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

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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 Bachelor of Chemical Engineering.

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I hereby declare that that the work in this thesis is my own except for quotations 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

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Dedicated to my parents

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ABSTRACT

Packed bed chromatography is widely used for protein separation and purification. However some limitation of packed bed chromatography has been identified such as high-pressure drop, internal diffusion limitation and compaction for the soft beads. This encourages people to use membrane chromatography as alternative to the packed bed chromatography. In this study, membrane chromatography was developed from polyamide-based membrane by grafting acrylic acid (AA) monomer to produce cation-exchanger membrane chromatography. During grafting process, the concentration of AA monomer from 0.1 M to 1.0 M and grafting time from 15 min to 120 min was studied. The optimum monomer concentration was achieved at 0.5 M with a binding capacity of 443.39 mg BSA/g membrane. The optimum reaction time is 45 minutes which gives a binding capacity of 533.40 BSA mg/g membranes. The BSA adsorption capacity can be related to the amount of carboxyl group present in the modified membrane surface after grafting process. The successful of membrane chromatography preparation depend on the careful optimization the chemical reaction parameters involve.

ABSTRAK

Kromatografi turus terpadat digunakan secara meluas untuk pengasingan dan penapisan protein. Walaubagaimanapun, beberapa kelemahan kromatografi turus terpadat telah dikenal pasti seperti penurunan tekanan yang tinggi, resapan dalaman yang terbatas dan pemadatan bahan kromatografi yang lembut. Kelemahan tersebut menggalakan penggunaan membran kromatografi sebagai alternatif kepada kromatografi turus terpadat. Dalam kajian ini, membran kromatografi telah dibangunkan berasaskan membran jenis *polyamide* yang dicantum dengan monomer asid akrilik (AA) untuk menghasilkan membran kromatografi jenis penukar kation. Semasa proses cantuman, kepekatan monomer AA yang digunakan adalah dari 0.1 M hingga 1.0 M dan masa cantuman adalah dari 15 minit hingga 120 minit telah dikaji. Kepekatan monomer yang optimum telah dicapai pada kepekatan 0.5 M dengan kapasiti 443.39 mg BSA / g membran. Masa tindak balas yang optimum adalah pada minit ke 45 yang memberikan kapasiti sebanyak 533.40 BSA mg / g membran. Kapasiti BSA terjerap boleh dikaitkan dengan jumlah kumpulan *carboxyl* yang hadir di permukaan membran selepas proses cantuman berlaku. Keberhasilan penyediaan kromatografi membran bergantung kepada pengoptimuman parameter tindak balas kimia yang terlibat.

TABLE OF CONTENTS

	Page
SUPERVISOR'S DECLARATION	ii
STUDENT'S DECLARATION	iii
DEDICATION	iv
ACKNOWLEDGEMENT	v
ABSTRACT	vi
ABSTRAK	vii
TABLE OF CONTENTS	viii
LIST OF TABLES	xi
LIST OF FIGURES	xii
LIST OF SYMBOLS	xiii
LIST OF ABBREVIATIONS	xiv

CHAPTER 1 INTRODUCTION

1.1	Background of research	1
1.2	Problem Statement	3
1.3	Objective of the Research	4
1.4	Scope of Research	4

CHAPTER 2 LITERATURE REVIEW

2.1 Membranes 5

2.1.1 Polyamide membrane 6

2.2 Chromatography 7

2.2.1 Chromatography system 8

2.3 Packed bed chromatography 10

2.4 Membrane chromatography 12

2.5 Development of cation exchange membrane chromatography 14

CHAPTER 3 METHODOLOGY

3.1 Material 16

3.2 Preparation of Sodium Hydroxide (NaOH) and Hydrochloric Acid (HCl) 16

3.3 Preparation of Buffer Solution 17

3.4 Grafting process 17

3.5 Protein Binding Experiment

3.5.1 Preparation of standard curve 18
3.5.2 Protein Binding 18

3.6 Membrane characterization

3.6.1 Fourier Transform Infrared (FTIR) protocol 19
3.6.2 Water content 19

CHAPTER 4 RESULT AND DISCUSSION

4.1 Standard curve 20

4.2 Effect of Monomer Concentration and Reaction Time 21

4.3 Water content 22

4.4 Effect of different monomer concentration on BSA adsorption capacity 23

4.5 Effect of different reaction time on BSA adsorption capacity 25

4.6 FTIR spectroscopy 27

CHAPTER 5 CONCLUSION AND RECOMMENDATION

5.1 Conclusion 33

5.2 Recommendation 34

REFERENCES 35

APPENDICES 38

LIST OF TABLES

Table No.	Title	Page
2.1	Development of cation exchange membrane chromatography	14
4.1	Effect of monomer concentration and reaction time	21
4.2	Water content of membrane	22

LIST OF FIGURES

Figure No.	Title	Page
1.1	Schematic representation of the methods of polymer modification	2
2.1	Schematic representation of membrane processes	5
2.2	Cross flow filtration	6
2.3	Dead-end filtration	6
2.4	Formula structure of polyamide	7
2.5	The matrix of the chromatography for protein separation	8
2.6	Chromatographic system	9
2.7	Different configuration of chromatographic columns	10
2.8	Packed bed column operation	11
2.9	Solute transportation in packed bed chromatography	12
2.10	Solute transportation in membrane chromatography	13
3.1	Vacuum pump	17
4.1	Standard curve graph for BSA protein	20
4.2	Effect of different monomer concentration on BSA adsorption capacity	24
4.3	Effect of different reaction time on BSA adsorption capacity	26
4.4	FTIR spectra at 0.1 M of acrylic acid (AA) monomer	28
4.5	FTIR spectra at 0.3 M of acrylic acid (AA) monomer	28
4.6	FTIR spectra at 0.5 M of acrylic acid (AA) monomer	29
4.7	FTIR spectra at 0.7 M of acrylic acid (AA) monomer.	30
4.8	FTIR spectra at 1.0 M of acrylic acid (AA) monomer	30
4.9	Water content with different monomer concentration and reaction time	31

LIST OF SYMBOLS

C_i	Initial concentration
C_f	Final concentration
Q	Binding capacity
W	Weight of dry membrane
W_W	Weight of wet membrane
W_C	Water content

LIST OF ABBREVIATIONS

AA	Acrylic Acid
HIC	Hydrophobic Interaction Chromatography
SEC	Size Exclusion
AC	Affinity Chromatography
GC	Gas Chromatography
LC	Liquid Chromatography
PA	Polyamide
PS	Polysulfone
PES	Polyethersulfone
PVA	Polyvinyl Alcohol
EGDMA	Ethyleneglycol Dimethacrylate
BSA	Bovine Serum Albumin
PPSS	Polyphenylenesul®desulfone
PP	Polypropylene
BP	Benzophenone
FTIR	Fourier Transform Infrared
ATR-FTIR	Attenuated Total Reflectance- Fourier Transform Infrared

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF RESEARCH

Proteins are difficult to isolate and protein purification is technically demanding, given their complex physico-chemical properties, and possibility of unfolding and denaturation. In the early days of protein chemistry, the only practical way to separate different types of proteins was by taking advantage of their relative solubility. Part of a mixture was caused to precipitate through alteration of some properties of the solvent e.g. addition of salts, organic solvents or polymers, or varying the pH or temperature. Fractional precipitation is still frequently used for separation of gross impurities, membrane proteins and nucleic acids. Almost every step in a protein purification process has the potential to alter protein structure and reduce biological activity. An efficient purification scheme needs to result in high activity and high yield of isolated proteins.

The increasing demand for biologics requires extension of the current production facilities or more efficient processing. In particular separation and purification processes require better process concepts. Precipitation, filtration, and chromatography are commonly used techniques for protein purification. There are various modes for column chromatography of proteins which including ion-exchange chromatography (IEC), hydrophobic interaction chromatography (HIC), size exclusion (SEC), and affinity chromatography (AC). Almost all large scale process configurations are based on packed bed chromatographic systems. The separation and purification processes are dictated by the hydrodynamics of these systems.

Recently a lot of research is carried out to optimize the isolation and purification processes for the production of biologics. One of the most promising techniques is by using membrane chromatography technology for which the target components are not separated and isolated by slow diffusion to control processes but by fast convective flows (Brandt, 1988).

Until now, most of the reported work more focused on modification of existing macroporous membranes mainly by chemical modification and radiation grafting methods (Ghosh, 2002; Klein, 2000). There are several articles regarding the preparation of membrane by using blending, mixing and coating methods were recently published as shown in Figure 1.1 (Lingeman, 1997; Markell, 1991).

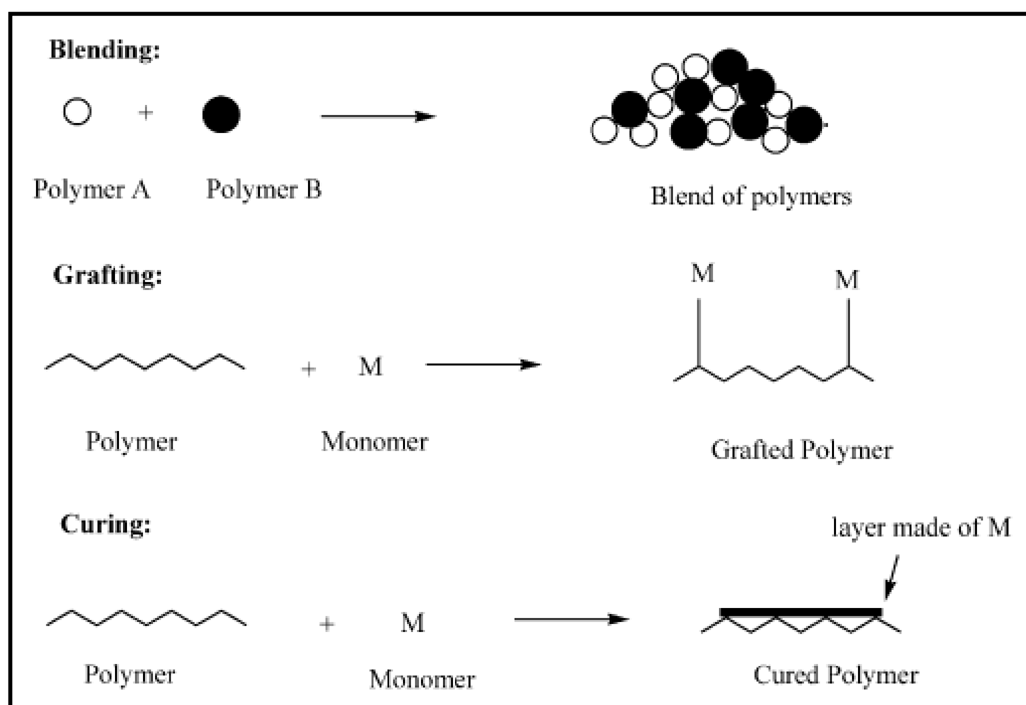


Figure 1.1: Schematic representation of the methods of polymer modification (Bhatthacharya, 2004)

1.2 PROBLEM STATEMENT

In a typical protein production process, the cost of protein isolation and purification contributed to 50 - 90% of the total production cost (Kawai, et al., 2003; Karumanchi, et al., 2002). Chromatography process is widely used for high-resolution separation and analysis of proteins. Traditional chromatography process is carried out by using packed beds column which have several major limitations such as low throughput, complex scale-up and high pressure drop. The pressure drop also tends to increase during a process due to the combined effects of bed consolidation caused by media deformation, and column blinding caused by accumulated colloidal material (Levine, 2002).

Some of the limitations of packed bed chromatography have been overcome by using newly developed monodisperse, non-porous, rigid chromatographic media or known as membrane chromatography. However, these media are generally expensive and the solute binding capacity is greatly reduced since binding can now only take place on the external surfaces.

A radically different approach to overcome the limitations associated with packed beds is to use synthetic microporous or macroporous membranes as chromatographic media. In membrane chromatographic processes the transport of solutes to their binding sites takes place predominantly by convection. It will reduce both process time and recovery liquid volume. The binding efficiency is generally independent of the feed flow-rate over a wide range and therefore very high flow-rates may be used. The pressure drop is also significantly lower than with packed beds. Another major advantage of membrane adsorbers is easy to scale-up compared with packed beds. Membrane chromatography is particularly suitable for larger proteins (i.e. M .250 000). Such proteins rarely enter pores present in particulate chromatographic media and only bind on the available surface area of such media.

1.3 OBJECTIVE

The main objective of this study was to produce cation-exchange membrane chromatography from commercial microfiltration polyamide membrane by grafting of acrylic acid monomer.

1.4 SCOPE OF RESEARCH

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

i. Study the effect of reaction time from 15 to 120 minutes to the polymer carboxyl group formation at the polyamide membrane surface.

ii. Study the effect of acrylic acid monomer concentration from 0.1 to 1.0 M during the grafting process on the polyamide membrane.

CHAPTER 2

LITERATURE REVIEW

2.1 MEMBRANES

Membrane is a perm selective barrier or interface between two phases that capable of transporting certain component from the one phase (i.e. feed side) to other phase (i.e. permeate side) while retaining others components as showed in Figure 2.1. This selective transport occurs in response to a driving force such as a gradient in concentration, pressure, temperature, or electrical potential. Membrane can be classified as natural, or biological as for example in bacteria, and synthetic. Synthetic membranes can be further divided between organic such as polymeric or liquid and inorganic such as ceramic and metal membranes.

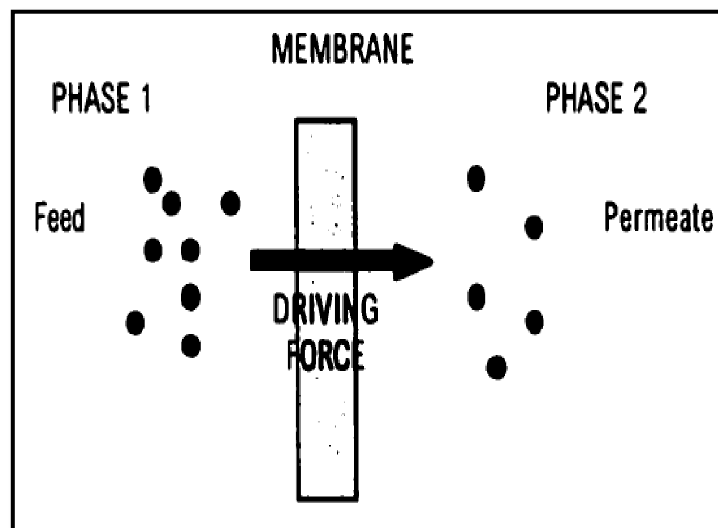


Figure 2.1: Schematic representation of membrane processes (Fidalgo, 2001)

Membrane processes can be operated in two different ways with respect to the direction of flow which is cross flow and dead-end filtration. In cross flow filtration, the feed stream flows parallel to the membrane surface, permeate penetrates through the membrane in the direction normal to the surface as showed in Figure 2.2. Figure 2.3 showed the operation of dead-end filtration. In this configuration, the flow of feed stream and permeate are both perpendicular to the membrane surface.

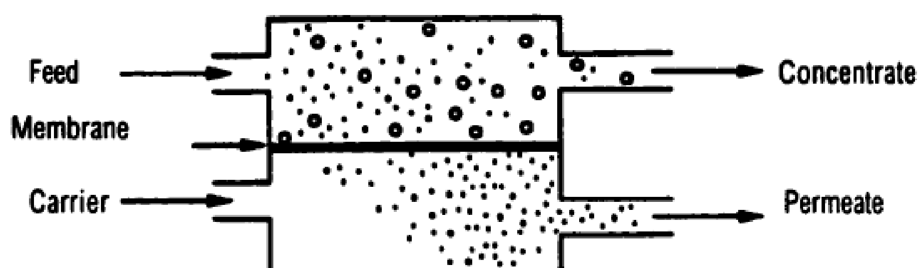


Figure 2.2: Cross flow filtration (Fidalgo 2001)

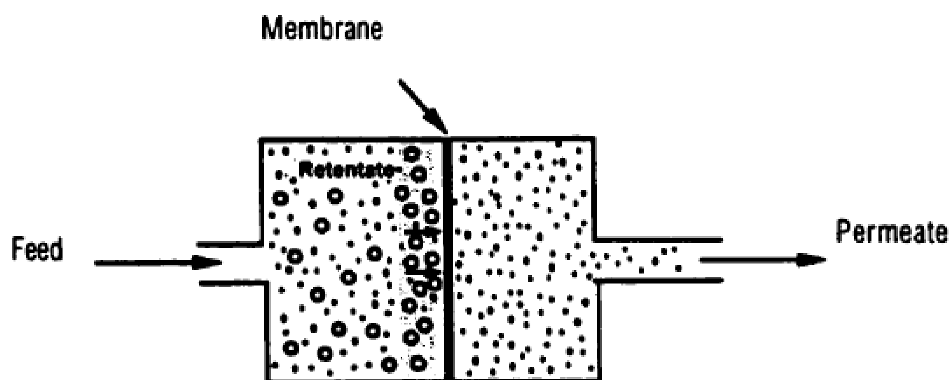


Figure 2.3: Dead-end filtration (Fidalgo 2001)

2.1.1 Polyamide Membrane

Polyamide membrane is mechanically strong and exhibit great wet strength and dry strength. They are hydrophilic which makes them suitable for aqueous and organic

solutions. The membrane filters can be used at temperature up to 135ºC and can be autoclaved at up to 134ºC. The formula structure of polyamide is showed in Figure 2.4.

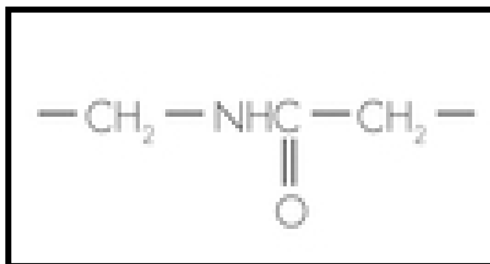
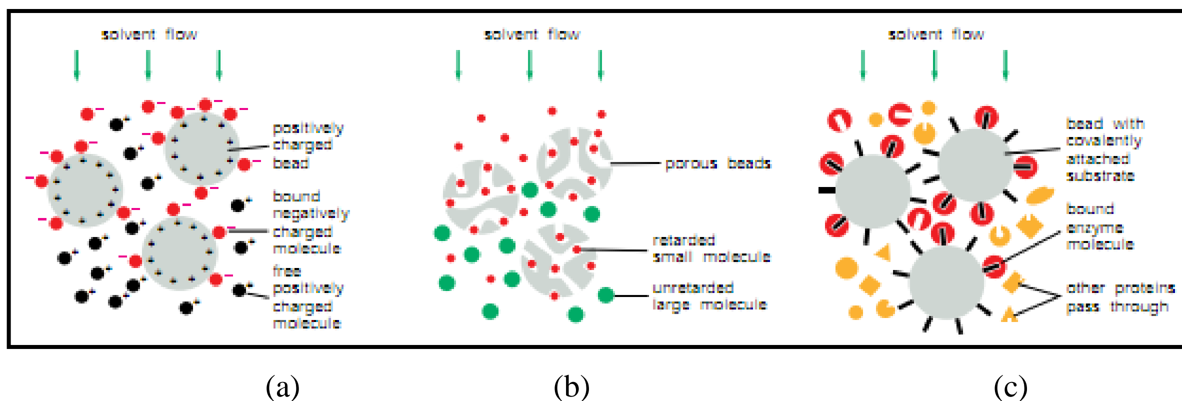


Figure 2.4: Formula structure of polyamide

2.2 CHROMATOGRAPHY

Chromatography is essentially a physical techniques of separation which the components to be separated are distributed between two phases. The first phase is stationary which can be solid, liquid or solid liquid mixture. The second phase is mobile phase which is can be either gas or liquid which gives rise to the two basic forms of chromatography, namely, gas chromatography (GC) and liquid chromatography (LC).

Chromatography is available in many types of matrix but it is usually packed in the column in the form of small beads. A typical protein purification strategy might employ three kinds of interaction mechanism which are ion-exchange chromatography, gel-filtration chromatography and affinity chromatography as showed in Figure 2.5.



(a) (b) (c)

Figure 2.5: The matrix of the chromatography for protein separation of (a) ion-exchange chromatography (b) gel-filtration chromatography (c) affinity chromatography (Alberts et al. 1998)

Ion-exchange column are packed with small beads that basically carry positive or negative charges that slow down the proteins of the opposite charge. The relationship between a protein and the matrix depends on the pH and ionic strength of the solution passing down the column. These can have a difference in a controlled way to achieve an effective separation. The separation of proteins in gel-filtration columns is according to their size. The matrix consists of tiny porous beads. The small molecules that enter the porous beads is delay and it travel more slowly compared to the molecules which is not enter the porous beads. Such columns also allow an estimate of protein size. Then, affinity columns contain a matrix covalently coupled to a molecule that interacts specifically with the protein of interest. For examples is an antibody, or an enzyme substrate. The proteins binding can finally be released by a pH change or by concentrated salt solutions. They also emerge highly purified.

2.2.1 Chromatography System

Basically, a chromatography separation system consists of a column, mobile phase reservoirs, pump, sample injector, detector and sometimes chromatography have a fraction collector. Figure 2.6 shows a simple chromatographic separation set-up.

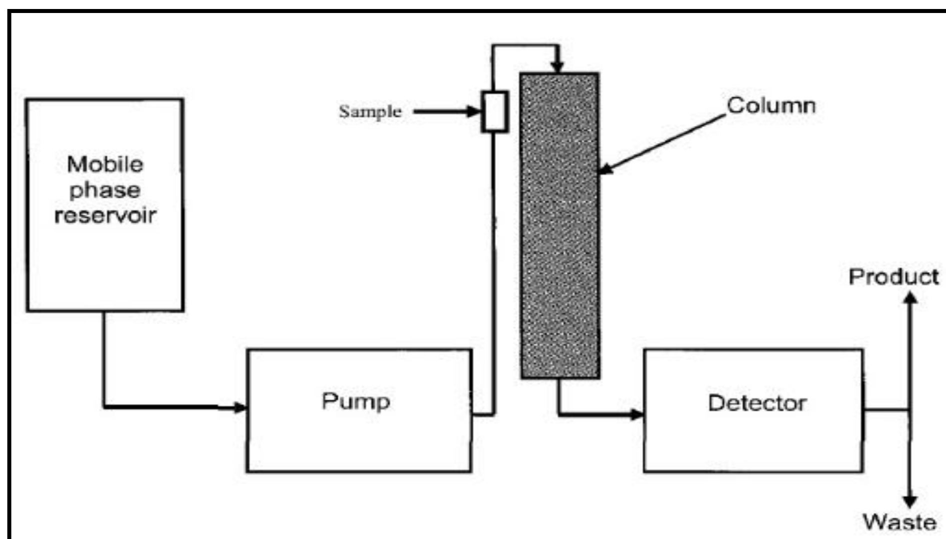


Figure 2.6: Chromatographic system (Ghosh 2006)

In chromatography, there are many types of columns that can be used for separation process. There are packed bed column, packed capillary column, open tubular column, membrane and also monolith. Usually packed bed columns are the most widely types used in chromatography. But for packed capillary and open tubular columns are mainly used for analytical chromatography of synthetic chemicals. In Figure 2.7, membrane columns and monolith columns it's seem to be the same but different in height. Membrane columns have very low bed height compared to monolith columns. However, monolith columns are prepared using rod-shaped porous structures and it is differ from membrane columns in terms of their material of construction and morphology. Also, monolith is used in analytical separation.

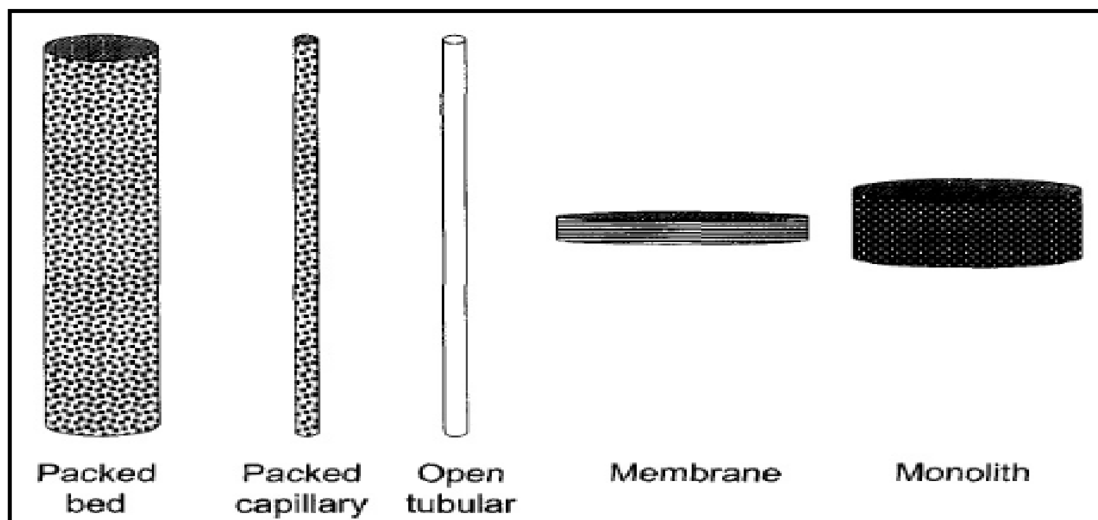


Figure 2.7: Different configuration of chromatographic columns (Ghosh 2006)

2.3 PACKED BED CHROMATOGRAPHY

Packed bed column chromatography is one of the common techniques that used in protein fractionation. Figure 2.8 shows the operation of packed bed column. A mixture of proteins in solution is applied to the top of a cylindrical column filled with chromatography media or matrix. A large amount of solvent is then pumped through the column. Because different proteins are retarded to different extents by their interaction with the matrix, they can be collected separately as they flow out from the bottom. Proteins can be separated according to their charge, hydrophobicity, size, or ability to bind to particular chemical groups.

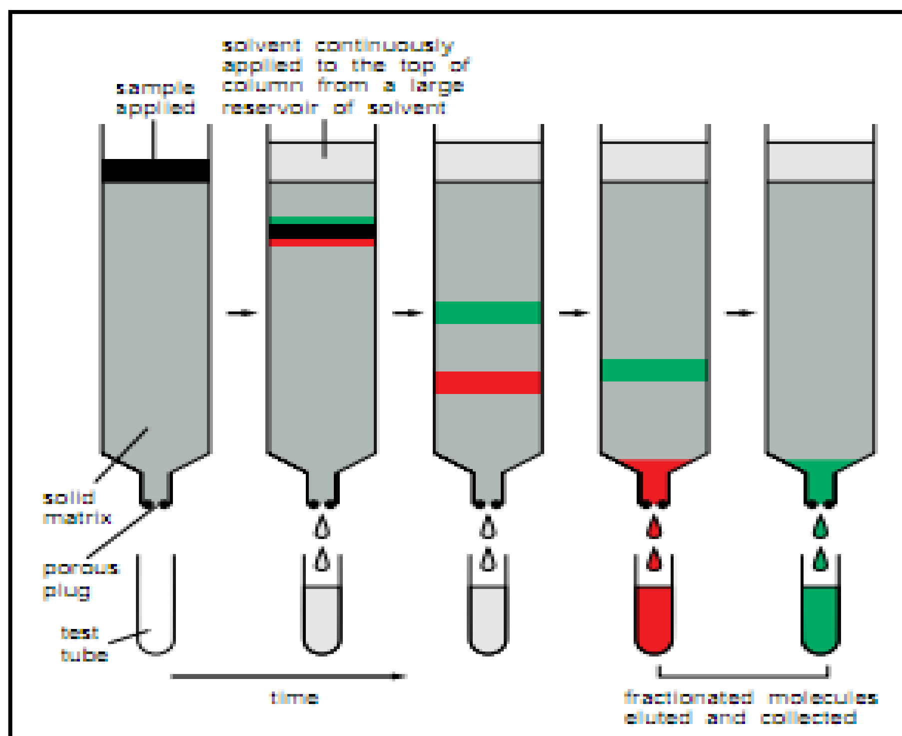


Figure 2.8: Packed bed column operation (Albert et al., 1994)

Packed bed chromatography has some limitations such as high pressure drop, internal diffusion limitation and compaction for the soft beads. In packed bed chromatography, the pressure drop is high and it tends to increase during the process. It is due to the combination of effects between bed consolidation which caused by media deformation and column blinding which caused by accumulated colloidal material. Since the pore is too small for large size protein to enter, the large size proteins is rarely enter pores and it can only bind in the surface area. Packed bed chromatography also has difficulty in scaling-up.

Another major limitation of packed bed chromatography is due to employed soft chromatographic media. The compaction for the soft beads is depends on intra-particle diffusion for the transport of solute molecules to their binding sites within the pores of the media. As showed in Figure 2.9, the process time may increases in packed bed configuration

since the transport of macromolecules by diffusion is slow, and particularly so when it is blocked.

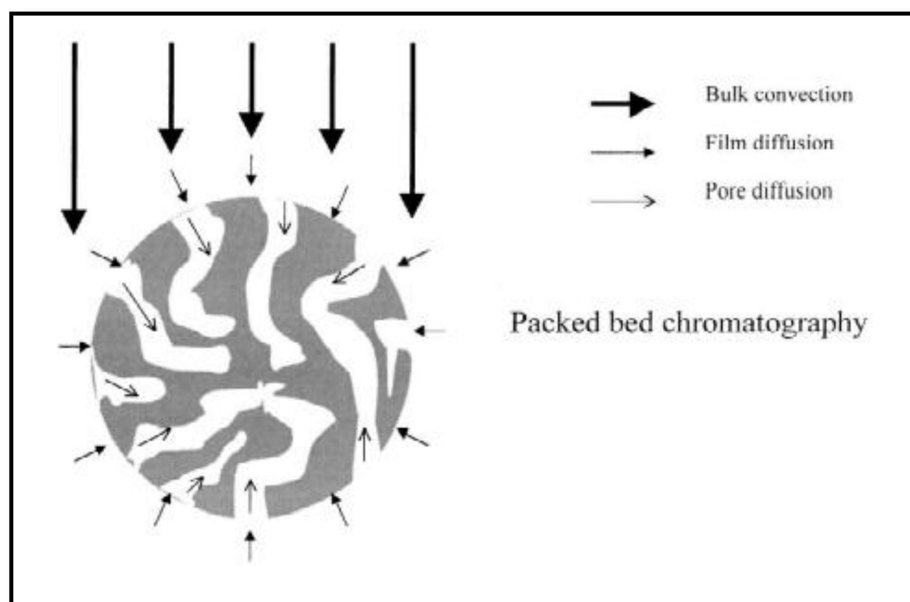


Figure 2.9: Solute transportation in packed bed chromatography (Ghosh, 2002)

2.4 MEMBRANE CHROMATOGRAPHY

In order to overcome the limitations of traditional beads column, synthetic microporous or macroporous membranes have been used as chromatography media. This method is called membrane chromatography. Membrane chromatography has functional ligands attached to their inner pore surface as adsorbents. There are many types of adsorptive membranes including ion-exchange membranes, affinity membranes, reverse-phase membranes and hydrophobic interaction membranes. All these membranes have been developed for the purification of proteins, enzymes, and antibodies from various sources (Thommes and Kula, 1995). In membrane chromatography, the transport of solutes to their binding sites occurs predominantly by convection process. The pore diffusion in membrane chromatography is very small comparing with the beads column, thereby the mass transfer resistance is hugely reduced (Ghosh, 2002).

. Because of the macroporous structure of the membrane support, membrane chromatography has a lower pressure drop due to very low bed height, can be operated at higher flow rate and give higher productivity (Ghosh, 2002; Zheng&Ruckenstein, 1998; Podgornik et al., 1999; Yang et al., 2002). High flow rate operation will reduce the process time taken for adsorption, washing, elution and regeneration time, which save time and improve efficiency (Briefs, 1992; Ghosh, 2001). In addition, fast process can avoid the inactivity of biomolecules.

In membrane chromatographic processes, the transport of solutes to their binding sites takes place predominantly by convection as showed Figure 2.10. So that it will reduce both of the process time and recovery liquid volume.

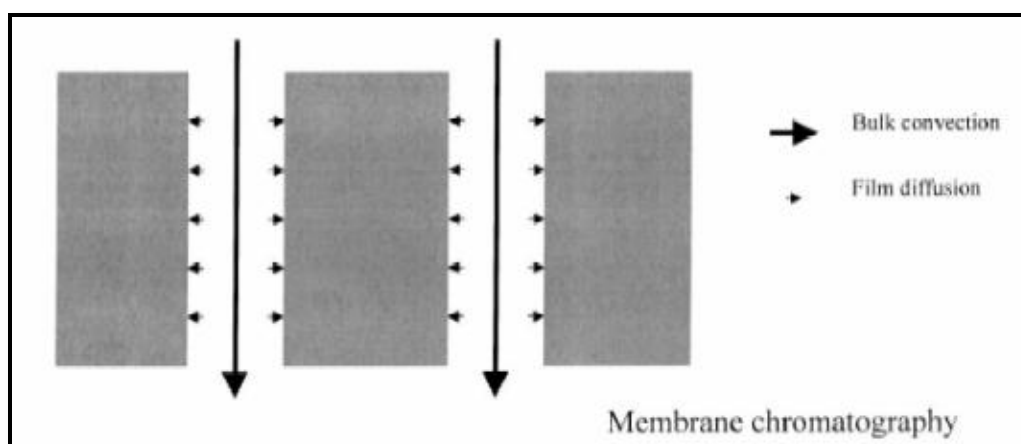


Figure 2.10: Solute transportation in membrane chromatography (Ghosh, 2002)

Membrane chromatography also is particularly suitable for large proteins which are having molecular weight larger than 250 kDa (Podgornik, 1999). Since the pore size on membrane is rather big comparing with that of beads, large size proteins can bind on it easily. The binding capacity of membrane adsorbents for large size protein is much bigger than that of traditional beads. Membrane chromatography provides easy set-up and scale-up (Teeters, Root & Lightfoot, 2002; Zeng, 1999).

On the other hand, membrane chromatography still has some disadvantages that need to be overcome. It is difficult to get uniform flow for the short wide beds of the membranes chromatography module. This happens in many cases and makes the membrane efficiency decrease (Teeters, Root & Lightfoot, 2002). Adequate flow distribution is necessary to maintain the membrane efficiency when doing a scale-up.

There are three basic steps in preparation of membrane chromatography which are preparation of base membrane, chemical activation of selected functional group in base membrane and coupling ligands which either charge or affinity into activated membrane.

2.5 DEVELOPEMENT OF CATION-EXCHANGE MEMBRANE CHROMATOGRAPHY

Various methods have been used in preparing membrane chromatography as showed in Table 2.1.

Table 2.1: Development of cation-exchange membrane chromatography.

Author, Year	Polymer	Summary
Fang et al., 2004	Polysulfone	- Prepared membrane via phase inversion method. - The saturation capacity of cationic lysozyme was 0.0098 µmol/cm^2. - The lysozyme recovery was higher than 93%.
Hwang et al., 1998	Polysulfone (PSf) and polyphenylenesul®desulfone (PPSS).	- Prepared membrane via sulfonation of the home-made block copolymers. - Measured the ion-exchange capacity, and membrane area resistance. - The lowest measured membrane area

		resistance, equilibrated in 2 M (mol/dm^3) KCl aqueous solution, was 2.5Ω cm^2. The cation exchange capacity reached up to 1.9 meq/ (g-dry-resin).
Ulbricht and Yang, 2005	Polypropylene (PP) microfiltration membranes	- Used photoinitiated surface-selective graft copolymerization by two different methods for coating the photoinitiator, benzophenone (BP), on the membrane surface. - Measure the membrane permeability at different pH as well as the reversible binding of a protein (lysozyme) under membrane chromatography conditions.
Zhang et al., 2006	Polyethersulfone (PES)	- Prepared membrane by the cation-exchange resin-mixed polyethersulfone (PES)-based fibrous adsorbents. - Measured the adsorption capacity of BSA protein. - The adsorption capacity was in the range of 68.2 to 93.2 mg BSA/g adsorbent for 50% resin loading.

CHAPTER 3

METHODOLOGY

3.1 MATERIAL

Ready-made polyamide (PA) membrane was purchased from Sartorius Stedim Biotech (Goettingen, Germany). Redox initiators which are potassium persulfate, $K_2S_2O_5$and potassium metabisulfite $K_2S_2O_8$, acrylic acid monomer, ethyleneglycoldimethacrylate (EGDMA), sodium hydroxide and hydrochloric acid were purchased from Mercks Chemicals (Darmstadt, Germany). For binding experiment, bovine serum albumin (BSA) with molecular weight of 66kDa and isoelectric point of 4.9 was purchased from Fisher Bioreagents. For acetate buffer preparation, sodium acetate (Merck) and acetic acid (J. T. Baker Philipsburg, NJ, USA) were used. All the solutions and the buffer solution were prepared using ultrapure water.

3.2 PREPARATION OF SODIUM HYDROXIDE AND HYDROCHLORIC ACID SOLUTION

The concentration of $[H]^+$ and $[OH]^-$ was calculated to get the desired pH and double checked with the pH meter. The solution of NaOH with pH 10 was prepared by diluted 0.04 g of sodium hydroxide in 1000 ml of distilled water. Meanwhile, HCl solution with pH 4 was prepared in 1000 ml of distilled water with 0.084 ml of hydrochloric acid.

3.3 PREPARATION OF BUFFER SOLUTION

A buffer solution is one which resists change in pH when small quantities of an alkali or acid are added to it. Acetate buffer pH 4.00 was prepared by dissolving 0.94 ml of acetic acid and 0.49 g of sodium acetate in 1000 ml of distilled water to obtain the concentration of 0.02 M. Then the buffer's pH was determined by pH meter. Vacuum pump was used to filter the impurities inside the buffer solution.

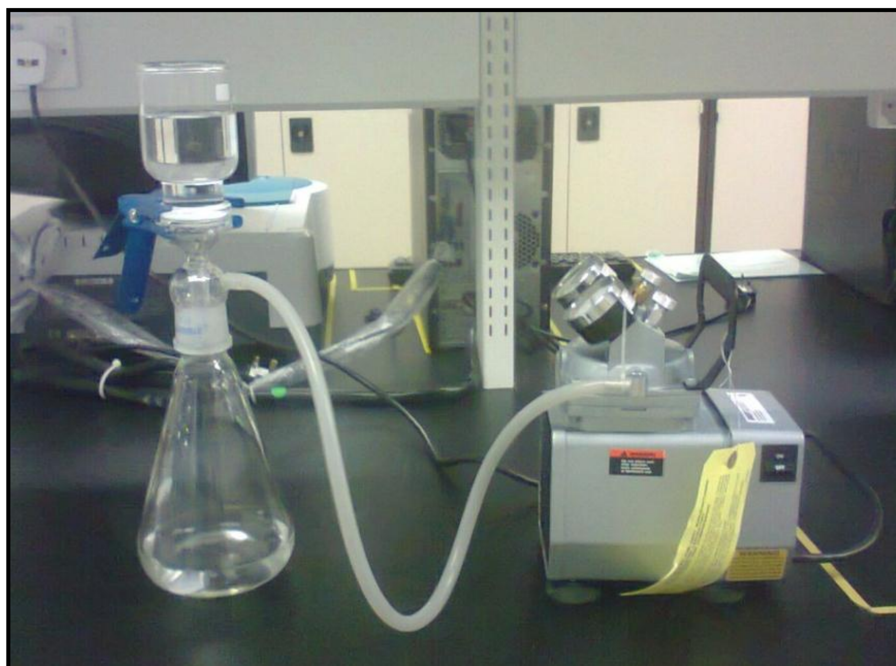


Figure 3.1: Vacuum pump

3.4 GRAFTING PROCESS

In radicals generation process, the concentration of redox initiator of potassium persulfate, $K_2S_2O_8$ and potassium metabisulfite, $K_2S_2O_5$ was set constant at 0.01 M with reaction time of 30 minutes. A membrane with area of 1 cm x 1 cm was immersed in 10 ml of mixture of $K_2S_2O_8$ and $K_2S_2O_5$ in centrifuge tube wan mixed by using rotator with 30 minutes reaction time.

The polyamide membrane from the redox initiator reaction was take out from the centrifuge tube and placed in the mixture of AA monomer and 0.01 EGDMA with different monomer concentration and reaction time. The concentrations of AA monomer of 0.1 M, 0.3 M, 0.5 M, 0.7 M and 1.0 M were varied in grafting process. During the grafting, 0.01 M EGDMA was used as a cross-linker. The reaction times also studied from 15 minutes, 30 minutes, 45 minutes, 60 minutes and 120 minutes.

After grafting, the polyamide membrane was removed from the centrifuge tube and washed with NaOH solution, pH 10 overnight followed with HCl solution, pH 4 another overnight. After washing process, the membrane was kept wet in water at temperature of 40 ºC. The purpose of doing this was to remove loosely bound homopolymer.

3.5 PROTEIN BINDING EXPERIMENT

3.5.1 Preparations of Standard Curve

Seven set of different BSA concentration in the range of 2 to 0.03125 mg/ml was prepared for the development of standard curve. The absorbance of BSA solution at 280nm was determined by using an UV-VIS spectrophotometer Model U-1800 HITHACHI. An absorbance-concentration standard curve was developed and samples were diluted with acetate buffer to within the absorbance range of the standard curve.

3.5.2 Protein Binding

1 cm x 1 cm of membrane was equilibrated in the 2 ml of acetate buffer for 2 hours. The membrane then is placed into the 2 ml of 2 mg/ml BSA solution dissolved in acetate buffer and incubated for 4 hours. The concentration of protein before and after the binding was determined by measuring UV absorbance at wavelength of 280nm. The amount of protein adsorbed can be determined by using the Equation 3.1:

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

where C_o is the initial concentration of protein, C_f is the final concentration of protein, W is the weight of membrane before binding and V is the volume of protein.

3.6 MEMBRANE CHARACTERIZATION

3.6.1 Fourier Transform Infrared (FTIR) protocol

Fourier Transform Infrared (FTIR) (Thermo Scientific Model) was used to identify the functional group of the unmodified and modified membrane. By using thin film plate, the functional groups of the membranes surface were identified. Before analyzing the sample, the plate was cleaned with acetone to remove any dust or substances that may affect the reading of the machine.

3.6.2 Water content

In water content experiment, the membrane was immersed in distilled water for 24 hours. Then its surface moisture was wiped and the wet membrane was weighed. The water content can be calculated from the Equation 3.2;

$$W_C(\%) = \frac{(W_W - W)}{W_W} \times 100 \qquad \text{Equation 3.2}$$

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

CHAPTER 4

RESULT AND DISCUSSION

4.1 STANDARD CURVES

An absorbance-concentration standard curve as shown in Figure 4.1 was developed from seven standard concentrations from 2 to 0.03125 mg/ml BSA.By using this standard curve, unknown protein concentration after binding can be determined by measuring the absorbance at 280nm.

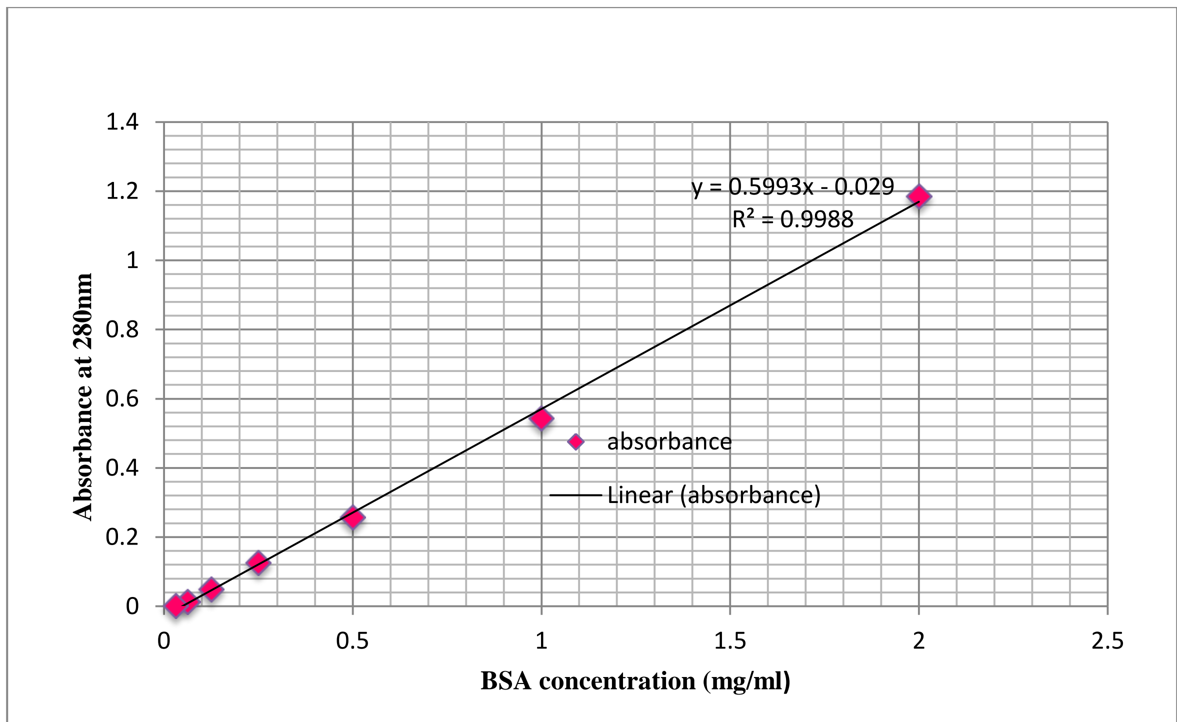


Figure 4.1: Standard curve graph for BSA protein

4.2 EFFECT OF MONOMER CONCENTRATION AND REACTION TIME

The binding capacity of BSA with different monomer concentration and reaction was showed in Table 4.1.

Table 4.1: Effect of monomer concentration and reaction time.

Monomer concentration (g/ml)	Reaction time (min)	Binding capacity, x 10^{-3} (mg/g)
Polyamide membrane	-	110.4319
0.1 M	15	218.4902
	30	189.4946
	45	533.4001
	60	260.8024
	120	168.4682
0.3 M	15	251.9606
	30	144.2794
	45	260.6091
	60	170.3972
	120	159.1756
0.5 M	15	315.5904
	30	163.2769
	45	443.3931
	60	223.1174
	120	330.1026
0.7 M	15	156.6579
	30	147.7807
	45	164.3540
	60	219.9211

	120	583.8336
1.0 M	15	246.1390
	30	148.0158
	45	318.3588
	60	219.3670
	120	80.7989

4.3 WATER CONTENT

The percentage of water content with different monomer concentration and reaction time was showed in Table 4.2.

Table 4.2: Water content of membrane

Monomer concentration (g/ml)	Reaction time (min)	Water content, W_c (%)
Polyamide membrane	-	58.49
0.1 M	15	71.67
	30	59.06
	45	68.34
	60	57.04
	120	58.46
0.3 M	15	61.48
	30	68.00
	45	61.07
	60	57.25
	120	42.96
0.5 M	15	66.39
	30	41.94
	45	62.22

	60	58.12
	120	54.96
0.7 M	15	54.33
	30	53.17
	45	55.63
	60	59.54
	120	66.14
1.0 M	15	71.32
	30	44.72
	45	52.68
	60	52.21
	120	67.97

4.4 EFFECT OF DIFFERENT MONOMER CONCENTRATION ON BSA ADSORPTION CAPACITY

For better understanding of the monomer concentration on adsorption capacity, five different experiments were performed with concentration of 0.1 M, 0.3 M, 0.5 M, 0.7 M and 1.0 M. The monomer concentration dependence of BSA adsorption with different time was shown in Figure 4.2.

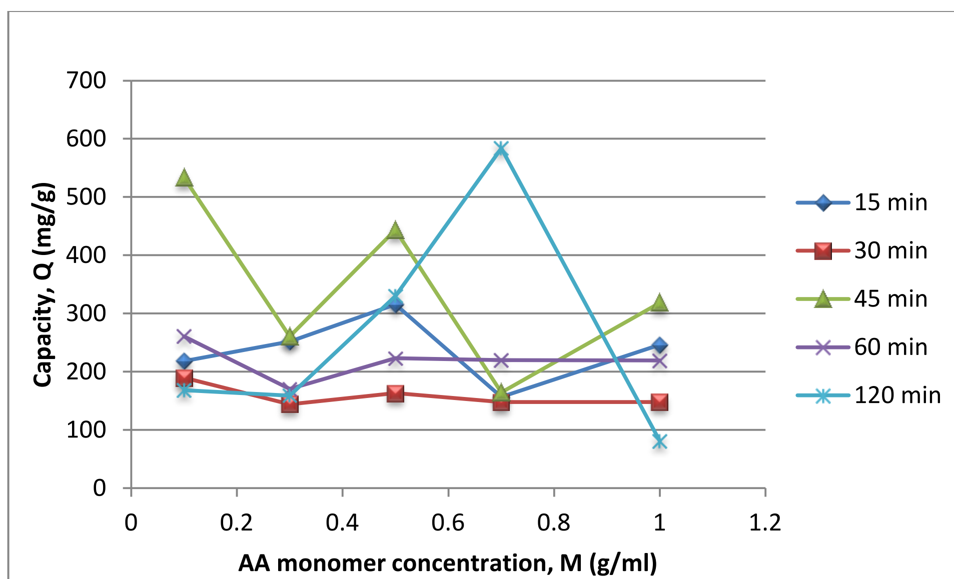


Figure 4.2: Effect of different monomer concentration on BSA adsorption capacity

For 15 minutes reaction time, as the concentration of monomer increases, the BSA adsorption capacity was also increased, from concentration of 0.1 M to 0.5 M. Then, BSA adsorption capacity was decreased at concentration of 0.7 M. However, at concentration 1.0 M, BSA adsorption capacity was increased again.

As shown in Figure 4.2, for 30 minutes reaction time, the trend of BSA adsorption capacity almost cannot see through the concentration. The BSA adsorption capacity was low and almost the same when increasing the monomer concentration. The highest binding capacity was at concentration of 0.1 M which is 189.5 mg/g and the lowest binding capacity was about 144.3 mg/g at concentration of 0.3 M. It was shows that for reaction time at 30 minutes, there was low BSA adsorption in the polyamide (PA) membrane surface.

For reaction time 45 minutes, the BSA adsorption capacity increased at concentration 0.1 M, 0.5 M and 1.0 M while it is decreased at concentration of 0.3 M and 0.7 M. The highest BSA adsorption capacity was at concentration 0.1 M which is about 533.4 mg/g. The increasing and decreasing of the BSA adsorption capacity for 45 minutes reaction time was obviously seen in the figure above.

The BSA adsorption capacity was decreasing when the concentration increased from 0.1 M to 0.3 M at 60 minutes reaction time. But at concentration 0.5 M, the BSA adsorption was increased and then almost constant with BSA adsorption capacity of 223.1 mg/g, 219.9 mg/g and 219.4 mg/g as increasing the monomer concentration. For 120 minutes, at concentration 0.1 M and 0.3 M, the BSA adsorption capacity was almost the same before it increased until concentration of 0.7 M. However, the BSA adsorption capacity decreased drastically from 583.8 mg/g to 80.8 mg/g at concentration of 1.0 M.

So, it can conclude that the effect of monomer concentration on BSA adsorption capacity which gives an optimum concentration was at 0.5 M for all reaction time except for 60 minutes reaction time. At 0.5 M they have reached the maximum concentration to get high binding capacity and if we can see from the figure above, after concentration of 0.5 M, the BSA adsorption capacity was decreased. Highest binding capacity means more carboxyl groups present in the modified membrane. But for reaction time of 60 minutes the optimum concentration was at 0.7 M. It is because at reaction time 60 minutes they need more concentrated Acrylic Acid (AA) monomer to get higher binding capacity.

4.5 EFFECT OF DIFFERENT REACTION TIME ON BSA ADSORPTION CAPACITY

To really determine the effect of reaction time on BSA adsorption capacity, five different experiments were performed with different reaction time which is 15 minutes, 30 minutes, 45 minutes, 60 minutes, and 120 minutes. Figure 4.3 shows the relationship between the BSA adsorption capacity and reaction time with different monomer concentration.

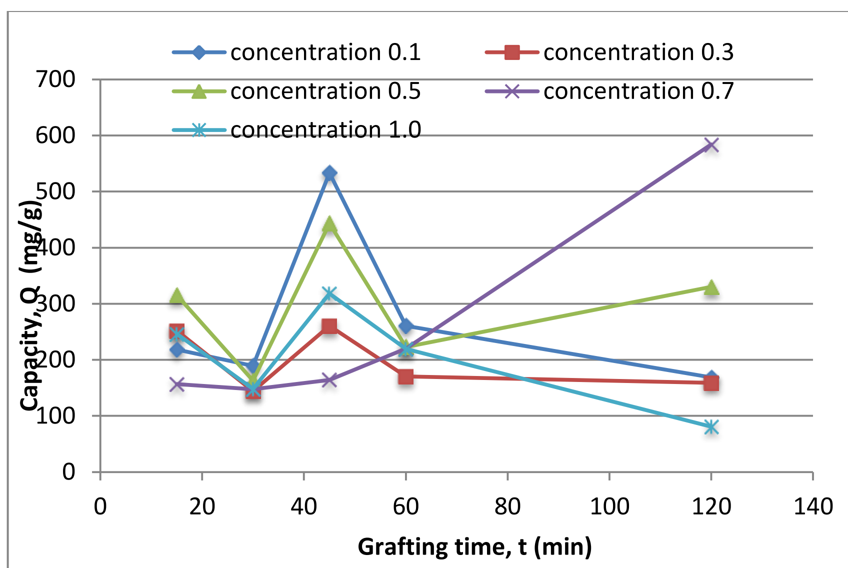


Figure 4.3: Effect of reaction time on BSA adsorption capacity

From Figure 4.3, it is show that all concentration almost has the same trend except for concentration at 0.7 M. At concentration of 0.1 M, 0.3 M and 1.0 M, BSA adsorption capacity was decreased at 30 minutes reaction time before it was increased to reach maximum binding capacity at 45 minutes reaction time. The BSA adsorption capacity then decreased as increasing the reaction time. By comparing with the three different monomer concentration, at concentration 0.1 M gives the highest binding capacity at 45 minutes reaction time which is 533.4 mg/g.

For the monomer concentration at 0.5 M, the BSA adsorption capacity was decreased at 30 minutes reaction time and drastically increased at 45 minutes reaction time. However the binding capacity then decreased again at 60 minutes before it increased back at reaction time of 120 minutes. The highest BSA adsorption capacity was at 45 minutes which is 443.4 mg/g while at 30 minutes reaction time, it gives the lowest binding capacity which is 163.3 mg/g.

At Acrylic Acid (AA) monomer concentration of 0.7 M, there was slightly decreased on BSA adsorption capacity at 30 minutes and then there were increasing the BSA adsorption capacity as increasing the reaction time. At this concentration, the trend was

totally different from the other four monomer concentration. It is because might be the experiment not handle properly or during the preparation of monomer concentration. So, the BSA adsorption capacity might be affected.

However, increasing the reaction time doesn't give an effect to the BSA adsorption capacity. Based on the Figure 4.3, it can conclude that an optimum reaction time which gives the highest BSA adsorption capacity was at 45 minutes of reaction time which means that the maximum protein adsorption on the polyamide (PA) membrane surface was at 45 minutes reaction time. From the figure, after it reaches an optimum reaction time, the BSA adsorption capacity was decreased. That is means after the maximum binding capacity was reached, the rate of polymerization will become slower. But at concentration 0.7 M, an optimum reaction time was at 120 minutes.

4.6 FTIR SPECTROSCOPY

The spectrum of the AA-grafted membrane is characterized by the appearance of a peak around 1709 cm^{-1} which associated with the C=O group of dimers of carboxylic acids (Socrates, 1994). The intensity of this peak increases as the grafting proceeds. Moreover, a slight but distinct shift towards lower frequencies (from 1712 to 1708 cm^{-1}) is observed. This might be the result of hydrogen bonding between the OH groups of acid carbonyl as their population increases.

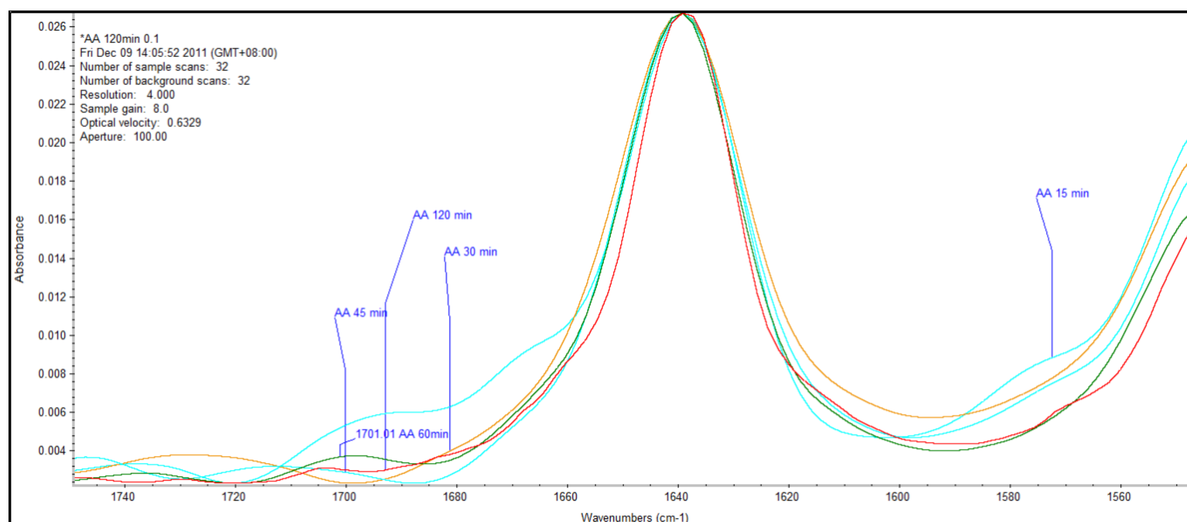


Figure 4.4: FTIR spectra at 0.1 M of acrylic acid (AA) monomer.

From Figure 4.4 above, for AA concentration at 0.1 M, by comparing to the other reaction time, the highest absorbance was at reaction time of 15 minutes. The highest of absorbance means the existence of carboxyl group in the membrane surface after grafting was high. From the figure, the peak not obviously seen and there were slightly different between the absorbance and the other reaction time.

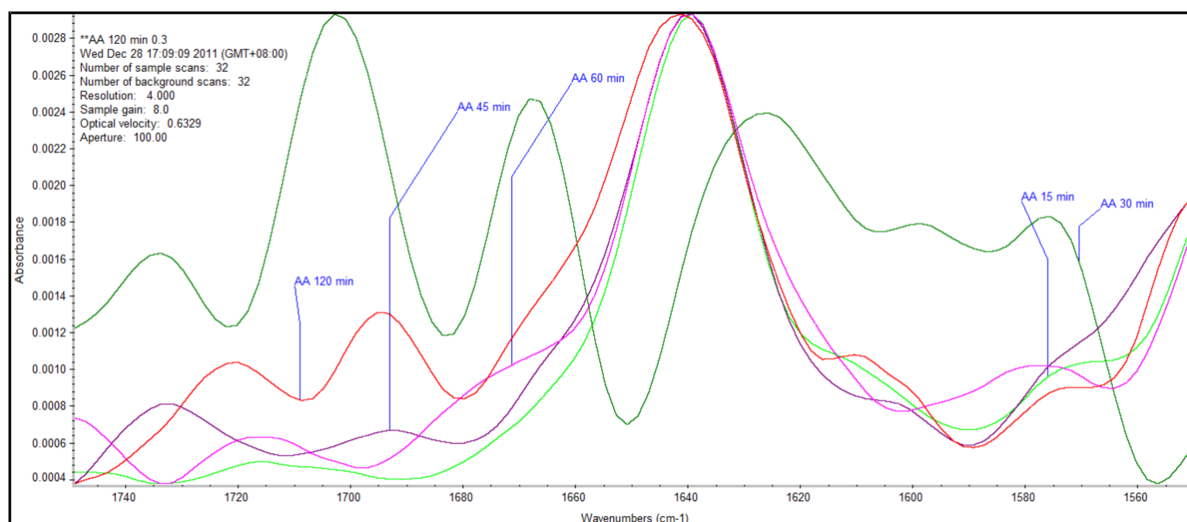


Figure 4.5: FTIR spectra at 0.3 M of acrylic acid (AA) monomer

At AA concentration of 0.3 M, even at 30 minutes reaction time have the highest peak of absorbance but it can't be consider due to the intensity which totally different with other reaction time. It is because during the analysis, the detector not properly penetrated due to unclean plate. For concentration of 0.3 M, the lowest absorbance was at 15 minutes reaction time.

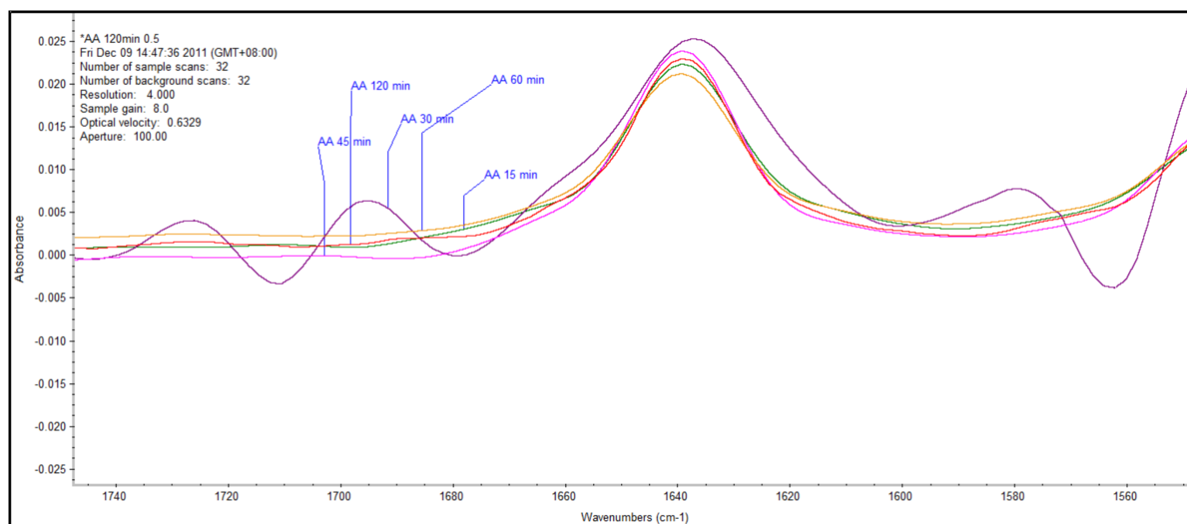


Figure 4.6: FTIR spectra at 0.5 M of acrylic acid (AA) monomer.

We can see from Figure 4.6, for AA concentration at 0.5 M, the highest absorbance was at reaction time of 60 minutes. At 60 minutes reaction time, there is more carboxyl group presence in the polyamide (PA) membrane surface. From the figure, the peak not obviously seen and there were slightly different between the absorbance and the other four reaction time.

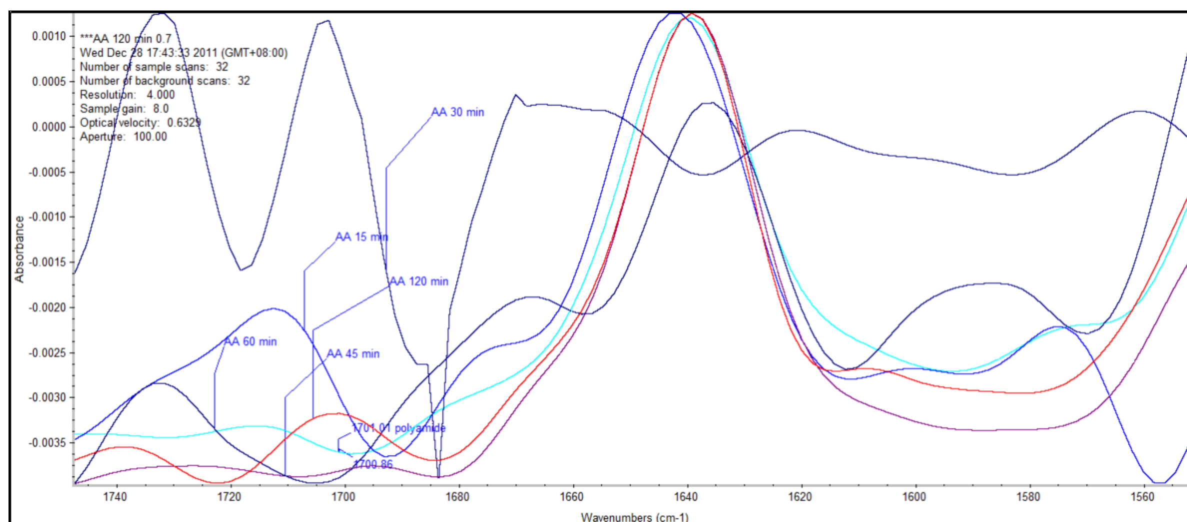


Figure 4.7: FTIR spectra at 0.7 M of acrylic acid (AA) monomer.

This case was same at concentration 0.3 M where at this concentration, even at 30 minutes reaction time has the highest peak of absorbance but it can't be consider due to the intensity which is totally different with other reaction time. It is because during the analysis, the detector not properly penetrated due to unclean plate. For concentration of 0.7 M, the highest absorbance was at 15 minutes reaction time.

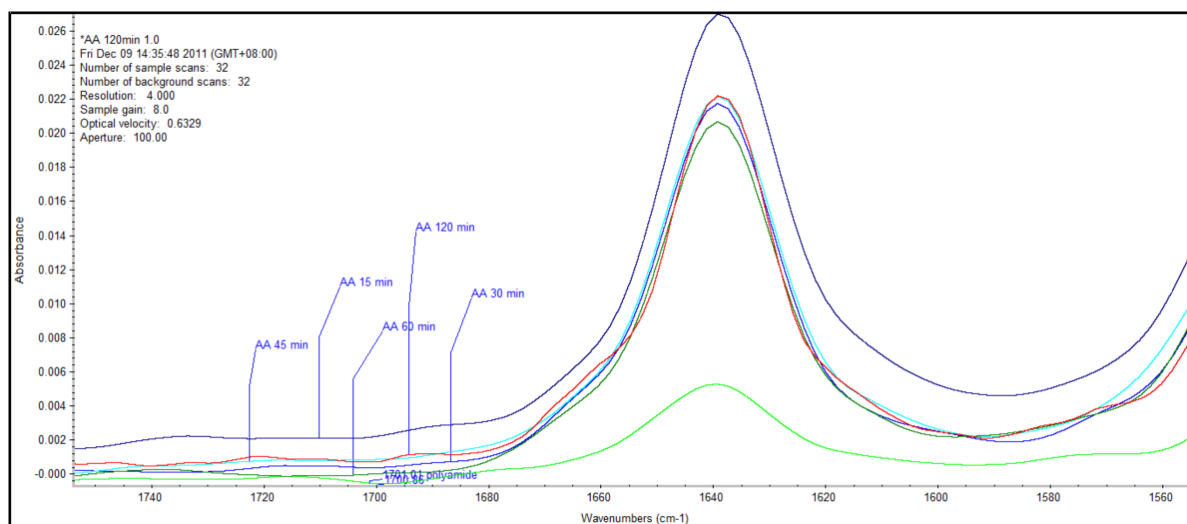


Figure 4.8: FTIR spectra at 1.0 M of acrylic acid (AA) monomer.

From Figure 4.8, at concentration 1.0 M, by comparing to the other reaction time, the highest absorbance was at reaction time of 15 minutes. The highest of absorbance means the existence of carboxyl group in the membrane surface after grafting was high. From the figure, the peak not obviously seen and there were slightly different between the absorbance and the other reaction time.

The water content is an important parameter for the membrane characterization and it is indirectly related to the hydrophilicity degree and directly related to the porosity of the membrane (Lin *et al*., 2006). The water content was actually represents the fraction of water molecules occupied in the pores of the membrane.

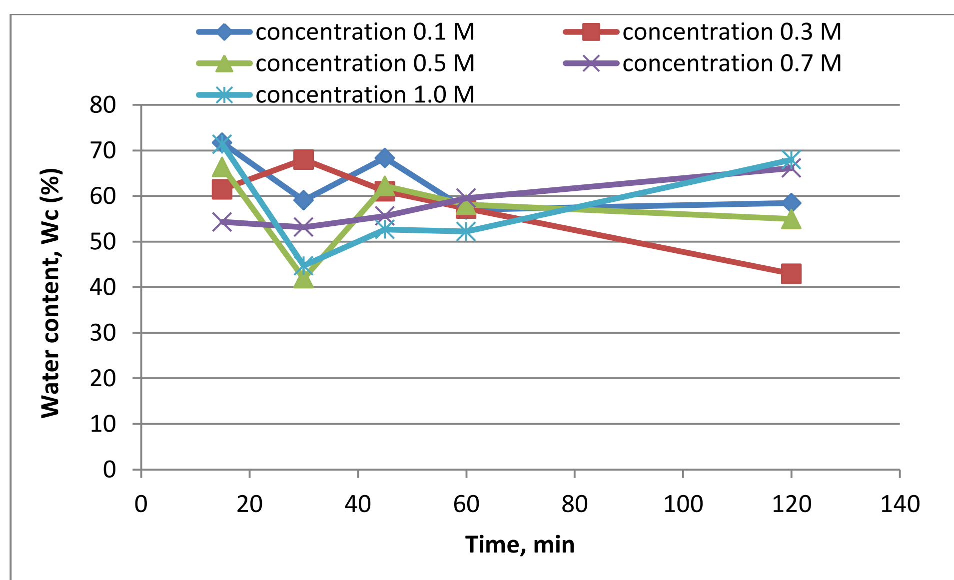


Figure 4.9: Water content with different monomer concentration and reaction time

At concentration 0.1 M, the highest water content in the membrane was at 15 minutes reaction time which is 71.67 % and the lowest water content was at 60 minutes which is 57.04 %. From the figure above, the increasing or decreasing of monomer concentration and reaction time was not affecting the water content in the membrane.

At concentration 0.3 M, the water content was increasing at 30 minutes reaction time and then was decreased as increasing the reaction time. The highest water content in the membrane was at 30 minutes reaction time which is 68 %. If we can see from the figure, at 0.5 M the water content was decreased at 30 minutes before it increased at 45 minutes. However, the water content was decreased again as increasing the reaction time.

There were slightly decreased on water content at concentration 0.7 M where then it was increased as increasing the reaction time. About 71% of water absorbed at 15 minutes reaction time which gives highest water content at concentration 1.0 M. Meanwhile, the lowest water content was 52.21 % at 60 minutes reaction time. The higher the water content indicates that the membrane has become more porous.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

The success of membrane chromatography preparation depends on the careful optimization of the chemical reaction involves. Detail study is necessary in order to produce high performance membrane chromatography for protein binding.

The effect of AA monomer concentration during grafting process was studied at range from 0.1 M to 1.0 M. The optimum monomer concentration was at 0.5 M which gives the binding capacity of 443.39 mg BSA/g membrane. The effect of reaction time from 15 minutes to 120 minutes was also studied. Highest BSA binding capacity achieved at 45 minutes reaction time which gives the binding capacity of 533.40 mg BSA/g membrane.

5.2 RECOMMENDATION

In order to get a better result, there are several recommendations that can take into consideration, as listed below:

i. In the present study, the equipment used to determine the functional group of the modified polyamide (PA) membrane was Fourier transform infrared spectroscopy (FTIR). In the further study, it is proposed to use the FTIR attenuated total reflectance (ATR). FTIR_ATR provides an easy and convenient way for determination of the relative amounts of different polymeric species present at the outmost part of PA membrane. The depth of penetration of the reflected IR beam in the ATR-FTIR is typically somewhat below 1 µm. Since the thickness is below 1 µm, this method proved highly suitable for analyzing the membranes. One advantage of ATR-FTIR over the FTIR is the limited path length into the sample. This avoids the problem of strong attenuation of the IR signal in highly absorbing media, such as aqueous solutions.

ii. An established polymer for membrane materials, especially hydrophilic polymers like cellulose, chitosan, cellulose acetate and etc., can also be a good candidate as a base polymer for preparing anion exchange membrane chromatography.

iii. Binding parameter can also be studied in the future such as pH or tested with different type of protein.

iv. For the future study, optimization the radical generation process during the redox initiation by getting the optimum reaction time and optimum concentration.

REFERENCES

Tongwen, X., (2005). Ion exchange membranes: State of their development and perspective. *Journal of Membrane Science*, 263,1–29.

Saxena, A., et. al. (2009). Membrane-based techniques for the separation and purification of proteins: An overview. *Advances in Colloid and Interface Science*, 145, 1-22.

Raja Ghosh, (2002). Protein separation using membrane chromatography: opportunities and challenges. *Journal of chromatography*, 952, 13-27.

Liming Cao, (2005). *Protein Separation with Ion-exchange Membrane Chromatography*. Degree of Master. Thesis. Worcester Polytechnic Institute.

Raja Ghosh, (2006). Principles of Bioseparations Engineering: 11.8.Singapore: World Scientific Publishing Co. Pte. Ltd.

Dr. Ehab Aboueladab. Chromatography course. Assistance Professor of Biochemistry, Mansoura University, Egypt.

Alberts, et al., (1998). Protein Separation: Part 1. Garland Publishing, a member of the Taylor & Francis Group.

Qin, J. J., Li, Y., Lee, L. S., & Lee, H., (2003). Cellulose acetate hollow fiber ultrafiltration membranes made from CA/PVP 360K/NMP/water. *Journal of Membrane Science*, 218, 173–183.

Fang *et al*., (2004). Preparation of Polysulfone-Based Cation-Exchange Membranes and Their Application in Protein Separation with a Plate-and-Frame Modul. *Journal of Reactive and Functional Polymer*, 59, 171–183.

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

Ulbricht M. and Yang H., (2005). Porous Polypropylene Membranes with Different Carboxyl Polymer Brush Layers for Reversible Protein Binding via Surface-Initiated Graft Copolymerization. *Chemical Material,* 17, 2622-2631.

Zhang *et al*., (2006). Adsorption behavior of cation-exchange resin-mixed polyethersulfone-based fibrous adsorbents with bovine serum albumin. *Desalinatio*n, 192, 224-233.

Freger, V., Gilron, J., & Belfer, S., (2002). TFC polyamide membranes modified by grafting of hydrophilic polymers: an FT-IR/AFM/ TEM study. *Journal of Membrane Science*, 209, 283-292.

Belfer, S., Purinson, Y., Fainshtein, R., Radchenko, Y., & Kedem, O., (1998). Surface modification of commercial composite polyamide reverse osmosis membranes. *Journal of Membrane Science*, 139, 175-181.

Bhattacharya, A., & Misra, B. N., (2004). Grafting: a versatile means to modify polymers: Techniques, factors and applications. *Program Polymer Science*, 29, 767-814.

Tai, H. C., (2004). *X-Ray crystallographic studies of bovine serum albumin and helicobacter pylori thioredoxin-2*. Degree of Master. Thesis. University of Saskatchewan, Saskatoon, Canada.

Ba, C., (2010). *Design of advanced reverse osmosis and nanofiltration membranes for water purification.* Ph.D. Thesis. University of Illinois, Urbana.

Dalwani, M. R., (2011). *Thin film composite nanofiltration membranes for extreme conditions*. Ph.D. Thesis. University of Twente.

Fidalgo, M. M., (2001). *Characteristics of ceramic membranes derived from metal-oxide nanoparticles.* Degree of Master. Thesis. Rice University, Texas.

Yang, H, (2008). *Fc-Binding Hexamer Peptide Ligands for Immunoglobulin Purification.* Degree of Doctor of Philosophy. Thesis. North Carolina State University, Raleigh.

Hedhammar, Karlstrom, A. E., & Hober, S.. *Chromatographic methods for protein purification.*Thesis.Royal Institute of Technology, Alba Nova University Center, Dept. of Biotechnology, Stockholm, Sweden.

Hu, J. Nicolet FTIR procedure. Materials Research Laboratory. University of California, Santa Barbara.

APPENDIX

APPENDIX A

A.1 Preparation of redox initiator

A.1.1 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

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

A.1.2 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

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

A.2 Preparation of monomer and cross-linker

A.2.1 Preparation of 500 ml of 0.01 M EthyleneglycolDimetacrylate, EGDMA

Molecular weight, MW of EGDMA = 198.22 g/mol

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

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

$$m = 0.9911\ g$$

$$1.05\ kg \equiv 1\ l$$

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

$$= 0.9440\ ml$$

A.2.2 Preparation of 100 ml of 0.1 M Acrylic Acid, AA

Molecular weight, MW of Acrylic Acid, AA = 72.06 g/mol

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

$$m = \frac{(0.1)(100)}{1000}(72.06\ )$$

$$m = 0.7206\ g$$

$$1.05\ kg \equiv 1\ l$$

$$0.7206 \times 10^{-3}\ kg \equiv \frac{(0.7206 \times 10^{-3})\ 1000\ ml}{1.05}$$

$$= 0.6863\ ml$$

A.2.3 Preparation of 100 ml of 0.3 M Acrylic Acid, AA

Molecular weight, MW of Acrylic Acid, AA = 72.06 g/mol

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

$$m = \frac{(0.3)(100)}{1000}(72.06\ )$$

$$m = 2.1618\ \text{g}$$

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

$$2.1618 \times 10^{-3}\ \text{kg} \equiv \frac{(2.1618 \times 10^{-3})\ 1000\ \text{ml}}{1.05}$$

$$= 2.0589\ \text{ml}$$

A.2.4 Preparation of 100 ml of 0.5 M Acrylic Acid, AA

Molecular weight, MW of Acrylic Acid, AA = 72.06 g/mol

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

$$m = \frac{(0.5)(100)}{1000}(72.06\ )$$

$$m = 3.6030\ \text{g}$$

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

$$3.6030 \times 10^{-3}\ \text{kg} \equiv \frac{(3.6030 \times 10^{-3})\ 1000\ \text{ml}}{1.05}$$

$$= 3.4314\ \text{ml}$$

A.2.5 Preparation of 100 ml of 0.7 M Acrylic Acid, AA

Molecular weight, MW of Acrylic Acid, AA = 72.06 g/mol

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

$$m = \frac{(0.7)(100)}{1000}(72.06\ )$$

$$m = 5.0442\ \text{g}$$

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

$$5.0442 \times 10^{-3} \text{ kg} \equiv \frac{(5.0442 \times 10^{-3})\ 1000 \text{ ml}}{1.05}$$

$$= 4.8040 \text{ ml}$$

A.2.6 Preparation of 100 ml of 1.0 M Acrylic Acid, AA

Molecular weight, MW of Acrylic Acid, AA = 72.06 g/mol

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

$$m = \frac{(1.0)(100)}{1000}(72.06)$$

$$m = 7.2060 \text{ g}$$

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

$$7.2060 \times 10^{-3} \text{ kg} \equiv \frac{(7.2060 \times 10^{-3})\ 1000 \text{ ml}}{1.05}$$

$$= 6.8629 \text{ ml}$$

A.3 Preparation of 1000 ml of 0.02M Acetate buffer with pH 4.0

Table 1: Buffer Recipe for 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,

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

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

$$= 0.0985 \text{ g} \times 10$$

$$= 0.985 \text{ g}$$

$$= \frac{0.985\text{g}}{1.049\ {}^{\text{g}}/_{\text{ml}}}$$

= 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

A.4 Preparation of Sodium Hydroxide (NaOH) with pH 10.0

At 20°C,

pH = 10

pOH = 14 – pH

= 14 – 10

= 4

$\log[\text{OH}^-] = -4$

Therefore,

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

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

Where, M is equal to mol/L

$$\frac{\text{No of mole}}{1\text{L}} = [\text{OH}^-]$$

$$\frac{\text{No of mole}}{1\text{L}} = 10^{-4}\text{M}$$

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

$\text{No of mole} = 10^{-4}\text{mol}$

So,

$\text{mass of NaOH} = \text{mol of NaOH x molecular weight of NaOH}$

$\text{mass of NaOH} = 10^{-4}\text{mol (40 g/mol)}$

$\text{mass of NaOH} = 4\text{x}10^{-3}\text{g}$

A.5 Preparation of Hydrochloric Acid (HCl) with pH 4.0

$\text{pH} = \log[\text{H}^+]$

$4 = \log[\text{H}^+]$

$[\text{H}^+] = 1\text{x}10^{-4}$

$$\frac{\text{No of mole HCl}}{1\text{L}} = [\text{H}^+]$$

$$\frac{\text{No of mole HCl}}{1\text{L}} = 10^{-4}\text{M}$$

Where, M is equal to mol/L

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

$\text{No of mole HCl} = 10^{-4}\text{mol}$

So,

$\text{mass of HCl} = \text{mol of NaOH x molecular weight of NaOH}$

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

$\text{mass of HCl} = 3.65\text{x}10^{-3}\text{g}$

$\text{mass of HCl} = 3.65\text{x}10^{-6}\text{kg}$

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

$\text{Specific Gravity of HCl, S. G}_{\text{HCl}} = 1.18$

$\rho_{\text{HCl}} = \text{S. G}_{\text{HCl}}(\rho_{\text{w}})$

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

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

The volume of HCL calculated by using equation

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

$$\text{volume of HCl, v} = \frac{3.65\text{x}10^{-6}\text{kg}}{1180\ \text{kg/m}^3}$$

$\text{volume of HCl, v} = 8.474\text{x}10^{-8}\text{m}^3$

$\text{volume of HCl, v} = 0.084\ \text{ml}$

APPENDIX B

B.1 Effect on unmodified Polyamide membrane

Table B1: Absorbance and binding capacity of unmodified Polyamide membrane

Absorbance			Average	Initial Concentration, C_i (mg/ml)	Final Concentration, C_f (mg/ml)	Volume , V (ml)	Weight, W (g)	Binding Capacity, Q $\times 10^{-3}$
1	2	3						
1.018	1.023	1.031	1.024	2	1.7570	2	0.0044	110.4319

B.2 Effect of Monomer Concentration

B.2.1 Concentration of 0.1 M of Acrylic Acid

Table B2: Absorbance and binding capacity of 0.1 M of Acrylic Acid

Time (min)	Absorbance			Average	Initial Concentration, C_i (mg/ml)	Final Concentration, C_f (mg/ml)	Volume , V (ml)	Weight , W (g)	Binding Capacity, $Q \times 10^{-3}$
	1	2	3						
15	0.864	0.983	0.994	0.947	2	1.6286	2	0.0034	218.4902
30	0.863	0.884	0.876	0.874	2	1.5073	2	0.0052	189.4946
45	0.481	0.436	0.482	0.466	2	0.8265	2	0.0044	533.4001
60	0.597	0.682	0.87	0.716	2	1.2437	2	0.0058	260.8024
120	0.983	0.78	0.928	0.897	2	1.5451	2	0.0054	168.4682

B.2.2 Concentration of 0.3 M of Acrylic Acid

Table B3: Absorbance and binding capacity of 0.3 M of Acrylic Acid

Time (min)	Absorbance			Average	Initial Concentration, C_i (mg/ml)	Final Concentration, C_f (mg/ml)	Volume , V (ml)	Weight, W (g)	Binding Capacity, $Q \times 10^{-3}$
	1	2	3						
15	0.695	0.858	0.778	0.777	2	1.3449	2	0.0052	251.9606
30	0.934	0.971	1.085	0.997	2	1.7114	2	0.0040	144.2794
45	0.465	0.95	0.899	0.771	2	1.3354	2	0.0051	260.6091
60	0.855	0.969	0.827	0.884	2	1.5229	2	0.0056	170.3972
120	0.774	0.858	0.775	0.802	2	1.3872	2	0.0077	159.1756

B.2.3 Concentration of 0.5 M of Acrylic Acid

Table B4: Absorbance and binding capacity of 0.5 M of Acrylic Acid

Time (min)	Absorbance			Average	Initial Concentration, C_i (mg/ml)	Final Concentration, C_f (mg/ml)	Volume , V (ml)	Weight, W (g)	Binding Capacity, $Q \times 10^{-3}$
	1	2	3						
15	0.882	0.844	0.648	0.791	2	1.3688	2	0.0040	315.5904
30	0.677	0.802	0.973	0.817	2	1.4122	2	0.0072	163.2769
45	0.512	0.478	0.486	0.492	2	0.8693	2	0.0051	443.3931
60	0.976	0.796	0.754	0.842	2	1.4534	2	0.0049	223.1174
120	0.549	0.58	0.629	0.586	2	1.0262	2	0.0059	330.1026

B.2.4 Concentration of 0.7 M of Acrylic Acid

Table B5: Absorbance and binding capacity of 0.7 M of Acrylic Acid

Time (min)	Absorbance			Average	Initial Concentration, C_i (mg/ml)	Final Concentration, C_f (mg/ml)	Volume , V (ml)	Weight, W (g)	Binding Capacity, $Q \times 10^{-3}$
	1	2	3						
15	0.835	0.946	0.911	0.897	2	1.5457	2	0.0058	156.6579
30	0.934	0.877	0.914	0.908	2	1.5640	2	0.0059	147.7807
45	0.77	0.882	0.926	0.859	2	1.4823	2	0.0063	164.3540
60	0.631	0.833	0.997	0.820	2	1.4172	2	0.0053	219.9211
120	0.451	0.378	0.423	0.417	2	0.7448	2	0.0043	583.8336

B.2.5 Concentration of 1.0 M of Acrylic Acid

Table B6: Absorbance and binding capacity of 1.0 M of Acrylic Acid

Time (min)	Absorbance			Average	Initial Concentration, C_i (mg/ml)	Final Concentration, C_f (mg/ml)	Volume , V (ml)	Weight, W (g)	Binding Capacity, $Q \times 10^{-3}$
	1	2	3						
15	0.924	0.993	0.928	0.948	2	1.6308	2	0.0030	246.1390
30	0.952	0.755	0.897	0.868	2	1.4967	2	0.0068	148.0158
45	0.661	0.674	0.657	0.664	2	1.1563	2	0.0053	318.3588
60	0.642	0.701	0.884	0.742	2	1.2871	2	0.0065	219.3670
120	1.095	1.025	1.091	1.070	2	1.8344	2	0.0041	80.7989

B.3 Effect on Reaction Time

B.3.1 Reaction Time of 15 minutes

Table B7: Absorbance and binding capacity at reaction time of 15 minutes

Concentration, (g/ml)	Absorbance			Average	Initial Concentration, C_i (mg/ml)	Final Concentration, C_f (mg/ml)	Volume , V (ml)	Weight , W (g)	Binding Capacity, Q $\times 10^{-3}$
	1	2	3						
0.1	0.864	0.983	0.994	0.947	2	1.6286	2	0.0034	218.4902
0.3	0.695	0.858	0.778	0.777	2	1.3449	2	0.0052	251.9606
0.5	0.882	0.844	0.648	0.791	2	1.3688	2	0.004	315.5904
0.7	0.835	0.946	0.911	0.897	2	1.5457	2	0.0058	156.6579
1.0	0.924	0.993	0.928	0.948	2	1.6308	2	0.003	246.1390

B.3.2 Reaction Time of 30 minutes

Table B8: Absorbance and binding capacity at reaction time of 30 minutes

Concentration, (g/ml)	Absorbance			Average	Initial Concentration, C_i (mg/ml)	Final Concentration, C_f (mg/ml)	Volume , V (ml)	Weight , W (g)	Binding Capacity, Q $\times 10^{-3}$
	1	2	3						
0.1	0.863	0.884	0.876	0.874	2	1.507314089	2	0.0052	189.4946
0.3	0.934	0.971	1.085	0.997	2	1.711441126	2	0.004	144.2794
0.5	0.677	0.802	0.973	0.817	2	1.412203126	2	0.0072	163.2769
0.7	0.934	0.877	0.914	0.908	2	1.564046944	2	0.0059	147.7807
1.0	0.952	0.755	0.897	0.868	2	1.496746204	2	0.0068	148.0158

B.3.3 Reaction Time of 45 minutes

Table B9: Absorbance and binding capacity at reaction time of 45 minutes

Concentration, (g/ml)	Absorbance			Average	Initial Concentration, C_i (mg/ml)	Final Concentration, C_f (mg/ml)	Volume , V (ml)	Weight , W (g)	Binding Capacity, Q $\times 10^{-3}$
	1	2	3						
0.1	0.481	0.436	0.482	0.466	2	0.8265	2	0.0044	533.4001
0.3	0.465	0.95	0.899	0.771	2	1.3354	2	0.0051	260.6091
0.5	0.512	0.478	0.486	0.492	2	0.8693	2	0.0051	443.3931
0.7	0.77	0.882	0.926	0.859	2	1.4823	2	0.0063	164.3540
1.0	0.661	0.674	0.657	0.664	2	1.1563	2	0.0053	318.3588

B.3.4 Reaction Time of 60 minutes

Table B10: Absorbance and binding capacity at reaction time of 60 minutes

Concentration, (g/ml)	Absorbance			Average	Initial Concentration, C_i (mg/ml)	Final Concentration, C_f (mg/ml)	Volume , V (ml)	Weight , W(g)	Binding Capacity, Q $\times 10^{-3}$
	1	2	3						
0.1	0.597	0.682	0.87	0.716	2	1.2437	2	0.0058	260.8024
0.3	0.855	0.969	0.827	0.884	2	1.5229	2	0.0056	170.3972
0.5	0.976	0.796	0.754	0.842	2	1.4534	2	0.0049	223.1174
0.7	0.631	0.833	0.997	0.820	2	1.4172	2	0.0053	219.9211
1.0	0.642	0.701	0.884	0.742	2	1.2871	2	0.0065	219.3670

B.3.5 Reaction Time of 120 minutes

Table B11: Absorbance and binding capacity at reaction time of 120 minutes

Concentration, (g/ml)	Absorbance			Average	Initial Concentration, C_i (mg/ml)	Final Concentration, C_f (mg/ml)	Volume , V (ml)	Weight , W (g)	Binding Capacity, Q $\times 10^{-3}$
	1	2	3						
0.1	0.983	0.78	0.928	0.897	2	1.5451	2	0.0054	168.4682
0.3	0.774	0.858	0.775	0.802	2	1.3872	2	0.0077	159.1756
0.5	0.549	0.58	0.629	0.586	2	1.0262	2	0.0059	330.1026
0.7	0.451	0.378	0.423	0.417	2	0.7448	2	0.0043	583.8336
1.0	1.095	1.025	1.091	1.070	2	1.8344	2	0.0041	80.7989

APPENDIX C

C.1 Water content

C.1.1 Unmodified polyamide (PA) membrane

Table C1: Water content at 0.1 M AA

W_w	W	W_c(%)
0.0106	0.0044	58.49

C.1.2 Monomer concentration 0.1 M

Table C2: Water content at 0.1 M AA

Time (min)	W_w	W	W_c(%)
15	0.0120	0.0034	71.67
30	0.0127	0.0052	59.06
45	0.0139	0.0044	68.34
60	0.0135	0.0058	57.04
120	0.0130	0.0054	58.46

C.1.3 Monomer concentration 0.3 M

Table C3: Water content at 0.3 M AA

Time (min)	W_w	W	W_c(%)
15	0.0135	0.0052	61.48
30	0.0125	0.004	68.00
45	0.0131	0.0051	61.07
60	0.0131	0.0056	57.25
120	0.0135	0.0077	42.96

C.1.4 Monomer concentration 0.5 M

Table C4: Water content at 0.5 M AA

Time (min)	W_w	W	W_c(%)
15	0.0119	0.004	66.39
30	0.0124	0.0072	41.94
45	0.0135	0.0051	62.22
60	0.0117	0.0049	58.12
120	0.0131	0.0059	54.96

C.1.5 Monomer concentration 0.7 M

Table C5: Water content at 0.7 M AA

Time (min)	W_w	W	W_c(%)
15	0.0127	0.0058	54.33
30	0.0126	0.0059	53.17
45	0.0142	0.0063	55.63
60	0.0131	0.0053	59.54
120	0.0127	0.0043	66.14

C.1.6 Monomer concentration 1.0 M

Table C6: Water content at 1.0 M AA

Time (min)	W_w	W	W_c(%)
15	0.0136	0.0039	71.32
30	0.0123	0.0068	44.72
45	0.0112	0.0053	52.68
60	0.0136	0.0065	52.21
120	0.0128	0.0041	67.97

APPENDIX D

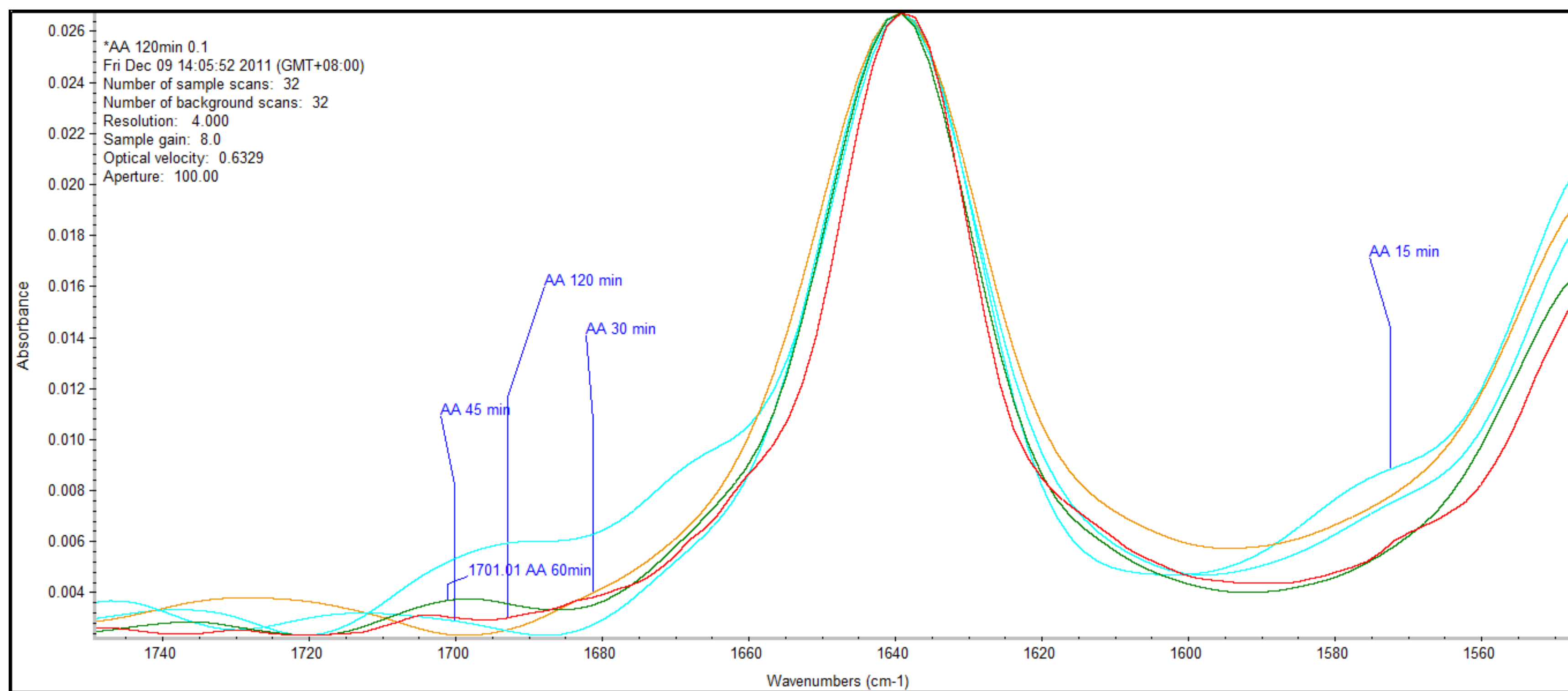


Figure D1: FTIR spectra at AA concentration of 0.1 M

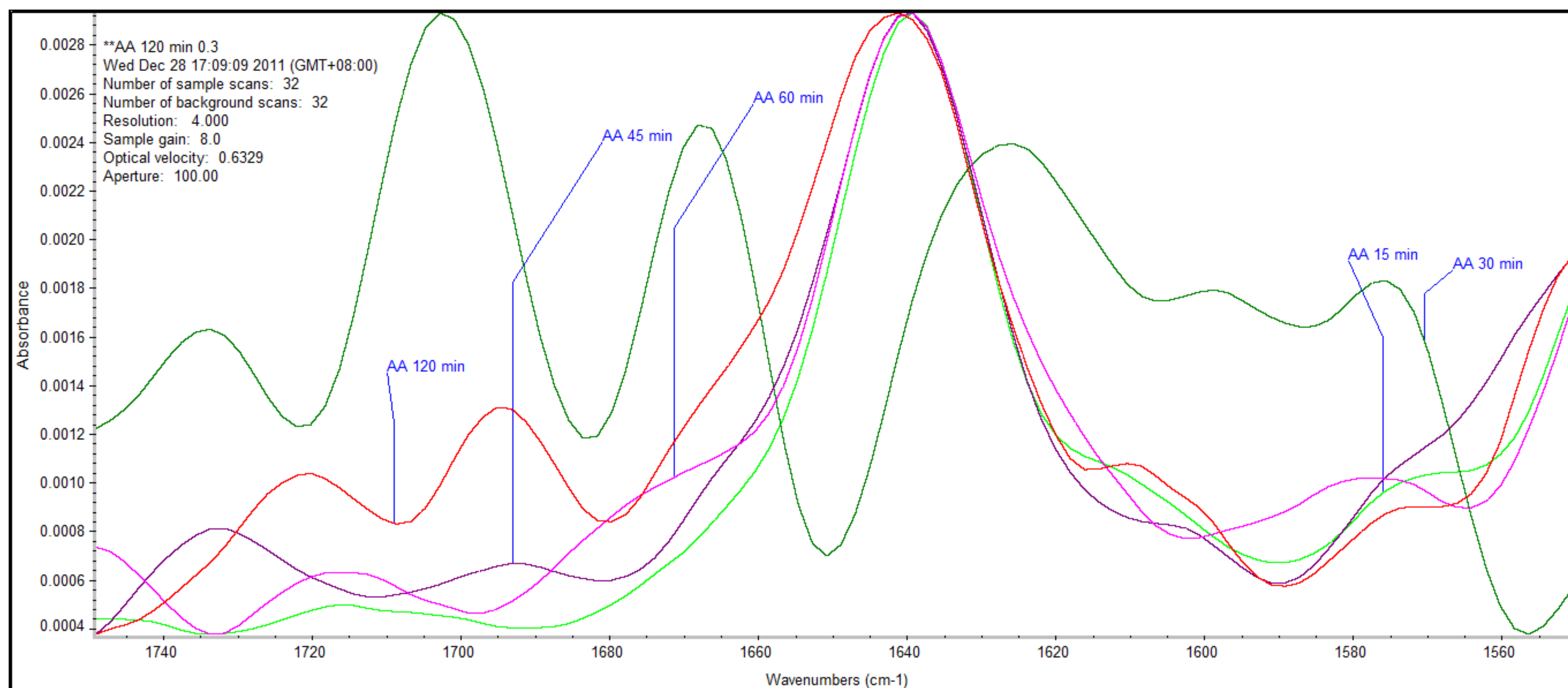


Figure D2: FTIR spectra at AA concentration of 0.3 M

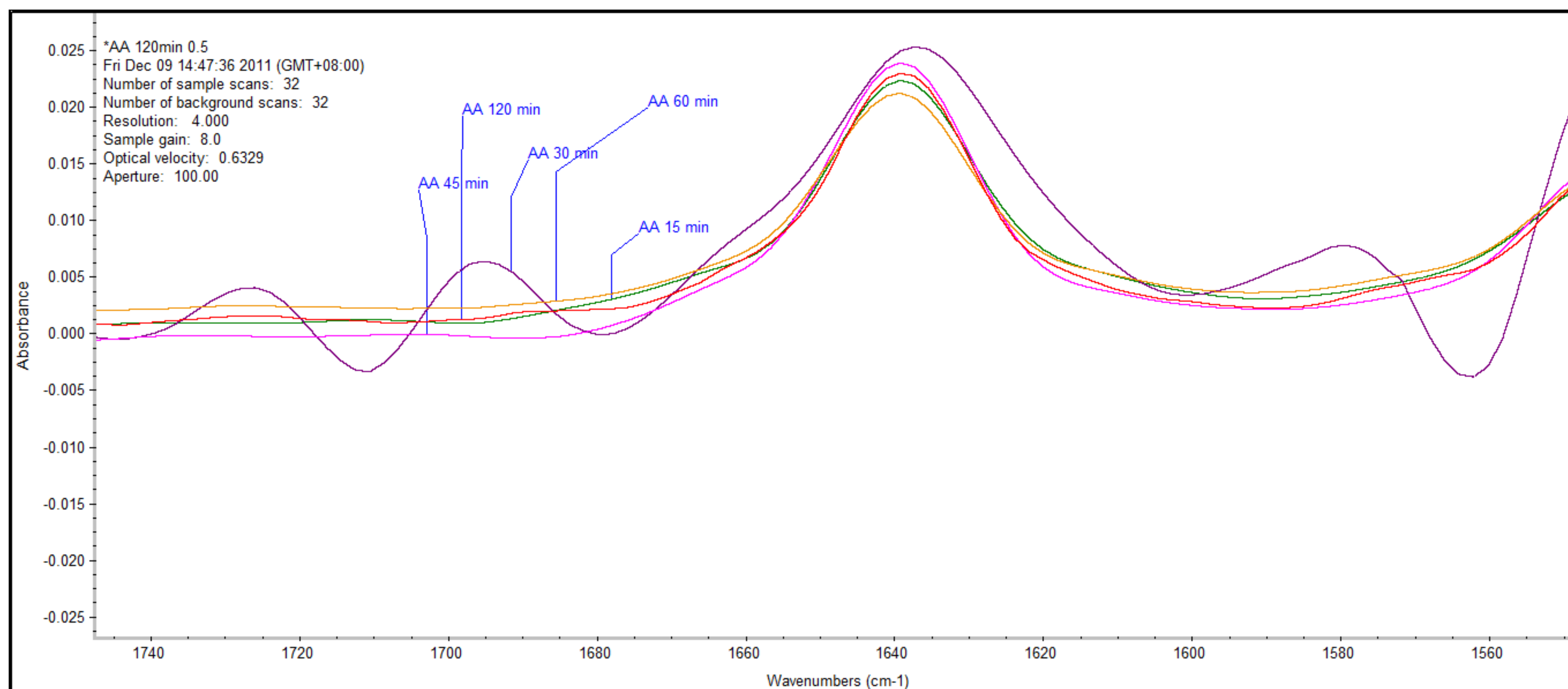


Figure D3: FTIR spectra at AA concentration of 0.5 M

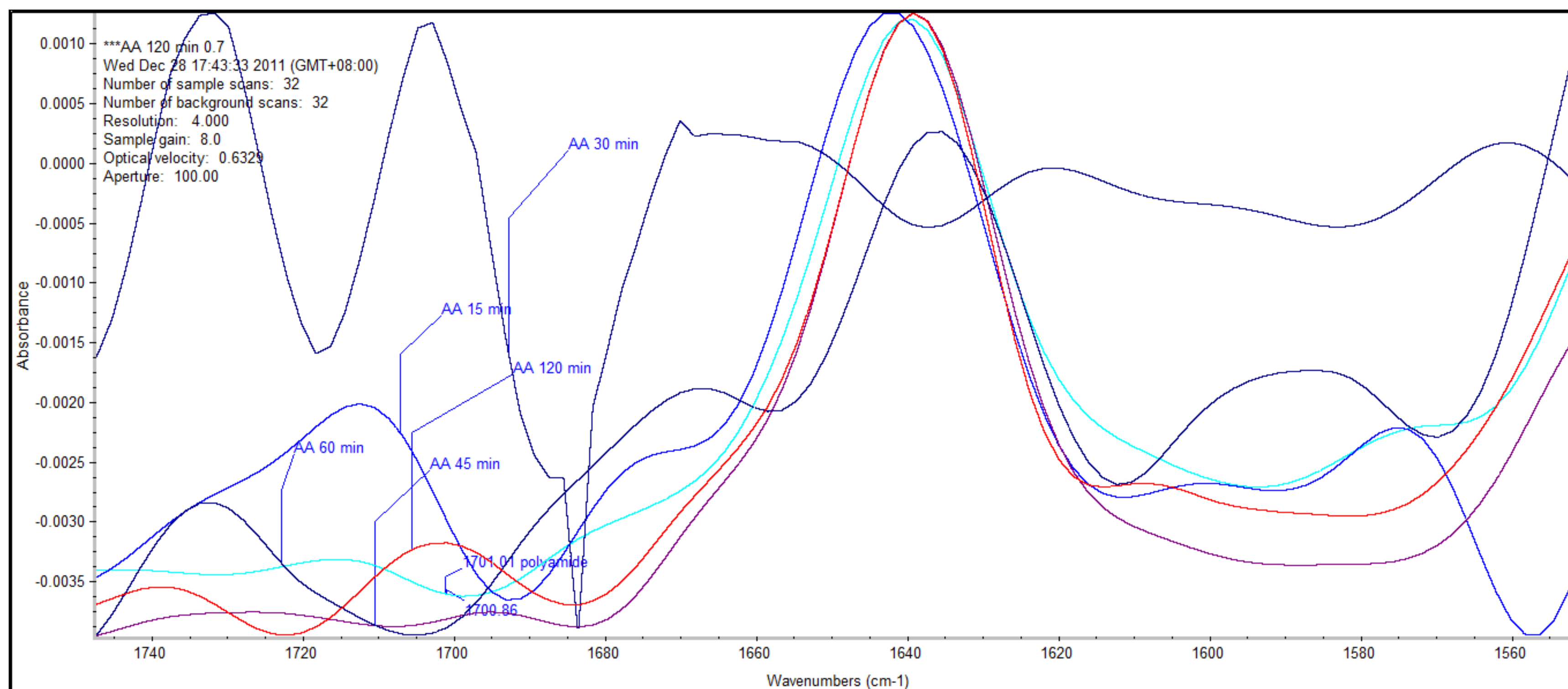


Figure D4: FTIR spectra at AA concentration of 0.7 M

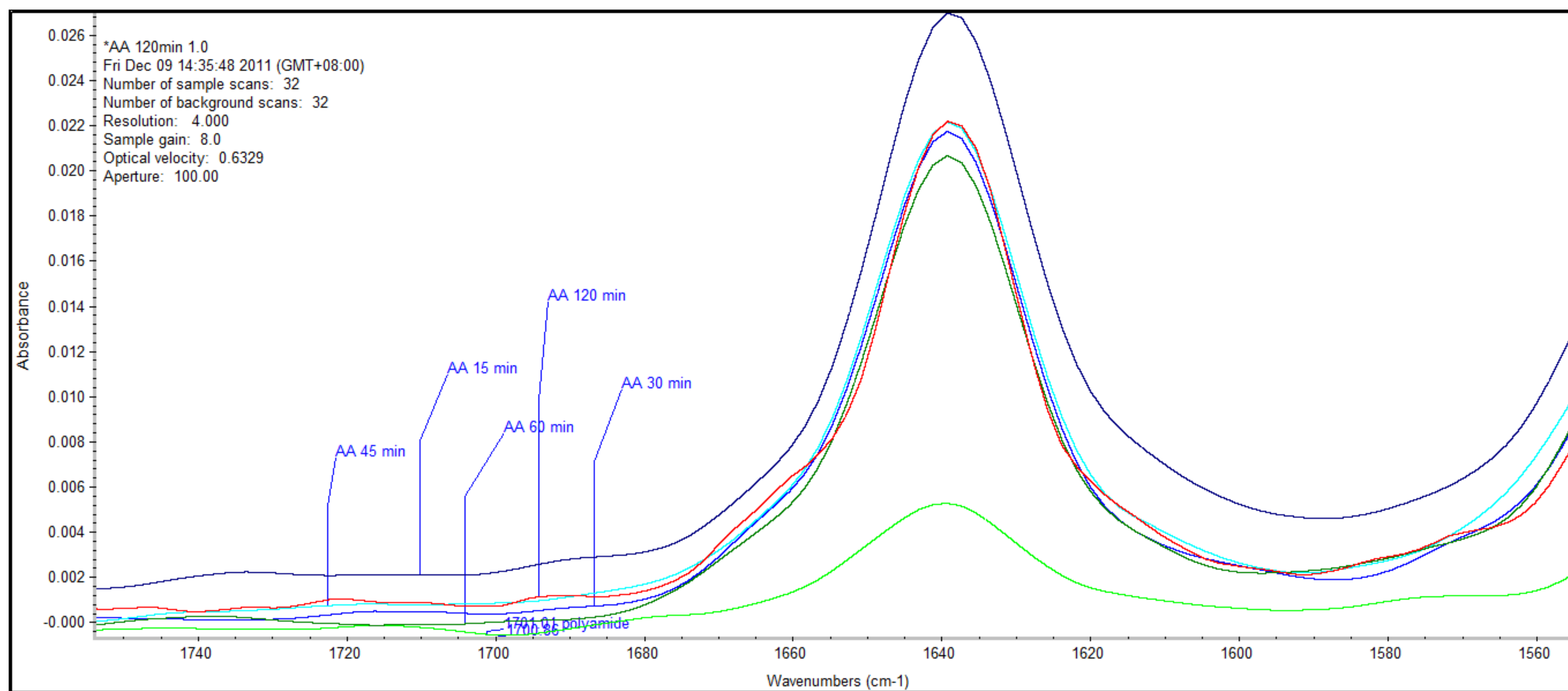


Figure D5: FTIR spectra at AA concentration of 1.0 M