# PREPARATION OF ION EXCHANGE MEMBRANE CHROMATOGRAPHY BY MODIFICATION OF POLYETHERSULFONE MEMBRANE THROUGH UV GRAFTING OF [2-(ACRYLOYLOXY) ETHYL] TRIMETHYL AMMONIUM CHLORIDE AND ACRYLIC ACID MONOMER.

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## SUPERVISOR'S DECLARATION

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

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## STUDENT'S DECLARATION

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

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*Special dedication to my beloved family especially my mother and*

*my sisters and brothers for their love and encouragement.*

*And,*

*Thanks to my friends, my fellow course mates and all faculty members.*

*For all your care, support and best wishes*

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# PREPARATION OF ION EXCHANGE MEMBRANE CHROMATOGRAPHY BY MODIFICATION OF POLYETHERSULFONE MEMBRANE THROUGH UV GRAFTING OF [2-(ACRYLOYLOXY) ETHYL] TRIMETHYL AMMONIUM CHLORIDE AND ACRYLIC ACID MONOMER.

## ABSTRACT

Chromatographic separation of protein mixtures has become one of the most effective widely used means of techniques to purifying individual proteins. Packed bed chromatography is the common technique that is used configuration for the protein separation. However, packed bed chromatography has its some limitations during separation process such as high pressure drop and time consuming. Membrane chromatography then introduced to overcome the limitations of the packed bed chromatography. In the current research, polyethersulfone (PES) commercial membrane was converted into ion exchange (IEX) membrane chromatography by attaching [2-(acryloyloxy)ethyl] trimethyl ammonium chloride (AETMA) and acrylic acid (AA) monomer using UV light irradiation technique. The effect of AETMA and AA monomer concentration from 1.5 M to 2.0 M was studied. The IEX membrane was characterized in term of degree of grafting, changed of functional group as well as protein binding capacity using pure bovine serum albumin. For AETMA-grafted membrane, the binding capacity increase about the 65.32 % as the monomer concentration increase from 1.5 M to 2.0 M. While, for AA-grafted membrane, the binding capacity shows a huge increment when the monomer concentration was increase from 1.5 M to 2.0 M with amount 78.65 %.

# PENYEDIAAN MEMBRAN KROMATOGRAFI PERTUKARAN ION MELALUI PENGUBAHSUAIAN MEMBRAN POLIETERSULFON BERDASARKAN CANTUMAN UV UNTUK MONOMER [2 - (ACRYLOYLOXY) ETIL] TRIMETHYL AMMONIUM KLORIDA DAN MONOMER ACRYLIC ACID.

## ABSTRAK

Pengasingan kromatografi campuran protein telah menjadi salah satu cara yang digunakan secara meluas dan paling berkesan untuk menulenkan protein individu. Kromatografi turus terpadat adalah teknik biasa yang digunakan untuk pemisahan protein. Walau bagaimanapun, kromatografi turus terpadat mempunyai beberapa kekangan dalam proses pemisahan seperti penurunan tekanan yang tinggi dan memakan masa. Membran kromatografi kemudian diperkenalkan untuk mengatasi kekangan kromatografi turus terpadat tersebut. Dalam kajian pada masa kini, komersial polietersulfon (PES) membran telah diubahsuai menjadi membran kromatografi pertukaran ion (IEX) dengan menggunakan [2 - (acryloyloxy) etil] ammonium klorida trimethyl (AETMA) dan asid akrilik (AA) sebagai monomer dan menggunakan teknik penyinaran cahaya UV. Kesan kepekatan AETMA dan AA monomer dari 1.5 M kepada 2.0 M telah dikaji. Membran IEX telah dicirikan dalam darjah cantuman, perubahan kumpulan berfungsi serta keupayaan mengikat protein menggunakan serum albumin lembu tulen. Untuk AETMA-dicantumkan membran, peningkatan kapasiti mengikat sebanyak 65.32% sebagai peningkatan kepekatan monomer dari 1.5 M kepada 2.0 M. Sementara itu, untuk AA-dicantumkan membran, kapasiti mengikat menunjukkan kenaikan yang besar apabila kepekatan monomer meningkat dari 1.5 M kepada 2.0 M dengan jumlah 78.65%.

# TABLE OF CONTENT

**CHAPTER 3 METHODOLOGY**



**CHAPTER 4 RESULTS AND DISCUSSION**



**CHAPTER 5 CONCLUSION AND RECOMMENDATION**

# LIST OF TABLES

**PAGE**

# LIST OF FIGURE

**PAGE**

## LIST OF SYMBOLS

| | |
|---|---|
| µm | Micrometres |
| M | Molar |
| mg | Miligram |
| g | Grams |
| hr | Hour |
| L | Litre |
| ml | Millilitre |
| m | Meter |
| cm | Centimeter |
| % | percentage |
| MW | Molecular weight |

## LIST OF ABBREVIATIONS

| | |
|---|---|
| PES | Polyethersulfone |
| AETMA | [2-(Acryloyloxy)Ethyl] Trimethyl Ammonium Chloride |
| AA | Acrylic Acid |
| MF | Microfiltration |
| UF | Ultrafiltration |
| RO | Revers Osmosis |
| NF | Nanofiltration |
| HPTFF | High-Performance Tangential Flow Filltration |
| IEXC | Ion Exchange Chromatography |
| S | Sulfonic |
| SP | Sulfopropyl |
| DEAE | Diethylaminoethyl |
| Q | Quaternary Ammonium |
| $NaH_2PO_4.1H_2O$ | Sodium Phosphate, Mono-Sodium Salt |
| $NaH_2PO_4.7H_2O$ | Sodium Phosphate, Disodium Salt |
| NaCl | Sodium Chloride |
| FTIR | Fourier Transform Infrared Spectroscopy |
| BSA | Bovine Serum Albumin |
| PI | Isoelectric Point |

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background of Study

Protein separation or protein purification is a process that isolates a single protein from complex protein mixture.

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

The packed bed chromatography is previously used to separate the protein. The absorbent normally packed into a cylindrical column. 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 this study, microfiltration membrane is chosen as a membrane process to separate the protein. Microfiltration is a membrane filtration process which discharges insanitary from a liquid or gas by passage through a microporous membrane. The range of the pore size of the microfiltration membrane is between 0.1 to 10 micrometres (μm). Method of protein separation for this study is ion-exchange chromatography. Ion-exchange chromatography is a process that permits the separation of ion and polar molecules depend on their charge. Ion-exchange chromatography separates compound based on the nature and degree of their ionic charge. Anion exchange resins have a positive charge and are used to retain and separate negatively charged compounds. On the other hand, the positively charge of compound will be separated by cation exchange resins. For this study, polyethersulfone (PES) microfiltarion membrane is modified by using ultaraviolet radiation grafting method (Malaisamy et al., 2010). In the current study, membrane chromatography was prepared through modification of PES by UV-grafting technique of AETMA and AA monomers.

## 1.2 Problem Statement

Chromatography is widely used for the separation and analysis of protein and nucleic acid. Chromatographic processes are traditionally carried out using packed beds. However, packed bed chromatography using conventional chromatographic media has several major disadvantages. The pressure drop tends to increase during the process due to bed consolidation. In addition to this, there are major diffusion limitations to the transport of solute molecules to their binding sites within the pores

of the chromatographic media. Besides, relatively time consuming process due to restricted flow rate operation. An alternative approach to solving some these problems is to use membrane chromatography, the transport of solutes to the binding sites take place by convection and hence the process is very fast (Ghosh, 2001). During this study, ion exchange membrane chromatography will be developed by modification of commercial microfiltration membrane.

## 1.3 Research Objective

The main purpose of this study is to study the effect of [2-(acryloyloxy)ethyl] trimethyl ammonium chloride (AETMA) and acrylic acid (AA) monomer concentration during preparation of polyethersulfone ion exchange membrane chromatography.

## 1.4 Scope of Study

In order to achieve the research objective, the following scopes was outlined:

i) To study on the effect of AETMA monomer concentration from 1.5 to 2 mg/ml during UV-grafting process.

ii) To study on the effect of AA monomer concentration from 1.5 to 2 mg/ml during UV-grafting process.

iii) To characterize the modified membrane by using degree of grafting and Fourier Transform Infrared Spectroscopy (FTIR) analysis.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Membrane

Membrane is an interphase, which act as barrier of the flow of molecular and ionic species in the liquids or vapour that commonly in heterogeneous and contacting the two surfaces. It is also shows different selectivity as a semi-permeable barrier between species. Function of the membrane is to selectively allow a species to permeate through the membrane freely whilst hindering the permeation of other component (Silva, 2007). The unique separation principle of the membranes was attracted the attention of chemical, chemist and biotechnical engineer. Membrane separation can be operated isothermally at low temperature with less consumption of energy and do not need additives compared to the other separation process. Therefore, reaction of the process and the up scaling and downscaling membrane separation are easy. Lately, the benefit of membrane-based process was realizing in biotechnology due to their ability for size and charge based separation of protein with high purity and throughput (Ahmed. 2005).

Membranes have previously been used for size-based separations with high-throughput but relatively low-resolution requirements (Saxena et al., 2009). Current research and development efforts are directed toward drastic improvements in selectivity while maintaining the inherent high-throughput characteristics of membranes. Although, essentially all membrane processes are used for bioseparation, but greatest interests have been shown in the pressure-driven technologies such as MF or UF. Recently, electric or ultrasonic fields were imposed simultaneously to increase throughput and membrane selectivity as well as reducing membrane fouling which is a common phenomenon in pressure-driven membrane separation technologies. During last two decades, membrane technologies were frequently used for the size or charge based protein separation/fractionation. MF membranes were tailored to retain cells and cell debris while allowing proteins and smaller molecules to pass into filtrate. UF membranes were designed to provide high retention of proteins and other macromolecules. These membrane processes involve the filtration of biological solutions containing proteins, peptides, amino acids, salts and other compounds like organic acids, sugars, vitamins, etc. Some examples include concentration of whey proteins during the production of a variety of dairy products, filtration of wine or the purification of downstream solutions in biotechnology. Nanofiltration (NF) was defined as a process that separates solvent, monovalent salts, small organics from divalent ions and larger species. Conventional UF is limited to separation of solutes that differ in 10 fold in size (Saxena et al., 2009). High-performance tangential flow filltration (HPTFF) is an emerging technology that enables the separation of proteins with similar both size and charge characteristic. HPTFF technology has become possible by exploiting several new discoveries. It has been demonstrated that optimum selectivity and throughput are

obtained in the pressure-dependent flux regime. Selectivity and throughput can also be enhanced through module design and process configurations that reduce the transmembrane pressure gradient. HPTFF obtained high selectivity by control of filtrate flux and device fluid mechanics in order to minimize fouling and exploit the effects of concentration polarization. Increasing the concentration of a solute at the membrane wall increases the effective sieving of the solute in the absence of fouling. At higher wall a concentration fouling occurs, resulting in a reduction in the effective pore size (Reis et al., 1999).

## 2.2 Membrane Technology

Membrane technology is used in many fields application, due to the less energy consumption. The removal of suspended solids such as microorganisms and a fraction of dissolved solids by using membrane technology are very commonly (Choi et al., 2005). The high separation efficiency of these membranes cause the industrially viable based on this technology, separation, concentration, and purification (Celik et al., 2010). Moreover, their low energy requirement, low space requirement, and simplicity of operation promote their use in separation processes (Arthanareeswaran et al., 2004).

Membrane technology is better than traditional separation technology that runs under the usual temperature due to;

i) The good in heat sensitive material concentration and concentration.
ii) The physical characteristic will be change during the membrane separation process and same as the consumption of energy and
iii) Low operation cost. Usually, the pressure will be stated to operate the membrane separating process and the process can be done in short.
iv) Convenient to handle.
v) Simple, compact and automatic control.

In the abundant fields, the traditional separation is replaced by the membrane technology in order to exquisite the productivity, lowering and simplify the operating cost.

In addition, Ahmed (2007) explained the process that used in discrimination between different phases coexist in one system is called as membrane separation, which is included in the membrane technology. This technology can say as a replacement of conventional separation processes such as distillation, extraction and also absorption. There are many benefits by using this technology, which are more energy saving and cost saving although its efficiency is not compared with other processes.

## 2.3 Membrane Filtration

Microfiltration is one of the most general types of membrane separation processes. In general, two types of fluid movement regimes have been reported; dead end and crossflow. The driving force in the microfiltration is the pressure gradient across the membrane (Rahimi et al., 2005).

In dead-end filtration, the applied pressure used to force all the feed solution to pass through the membrane. The particles on the membrane or residue will be collected. Direction of the feed flow is vertically to the filtration membrane's surface and as same as the penetrate direction that pass the membrane. Surface membrane will be fit together with the retained particle in the feed solution and it is a sign to change the cartridge often, indirectly the time is shorter. Cleaning cannot be used for the almost cartridge filter types.

In cross-flow filtration, the fluid to be filtered is pumped across the membrane parallel to its surface as shown in figure 2.1. Cross-flow produces two solutions; a clear filtrate (permeate) and a retentate containing most of the retained particles in the solution. By maintaining a high velocity across the membrane, the retained material is swept off the membrane surface. Thus, cross-flow is used when significant quantities of material will be retained by the membrane, resulting in plugging and fouling. The life of the module will be longer, maybe 12 months to 3 three years according to different material of membrane.

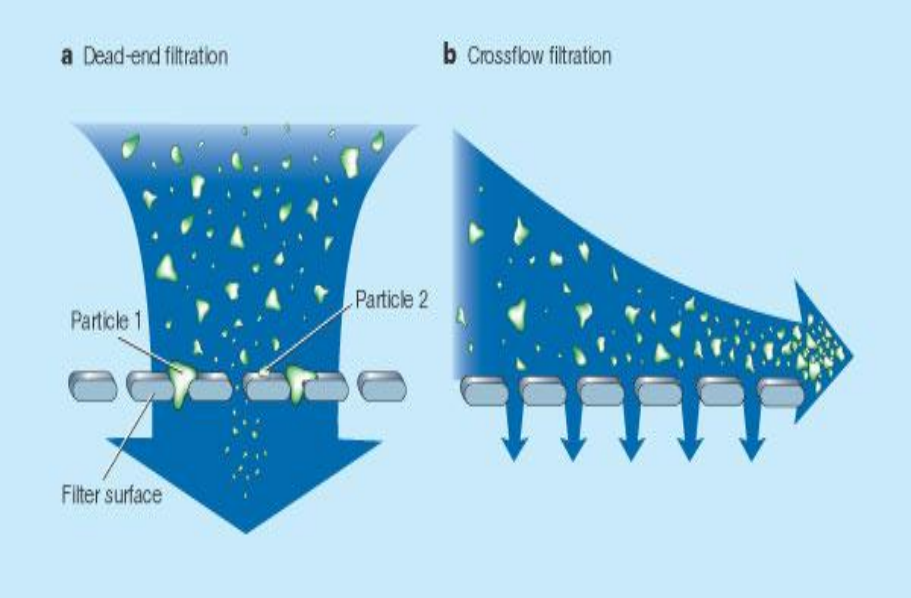


**Figure 2.1 (a)** In dead-end filtration, fluid flow is perpendicular to the filter surface and the filter rapidly becomes clogged with particles. **(b)** In cross flow filtration, fluid flows parallel to the filter surface and particles become more concentrated as filtrate leaves through the filter's pores.
(Source: Elizabeth, 2001).

## 2.4 Protein Separation

Membranes have traditionally been used for size-based separations with high-throughput but relatively low-resolution requirements. Although, essentially all membrane processes are used for bioseparation, but greatest interests have been shown in the pressure-driven technologies such as MF or UF. Recently, electric or ultrasonic fields were imposed simultaneously to increase throughput and membrane selectivity as well as reducing membrane fouling which is a common phenomenon in pressure-driven membrane separation technologies (Saxena et al., 2009).

### 2.4.1 Microfiltration

MF is widely used for the separation, purification and clarifying of protein containing solutions, e.g. for the recovery of extracellular proteins produced via fermentation and for the removal of bacteria and viruses in the final formulation of therapeutic proteins. The basic operational concept of MF leads to a solute concentration that is higher and close to the membrane surface than it is in the bulk feed stream. This is so called concentration polarization, which causes due to diffusive flow of solute back to the bulk feed. After a given period steady state conditions will be achieved. The effect of concentration polarization can be very served in MF applications because the fluxes are high and the mass-transfer coefficients are low as a result of the low diffusion coefficients of macromolecular solutes and of small particulates, colloids and emulsions. Module configuration of MF include hollow fiber, tubular, flat plate, spiral-wound and rotating devices. MF is commonly used to recover macromolecules and retain suspended colloidal particles, and is being integrated into both upstream and downstream processes. A large range of MF applications is reported to pretreatment steps, removal of small molecules from bigger protein molecules, clarify suspensions for cell harvesting, and sterilize liquids to remove viruses and bacteria.

### 2.4.2 Ultrafiltration

UF has been widely used as preferred method for protein concentration and buffer exchange, and replaced size exclusion chromatography in these applications. UF membranes, based on variety of synthetic polymers, have high thermal stability,

chemical resistivity, and restricted the use of fairly harsh cleaning chemicals. Figure 2.2 below showed the membrane size-based separations type.

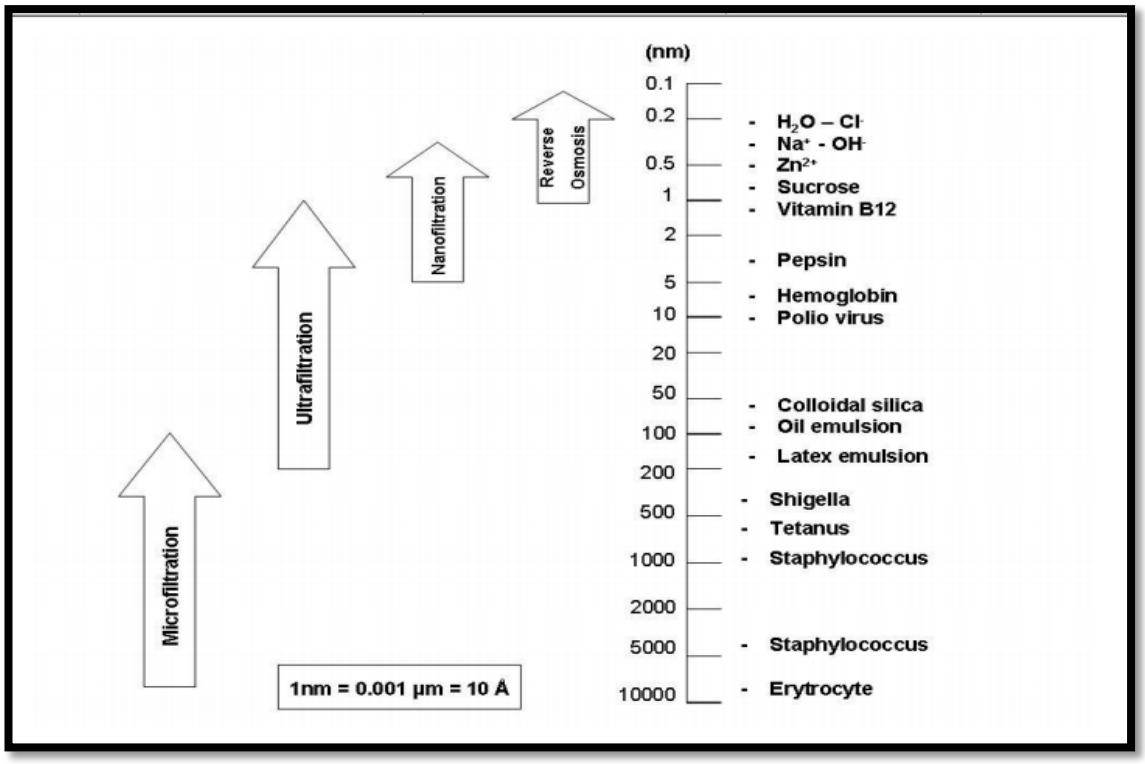


**Figure 2.2** Reverse osmosis, ultrafiltration, microfiltration and conventional filtration are related processes differing principally in the average pore diameter of the membrane filter.
(Source: Mulder, 1996).

## 2.5 Membrane Chromatography

Membrane chromatography is used as an alternative to conventional resin based chromatography columns for a large range of chromatographic purification. Various type of membrane chromatography has been used for protein separation such as ion-exchange, hydrophobic, reversed phase, and affinity chromatography.

Ionic interactions are the basis for purification of proteins by Ion Exchange Chromatography. The separation is due to competition between proteins with different surface charges for oppositely charged groups on an ion exchanger adsorbent (Karlsson et al., 1998). Ion-exchange membranes represent major segment of media used in membrane chromatography. A large number of membranes used microfiltaration are known to have ion-exchange properties. In many applications this was considered to be a major advantage. However, this property proved to be potentially useful for carrying out chromatographic separations. Some of this membrane was modified to enhance their ion-exchange capacity. Different charged group such as sulfonic acid (S), sulfopropyl (SP), diethylaminoethyl (DEAE) and quaternary ammonium (Q) were introduce to obtain high protein binding membranes (Ghosh, 2002).

Jungbauer et al., (2005), states that the reverse phases and hydrophobic interaction based separation in membrane chromatography is most available synthetic are incompatible with organic solvents. This probably explains why there are few reports on reversed-phase membrane chromatography. Hydrophobic interaction is known to have several advantages over other separation chemistries, particularly from the point of view of protein stability. The general approach in hydrophobic interaction membrane chromatography has been to attach hydrophobic ligands which are usually hydrocarbon chains or rings to various membranes.

In affinity membrane, ligands that have specific interactions with other molecules is used. These interactions might occur with low molecular is used. An interacting protein has binding sites with complementary surfaces to its ligand. The

binding can involve a combination of electrostatic or hydrophobic interactions as well as short-range molecular interactions such as van der Waals forces and hydrogen bonds (Lee et al., 2004).

The term affinity chromatography referred originally to the use of an immobilized natural ligand, which specifically interacts with the desired protein, but has then been given quite different connotations by different authors. Sometimes it is very broad and includes all kinds of adsorption chromatography techniques based on non-traditional ligands, and is thus used in a more general sense of attraction. In other cases it refers only to specific interactions between biologically functional pairs which interact at natural binding sites.

## 2.6 Advantages of Membrane Chromatography.

The advantage of membrane chromatography lies in;

i) The predominance of convective material transport.
ii) Efficiency is not necessarily guarantee for the predominance of convection alone.
iii) Convective flow of inappropriate type can be a serious disadvantage.
iv) Flow distribution is a major concern in chromatographic and indeed most types of separation processes.

v) Rational design of the membrane chromatographic process and equipment is possible only when the transport phenomena involved are properly understood (Ghosh, 2002).

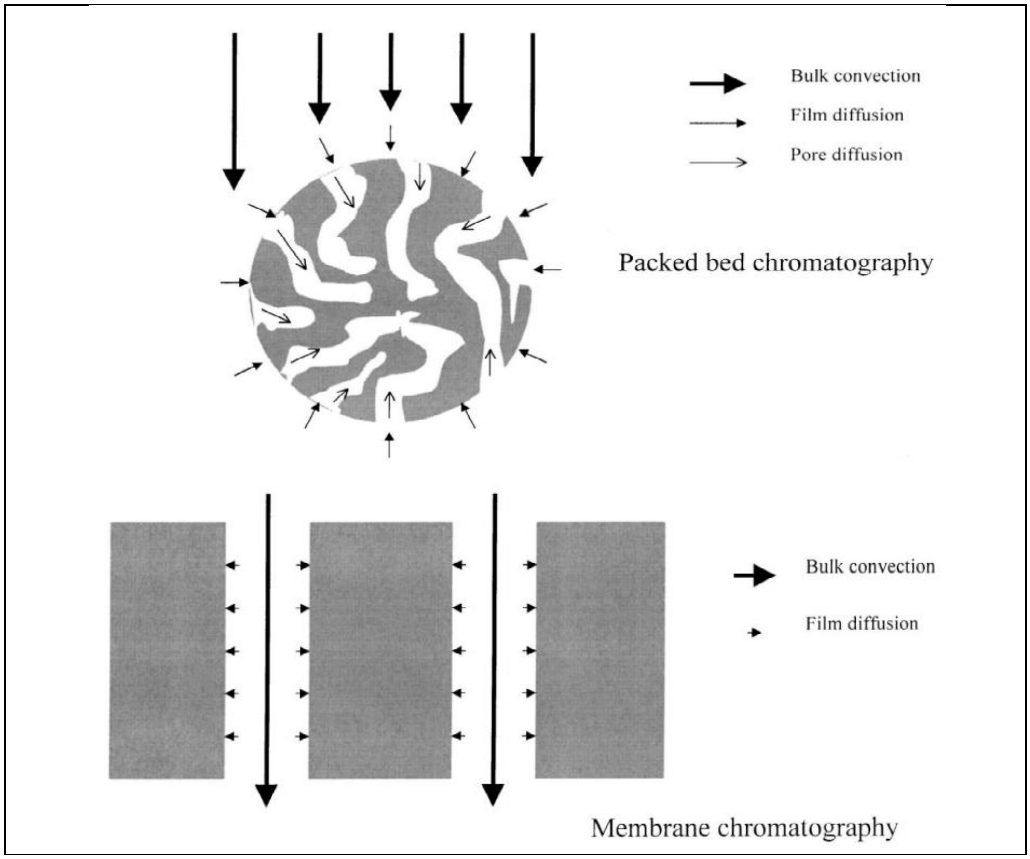


**Figure 2.3** Solute transports in packed bed chromatography and membrane chromatography.
(Source: Ghosh, 2002).

## 2.7 Preparation of Charged Membrane chromatography.

Charged membrane can be formed by using chemical grafting, and UV photografting.

Chemical Grafting technology is defined as the attaching and continued renewal of more useful properties to surfaces of working metal parts than were present originally.

Photografting provided a simple and feasible method for synthesis of new well-defined pore covering composite membranes.

## 2.8 Protein

Protein are macromolecules and made from amino acids which linked by covalent peptide bond (Chang and Raymond, 2003) Every protein have it unique and genetically amino acids sequence that determining it specific shape and function such as coordinated motion, enzyme catalyst and generation and 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 protein are hydrophobic because of that, these protein roles are giving the protection to the tissue and cells and also the giving of strength.

Second class of protein is globular protein which having hydrophilic at their outer surface that makes them water soluble. Example of this second class of protein are enzymes 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 the cell membrane.

Protein contain positive and negative charge group depending on the amino acid sequence. Isoelectric point (PI) is pH of the protein where the number of positive and negative charge is equal or it carries zero net charge. If the PI value of the protein below the pH of solution, the protein will has negative charge and bind to anion-exchange, and its will have positive charge and bind to cation-exchange when the PI value of the protein above the pH solution.

### 2.8.1 Bovine Serum Albumin (BSA)

BSA is a single peptide chain that contains no carbohydrate and consisting 583 amino acids residue. BSA is soluble in the water but can be precipitate at the high concentration of neutrals salt such as ammonium sulphate. BSA has very good solution stability and because of that, BSA was used as stabilizer for other solubilized proteins such an enzyme.

BSA is an acidic group which occurs plentifully in the body fluids and tissue of mammals and in some plant seed (Benedek, 1999).

# CHAPTER THREE

# METHODOLOGY

## 3.1 Materials

Commercial PES membrane with 0.1 micrometer pore size was purchased from Merck. Monomer used in grafting process are [2-(acryloyloxy)ethyl] trimethylammonium chloride (AETMA) and acrylic acid (AA) were purchased from Sigma Aldrich, Sodium hydroxide (NaOH) was purchased from Sigma Aldrich. Sodium phosphate, mono-sodium salt ($NaH_2PO_4.1H_2O$) and sodium phosphate, disodium salt ($NaH_2PO_4.7H_2O$) for preparation of the pH 7 buffer solution which both was purchased from Merck. . Besides, 0.2 M acetic acid and 0.2 M sodium acetate to form 0.1 stock solutions for preparation of the pH 3.6 buffer solution were purchased from Sigma Aldrich. Sodium chloride (NaCl) was purchased from Sigma Aldrich.

## 3.2 Preparation of Elution Buffer.

A buffer solution is one which resists change in pH when small quantities of an alkali or acid are added to it. 0.1 M sodium phosphate buffer pH 7.0 was prepared by dissolving 0.2153 g of $NaH_2PO_4.1H_2O$ and 0.6542 g of $NaH_2PO_4.7H_2O$ with 400 ml of ultrapure water to get 400 ml of phosphate buffer. Then the buffer's pH was determined by pH meter. Vacuum pump was used to filter the impurities inside the buffer solution.

While, 0.01 acetate buffer pH 3.6 was prepared by dissolving with the 40 ml of stock solution with 0.1 M acetic acid. The stock solution was prepared by adding 50 ml ultrapure water into the 50 ml of 0.2 M acetic acid and 0.2 M sodium acetate solution.

Elution buffer was used to recoverthe bound protein from the membrane. 1M NaCl was added to the binding buffer above as an elution buffer.

## 3.3 Preparation of Standard Curve.

Six set of different BSA concentrations in range of 0.0625 mg/ml to 2 mg/ml was prepared to development of standard curve. The absorbance of BSA solution at 280 nm was determined by using UV-VIS spectrophotometer Hitachi U 1800. An absorbance-concentration standard curve was developed and samples were diluted with buffer to within the absorbance range of the standard curve.

### 3.4 Membrane Grafting Process.

Unmodified PES membrane was initially soaked in water for washing and rinsing any chemical or particles on the surface of membrane. This flushing was done on ultrasonic machine several times by replacing fresh water for each treatment. Cleaned membrane was air-dries and the weight of membrane was measured before the modification process.

Membrane with 1 cm x 1.5 cm dimension was immersed in the monomer solution of AETMA or acrylic acid for 12 h. After that, the membrane was placed into glass petri dish for UV radiation grafting. Before that, all the UV radiation equipment as shown in Figure 3.1 was warm up for 30 minutes. Then, the reaction was initiated by turning on the UV lamps, which reach their highest intensity within a few seconds. After the 5 minutes reaction time, the modified membranes were carefully taken out of the chamber, rinsed to remove the free AETMA and acrylic acid monomer from the surface.

In order to remove strongly adhered monomer, the modified membrane was shaken in water for 4 h in a mechanical shaker at 250 rpm. Then, to remove any homo polymer formed the membranes were then placed in 0.1M NaOH solution and shaken for 30min. Afterwards, the modified membrane was rinsed with water and shaken in water for1 h to remove the excess NaOH from the membrane. Lastly, all the modified membranes were air-dried at room temperature and further stored in dessicator containing dried calcium carbonate as the desiccant for 12 h before characterization (Malaysamy et al., 2010).

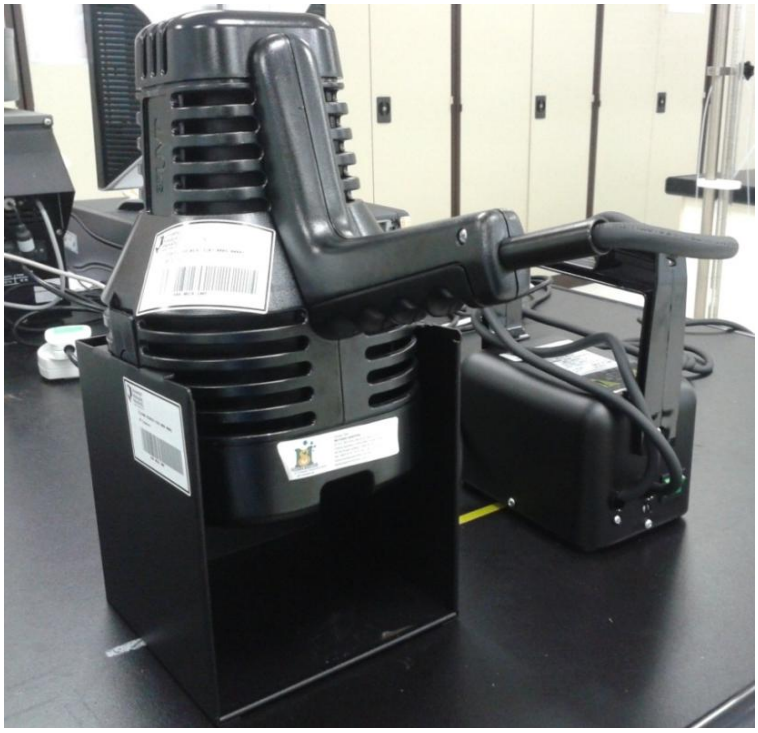

**Figure 3.1** UV-Light Cell, Wave Length 365 nm (B-100AP Lamp. Brand: Ultraviolet Product (RDU100111) with Exposure Box for B-100 AP.

## 3.5 Protein Binding Experiment

1 cm x 1.5 cm of grafted membrane was equilibrated in the 1.5 ml of binding buffer for three hours. All the membranes were then placed into the 1.5 ml of 2 mg/ml BSA solution dissolved in binding buffer for 12 hours. The concentration before and after the binding was determined using UV-VIS spectrophotometer method. Bound protein from membrane was eluted by incubating in 1.5 ml of elution buffer for 12 hours to get the recovery of the protein binding. All the binding and elution process conducted by placing the 1.5 ml of centrifuge tube in the rotator as shown in Figure 3.2 to make sure the mixing was done effectively.


**Figure 3.2** Rotator which the binding process was takes place.

## 3.6 Membrane Characterization

### 3.6.1. Degree of Grafting (DG)

The degrees of grafting (DG) express the extent of the modification process. DG can be determined various way such as based on the percentage of weight increase, the number of moles of monomer grafted, ion exchange method or by spectroscopic analysis. In this study, the DG is calculated based on the amount of the polymer grafted on the membrane per unit area of the membrane as shown in Equation 3.1:

$$DG\ (mg/cm^2) = \frac{W_1 - W_0}{A} \quad (3.1)$$

$W_0$is the weight of the unmodified membrane, $W_1$ is the weight of the membrane after modification and A is the area of the membrane. Table 4.2 shows the degree of grafting of the membrane grafted with AETMA monomer and AA monomer at concentration of 1.5 M and 2.0 M.

### 3.6.2. Fourier Transform Infrared Spectroscopy (FTIR) analysis

Surface chemical functionality of the unmodified and modified PES membranes was obtained using a FTIR spectrometer model Thermo Nicolat Avatar 370. Clean, dry membrane pieces were mountedon the sample chamber. IR scans were performed at a resolution of 4 $cm^{-1}$ at an incident angle of 45◦. The IR penetration depth for this incident angle is 2.0–4.36 micrometer. A base line was obtained for each membrane and the sample chamber maintained under vacuum at 2.30 hPa to avoid interferences from air and moisture (Malaysamy et al., 2010).

### 3.6.3. Pure Water Flux.

Water flux is a measure of permeate flow rate per unit area membrane per unit time.

Pure water flux was measured using Amicon stirred cell Model 8010 as shown in Figure 3.3, which can fix with 1.3 cm diameter of membrane size. Before water flux measurement, the modified membrane was flushed with distilled water at

pressure 4bar. The purpose was to clean the modified membrane and to make sure the pores of the surface membrane were opened. Then water flux process was run at different pressure of 4, 3, 2, and 1 bar. The time taken for reach 1 mL pearmeate volume was recorded. Water flux was calculated using Equation 3.2

$$J = \frac{V}{At} \qquad (3.2)$$

Where V is total volume of pure permeates during the experiment, A is the membrane area and t is the operation time.


**Figure 3.3** Amicon Stirred Cell model 8010 for measuring water flux.

# CHAPTER FOUR

# RESULTS AND DISCUSSION

## 4.1 Standard Curve

An absorbance-concentration standard curve for BSA dissolved in 0.1 M Sodium phosphate pH 7 and 0.01 M sodium acetate pH 3.6 are shown in Figure 4.1 and Figure 4.2 respectively. This standard curve was used to calculate the unknown protein concentration after binding by absorbance at 280 nm. The standard curve for BSA in sodium phosphate pH 7 is followed the linear equation of $y = 0.658x + 0.013$ with $R^2$ value is 0.996.

For BSA in sodium acetate pH 3.6 an equation of $y = 0.569x + 0.005$, $R^2$ value of 0.999 was obtained. The absorbance was measured least three time to get the average value for every samples.

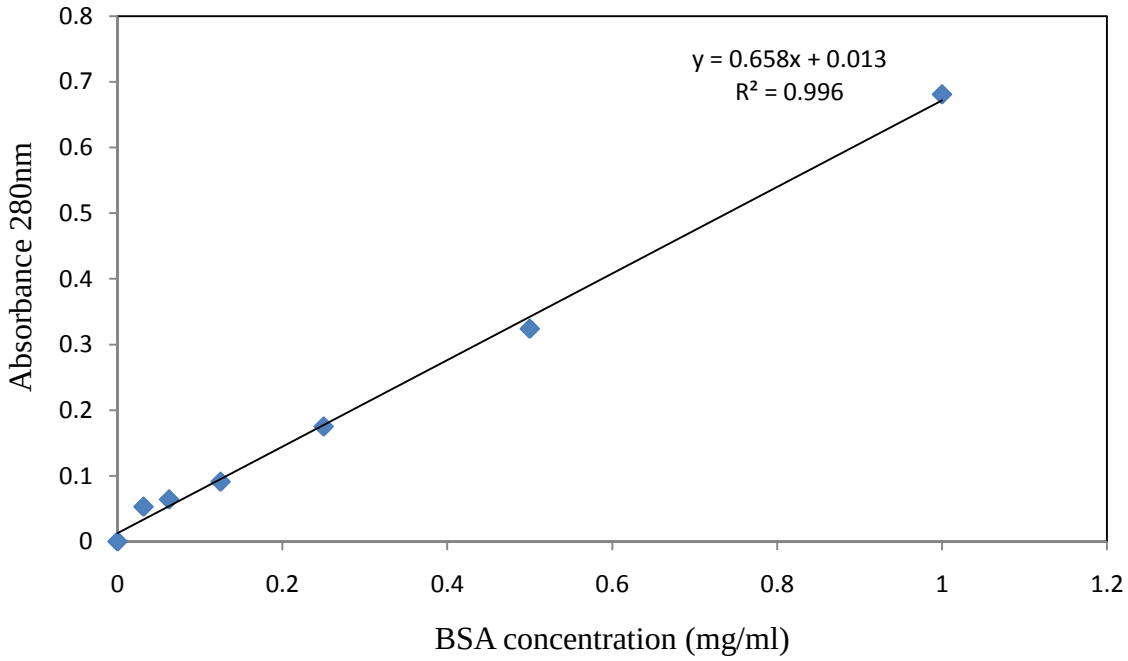


**Figure 4.1** Absorbance-concentration standard curve graphs for BSA in 0.1 M sodium phosphate pH 7.

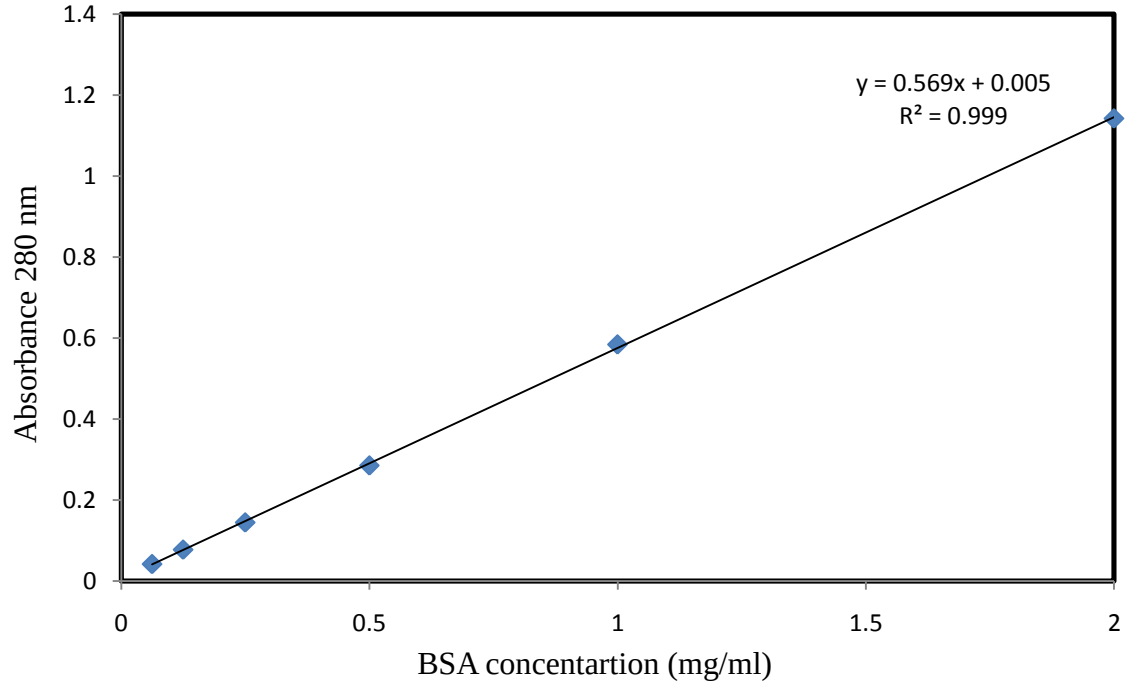


**Figure 4.2** Absorbance-concentration standard curve graphs for BSA in 0.01 M sodium acetate pH 3.6.

## 4.2 Effect of Monomer Concentration on BSA Binding Capacity.

Table 4.1 shows the monomer at different monomer concentration on the BSA binding membrane grafted with AEMA and AA.

**Table 4.1** Effect of monomer concentration on BSA binding membrane grafted with AETMA and AA.

| Membrane type | AETMA | | AA | |
|---|---|---|---|---|
| Concentration (M) | 1.5 | 2.0 | 1.5 | 2.0 |
| Area ($cm^2$) | 1.5 | 1.5 | 1.5 | 1.5 |
| Feed BSA (mg) | 2.252 | 2.052 | 2.664 | 2.002 |
| BSA Bound (mg) | 0.233 | 0.438 | 0.219 | 0.807 |
| Capasity (mg BSA/$cm^2$ membrane) | 0.155 | 0.292 | 0.146 | 0.538 |
| Eluted (mg) | 0.188 | 0.249 | 0.259 | 0.152 |
| Recovery (%) | 80.69 | 57.90 | 80.37 | 19.80 |

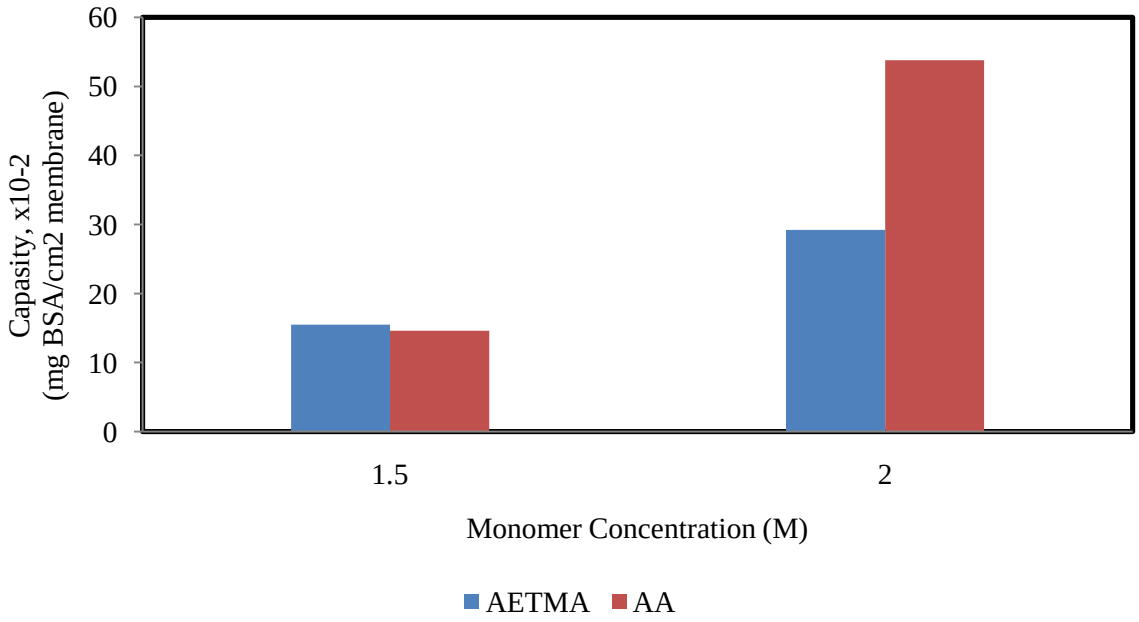


**Figure 4.3** Binding capacity for PES membrane grafted with AETMA and AA.

Both grafted membrane show an increase of binding capacity when the monomer concentration increases. For AETMA-grafted membrane, the binding capacity increase about 65.32 %.

When the AETMA concentrations increase, the numbers of positively charge group grafted on the membrane will increase proportionally. Consequently, the membrane can attract more negatively charge BSA, thus achieved high binding capacity.

Huge percent increment was obtained in AA-grafted membrane which is about 78.65 %. The binding capacity of membrane grafted with 2.0 M monomer is 53.8 x $10^{-2}$ mg BSA/$cm^2$ $cm^2$for AA-grafted membrane, which is higher than the binding capacity of AETMA-grafted membrane that show the binding capacity of 29.2 x $10^{-2}$ mg BSA/$cm^2$.

## 4.3 Effect of Monomer Concentration towards Water Flux.

Figure 4.4 shows the normalized water flux for membrane grafted at different monomer.

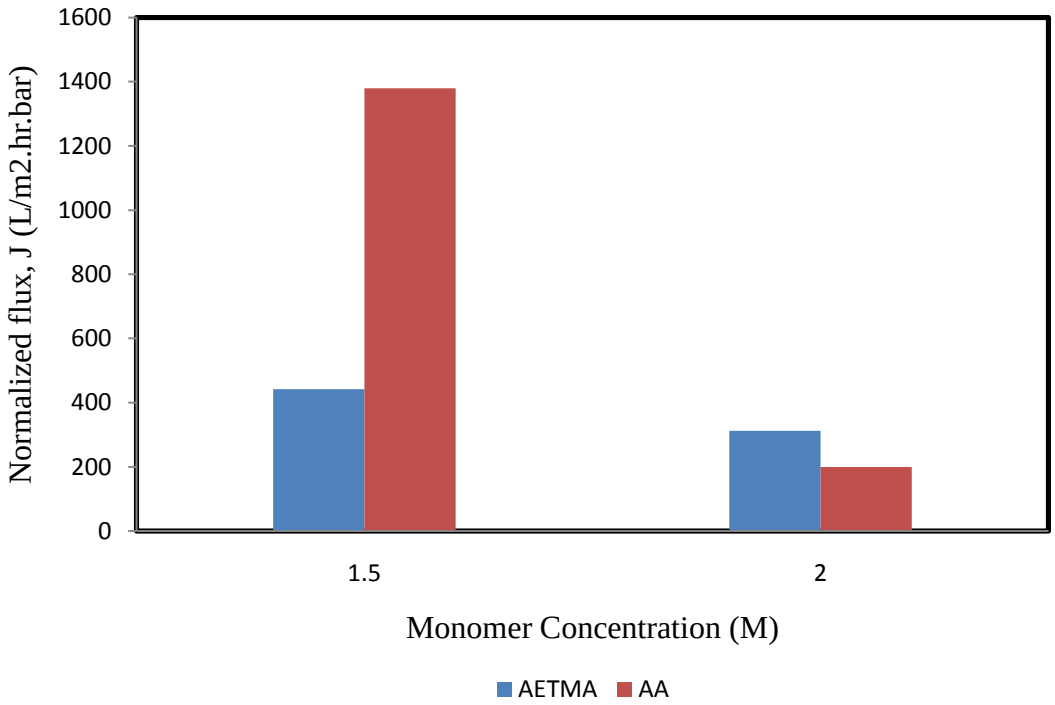


**Figure 4.4** Normalized water flux AETMA- and AA-grafted membrane at different monomer concentration.

Both membranes grafted with AETMA and AA at high monomer gave low water flux. According to Baker (2004), flux is inversely proportional to pores size of the membrane where the pores size of the membrane will give a lower flux. Therefore, it is shown that, the higher value of monomer concentration, the large the pore size of the membrane. Consequently, the lower flux will achieve.

## 4.4 Surface Characterization of Modified Membranes.

### 4.4.1 Degree of Grafting (DG).

**Table 4.2** The degree of grafting of the AETMA monomer and AAmonomer with concentration of 1.5 Mand 2.0 M.

| | CONCENTRATION (M) | DEGREE OF GRAFTING $x10^{-4}$ ($mg/cm^2$) |
|---|---|---|
| AETMA | 1.5 | 583.058 |
| | 2.0 | 586.386 |
| AA | 1.5 | 585.212 |
| | 2.0 | 587.970 |

Based on Table 4.2, it shows that DG is depends on monomer concentration. When the monomer concentration is increase, the DG value also increases. Both AETMA and AA concentration showed the same condition.

The DG of the AETMA monomer is 583.058 x $10^{-4}$ $mg/cm^2$and 586.386 x $10^{-4}$ $mg/cm^2$for the concentration of 1.5 M and 2.0 M respectively. Meanwhile for the AA monomer gave the value of DG for the 1.5 M and 2.0 M monomer concentration are 585.212 x $10^{-4}$ $mg/cm^2$and 587.97 x $10^{-4}$ $mg/cm^2$respectively.

Taniguchi et al, (2004) said that, AA has the higher diffusivity through the pores. It is because the AA's is relatively smaller size and higher reactivity. As shown in the figure above, the DG of AA for both concentrations is higher compared to the DG of AETMA monomer.

### 4.4.2 Fourier Transform Infrared Spectroscopy (FTIR) analysis.

Figure 4.5 illustrate the graph corresponds to the unmodified PES membrane surface.

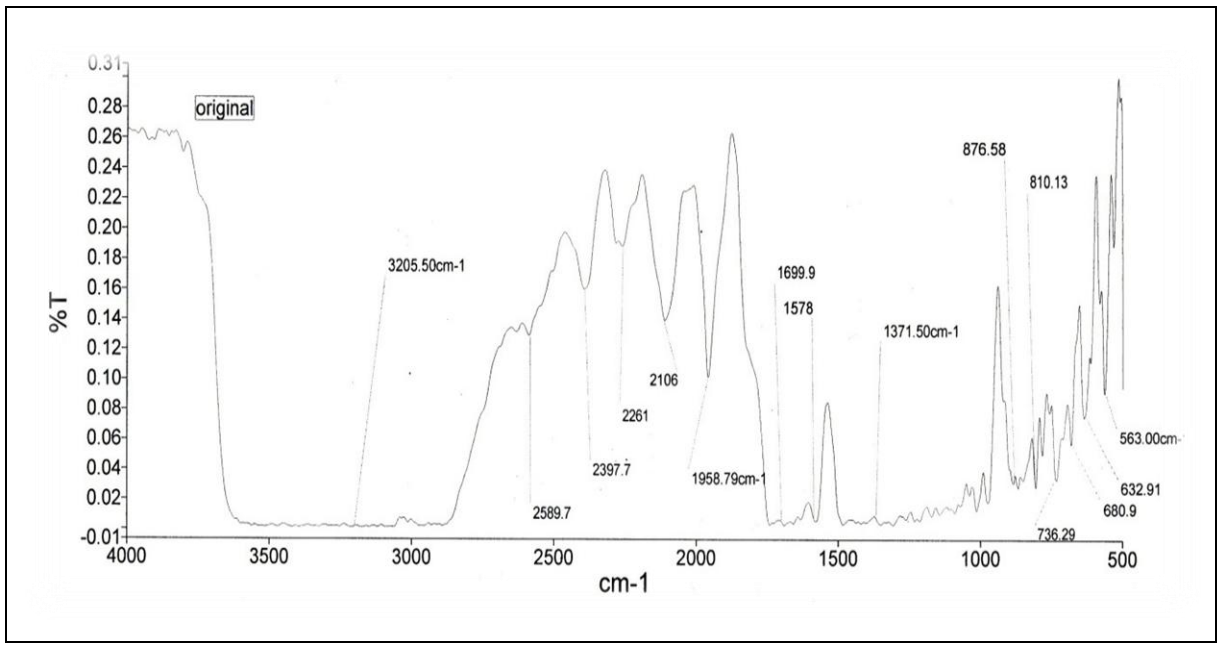


**Figure 4.5** Graph of Absorbance against the Wavenumbers for the Unmodified PES Membrane.

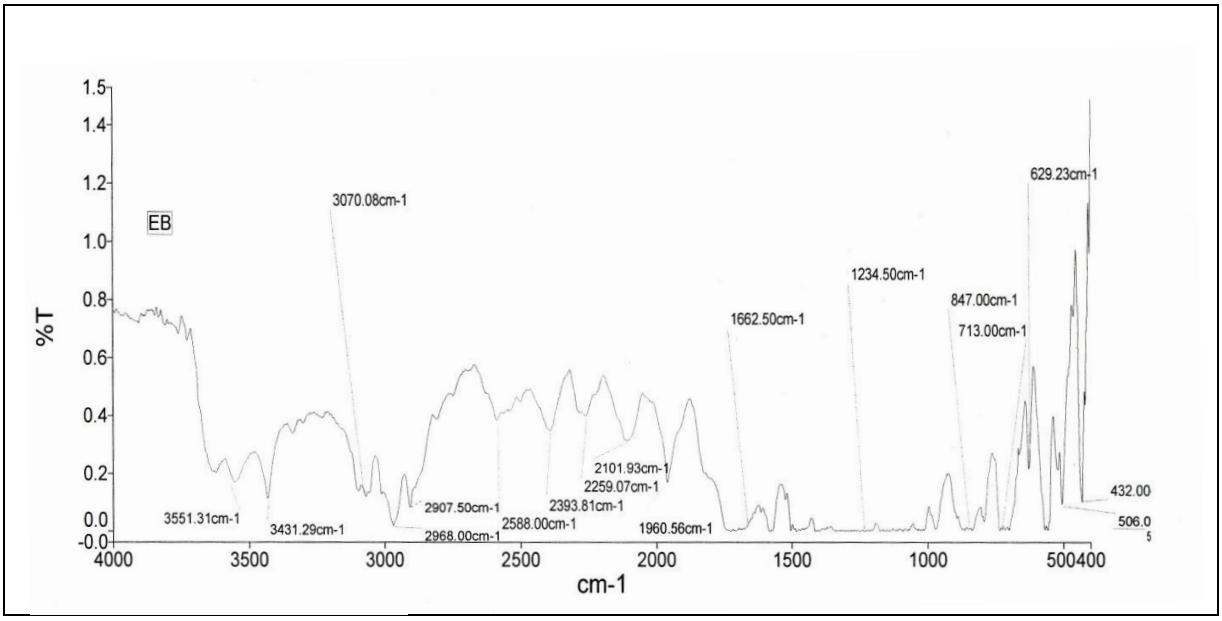


**Figure 4.6** The Graph of Absorbance against the Wavenumbers for the Modified PES Membrane with 1.5 M Concentration of ETMA.

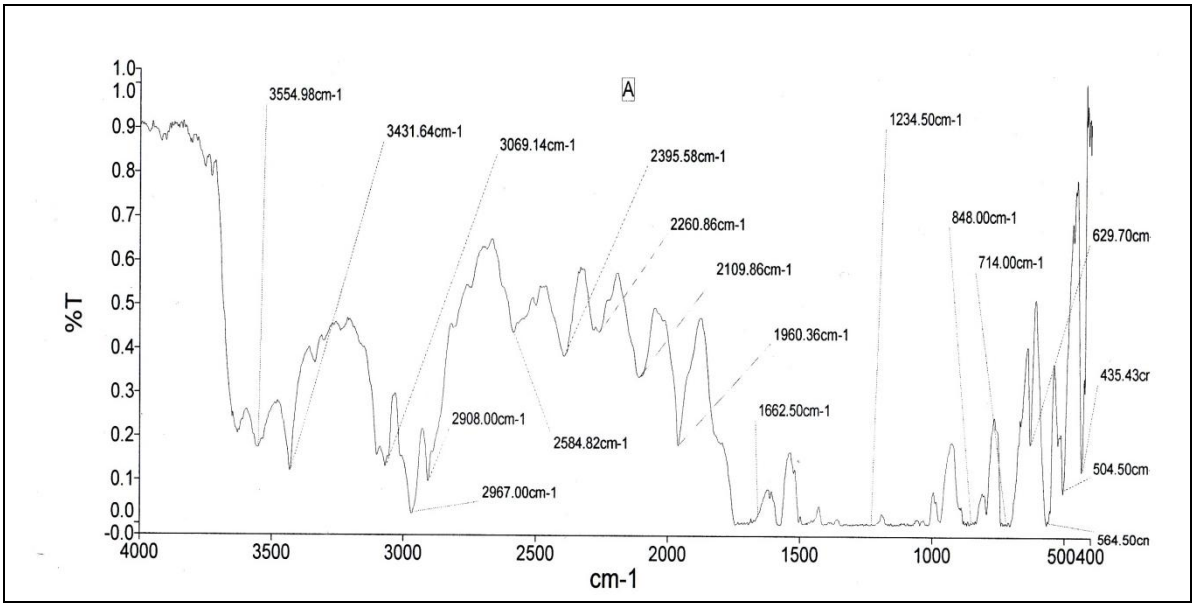


**Figure 4.7** The Graph of Absorbance against the Wavenumbers for the Modified PES Membrane with 2.0 M Concentration of ETMA.

As a consequence, the Figure 4.6 and Figure 4.7 above illustrate the modified of PES membrane with 1.5 M and 2.0 M of AETMA monomer respectively.

Compared to Figure 4.5, Figure 4.6 shown the changes of the peaks of membranes surface. As the present of carbonyl group within the AETMA monomer (Malaysamy, 2010), the Figure 4.7 demonstrate there is a peak between 1670 to 1820 $cm^{-1}$ that show the present of the carbonyl group within this modified of PES membrane. Indirectly, it is give the positively charge to the membrane.

Meanwhile, for the Graph of Absorbance against the Wavenumbers for the Modified PES Membrane with 2.0 M Concentration of AETMA above, it prove that the present of these two carbonyl group within the modified of PES membrane. As increase the AETMA concentration, the peaks become higher.

These FTIR measurements give additional confirmation of successful of modification of PES membrane with AETMA monomer. It should be emphasize on that the peak height increase proportionally to the DG.

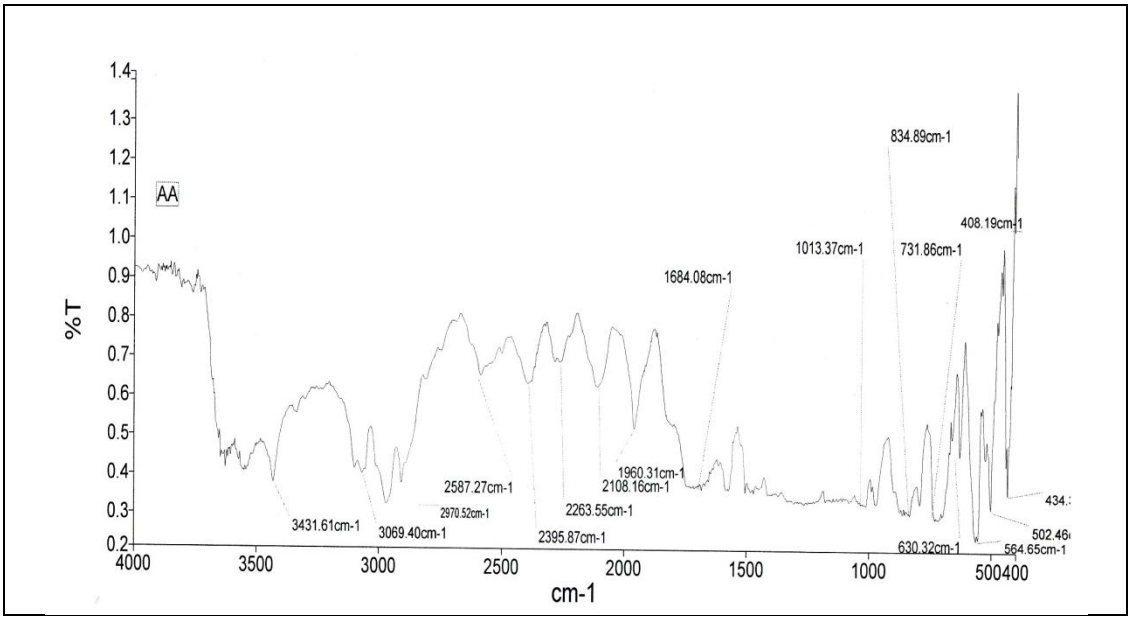


**Figure 4.8** The Graph of Absorbance against the Wavenumbers for the Modified PES Membrane with 1.5 M Concentration of AA.

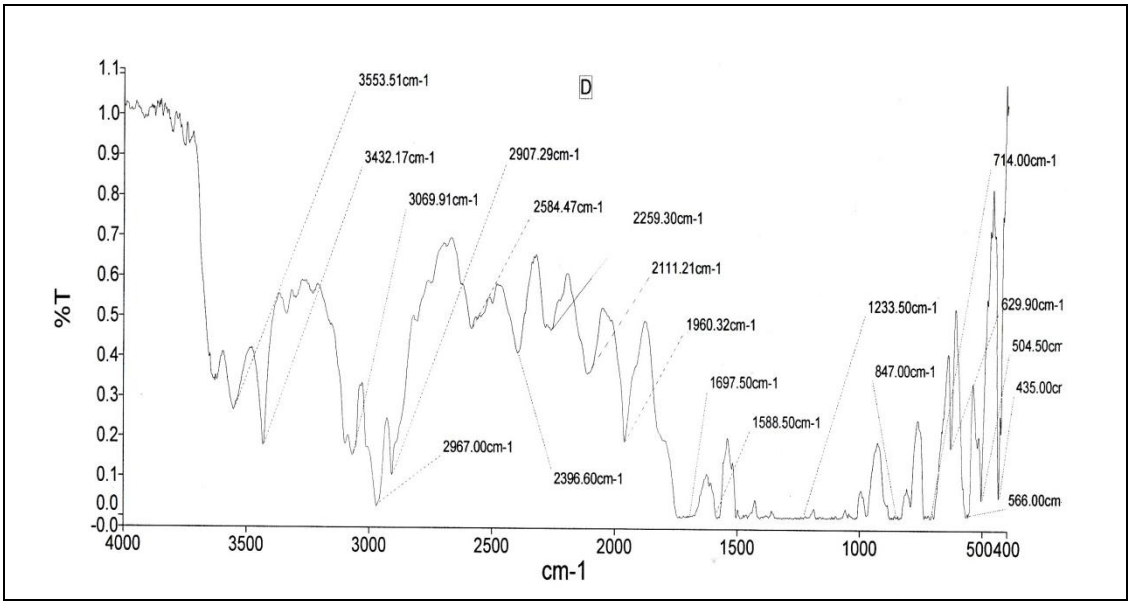


**Figure 4.9** The Graph of Absorbance against the Wavenumbers for the Modified PES Membrane with 2.0 M Concentration of AA.

Likewise, the Figure 4.8 and Figure 4.9 above show the modified of PES membrane with 1.5 M and 2.0 M of AA monomer respectively. Compared to Figure 4.5, Figure 4.8 shown the changes of the peaks of membranes surface. The present of

alcohol (OH) group within the modified membrane shown at tall peak between 3200 to 3700 $cm^{-1}$ in Figure 4.9 that absent within the Figure 4.5. Indirectly, it is give a negatively charge to the membrane.

Meanwhile, for the Graph of Absorbance against the Wavenumbers for the Modified PES Membrane with 2.0 M Concentration of AA above, it prove that the present of these two carbonyl group and alcohol group within the modified of PES membrane.

Therefore, it is same goes to AETMA; these FTIR measurements give additional confirmation of successful of modification of PES membrane with AA monomer. It should be confirmed on that the peak height increase proportionally to the DG.

# CHAPTER FIVE

# CONCLUSION AND RECOMMENDATION

## 5.1 Conclusion

PES membrane was successfully grafted with AETMA monomer and AA monomer to membrane chromatography.

The binding capacity of both monomer increases as the concentration of the monomer increase. It is due to the amount of the ion exchange that occurs between the monomer and the membrane. Besides, the water flux testing also gave the acceptable result, which is the flux is inversely proportional to the pores size of the membrane. The more concentrate of monomer, the larger pores size of the membrane, thus, lower the flux value.

In addition, for the membrane characterization, the degree of grafting of the membrane point out the higher value of the DG can be achieved when the monomer concentration was increased. Lastly, for the FTIR analysis, the results illustrate that

the present of the carbonyl group within the modified of PES membrane with the AETMA monomer. Meanwhile, the alcohol group was present within the modified of PES membrane with the AA monomer.

## 5.2 Recommendation

Several recommendations that can take into consideration for future study to expand this research, as followed:

I. Use another type of monomer such as sulfonic acid and methacrylic acid during preparation of membrane chromatography.

II. Study on different type of base polymer for preparing ion exchange membrane chromatography such as cellulose acetate, polyamide and chitosen.

III. Use an Attenuated Total Reflectance (ATR-FTIR) for characterization of the modified PES membrane. ATR-FTIR provides an easy and convenient way for determination of the relative amounts of different functional group present at the outmost part of the membrane.

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# APPENDIX

## APPENDIX A

Figure A-1: Unmodified PES membrane with diameter of 4.7 cm.


Figure A-2: The unmodified PES membrane was soaking within the ultrasonic.


Figure A-3: The unmodified membrane was immersed into the monomer for 12 hours.


Figure A-4: The protein binding process.


Figure A-5: Water flux testing.

**APPENDIX B**

CALCULATION

B.1 Calculation of monomer solution.

B.1.1 100 ml of 1.5 M AETMA monomer.

$$M = \frac{\rho \times \% \times 1000}{MW}$$

$$M = \frac{1.132 \times 0.8 \times 1000}{193.67}$$

$$M = 4.676\ M\ of\ AETMA$$

$$M_1V_1 = M_2V_2$$

$$(4.676M)(V_{1)} = (1.5M)(100ml)$$

$$V_1 = 32.078\ ml \text{ of AA}$$

B.1.1 100 ml of 1.5 M AA monomer.

$$M = \frac{\rho \times \% \times 1000}{MW}$$

$$M = \frac{1.049 \times 0.99 \times 1000}{72.06}$$

$$M = 14.412\ M\ of\ AA$$

$$M_1V_1 = M_2V_2$$

$$(14.412M)(V_{1)} = (1.5M)(100ml)$$

$$V_1 = 10.408\, ml\ of\ AA$$

B.2 Calculation for 36 ml of 2 mg/ml of BSA

$$\frac{2\ mg}{ml} \times \frac{40ml}{40ml} = \frac{80\ mg\ of\ BSA}{40\ ml\ biffer}$$

B.3 Calculation for 200ml of elution buffer

$$1\ mol\ NaCl \rightarrow 200\ ml$$

$$mol = \frac{1\ m}{l} \times 0.2\ l = 0.2\ mol$$

$$mol = \frac{mass}{JMR}$$

$$mass = 0.2\ mol \times 58.44 \frac{g}{mol} = 11.688\ g\ NaCl$$

B.4 Calculation for water flux

Time taken for 1 mL permeates at different operating pressure was recorded as on B-1.

Permeate volume = 0.001 L

Membrane area = 0.00531 $m^2$

The water flux was calculated using Eq. (3.1),

$$J = \frac{V}{A\Delta t}$$

Where, $J$ = Membrane flux, L/m2.hr

$A$ = Membrane area, $m^2$

$\Delta t$ = Filtration time, $\Delta t$

$$\mathrm{J} = \frac{0.001\ \mathrm{L}}{(0.00531\ \mathrm{m^2})(0.373\ \mathrm{hr})} = 50.489\frac{\mathrm{L}}{\mathrm{m^2}}.\mathrm{hr}$$

The calculated water flux at each pressure were tabulated in table B-1

B-1: Flux calculated at different pressure.

| | | AETMA | | AA | |
|---|---|---|---|---|---|
| Concentration (M) | Pressure (bar) | Time, t (hr) | Flux, J ($L/m^2.hr$) | Time, t (hr) | Flux, J ($L/m^2.hr$) |
| 2.0 | 1 | 0.5565 | 0.338408 | 0.373 | 0.50489 |
| | 2 | 0.303 | 0.310766 | 0.219 | 0.429963 |
| | 3 | 0.207 | 0.303259 | 0.143 | 0.438983 |
| | 4 | 0.158 | 0.297981 | 0.257 | 0.183194 |
| 1.5 | 1 | 0.373 | 0.50489 | 0.126 | 1.494634 |
| | 2 | 0.219 | 0.429963 | 0.071 | 1.326225 |
| | 3 | 0.143 | 0.438983 | 0.043 | 1.459875 |
| | 4 | 0.119 | 0.395638 | 0.038 | 1.238973 |

**APPENDIX C**

C.1 Effect of monomer concentration

C.1.1 1.5 M of AETMA

Table C-1: Absorbance and binding capacity for the modified membrane with 1.5 M of AETMA.

| | Membrane area $cm^2$ | Volume (mL) | Absorbance | [BSA] (mg/mL) | Feed BSA (mg) | BSA bound (mg) | Volume (mL) | Absorbance | [bsa] (mg/mL) | Elute (mg) | Recovery (%) | Capacity (mg BSA/$cm^2$ membrane) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| original | | 1.500 | 1.090 | 1.657 | 2.485 | | | | | | | |
| A1 | 1.500 | 1.500 | 0.987 | 1.500 | 2.250 | 0.235 | 1.500 | 0.089 | 0.135 | 0.203 | 0.864 | 0.157 |
| A2 | 1.500 | 1.500 | 0.999 | 1.518 | 2.277 | 0.207 | 1.500 | 0.071 | 0.108 | 0.162 | 0.780 | 0.138 |
| A3 | 1.500 | 1.500 | 0.989 | 1.503 | 2.255 | 0.230 | 1.500 | 0.089 | 0.135 | 0.203 | 0.881 | 0.153 |
| B1 | 1.500 | 1.500 | 0.989 | 1.503 | 2.255 | 0.230 | 1.500 | 0.094 | 0.143 | 0.214 | 0.931 | 0.153 |
| B2 | 1.500 | 1.500 | 0.987 | 1.500 | 2.250 | 0.235 | 1.500 | 0.065 | 0.099 | 0.148 | 0.631 | 0.157 |
| B3 | 1.500 | 1.500 | 0.976 | 1.483 | 2.225 | 0.260 | 1.500 | 0.086 | 0.131 | 0.196 | 0.754 | 0.173 |
| **Ave** | **1.500** | **1.500** | **0.988** | **1.501** | **2.252** | **0.233** | **1.500** | **0.082** | | **0.188** | **0.807** | **0.155** |
| **STD** | **0.000** | | | | | **0.017** | | | | **0.026** | **0.108** | **0.011** |

C.1.2 2 M of AETMA

Table C-2: Absorbance and binding capacity for the modified membrane with 2.0 M of AETMA.

| | Membrane area $cm^2$ | Volume (mL) | Absorbance | [BSA] (mg/mL) | Feed BSA (mg) | BSA bound (mg) | Volume (mL) | Absorbance | [bsa] (mg/mL) | Elute (mg) | Recovery (%) | Capacity (mg BSA/$cm^2$ membrane) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| original | | 1.500 | 1.092 | 1.660 | 2.489 | | | | | | | |
| A1 | 1.500 | 1.500 | 0.920 | 1.398 | 2.097 | 0.392 | 1.500 | 0.140 | 0.213 | 0.319 | 0.814 | 0.261 |
| A2 | 1.500 | 1.500 | 0.880 | 1.337 | 2.006 | 0.483 | 1.500 | 0.101 | 0.153 | 0.230 | 0.476 | 0.322 |
| A3 | 1.500 | 1.500 | 0.890 | 1.353 | 2.029 | 0.460 | 1.500 | 0.120 | 0.182 | 0.274 | 0.594 | 0.307 |
| B1 | 1.500 | 1.500 | 0.940 | 1.429 | 2.143 | 0.347 | 1.500 | 0.094 | 0.143 | 0.214 | 0.618 | 0.231 |
| B2 | 1.500 | 1.500 | 0.900 | 1.368 | 2.052 | 0.438 | 1.500 | 0.100 | 0.152 | 0.228 | 0.521 | 0.292 |
| B3 | 1.500 | 1.500 | 0.870 | 1.322 | 1.983 | 0.506 | 1.500 | 0.100 | 0.152 | 0.228 | 0.450 | 0.337 |
| Ave | 1.500 | 1.500 | 0.900 | 1.368 | 2.052 | 0.438 | 1.500 | 0.109 | 0.166 | 0.249 | 0.579 | 0.292 |
| STD | 0.000 | | | | | 0.059 | | | | 0.040 | 0.132 | 0.040 |

C.1.3 1.5 M of AA

Table C-3: Absorbance and binding capacity for the modified membrane with 1.5 M of AA.

| | Membrane area $cm^2$ | Volume (mL) | Absorbance | [BSA] (mg/mL) | Feed BSA (mg) | BSA bound (mg) | Volume (mL) | Absorbance | [bsa] (mg/mL) | Elute (mg) | Recovery (%) | Capacity (mg BSA/$cm^2$ membrane) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| original | | 1.500 | 1.101 | 1.921 | 2.882 | | | | | | | |
| A2 | 1.500 | 1.500 | 1.030 | 1.798 | 2.696 | 0.186 | 1.500 | 0.101 | 0.176 | 0.264 | 0.948 | 0.124 |
| B1 | 1.500 | 1.500 | 1.000 | 1.745 | 2.618 | 0.264 | 1.500 | 0.094 | 0.164 | 0.246 | 0.620 | 0.176 |
| B2 | 1.500 | 1.500 | 1.020 | 1.780 | 2.670 | 0.212 | 1.500 | 0.100 | 0.175 | 0.262 | 0.823 | 0.141 |
| B3 | 1.500 | 1.500 | 1.020 | 1.780 | 2.670 | 0.212 | 1.500 | 0.100 | 0.175 | 0.262 | 0.823 | 0.141 |
| Ave | 1.500 | 1.500 | 1.018 | 1.776 | 2.664 | 0.219 | 1.500 | 0.099 | 0.172 | 0.259 | 0.804 | 0.146 |
| STD | 0.000 | | | | | 0.033 | | | | 0.008 | 0.136 | 0.022 |

C.1.4 2 M of AA

Table C-4: Absorbance and binding capacity for the modified membrane with 2.0 M of AA.

| | Membrane area $cm^2$ | Volume (mL) | Absorbance | [BSA] (mg/mL) | Feed BSA (mg) | BSA bound (mg) | Volume (mL) | Absorbance | [bsa] (mg/mL) | Elute (mg) | Recovery (%) | Capacity (mg BSA/$cm^2$ membrane) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| original | | 1.500 | 1.073 | 1.873 | 2.809 | | | | | | | |
| A1 | 1.500 | 1.500 | 0.801 | 1.398 | 2.097 | 0.712 | 1.500 | 0.058 | 0.101 | 0.152 | 0.213 | 0.475 |
| A2 | 1.500 | 1.500 | 0.766 | 1.337 | 2.005 | 0.804 | 1.500 | 0.061 | 0.106 | 0.160 | 0.199 | 0.536 |
| A3 | 1.500 | 1.500 | 0.789 | 1.377 | 2.065 | 0.743 | 1.500 | 0.080 | 0.140 | 0.209 | 0.282 | 0.496 |
| B1 | 1.500 | 1.500 | 0.756 | 1.319 | 1.979 | 0.830 | 1.500 | 0.046 | 0.080 | 0.120 | 0.145 | 0.553 |
| B2 | 1.500 | 1.500 | 0.856 | 1.494 | 2.241 | 0.568 | 1.500 | 0.053 | 0.092 | 0.139 | 0.244 | 0.379 |
| B3 | 1.500 | 1.500 | 0.657 | 1.147 | 1.720 | 1.089 | 1.500 | 0.050 | 0.087 | 0.131 | 0.120 | 0.726 |
| Ave | 1.500 | 1.500 | 0.765 | 1.335 | 2.002 | 0.807 | 1.500 | 0.058 | 0.101 | 0.152 | 0.198 | 0.538 |
| STD | 0.000 | | | | | 0.188 | | | | 0.035 | 0.067 | 0.125 |

**APPENDIX D**

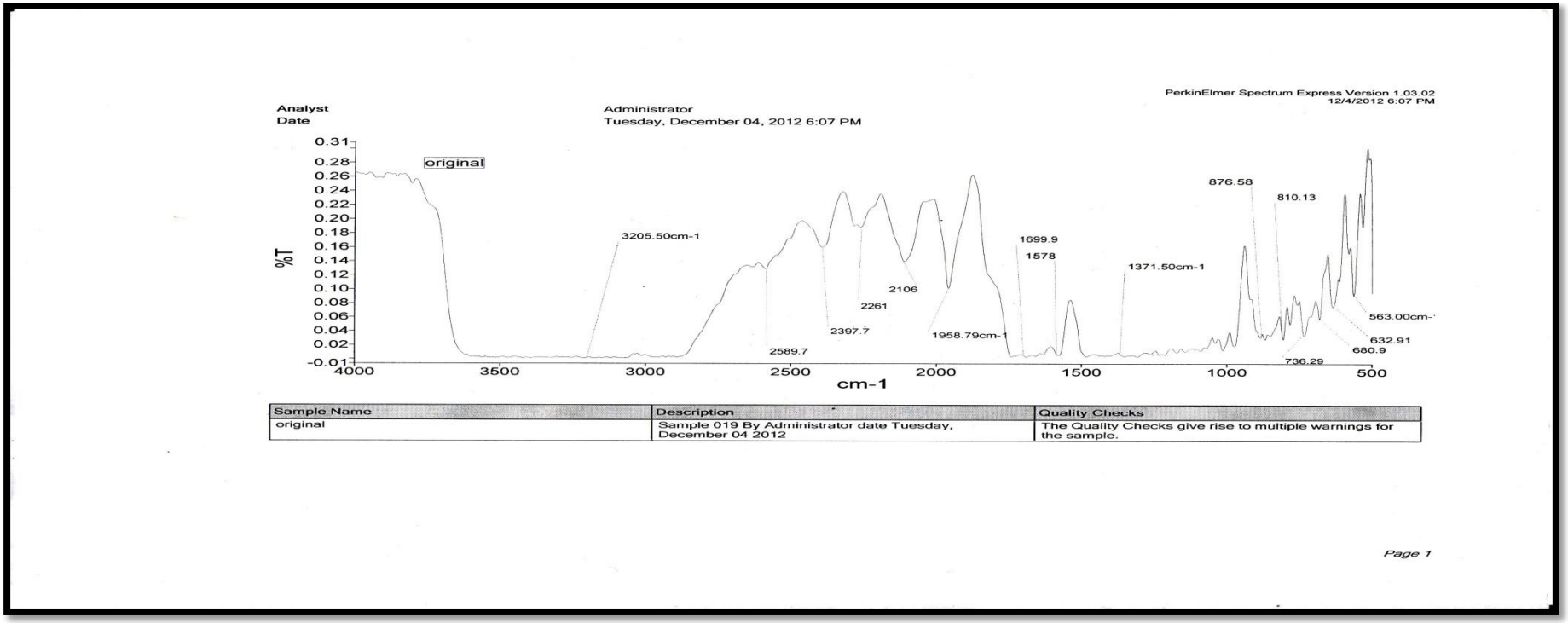


**Figure D-1:** Graph of Absorbance against the Wavenumbers for the Unmodified PES Membrane.

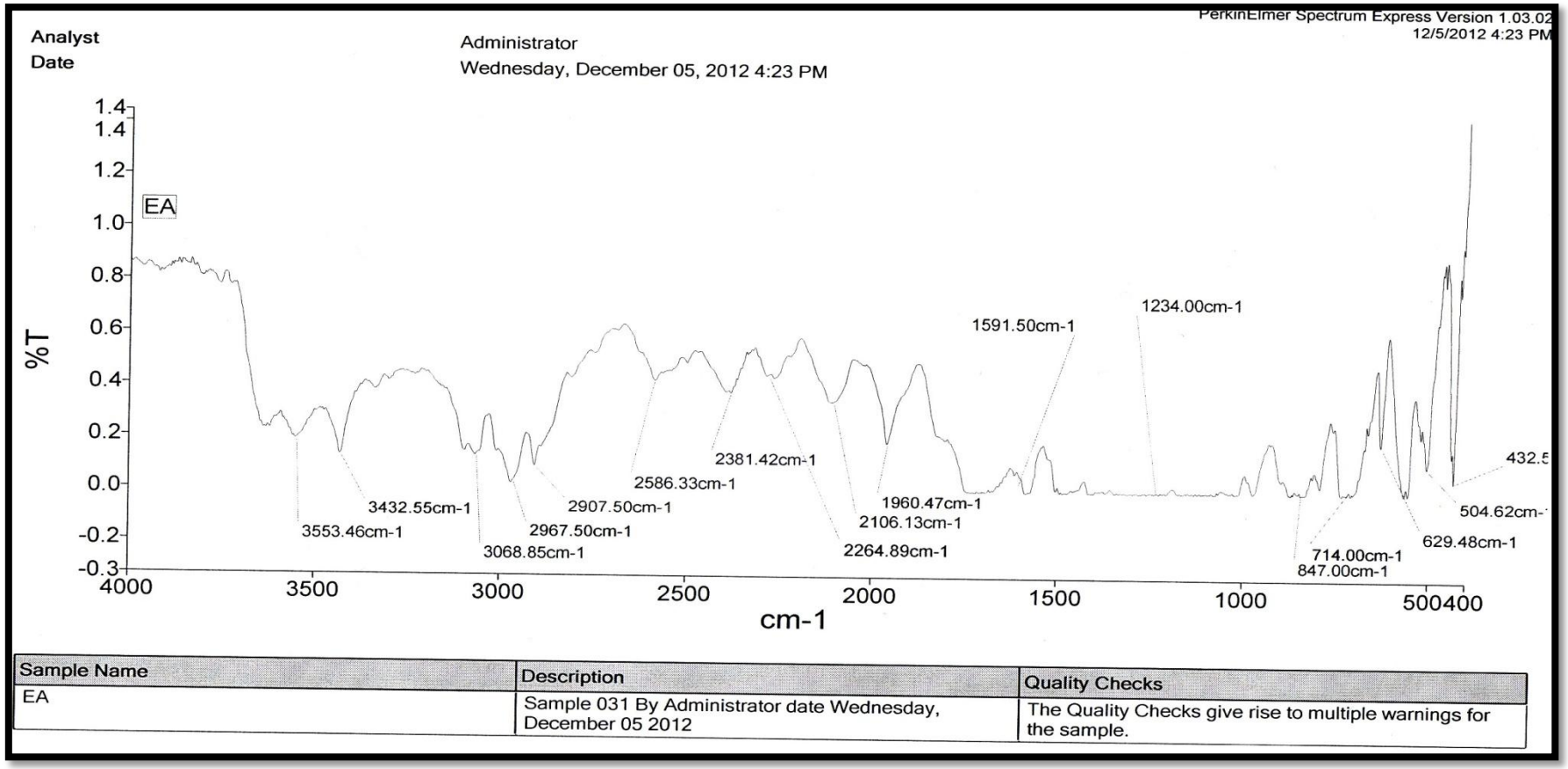


**Figure D-2:** Graph of Absorbance against the Wavenumbers for the modified membrane with 1.5 M of AETMA.

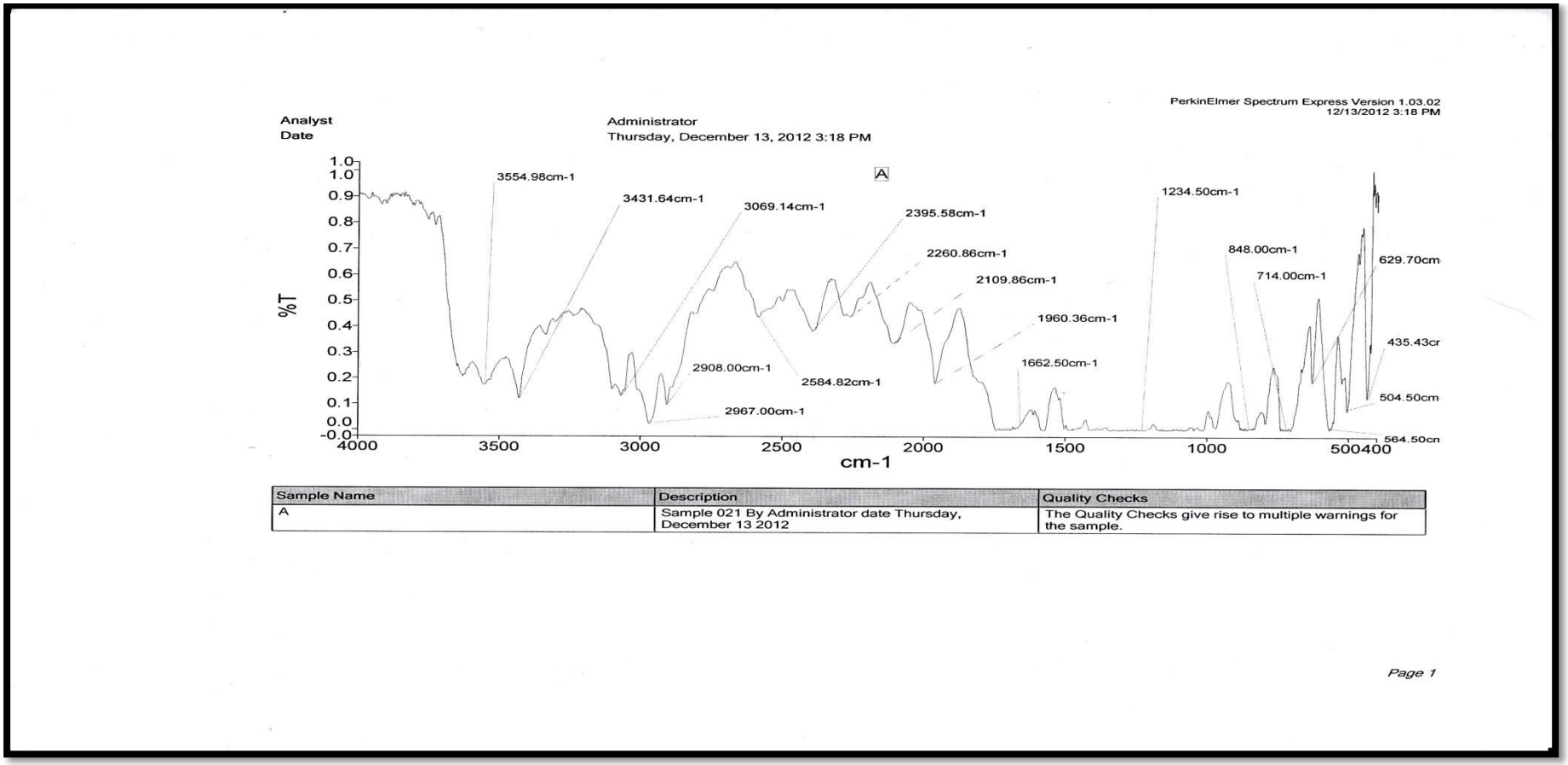


**Figure D-3:** Graph of Absorbance against the Wavenumbers for the modified membrane with 2.0 M of AETMA.

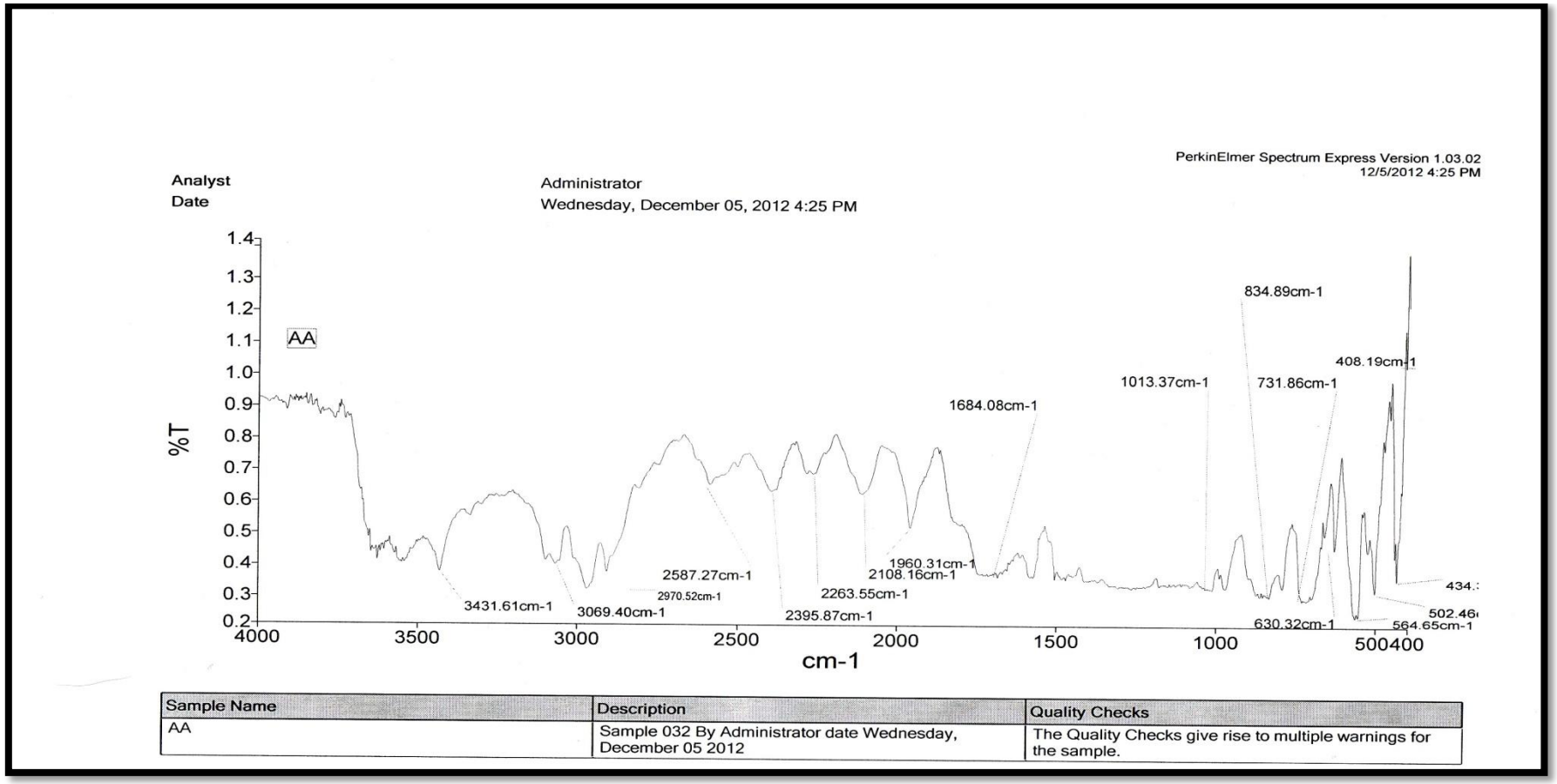


**Figure D-4:** Graph of Absorbance against the Wavenumbers for the modified membrane with 1.5 M of AA..

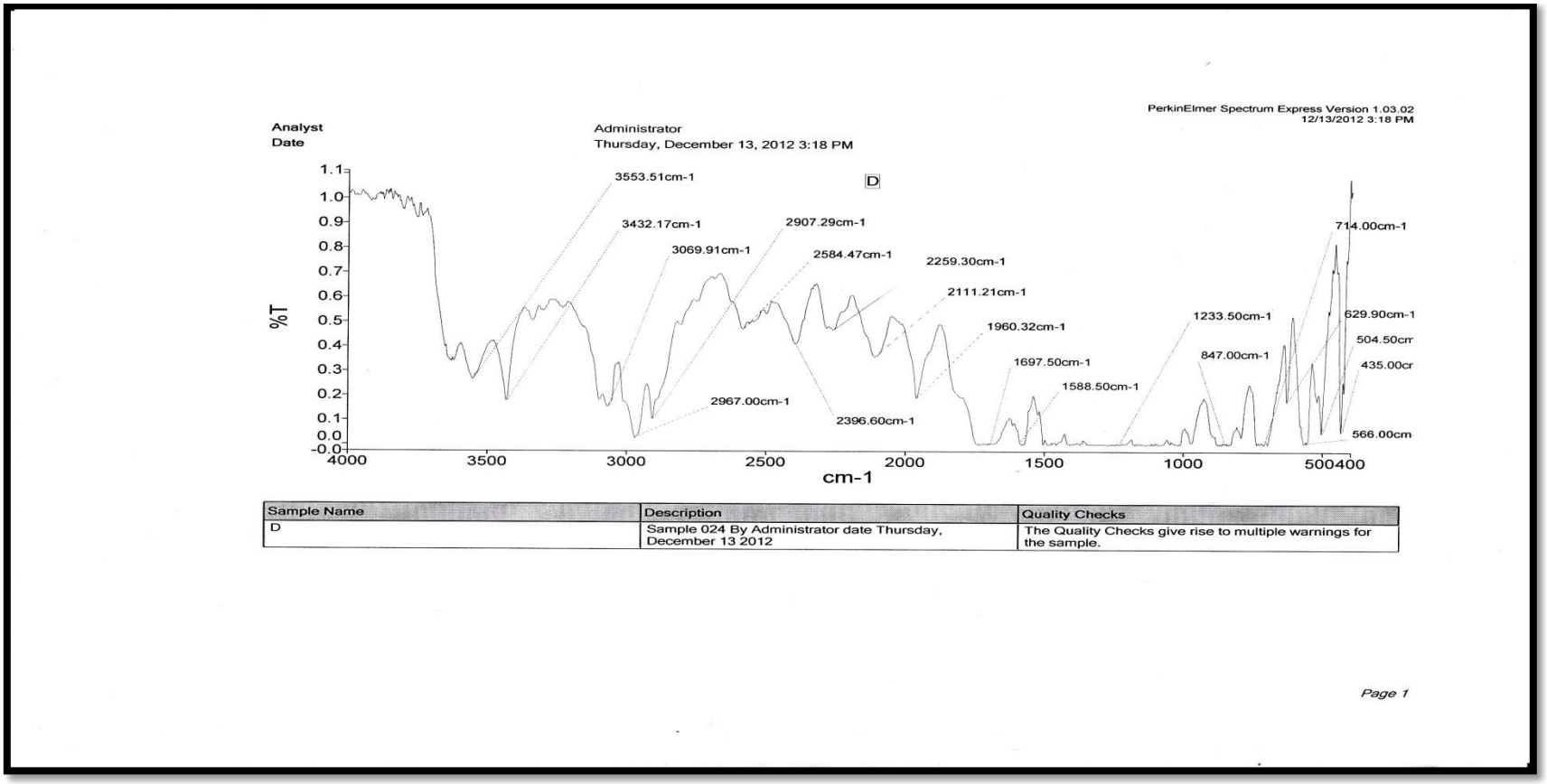


**Figure D-5:** Graph of Absorbance against the Wavenumbers for the modified membrane with 2.0 M of AA.