ADSORPTIVE STUDIES OF BOVINE SERUM ALBUMIN (BSA) BY PES/HAP/PVP MIXED MATRIX MEMBRANE

NURUL HUSNA ELYANA BT HANIFA

UNIVERSITI SAINS MALAYSIA

2017

ADSORPTIVE STUDIES OF BOVINE SERUM ALBUMIN (BSA) BY PES/HAP/PVP MIXED MATRIX MEMBRANE

by

NURUL HUSNA ELYANA BT HANIFA

Thesis submitted in partial fulfilment of the requirement

for the degree of Bachelor of Chemical Engineering

June 2017

ACKNOWLEDGEMENTS

In the name of Allah, the Most Beneficent and the Most Merciful. All praises to Allah the Almighty for giving me the strengths, guidance and patience in completing this thesis.

First, I would like to express my genuine gratitude to my beloved father and mother, Hanifa bin Awang and Zahrah bt Ahmad for their endless love, prayers and tolerance. To my wonderful younger sister and younger brother, thank you for your persevering support and encouragement.

My sincere thanks to both my dedicated supervisors and co-supervisor, Professor Dr. Abdul Latif Ahmad and Dr. Noorfazliani Shoparwe for their support and encouragement during period of my studies. Thank you very much for the unending help throughout the course of my research.

My special acknowledgement goes to the Dean of Chemical Engineering School, Professor Dr. Azlina bt Harun for her grateful support towards my undergraduate studies. Thanks to Associated Professor Dr. Mohd Azmier bin Ahmad as Final Year Project coordinator and staff of Chemical Engineering School for giving me full support in the success of my research.

I would like to express my deepest gratitude to all my friends especially my coursemates of Chemical Engineering School for their motivation, encouragement and moral support during my research work. Last but not least, I would like to thank to all the people who have helped me throughout my research, directly or indirectly; your contribution shall not be forgotten. Thank you so much.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

C_{eq}	Equilibrium concentration of BSA	g/L
C_0	Initial concentration of BSA	g/L
dw	Water density at room temperature	kg/m^3
l	Membrane thickness	m
K_D	Dissociation constant	L/g
K_F	Indicator for adsorption capacity	-
k_1	Pseudo-first-order rate	hr^{-1}
k_2	Pseudo-seconder-order rate	g BSA/g membrane.hr
n	Heterogeneity factor	-
Pr	Membrane porosity	-
q_{eq}	Equilibrium adsorption capacity	g BSA/g membrane
q_{eq1}	Equilibrium adsorption capacity by pseudo-first-order kinetic model	g BSA/g membrane
q_{eq2}	Equilibrium adsorption capacity by pseudo-second-order kinetic model	g BSA/g membrane
q_m	Maximum adsorption capacity	g BSA/g membrane
q_t	Amount of BSA adsorbed during time	g BSA/g membrane
S	Membrane area	m^2
t	Time	hr
V	Volume of BSA	L
W	Weight of membrane	g
W_w	Weight of weight membrane	g
W_d	Weight of dry membrane	g

LIST OF ABBREVATION

BSA	Bovine serum albumin
CA	Contact angle
DMAc	Diemthyl Acetamide
FTIR	Fourier Transform Infrared Spectroscopy
HAP	Hydroxyapatite
MABs	Monoclonal Antibodies
MF	Microfiltration
MMMs	Mixed matrix membranes
NF	Nanofiltration
OH	Hydroxide ion
PES	Polyethersulfone
PVP	Polyvinylpyrrolidone
RO	Reverse osmosis
SEM	Scanning Electron Microscope

KAJIAN SERAPAN DARIPADA BOVINE SERUM ALBUMIN (BSA) DENGAN PES/HAP/PVP CAMPURAN MATRIX MEMBRANE

ABSTRAK

Zarah Hydroxyapatite, HAP yang tertanam dalam Polyethersulfon, PES Membran Matrix Campuran (MMMs) telah berjaya disediakan, bagi mengkaji fonomena penjerapan ke arah protein Bovine Serum Albumin (BSA). BSA dipilih sebagai model protein dalam sistem ini. Membran dengan kepekatan PES berbeza dan kepekatan HAP berbeza telah disediakan dengan menggunakan kaedah penyongsangan fasa. Semua membran yang disediakan dianalisis melalui Fourier Transform Infrared Spektroskopi analisis, analisis Mikroskop Imbasan Elektron, pengukuran sudut air, kandungan air di dalam membran dan keliangan keseluruhan membran. Dari FTIS spektrum, puncak menunjukkan pada 1011.04 cm-1 disebabkan oleh sifat kimia zarah HAP. Ini menunjukkan bahawa zarah HAP telah berjaya dilarutkan ke dalam PES membran matriks. Struktur membran oleh MIE mengesahkan bahawa liang permukaan menjadi lebih besar dengan mengurangkan kepekatan PES dan permukaan liang menjadi semakin kecil dengan menambahkan zarah HAP ke dalam PES membran matriks campuran. Kandungan air bertambah apabila kepekatan PES ditambah dan meningkatkan hidrofilik MMMs. Hal ini juga menyebabkan saiz liang membran bertambah. Eksperimen penjerapan telah dijalankan bagi mengkaji kesan kepekatan PES dan HAP ke atas penjerapan BSA. Membran dengan kepekatan PES paling rendah iaitu 14 % berat kepekatan PES menunjukkan kapasiti penjerapan yang tinggi ke arah BSA. Zarah HAP paling banyak dilarutkan ke dalam 14 % berat memberi kapasiti penjerapan tertinggi iaitu 0.240 g BSA/g membran kapasiti penjerapan maksimum. Membran yang mengandungi 14 % berat PES kepekatan dengan 60 % berat dan 80 % berat kepekatan HAP daripada kepekatan PES telah dipilih untuk penjerapan BSA dinamik ke membran. Kepekatan

optimum untuk kepekatan BSA awal pada 1.5 g/L telah dipilih sebagai kepekatan keseimbangan BSA. Untuk kajian keseimbangan, dua isoterma penjerapan analisis iaitu Langmuir dan Freundlich model telah digunakan. PES/HAP/PVP MMMs menunjukkan bahawa Langmuir isoterma model adalah data yang sesuai dilengkapi untuk menerangkan tentang proses penjerapan BSA. Untuk kajian kinetik, 14% berat kepekatan PES dengan 60% berat dan 80% berat kepekatan HAP dari kepekatan PES didapati sesuai dengan model kinetik pseudo-tertib kedua. Pengunaan semula membran telah dijalankan dengan merendam membran yang telah digunakan dalam larutan buffer asetat pada pH 4.2 dan hasilnya menunjukkan bahawa 14% berat kepekatan PES dengan 60% berat dan 80% berat HAP daripada kepekatan PES, tidak ada pengurangan yang ketara dalam kapasiti penjerapan

ADSORTIVE STUDIES OF BOVINE SERUM ALBUMIN (BSA) BY PES/HAP/PVP MIXED MATRIX MEMBRANE

ABSTRACT

Hydroxyapatite (HAP) as adsorptive particles embedded into Polyethersulfone (PES) Mixed Matrix Membranes (MMMs) were successfully prepared, in order to study the adsorption behavior of Bovine Serum Albumin (BSA) as protein model in this system. Membranes with different PES and different HAP concentrations were prepared by phase inversion method. All the prepared membranes were characterized through Fourier Transform Infrared Spectroscopy (FTIR) analysis, Scanning Electron Microscope (SEM) analysis, Contact Angle measurement (CA), water content and overall porosity. From the FTIR spectra, peak at 1011.04 cm^{-1} was attributed to chemical properties of HAP particle which indicates that the HAP particle was successfully impregnated into PES mixed matrix membranes. Membranes structure analysed through SEM confirmed that the surface pores enlarged with reducing the PES concentration and the surface pores became smaller with the addition of HAP particles into PES mixed matrix membranes. Water content was increased with increasing of PES concentration and increased the hydrophilicity of the MMMs, which partly caused by the increase in overall porosity of the MMMs. Adsorption experiment was carried out to investigate the effect of PES and HAP concentrations on BSA adsorption. The initial concentration and contact time in a batch system was varied with constant shaking rate of 90 rpm for all the prepared membranes. The membranes with lowest PES concentration at 14 wt% PES concentration showed the highest adsorption capacity towards BSA. The highest HAP of 80 wt% gave the highest adsorption capacity of 0.240 g BSA/g membrane of maximum adsorption capacity. The 14 wt% PES concentration with 60 wt% and 80 wt% of HAP concentration of PES concentration were chosen to dynamic BSA adsorption onto membrane. The

optimum concentration for initial BSA concentration at 1.5 g/L was chosen as equilibrium BSA concentrations. For equilibrium studies, two analytical adsorption isotherms, Langmuir and Freundlich models were fitted to equilibrium adsorption data. PES/HAP/PVP MMMs shows that The Langmuir isotherms model was the best fitted data to describe the BSA adsorption process. For kinetic studies, the 14 wt% PES concentration with 60 wt% and 80 wt% of HAP concentration of PES concentration were found to agree with pseudo-second-order kinetics model. Regeneration of membranes were carried out by shaking the used membrane in acetate buffer solution at pH 4.2 and the result demonstrated for 14 wt% PES concentration with 60 wt% and 80 wt% of HAP concentration of PES concentration that, there is no significant reduction in the adsorption capacity.

CHAPTER ONE

INTRODUCTION

1.1 Research Background

Proteins are known as complex structures because it consists of one or more long chains of amino acid residues. Protein have play important roles in the food technology and also in pharmacological technology, it serve as a drug for treating ulcers and infections and a high level of purity. Therefore, the purification processes are required to determine its unique characteristics such as size, charge, shape, and functions.

In the recent years, there has been increasing interest in the research of separation and purification of proteins such as BSA, lysozyme, and milk proteins, etc. BSA is widely used as a model protein as it has similar properties, molecular weight, and an amino acid sequence to its human variant, human serum albumin. Moreover, this type of model protein is easily available and large amount of albumin contains in the body fluids (Givens et al., 2017). It is also taken as a model protein in the drug development because of the abundance of binding constant with organic chemicals (Ma et al., 2017).

The adsorption of proteins onto solid surfaces is an extremely important as it is first step in many biological processes. In advance, protein adsorption also can activate adhesion of particles, bacteria or cells possibly promoting inflammation cascades, or fouling processes. Therefore, protein adsorption is a common phenomenon, wherever proteins in contact with a solid interface they will adsorb to it.

Mixed Matrix Membranes, MMMs is a type of membrane formed by incorporating fillers in polymer matrix. The fillers employed for improving the MMMs performances and types of fillers that usually used is inorganic materials with specific features such as surface interaction, shape, pore size and so on (Suen, 2015). The MMMs

have several advantages for adsorption purpose such as high porosity, large pores for convective flow, reduce mass transfer resistances, and operation at lower pressure drop.

In this research study, the concept of MMMs incorporated with adsorptive particles are used as the solid surfaces towards adsorption of BSA as the flow process using MMMs is more efficient for adsorption application . Not only that, the principle of ion exchange chromatography is applied in this current research study. This research focused on adsorption studies of BSA by MMMs.

1.2 Problem Statement

Protein separation by packed column chromatography is commonly used in downstream processing. The resins are provided in this chromatographic technique to achieve high binding capacity with cost effectively. However, there is still having the limitations of packed column chromatography included high pressure drop, relatively slow intra-bead mass transport, difficulty in column packing and complicated in scale-up procedures (Saufi and Fee, 2013). Furthermore, this method requires long process time, bed compression, and clogging might be occurred due to low bed porosity and long bed height. The non-uniform packing typically happens in this case with a wide and short bed. Therefore, these limitations have led to the development of membrane chromatography.

Membrane chromatography has widely used for adsorption application. It is also known as membrane adsorption. Membranes chromatography process provide high adsorption capacity , convective mass transfer properties, low clogging tendency, and leading to the operation at low pressure drop and shorter residence times. Thus, the combination of membranes filtration and ion exchange chromatography are applied. Besides, the introduction of Hydroxyapatite, HAP into the membranes will enhances the adsorption process towards protein. It have been proven that electrostatic interaction between HAP and protein molecules is the dominating factor in adsorption process (Yin

et al., 2002). Also, the protein adsorption in ion exchange chromatography is mainly determined by the electrostatic interaction between the solute and the oppositely charged of the surface stationary phase. Therefore, the addition of HAP as an adsorptive particles incorporated in a membrane matrix are applied in this study toward protein adsorption.

1.3 Research objectives

The objectives of this research are as follow:

i. To fabricate and characterize the PES/HAP/PVP MMMs at different concentration of PES and HAP.
ii. To elucidate the adsorption behavior of BSA on the PES/HAP/PVP MMMs in a batch system.
iii. To evaluate the equilibrium and kinetic adsorption model of BSA into PES/HAP/PVP MMMs.

1.4 Scope of research

In this work, the MMMs were fabricated by varying the PES and HAP concentration for adsorption studies on BSA. Flat sheet membranes were prepared by phase inversion method. The MMMs were characterized in terms of morphology, chemical properties, hydrophilicity and hydrophobicity, water content and membrane porosity.

Batch adsorption system was performed in this research studies. Static BSA adsorption, dynamic BSA adsorption equilibrium studies and kinetic studies were accomplished in order to study the adsorption behavior of MMMs on BSA. The optimized MMMs were determined from static adsorption capacity. The equilibrium and kinetics were performed in this research. The optimum BSA concentration was determined from the equilibrium studies by performed Langmuir and Freundlich isotherms. The optimized

MMMs and BSA concentration was further used for dynamic adsorption capacity with different time and kinetic studies to investigate the adsorption behavior of the MMMs toward BSA. Regeneration of the MMMs was carried out using acetate buffer solution at pH 4.2 for the membrane reusability.

1.5 Organization of thesis

There are five chapters in this thesis and each chapter describes the sequence of this research.

Chapter 1 presents the overall background of this research study. This chapter also presents the problem statement, research objectives, scope of research and thesis organization.

Chapter 2 covers an overview of membrane technology. The membrane adsorber, principle of ion exchange, mixed matrix membranes, selection of hydroxyapatite as adsorptive particles and PES/PVP/DMAc as polymeric membranes are discussed in detail.

Chapter 3 refers to the material and methods describing the experimental procedure in the research for batch adsorption system. This chapter also covers the characterization of MMMs.

Chapter 4 refers to the experimental results and discussions of the data obtained. The membranes characterization and analysis obtain are showed in detail. Further elaboration on the effect of PES and HAP concentration of the synthesized membranes toward BSA adsorption. The adsorption studies on the results on equilibrium, kinetic studies and membrane regeneration are also provided in this chapter

Chapter 5 refers to overall conclusions that are based on the findings obtained in the results and discussion (Chapter 4). Recommendations for future research are also given in the chapter.

CHAPTER TWO

LITERATURE REVIEW

2.1 Membrane technology

Membrane technology is well-established technology and widely used in the biotechnology industry and wastewater treatment industry. This technology has wide range for the separation, removal, recovery and purification processes. It is also proven that the membrane technology processes have high stability and efficiency, low energy requirements, easy operation, most economical processes and potentially better for the environment since the membrane approach require the use of relatively simple and non-harmful materials (Chai et al., 2017).

Biotechnology industry have play major role in the field of separation and purification of biotechnological products. Over the last three decades, the membrane technology attracted the attention of chemists, chemical and biotechnical engineers due to their unique separation principle. There are various applications in the membrane processes in both upstream and downstream technology for the purification of proteins, such as microfiltration, ultrafiltration, emerging processes as membrane chromatography, high performance tangential flow filtration and electrophoretic membrane contactor (Saxena et al., 2009). The commercial manufactures in this industry are to produce high purity and throughput of protein such as BSA, therapeutic proteins, monoclonal antibodies (MABs) with cost effectively.

According to the perspective that highlighted from biotechnology and bioengineering article Zydney (2009), there have an improvements in membrane

technology for bio-processing applications. The improvements are based on fundamental understanding of the effects of electrostatic interactions and concentration polarization on protein transmission during ultrafiltration and the role of membrane morphology on protein fouling during the membrane filtration processes.

Generally, the membrane technology has several types of filtration processes in order to remove large impurities and to retain the desired products. Driving force is applied during the filtration processes to deliver energy for separation process. Commonly, the driven force is introduced in terms of pressure, concentration, temperature or electrical potential. The most commonly used in the filtration processes is the pressure driven processes. The types of pressure-driven processes are classified as microfiltration, ultrafiltration, nanofiltration, and reverse osmosis. Figure 2.1 shows the filtration spectrum of microfiltration, ultrafiltration, nanofiltration, reverse osmosis and particulate filtration according to the particle sizes. Each and every type of pressure-driven processes has its own limitations in the separation, removal and purification processes. Hence, the type of membrane processes is selected based on the size of particle to be separated.

Microfiltration (MF) membranes are capable to remove particles size larger than 0.1 micrometers. Commonly, MF membranes are applied for the separation, purification and clarifying of protein-containing solutions, such as for the recovery of extracellular proteins produced via fermentation and for the removal of bacteria and viruses in the final formulation of therapeutic proteins (Saxena et al., 2009). Other than that, MF membranes are also popular used in wastewater treatment industry for removal of heavy metals, organic and chemicals. In the bio-separation processes, the size of macromolecules and proteins involved much smaller than the pores size of MF membrane. As a resulted, high fluxes and low the mass-transfer coefficients.

Ultrafiltration (UF) membranes have ability to remove the particle sizes is less than 0.1 micrometers. In this currently years, UF is used as preferred method for protein concentration and buffer exchange, and replaced size exclusion chromatography in the biotechnological industry (Saxena et al., 2009). This is due to its ability to trace the impurities such as virus, and purification steps for protein. Important characteristic of UF membrane for achieving high purity of protein are high thermal stability, chemical resistivity, and restricted the use of fairly harsh cleaning chemicals (Saxena et al., 2009). Membranes for nanofiltration (NF) and reverse osmosis (RO) are for partially retain molecular and ionic substances, which the capability to remove less than 0.01 micrometer. However, high pressures are necessary for this which requires high investment and operating costs.

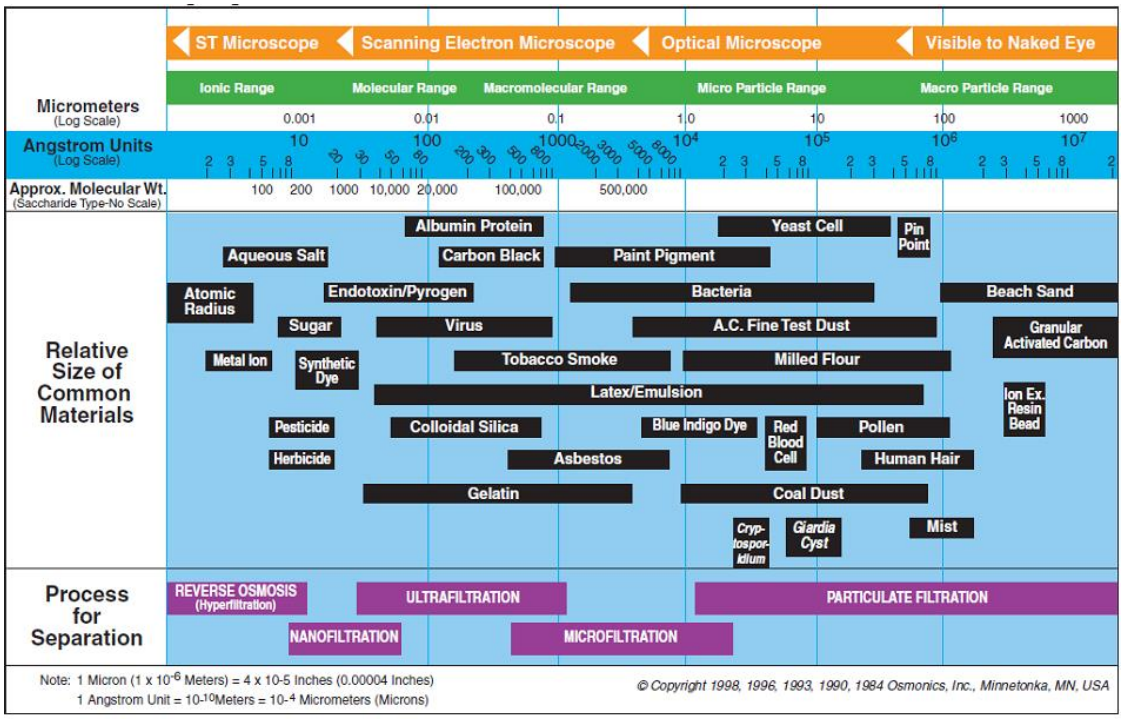


Figure 2.1 Filtration spectrum of microfiltration, ultrafiltration, nanofiltration, reverse osmosis and particulate filtration according to size of materials (Source RADCLIFF 2004)

Other than that, membrane technology has widely used in the adsorption application. The flow process is more efficient for practical adsorption applications (Suen, 2015). Compared to packed column chromatography, the used of the membrane as adsorber give more benefits and efficient method towards separation processes. A few crucial problems encountered in packed column chromatography such as high pressure drop, slow mass transfer, long process time, bed compression, and clogging may associate with low bed porosity and long bed height. Moreover, with a wide and short bed, non-uniform packing were frequently happened (Suen, 2015).

Therefore, these problems could be intrinsically offers with the use of adsorbent in the membranes which provides shorter bed height, higher bed porosity and larger pores (Suen, 2015). The larger pores of membranes morphology could leads to higher adsorption rate due to the larger surface area (Mangun et al., 1998). Consequently, the membranes adsorber give the benefits in time and energy savings with respect to the packed column chromatography.

2.2 Membrane adsorption

Membrane adsorption is known as one of the efficient methods for the purification and separation process in biotechnology industry compared to packed bed column chromatography. There are limitations in protein separation using packed column include a high pressure drop, difficulty in column packing, relatively slow intra-bed mass transport and complicated scaled up procedures (Saufi and Fee, 2013). The review from Sun and Wu (2014), Avramescu et al. (2003), Yuzhong Zhang (2006) had proven that the adsorptive membranes potentially give high efficiency in protein separation and purification.

Several advantages of membranes adsorber which are the liquid flows through the membrane provide very short time and wide bed, and the elimination of diffusion resistance leaves a system controlled by much faster binding kinetics, thereby enabling adsorptive separation of proteins one-tenth the time common for packed bed column chromatography (Yuzhong Zhang, 2006).

Mass transfer mechanism is the key factors governing the adsorption performances. The mass transfer mechanism steps are external mass transfer from bulk liquid to frontal membranes surface which could accelerated by shaking of the container, radial diffusion in membrane pores, diffusion inside the particle pores, and followed by intrinsic adsorption onto the active sites of particle surface (Suen, 2015). The mass transfer mechanism is basically between the adsorbate and adsorptive particles that filled in the membrane. As mentioned by Yuzhong Zhang (2006), the use of membranes in the adsorption application can reduces the mass transfer resistance for the solute to the matrix as it provide larger pore size.

Basically, the general definition described adsorption as an enrichment of chemical species from a fluid phase on the surface of a liquid or a solid. The molecules or ions will be removed from the aqueous solution by adsorption onto solid surfaces. The adsorption processes are also known as a surface phenomenon. The influence parameters that affect the adsorption behavior are pH, concentration, temperature, ionic strength and buffer composition. The basic terms of adsorption process are shown in Figure 2.2.

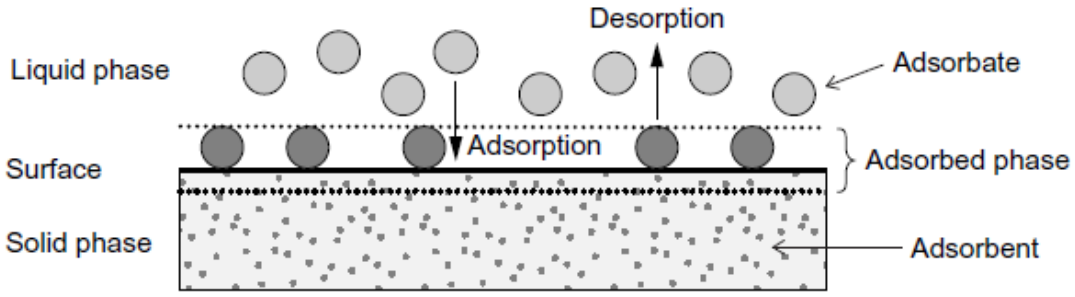


Figure 2.2 Adsorption process (Worch, 2012)

According to Esfahani et al. (2015), the mechanism adsorption of protein is actually highly complex, and the interaction is depends on the physicochemical properties of the protein itself such as surface area, electrostatic charges, polar/non polar groups, chemical structure and hydrophobicity/ hydrophilicity. However, the protein is easily to adsorb to the membrane surfaces. Several phenomena of membrane adsorption on adsorbent surfaces are recognized such as resin (Yuzhong Zhang, 2006), carbon nanotubes (Salehi et al., 2012), nanoparticles (Sun and Wu, 2014), zeolites.

The mechanism of protein adsorption is based on the ion exchange process which there is a reversible exchange of ions with ions in protein solution that electrostatically bounded to the membrane adsorbers. The electrostatic force between the adsorbent and adsorbate play an important role in this process. The mechanism of the adsorption of protein with ion exchange principle is further described below.

2.3 Principle of ion exchange

Ion exchange is one the method that widely used for the separation of various kinds of proteins (Murat Akgül1 and Karabakan1, 2008), due to high resolution of proteins, low relative costs, and it can maintain the native configuration and biological activity of proteins during the process (Sun and Wu, 2014). This process is adsorption phenomenon where the mobile ions from an external solution are exchanged for ions that are electrostatically bounded to the functional groups that contained within a solid matrix.

Ion exchangers are divided into two types which are cation exchanger (negative charge) and anion exchanger (positive charge) as shown in Figure 2.3. These two types of ion exchangers can be separated using ion exchange method. The external solution is containing an aqueous buffer system which is protein solution. The stationary phase represents as solid matrix and it is usually made from inert organic matrix which carry displaceable oppositely charged ion (Acikara, 2013b).

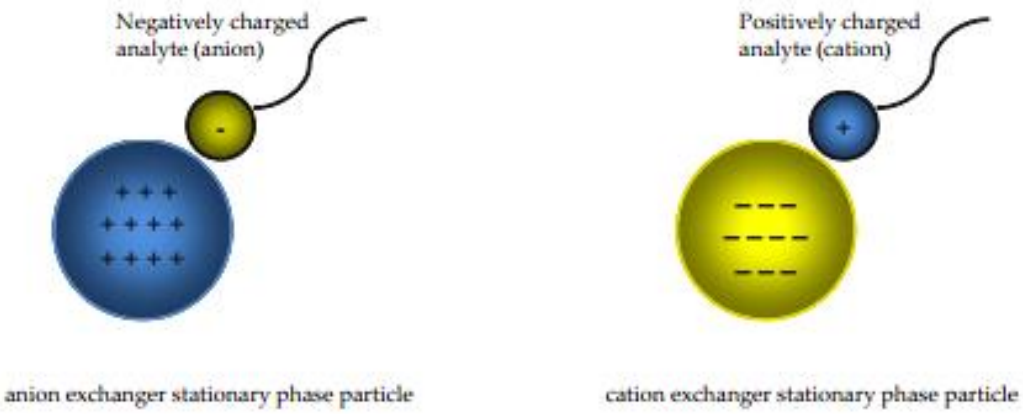


Figure 2.3 Types of ion exchangers (Acikara, 2013a)

Generally, the mechanisms of ion exchanges have five stages as illustrated in Figure 2.4. The first stages is starting conditions in aqueous buffer solution, followed by adsorption of samples, starting to desorption process, end of desorption and the last stages is regeneration.

At the initial stage, the important consideration is maintaining the pH and ionic strength of the buffers solution; so that the charged group of ion exchanger will easily bind to the active site of the membranes surface. According to Avramescu et al. (2003), for protein, the charge is based on the isoelectric point, pI, because of the protein is neutrally charged, and thus it is measure as pI. For the cation exchange adsorber membranes, which operating at lower pH than the isoelectric point of protein used, the

protein is positively charged, while the ion exchange particles is possess a negative net charge. Thus, the pH adjustment of the buffer solution is important consideration in this process in order to keep good protein adsorption efficiency in the membrane adsorbers.

The second stage is the adsorption of samples (protein solution) whereby the charged group of ion in the protein solution is reversible binding to the active sites of the membrane adsorbers. Since protein is known as complex structures, there are some ions, impurities and hydrogen ions that do not bind to the membrane adsorbers, therefore these can be removed by washing the membranes with buffer solution.

For the third and fourth stages are retained proteins are eluted from the membrane adsorbers. This stages is known as a desorption process. Desorption can be achieved by treated the membrane adsorbers with the starting buffer solution. Under these conditions, the proteins will have the same charge as the membrane adsorbers thus, the ion molecules are released from the membrane adsorbers (Avramescu et al., 2003). Regenaration steps is the regenerated membrane, the membrane can be reuse for next adsorption stages.

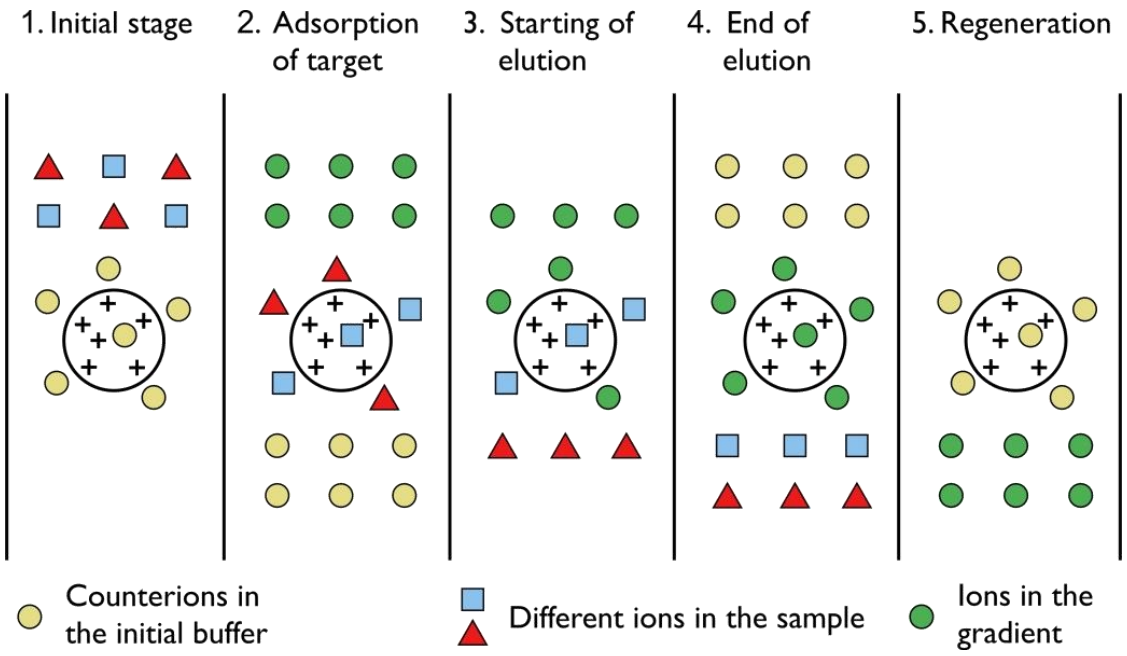


Figure 2.4 Mechanism of ion exchange chromatography (Gyorgy Hegyi, 2017)

2.4 Mixed Matrix Membranes

In this currently years, there is increasing number of people pay high attention to the research of mixed matrix membranes (MMMs, due to lower costs, manufacturing ease and good separation process. MMMs have wide variety of applications such as protein adsorption (Sun and Wu, 2014), removal of enzyme (Sun et al., 2015), gas separation(Chung et al., 2007), and wastewater treatment.

According to Sun and Wu (2014), MMMs embedded with adsorptive particles can improve the purification steps of protein while by combined the selective organic or inorganic particles with MMMs are applied for separation and recovery of proteins or enzymes. Furthermore, combination of ion exchange resins and MMMs can be applied in protein capturing, purifying and polishing steps. Furthermore, the adsorptive particles embedded in MMMs contain ionic groups that could provide charge interaction with proteins. The interaction rate of ion exchange mode is normally faster than other adsorption modes.

There are several types of configuration membrane adsorbers that have been developed in protein separation which are flat sheet, hollow fiber and radial flow as shown in the Figure 2.5. For the flat sheet membrane adsorbers, the liquid was introduced to the membrane surface. The hollow fiber type, the liquid flows parallel to the membrane surface and the liquid moves through the pore of membranes. The radial flow adsorbers were commonly used for large scale application (Saxena et al., 2009).

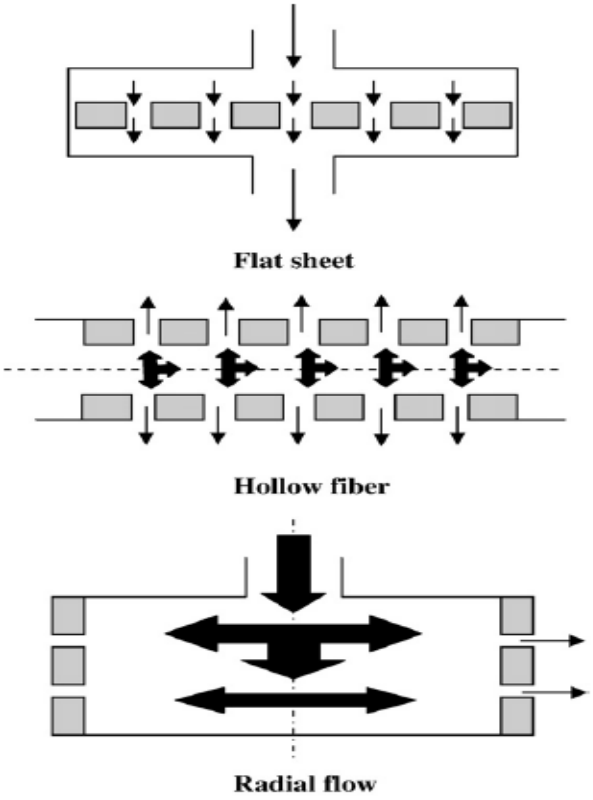


Figure 2.5 Types of membranes configuration with the distribution of liquid flow inside the membranes (Saxena et al., 2009)

The advantages of MMMs are maintaining the native conformation and biological activity of proteins enzyme during the isolation and purification process. This is due to lower resistance and mild process condition (Sun and Wu, 2014).The MMMs are consisting of organic polymer and inorganic particle phases as shown in Figure 2.6.

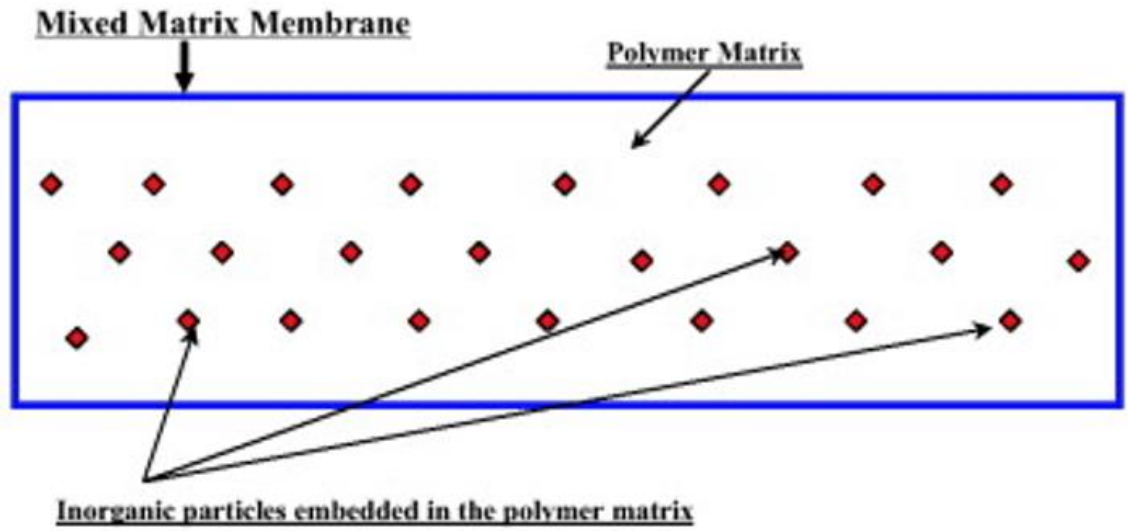


Figure 2.6 Schematic of Mixed Matrix Membranes (MMMs) (Aroon et al., 2010)

MMMs preparation procedure is recognized as simple method for adsorptive membrane since it is involved with only blending and inversion method. This concept has successfully been applied to make a variety of anionic and cationic membranes for protein purification (Saufi and Fee, 2013). Therefore, MMMs that embedded adsorptive particles with combining ion exchange technology is study in this research because it is time efficiency, energy saving, minimum membrane fouling, flux decline, simple and flexible for large scale operation (Suen, 2015).

2.5 Hydroxyapatite (HAP) as adsorptive particle on protein adsorption

Hydroxyapatite, (Ca_{10} (PO_4)$_6$(OH)$_2$) is inorganic component of the hard tissues in bones. HAP are usually observed to be carbonate-substituted and calcium-deficient (Cüneyt Tas, 2000). According to Yin et al. (2002), HAP has successfully applied in separation of proteins, enzymes, nuclei acid, virus and other biological molecules. Not only that, it has also been widely used to make medical implant as HAP is the mineral prototype for bones and teeth and with proven compatibility (Yin et al., 2002).

Some researchers prepared MMMs embedding with adsorptive particles to enhance protein purification (Sun and Wu, 2014). Other than that, utilizing the inorganic particles as adsorptive particles into the polymeric materials can improve the separation process as well as increasing adsorption capacity (Hosseini et al., 2014). Therefore, the adsorption capacity for different type of adsorbent used is summarized in Table 2.1.

Table 2.1 Comparison of Adsorption Capacity using different type of adsorbent and adsorber in biotechnological industry

Type of Adsorber	Type of Adsorbents	Adsorption Capacity	Applications	References
Ethylene-vinyl alcohol(EVAL)	Lewatit ion-exchange Resin	34 mg BSA/ml adsorber	Adsorption of BSA and separation of BSA and bovine hemoglobin	Avramescu et al. (2003)
Ethylene-vinyl alcohol(EVAL)	Ion exchange Resin	147 mg LZ/ml	Capturing Lysozyme (LZ)	Saiful et al. (2006)
Ethylene-vinyl alcohol(EVAL)	Phenyl Sepharose Resin	18.4 mg/ml ß-Lactoglobulin 45.9 mg/ml α-lactalbumin 41.1 mg/ml BSA 42.5 mg/ml lactoferin	Binding whey protein	Saufi and Fee (2013)
Polyethersulfone (PES)	HAP particles	61.2 mg BSA/g HAP	Adsorption Of BSA	Sun and Wu (2014)
Mixed matrix polyvinylidene fluoride	Micro/nano polyethyleneimine particles	105 mg protein/ml membrane	Adsorption of BSA	Sun and Wu (2014)

According to Table 2.1, most of the researchers used ion exchange resin as adsorbent for the protein purifications. Nevertheless, the adsorption capacity of ion exchange resin is lower compared to nanoparticles. As seen in Table 2.1, the adsorption capacity of HAP on BSA is higher compared to ion exchange resin.

HAP has good adsorption capacity towards protein. HAP is major used for repairing bone tissues and culturing sclerotin in the medical field due to its biocompatibility, bioactivity and osteoconductivity (Sun and Wu, 2014). Beside, HAP is also capable in capturing various types of protein such as milk proteins, albumin and lysozyme. It is because HAP has selective adsorption property to protein.

Moreover, the introduction of HAP to separation of proteins by Tiselius et al. (1956) has been successfully applied to separation of proteins, enzymes, nuclei acid, virus and other biological molecules. The performances of HAP particles shows the great efforts on the adsorption process of proteins. Also, it have been proven that electrostatic interaction between HAP and protein molecules is the dominating factor in adsorption process (Yin et al., 2002).

In addition, HAP shows great efforts in adsorption mechanism of biological molecules. It is well known that there is strong interaction between biological molecule and HAP surface which could enhance the adsorption process. Therefore, HAP is chosen as an adsorptive particles by embedding in MMMs for the adsorption of protein. Since this research project is mainly about the adsorption studies, the addition of HAP as an adsorptive particles incorporated in MMMs are applied toward protein adsorption in order to enhance the adsorption process.

2.6 Selection of PES, PVP and solvent for adsorptive membrane preparation

In the membrane preparation, the MMMs incorporation with HAP particles is chosen as the membrane adsorbers on BSA adsorption. For the membrane based content, PES, PVP, and DMAc were being selected to be used in this research studies.

According to T.Balamurali (2014), PES is the material of choice for the various application of polymeric as it has high mechanical strength, thermal stability, and formability. Other than that, this polymer has widely used in the UF separation technology. Moreover, PES is used as membrane material due to pore size and distributions of the PES membranes can easily controlled by varying the composition of the casting solution (Sun and Chen, 2016). Since, the main disadvantages of PES membranes is its low hydrophilicity and permeation, which resulted low membrane flux thus, the addition of DMAc as solvent can significantly improve the hydrophilicity and antifouling property of the membrane.

The addition of the additives of PVP to PES membrane will causes an expected increase in viscosity (Greenlee and Rentz, 2016), resulting high mechanical strength of PES membrane and tends to produce high permeation flux and rejection. Therefore, the combination of PES/PVP/DMAc is selected as polymeric membrane content in this research study.

2.7 Kinetic Studies of Adsorption Process

Adsorption studies were concern with equilibrium experimentation and modeling, as well as kinetic studies (Al-Jabari, 2016). The kinetic models handle for batch adsorption process and fixed bed adsorption column. In this research study, the batch adsorption process is carried out in determination of adsorption capacity of MMMs towards BSA. Therefore, kinetic model equations can be applied.

Adsorption isotherm is a method used to describe equilibrium relationship between the concentration of adsorbate and adsorbent phase. From the adsorption curves, it allows to determine the maximum adsorption capacity and dissociation constant (Kd). There are two types of adsorption isotherm which are Langmuir and Freundlich. It is known that Langmuir model can be used for the protein adsorption in batch adsorption process (Sun and Wu, 2014). Theoretically, the Langmuir equation can be expressed as:

$$q_{eq} = \frac{q_m K_D C_{eq}}{1 + K_D C_{eq}} \tag{2.7.1}$$

Where C_{eq} is equilibrium concentration of adsorbate (mg/L), q_{eq} is the amount of adsorbate adsorbed at equilibrium concentration (mg/g), q_m is the maximum adsorption capacity of the adsorbent (mg/g), and K_D is dissociation constant (L/mg).

By rearrange the equation, we get;

$$\frac{1}{q_{eq}} = \frac{1}{q_m} + \frac{K_d}{q_m} \times \frac{1}{C_{eq}} \tag{2.7.2}$$

Therefore, by using linear curve fitting, a plot of $1/C_{eq}$ against $1/q_{eq}$ allows the determination of the dissociation constant (K_D) and maximum adsorption capacity (q_m).

Kinetic adsorption studies helps to understand the mechanism of the adsorption and determine the rate determining steps of adsorption process. Pseudo-first-order kinetic model and pseudo-second-order kinetic model is the most simple and basic kinetics model in describing the adsorption reaction. Equation 2.7.3 illustrates the pseudo-first-order kinetic model and Equation 2.7.4 illustrates the pseudo-second-order kinetic model.

$$q_t = q_{eq1}(1 - e^{k1t}) \quad (2.7.3)$$

$$q_t = \frac{t}{(\frac{1}{(k_2 q_{eq2}{}^2)} + (\frac{t}{q_{eq2}})} \quad (2.7.4)$$

Where k_1 and k_2 (min^{-1} and g/mg min) are the pseudo-first and pseudo-second order rate constant respectively, q_{eq1} and q_{eq2} (mg/g) are the calculated values of the amount of BSA adsorbed in equilibrium state by pseudo-first and pseudo-second order kinetic models, q_t (mg/g) is amount of BSA adsorbed during time, t.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Introduction

This chapter presents a production of flat sheet membranes for the adsorption of BSA. Various composition of flat sheet mixed matrix membranes were produced from PES/HAP/PVP MMMs. Types of adsorbent that used is Hydroxyapatite (HAP) particles. The MMMs were fabricated by varying PES and HAP concentration. The synthesized membranes were undergo characterization and batch adsorption experiments for 24 hours. After that, determination of optimum membranes with highest maximum adsorption capacity towards BSA according to adsorption isotherms. Hence, the optimum MMMs were further used for the batch adsorption experiment with function of time.

The overall methodology for preparation of MMMs, preparation of BSA solution was presented in this chapter. All the chemicals, characterization and performances test method were briefly explained on how to accomplish the experiment.

3.2 Materials

Materials that used in this study were listed in Table 3.1.

Table 3.1 List of Materials

Materials	Supplier	Usage
Polyether Sulfone (PES)	Sigma-Aldrich	Base membrane polymer
Dimethyl Acetamide (DMAc)	Sigma-Aldrich	Solvent membrane polymer
Polyvinylpyrrolidone (PVP)	Sigma-Aldrich	Additive membrane polymer
Hydroxyapatite (HAP)	Acros Organics	Adsorptive particles
Bovine Serum Albumin (BSA)	Sigma-Aldrich	Protein model
Acetic Acid	Sigma-Aldrich	Monobasic solution in acetate buffer
Sodium Acetate	Sigma-Aldrich	Dibasic solution in acetate buffer
BCA solution	Novagen	Reagent for to detect BSA protein
4% Cupric Sulphate	Novagen	Reagent for detect BSA protein

The properties of chemicals used were summarized in Table below:

Table 3.2 N,N-Dimethylacetamide (DMAc) Properties

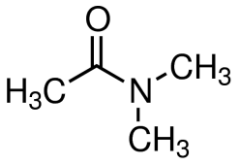
	Properties
Chemical Name	*N*,*N*-Dimethylacetamide
Chemical Structure	
CAS No	127-19-5
Molecular Formula	$CH_3CON(CH_3)_2$
Molecular Weight	87.12
Assay	99.8%
Grade	Anhydrous

Table 3.3 Bovine Serum Albumin (BSA) Properties

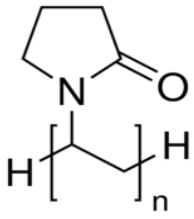
	Properties
Chemical Name	Bovine Serum Albumin
Chemical Structure	
CAS No	9048-46-8
Molecular Weight	mol wt ~66 kDa
assay	≥96% (agarose gel electrophoresis)
form	lyophilized powder

Table 3.4 Hydroxylapatite (HAP) Properties

Properties	
Chemical Name	Hydroxylapatite
CAS No	1306-06-5
Molecular Formula	$HCa_5O_{13}P_3$
Molecular Weight	502.31
Color	White
Assay	35 to 40 % (Ca)

3.3 Equipment and Instrumentations

Table 3.5 shown the list of equipment used in the membrane casting process, membrane characterization and adsorbance.

Table 3.5 List of Equipment

Equipment	Model/Brand	Usage
Elcometer	Elcometer 4340	Membrane Casting
Scanning Electron Microscopy (SEM)	TM 3000 SEM	Surfaces and cross-sectional morphologies
Fourier-Transform Infrared Spectroscopy (FTIR)	NICOLEST iS10, USA	Identification of functional group
Contact Angle Goniometer	250-F1	Contact angle measurement
Dead-End Stirred Cell	Sterlitech HP4750	Membrane water flux
UV-VIS Spectrophotometer	Spectroquant Pharo 300	Absorbance/Transmission
Sonicator	Elmasonic	Sonicate casting solution