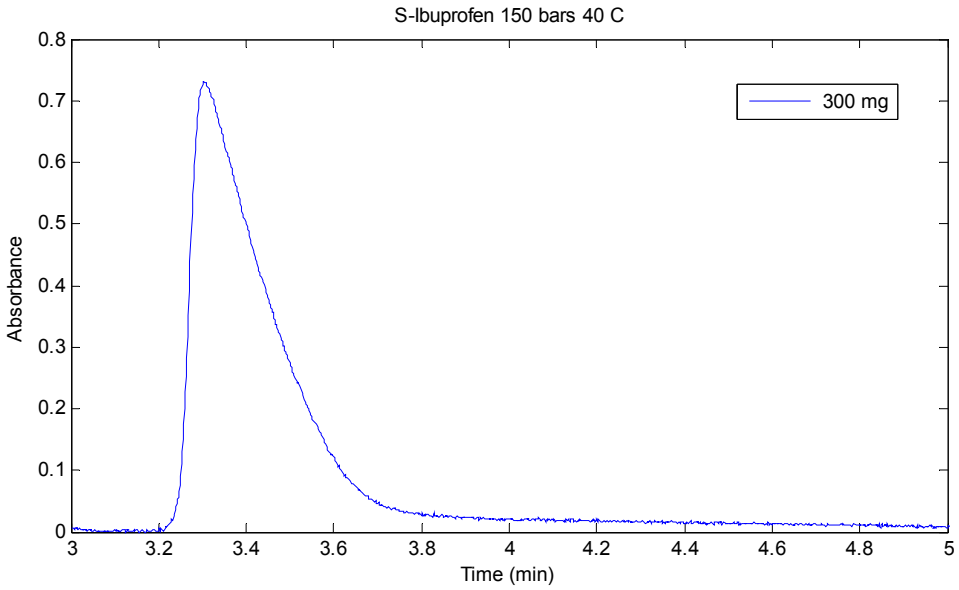


Figure 4.1 S-Ibuprofen Chromatogram at 150 Bars and 40℃

Concentration is given by (Martin, 2004)

$$c = \frac{absorbance * N}{A * F_C} = \frac{abs * (mmol)}{\min(abs)\frac{mL}{min}} \tag{4.3}$$

$$c = \frac{mmol}{mL}$$

For the given concentration of the 10 μl sample injected, the number of moles injected is (Martin, 2004):

$$N = \frac{300\ mg\ Ibuprofen}{1\ ml\ ethanol} * 10\ \mu l\ injected * \frac{1\ ml}{1000\ \mu l} * \frac{1\ mmol}{206\ mg} \tag{4.4}$$

$$N = 0.0146\ mmol\ of\ Ibuprofen\ injected$$

A is the area under the chromatogram after the peak maximum. The area for the sample peak is equal to 0.12 $Au * min$ and calculated concentration values are given in Appendix B. Figure 4.1 shows the chromatogram of S-Ibuprofen at 150 bars, 40℃, and 300 mg.

Retention volume vs. the concentration of the sample in the mobile phase is given in Figure 4.2.

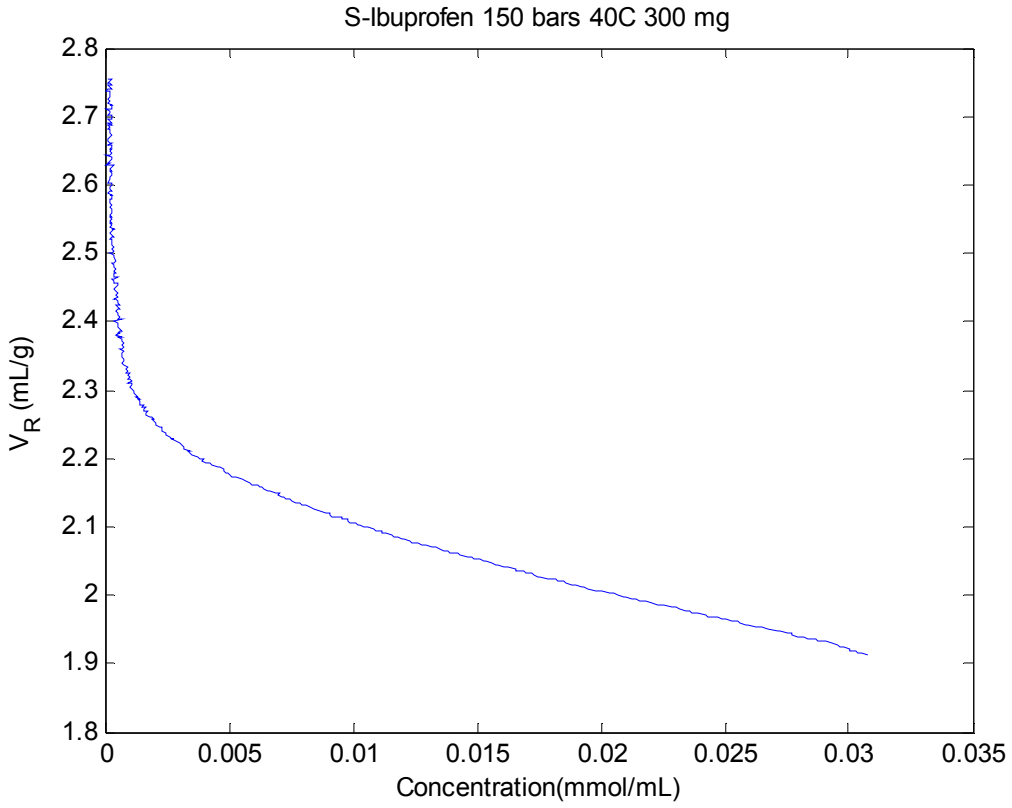


Figure 4.2 Concentrations vs. Retention Volume for S-Ibuprofen at 150 Bars and 40 ℃

By integrating $V_R\ vs. c$ with the following equation (Guiochon, et al., 2006)

$$q = \frac{1}{V} \int_{C(t_M)}^{C(t_R)} V_R * dc \tag{4.5}$$

The equation gives the isotherm. The integration is done using Matlab cumulative trapezoidal rule. The isotherm is shown in Figure 4.3 for S-Ibuprofen at 150 bars, 40℃, and 300 mg.

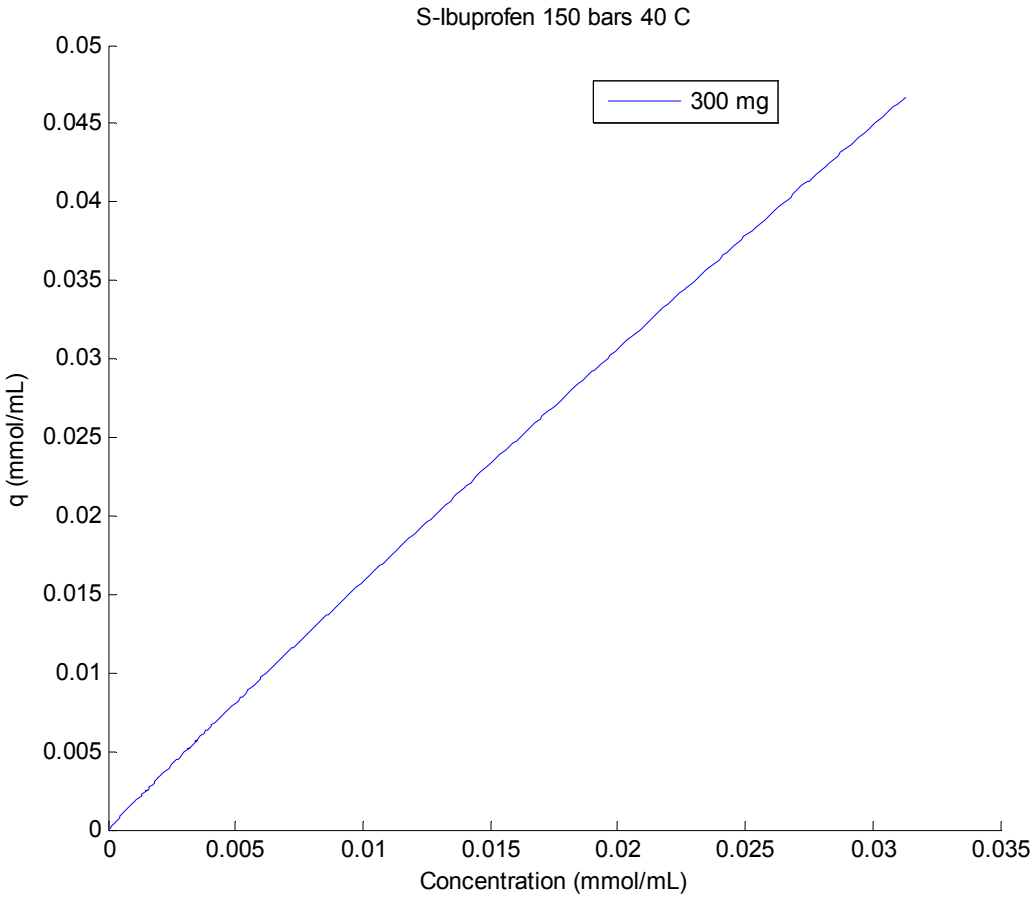


Figure 4.3 S-Ibuprofen Isotherm at 150 Bars, 40℃, 300 mg

The isotherms obtained are fit to the Langmuir equation and isotherm constants “a” and “b” are determined by linearized equation 4.6. The Langmuir Equation is in the following form (Guiochon, et al., 2006)

$$q = \frac{ac}{1 + bc} \tag{4.6}$$

$$q + qbc = ac$$

$$\frac{q}{a} + \frac{qbc}{a} = c$$

Constants “a” and “b” are 1.67 and 0.4 respectively for the S-Ibuprofen isotherm at 150 bars, 40 ℃, and 300 mg. Experimentally determined isotherms plotted using the regressed “a” and “b” values from the Langmuir equation are plotted in Figure 4.4.

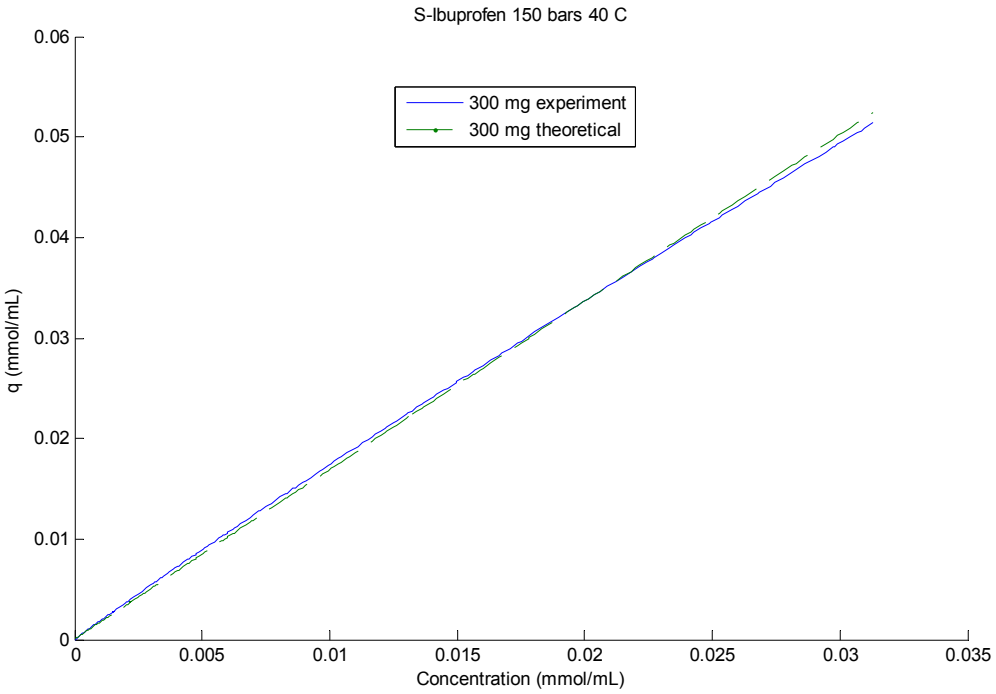


Figure 4.4 Comparison of Isotherms from ECP Method with its Langmuir Model Representation

The sum of the residual

$$SSE = \sum_{i=1}^{n} w_i * (y_i - \acute{y_i})^2 \tag{4.7}$$

which is the difference between the experimental values and the fitted values, for the regression using the method of linear least squares in Matlab gave the goodness of fit of 0.99 and with a 95 % confidence interval for the “a” value of

(1.674, 1.68) and “b” value of 0.4 for the S-Ibuprofen isotherm to the Langmuir model, as shown in Figure 4.5.

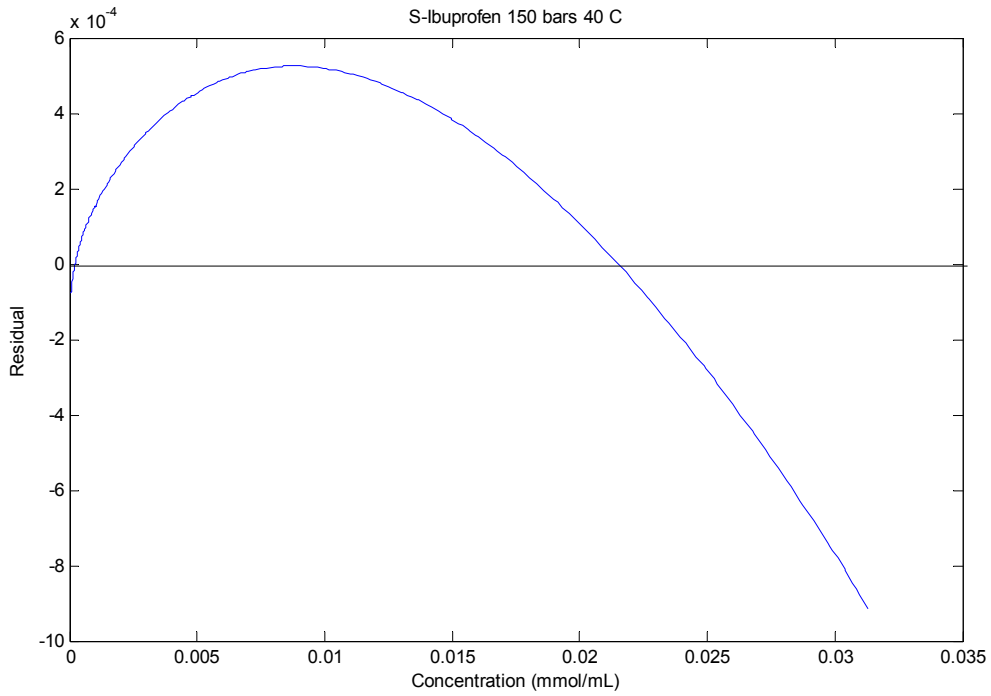


Figure 4.5 Residual Langmuir Model in Comparison to Experiment for S-Ibuprofen 150 Bars 40 ℃ 300 mg

4.1.2 Evaluation of Data for Adsorption Isotherms Using Absorbance Versus Concentration Data for Concentration Calibrations

When using this method, the retention volume is evaluated the same way as described in section 4.1.1. For converting absorbance into concentration, calibration experiments were carried out (Guiochon, et al., 2006). In those experiments, carrier fluid was switched from pure carbon dioxide-Ethanol mixture (corresponding to zero absorbance) to a carbon dioxide-Ethanol-ibuprofen mixture (Martin, 2004). The absorbance for this known concentration is recorded. The calibration experiment is carried out at different concentrations (Gonenc, 1985). The absorbance values obtained at different concentrations are

shown in Figure 4.6 where concentrations used were 10, 40, 50, and 75 mg of Ibuprofen dissolved in 10 ml of Ethanol. For the first front in Figure 4.7 (10 mg ibuprofen in 10 ml of Ethanol), the concentration of the ibuprofen in the mobile phase is calculated as follows (Gonenc, 1985):

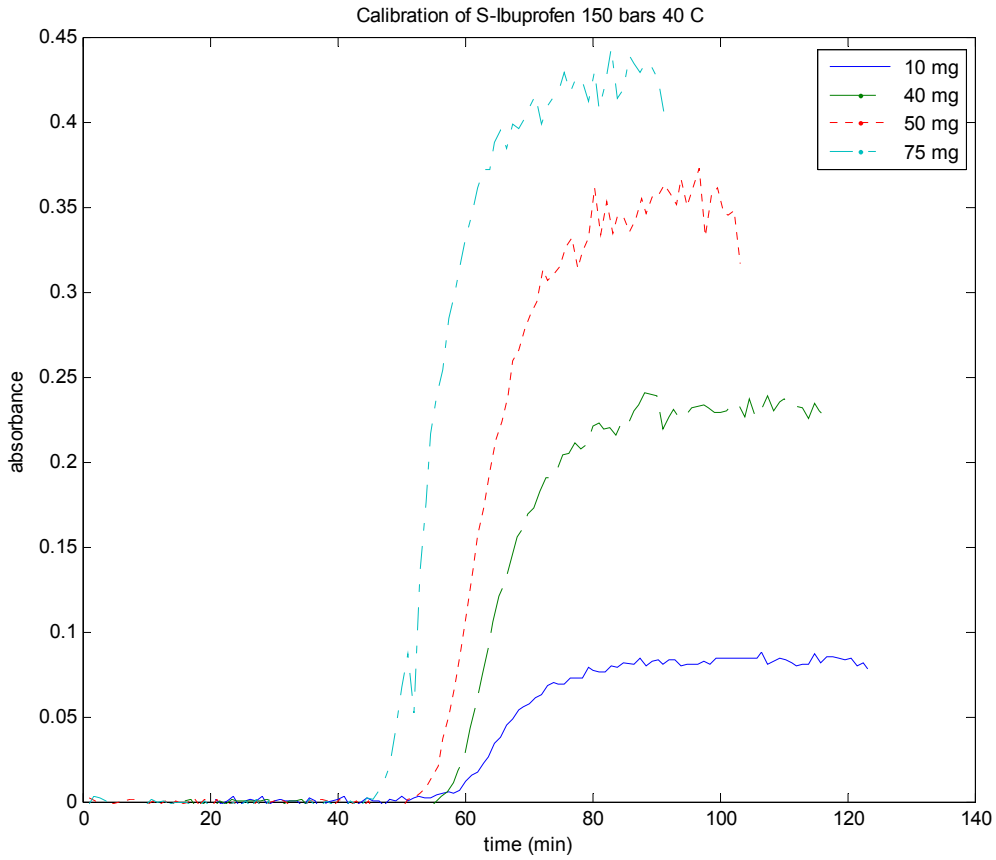


Figure 4.6 Calibration Chromatogram

The flow rate of the mobile phase at room temperature out of the pump and at a pressure of 150 bars is $3\ \frac{ml}{min}$. The mobile phase consists of $2.85\frac{ml}{min}$ carbon dioxide and $0.15\frac{ml}{min}$ of Ethanol-Ibuprofen mixture. Ibuprofen concentration is

$$c = \frac{0.15\ ml}{min}\frac{EtOH\ min}{3\ ml\ mix} * \frac{10\ mg\ IP}{1\ ml\ EtOH} * \frac{1\ mmol}{206\ mg} \tag{4.8}$$

$$c = \frac{0.15 * (10)}{3 * 206}\frac{mmol}{ml} = .002\ \frac{mmol}{ml\ mix}$$

Conversion of absorbance to concentration for ibuprofen at 150 bars, 40℃, and different concentrations are summarized in Table 4.1.

Table 4.1 Calibration vs. Absorbance

Sample Size (mg)	Absorbance	Absorbance shift to zero	Concentration ($\frac{mmol}{ml}$)
0	0	0	0
10	0.132	0.082	0.0024
40	0.280	0.23	0.0097
50	0.410	0.36	0.0121
75	0.483	0.433	0.0182

Absorbance versus concentration values are then plotted and fitted into a polynomial equation for Figure 4.7 using Matlab.

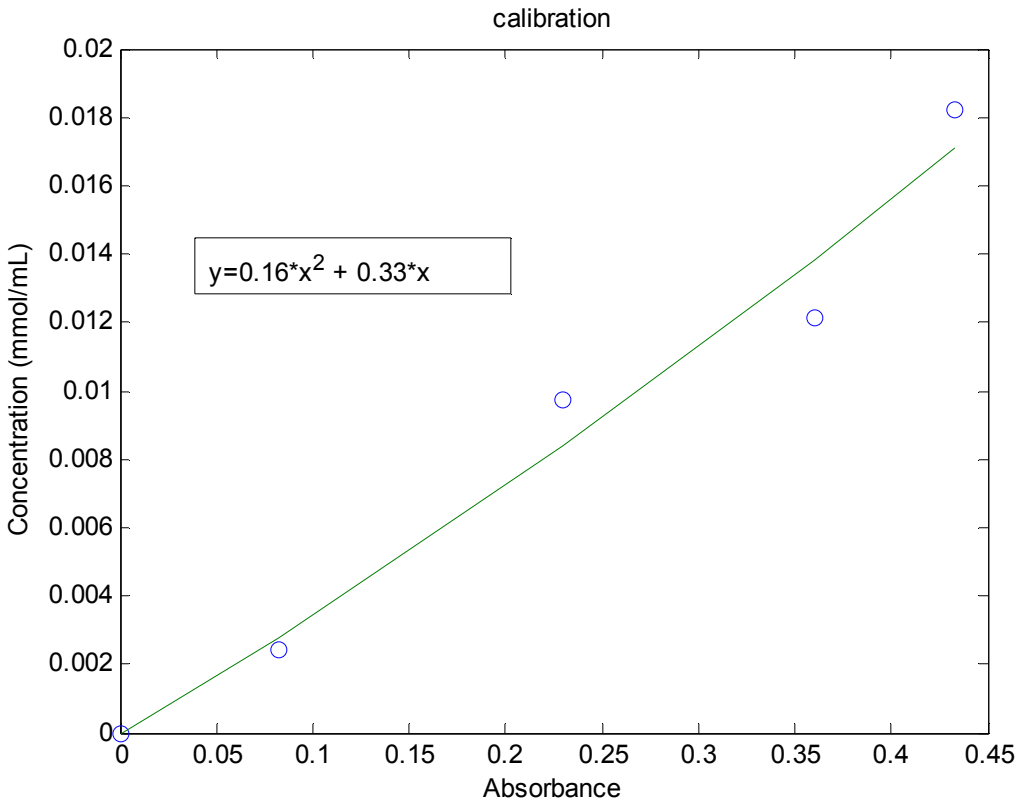


Figure 4.7 Calibration Data Point

4.1.3 Evaluation of Data for Adsorption Isotherms Using Peak Maxima (ECP) Method

Adsorption isotherms can be determined from the retention time (corresponding to peak maxima) of chromatographic peaks, that are obtained by injecting different amounts of solute into the mobile phase (Martin, 2004). Using the material balance equation on the column and the solute injected into the column, one can determine concentration of the solute on the stationary phase as a function of the amount of solute injected, the mobile phase flow rate in the column and the retention time (Guiochon, et al., 2006).

The number of moles of ibuprofen in a $10\ \mu l$ column injection is calculated as follows for a sample containing 300 mg of ibuprofen dissolved in 1 ml of Ethanol (Martin, 2006).

$$N = \frac{300\ mg\ ibuprofen}{1\ ml\ ethanol} * 10\ \mu l\ injected\ \frac{1\ ml}{1000\ \mu l} * \frac{mmol}{206\ mg} \tag{4.9}$$

$$N = 0.0146\ mmol\ ibuprofen\ injected$$

The phase ratio of the column is (Guiochon, et al., 2006)

$$\varphi = \frac{(1-\varepsilon)}{\varepsilon} = \frac{1-0.6}{0.6} = 0.67 \tag{4.10}$$

where ε is the porosity of the silica gel. The porosity is 0.6 and it was determined experimentally by Whelan (Whelan, 2003).

The flow rate of the CO_2 and Ethanol was calculated using the Rackett Equation for liquid and the Peng-Robinson Equation of State is used to determine the compressibility factor at the experimental conditions (Saleh, 2002). The flow rate needs to be converted to linear velocity in [mm/s]. The weight of the silica is 3.5 g (Regis, 2000).

The constant “a” is calculated by the following formula (Guiochon, et al., 2006):

$$a = \frac{t_r - t_o}{F * t_o} \tag{4.11}$$

where t_r is the retention time, t_o is the dead time, and F is the linear velocity.

The next step is to calculate the loading factor (L_f) with the following formula (Guiochon, et al., 2006)

$$L_f = (1 - \sqrt{\frac{t_f - t_o - t_p}{t_r - t_o}})^2 \tag{4.12}$$

The next constant that needs to be calculated is “b”, using the following formula (Guiochon, et al., 2006):

$$b = \frac{L_f * F * (t_r - t_o)}{N} \tag{4.13}$$

Determine the concentration of the analytes on the stationary phase with the following equation (Gonenc, 1985):

$$q_M = \sqrt{\frac{N * a}{F * t_0 * \varphi * b}} = \sqrt{\frac{N * a * \varepsilon}{F * t_0 * b * (1 - \varepsilon)}} \tag{4.14}$$

Solute samples are prepared by dissolving 300 mg, 150 mg, 100 mg, and 75 mg samples in 1 ml of Ethanol. Chromatograms obtained by injecting solute samples of different amounts are shown in Figure 4.8.

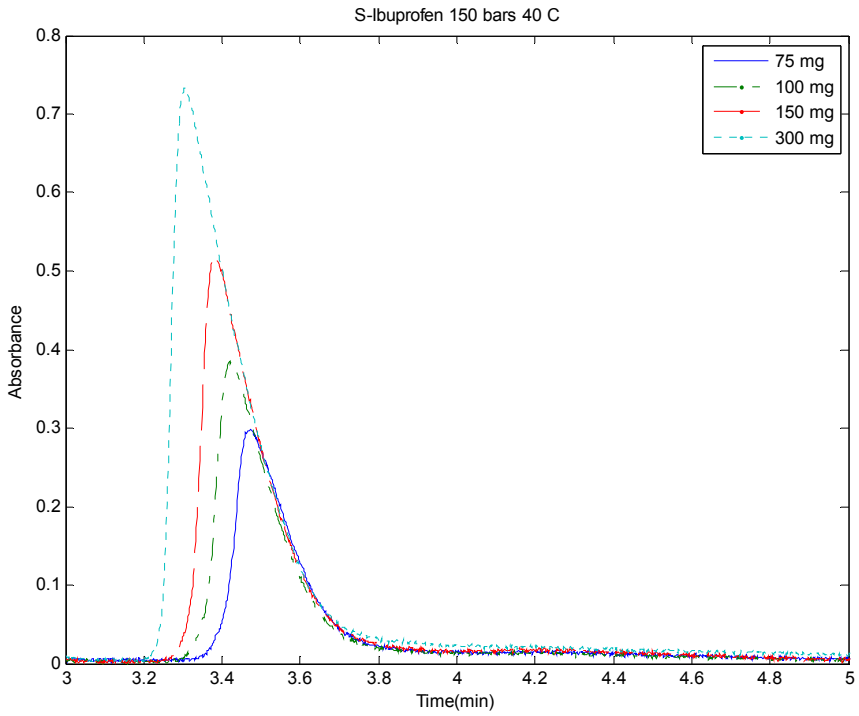


Figure 4.8 S-Ibuprofen Chromatogram at 150 Bars and 40 ℃

Concentration of the solute in the mobile phase is calculated using the Langmuir equation (Guiochon, et al., 2006)

$$q = \frac{a * c}{1 + b * c} \tag{4.15}$$

$$c = \frac{q}{a - bq} \tag{4.16}$$

The procedure described above was performed using the Langmuir constants obtained from equation (4.11and 4.13). Also, the isotherm constants “a” and “b” are regressed from the following equations (Guiochon, et al., 2006)

$$t_r = t_0\left(1 + \varphi\frac{\left(a - \frac{bac}{1+bc}\right)^2}{a}\right) \tag{4.17}$$

In the above equation, t_r is the retention time at the peak maxima.

4.2 Determination of Isotherm Constants Through Regression of Chromatograph Models

Under equilibrium conditions, the concentration profile for the solute is given by (Guiochon, et al., 2006):

$$\frac{\partial c}{\partial t} + \varphi\frac{\partial q}{\partial t} + u * \frac{\partial c}{\partial z} = 0 \tag{4.18}$$

where

$$q = \frac{a * c}{1 + b * c}$$

Equation 4.18 then become (Guiochon, et al., 2006)

$$\frac{\partial c}{\partial t} + \varphi\frac{\partial\left(\frac{a*c}{1+b*c}\right)}{\partial t} + u_0 * \frac{\partial c}{\partial z} = 0 \tag{4.19}$$

The chromatogram consists of absorbance versus time data where absorbance can be converted to concentration (Section 3.3). A numerical method is needed to solve the chromatogram model using Crank-Nicholson.

4.3 Determination of Isotherms from Mixture Data

The determination of isotherm from mixture data is the same procedure for the single data.

4.3.1 Evaluation of Data for Adsorption Isotherms for Mixture Using Peak Area Method

The adsorption isotherms were obtained from analysis of the mixture elution chromatogram. Specific retention volume,$V_{R_1} and\ V_{R_2}$, and concentration of the adsorbate in the mobile phase of each solute, $C_1\ and\ C_2$, are calculated using Equation (2.26) and (2.27), respectively. As discussed in Chapter 2, the adsorption isotherm was obtained by integrating the $V_R\ vs. c$ curve. A sample calculation is given below for the isotherms of the Ibuprofen mixture analytes, which consist of R- and S-Ibuprofen, with adsorptions measured at experimental conditions of 150 bars and $40\ ℃$. The sample to be injected was prepared by dissolving 300 mg of Ibuprofen mixture in 1 ml of Ethanol. 10 μl of these samples were injected into the column. The chromatograph is shown in Figure 4.9.

Retention volumes and corresponding concentrations of solute in the mobile phase were calculated for each peak on the abscissa of the chromatogram, which is equal or greater than the retention time corresponding to the maximum of the sample peak.

Retention volume of each concentration is described by (Martin, 2004)

$$V_{R_1} = \frac{[F_{c_1} * (t_{c_1} - t_{0_1})]}{w} \tag{4.20}$$

$$V_{R_2} = \frac{[F_{c_2} * (t_{c_2} - t_{0_2})]}{w} \tag{4.21}$$

For the given conditions of temperature and pressure, t_0, the hold-up time was measured as being equal to 1.28 min. The concentration, $C_1\ and\ C_2$, are for the S and R-Ibuprofen, respectively. The weight of the adsorbent, which is silica,

(column material) was 3.5 g (Regis, 2000). The flow rate of the mobile phase (2.85$\frac{ml}{min}$ of CO_2 and 0.15 $\frac{ml}{min}$ of Ethanol mixture at 4 ℃ and 100 bar) at 150 bars and 40 ℃ is determined as described in Chapter 2 (Martin, 2004). The compressibility factor was calculated using the compressibility program (Patel, 2008).

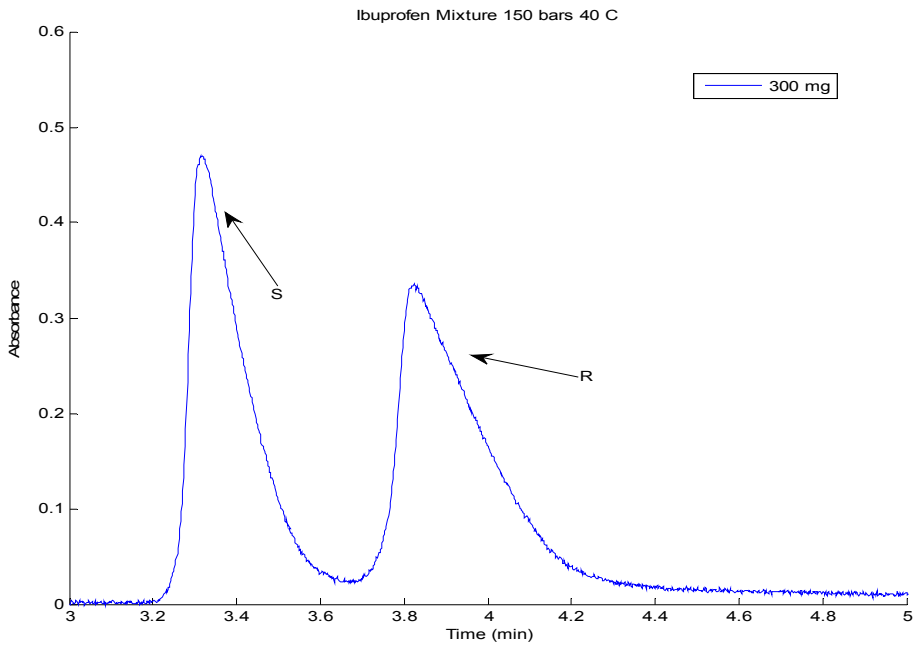


Figure 4.9 Chromatogram of Ibuprofen Mixture at 150 Bars, 40℃, and 300 mg

Concentrations are given by (Martin, 2004):

$$c_s = \frac{absorbance_1 * N}{A * F_C} \tag{4.22}$$

$$c_R = \frac{absorbance_2 * N}{A * F_C} \tag{4.23}$$

The equations are for concentration of the S and R Ibuprofen, respectively. The first absorbance in the chromatogram is the S-Ibuprofen and the second is the R-Ibuprofen.

For the given concentration of the 10 μl sample injected, the number of moles injected is (Martin, 2004)

$$N = \frac{300\ mg\ Ibuprofen}{1\ ml\ ethanol} * 10\ \mu l\ injected * \frac{1\ ml}{1000\ \mu l} * \frac{1\ mmol}{206\ mg} \qquad (4.24)$$

$$N = 0.0146\ \ Ibuprofen\ injected$$

A is the area under the chromatogram after the peak maximum. The area for the sample peak is equal to 0.12 Au*min for each point on the graph and calculated concentration values are given in Appendix A.

Retention volume vs. the concentration of the sample in the mobile phase is given in Figure 4.10.

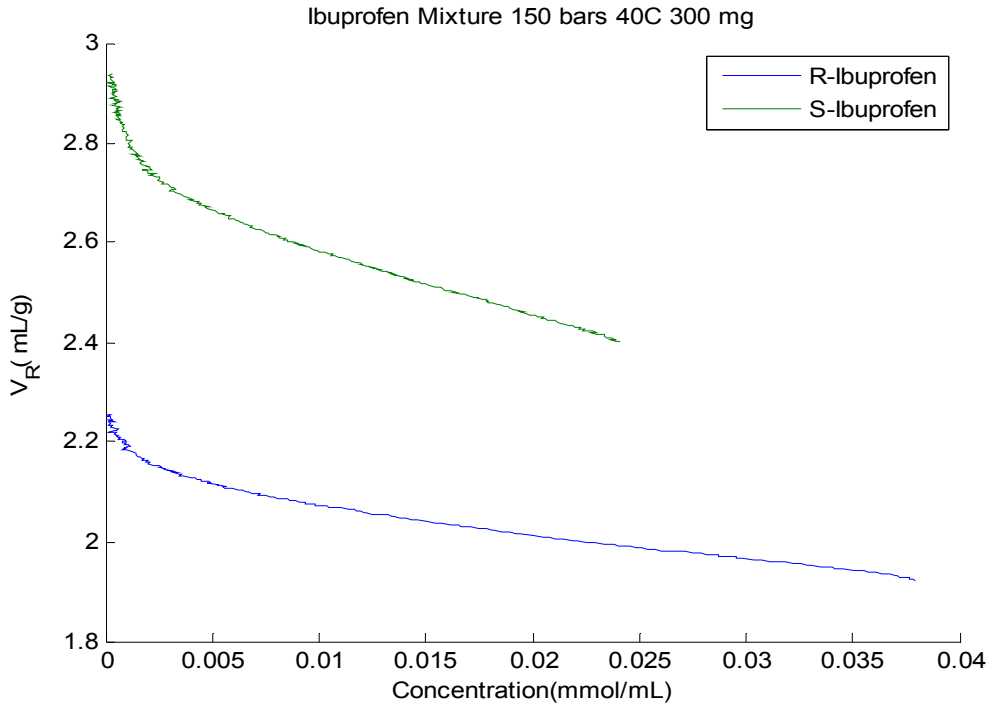


Figure 4.10 Retention Volumes of Ibuprofen Mixture vs. Concentration

4.3.2 Competitive Langmuir Isotherms

The Langmuir competitive isotherm is used for calculating the isotherms for the mixture components for the column. The equations for the competitive isotherms where the number of component is two are as follows (Guiochon, et al., 2006):

$$q_1 = \frac{a_1 c_1}{1 + b_1 c_1 + b_2 c_2} \tag{4.25}$$

$$q_2 = \frac{a_2 c_2}{1 + b_1 c_1 + b_2 c_2} \tag{4.26}$$

In equation 4.25 and 4.26, Langmuir the constant are,a_1 ,b_1,a_2,b_2.If there are no competing components for the active site, the equation will reduce to Langmuir single isotherm equation (Guiochon, et al., 2006).

In Chapter 5, we will discuss the results of the elution chromatogram at 150 bars, with temperatures of 40℃ and 45℃, and concentration of S-Ibuprofen from 75 mg to 300 mg. Also, three different models will be use to predict the isotherm.

Chapter 5

Results

In this chapter, result of Ibuprofen experiments with single and multiple solutes are discussed. The effect of temperature, pressure, and concentration on isotherms is also discussed.

5.1 Single Solute Isotherms for Ibuprofen

The Figure 5.1 below shows the experimental chromatograph of absorbance vs. time. The detector has a noise level of 0.05 nm. The graph is only taken from the maximum retention time to when the absorbance has reached the minimum point. The graph is the concentration of S-Ibuprofen at 300 mg with 150 bars and 40℃. It took about 3 minutes for the solute to desorb from the column and exit the column in about one minute. The absorbance for the S-Ibuprofen is at 0.77. The absorbance decreases when the solute reaches a maximum and then decreases as the solute moves through the column.

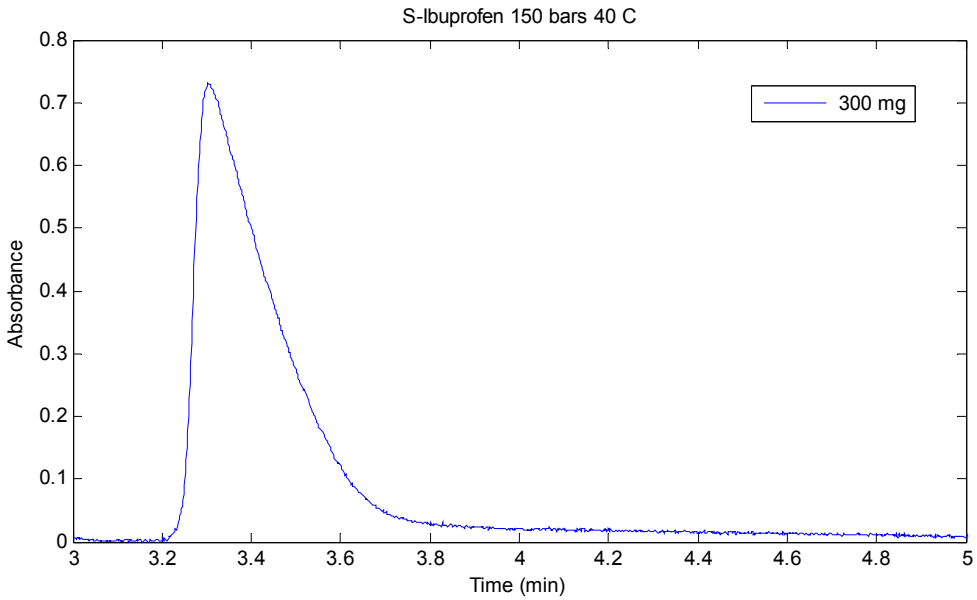


Figure 5.1 S-Ibuprofen Chromatogram at 150 Bars, 40 ℃ , 75mg to 300 mg

Figure 5.2 below shows the data point being taken from the peak of the chromatogram where the absorbance levels off. The maximum peak absorbance for 150 bars and 40℃ is at 0.72 after taking into account the noise level of 0.05 Au. The time it took for the solute to enter and exit the column was about 3 minutes. The graph also accounts for the inert time inside the column which is 1.28 minute.

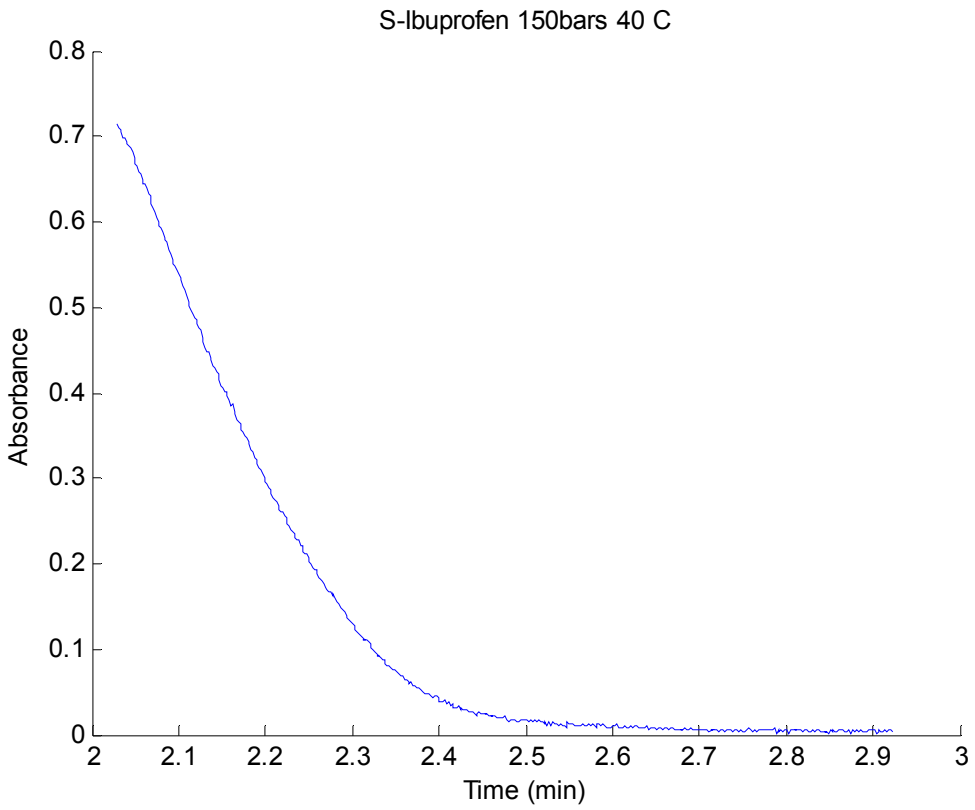


Figure 5.2 Absorbance vs. Time for S-Ibuprofen 150 Bars, 40 ℃, and 300 mg

Figure 5.3 below shows the volume of the retention gas vs. the concentration of the sample. The time from the above graph was converted into the volume of the gas while the absorbance was converted to the concentration in the mobile phase. The volume of the retention gas at 150 bars and 40 ℃ at 300 mg is $2.75\ \frac{mL}{mL}$. The concentration in the mobile phase is $0.0316\ \frac{mmol}{mL}$. The volume of the retention gas decreases when the concentration in the mobile phase increases.

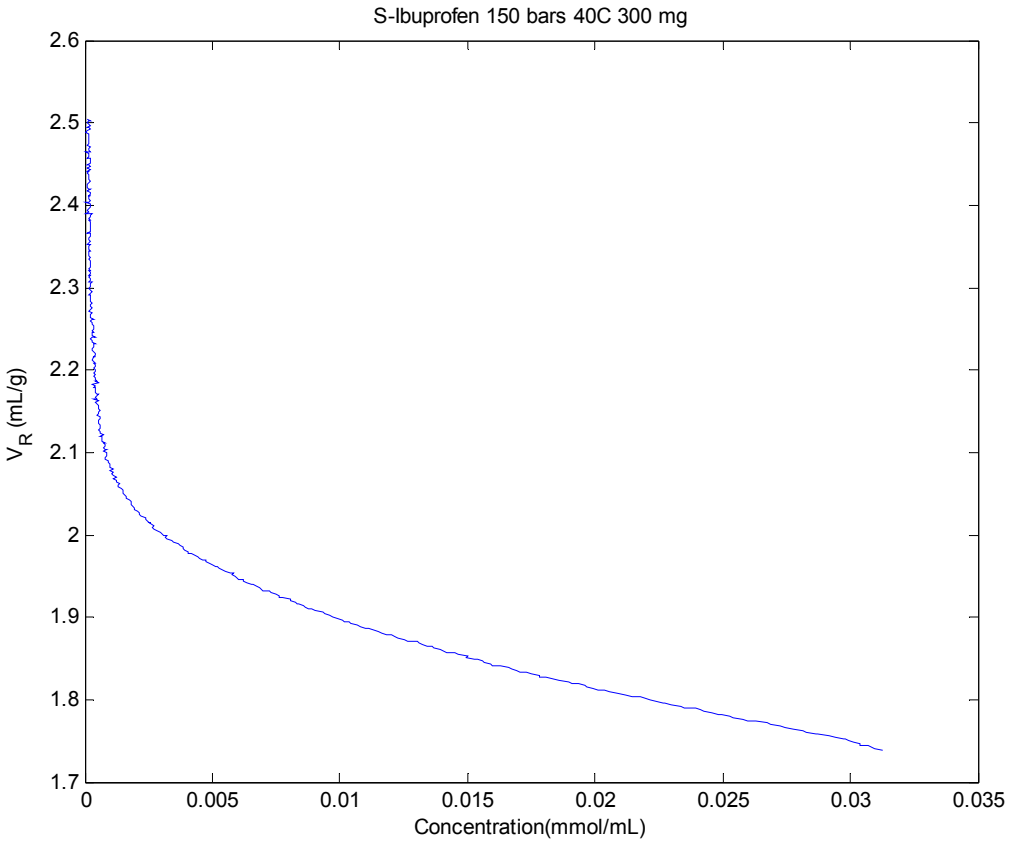


Figure 5.3 Concentration vs. V_R for S-Ibuprofen at 150 Bars, 40℃, and 300 mg

Figure 5.4 below was obtained from the integration of the above graph for S-Ibuprofen at 300 mg, 150 bars, and 40℃. The integration use is cumulative trapezoidal that took each points numerically of the retention volume of gas vs. concentration and calculates the area under the graph. Then, each of the small area is then adds up to get the isotherm. The concentration in the mobile phase is $0.0313\,\frac{mmol}{mL}$ and the concentration on the stationary phase is $0.052\,\frac{mmol}{mL}$.

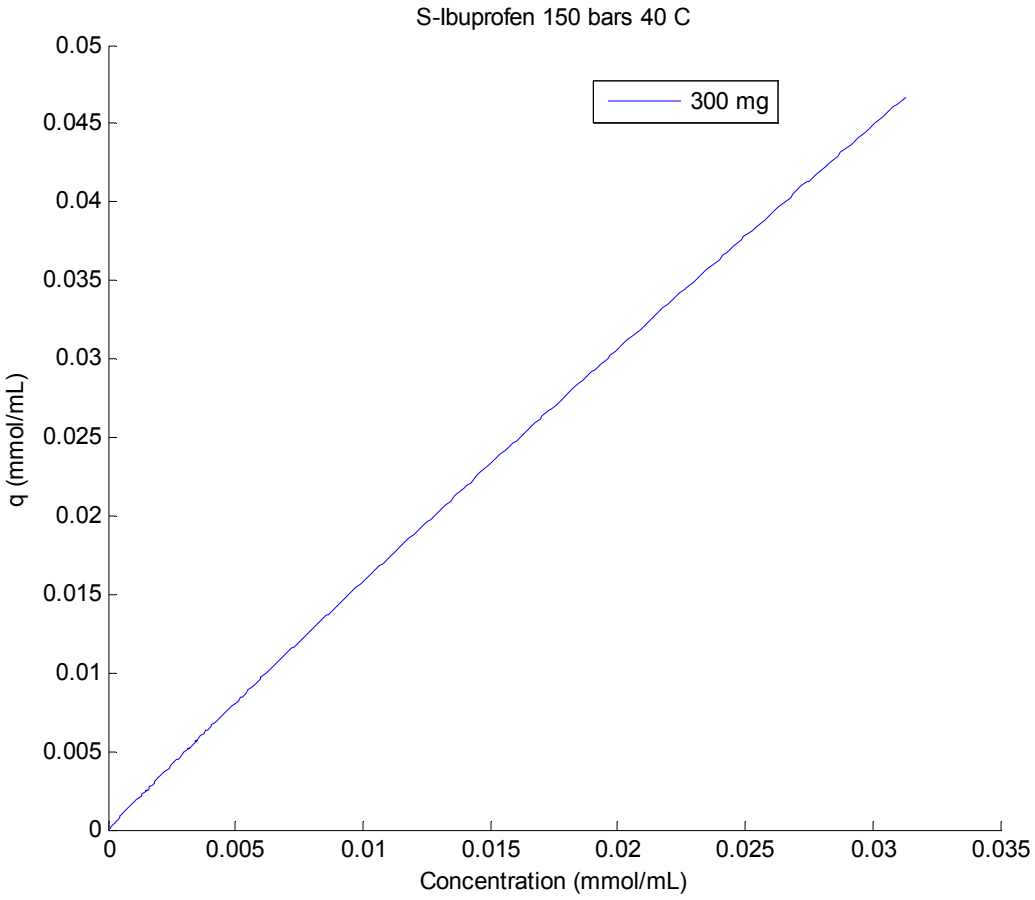


Figure 5.4 Concentration vs. q for S-Ibuprofen at 150 Bars, 40℃, and 300 mg

Figure 5.5 below shows the Langmuir, Toth, and Freundlich model prediction vs. experiment for S-ibuprofen at 150 bars and 40 ℃ at 300 mg. The Langmuir isotherm model makes many assumptions, such as that the concentration behave in a linear manner, that all the Ibuprofen molecules have the same chance to absorb onto the surface of the stationary phase, and that the column is homogenous, and ideal (Guiochon, et al., 2006). The equation takes into account the amount of coverage the silica gel can get and how much void fraction it is allowed to have. The constants “a” and “b” can be calculated through linear regression (slope and intercept).

The experiment does fit closely to the Langmuir, Toth, and Freundlich model isotherms. The concentrations of analyte in the mobile and stationary phases for the experiment are 0.0313$\frac{mmol}{mL}$ and 0.051$\frac{mmol}{mL}$, respectively. The concentrations in the mobile and stationary phases for the Langmuir model are 0.0313$\frac{mmol}{mL}$ and 0.052$\frac{mmol}{mL}$, respectively.

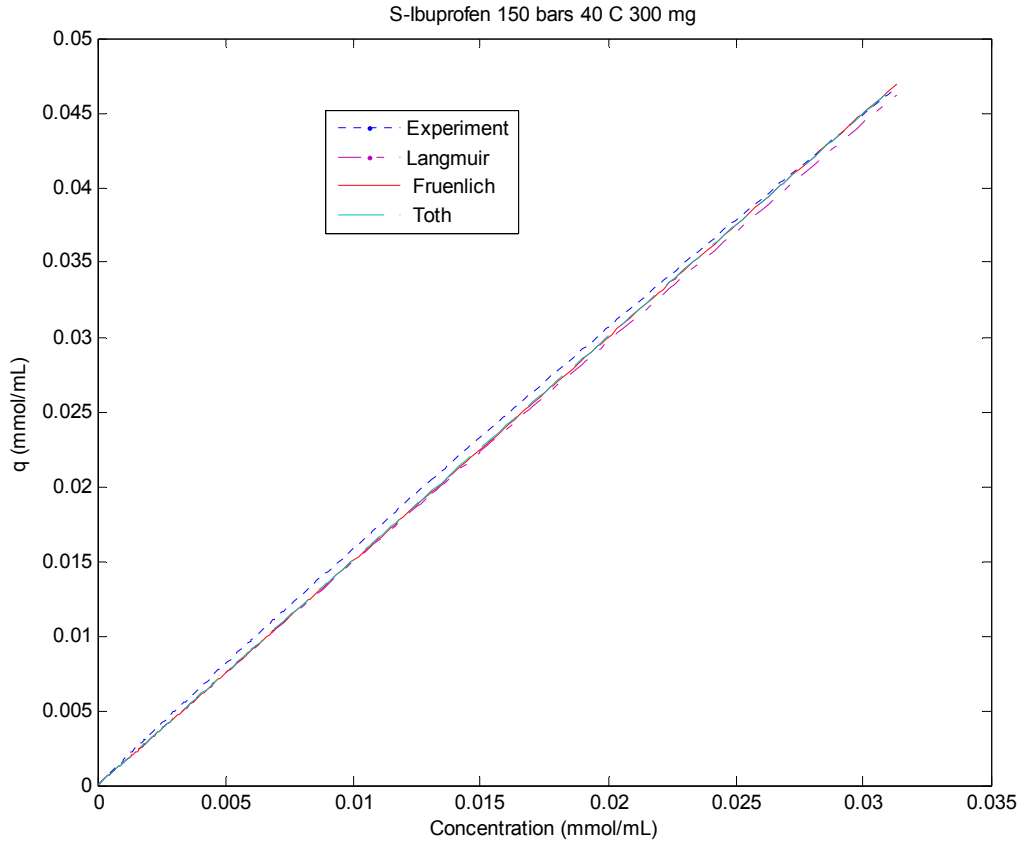


Figure 5.5 Comparison of Isotherm Models with ECP Based Isotherm for S-Ibuprofen at 150 bars, 40℃, and 300 mg

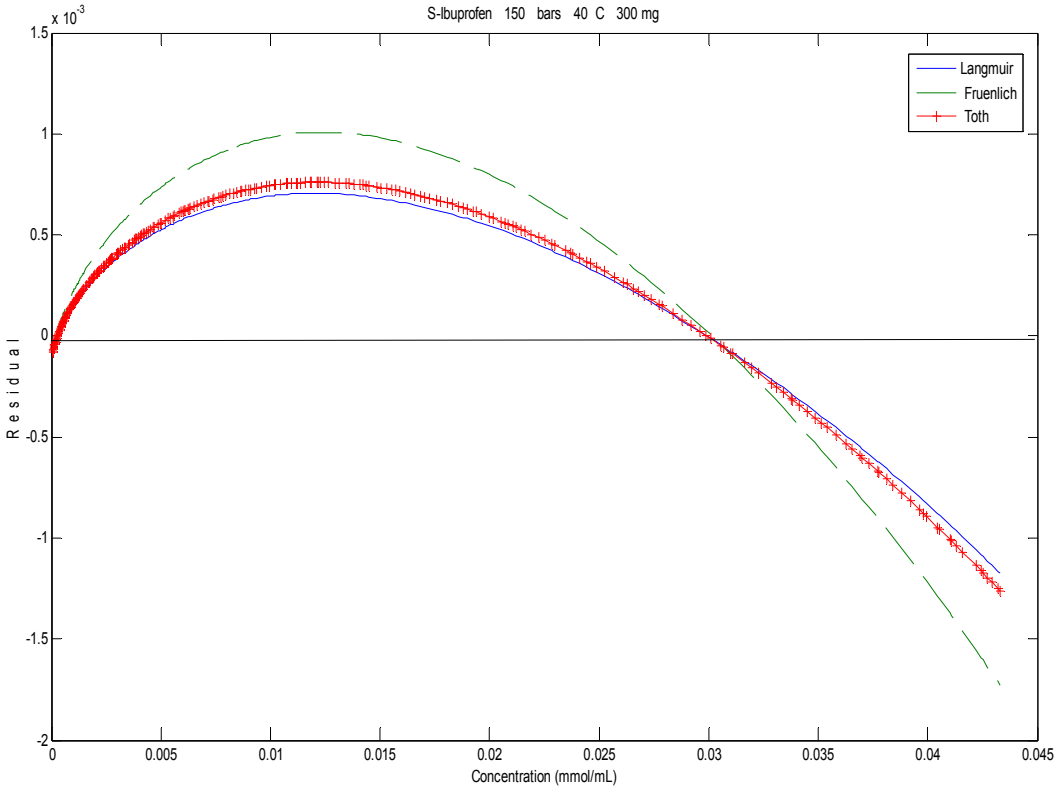


Figure 5.6 Residual of Langmuir, Freundlich, and Toth Isotherms of S-Ibuprofen at 150 Bars, 40℃ , and 300 mg

Figure 5.6 above shows the residuals using the method of least squares for S-Ibuprofen at 150 bars 40 ℃ and 300 mg between the three isotherms and experiment. We can see that the error between the experiment and theory is very small compare to the original data so predictions using either the Langmuir, Freundlich, or Toth isotherm model are in very good agreement with experiment.

Figure 5.7 below shows the S-Ibuprofen isotherm from 75 mg to 300 mg at 150 bars and 40℃. We can see from the graph that the isotherm for the given concentration come out to be linear with an r^2 of 0.98.

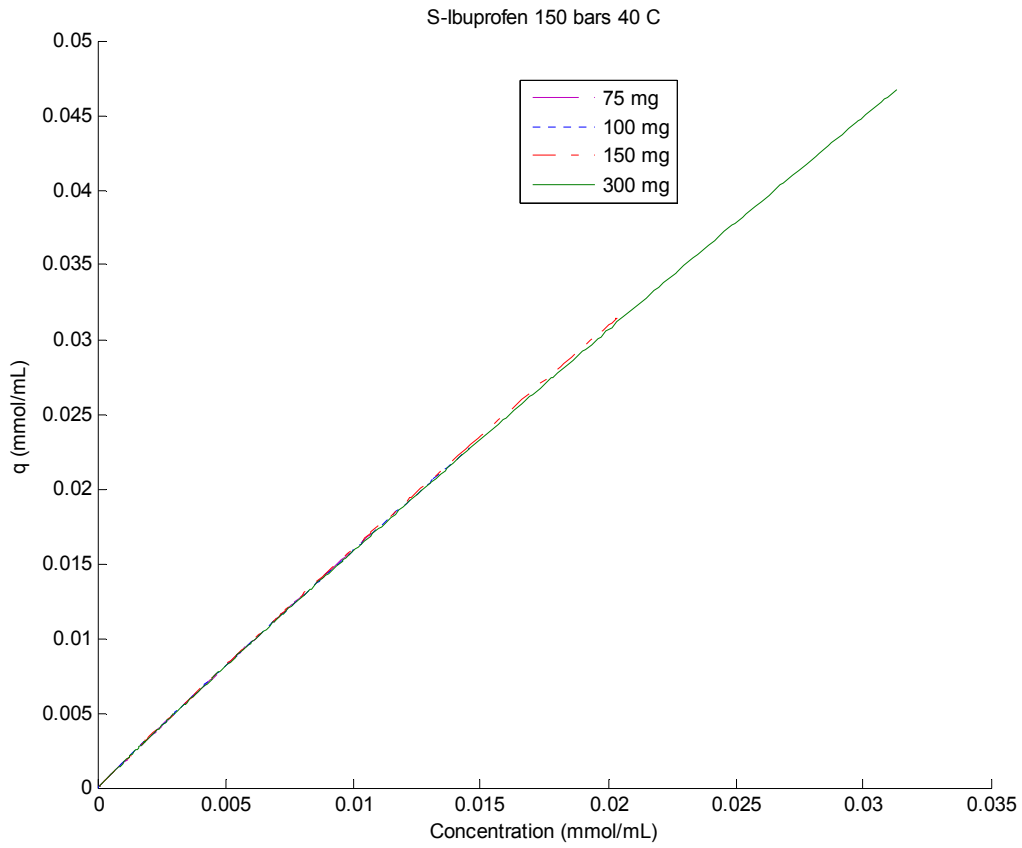


Figure 5.7 Isotherms for S-Ibuprofen at 150 Bars, 40℃, and from 75 to 300 mg

Figure 5.8 below shows the S-Ibuprofen of 150 bars 40 ℃ and concentrations from 75 mg to 300 mg for both the experiment and Langmuir isotherms together. We can see that the experiment fits closely to the model. So, by using the Langmuir theory for the smaller concentration, we can obtain the constants “a” and “b” which would equally apply to those at the larger concentrations because the relationship is assumed to be linear (Guiochon, et al., 2006).

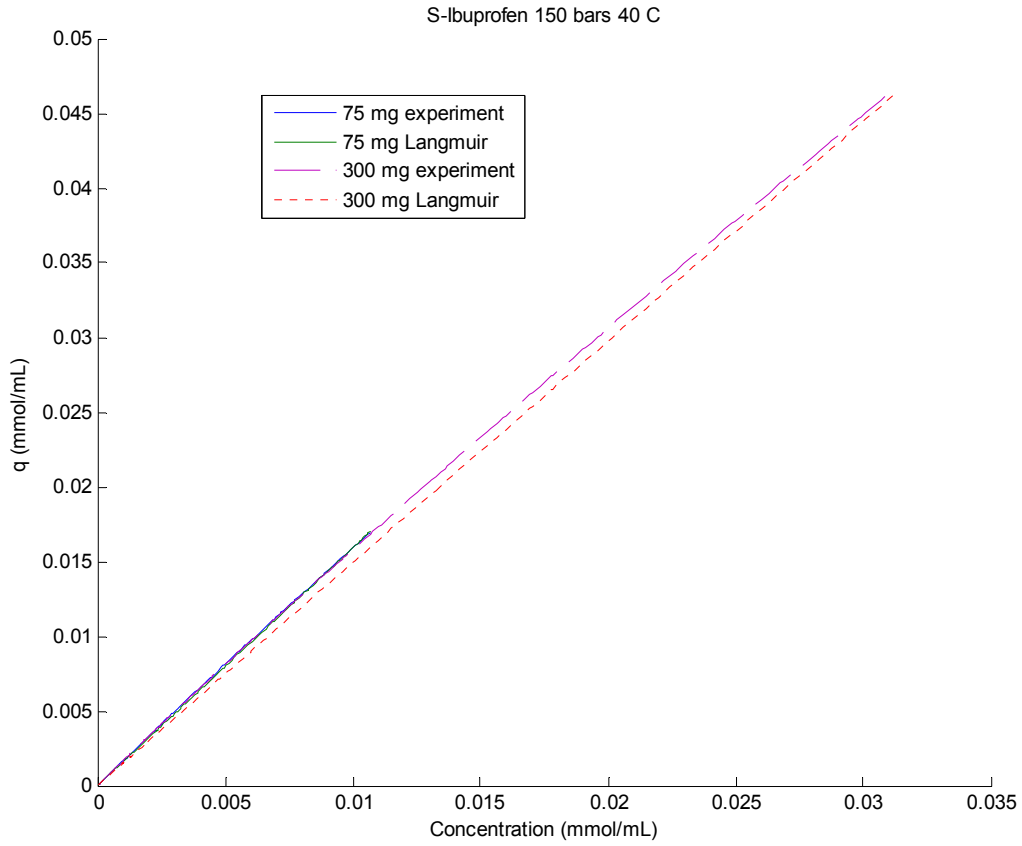


Figure 5.8 Isotherms for S-Ibuprofen and Langmuir Model Fit at 150 Bars, 40℃

5.2 Mixture Solute Isotherms for Ibuprofen

Figure 5.9 below shows the chromatogram for mixture of S-Ibuprofen and R-Ibuprofen over the concentration range from 75 mg to 300 mg at 150 bars and 40℃. The S and R-Ibuprofen is the first and second peak, respectively. The absorbance for S-Ibuprofen is at 0.55 and R-Ibuprofen is at 0.4 at 300 mg. It took about four minutes and five minutes, respectively, for the S and R-Ibuprofen to exit the column. In the mixture, the S-Ibuprofen is the less retained solute so it always elutes first because the Whelk column is a R,R type (Regis, 2000) and it's coating was selected to retained the R-Ibuprofen more.

We can use a different Whelk column such as an S, S so that the R-Ibuprofen elutes first, followed by the S-Ibuprofen (Regis, 2000).

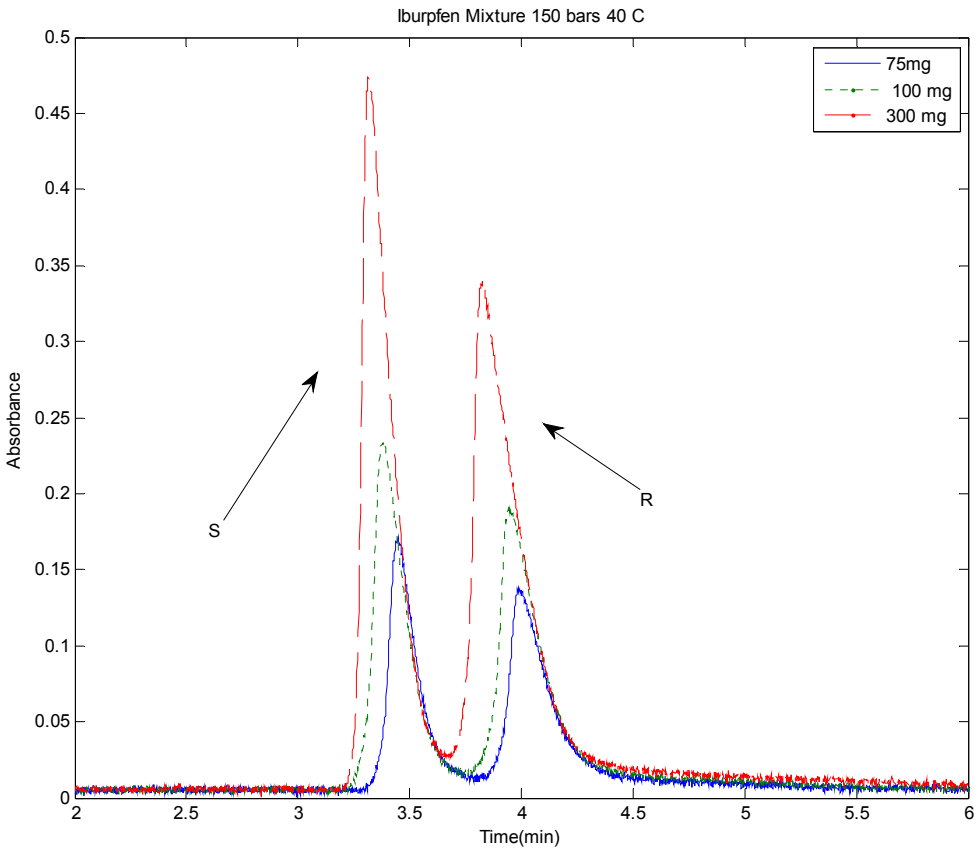


Figure 5.9 Ibuprofen Enantiomer Mixtures at 150 Bars and 40 ℃ with Concentration from 75 to 300 mg

Figure 5.10 below shows the isotherm for the Ibuprofen mixture at 150 bars and 40 ℃ over a concentration range from 75 mg to 300 mg. The concentrations for the S-Ibuprofen in the mobile and stationary phase are 0.0178 $\frac{mmol}{mL}$ and $0.036\frac{mmol}{mL}$, respectively at 300 mg. The concentrations for the R-Ibuprofen in the mobile and stationary phase are $0.0121\frac{mmol}{mL}$ and $0.031\frac{mmol}{mL}$, respectively at 300 mg.

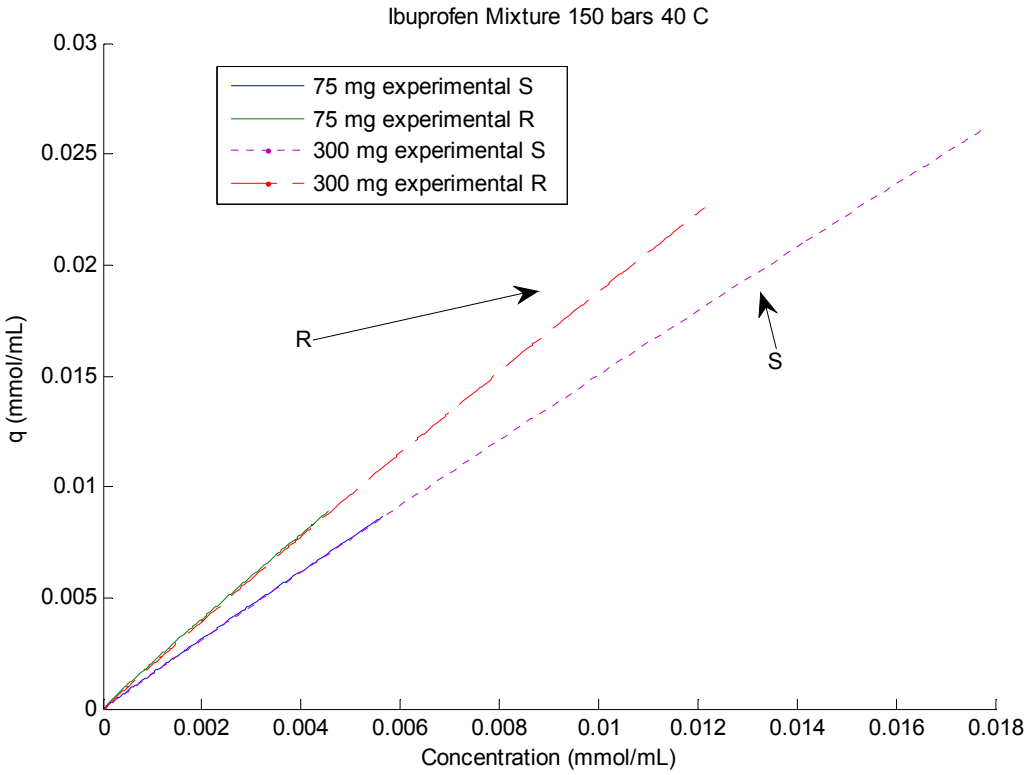


Figure 5.10 Isotherms of Ibuprofen Mixtures at 150 Bars, 40℃, with Concentration from 75 to 300 mg

Figure 5.11 below shows the isotherm for the Ibuprofen mixture at 150 bars and 40℃. The experimental and model concentration range is from 75 mg to 300 mg. We can see from the graph that the higher concentration is the S-Ibuprofen and the lower concentration is the R-Ibuprofen.

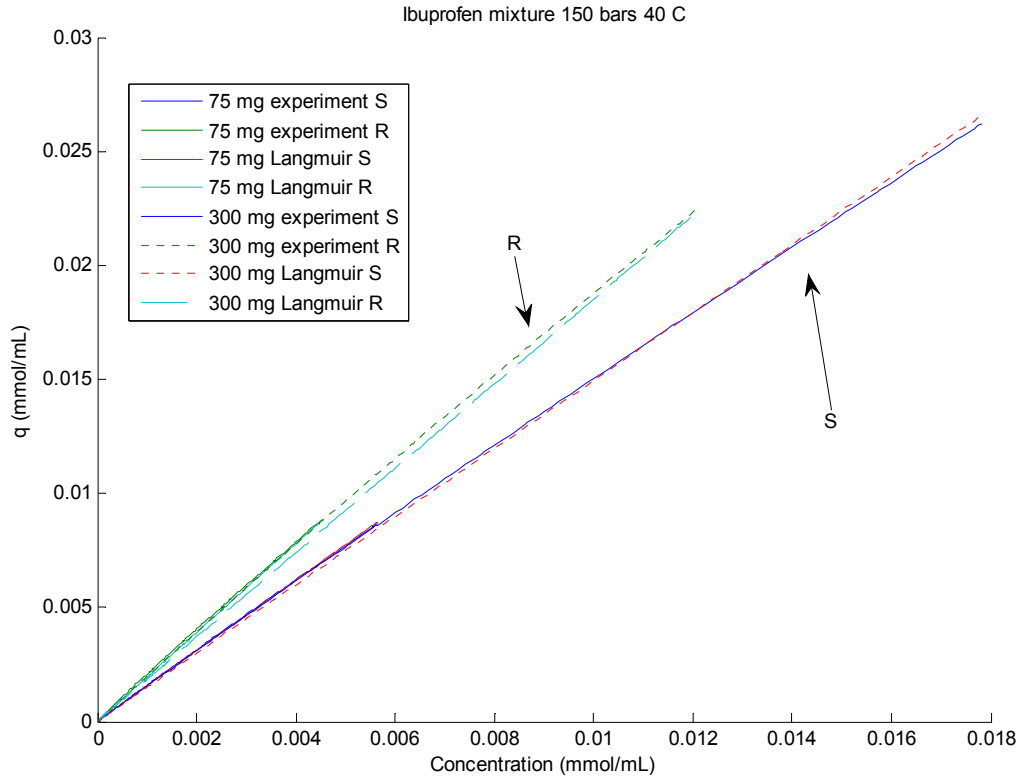


Figure 5.11 Isotherms of Ibuprofen Enantiomer Mixture and Langmuir Model Fit at 150 Bars, 40℃, with Concentration from 75 to 300 mg

Figure 5.12 below shows the single and S- and R-Ibuprofen together at 150 bars, 40℃, and 300 mg. We can see from the graph that we can just use the mixture to be able to predict the single solute accurately. The single solute seems to have a bigger isotherm than the mixture because the single solute has nothing to compete with when it is in the column. In the mixture, the S and R-Ibuprofen has to compete for the active site, so that is why the isotherm seems to be shorter (Guiochon, et al., 2006).

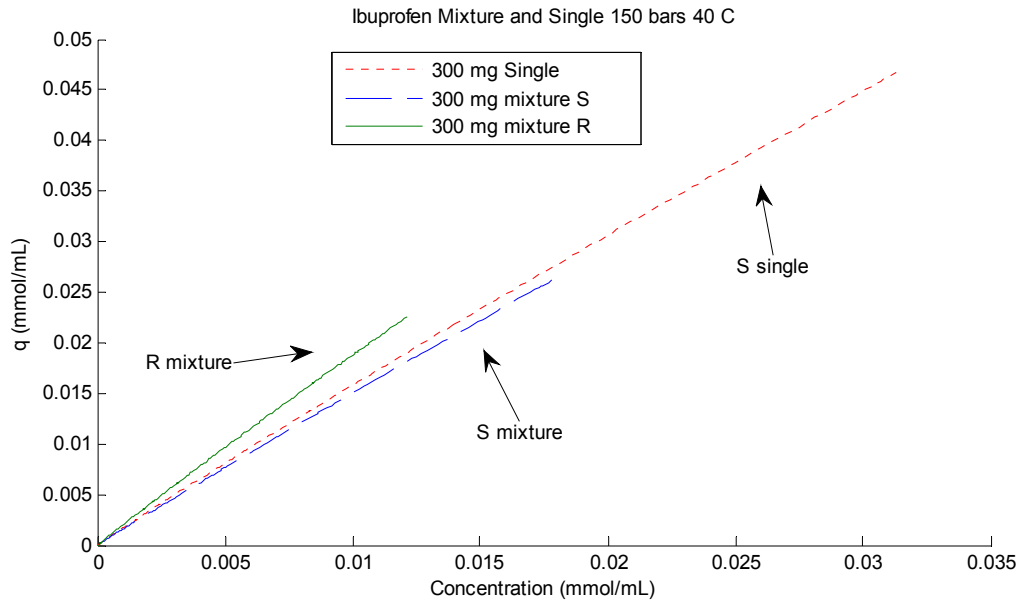


Figure 5.12 Isotherms of Ibuprofen Enantiomer Mixture and Single Adsorbate at 150 Bars, 40℃, and 300 mg

Figure 5.13 below shows the isotherm of S-Ibuprofen at 150 bars, 45℃, and 300 mg. The concentration in the mobile phase is 0.043 $\frac{mmol}{mL}$ and the concentration in the stationary phase is 0.066 $\frac{mmol}{mL}$.

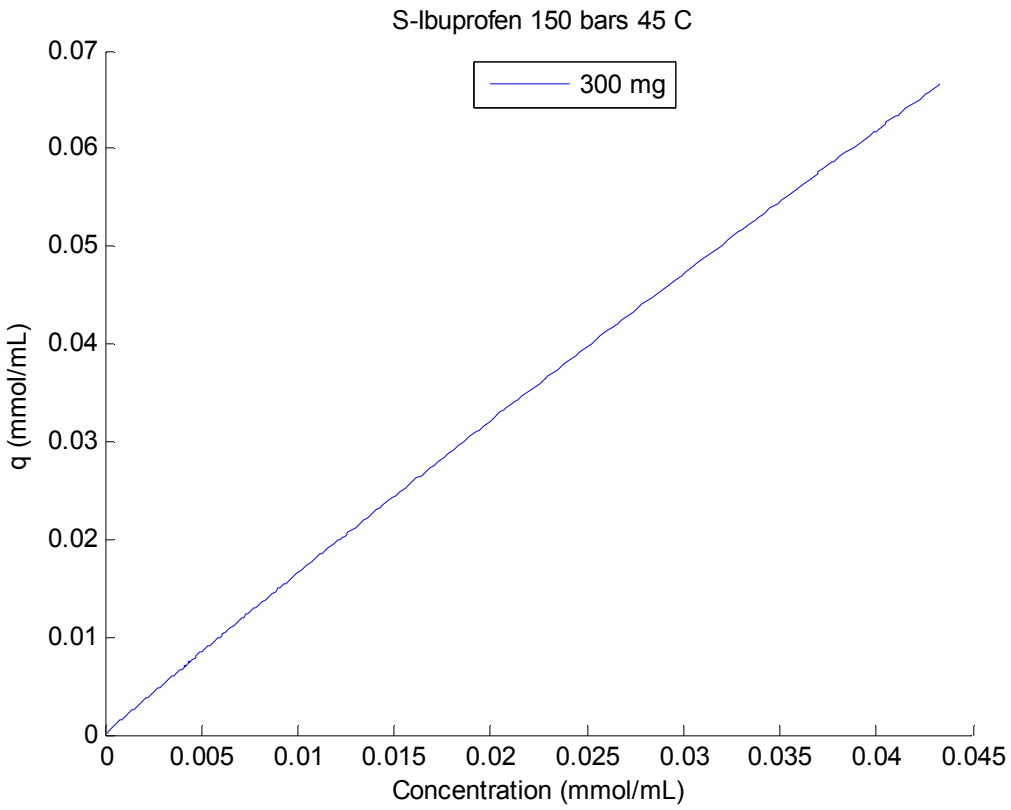


Figure 5.13 Isotherm of S-Ibuprofen at 150 Bars, 45℃, and 300 mg

Figure 5.14 below shows the experimental and Langmuir isotherms for S-Ibuprofen at 150 bars, 45℃, and 300 mg.

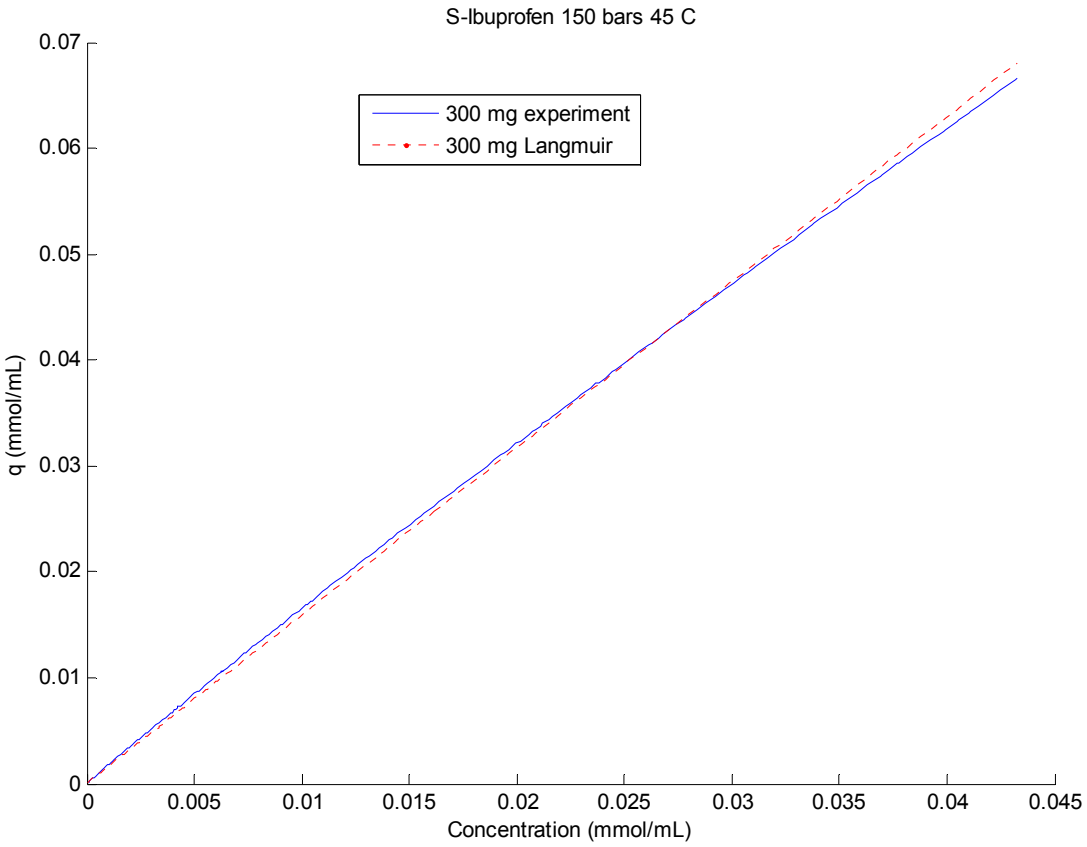


Figure 5.14 Isotherms of S-Ibuprofen and Langmuir Model Fit at 150 Bars, 45 ℃, and 300 mg

Figure 5.15 below shows the S-Ibuprofen at 150 bars, 45 ℃, and 300 mg of the experiment, compared to the Langmuir, Toth, and Freundlich isotherms. We see that all three models give almost the same isotherm.

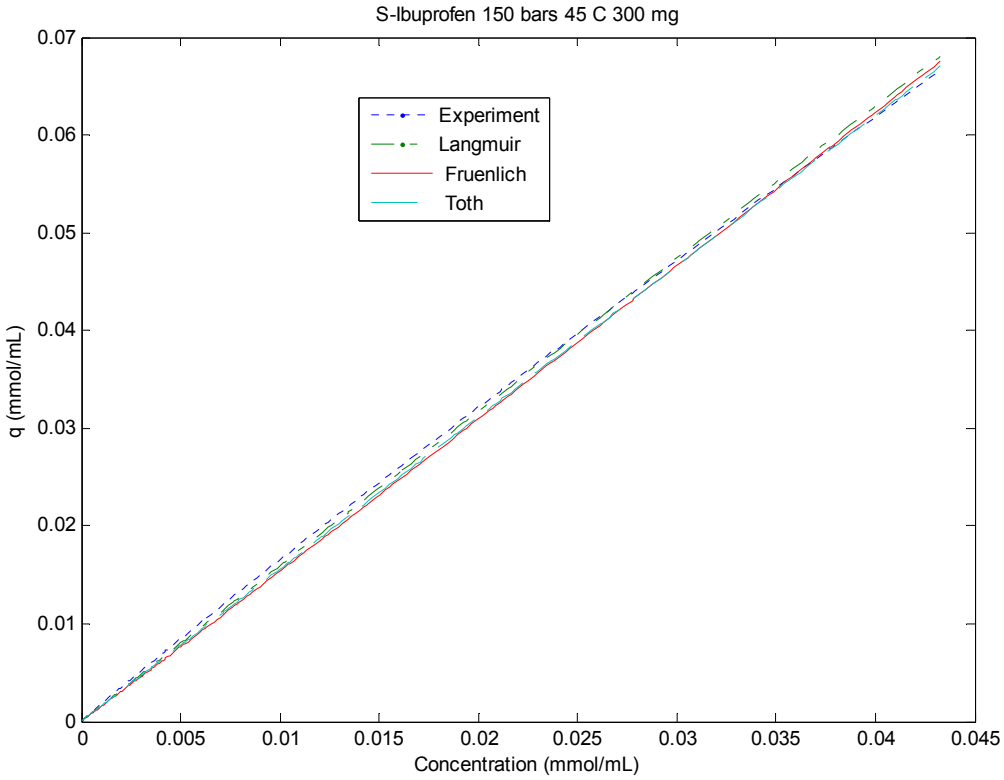


Figure 5.15 Comparison of Different Isotherm Models for S-Ibuprofen at 150 Bars, 45℃, and 300 mg

Figure 5.16 below shows the residual of the Langmuir, Freundlich, and Toth isotherms data of S-Ibuprofen at 150 bars, 45 ℃, and 300 mg. The residual between the experiment and the Langmuir isotherm is very small.

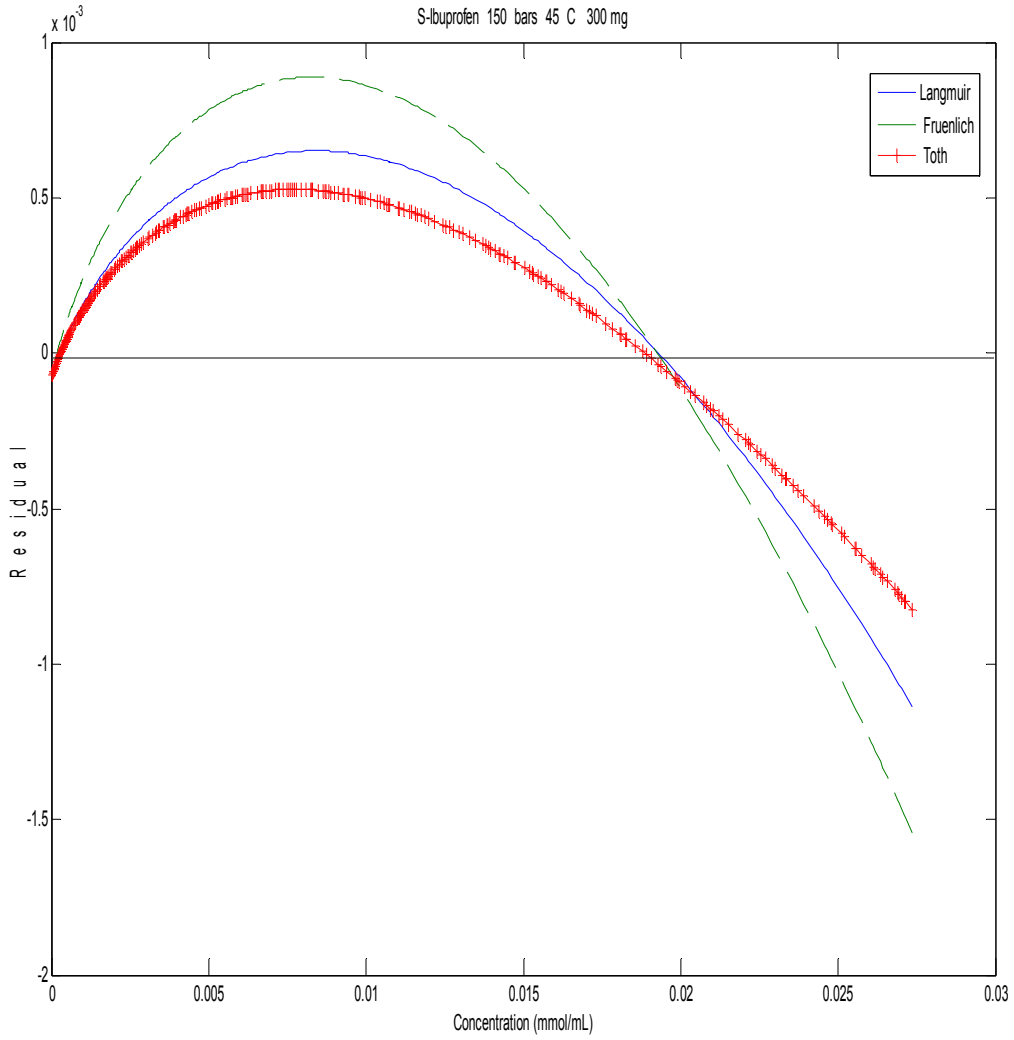


Figure 5.16 Residual of Langmuir, Freundlich, and Toth Isotherms of S-Ibuprofen at 150 Bars, 40℃ , and 300 mg

Figure 5.17 below shows the isotherm for S-Ibuprofen at 150 bars, 300 mg, obtained for experimental temperature of 40 ℃ and 45 ℃. As the temperature increase, the concentration of the S-Ibuprofen in the stationary phase appears to increases.

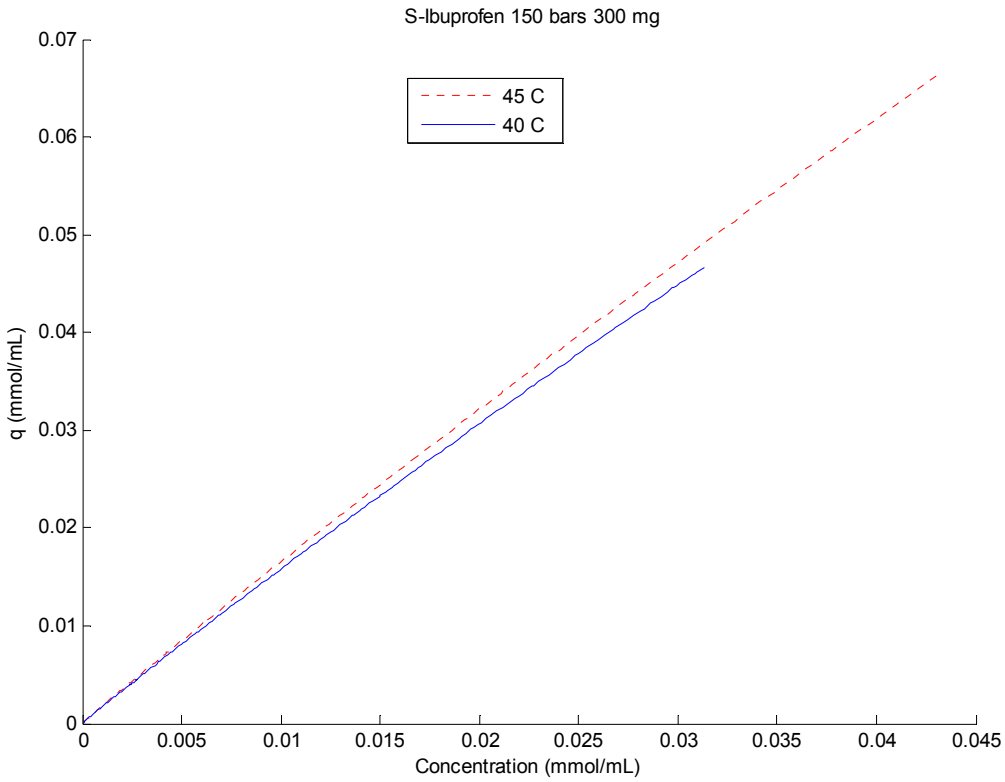


Figure 5.17 Isotherms of S-Ibuprofen 150 Bars, 300 mg as a Function of Temperature

In Figure 5.18 below we compare the experimental results with the diffusion equation model in COMSOL (COMSOL, 2010) to see if we are able to simulate the chromatogram by using finite difference element. The assumption is that the axial dispersion in the column is negligible. The model has 10700 degrees of freedom and 2673 number of mesh points.

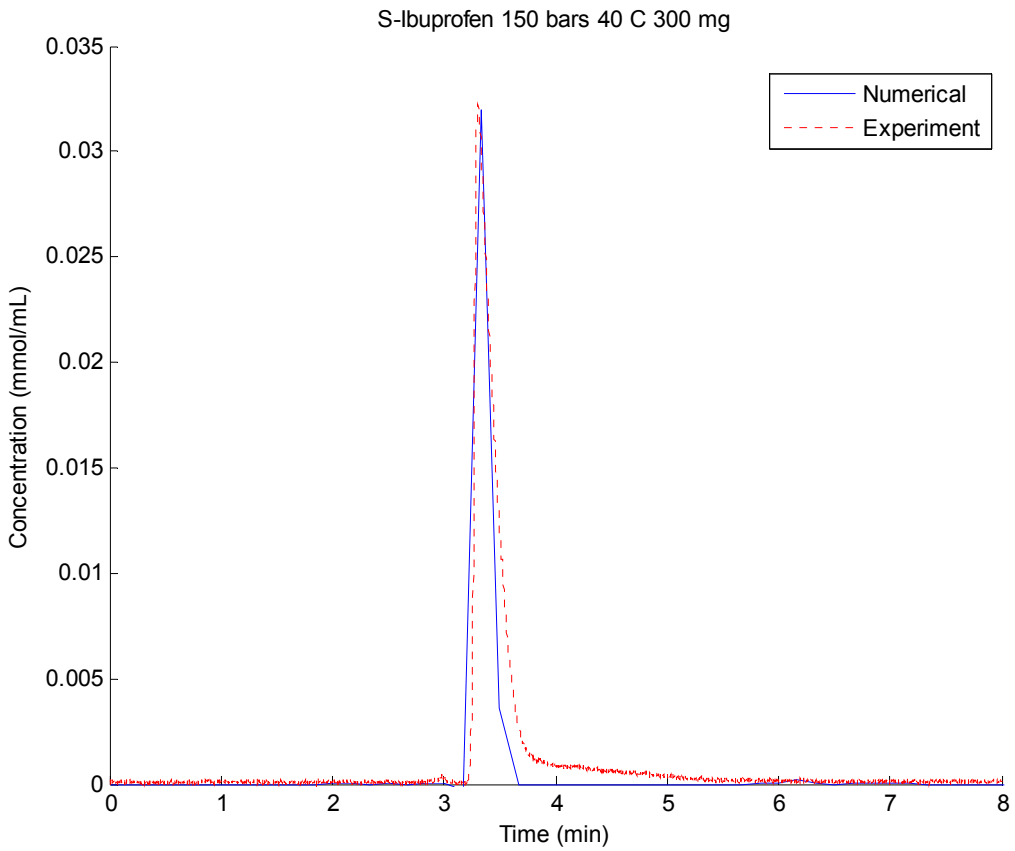


Figure 5.18 Simulated Chromatogram of S-Ibuprofen at 150 Bars,40℃, and 300 mg

Figure 5.19 compares the result of the Ibuprofen mixture with the simulated result from COMSOL (COMSOL, 2010) using the convection and diffusion model.

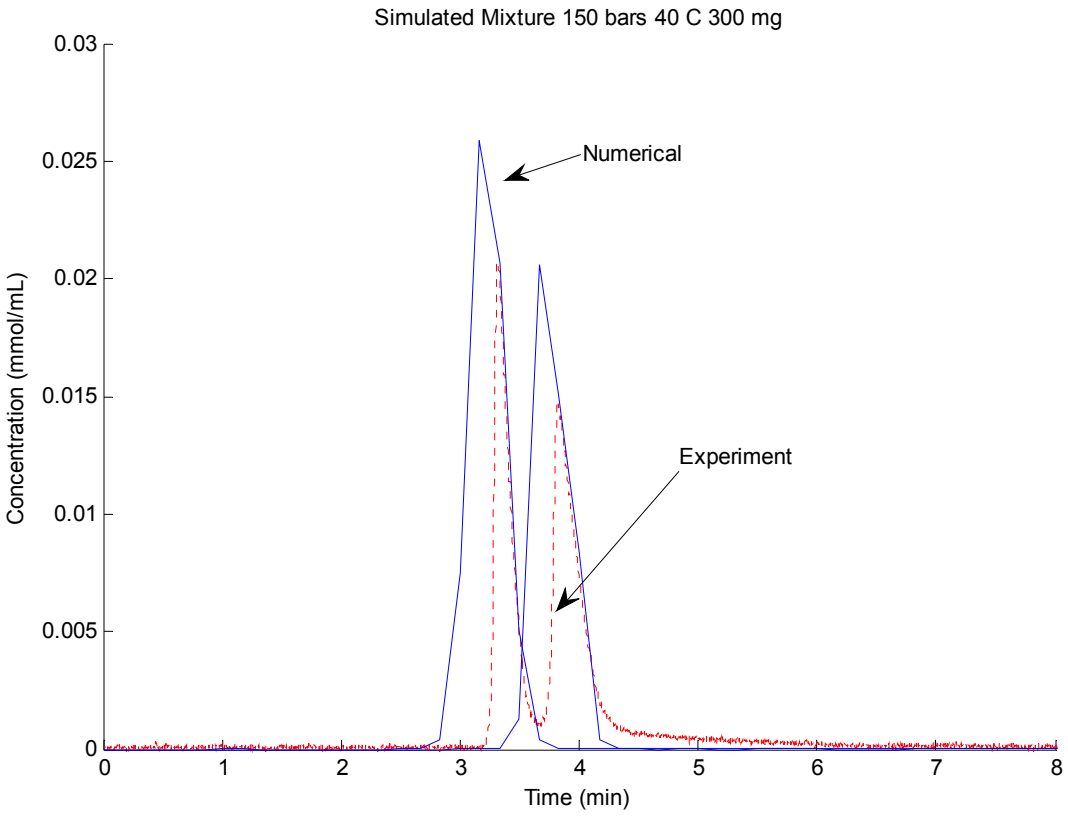


Figure 5.19 Simulated Chromatograms of Ibuprofen Enantiomer Mixture at 150 Bars,40℃, 300 mg

Chapter 6

Conclusions and Recommendations

In the conclusion, we will discuss the results of the experiment and what can be done to reduce the cost, improve on the temperature, and recommend other software that can help with development of a rigorous partial differential model.

6.1 Conclusions

We can see from the results that laboratory evaluation of mixtures of Ibuprofen appears to be effective in predicting Ibuprofen isotherms to save on cost. The Langmuir constant is affected by temperatures and pressures, as the temperature increases, the constant increases and vice versa. The column temperature cannot be too high because it can cause the silica gel inside the column to be denatured and would give an error in reading the chromatogram. It is also cost effective by using low concentration to predict the higher concentration isotherms and using less energy to run the experiment. The COMSOL model can be use to predict the chromatogram so that less time is wasted on running experiment and material cost. Any of the three models such as Langmuir, Toth, or Freundlich can be use to predict the isotherm that gave a small residual from experiment. The Langmuir parameter obtained from the experiment can be use to for industrial separation of the (S)-(+)-ibuprofen.

6.2 Recommendations

The recommendation for this work is to use a better detector to minimize the white noise when collecting the data using Labview. The use of other mobile phase solvent modifiers such as Methanol should also be evaluated to potentially improve analyte the peak resolutions. Better methods to evaluate more accurate isotherm parameters, and more flexible simulation models requiring less assumption for prediction of isotherms could reduce or eliminate the need for more costly and time consuming laboratory experimentation. Labview further has the capability of using more complex data acquisition and mathematical models allowing more automation of the data analysis potentially allowing Labview to "read" the data for peak recognition, allowing real-time calculation of, for instance, retention time and retention volume, which could then be used to directly calculate and report the isotherm, without doing any additional calculation. We can also use COMSOL to model the chromatogram using a convection and diffusion model so that it would require less time and material in running the experiment.

References

Ager, D. “Synthesis of Large-Volume Product”.A Handbook of Chiral Chemicals.Ed.Agar, D. New York: Marcel Dekker, 1999. 33-48. Print.

Agilent Technologies.Service Handbook for 1050 Series of HPLC Modules. Variable Wavelength Detector. 2001.

ASHRAE, n.p. n.d. Web. 7 Oct. 2010. < www.ashrae.org>

Ahuja, Satinder .Chiral Separations by Chromatography. Washington, D.C. :AmericanChemical Society, c2000.

Ahuja, Satinder, editor.Chiral Separations by Liquid Chromatography. Washington, D.C. :American Chemical Society, 1991.

Anton,K, Berger, C. Supercritical Fluid Chromatography with Packed Columns : Techniques and Applications. New York : Marcel Dekker, 1998.

Bracey, W. Fractionation of Aqueous Fructose/Glucose Mixtures by Adsorption and Supercritical Desorption .MS Thesis. University of South Florida,1989.

Bright,F, editor, McNally,M, editor. Supercritical Fluid Technology: Theoretical and Applied Approaches to Analytical Chemistry. Washington, DC : American Chemical Society, 1992.

Brown, J. Subramanian, G. “Regulatory Implications and Chiral Separations”. Ed. Subramanian, G. A Practical Approach to Chiral Separations by Liquid Chromatography.Weinheim , New York : VCH, c1994.

Canaparo,R,Mutoni,E, Zara,P, Pepa C, Berno,E, Costa,M,Eandi,M. “Determination of Ibuprofen in Human Plasma by High Performance Liquid Chromatography: Validation and Application in Pharmacokinetic Study”. University of Torino,Torino Italy 1999.

Charpentier,B.,editor,Sevenants,M.,editor, Marentis,R. Supercritical Fluid Extraction and Chromatography: Techniques and Applications. Washington, D.C. : American Chemical Society, 1988.

ChemBioDraw.CambridgeSoft Corporation. Cambrigde , MA. V2008. 2008.

CHEMCAD.ChemStation. University of South Florida.V6.1. 2010.

ChemSketch.Advanced Chemistry Development, Inc. V11.02. 2008.

Choi, Y., Han S., Chung, S., Row K., “Chromatographic Separation of Bupivacaine Racemate by Mathematical Model with Competitive Langmuir Isotherm”. Korean Journal of Chem.Eng. ,21 (4), 829-835 (2004).

COMSOL.COMSOL Multiphysics. University of South Florida. V 3.5. 2010.

Condor, J.R., Young, C.L. Physicochemical Measurement by Gas Chromatography. Chichester, New York: Wiley c1979.

Cox,G. ‘Simulated Moving Bed Chromatography and Chiral Compounds’. Toronto, 2005.

Cremer, E., Huber, H.F., ISA Proc: 1961 Intl. Gas Chromatography Symp., p.117.,Angew. Chem.73, 461, 1961.

Gonenc, S. Gas Chromatographic Study of Hydrogen and Hydrocarbon Adsorption on Platinum- Alumina Catalyst. MS Thesis. Bogazici University, 1985.

Guiochon,G.,Shirazi S.,Katti, A. Fundamentals of Preparative and Nonlinear Chromatography. Boston: Academic Press, 2006.

Guiochon,G., Zhong. G. Simulated Moving Bed Chromatography. Effect of Axial Dispersion and Mass Transfer Under Linear Conditions. University of Tennessee, Chemical Engineering Science, Vol.52 No.18, 3117-3132 (1997). Reproduced with Permission.

Hassan Y.A.E, Ali, I. Chiral Separations by Liquid Chromatography and Related Technologies New York : M. Dekker, Inc., c2003.

Hubber, A, editor.,Shafei, G.M.S. Encyclopedia of Surface and Colloid Science: Vol. 1, p. 618 (2002).

Hyun-Ku Rhee, Rutherford Aris, Neal R. Amundson.First-Order Partial Differential equations.Englewood Cliffs, N.J. : Prentice-Hall, c1986-

Jenning,W . Analytical gas chromatography.Jennings.Orlando : Academic Press, 1987.

Jenson V. G. , Jeffreys, G. V. Mathematical Methods in Chemical Engineering. London ; New York : Academic Press, 1977.

Johannsen,M. “Separation of Enantiomer of Ibuprofen on Chiral Stationary Phases by Packed Column Supercritical Fluid Chromatography”.TechnischeUniversitat Hamburg, Germany Journal of Chromatography, Vol. 937 Issue: 1-2 p.135-138(2001).

Lough,W,J. Chiral Liquid Chromatography . Glasgow ; Blackie, 1989.

Martin, M. Determination of Adsorption Isotherms for Flurbiprofen with Supercritical Fluid Conditions. University of South Florida,2004.

Matlab.Mathworks. University of South Florida. V2009a. 2010.

McHardy ,J, Sawan,S. Supercritical Fluid Cleaning: Fundamentals, Technology and Applications . Westwood, N.J. : Noyes Publications, c1998.

Meyer ,V. Practical High-Performance Liquid Chromatography . Chichester [Chichestershire] ;New York : Wiley, c1998. Reproduced with Permission.

Paint.Microsoft.Microsoft Corporation. 2007.

Paulaitis,M . [et al.]. Chemical Engineering at Supercritical Fluid Conditions. Ann Arbor, Mich. :Ann Arbor Science, c1983.

Patel, K. Automatic Generation of Global Phase Equilibrium Diagram from Equation of State. Dissertation, Florida, University of South Florida, 2007.

Perry, S. G., Amos,R, Brewer, P. I. .Practical Liquid Chromatography. New York, Plenum Press, 1972.

Plakogiannis,F,Kazmi S, Ali A. “High Pressure Liquid Chromatographic Determination of Ibuprofen in Plasma”. Long Island University , Brooklyn, NY. J Pharm. Sci. 70(8):944-5 (1981).

Saleh, J. Fluid Flow Handbook. McGraw, New York, 2002.

Sivasankar, B. Bioseparations: Principles and Techniques. Prentice Hall, New Delhi, 2005.

Skoog,D , West,D. Fundamentals of Analytical Chemistry . New York : Holt, Rinehart, and Winston, c1976.

Smith,R,Ma,L. “Chiral Supercritical Fluid Chromatography of Phenylpropanols and Related Compounds”.Journal of Chromatography A.785 179-184 (1997).

Subramanian, G. An Introduction to Enantioseparation by Liquid Chromatography. Ed. Subramanian, G. A Practical Approach to Chiral Separations by Liquid Chromatography.Weinheim , New York : VCH, c1994. Reproduced with Permission.

Suleiman,D,Estevez,L,Pulido,J, Garcia,J,Mojica,C. “Solubility of Anti-Inflammatory, Anti-Cancer in Supercritical Carbon Dioxide”. University of Puerto Rico, Mayaguex, Puerto Rico. J. Chem. Eng. Data. , 50 1234-1241 (2005).

Sunol ,A., Sunol S. “Substitution of Solvents by Safer Products and Process”. Handbook of Solvents. Ed. Wypych,G., Wypych, J. Toronto:ChemTec, 2001. 1419-1484. Print.

Sunol, S., Mierau, B. Serifoglu I., and Sunol, A. K. “Estimation of Physicochemical Properties using Supercritical Fluid Chromatography” in Martin Abraham and Aydin K. Sunol (ed.); Supercritical Fluids: Extraction and Pollution Prevention, ACS Symposium Series, 1997.

Székely,E. Supercritical Fluid Extraction. Budapest University of Technology and Economics. Chemical Technology and Biotechnology, Department of Chemical and Environmental Process Engineering. 2009. Reproduced with permission.

Visio.Mircosoft. Microsoft Corporation. 2007.

Whelan, J. Separation of Ibuprofen Enantiomers on a Chiral Stationary Phase by Supercritical and Sub-Critical Fluid Chromatography. Master’s Thesis, University of South Florida, 2003.

White, C. Subramanian, G. “ An Introduction to Enantioseparation by Liquid Chromatography”. Ed. Subramanian, G. A Practical Approach to Chiral Separations by Liquid Chromatography.Weinheim , New York : VCH, c1994.

Wilson, W. “Direct Enantiomer Resolution of Ibuprofen and Flurbiprofen by Packed Column SFC”.Hewlett Packard Co., Wilmington, Delaware, Chirality 6: 216-219 (1994).

Wischumerski, R., Turk, M., Wahl, M. Direct Drug Loading into Preformed Porous Solid Dosage Units by the Controlled Particle Deposition, A New Concept for Improved Dissolution Using SFC Technology. Wiley Interscience, Journal of Pharmaceutical Sciences, Vol.97, 4416-4424 (2008).

Appendices

Appendix A: Sample Calculation for S-Ibuprofen

This is a sample output of the retention volume, V_R, concentration in the mobile phase, c, and the concentration in the stationary phase, q.

$$V_R = F_C t_R = F_C * \frac{t_C - t_0}{w}$$

$$c = \frac{a * N}{A * F_c}$$

$$q = \int_{C(t_M)}^{C(t_R)} V_R * dc$$

Table A.1 Sample Calculation for S-Ibuprofen

T=40℃ , P= 150 Bars, Inert Retention time=1.28 min, flow rate=3.3 mL/min Concentration=300 mg						
Time(min)	Corrected Time (min)	Absorbance	Adjusted Absoraba nce	Concentration (mmol/mL)	VR(mL/g)	q (mmol/mL)
3.3087	2.0287	0.7786	0.7156	0.0262	1.9128	0
3.3178	2.0378	0.7617	0.6987	0.0256	1.9214	0.0001
3.3269	2.0469	0.7442	0.6812	0.0249	1.93	0.0002
3.336	2.056	0.7194	0.6564	0.024	1.9385	0.0002
3.3452	2.0652	0.6952	0.6322	0.0231	1.9471	0.0002
3.3543	2.0743	0.6668	0.6038	0.0221	1.9557	0.0003
3.3634	2.0834	0.6448	0.5818	0.0213	1.9643	0.0003
3.3725	2.0925	0.6187	0.5557	0.0203	1.9729	0.0003
3.3816	2.1016	0.5986	0.5356	0.0196	1.9815	0.0003
3.3907	2.1107	0.57	0.507	0.0186	1.9901	0.0003
3.3998	2.1198	0.5493	0.4863	0.0178	1.9987	0.0003
3.409	2.129	0.5225	0.4595	0.0168	2.0073	0.0003
3.4181	2.1381	0.5011	0.4381	0.016	2.0159	0.0004
3.4272	2.1472	0.4782	0.4152	0.0152	2.0245	0.0004
3.4363	2.1563	0.4585	0.3955	0.0145	2.0331	0.0004
3.4454	2.1654	0.4378	0.3748	0.0137	2.0417	0.0004
3.4545	2.1745	0.418	0.355	0.013	2.0503	0.0004

Appendix A (Continued)

Table A.1 (Continued)

3.4636	2.1836	0.3957	0.3327	0.0122	2.0589	0.0004
3.4728	2.1928	0.3763	0.3133	0.0115	2.0675	0.0004
3.4819	2.2019	0.3572	0.2942	0.0108	2.076	
3.491	2.211	0.3377	0.2747	0.0101	2.0846	0.0004
3.5001	2.2201	0.3234	0.2604	0.0095	2.0932	0.0005
3.5092	2.2292	0.303	0.24	0.0088	2.1018	0.0005
3.5183	2.2383	0.2906	0.2276	0.0083	2.1104	0.0005
3.5275	2.2474	0.2737	0.2107	0.0077	2.119	0.0005
3.5366	2.2566	0.2562	0.1932	0.0071	2.1276	0.0005
3.5457	2.2657	0.2412	0.1782	0.0065	2.1362	0.0005
3.5548	2.2748	0.2291	0.1661	0.0061	2.1448	0.0005
3.5639	2.2839	0.2157	0.1527	0.0056	2.1534	0.0005
3.573	2.293	0.2014	0.1384	0.0051	2.162	0.0006
3.5821	2.3021	0.1905	0.1275	0.0047	2.1706	0.0006
3.5913	2.3113	0.1768	0.1138	0.0042	2.1792	0.0006
3.6004	2.3204	0.1686	0.1056	0.0039	2.1878	0.0006
3.6095	2.3295	0.1545	0.0915	0.0034	2.1964	0.0007
3.6186	2.3386	0.1453	0.0823	0.003	2.205	0.0007
3.6277	2.3477	0.138	0.075	0.0027	2.2135	0.0008
3.6368	2.3568	0.1326	0.0696	0.0025	2.2221	0.0008
3.646	2.3659	0.1233	0.0603	0.0022	2.2308	0.0008
3.6551	2.3751	0.117	0.054	0.002	2.2393	0.0009
3.6642	2.3842	0.1109	0.0479	0.0018	2.2479	0.0009
3.6733	2.3933	0.1074	0.0444	0.0016	2.2565	0.001
3.6824	2.4024	0.1023	0.0393	0.0014	2.2651	0.001
3.6915	2.4115	0.1007	0.0377	0.0014	2.2737	0.001
3.7007	2.4207	0.0953	0.0323	0.0012	2.2823	0.0011
3.7098	2.4298	0.0931	0.0301	0.0011	2.2909	0.0012
3.7189	2.4389	0.0905	0.0275	0.001	2.2995	0.0013
3.728	2.448	0.088	0.025	0.0009	2.3081	0.0013
3.7371	2.4571	0.0848	0.0218	0.0008	2.3167	0.0014
3.7462	2.4662	0.0835	0.0205	0.0008	2.3253	0.0015
3.7553	2.4753	0.0845	0.0215	0.0008	2.3339	0.0015
3.7644	2.4844	0.0813	0.0183	0.0007	2.3425	0.0016
3.7736	2.4935	0.0794	0.0164	0.0006	2.3511	0.0018
3.7827	2.5027	0.0778	0.0148	0.0005	2.3596	0.002

Appendix A (Continued)

Table A.1 (Continued)

3.81	2.53	0.0774	0.0144	0.0005	2.3854	0.0025
3.8191	2.5391	0.0746	0.0116	0.0004	2.394	0.0028
3.8282	2.5482	0.0787	0.0157	0.0006	2.4026	0.0031
3.8374	2.5574	0.0743	0.0113	0.0004	2.4112	0.0033
3.8465	2.5665	0.0762	0.0132	0.0005	2.4198	0.0038
3.8556	2.5756	0.0743	0.0113	0.0004	2.4284	0.0041
3.8647	2.5847	0.0752	0.0122	0.0004	2.437	0.0045
3.8738	2.5938	0.0717	0.0087	0.0003	2.4456	0.0051
3.8829	2.6029	0.0743	0.0113	0.0004	2.4542	0.0058
3.8921	2.6121	0.0724	0.0094	0.0003	2.4628	0.0062
3.9012	2.6212	0.073	0.01	0.0004	2.4714	0.0068
3.9103	2.6303	0.0724	0.0094	0.0003	2.48	0.0077
3.9194	2.6394	0.0733	0.0103	0.0004	2.4886	0.0087
3.9285	2.6485	0.0714	0.0084	0.0003	2.4972	0.0094
3.9376	2.6576	0.0688	0.0058	0.0002	2.5058	0.0104
3.9467	2.6667	0.0701	0.0071	0.0003	2.5143	0.0113
3.9558	2.6758	0.0695	0.0065	0.0002	2.5229	0.0124
3.965	2.685	0.0698	0.0068	0.0002	2.5315	0.0135
3.9741	2.6941	0.0695	0.0065	0.0002	2.5401	0.0144
3.9832	2.7032	0.0692	0.0062	0.0002	2.5487	0.0156
3.9923	2.7123	0.0704	0.0074	0.0003	2.5573	0.017
4.0014	2.7214	0.0695	0.0065	0.0002	2.5659	0.0183
4.0106	2.7306	0.0673	0.0043	0.0002	2.5745	0.0192
4.0197	2.7397	0.0679	0.0049	0.0002	2.5831	0.0208
4.0288	2.7488	0.0688	0.0058	0.0002	2.5917	0.0219
4.0379	2.7579	0.0704	0.0074	0.0003	2.6003	0.0234
4.047	2.767	0.0669	0.0039	0.0001	2.6089	0.0248
4.0561	2.7761	0.0695	0.0065	0.0002	2.6174	0.0263
4.0653	2.7853	0.0692	0.0062	0.0002	2.6261	0.028
4.0743	2.7943	0.0685	0.0055	0.0002	2.6347	0.0294
4.0835	2.8034	0.0647	0.0017	0.0001	2.6433	0.031
4.0926	2.8126	0.0695	0.0065	0.0002	2.6518	0.0325
4.1017	2.8217	0.0688	0.0058	0.0002	2.6605	0.0342
4.1108	2.8308	0.0679	0.0049	0.0002	2.6691	0.0357
4.1199	2.8399	0.0669	0.0039	0.0001	2.6776	0.0377

Appendix A (Continued)

Table A.1 (Continued)

4.1655	2.8855	0.0679	0.0049	0.0002	2.7206	0.0462
4.1746	2.8946	0.0685	0.0055	0.0002	2.7292	0.0482
4.1837	2.9037	0.0647	0.0017	0.0001	2.7378	0.0499
4.1928	2.9128	0.0695	0.0065	0.0002	2.7464	0.0517
4.2019	2.9219	0.066	0.003	0.0001	2.755	0.0529
						0.0541

Appendix B: Sample Calculation using Peak Maxima Method

This is a sample calculation of the peak maxima.

$$a = \frac{tr - to}{v * to}$$

$$a = \frac{3.30 - 1.28}{0.7 * 1.28} = 2.25$$

$$Lf = ((1 - \left(\frac{tf - to - tp}{tr - to}\right)^{.5})\text{^}2$$

$$Lf = (\left(1 - \left(\frac{4 - 1.28 - 0.8}{3.3 - 1.28}\right)^{.5}\right)^{2} = 0.0006$$

$$b = \frac{Lf * v * (tr - to)}{Nm}$$

$$b = \frac{.0006 * 0.7 * (3.3 - 1.28)}{0.0146} = 0.0610$$

$$Qm = \sqrt{\frac{Na}{F\ to\ \varphi\ b}}$$

$$Qm = \sqrt{\frac{0.0146 * 2.25}{3.5 * 1.28 * 0.67 * 0.061}} = 0.9492$$

$$t_r = t_0(1 + \varphi \frac{\left(a - \frac{bac}{1+bc}\right)^2}{a})$$

$$t_r = 1.28\left(1 + 0.67 \frac{\left(2.25 - \frac{0.061*2.25*0.4321}{1+0.061*0.4321}\right)^2}{2.25}\right) = 3.29$$

Appendix C: Sample Calculation for an Isotherm

This is the sample of the matlab program to calculate the isotherm.

```
F=3;        %ml/min
w=3.5;  % gram
vg=(((F.*tr)./w))
%vgg=vg(1:5:495);
% trr=tr(1:5:495);
% trr2=tr(1:5:495) + 1.28;
% absorbancea=absorbance(1:5:495);
% absorbanceb=absorbance(1:5:495) +.063;
%amount=input('amount of component');  % mg/ml
%amount=input('amount of concentration');
N=(300./(206.*100));
%area=input('area under curve');
% area1=trapz(tr,absorbance);
area1=.1;
figure(1)
holdon
plot(tr,absorbance)
xlabel('Time (min)')
ylabel('Absorbance(nm)')
title(' S-Ibuprofen 150bars 40 C ')
 c=(absorbance.*N./(area1.*F))
figure(10)
plot(tr,c)
xlabel('Time (min)')
ylabel('Concentration(mmol/mL)')
title(' S-Ibuprofen 150bars 40 C ')
%plot(vg,c)
figure(2)
holdon
plot(c,vg)
xlabel('Concentration(mmol/mL)')
ylabel('V_R (ml/g)')
title('S-Ibuprofen 150 bars 40C 300 mg')
vg1=-sort(vg);
vg2=-sort(vg1);
  a=sort(c)
%  b=[a vg2];
dd=[a c vg2]
%   ee=a(1:5:495)
% ff=c(1:5:495)
%   aa=a(1:5:495)
%   vg22=vg2(1:5:495)
holdon
figure(33)
    q1=cumtrapz(a,vg2)
```

Appendix C (Continued)

```
q3=(3.3.*a)./(1+2.*a);
holdon
plot(a,q1)
figure(3)
holdon
plot(a,q1)
aaa=cumtrapz(tr,absorbance)
xlabel('Concentration (mmol/mL)')
ylabel('q (mL/g)')
legend('75 mg', '100 mg', '150 mg', '300 mg')
title(' S-Ibuprofen 150 bars 40 C ')
figure(8)
plot(a,q1)

     y=.015*absorbance.^2 + .033.*absorbance
figure(13)
plot(tr,y)
figure(98)
plot(y,vg)
xlabel('Concentration(mmol/mL)')
ylabel('V_R (mL/g)')
title('S-Ibuprofen 150 bars 40C 300 mg')
y2=sort(y)
%y3=y2([1:5:495])
oo=[y2 vg2];
q2=(1/1.25).*(cumtrapz(y2,vg2))
figure(99)
holdon
plot(y2,q2)

xlabel('Concentration (mmol/mL)')
ylabel('q (mmol/mL)')
legend('75 mg', '100 mg', '150 mg', '300 mg')
title(' S-Ibuprofen 150 bars 40 C ')
figure(101)
holdon
plot(y2,q2)
xlabel('Concentration (mmol/mL)')
ylabel('q (mmol/mL)')
legend('30 C','40 C', '45 C')
title(' S-Ibuprofen 300 mg 150 bars')
figure(103)
holdon
     q3=(1.5.*y2)./(1+.4.*y2)
plot(y2,q2,y2,q3)
xlabel('Concentration (mmol/mL)')
ylabel('q (mmol/mL)')
legend('75 mg experiment','75 mg theoretical',...
```

Appendix C (Continued)

```
'100 mg experiment','100 mg theoretical',...
'150 mg experiment','150 mg theoretical',...
'300 mg experiment','300 mg theoretical')
title(' S-Ibuprofen 150 bars 40 C ')

%freundlich isotherm
% a=1.5;
% n=1;
%  q12=a.*y2.^(1./n)
% figure(108)
% plot(y2,q2,y2,q12,y2,q3)
%
figure(109)
a=1.5;
n=1;
 q12=a.*y2.^(1./n)
plot(y2,q2,y2,q12)
%least square method
[x,resnorm,residual,exitflag,output] = ...
lsqlin(q2,q12,[],[],[],[],[],[])
figure(107)
plot(y2,residual)
% %
% % figure(122)
% % plot(y,vg)
% %
% % figure(110)
% %q22=(1/1.2).*(cumtrapz(-y,vg2))
% % plot(y,q22)
% %
% %
% %      figure(34)
% %      plot(y2,q2)
% %
% toth isotherm
     t=1.5;
qt=(y2)./(.54+y2.^t).^(1./t)
figure(111)
plot(y2,q2,y2,qt)
     [x,resnorm,residual,exitflag,output] = ...
lsqlin(q2,qt,[],[],[],[],[],[])
figure(110)
plot(y2,residual)
%
      %all langmuir, fruenlich, and toth isotherm together
figure(112)
plot(y2,q2,'o',y2,q3,'+',y2,q12,'-',y2,qt,'--')
xlabel('Concentration (mmol/mL)')
```

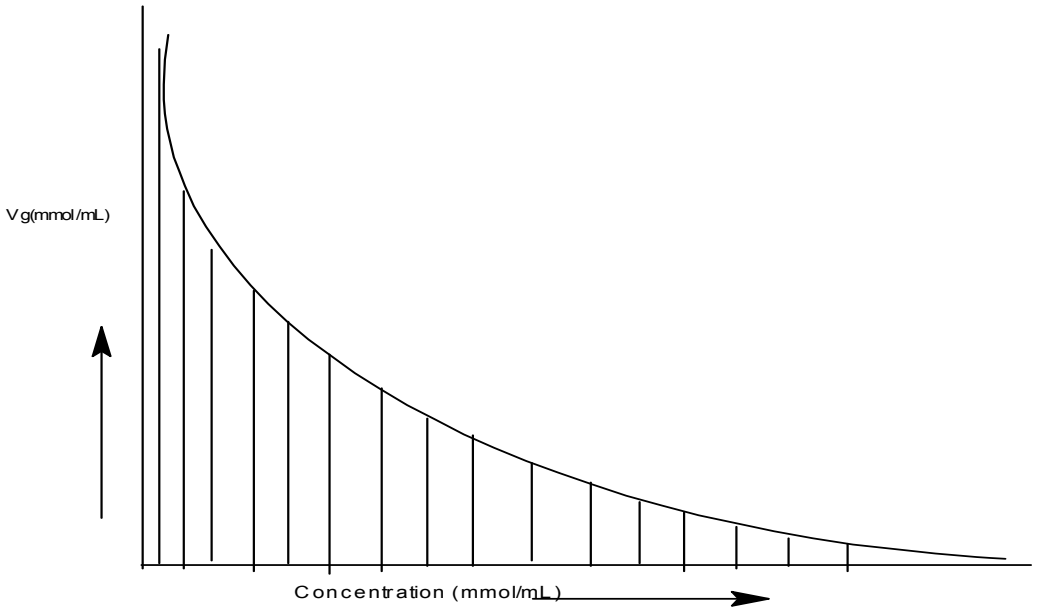
Appendix C (Continued)

```
ylabel('q (mmol/mL)')
legend('Experiment','Langmuir', ' Fruenlich', ' Toth')
title('S-Ibuprofen 150 bars 40 C 300 mg')
confidence=.05;  % I chose i 99% confidence interval
%non-linear

 [theta,theta_CI,resid1,res_CI,stats]=regress(q2,y2,confidence)
 %linear
 [theta2,theta2_CI,resid2,res2_CI,stats2]=regress(q2,y2,confidence)
 X=[ones(size(a)) a]
 %a3=X\q1
theta
theta_CI
stats
%
 X=[ones(size(a)) a]
 a4=X\q3
 theta2
 theta2_CI
 stats2

% qq7=resid1
% figure(106)
% plot(y2,qq7)
%
%
% %least square method
[x,resnorm,residual,exitflag,output] = ...
%               lsqlin(q2,q3,[],[],[],[],[],[])
figure(107)
plot(y2,residual)
sum(residual)
```

Appendix D: Integration Curve



Appendix E: Nomenclatures

α = Separation Factor
ε = Void Fraction of the Column
φ = Phase Ratio
ρ= Density of the Solid Particles [$\frac{kg}{m^3}$]
A= Inner area of the Column Tube [m^2]
A_1= Absorbance
a= Langmuir Parameter
b= Langmuir Parameter
b_1= Path Length of the Sample [cm]
c= Concentration of the Solute in the Mobile Phase [mmol/mL]
c_i= Equilibrium Concentration [$\frac{mol}{m^3}$]
c_i^*= Local Concentration in the Stationary Phase [$\frac{mol}{m^3}$]
c_M = Maximum Concentration [$\frac{mol}{m^3}$]
D_{eff}= Diffusion Effective Constant [$\frac{m^2}{s}$]
$D_{L,i}$= The Axial Dispersion Coefficient [$\frac{m^2}{s}$]
e= Molar Absorbtivity [$\frac{L}{mol*cm}$]
F= Volumetric Flow Rate in the Column [$\frac{mL}{min}$]
F_C = Mobile Phase Flow Rate in the Column [$\frac{mL}{min}$]
H= Height Equivalent of a Theoretical Plate [m]
I_0= Bessel Function
k= Rate Constant
K_i= Adsorption Constant for the Component i [$\frac{m^3}{mol}$]
L= Length of Column [L]
Lf= Loading Factor
N= Number of Mole Injected [mol]
n_i= Adsorbate Concentrations in the Stationary Phase of Component i [$\frac{mol}{m^3}$]
n_{0i}= Monolayer Coverage Capacity of the Stationary Phase for Component i [$\frac{mol}{m^2}$]
q = Concentration of the Solute on the Stationary Phase [mmol/mL]
R_s = Peak Resolution
S_1= Cross sectional Area [m^2]
S= Surface Area of Particles in Column [$\frac{m^2}{kg}$]
t= Time spent in the Column [min]
t_f= Final Time [min]
t_0 = Hold Up Time [min]
t_p= Width of the Peak [min]
t_R = Adjusted Retention Time [min]
u= Velocity of the Mobile Phase [mm/s]

Appendix E (Continued)

$v =$ Linear Velocity Inside the Column [mm]
v= Mobile Phase Flow Rate [$\frac{m^3}{s}$]
$V_R =$ Retention Volume [ml/g]
V= Volume of the Column [m^3]
v_i= Linear Velocity of the Mobile Phase in the Column [$\frac{m}{s}$]
$V_m =$ Volume of the Mobile Phase [ml/g]
$V_s =$ Volume of the Stationary Phase [ml/g]
v_{zi}= Migration Velocity of the Adsorbate Zone through the Column [m]
w= Weight of the Adsorbent [kg]
$w_i =$ Weighted Value [kg]
y= Response Value
$\acute{y}_i =$ Predicted Response Value
z= Axial Dimension of the Column [mm]
Z= Compressibility of the Component [unitless]

Appendix F: Figures and Tables

Software Used	Figures/ Table
Matlab	Figures 2.2, 3.11, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 4.10, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 5.10, 5.11, 5.12, 5.13, 5.14, 5.15, 5.16, 5.17, 5.18, 5.19
ChemDraw	Figures 1.2 , 2.1 (a-c) , 2.3 (a-c), 3.2, 3.6, 3.9
ChemiBiodraw	Figure 1.1, 2.4
CHEMCAD	Table 1.2
COMSOL	Figures 5.22, 5.23

Appendix G: Parameter of Single Ibuprofen at 150 Bars and 300 mg

40 ℃	
Isotherm Model	Parameters
Langmuir	a= 1.5 b= 0.4
Freundlich	a=1.5 n= 1.0
Toth	b= 0.54 t= 1.5

45 ℃	
Isotherm Model	Parameters
Langmuir	a= 1.60 b= 0.4
Freundlich	a=1.61 n= 0.99
Toth	b= 0.49 t= 1.6

Appendix H: Parameter of Competitive Ibuprofen at 150 Bars and 300 mg

40 ℃	
Isotherm Model	Parameters
Langmuir	R a b 1.85 0.15 S a b 1.5 0.4
Freundlich	R a t 1.9 1.0 S a t 1.5 1.0
Toth	R b t 1.3 0.44 S b t 0.49 1.6

Appendix I: Permissions

I.1 Permission to Use Figure 1.3

Dear Sir or Madame,
My name is Loi Ho and I am a graduate student at the University of South Florida. I am writing to seek permissions to use in my thesis the following: The title of the book is Bioseparations: Principles and Techniques by B. Sivasankar with ISBN: 81-203-2649-0 to use the table 6.3 and figure 6.11 (a).
Sincerely,
Loi Ho
from: Permissions - US permissionsus@wiley.com
to: Loi Ho <ldho@mail.usf.edu>
date: Tue, Feb 14, 2012 at 9:36 AM
subject: RE: permissions to use in thesismailed-by: wiley.com
Dear Loi Ho:
Thank you for your query. Permission is granted to use the content from Bioseparations in your thesis. Please credit accordingly.
Best wishes,
Paulette Goldweber
Associate Manager, Permissions
Global Rights
John Wiley & Sons, Inc.
ph: 201-748-8765
f: 201-748-6008
pgoldweb@wiley.com

Appendix I (Continued)

I.2 Permission to Use Figure 2.4

Dear Sir or Madame,
My name Loi Ho and I am a graduate student at the University of South Florida and I would like to seek permission to use the Whelk 0 1 image to be included in my thesis.
Sincerely,
Loi Ho
Jelena Kocergin JKocergin@registech.com to: "ldho@mail.usf.edu" <ldho@mail.usf.edu>
cc: Diane Richards <DRichards@registech.com>,
Toria Evans <TEvans@registech.com>,
Louis Glunz <lglunziv@registech.com>
date: Mon, Feb 6, 2012 at 4:28 PMsubject: RE: Permission to use Whelk 0 1 image

Hello Loi,

My name is Jelena Kocergin and I am Business Manager for Chromatography Department at Regis Technologies.

You are welcome to use Whelk-O1 image in your thesis work. Did you work with Whelk-O1? Hope you had good success in chiral work and let us know if you continue to be involved with chiral columns in future.

Let us know if there is anything else I can do to help.

Best regards,

Jelena

Jelena Kocergin

Business Manager

Chromatography Products

Regis Technologies, Inc.

847.583.7649 Fax 847.967.1214

Email: jkocergin@registech.com

Appendix I (Continued)

I.3 Permission to Use Table 1.1 and 1.2

Dear Sir or Madame,
My name is Loi Ho and I am a graduate student at the University of South Florida. I am writing to request for permission the use of two tables for my thesis from the following book:
The title of the book is " A Practical Approach to Chiral Separations by Liquid Chromatography by G. Subramanian with ISBN: 3-527-28288-2 to use tables 1.1 and 1.2.
Sincerely,
Loi Ho

Dear Loi Ho,

Thank you for your email.

We hereby grant permission for the requested use expected that due credit is given to the original source.

With kind regards

Bettina Loycke

Bettina Loycke

Senior Rights Manager

Wiley-VCH Verlag GmbH & Co. KGaA

Boschstr. 12

69469 Weinheim

Germany

Phone: +49 (0) 62 01- 606 - 280

Fax: +49 (0) 62 01 - 606 - 332

Email: rights@wiley-vch.de

Appendix I (Continued)

I.4 Permission to Use Figure 1.2

License Details
This is a License Agreement between Loi Ho ("You") and Elsevier ("Elsevier"). The license consists of your order details, the terms and conditions provided by Elsevier, and the payment terms and conditions.
Get the printable license.
License Number 2842880366582
License date Feb 06, 2012
Licensed content publisher Elsevier
Licensed content publication Chemical Engineering Science
Licensed content title Simulated moving bed chromatography. Effects of axial dispersion and mass transfer under linear conditions
Licensed content author Guoming Zhong,Georges Guiochon
Licensed content date September 1997
Licensed content volume number 52
Licensed content issue number 18
Number of pages 16
Type of Use reuse in a thesis/dissertation
Portion figures/tables/illustrations
Number of figures/tables/illustrations 1
Format print
Are you the author of this Elsevier article? No
Will you be translating? No
Order reference number None
Title of your thesis/dissertation Determination of Ibuprofen Isotherms Using Supercritical Fluid Chromatography
Expected completion date May 2012
Estimated size (number of pages) 131
Elsevier VAT number GB 494 6272 12
Permissions price 0.00 USD
VAT/Local Sales Tax 0.0 USD / 0.0 GBP
Total 0.00 USD

Appendix I (Continued)

I.5 Permission to Use Table 1.5

License Details

This is a License Agreement between Loi Ho ("You") and John Wiley and Sons ("John Wiley and Sons"). The license consists of your order details, the terms and conditions provided by John Wiley and Sons, and the payment terms and conditions.

Get the printable license.

License Number 2843170854613

License date Feb 06, 2012

Licensed content publisher John Wiley and Sons

Licensed content publication Wiley Books

Licensed content title Practical High-Performance Liquid Chromatography, 5th Edition

Licensed content author Veronika R. Meyer

Licensed content date May 1, 2010

Type of use Dissertation/Thesis

Requestor type University/Academic

Format Print

Portion Figure/table

Number of figures/tables 1

Original Wiley figure/table number(s) table 2.1 effect of the number of theoretical plates on resolution for different separation factors

Will you be translating? No

Order reference number None

Total 0.00 USD

Appendix I (Continued)

I.6 Permission to Use Table 1.3

Dear Dr. Székely,

My name is Loi Ho and I am a graduate student at the University of South Florida. I would like to ask permission to use the table 1 in your supercritical fluid extraction properties of liquids, gases, and sfcs.
Sincerely,
Loi Ho

sz-edit@mail.bme.hu

to: Loi Ho ldho@mail.usf.edu
date: Sat, Jun 30, 2012 at 11:53 AM

subject: Re: request to use table for thesismailed-

by: mail.bme.h

Dear Loi Ho,
I principle you can use anything from our published works if you give the reference there. May i ask which Table 1 you mean?

Best regards,

Edit Székely PhD.
associate professor
BME Department of Chemical and Environmental Process Engineering
Budapest, Budafoki ut 8., H-1111, Hungary
tel: +36-1-4633096 fax: +36-1-4633197 handy: 3670-392-5567
sz-edit@mail.bme.hu

You may use it.
Best regards,