PREPARATION OF CATION-EXCHANGE MEMBRANE CHROMATOGRAPHY BY MODIFICATION OF POLYAMIDE MEMBRANE THROUGH GRAFTING OF METHACRYLIC ACID MONOMER

NURUL ALIA BINTI KHALIL

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for the award of the degree of
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SUPERVISOR'S DECLARATION

I hereby declare that I have checked this thesis and in my opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering.

Signature

Name of Supervisor: Dr. Syed Mohd Saufi b Tuan Chik

Position: Senior Lecturer

Date:

STUDENT'S DECLARATION

I hereby declare that the work in this thesis is my own except for quotation and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

Signature

Name: Nurul Alia binti Khalil

ID Number: KA08125

Date:

This project is dedicated to my parents who continuously support me in term of financial and moral, and constantly provide everything that I need during conducting and finishing my thesis.

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ABSTRACT

Nowadays, with the increase demand of protein production and purity, the purification cost of the protein also increases about 50 to 90%. Packed bed chromatography is widely used in protein separation. However, there are some limitations of the packed bed chromatography such as high-pressure drop, channeling problem and complicated scale up process. Most of these limitations can be overcome by membrane chromatography. The main objective of this research is to prepare cation-exchange membrane chromatography from polyamide membrane by chemical grafting of methacrylic acid monomer. Potassium persulfate and potassium metabisulfite was used to generate the radicals in the polyamide membrane and grafted with methacrylic acid monomer together with ethylene glycol dimethacrylate as a cross-linker. Different monomer concentration between 0.1 and 1.0M and monomer grafting duration from 15 – 120 min was studied toward producing high protein binding capacity membrane. Optimum time for grafting methacrylic acid onto polyamide membrane was 45 minutes with the binding capacity 599.6390 mg/g BSA at the monomer concentration of 0.1 M. Identification and optimization of critical parameter in grafting process is crucial for the development of high performance membrane chromatography materials.

ABSTRAK

Pada masa kini, dengan meningkatnya permintaan pengeluaran protein dan juga ketulenan protein, kos pemisahan protein juga meningkat kira-kira 50 hingga 90%. Kromatografi turus terpadat digunakan secara meluas dalam proses pengasingan protein. Walau bagaimanapun, terdapat beberapa kekangan bagi kromatografi turus terpadat seperti penurunan tekanan yang tinggi, masalah penyaluran aliran dan juga proses pengembangan skala yang rumit. Kebanyakan kekangan ini dapat diatasi dengan penggunaan membran kromatografi yang mempunyai kejatuhan tekanan yang rendah, proses pengembangan skal yang mudah dan boleh beroperasi pada kadar pemprosesan yang tinggi. Objektif utama kajian ini adalah untuk menyediakan membran kromatografi jenis penukar cas positif dari membran poliamide menggunakan asid metakrilik sebagai monomer. Kalium persulfate ($K_2S_2O_8$) dan kalium metabisulfite ($K_2S_2O_5$) digunakan untuk menjana radikal dalam membran poliamide dan ditambah dengan monomer asid metakrilik bersama-sama dengan dimetilkrilik glikol etilena sebagai agen pemaut. Kepekatan monomer yang berbeza iaitu diantara 0.1 dan 1.0M dan tempoh cantuman monomer dari 15 - 120 minit telah dikaji kearah menghasilkan pengikatan protein yang berkapasiti tinggi. Keputusannya, masa optimum bagi poliamide yang telah diubahsuai permukaanya adalah 45 minit dengan kapasiti penjerapan sebanyak 599.6390 mg/g BSA pada kepekatan monomer 0.1 M. Penentuan dan pengoptimuman parameter penting semasa proses *grafting* adalah mustahak dalam menghasilkan membrane kromatografi berkapasiti terbaik.

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LIST OF ABBREVIATIONS

AA	Acrylic Acid
ATR-FTIR	Attenuated Total Reflection Fourier Transform Infrared
BSA	Bovine Serum Albumin
C_o	Initial Concentration
C_f	Final Concentration
Ca	Calcium
cm	Centimeter
DI	Deionized Water
EGDMA	Ethylene Glycol Dimethacrylate
ELISA	Enzyme-Linked Immunosorbent Assay
HCl	Hydrochloric Acid
K	Potassium
kPa	kiloPascal
$K_2S_2O_5$	Potassium Metabisulfite
$K_2S_2O_8$	Potassium Persulfate
$LiNO_3$	Lithium Nitrate
m	Mass of Substance
M	Molarity
MA	Methacrylic Acid
meq	miliEquivalent
mg	Milligram
ml	Milliliter
M_w	Molecular Weight
Na	Sodium
NaOH	Sodium Hydroxide
nm	Nanometer
NMP	N-Methylatedpyrrolidone
PA	Polyamide
PEG	Polyethylene Glycol
PEGMA	Polyethylene Glycol Methacrylate
PI	Isoelectric Point
pKa	Acid Dissociation Constant
Q	Binding Capacity
Redox	Reduction and Oxidation
RO	Reverse Osmosis
rpm	Rotation Per Minute
SPM	Sulfo-Propyl Metacrylate
SPS	Sulfonated Polysufone
TFC	Thin-Film Composite
UF	Ultrafiltration
UV	Ultra Violet
V	Volume
W	Weight of Dry Membrane

W_C	Water Content
W_W	Weight of Wet Membrane

CHAPTER 1

INTRODUCTION

1.1 Background

Large scale separation and purification step may contribute a large extend of the total product manufacturing cost. Sometimes, the cost of protein purification and protein separation can be as high as 50 to 90% of the total production cost (Saiful et al, 2006).

Several techniques are available in protein separation such as chromatography, membrane filtration, centrifugation, precipitation and membrane chromatography. Among them, chromatography based separation is widely used and more specifically using packed bed chromatography (Saufi, 2010).

Chromatography is the separation process in which a mixture of components to be separate is carried by a mobile phase (i.e. solvent) to pass through a stationary phase (adsorbent) (Berezkin et al, 2006). Each component in the mixture will interact differentially between the mobile phase and adsorbent in the column. The smaller the affinity of molecule had for the stationary phase, the shorter time it spent in the column.

The adsorbent normally packed into a cylindrical column or known as packed bed chromatography. However, there are several major limitations of packed bed chromatography such as high pressure drop, flow channeling and long processing time due to limited flow rate operation. Most of this limitation can be overcome by using membrane chromatography (Ghosh, 2002). In the current study, membrane chromatography was

prepared through modification of commercial polyamide membrane by grafting methacrylic acid monomer.

1.2 Problem Statement

Packed bed chromatography used in protein separation have several limitations such as high pressure drop, increasing process time due to slow flow rate capability and flow channeling problem. Membrane chromatography is an alternative for packed bed chromatography for protein separation. Commercial and ready-made microfiltration can be transformed into membrane chromatography material by introducing specific functional group that can interact with the protein during the separation process.

In this study, the polyamide membrane was grafting with methacrylic acid to introduce carboxyl group, which can interact with the positive protein, becoming cation exchange membrane chromatography.

1.3 Research Objectives

The main objective of this study is to prepare cation exchange membrane chromatography by introducing the carboxyl group into the polyamide membrane by grafting methacrylic acid monomer.

1.4 Scope of Research

In order to fulfill the research objective, the following scopes were outlined:

i. Optimizing the attachment of carboxyl group that come from the methacrylic acid which is act as monomer by study the effect of the five different methacrylic acid concentration which is 0.1, 0.3, 0.5, 0.7 and 1.0 M during the grafting of polymer at the surface of the polyamide membrane.

ii. Study the effect reaction time to the polymer carboxyl group formation at the polyamide membrane surface with six different times which is between 15 to 120 min.

iii. Characterize the performance of the cation-exchange membrane chromatography by measuring the maximum protein binding capacity using bovine serum albumin (BSA) as the model protein.

CHAPTER 2

LITERATURE REVIEW

2.1 Membrane

Membrane can be defined as a barrier which separate two phase and restrict the transport of various chemical species in a specific manner. A membrane can be homogeneous or heterogeneous, symmetric or asymmetric in structure; it may be solid or liquid; it may be neutral, may vary between less than 100 nm to more than a centimeter of pore size. Mass transport through a membrane may be caused by convection or by diffusion or by diffusion of individual molecules, induced by electric field, or concentration, pressure or temperature gradient (Noh, 2008).

Polyamide membrane is a type of asymmetric membrane which is porous support layer from polysulfone. Polyamide membranes also comprise of a fabric and it was an ultra-thin salt rejection barrier layer. Usually polyamide membrane was used in water treatment and seawater desalination (Tarboush, 2010). The formula structure of polyamide and example of commercial polyamide membrane is showed in Figure 2.1.

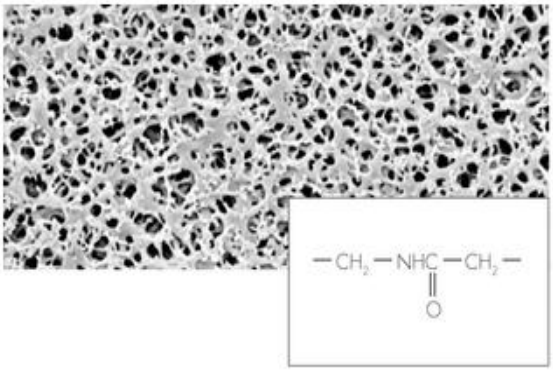

Figure 2.1: Structure of Polyamide Membrane

Polyamide membrane is a hydrophilic membrane and it also resistance to a chemical solution such as alkaline solution and organic solvent (Kasher, 2009). The recommended application of polyamide membrane is for the particle removing from water, aqueous solution and solvent. Polyamide membrane also highly recommended for the isolation of Legionella bacteria.

2.2 Ion Exchange

Ion exchange can be dividing into two types which are cation and anion exchanger. Electrostatic interaction occurs between ions in a solutions and ion in insoluble solid phase as shown in Figure 2.2.

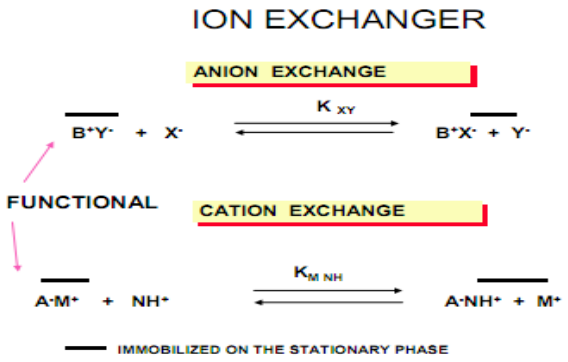

Figure 2.2: The Principle of Anion and Cation Exchanger Process

For cation exchange, the more electronegativity the cation elements in the solute, more easily the positively charge in the solid to be replaced and by following the cationic order that are $Ba^{2+} > Pb^{2+} > Sr^{2+} > Ca^{2+} > Ni^{2+} > Cd^{2+} > Cu^{2+} > Co^{2+} > Zn^{2+} > Mg^{2+} > Ag^{+} > Cs^{+} > K^{+} > NH_4^{+} > H^{+}$. The lowest ion were chosen for the solute in order to make the exchange of ion between solid and solute exist which means the more suitable cation is hydrogen ion (H^{+}) which have the lowest electropositive.

For anion exchange, more electropositive the anion elements in solute, the negatively charged in the solid will be removed easily and the place will replace with the negatively charged of solute. To choose the lowest electronegativity of solute for anion exchange are by following the anionic order that are $SO_4^{2-} > I^{-} > NO_3^{-} > HCrO_4^{-} > Br^{-} > Cl^{-} > OH^{-}$. From the anionic order, the best ion that can be choose in order to exist the exchange between solute and solid id hydroxide ion (OH^{-}) because it has the lowest electronegativity (Geankoplis, 2003).

2.3 Protein

Proteins are macromolecules and made from amino acids which linked by covalent peptide bond (Chang & Raymond, 2003). Every protein have it unique and genetically

amino acid sequences that determining it specific shape and function such as coordinated motion, enzyme catalysis and generation and transmission (Albert et al, 2002).

Protein can be divided into three classes (Smith and Janice, 2006). First class of protein composed of long linear polypeptide chain that bundled together and form a rods or sheets. These proteins are hydrophobic and because of that, these protein roles are giving the protection to the tissues and cells and also the giving the strength.

Second class of protein is globular protein which having hydrophilic at their outer surface that makes them water soluble. Examples of this second class of protein are enzyme and transport protein. This protein is soluble in the blood and other aqueous environment in cells.

The third class of protein is a membrane protein that having role as receptors or provide channel for polar or charge molecule to passing through cell membrane.

Proteins contains positive and negative charge group depending on the amino acid sequence. Isoelectric point (pI) is the pH of the protein where the number of positive and negative charge is equal or it carries zero net charge. Table 2.1 gives the pI value of several common proteins.

Table 2.1: Isoelectric Point of Several Common Proteins

Protein	**Isoelectric pH**
Pepsin	<1.0
Ovalbumin (hen)	4.6
Serum Albumin (human)	4.9
Tropomyosin	5.1
Insulin (bovine)	5.4
Fibrinogen (human)	5.8

γ-Globulin (human)	6.6
Collagen	6.6
Myoglobin (horse)	7.0
Hemoglobin (human)	7.1
Ribonuclease A (bovine)	7.8
Histone (bovine)	10.8
Lysozyme (hen)	11.0
Salmine (salmon)	12.1

The charge of the protein depend strongly on the pH of the solution relative to it pI value as showed in Figure 2.3. If the pI of the protein below the pH of solution, the protein will has net negative charge and bind to anion-exchanger (Cutler, 2004).

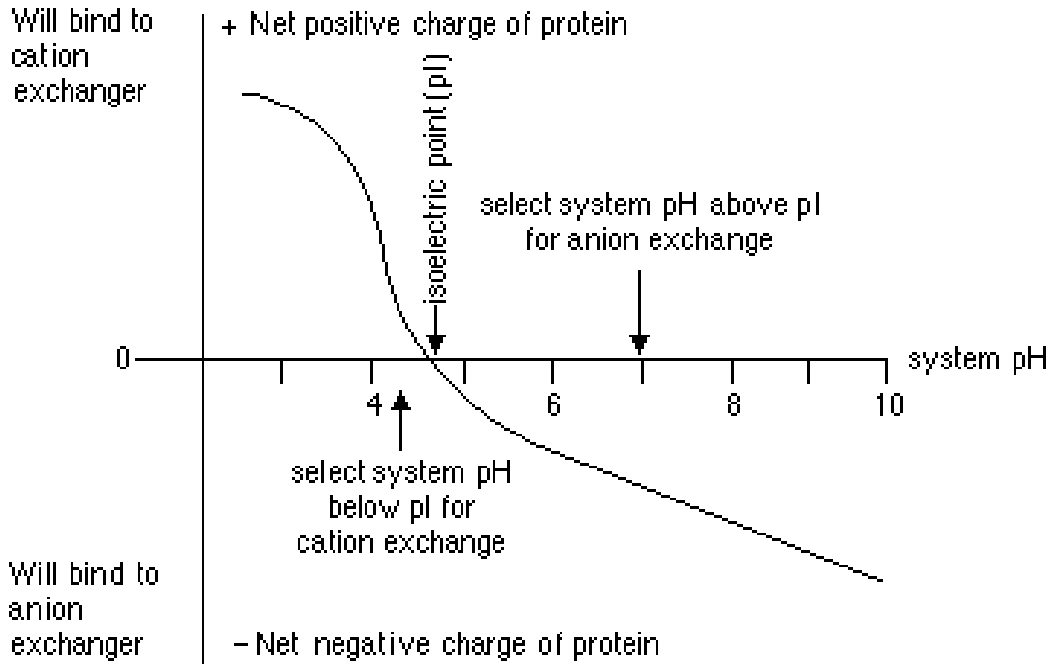

Figure 2.3: Relationship between System pH and Pi Related to the Protein Charged

2.3.1 Bovine Serum Albumin

Bovine serum albumin (BSA) is a single peptide chain that contains no carbohydrate and consisting 583 amino acids residues. The properties of BSA are showed in Table 2.2 (Haginaka, 2001).

Table 2.2: Physical Properties of Bovine Serum Albumin (Tra, 2007)

Properties	**Value**
Number of amino acid	583
Molecular Weight	69 000 Da
Isoelectric Point	4.7- 4.9
Molecular Size (Å)	40 x 40 x140

BSA is soluble in the water but can be precipitate at the high concentration of neutral salts such as ammonium sulphate. BSA has very good solution stability and because of that, BSA was used as stabilizer for other solubilized proteins such as enzyme. However, BSA will coagulate by heat starting from temperature 50°C and above and it rapidly forming hydrophobic aggregates which do not revert back to monomer upon cooling. At low temperature BSA also will coagulate but it relatively occurs in a slow rate (Burgess, 2008).

BSA is an acidic proteins group which occurs plentifully in the body fluids and tissues of mammals and in some plant seeds (Benedek, 1999). Due to the hydrophobic cleft of BSA, it can bind with water, Ca^{2+}, Na^{+}, K^{+}, fatty acids, hormones and drugs. In the biological, BSA was used to regulate the colloidal osmotic pressure blood. It also used to stabilized lipids and as blocking agent in enzyme-linked immunosorbent assay (ELISA) application.

2.4 Protein Purification

There are several techniques available for protein separation such as chromatography, membrane filtration, centrifugation, precipitation and membrane chromatography. The following section will discuss in detail about the chromatography based technique which commonly used in protein separation.

2.4.1 Chromatography

Chromatography process can be defined according to the interaction mechanism between the component and the stationary phase, or according to the type of the mobile phase used. Based on interaction mechanism, several types of chromatography such as ion exchange chromatography, hydrophobic interaction chromatography, affinity chromatography and reverse phase chromatography are available (Garret and Grisham, 2008). Figure 2.4 shows the different interaction mechanism in chromatography based separation process.

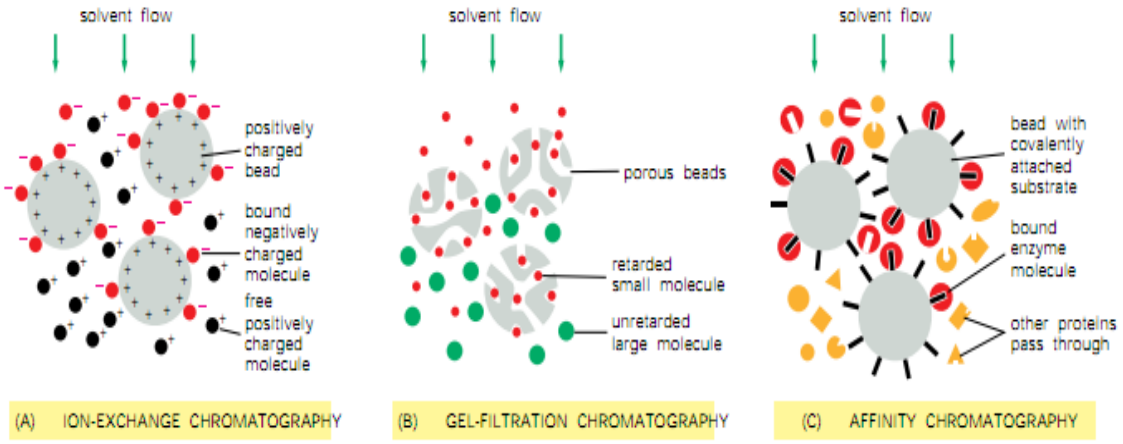

Figure 2.4: Interaction Mechanism in Chromatography Based Process

Ion-exchange chromatography media is carried certain charged either positive or negative charges. In Figure 2.4 as an example, the media had a positively charged group.

The negatively charged protein will be attracted with the media while the positively charged protein will freely flow through the chromatography media. At this condition, the positively and negatively protein can be separated.

In gel-filtration chromatography, the protein components are separated according to their size and pore size of the media. The small protein molecule can enter through the porous structure of the media beads and become delayed and travel slowly through the column. Bigger protein will excluded from entering the pore of the media and will elute early from the column.

For affinity chromatography, the ligand attached to the media had a specific interaction with the target protein molecule like a key-and-lock interaction. Therefore, only specific protein can bind to affinity chromatography and other protein will flow through the column.

2.4.2 Ion Exchange Chromatography

Two types of ion exchange chromatography available which is anion exchange chromatography and cation exchange chromatography (Stojiljkovic, 1999). Anion exchange chromatography will attract positively charged protein and cation exchange chromatography will attract negatively charged protein. The charged of the protein will depend on the isoelectric point (pI) and the pH of the operation.

Separation in ion exchange chromatography depends upon the reversible adsorption of charged solute molecules to immobilized ion exchange groups of opposite charge. Most ion exchange experiments are performed in five main stages (Harris, 2003) as shown in Figure 2.5.

In first step, an anion-exchange column was equilibrated with the suitable buffer solution. Anion-exchange was equilibrating with the buffer solution in order to increase the rate of transfer of the exchanger. The second stage is then negatively charged solute

components exchanging the ion position with the buffer solution and the salt gradient were applied. The protein component which have the lower affinity eluted first in the column by the lower concentration of the salt solution. In the fourth stage, the higher affinity of the protein component eluted after all the lower affinity of the protein component eluted. These high affinity protein components were eluted by a higher concentration of the salt solution. At the last stage, the column was equilibrated with the starting buffer and then was followed by the regeneration of the column.

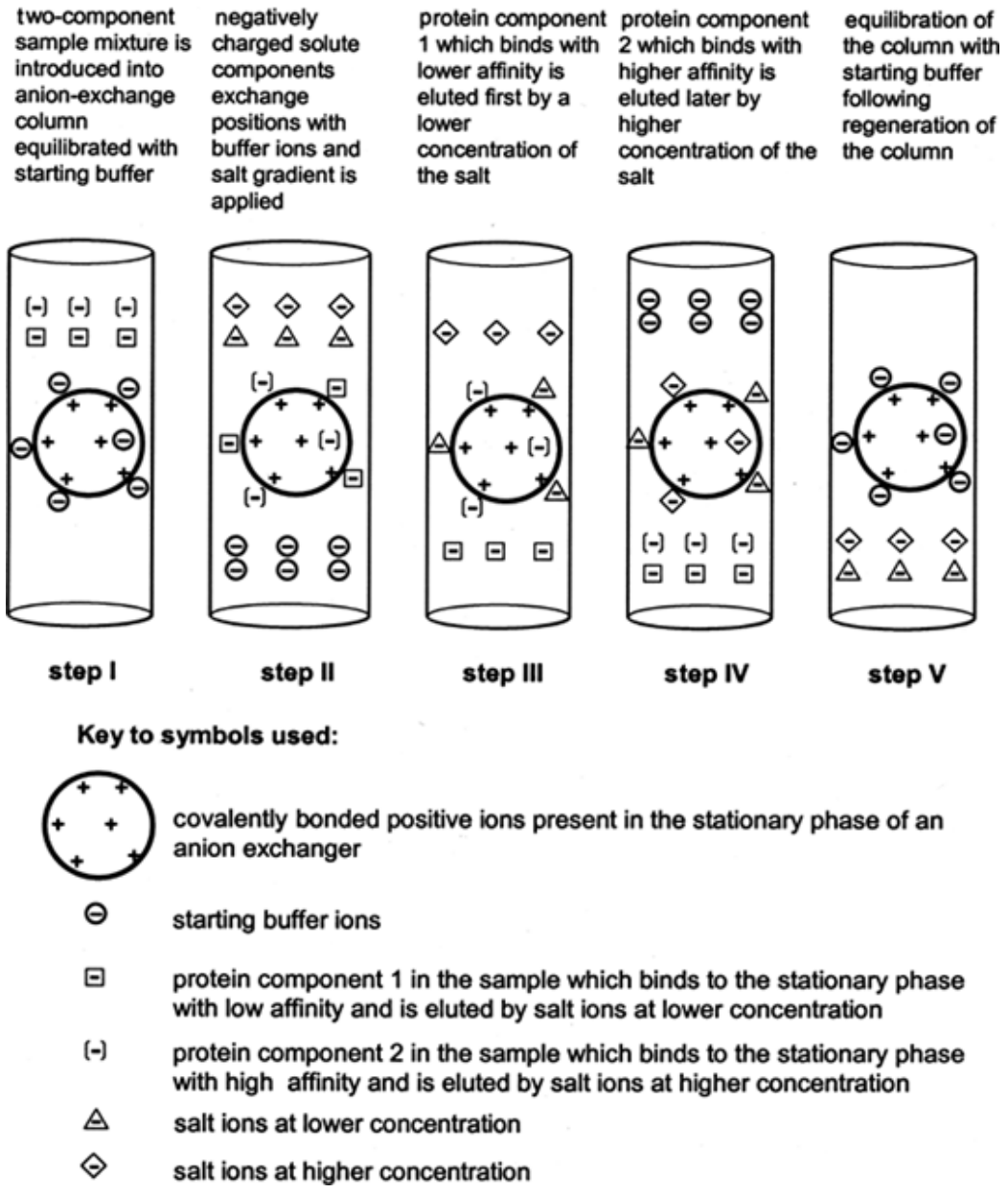

Figure 2.5: Principle of Ion Exchange Chromatography **(**Amersham Bioscience, 2008)

2.4.3 Packed Bed Chromatography

Chromatography media normally packed into cylindrical column or known as packed bed chromatography. However, there is several major limitation of packed bed configuration especially regarding to high pressure drop across the packed bed column

(Ghosh, 2002) and it tends to increase because of the combined effect of bed consolidation (Kawai et al, 2003).

Packed bed column also suffered from chanelling problem due to the flow passage through the cracked packed bed. This also will make the scale up process more complicated. The process time also increase in packed bed due to the limitation of using high flow rate to avoid excessive pressure drop. Moreover, the pore inside the packed bed was too samll and it cannot be used in the separation of the larger protein size (Zou et al, 2001).

2.4.4 Membrane Chromatography

The limitation of the packed bed chromatography can be overcome by using a membrane chromatography (Ghosh, 2002, Ghosh and Wong, 2006). The mass transfer resistance can be reduced by membrane chromatography but the diffusion transport is not totally absent. Normally, three main shapes of the membranes is used in membrane chromatography for the protein separation which are flat sheet, hollow fiber and radial flow (Cao, 2005). Membrane chromatography has an ability to purify a large biomolecules because of the open pores of the structure offers a three-dimensional structure. A channel was created from the large pore size for immediately available the active chemistry groups on the membrane surface and it will allow the high binding capacity of the large protein particles (Sellick, 2006). Other than that, the membrane chromatography can overcome the highly pressure drop in the packed bed chromatography because membrane chromatography can be operated at high flow rate and this will also reducing the process time (Ghosh, 2002)

Membrane chromatography can also be used for the larger scale of the separation process including isolation, purification and recover high purity of the protein and enzymes (Zeng and Ruckensein, 1989). Figure 2.6 shows the fluid flows inside the packed bed chromatography and membrane chromatography.

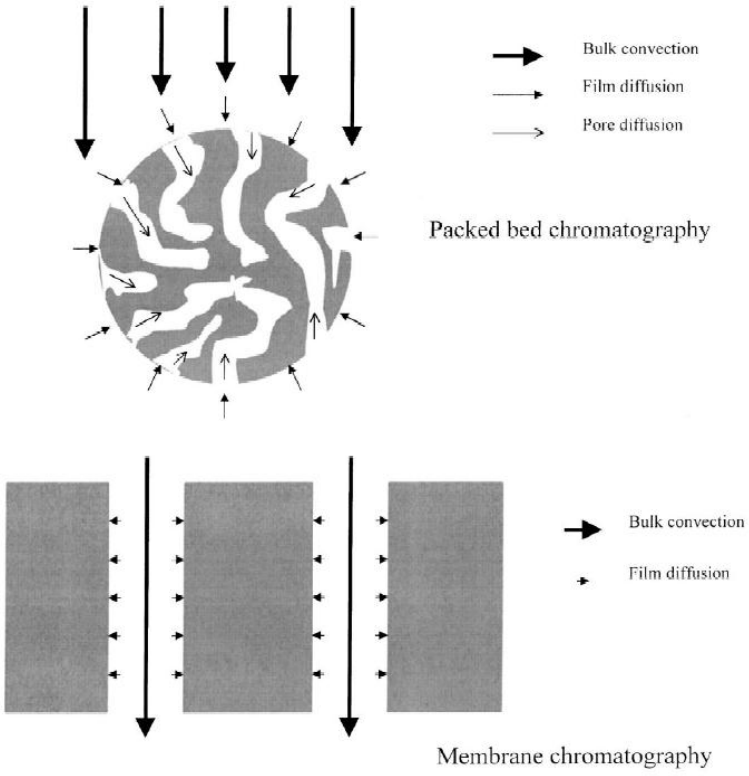

Figure 2.6: Solute Transport in Packed Bed Chromatography and Membrane Chromatography **(**Saxena et. al, 2009)

2.5 Development of Ion Exchange Membrane Chromatography

Several techniques have been used to prepare cation-exchange membrane chromatography. Some of them was summarized in Table 2.3. Hwang et al (1999) produced cation exchange membrane for electrodialysis process by sulfonation of polysulfone and polyphenylenesulfone. The membrane produced has a transport number of the cation in range 0.77 to 0.87 and ion exchange capacity of 1.9meq/(g-dry-resin).

Fang et al (2004) studied the sulfonation process and membrane preparation process during preparation of polysulfone cation-exchange membrane. In this study, they found the most favourable sulfonation condition was at 75ºC for 4 hours and the ratio of the

polyethylene glycol (PEG) and N-methylpyrrolidne (NMP) is 5:3. The membrane has a thickness of 90µm with an ion exchange capacity of 2.9µeq/cm^2.

Belfer et al (1998) introduced a charged carboxyl functional group on polyamide membrane in order to overcome fouling in thin-film composite (TFC) reverse osmosis membranes. Methacrylic acid (MA) and polyethyleneglycolmethacrylate (PEG-MA) was used as monomer to introduce this carboxyl functional. The modified membrane was characterized by using attenuated total reflection fourier transform infrared (ATR-FTIR) to confirm the C=O attachment on membrane.

Freger et al (2002) used acrylic acid (AA) as monomer in preparing charged TFC membrane. They study the effects of modification time, monomer concentration and concentration of initiator during the membrane preparation.

Table 2.3: Development of Ion Exchange Membrane Chromatography

Author / Year	Polymer	Summary
Hwang et al (1999)	-Chloromethylated Copolymer -Sulfonated Copolymer	- Investigate the purpose by applying electrodialysis process - Cation-exchange capacity reach up to 1.9 meq/ (g-dry-resin) - Develop the cation-exchange membrane chromatography.
Fang et al (2004)	-Polysulfone	- Prepare membrane using different condition - Found the water content of membrane is 0.68 and ion exchange capacity is 2.9µeq/cm^2 - Study the saturation capacity of lysonzyme - Develop the cation-exchange membrane chromatography.

S.Belfer et al (1999)	-Polyamide Composite Membrane	- Using polyamide membrane composite. - For reverse osmosis (RO) membrane. - Modifying the polyamide membrane surface by using graft polymerization to improving fouling for RO membrane. - Before grafting with the cross-linker and monomer, it will going through redox process. - The C=O groups bonding to the membrane were increasing with the time. - Develop the cation-exchange membrane chromatography. - Membrane surface was modified by using different reaction time and the checked by using ATR-FTIR.

V. Freger et al (2002)	- Thin-film Composite (TFC) polyamide membrane	- Develop cation-exchange membrane chromatography. - Modified membrane surface by using grafting method to fouling properties thin-film composite membrane. - Modified by using graft polymerization of acrylic (AA) monomer and introduce carboxyl functional group. - Modifying by using different concentration of Redox solution, monomer concentration and reaction time. - Modified membrane was analyzed by using ATR-FTIR.

CHAPTER 3

METHODOLOGY

3.1 Materials

Commercial polyamide membranes with pore size of 0.2µm were purchased from Sartorius Stedim. In redox reaction, potassium persulfate ($K_2S_2O_8$) and potassium metabisulfite ($K_2S_2O_5$) were purchased from Mercks. In the reaction of crosslinker and monomer, ethylene glycol dimethacrylate (EGDMA) and methacrylic acid respectively were bought from Mercks.

Sodium hydroxide (NaOH) and hydrochloric acid (HCl) used in washing step were purchased from Mercks and Fisher Scientific respectively. Bovine serum albumin (BSA) from Sigma was used as sample protein in binding experiment. Acetic acid and sodium acetate were used to prepare a buffer solution.

3.2 Solution Preparation

Redox solutions were prepared by from 0.01 M of potassium metabisulfite ($K_2S_2O_5$) and 0.01 M of potassium persulfate ($K_2S_2O_8$). These two solutions were mixed together. EGDMA which act as a cross-linker also prepared with a concentration 0.01 M. The concentration of methacrylic acid monomer was varied with five different concentration of 0.1, 0.3, 0.5, 0.7 and 1.0 M.

Sodium hydroxides (NaOH) pH 10 and hydrochloric acid (HCl) pH 4 were used as washing solution to the modified polyamide membrane in order to remove loosely bound homopolymer. Appropriate amount of sodium hydroxide (NaOH) and hydrochloric acid (HCl) was diluted in deionized (DI) water calculated using formulas shown in appendix A. The pH for both solutions were checked by using pH meter to make sure the targeted pH achieved.

20mM acetate buffer pH 4 for binding experiment was prepared by mixing 0.49 g of sodium acetate and 0.94 ml of acetic acid in 1000ml of distilled water according to the buffer recipe. This buffer solution was filtered with 0.2µm nylon membrane filter using vacuum filter as shown in Figure 3.1. Filtering is necessary to avoid any impurities inside the buffer solution that may affect the binding of the protein onto the modified polyamide membrane.

Figure 3.1: Vacuum Pump

3.3 Standard Curve

Stock solution BSA was prepared in acetate buffer pH 4.0 at concentration of 2 mg/ml. BSA stock solution was diluted in series to get another six standards with concentration of 1.0, 0.5, 0.25, 0.125, 0.0625 and 0.03125 mg/ml. The absorbance of each standard was measured at 280 nm wavelength using UV-Vis spectrophotometer. Standard curve was constructed by plotting the abs-concentration graph.

3.4 Preparation of Cation-Exchange Membrane Chromatography

Polyamide membrane was cut into square shape of 1 cm x 1 cm and immersed into 2 ml redox solution in test tube. The mixture was mixed on rotator at speed 25 rpm for 30 minutes as showed in Figure 3.2. During redox process, radicals will be generated to allow graft polymerization reaction in the next step.

Figure 3.2: Rotator

After the redox process, the membranes was added to the monomer solution together with the cross linker. At this step, the monomers of carbonyl group has been grows by propagation. Different reaction time was studied at 15, 30, 45, 60 and 120 minutes.

Next, the membranes were proceeding with the washing step in order to remove the loosely bound of homopolymer that exist at the surface of the membranes. The modified polyamide membrane were immersed into 2 ml NaOH at pH 10 for 24 hours at room temperature and then washed with deionized (DI) water. Second washing was done by washing the membrane with 2 ml hydrochloric acid (HCl) at pH 4.0 for another 24 hours at room temperature and followed by washing with DI water until the pH of the membrane became neutral. The neutral modified polyamide membrane was left inside the water bath for 24 hours with temperature between 30 and 40°C.

3.5 Protein Binding

Membrane was equilibrated in binding buffer in rotator for 2 hours. After that, the membrane was taken out from the solution and gently dried with tissue. The equilibrated membrane was incubated for 4 hours with 2 ml of 2 mg/ml of BSA dissolved in sodium acetate pH 4 for binding experiment. The remaining BSA concentration was measured by UV-vis spectrometer to calculate the amount of BSA bound to the membrane. The binding capacity of the polyamide and modified membrane was calculated by using Equation 3.1.

$$Q = \frac{(C_o - C_f)V}{w} \qquad \textbf{(Equation 3.1)}$$

Where

C_f = final concentration of the protein, mg/ml

C_o = initial concentration of the protein, mg/ml

V= volume of the protein, ml

W = weight of the membrane, mg

3.6 Membrane Characterization

3.6.1 Fourier Transform Infrared (FTIR)

Fourier Transform Infrared (FTIR), from Thermo Scientific was used to identify the functional group on original and modified membrane. By using the FTIR thin film plate, the functional group forms at the surface of the membrane were identified. Before the plate was used, the plate was cleaned by using acetone to remove any dust or substances that were affected the reading of the membrane.

3.6.2 Water Content

Modified membranes with a size of 1 cm x 1 cm were soaked in the DI water for 24 hours. Then, the wet membrane was removed and wiped with tissue to remove the excess surface water on the membrane. The weight of wet membrane was measured. This membrane was dried at 60°C for 30 minutes. After 30 minutes, the dry membranes weight was measured. The water content of membrane was calculated by using Equation 3.2.

$$W_C = \frac{(W_W - W)}{W_W} \qquad \textbf{(Equation 3.2)}$$

Where W_W is the weight of the wet membrane and W is the weight of dry membrane.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Standard Curve

Standard curve were prepared in order to calculate the final concentration of the protein after binding with the modified polyamide membrane. Figure 4.1 shows the standard curve of abs-concentration data determine by UV-Vis spectrophotometer. The standard curve gives an equation of $y = 0.5993x - 0.029$ with R^2 value of 0.9988.

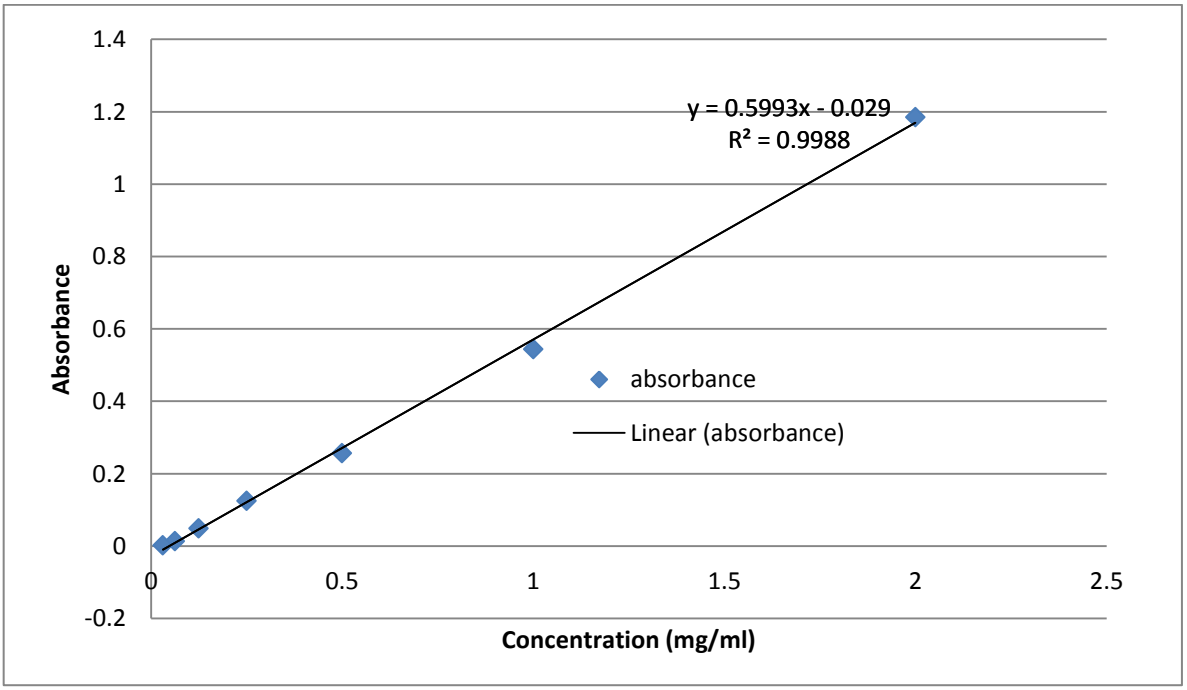

Figure 4.1: Standard Curve from the Seven Different Concentrations of BSA Solutions.

4.2 Effect of the Reaction Time and Monomer Concentration

The binding capacity of unmodified and modified polyamide membrane is shown in Table 4.1 and Table 4.2 respectively. In Table 4.2 different monomer concentration and reaction time was shown.

Table 4.1: Result from Binding of BSA Protein with the Unmodified Polyamide Membrane

Set	1	2	3
Mass membrane, mg	0.0115	0.0115	0.0115
Binding volume, ml	2	2	2
Initial BSA concentration, mg/ml	2	2	2
Abs after binding	1.018	1.023	1.031
Final BSA concentration, mg/ml	1.747	1.755	1.769
Binding capacity, mg BSA/g membrane	114.923	111.190	105.123

Since the monomer used is methacrylic acid, it introduced a carboxyl group to the polyamide membrane surface and formed a positive charged at the membrane surface. From FTIR analysis, the carboxyl group was identified within the wavelength between 1700 and 1720 cm^{-1}. The result of FTIR analysis is showed in the Appendix B. The results of FTIR analysis were compared with the binding capacity of the membrane. When the absorbance of the membrane is high, it means the total number of carboxyl group is high and the tendency to bind with more of protein is also high.

Table 4.2: Effect of the Reaction Time and the Monomer Concentration on the Protein Binding Capacity

Monomer Concentration, M	Reaction Time, min	Binding Capacity, mg/g
Polyamide membrane	-	110.43
0.1	15	313.36
	30	254.91
	45	599.64
	60	102.72
	120	242.44
0.3	15	143.93
	30	170.22
	45	179.97
	60	413.64
	120	240.49
0.5	15	100.31
	30	156.73
	45	259.83
	60	109.869
	120	167.87
0.7	15	150.35
	30	97.69
	45	159.64
	60	141.52
	120	107.03
1.0	15	233.06
	30	150.44
	45	301.09
	60	195.73
	120	177.45

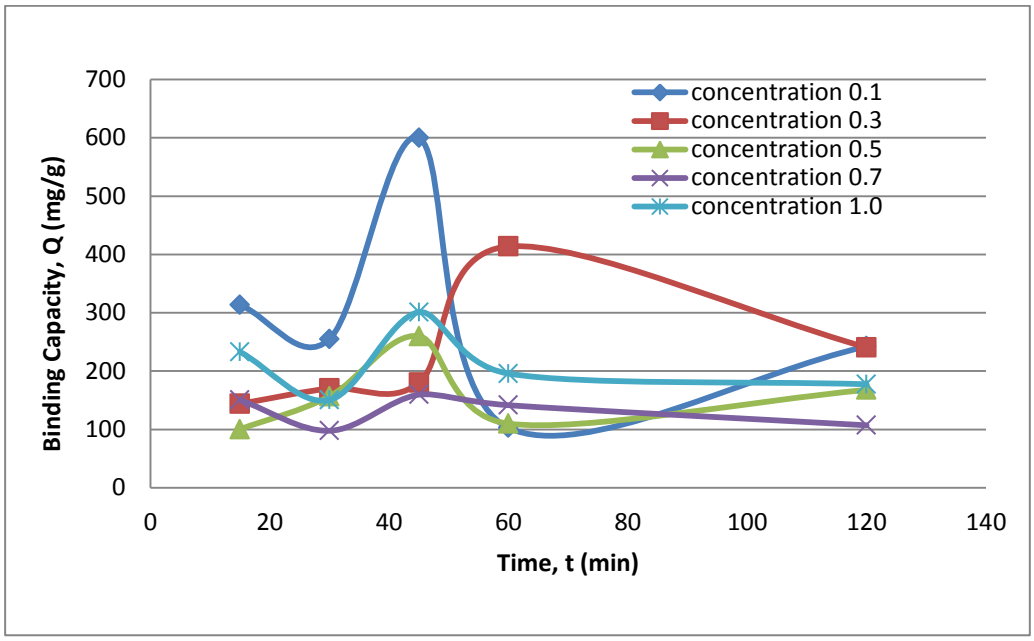

Figure 4.2: Effect of Time to the Binding Capacity of the Membrane at Different Concentration

In Figure 4.2, it can be seen that the increasing or decreasing of binding capacity of different monomer concentration does not affect by increasing of time. It happened when the optimum time for the polymerization of carboxyl group to grow on the membrane surface has been reach.

The optimum time for all monomer concentration is at 45 minutes of time reaction but for methacrylic acid with concentration 0.3 M has an optimum reaction time of 60 minutes. After reaching optimum time, binding capacity of the membrane are decreasing.

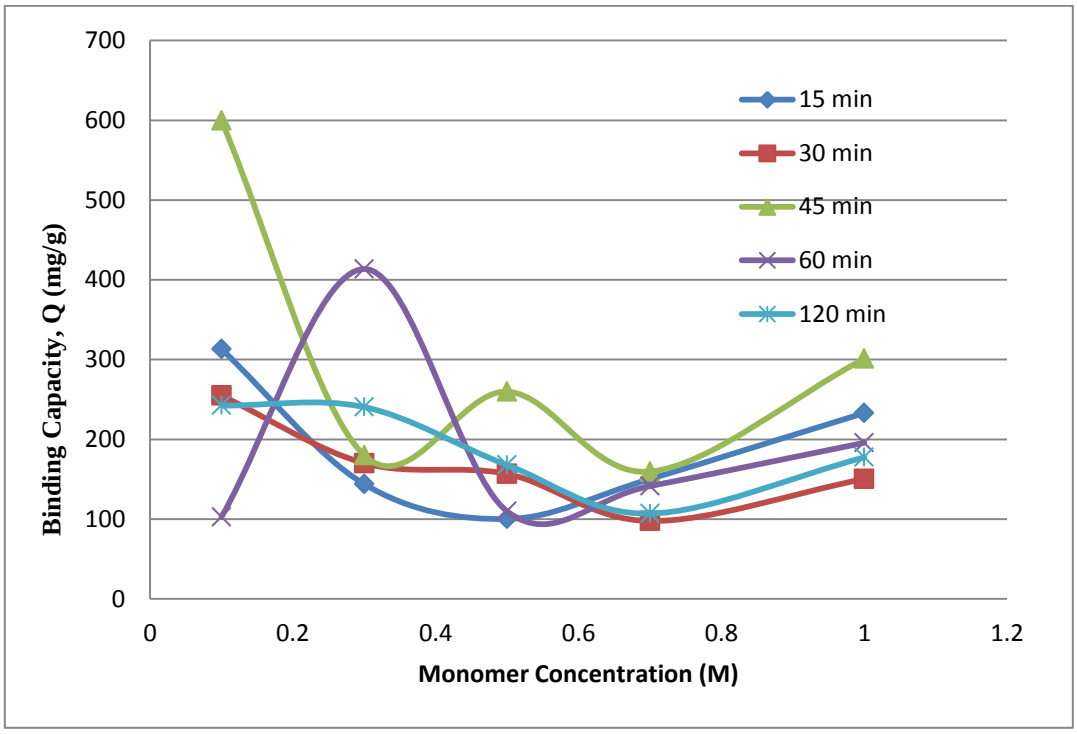

Figure 4.3: Effect of Different Monomer Concentration to the Binding Capacity of the Membrane at Different Time

For the effect of the monomer to the binding capacity of the BSA protein, the increasing or decreasing of the binding capacity is affected from the growth of carboxyl group that comes from the methacrylic acid. When carboxyl group at the surface of polyamide membrane was increase, the binding capacities of the membrane also increase.

From the Figure 4.3, it can be found that; monomer with concentration of 0.1 M at 45 minutes of reaction time has the highest binding capacity. Meanwhile, the modified membrane that has the lowest binding capacity is at the monomer concentration 0.1M which their reaction time 60 minutes. It means that the binding capacity, Q was not directly proportional to the monomer concentration.

4.3 Water Content

Table 4.3 below shows the comparison of the water content for the unmodified polyamide membrane and modified polyamide membrane.

Table 4.3: Water Content

Monomer Concentration, M	Reaction Time, min	Weight of Wet Membrane (W_w), g	Weight of Dry Membrane (W), g
Polyamide membrane	-	0.0106	0.0044
0.1	15	0.0128	0.0046
	30	0.0123	0.0061
	45	0.0137	0.0044
	60	0.0131	0.0076
	120	0.0118	0.0035
0.3	15	0.0142	0.0052
	30	0.0129	0.0049
	45	0.0134	0.0055
	60	0.0136	0.0037
	120	0.0137	0.0032
0.5	15	0.0124	0.0047
	30	0.0147	0.0054
	45	0.0121	0.0047
	60	0.0135	0.0058
	120	0.0126	0.0053
0.7	15	0.0129	0.0052

	30	0.0129	0.0079
	45	0.0134	0.0065
	60	0.0141	0.0051
	120	0.0137	0.0064
1.0	15	0.0129	0.0045
	30	0.0138	0.0055
	45	0.0121	0.0057
	60	0.0115	0.0045
	120	0.0111	0.0046

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

From this research, it can be conclude that the carboxylic group polymerization were increase when the times are increase until it meets their optimum concentration and optimum time. After this optimum point, the carboxylic group polymerization will became lower. When the monomer concentration increase, the binding capacity of the monomer also increase. But when it reaches at their limit, the binding capacities were become lower than binding capacity at the optimum.

5.2 Recommendation

There were several recommendations that can be taken for further study in order to improve the preparation of membrane chromatography as listed below:

.

i. Use an Attenuated Total Reflectance (ATR-FTIR) for characterization of modified polyamide membrane. ATR-FTIR can determine the changes at the surface of the material accurately compared to normal FTIR.
ii. Optimize the concentration of redox solution in radical generation process
iii. Used other monomer such as acrylic acid, sulfonic acid to form a charge group on polyamide membrane

REFFERENCES

Abu Tarboush, Matsuura, Takeshi, Belal, Rana, Dipak & Arafat, Hassan A. (2010, February 18). Thin Film Composite Membrane Preparation and Surface Modification. *SciTopics*. Retrieved January 12, 2012, from http://www.scitopics.com/Thin_Film_Composite_Membrane_Preparation_and_Surface_Modification.html

Albert, Bray, Johnson, Lewis, Raff, Roberts & Walter (1998). Protein Separation-Part 1. Garland Publishing.

Benedek (1999). Osmotic Pressure Contribution of Albumin to Colloidal Interactions. Retrived December 29, 2011 from http://www.pnas.org/content/96/12/6711.full

Chang and Raymond (2003). General Chemistry. 3rd Edition. New York: McGraw-Hill.

Fang J.-K , Chiu H.-C, Wu J.-Y , & Suen S.-Y (2004) "Preparation of Polysulfone-Based Cation-Exchange Membranes and Their Application in Protein Separation with a Plate-and-Frame Module". *Journal of Reactive and Functional Polymer*. 59, 171–183.

Geankoplis (2003). Transport Process and Separation Process Principle (4th ed.). USA:Bernard Goodwin.

Ghosh R. (2002). "Protein Separation Using Membrane Chromatography: Opportunities and Challenges". *Journal of Chromatography A*. 952, 13-27.

Ghosh R & Wong T (2006) "Effect of Module Design on the Effeciency of Membrane Chromatographic Separation Processes". *Journal of Membrane Science*. 281, 532-540

Haginaka Jun (2001). Protein-Based Chiral Stationary Phases for High-Performance Liquid Chromatography Enantioseparation. *Journal of Chromatography A*. 906, 253-273.

Hwang G.-J, Ohya H & Nagai T (1999) "Ion Exchange Membrane Basedon Block Copolymers. Part III: Preparation of Cation Exchange Membrane". *Journal of Membrane Science*. 156, 61-65.

Kawai T, Saito K, Lee W.(2003) "Protein binding to polymer brush, based on ion-exchange, hydrophobic, and affinity interactions."*Journal of Chromatography B*. 790, 131-142.

M.M. Soltys, Z.M. Yaremko, N.H. Tkachenko, V.D. Havryliv (Sept, 2002). "Poly (Methacrylic Acid) Adsorption and Electrical Surface Properties of Titanium Dioxide Suspensions". *Journal of Adsorption Science and Technology*. 20(7), 633-645.

Malito. E. Article of Separation Method. Retrieved October 20, 2011 from http://www.stampcollectingproject.org/random-stuff/biochem/separation-methods

Matsumoto Y, Sudoh M & Suzuki Y (1999) "Preparation of Composite UF Membranes of Sulfonated Polysulfone Coated on Ceramics". *Journal of Membrane Science*. 158, 55-62.

Md. Noh. N (May, 2008). Thesis Fabrication of Chitosan Membrane: The Effects of Different Chitosan Composition on Membrane Performance in Treating Oily Wastewater.

On-line Medical Dictionary. Article of Isoelectric Point. Retrieved October 17, 2011 from http://www.jcsg.org/help/robohelp/Definitions/Isoelectric_Point.htm

Reinald Garrett & Charles M. Grisham (2008). Biochemistry. USA: Mary Finch.

S. Belfer, J. Gilron, O. Kedem (1999). "Characterization of Commercial RO and UF modified and Fouled Membranes by Means of ATR/FTIR". *Journal of Desalination*. 124,175-180.

Saiful, Borneman Z, Wessling M (2006) "Enzyme Capturing and Concentration with Mixed Matrix Membrane Adsorbers". *Journal of Membrane Science*. 280, 406-417.

Sartorius Stedim Biotech. Article of Polyamide Membrane Filter. Retrieved January 1, 2012 from http://www.sartorius-stedim.com/WW/en/Membrane--Depth-Filters/Polyamide-Membrane-Filters/klbtfti3g8y/dhi6wxx/py8/mp.htm?view=desc

Saufi S.M (2010). Thesis of Mixed Matrix Membrane Chromatography for Bovine Whey Protein Fractionantion.

Sawai. F (May, 2009). Thesis of Protein Purification by Using Immobilized Metal Ion Affinity (IMA) Adsorbent.

Smith and Janice. G (2006). Organic Chemistry. New York: McGraw-Hill

V. Fregger, J. Gilron, S. Belfer (2002). "TFC Polyamide Membranes Modified by Grafting of Hydrophilic Polymers: an FTIR/AFM/TEM Study". *Journal of Membrane Science*. 209, 283-292.

Zeng X & Ruckenstein E (1999) "Membrane Chromatography: Preparation and Application to Protein". *Journal of Biotechnology Program*. 15, 1003–1019.

Zou H, Lou Q & Zhou D. (2001) "Affinity membrane chromatography for the analysis and purification of proteins." *Journal of Biochemical and Biophysical Methods*. 49, 199-240.

APPENDIX A
CALCULATION OF PREPARATION OF SOLUTIONS

Preparation of 500 ml of 0.01 M Potassium Persulfate, $K_2S_2O_8$

Molecular weight, MW of $K_2S_2O_8$ = 270.32 g/mol

$$m = \frac{MV}{1000}\,(MW)$$
$$m = \frac{(0.01)(500)}{1000}\,(270.32)$$
$$m = 1.3516\ g$$

Preparation of 500 ml of 0.01 M Potassium Metabisulfite, $K_2S_2O_5$

Molecular weight, MW of $K_2S_2O_5$ = 222.33 g/mol

$$m = \frac{MV}{1000}\,(MW)$$
$$m = \frac{(0.01)(500)}{1000}\,(222.33)$$
$$m = 1.1117\ g$$

Preparation of 500 ml of 0.01 M Ethyleneglycol Dimetacrylate, EGDMA

Molecular weight, MW of EGDMA = 198.22 g/mol

$$\mathrm{m} = \frac{\mathrm{MV}}{1000}\,(\mathrm{MW})$$

$$\mathrm{m} = \frac{(0.01)(500)}{1000}\,(198.22\,)$$

$$\mathrm{m} = 0.9911\ \mathrm{g}$$

$$1.05\ \mathrm{kg} \equiv 1\ \mathrm{l}$$

$$0.9911 \times 10^{-3}\ \mathrm{kg} \equiv \frac{(0.9911 \times 10^{-3})\ 1000\ \mathrm{ml}}{1.05}$$

$$= 0.9440\ \mathrm{ml}$$

Preparation of 100 ml of 0.1 M Methacrylic Acid, MA

Molecular weight, MW of Acrylic Acid, MA = 86.09 g/mol

$$\mathrm{m} = \frac{\mathrm{MV}}{1000}\,(\mathrm{MW})$$

$$\mathrm{m} = \frac{(0.1)(100)}{1000}\,(86.09\,\mathrm{g/mol}\,)$$

$$\mathrm{m} = 0.8609\ \mathrm{g}$$

$$1.015\ \mathrm{kg} \equiv 1\ \mathrm{l}$$

$$0.8609 \times 10^{-3}\ \mathrm{kg} \equiv \frac{(0.8609 \times 10^{-3}\mathrm{kg})\ 1000\ \mathrm{ml}}{1.015kg}$$

$$= 0.8482\ \mathrm{ml}$$

Preparation of 100 ml of 0.3 M Methacrylic Acid, MA

Molecular weight, MW of Acrylic Acid, MA = 86.09 g/mol

$$\mathrm{m} = \frac{\mathrm{MV}}{1000}\ (\mathrm{MW})$$

$$\mathrm{m} = \frac{(0.3)(100)}{1000}\ (86.09\ \mathrm{g/mol})$$

$$\mathrm{m} = 2.5827\ \mathrm{g}$$

$$1.015\ \mathrm{kg} \equiv 1\ \mathrm{l}$$

$$2.5827 \times 10^{-3}\ \mathrm{kg} \equiv \frac{(2.5827 \times 10^{-3}\mathrm{kg})\ 1000\ \mathrm{ml}}{1.015kg}$$

$$= 2.5445\ \mathrm{ml}$$

Preparation of 100 ml of 0.5 M Methacrylic Acid, MA

Molecular weight, MW of Acrylic Acid, MA = 86.09 g/mol

$$\mathrm{m} = \frac{\mathrm{MV}}{1000}\ (\mathrm{MW})$$

$$\mathrm{m} = \frac{(0.5)(100)}{1000}\ (86.09\ \mathrm{g/mol})$$

$$\mathrm{m} = 4.3045\ \mathrm{g}$$

$$1.015\ \mathrm{kg} \equiv 1\ \mathrm{l}$$

$$4.3045 \times 10^{-3}\ \mathrm{kg} \equiv \frac{(4.3045 \times 10^{-3}\mathrm{kg})\ 1000\ \mathrm{ml}}{1.015kg}$$

$$= 4.2409\ \mathrm{ml}$$

Preparation of 100 ml of 0.7 M Methacrylic Acid, MA

Molecular weight, MW of Acrylic Acid, MA = 86.09 g/mol

$$\mathrm{m} = \frac{\mathrm{MV}}{1000}\,(\mathrm{MW})$$

$$\mathrm{m} = \frac{(0.7)(100)}{1000}\,(86.09\,\mathrm{g/mol})$$

$$\mathrm{m} = 6.0263\,\mathrm{g}$$

$$1.015\,\mathrm{kg} \equiv 1\,\mathrm{l}$$

$$6.0263 \times 10^{-3}\,\mathrm{kg} \equiv \frac{(6.0263 \times 10^{-3}\mathrm{kg})\ 1000\ \mathrm{ml}}{1.015kg}$$

$$= 5.9372\ \mathrm{ml}$$

Preparation of 100 ml of 1.0 M Methacrylic Acid, MA

Molecular weight, MW of Acrylic Acid, MA = 86.09 g/mol

$$\mathrm{m} = \frac{\mathrm{MV}}{1000}\,(\mathrm{MW})$$

$$\mathrm{m} = \frac{(1.0)(100)}{1000}\,(86.09)$$

$$\mathrm{m} = 8.609\,\mathrm{g}$$

$$1.015\,\mathrm{kg} \equiv 1\,\mathrm{l}$$

$$8.609 \times 10^{-3}\,\mathrm{kg} \equiv \frac{(8.609 \times 10^{-3}\mathrm{kg})\ 1000\ \mathrm{ml}}{1.015kg}$$

$$= 8.4818\ \mathrm{ml}$$

Preparation of 1000 ml of 0.02M Acetate buffer with pH 4.0

Table 6.1: Buffer Recipe for 100ml Acetate Buffer

Desired pH[a]	0.2 M acetic acid solution (ml)	0.2 M sodium or potassium acetate solution (ml)	H_2O (ml)
3.6	46.3	3.7	50
3.8	44.0	6.0	50
4.0	41.0	9.0	50
4.2	36.8	13.2	50
4.4	30.5	19.5	50
4.6	25.5	24.5	50
4.8	20.0	30.0	50
5.0	14.8	35.2	50
5.2	10.5	39.5	50
5.4	8.8	41.2	50
5.6	4.8	45.2	50

Volume of acetic acid and sodium acetate were taken from the table above.

For 0.02 M Acetic Acid,

$$m = \frac{MV}{1000} \times MW$$

$$m = \frac{(0.04)(41)}{1000} \times 60.05$$

$= 0.0985\ g \times 10$

$= 0.985\ g$

$$volume\ of\ acetic\ acid, v = \frac{mass\ of\ acetic\ acid\ ,m}{density\ of\ acetic\ acid, \rho}$$

$$volume\ of\ acetic\ acid, v = \frac{0.985\text{g}}{1.049\ \text{g/ml}}$$

$$volume\ of\ acetic\ acid, v = 0.94\ ml$$

For 0.02 M Sodium Acetate,

$$\text{m} = \frac{\text{MV}}{1000} \times \text{MW}$$

$$\text{m} = \frac{(0.04)(9)}{1000} \times 136.08$$

= 0.049 g × 10

= 0.49 g

Preparation of Sodium Hydroxide (NaOH) with pH 10.0

At 20°C,

pH = 10

pOH = 14 – pH

= 14 – 10

= 4

$\log[OH^-] = -4$

Therefore,

$[OH^-] = log^{-1}(-4)$

$[OH^-] = 10^{-4}M$

Where, M is equal to mol/L

$$\frac{No\ of\ mole}{1L} = [OH^-]$$
$$\frac{No\ of\ mole}{1L} = \ 10^{-4}M$$
$$No\ of\ mole = 10^{-4}M\ (1L)$$
$$No\ of\ mole = \ 10^{-4}mol$$

So,

$$mass\ of\ NaOH, m = mol\ of\ NaOH\ x\ molecular\ weight\ of\ NaOH, MW$$
$$mass\ of\ NaOH, m = 10^{-4}mol\ (40\ g/mol)$$
$$mass\ of\ NaOH, m = 4x10^{-3}g$$

Preparation of Hydrocloric Acid (HCl) with pH 4.0

$$pH = log[H^+]$$
$$4 = \log[H^+]$$
$$[H^+] = 1x10^{-4}$$

$$\frac{No\ of\ mole\ HCl}{1L} = [H^+]$$
$$\frac{No\ of\ mole\ HCl}{1L} = \ 10^{-4}M$$

Where, M is equal to mol/L

$$No\ of\ mole\ HCl = 10^{-4}M\ (1L)$$
$$No\ of\ mole\ HCl = \ 10^{-4}mol$$

So,

$mass\ of\ HCl = mol\ of\ NaOH\ x\ molecular\ weight\ of\ NaOH$

$mass\ of\ HCl = 10^{-4} mol\ (36.5\ g/mol)$

$mass\ of\ HCl = 3.65x10^{-3} g$

$mass\ of\ HCl = 3.65x10^{-6} kg$

Since HCl is in liquid, we need to find the volume of the HCl using their density.

$Specific\ Gravity\ of\ HCl, S.G_{HCl} = 1.18$

$\rho_{HCl} = S.G_{HCl}(\rho_w)$

$\rho_{HCl} = 1.18\ (1000\ kg/m^3)$

$\rho_{HCl} = 1180\ kg/m^3$

The volume of HCL calculated by using equation

$volume\ of\ HCl, v = \frac{mass\ of\ HCl, m}{density\ of\ HCl, \rho}$

$volume\ of\ HCl, v = \frac{3.65x10^{-6} kg}{1180 kg/m^3}$

$volume\ of\ HCl, v = 8.474x10^{-8} m^3$

$volume\ of\ HCl, v = 0.084\ ml$

APPENDIX B

RESULTS FROM FTIR'S TESTING

Concentration of 0.1M of Methacrylic Acid

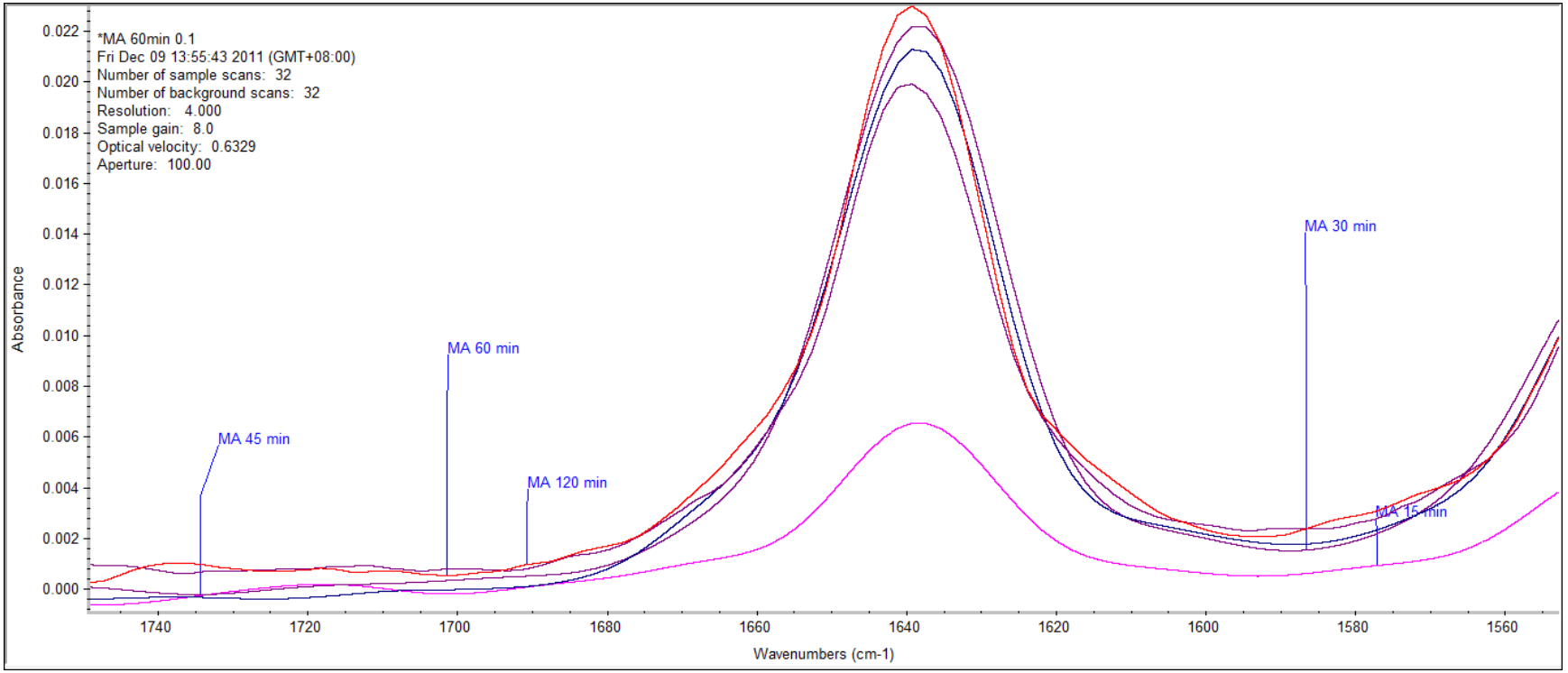

Figure 6.1: FTIR'S Result for Concentration of 0.1M Methacrylic Acid

Concentration of 0.3M of Methacrylic Acid

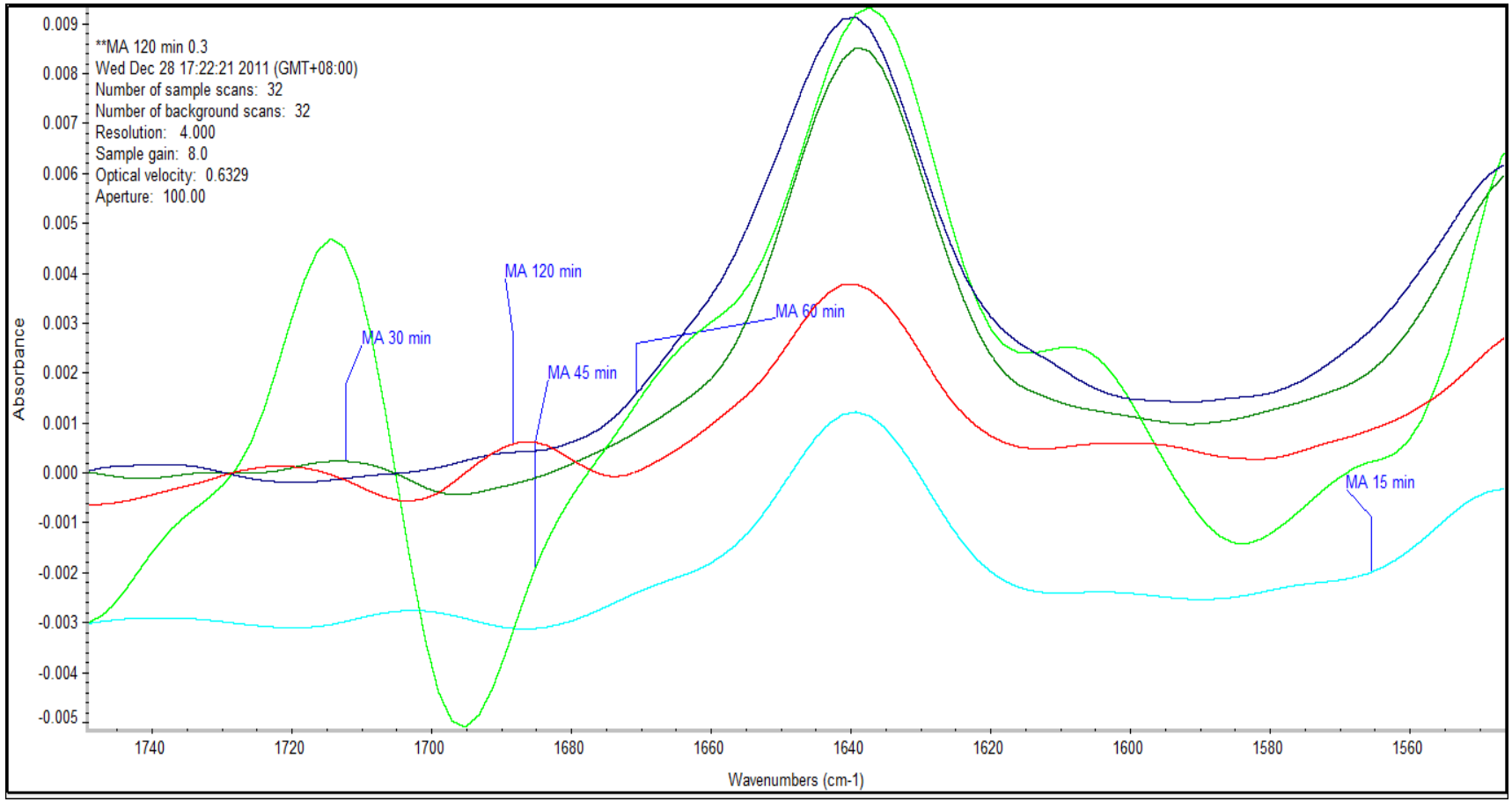

Figure 6.2: FTIR'S Result for Concentration of 0.3M Methacrylic Acid

Concentration of 0.5M of Methacrylic Acid

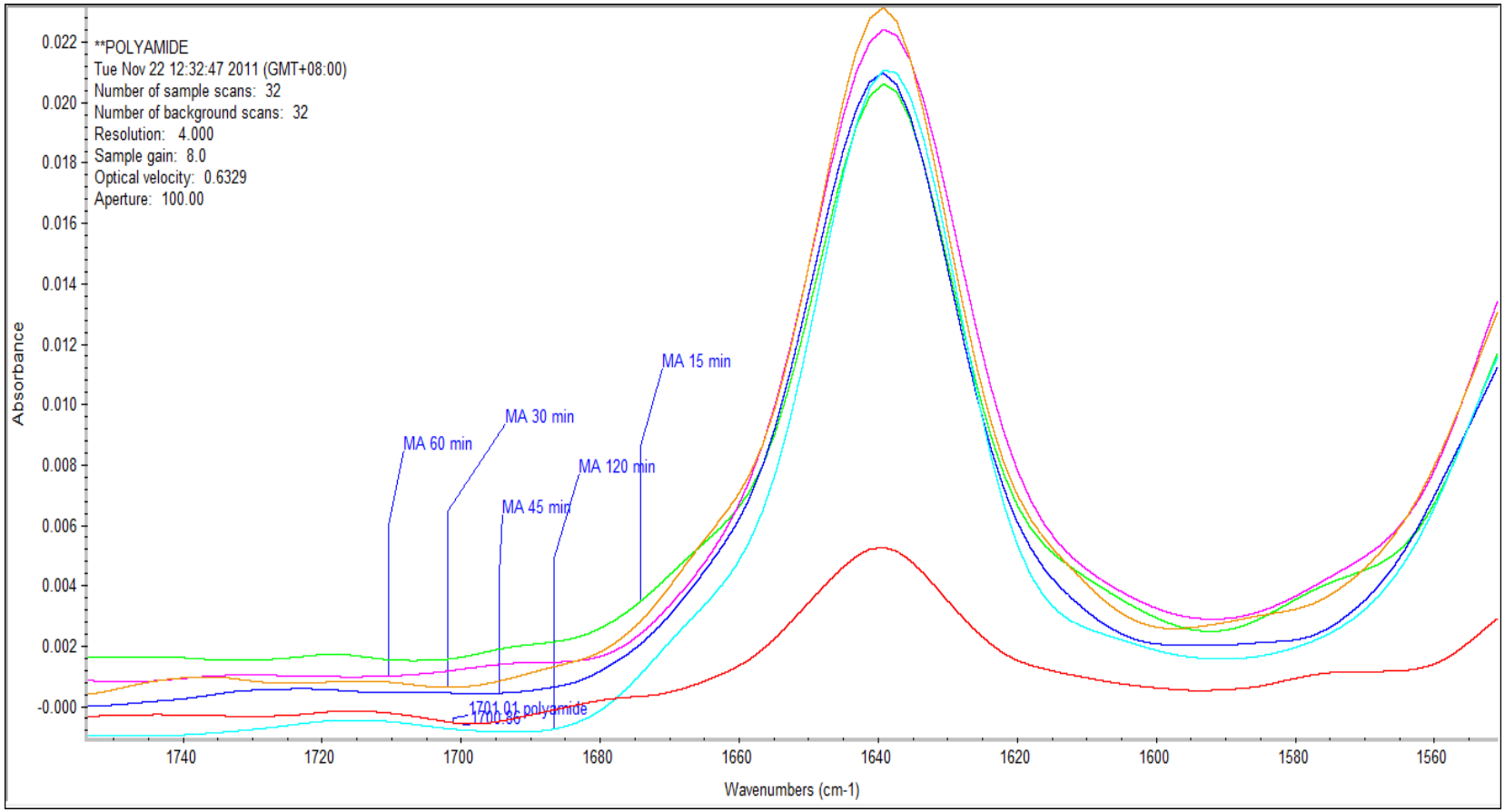

Figure 6.3: FTIR'S Result for Concentration of 0.5M Methacrylic Acid

Concentration of 0.7M of Methacrylic Acid

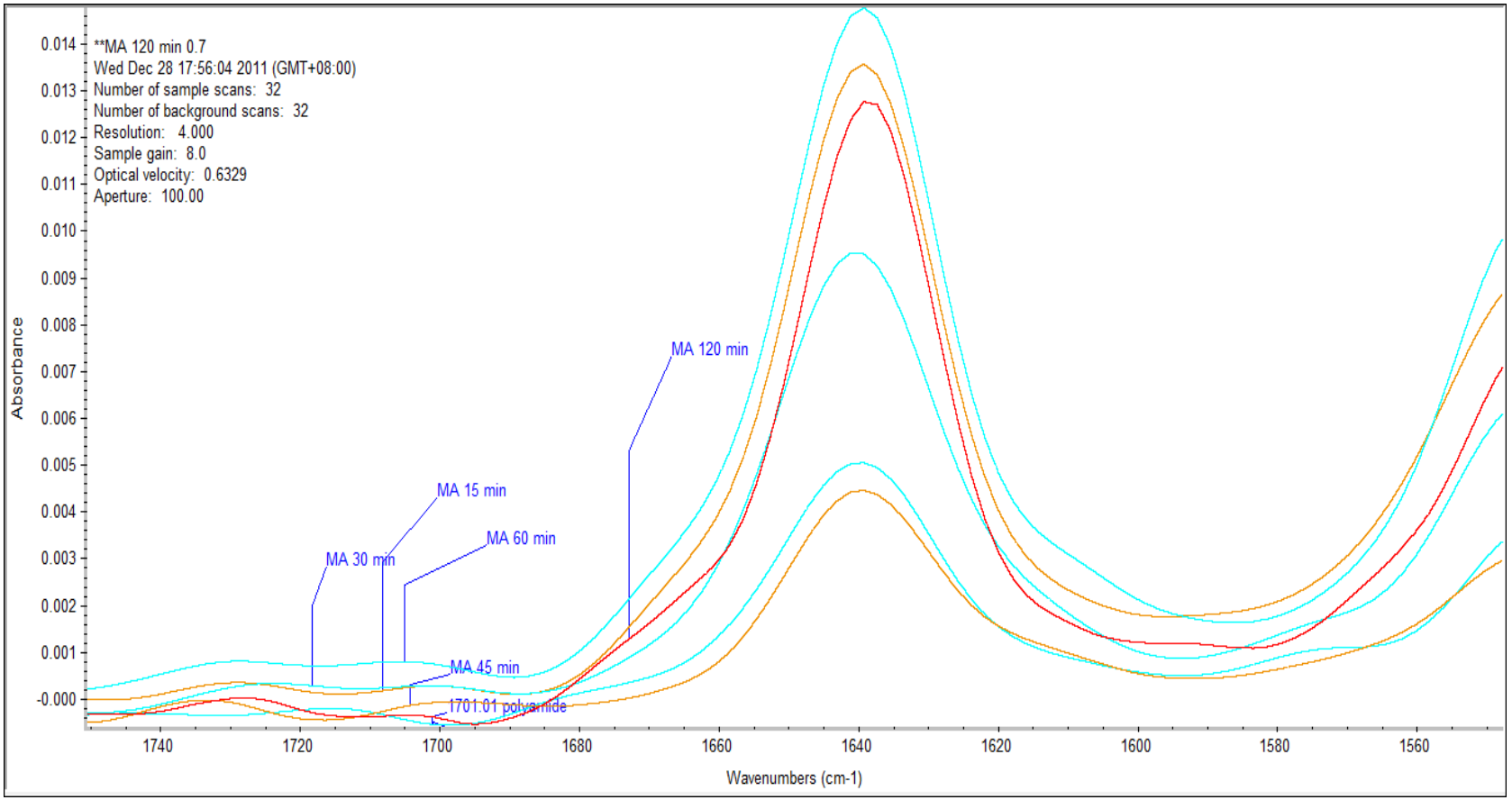

Figure 6.4: FTIR'S Result for Concentration of 0.7M Methacrylic Acid

Concentration of 1.0M of Methacrylic Acid

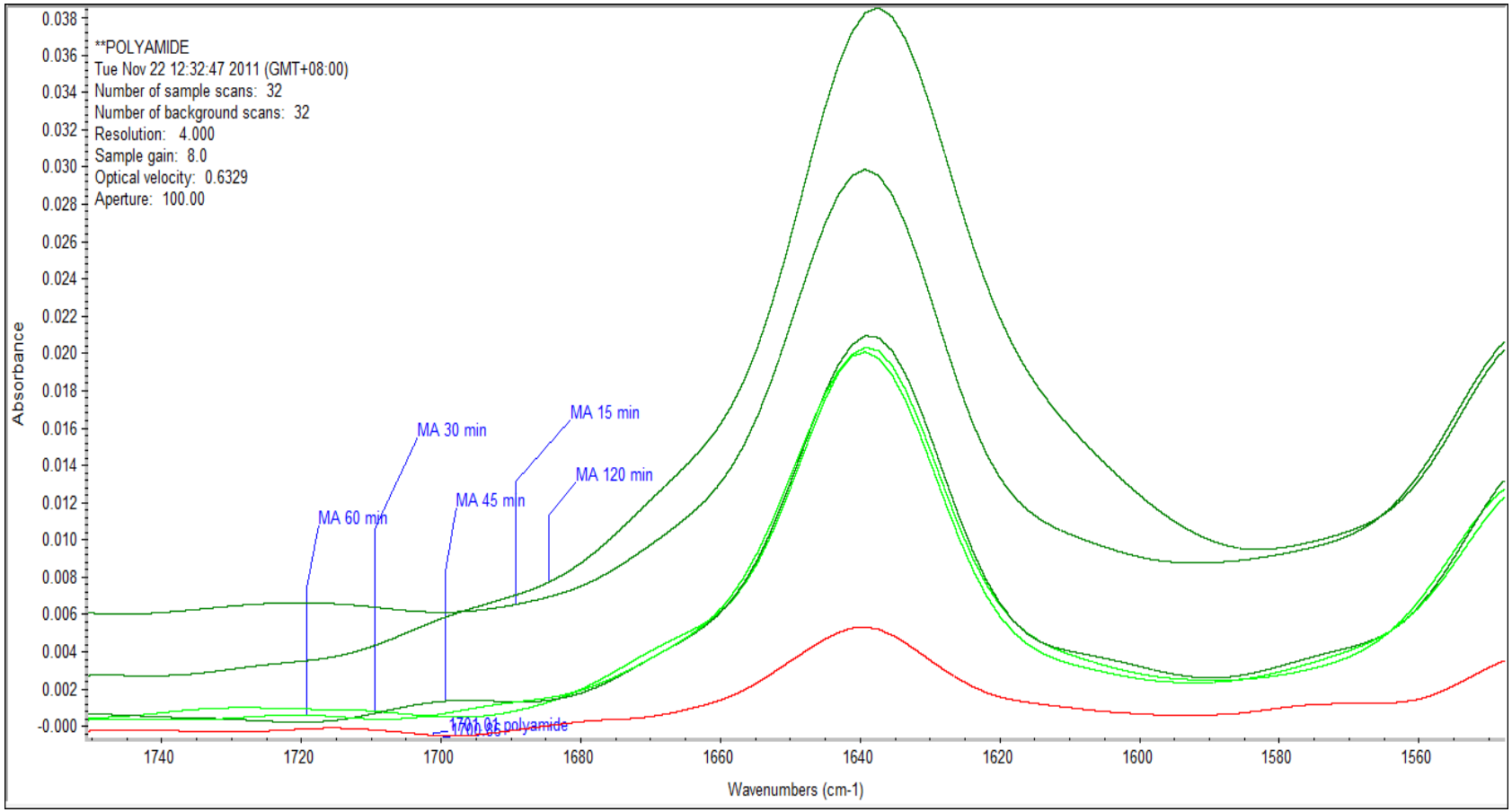

Figure 6.5: FTIR'S Result for Concentration of 1.0M Methacrylic Acid

APPENDIX C

EFFECT OF MONOMER CONCENTRATION AND REACTION TIME TO THE BINDING CAPACITY OF BSA PROTEIN

Table 6.2: Polyamide Membrane Absorbance

Absorbance			**Average**	**Initial Concentration, C_i (mg/ml)**	**Final Concentration, C_f (mg/ml)**	**Volume , v (ml)**	**Weight, w (mg)**	**Binding Capacity, Q (mg/g)**
1	**2**	**3**						
1.018	1.023	1.031	1.024	2	1.757049892	2	0.0044	110.4318675

Table 6.3: Effect of 0.1M Monomer Concentration with Different Time

Time (min)	Absorbance			Average	Initial Concentration, C_i (mg/ml)	Final Concentration, C_f (mg/ml)	Volume , v (ml)	Weight, w (mg)	Binding Capacity, Q (mg/g)
	1	2	3						
15	0.691	0.673	0.849	0.737667	2	1.27927026	2	0.0046	313.3607566
30	0.589	0.7	0.822	0.703667	2	1.222537405	2	0.0061	254.9057689
45	0.333	0.492	0.312	0.379	2	0.68079426	2	0.0044	599.6389727
60	0.928	0.903	0.976	0.935667	2	1.609655709	2	0.0076	102.7221817
120	0.822	0.96	0.964	0.915333	2	1.575727237	2	0.0035	242.4415787

Table 6.4: Effect of 0.3M Monomer Concentration with Different Time

Time (min)	Absorbance			Average	Initial Concentration, C_i (mg/ml)	Final Concentration, C_f (mg/ml)	Volume , v (ml)	Weight, w (mg)	Binding Capacity, Q (mg/g)
	1	2	3						
15	0.91	0.947	0.979	0.945333	2	1.625785639	2	0.0052	143.9286005
30	0.934	0.884	0.941	0.919667	2	1.582957895	2	0.0049	170.2212672
45	0.844	0.867	0.908	0.873	2	1.505089271	2	0.0055	179.9675379
60	0.675	0.707	0.751	0.711	2	1.234773903	2	0.0037	413.6357282
120	0.864	0.981	0.972	0.939	2	1.615217754	2	0.0032	240.4889037

Table 6.5: Effect of 0.5M Monomer Concentration with Different Time

Time (min)	**Absorbance**			**Average**	**Initial Concentration, C_i (mg/ml)**	**Final Concentration, C_f (mg/ml)**	**Volume , v (ml)**	**Weight, w (mg)**	**Binding Capacity, Q (mg/g)**
	1	**2**	**3**						
15	1.036	0.999	1.05	1.028333	2	1.76428055	2	0.0047	100.3061491
30	0.932	0.974	0.842	0.916	2	1.576839646	2	0.0054	156.7260569
45	0.88	0.751	0.78	0.803667	2	1.389398743	2	0.0047	259.8303221
60	0.883	1.024	1.029	0.978667	2	1.681406085	2	0.0058	109.8599707
120	0.915	0.96	0.834	0.903	2	1.555147672	2	0.0053	167.8688029

Table 6.6: Effect of 0.7M Monomer Concentration with Different Time

Time (min)	**Absorbance**			**Average**	**Initial Concentration, C_i (mg/ml)**	**Final Concentration, C_f (mg/ml)**	**Volume , v (ml)**	**Weight, w (mg)**	**Binding Capacity, Q (mg/g)**
	1	**2**	**3**						
15	0.921	0.973	0.912	0.935333	2	1.609099505	2	0.0052	150.3463442
30	0.913	0.985	0.917	0.938333	2	1.614105345	2	0.0079	97.69484934
45	0.791	0.9	0.885	0.858667	2	1.481172479	2	0.0065	159.6392372
60	0.99	0.923	0.947	0.953333	2	1.639134546	2	0.0051	141.5158644
120	0.954	0.962	0.977	0.964333	2	1.657489293	2	0.0064	107.0345959

Table 6.7: Effect of 1.0M Monomer Concentration with Different Time

Time (min)	Absorbance			Average	Initial Concentration, C_i (mg/ml)	Final Concentration, C_f (mg/ml)	Volume , v (ml)	Weight, w (mg)	Binding Capacity, Q (mg/g)
	1	2	3						
15	0.837	0.963	0.766	0.855333	2	1.475610434	2	0.0045	233.0620292
30	0.961	0.858	0.946	0.921667	2	1.586295122	2	0.0055	150.4381374
45	0.769	0.679	0.518	0.655333	2	1.141887758	2	0.0057	301.0920148
60	0.938	0.835	0.944	0.905667	2	1.559597308	2	0.0045	195.7345298
120	0.995	0.967	0.813	0.925	2	1.591857167	2	0.0046	177.4534058