MIXED-MODE INTERACTION MEMBRANE CHROMATOGRAPHY FOR PROTEIN FRACTIONATION

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ABSTRACT

Nowadays, membrane chromatography has widely use in protein separation. Most of the membrane chromatography is prepared through a chemical modification which sometimes can changes the structure and properties of the membrane itself. In this study, membrane chromatography was prepared through physical modification using the mixed matrix membrane preparation concept. Amberlite IR120 cation exchange resin (CEX) and Lewatit MP500 anion exchange resin (AEX) was used to formulate mixed mode interaction membrane chromatography (MMIMC) based on ethylene vinyl alcohol base membrane matrix. The total resin loading from 20-50% and the ratio between anion exchange resin and cation exchange resin was studied. This research found the optimum resin loading is 30%. Pure AEX MMM has the binding capacity of 340.26 mg BSA/g membrane and pure CEX MMM has a binding capacity of 3166.22 mg LZY/g membrane. The MMIMC with ratio CEX/AEX equal to 50/50 has a capacity of 895.898 mg LZY/g membrane and 3430.74 mg BSA/g membrane. A high performance MMIMC can be produced by optimization the loading percentage of ion exchanger resin and the ratio of CEX/AEX resin incorporate into the membrane.

ABSTRAK

Pada masa kini, membran kromatografi telah meluas digunakan dalam pengasingan protein. Pada kebiasaannya membran kromatografi disediakan melalui pengubahsuaian kimia yang kadang-kadang mengubah struktur dan sifat-sifat membranitu sendiri. Dalam kajian ini, membran kromatografi telah disediakan melalui pengubahsuaian fizikal yang menggunakan konsep penyediaan campuran matriks membran. Amberlite IR120 pertukaran kation resin (CEX) dan Lewatit MP500 penukaran anion resin (AEX) telah digunakan untuk menghasilkan membran interaksi mod campuran kromatografi (MMIMC), yang berasaskan etilena vinil alkohol membran matriks asas. Jumlah kandungan peratusan resin dari 20-50% dan nisbah antara resin pertukaran anion dan kation resin pertukaran telah dikaji. Kajian ini mendapati kandungan peratus resin optimum ialah 30%. Tulen AEX MMM mempunyai keupayaan mengikat 340.26 mg BSA / g membran dan tulen CEX MMM mempunyai keupayaan mengikat 3166.22 mg membran LZY / g. MMIMC dengan nisbah CEX / AEX bersamaan dengan 50/50 mempunyai kapasiti 895.898 mg membran LZY / g dan 3430.74 BSA mg / g membran. Satu MMIMC berprestasi tinggi boleh dihasilkan oleh pengoptimuman peratusan beban resin penukar ion dan nisbah resin CEX / AEX memasukkan ke dalam membran.

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Protein bioseparation is an operation to recover and purify protein products from various biological feed streams. It is an important unit operation in the pharmaceutical, biotechnological and food industry. The main concerns in production of bio-product is the cost of protein purification contributes major cost which is about 50%-90% of total cost production. Most products produced from a bioreactor contain a lot of impurities that need to be removing to get the final desired bio-product.

There are several steps normally applied to remove or separate the impurities from the crude product produced from the bio-reactor. These steps are aim for impurities removal, product isolation, product purification and final product polishing. Chromatography is considerably the most broadly unit operation in all bioseparation steps mentioned above. In chromatography process, the type of media and the principle of interaction between the media and the protein are important factors that must always be considered. However, there are several limitations have been found in the conventional packed bed chromatography such as relatively time-consuming process due to restricted flow rate operation, high-pressure drop in the columns and the slow diffusion of solutes within the pores of the chromatography media (Ghosh 2002). Most of the problem encounter in packed bed chromatography can be minimize by using membrane chromatography.

Membrane chromatography is one of the momentous chromatographic inventions during the past decade. The highly efficient process of membrane-chromatographic separation is based on the use of thin layers of finely organized and well-controlled macroporous polymeric stationary phases. As a result of the convective flow of the solution through the pores, the mass transfer resistance is tremendously reduced. Due to the macroporous structure of the membrane support, membrane chromatography has a lower pressure drop, higher flow-rate, and higher productivity than column chromatography (Zou, 2001).

Chemical modification required three steps for the preparation of membrane chromatography from the readymade membrane. These steps are 1) preparation of basic membrane. 2) Chemical activation of basic membrane and 3) coupling affinity ligands (or specific functionality) to the activated membrane (Zeng, 1999). However, these steps will change and sometimes will damage the structure and properties of the membrane if it is poorly control. In this study, physical modification is used to prepare membrane chromatography based on the preparation concept of mixed matrix membrane.

1.2 PROBLEM STATEMENT

Normal membrane filtration process based on size such as microfiltration, nanofiltration and ultrafiltration is not able to separate the mixture of protein very effectively. Chromatographic process is a prefer technique to separate these mixtures based on different interaction mechanism between protein and chromatography media such as ion exchange, hydrophobicity, afinity and etc. Chromatography media normally packed into a glass column which has several limitations such as high pressure drop, long processing times due to slow intraparticle diffusion and complicated scale up procedures. Membrane chromatography is developed to minimize the limitation of packed bed chromatography.

The normal procedure to prepare membrane chromatography through chemical modification can change and damage the properties of the membrane chromatography. Mixed matrix membrane preparation concept provides a physical modification and amore gentle way to produce membrane chromatography. In addition, the mixed-mode interaction membrane

chromatography which produced multiple interactions in single membrane material is still new and under developed in the area of membrane chromatography research.

1.3 RESEARH OBJECTIVES

The objective of this study is to produce high performance mixed-mode interaction membrane chromatography material for protein separation using mixed matrix membrane preparation technique.

1.4 SCOPE OF STUDY

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

i. To optimize the total loading percentage of resin to be incorporated into the membrane in the range of 20-50wt% respect to the polymer content.
ii. To test the performance of mixed-mode interaction membrane at different ratio of cation/anion resin in mixed matrix membrane.
iii. To apply the mixed-mode membrane chromatography in binding to single and binary protein mixture.

CHAPTER 2

LITERATURE REVIEW

2.1 PROTEIN SEPARATION TECHNIQUE

Nowadays, there are a lot of techniques used in protein separation field. The common techniques used are membrane filtration, chromatography and membrane chromatography. In this chapter, the modification of membrane chromatography technique will be discussed in details.

2.2 MEMBRANE CHROMATOGRAPHY

In protein separation, there are several steps normally used which are impurities removal, product isolation, product purification and polishing step. In the above steps, chromatography is by far the most widely used technique (Ghosh, 2002). Chromatography media is packed on column or known as packed bed chromatography. However, there are several limitations of packed bed chromatography such as the pressure drop across the packed bed is high and slow intra-particle diffusion for the transport of solutes molecules to their binding sites within the pores and long process time due to limited flow rate operation (Ghosh, 2002; Zou et al., 2001; Avramescu et al., 2003_a). Membrane chromatography has been introduced to overcome the limitation of packed bed chromatography in protein separation.

Membrane chromatography also known as membrane adsorber, is an adsorptive membrane which carries specific functionality similar to a chromatography resin. This

combines the principles of chromatography and membrane filtration in a single separation device (Kiyono, 2004; Saufi& Fee, 2009). Membrane chromatography is a promising technique for the isolation, the purification and the recovery of bio-molecules. When compared to traditional bead chromatography, the process is much faster, easier in processing and more robust. In addition it is also easier to set up and to scale up (Ghosh, 2002; Z. Borneman, 2010). Another advantage of membrane chromatography is that the transport through the porous structure is controlled by convective instead of diffusion transport. This results in shorter process time and minimizes the denaturation of the product (M. Avramescu, M. Wessling, 2003_b; R. Kiyono, 2004)

Membrane chromatography has a lower pressure drop, higher flow rate, and higher productivity as a result of the microporous/macroporous structure of the thin membrane (Zeng, 1999). Similar to conventional chromatography media, different types of interaction are also possible for membrane chromatography, such as anion exchange, cation exchange, hydrophobic interaction and affinity interaction.

Membrane chromatography is considered as an alternative to conventional resin-based adsorption columns. It is performed by ion-exchange, affinity, hydrophobic interaction and reversed-phase membrane adsorbers, and combines the advantages of conventional chromatography columns in terms of separation power and capacity with membrane technology in its mass transfer, high throughput and robustness. As the main advantage is the absence of long diffusive paths, a process performed with membrane adsorbers is faster than one with a traditional column configuration (J. Lamanda, 2011)

Membrane chromatography has very good characteristic for bio molecular purification. It is easy scale up and set up. Comparing with the traditional column, membrane has bigger pores, which makes the proteins can access the binding site on the membrane surface by directly bulk convection and with very little pore diffusion (L. Cao, 2005).

Membrane chromatography has emerged as a promising alternativeto conventional resin column chromatography for thepurification of biological molecules.Specifically, membrane chromatography offers volumetric flowrate independent dynamic capacities, higher separation speed, andeasier scale-up (V. Bharat, 2010).

2.2.1 ION EXCHANGE MEMBRANE CHROMATOGRAPHY

Ion exchangers are widely used for extraction, separation, and purification of organic. The most extensive use of ion exchangers in analytical chromatography nowadays is the separation of proteins and other large biological molecules. Thus, ion exchange chromatography of proteins requires sorbents with large pores while ion exchange capacity could be much less than the capacity of exchangers used in large-scale preparative separations. The bead size ranges from2 to 300 μm with the size distribution varying from mono-disperse up to 30% standard deviation substances (A. Zagorodni, 2007).

Ion-exchange chromatography is the most popular method for protein purification. The theory of it is to use the difference of charges on proteins at a given pH. The solid adsorbents are charged, positive or negative. Then the charged protein will be adsorbed by the charged adsorbents. According to the difference of the interaction forces between the protein and adsorbent, different protein is bounded differently by the adsorbent. Then, when some other buffer is used to replace the protein, they (the proteins) will be washed out of the adsorbents in different velocity: the less the interaction between the adsorbent and the proteins, the faster they will be washed out. Then, proteins can be separated according to the sequence of their elution. There are two kinds of ion exchangers: anion exchangers, which have positively charged matrix, and will adsorb the proteins with negative charge; cation exchanger, which have negative charged matrix, and will adsorb the proteins with positive charge. (L. Cao, 2005)

Protein separation in ion exchange chromatography is mainly determined by the electrostatic interaction between solute and the oppositely charged of the surface stationary phase (Saiful, 2006). Ion-exchange membrane adsorbers operate in convective mode, which

significantly reduces the diffusion and the pressure drop limitations commonly encountered in column separation processes (J. Lamanda, J. Sabate, and J. Llorens, 2011)

Ion exchange membrane chromatography is a high resolution separation technique most suited for protein purification protocols. The separation is based on the reversible electrostatic interaction between a charged protein molecule and the oppositely charged chromatographic membrane (ion exchanger). As proteins are usually handled in buffer media, one is usually interested in their isoelectric points (pI). The protein is positively charged below pI and it will bind to any cationic exchange membrane. At a pH higher than the pI of the protein, the target protein will be negatively charged and bind to anionic exchange membrane. (S. Bhattacharjee, C. Bhattacharjee and S. Datta, 2006)

The membrane adsorbers containing ion-exchange groups are useful for capturing proteins. A notable feature of the ion-exchange-group-containing polymer chain grafted onto a porous membrane is the extension of the polymer brush, i.e., the polymer chain from the pore surface toward the pore interior, due to electrostatic repulsion, which decreases the liquid permeability of the porous membrane (K. Miyoshi, 2005).

There are a few numbers of examples of protein purification using membrane chromatography with commercial ion-exchange membranes. Suck et al. demonstrated the application of membrane chromatography for separation of two model proteins, human serum albumin (HSA) and immunoglobulin G (IgG). That same group used anion-exchange membrane chromatography to separate enzyme penicillin acylase from the crude Escherichia coli supernatant. Santarelli et al. reported the separation of IgM from the supernatant of a human hybridoma cell culture using membrane ion-exchange chromatography.

There have a lots of application of ion exchange was use in purification of protein presently. Therefore, in the following section, various reaction schemes used to prepare different types of ion exchange and hydrophobic interaction membrane chromatography materials are reviewed.

2.2.2 MIXED-MATRIX MEMBRANE CHROMATOGRAPHY

In the early development of mixed matrix membrane (MMM) chromatography, the preparation concept is flexible and offers the possibility to easily adjust the geometry, the adsorption capacity as well as the functionality of the membranes (M. Avramescu et al. 2003_a). Avramescu et al. (2003a) prepared various geometries of mixed matrix materials from an EVAL polymer and a Lewatit CNP80WS cation resin in the form of flat sheet, solid fiber and hollow fiber membranes. In static adsorption experiments, a protein capacity of 135 mg BSA g^{-1} membrane or 45 mg BSA mL^{-1} membrane was achieved.

In a presently publication, different types of resin were incorporated into the EVAL base membrane to prepare cation exchange MMMs by incorporating Lewatit SP112 resin and anion exchange MMMs by incorporating Lewatit MP500 resin (M. Avramescu et al. 2003_b). They investigated the performance of these MMMs for separation of two similarly sized proteins, Bovine Serum Albumin (BSA) and hemoglobulin (Hb). By using a proper selection of the system pH, either anionic or cationic MMMs were able to separate the mixture of BSA and Hb effectively. Table 2.1 showed the published literature preparing MMM for protein separation using different materials with various formats..

Table 2.1: Mixed-matrix membrane chromatography using different materials with various formats (Saufi, 2009)

Authors	**Membrane matrix**	**Adsorbent resin**	**Adsorbent loading,%**	**Configuration**
Avramescu et al. 2008	Polyethersulfone	Lewatit CNP80WS-cation resin	50	Hollow fiber
Saiful et al. 2006	Ethylene vinyl alcohol	Lewatit CNP80WS-	65	Flat sheet

		cation resin		
Zhang et al. 2006	Polyethersulfone	Lewatit CNP80WS - cation resin Lewatit SP112WS - cation resin	10-85 50	Solid fiber
Kiyono et al. 2004	Polysulfone Amberlite	2004 IR120 - cation resin	20-50	Hollow fiber
Avramescu et al. 2003	Ethylene vinyl alcohol	Lewatit SP112WS - cation resin	65	Flat sheet

2.2.3 MIXED MODE INTERACTION MEMBRANE CHROMATOGRAPHY

Due to the simplicity of MMM preparation, there is a motivation to prepare mixed mode interaction or multi-mode membrane chromatography by combining hydrophobic and ionic interaction modes using MMM preparation technique (Saufi, 2011). Mixed mode interaction chromatography refers to the chromatographic method that utilizes more than one form of interaction between the stationary phase and solutes in the feed. In other words, many affinity chromatography adsorbents, all fall into the category of mixed mode chromatography (Guofeng Zhou, 2009).

Guofeng Zhou founds that mixed-mode interactions allowed the target protein to be adsorbed at a wide range of ionic strength, thus eliminating the requirement of prior salt addition to or removal from the feedstock. A rather new approached is based on the so-called mixed mode resins that offer more than on functionality for purification of protein.

CHAPTER 3

METHODOLOGY

3.1 MATERIALS

Ethylene vinyl alcohol (EVAL) (a random copolymer of ethylene and vinyl alcohol) with an average ethylene content of 44 mol% was purchased from Sigma Aldrich and was used as membrane material without further modification. Dimethysulfoxide (DMSO) was employed as the EVAL solvent and 1-octanol as a non-solvent additive in casting solutions. Water was used as non solvent in the coagulation bath. Lewatit MonoPlus ® MP500 (Fluka)for anionic MMM and Amberlite IR120 hydrogen form (Fluka)for cationic MMM were used as adsorbent particle. The particles were grinded and sieved to an average size of 45μm. The proteins used for the adsorption measurement was lysozyme (LZY) and bovine serum albumin (BSA). Sodium chloride (NaCl) is used for the regeneration or elution of MMM adsorbers.

Buffer solutions were freshly prepared in ultra pure water. The buffers used for adsorption were phosphate buffer at pH 7.0. The elution buffer was prepared by increasing the concentration of the adsorption buffer pH 7.0 to 1M using NaCl.

3.2 MIXED-MATRIX MEMBRANE CASTING SOLUTION

A homogenous polymer solution, consisting of 15 wt% EVAL polymer and 15 wt% 1- octanol in 70% DMSO was prepared by continuous stirring at about 60°C for 6 to 8 hours until all EVAL pellets were completely dissolved.

To obtain membrane with protein adsorptive properties, the adsorbent Lewatit and Amberlite ion exchanger particles with an average diameter 45µm were added to the prepared polymer solution at certain weight fraction which are from 20%-50% (relative to the EVAL content in the polymer solution) according to the resin loading equation showed in Equation 3.1.

$$R_{LOADING} = \frac{W_R}{W_R + W_P} \quad x \quad 100 \qquad \text{Eq. 3.1}$$

Where

W_R = weight of resin, (g)

W_P= weight of polymer, (g)

This mixture was stirred until homogeneous casting slurry was obtained at 50^0C to break down clusters of particles. In order to make in mixed-mode interaction membrane, anion resin and cation resin were incorporate into the polymer solution. The procedure to prepare the MMM is showed in Figure 3.1.

MMM polymer solution was cast on glass plate with a 400µm casting block and immediately immersed in a water coagulation bath at 40^0C and the membranes were formed a few moments after immersion. The membranes were immersed overnight in tap water bath at room temperature to remove residual solvent and 1-octanol.The membranes were dried in the freeze-dryer to remove water without affecting the membrane structure.

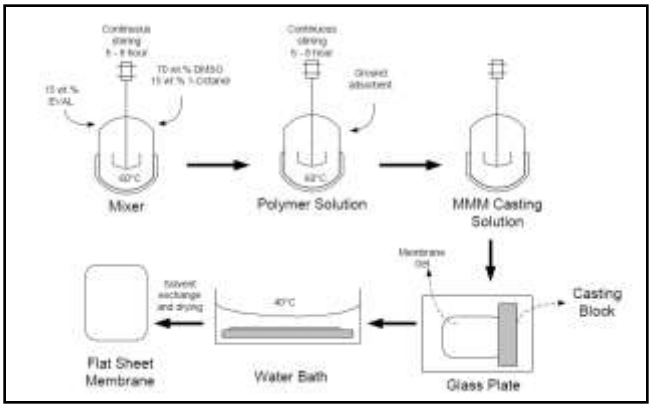


Figure 3.1: Preparation of mixed-matrix membrane chromatography (Saufi, 2010)

3.3 OPTIMIZATION RESIN LOADING EXPERIMENT

The optimization of loading percentage of ion exchange resin to be incorporated into the membrane was determined by using various resins loading. The resin loading were in the range of 20-50wt% respect to the polymer content while the fraction of anion exchange (AEX) resin and cation exchange (CEX) resin were fixed 50/50 in the first part of study.

In second part of experiment, the optimum resin loading obtained from previous experiment were used while the fraction of anion exchange (AEX) resin and cation exchange (CEX) resin are vary. The weight ratios CEX/AEX resin of the dope solutions of0/100, 50/50, and 100/0 were studied.

3.4 BATCH ADSORPTION EXPERIMENT

The adsorption capacity of mixed-mode interaction membranes was determined by batch adsorption experiments. A model protein was dissolved in phosphate buffer pH 7 with different initial concentrations of 1-8 mg/ml. A known weight of membrane (10mm x 20mm) was equilibrated in binding buffer for 3 hours to reconditioning the membrane. The equilibrated membrane was wiped with tissue and transferred to the model protein solution in centrifuge tube. Binding was done for 24 hour under rotator at room temperature. The equilibrium protein concentration was determined by spectrophotometric analysis by measuring absorbance at 280nm. The amount of protein adsorbed, q_{eq} (mg protein/g membrane), at equilibrium is calculated by Equation 3.2.

$$q_{eq} = \frac{(C_0 - C_{eq})V}{W_{membrane}}$$

Eq. 3.2

Where

C_0 = initial protein concentration (mg/ml)

C_{eq} = the protein concentration at equilibrium (mg/ml)

V = volume of the solution (ml)

$W_{membrane}$ = weight of dried membrane (g)

3.4.1 ADSORPTION ISOTHERM

In order to determine the adsorption isotherm, batch adsorption experiment was carried out with different initial protein concentration. The same area of membrane was used in each experiment to end up with different equilibrium concentrations. The binding capacity data were tested to fit either using Langmuir or Freundlich isotherm using a least square regression method. Langmuir isotherm and Freundlich isotherm were given in Equation 3.3 and Equation 3.4 respectively.

$$q_{eq} = \frac{q_m c_{eq}}{K_d + c_{eq}}$$

Eq. 3.3

$$q = Kc^n$$

Eq. 3.4

Where

q_{eq} = Equilibrium binding capacity, mg protein/g membrane

q_m = maximum binding capacity, mg protein/g membrane

K_d = Langmuir dissociation constant, mg/mL

C_{eq} = Equilibrium concentration

q = Binding capacity, mg protein/g membrane

K = Freundlich constant,

n = dimensionless exponent

3.5 PROTEIN ASSAY

3.5.1 UV-Visible Spectrophotometer

All the single protein concentrations were assayed by measuring absorbance of solution at 280 nm using an UV-Visible spectrophotometer (Model U-1800 Spectrometer, HITACHI). An absorbance-concentration standard curve was developed by series dilutionfrom seven standard concentrations from 0 to 2 $mgmL^{-1}$. Samples were diluted with phosphate buffer at pH 7.0 to within the absorbance range of the standard curve.

5.5.2 AKTA Explorer

Binary protein components were assayed using a reverse phase chromatography (RPC) column. The assay was run on an AKTA explorer liquid chromatography system controlled by Unicorn 5.01 software with samples manually injected through a sample injection loop. A 1 mL Resource™ RPC column (GE Healthcare Technologies) was used with 0.1% v/v trifluoroacetic acid (TFA) (Sigma)in DI water as buffer A and 90% v/v acetonitrile (Merck) in DI water as buffer B. The column was equilibrated with one CV's of 20% buffer B before a 500 μL sample was injected manually. A series of linear gradients were then applied as follows: 0-2 CV, 20% B; 2-11 CV, 20-100% B; 11-13 CV, 100% B; 13CV, 20% B; 13-14 CV, 20% B. Detection was by absorbance at 280 nm and a flow rate of 2 $mLmin^{-1}$ was used. The standard curve of peak area versus concentration was developed using a dilution series from a mixture of pure proteins corresponding to their composition in binary protein. Figure 3.6 shows an example of binary protein chromatogram assayed by this technique.

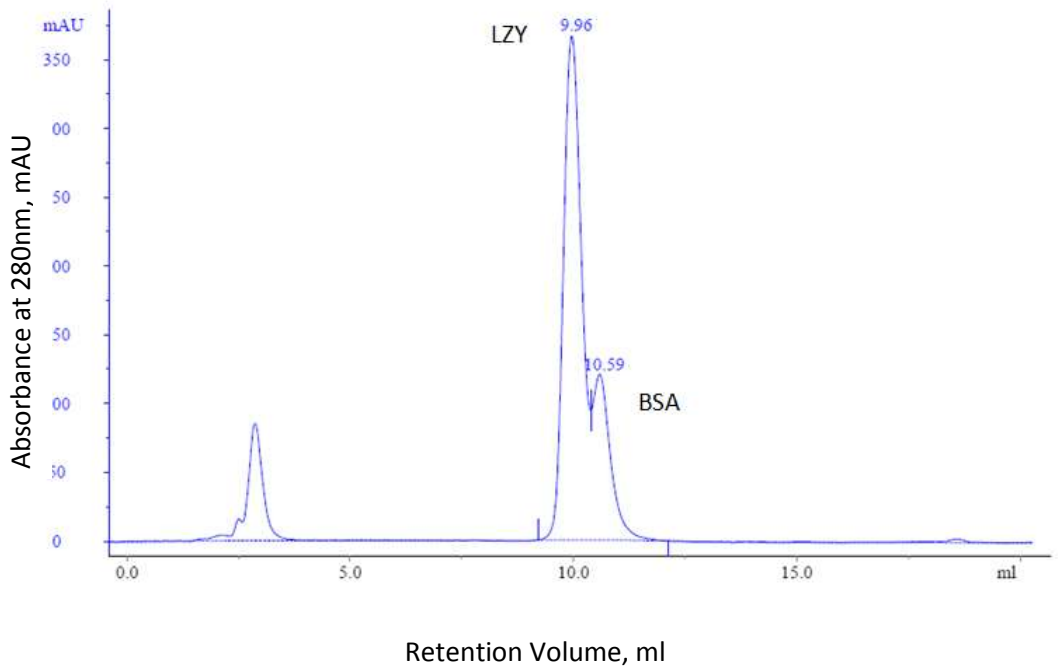


Figure 3.2: Chromatogram for binary protein assayed using Resource RPC1ml Reverse Phase Chromatography. Abbreviation: BSA- Bovine Serum Albumin, LZY-Lysozyme.

3.6 BATCH PROTEIN FRACTIONATION EXPERIMENT

Batch fractionation of protein was tested using small pieces of membrane (10mmx20mm) of known weight and small amount of resins. The adsorbent pure resins and various type of MMIMC were equilibrated for 3h in 20 mM sodium phosphate at pH 7.0. Then the adsorbents were incubated in single protein BSA and LZY at pH 7.0 overnight at room temperature using continuous rotating. The remaining protein solution was assayed to obtain the amount of protein bound. Afterwards, the membrane was removed from the protein solution and washed with equilibration buffer for 30 min and again the solution was assayed. For pure resins, the resins were sediment by using micro-centrifuge at 13rpm for 10 minutes then the liquid solution is separate to be assayed. The adsorbents were then incubated overnight in an elution buffer of 1 M NaCl in 20 mM sodium phosphate, pH 7.0, to

recover the adsorbed protein from the membrane. Thus, the percentages of protein recovery were calculated. The protein fractionation is then test by using binary protein solution. The procedure on binary protein testing is the same as static binding experiment. The different is just the protein solution used. For binary, the proteins of BSA with LZY at concentration 8mgmL-1 each were mixed together.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 EFFECT OF RESIN LOADING ON MIXED MATRIX MEMBRANE

Lewatit MP500 Monoplus, anion exchange resin and Amberlite IR120, cation exchange resin were employed in the preparation of MMM. Lewatit MP500 Monoplus has a positively quaternary amine groups which can bind to the acidic protein of BSA. Meanwhile, Amberlite IR120 has negatively sulfonic acid groups which can bind to the basic protein of LZY. The amount of cation or anion resin incorporated into the polymeric matrix play important parameters in preparing MMIM chromatography.

High ion exchange resin loading and the use of small particles resin favour a high adsorption capacity of the MMM chromatography.Small resin particles allow it to stick tightly within the MMM structure and also improves the homogeneity of resin particles distribution throughout the membrane matrix. A possible disadvantage of the MMM approach to creating adsorptive membranes, compared to chemically modified membranes, is that the embedded resin particles within the membrane present a diffusive path length that might slow down dynamic binding. However, resin particle size reduction by grinding also increases the interfacial surface area between permeate and resin thus decreases the diffusion path length to the internal ion exchange sites, both of which improve the dynamic ion exchange capacity.

The viscosity of the casting solution increases with increasing resin loading. Based on the preliminary experiment, the maximum resin loading is 30%. Above this value, the viscosity of the casting solution is too viscous and has a difficulty in casting into the membrane. The following section will explained the performance of MMIMC prepared using various ratios of CEX and AEX.

4.2 PROTEIN ADSORPTION ISOTHERM

The MMIMC was prepared with 30% resin loading. The single proteins used for this test were BSA or LZY. The results of binding capacity is showed in Figure 4.1.

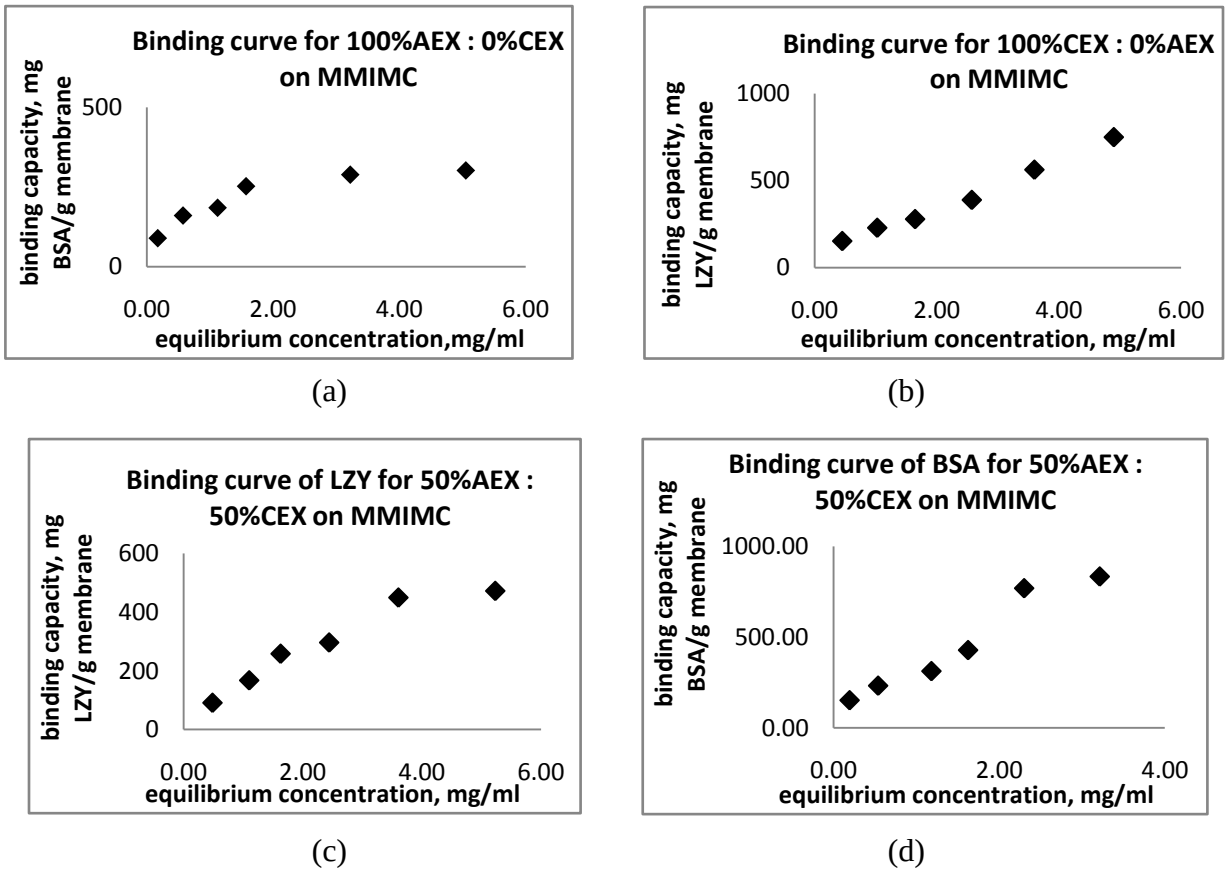


Figure 4.1: Binding capacity at various ratios. (a) 100%AEX: 0%CEX; (b) 100 CEX : 0%AEX; (c) 50%AEX : 50% CEX (LZY); (d) 50%AEX : 50%CEX (BSA).

The results obtained are fitted to the Langmuir adsorption isotherm Eq. (3.3)using a least-square regression method as shown in the following figure.As plotted on the reciprocal binding capacity versus reciprocal equilibrium concentration, the maximum binding capacity, q_m and constant $K_d$are obtained.

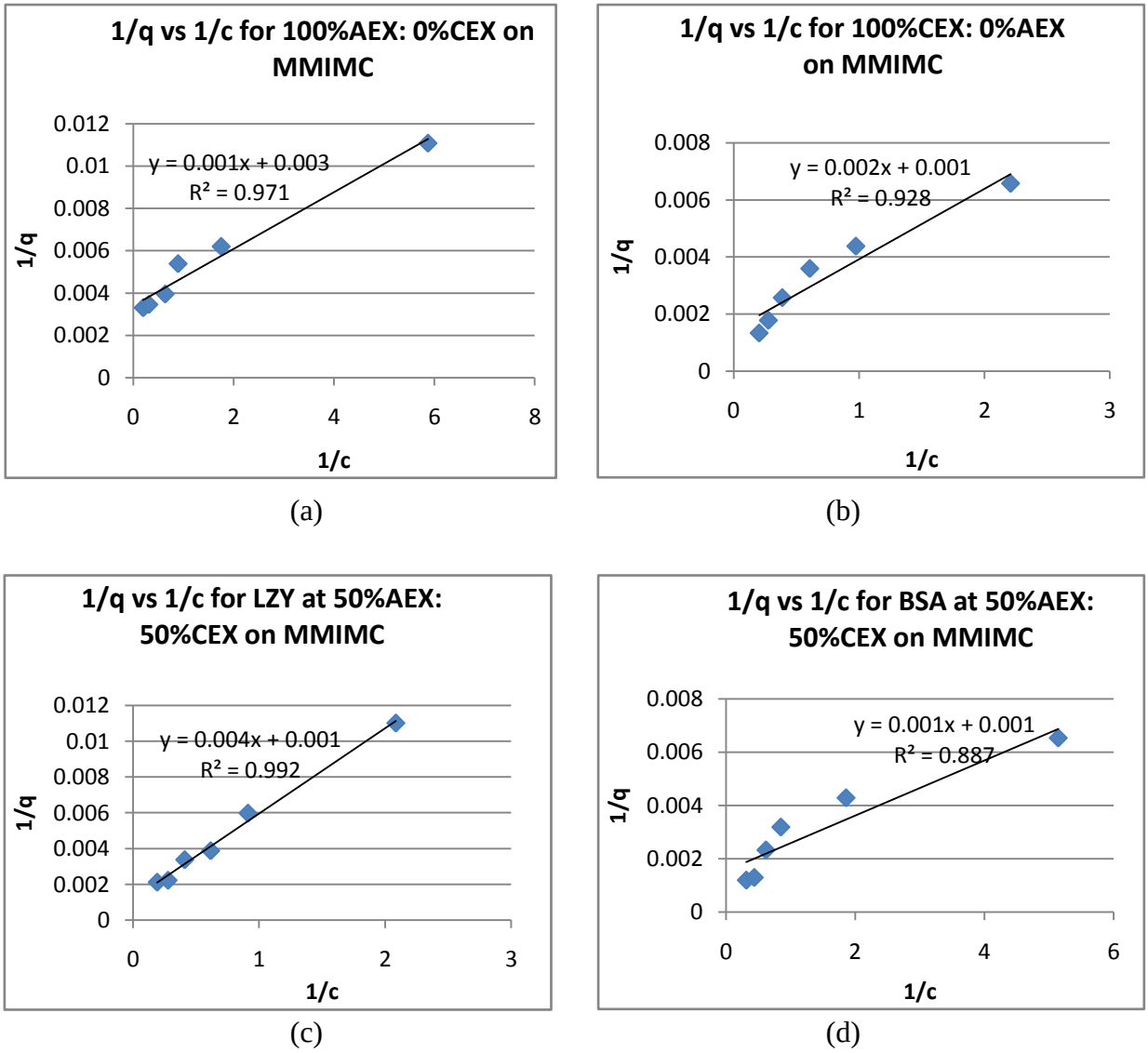
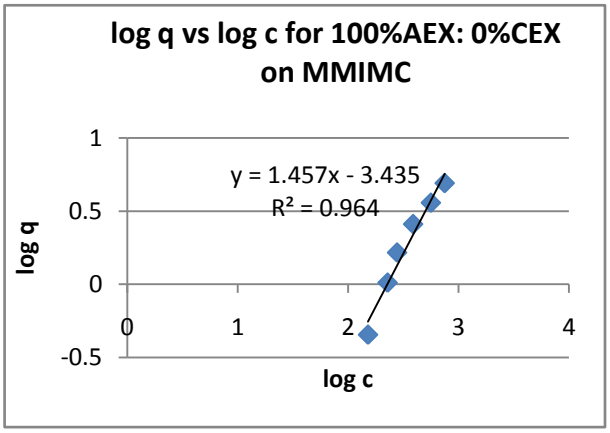
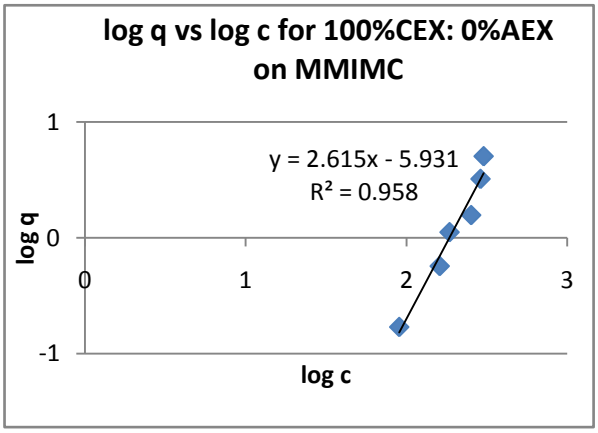


Figure 4.2: Determination of maximum binding capacity,q_m of protein with various ratios using langmuir adsorption isotherm. (a) 100%AEX: 0%CEX; (b) 100 CEX: 0%AEX; (c) 50%AEX: 50% CEX (LZY); (d) 50%AEX: 50%CEX (BSA).

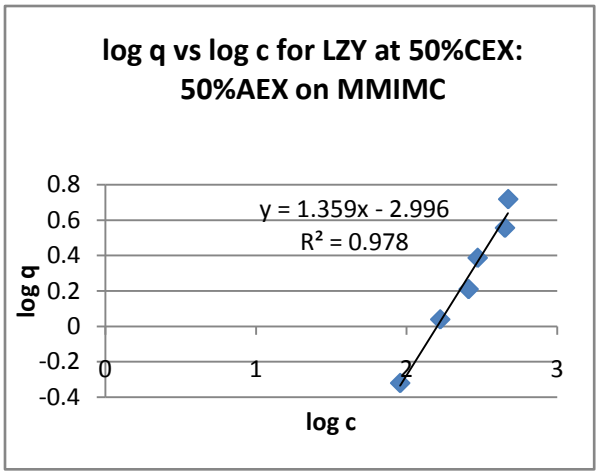
From the binding curve, the data also were fitted to the Freundlich adsorption isotherm Eq. (3.4) using least-square regression method as shown in the figure 4.3. As plotted on the log binding capacity versus log equilibrium concentration, the Freundlich constant, K and exponent, n are obtained.



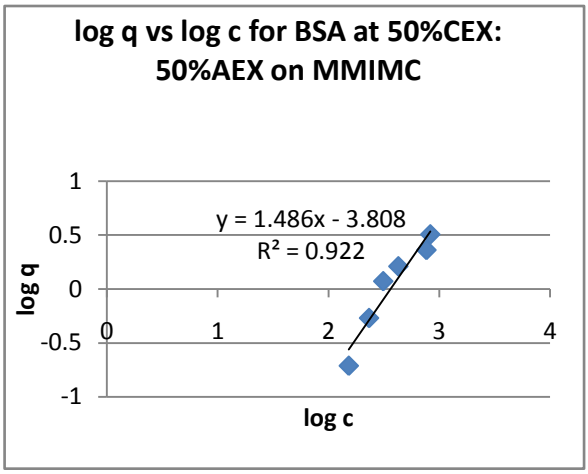
(a)



(b)



(c)



(d)

Figure 4.3: Determination of constant, K and value exponent, n at various ratios using Freundlich adsorption isotherm. (a) 100% AEX: 0%CEX; (b) 100 CEX: 0%AEX; (c) 50%AEX: 50% CEX (LZY); (d) 50%AEX: 50%CEX (BSA).

All the constant value obtained from Langmuir Isotherm (Eq. 3.3) and Freundlich Isotherm (Eq. 3.5) was summarized in Table 4.1.

Table 4.1: Langmuir Isotherm constant (Equation 3.3) and Freundlich Isotherm constant (Equation 3.5) on MMIMC at different ratio of CEX/AEX.

Ratio CEX/AEX of MMIMC	Maximum binding capacity, q_m (mg protein bound/g membrane)	Langmuir dissociation constant, K_d (mg/mL)	Freundlich dissociation constant, K	Freundlich exponent, n
0:100	340.260	0.646	191.044	0.321
100:0	3166.217	16.334	200.042	0.811
50:50 (LZY)	895.898	4.317	174.932	0.639
50:50 (BSA)	3430.739	9.551	336.594	0.805

From the entire constant, the data was compared with both Freundlich and Langmuir to obtain the best fitting for protein adsorption.

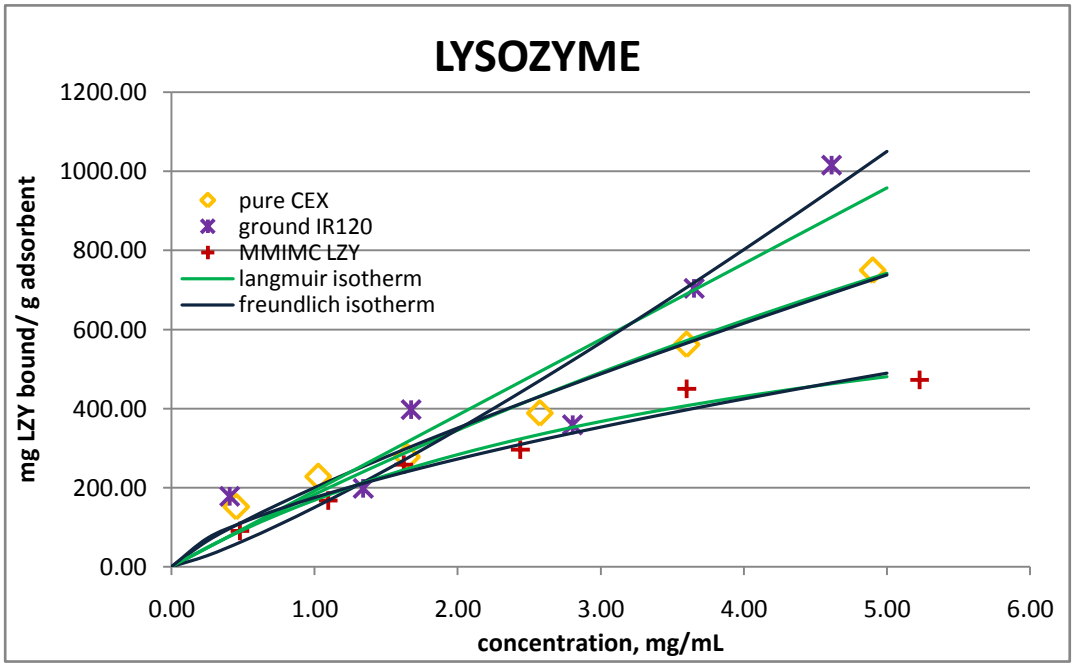


Figure 4.4: Static binding capacities of pure CEX, ground IR120 resin and a Mixed-Mode Interaction Membrane Chromatography (MMIMC) for lysozyme (LZY).

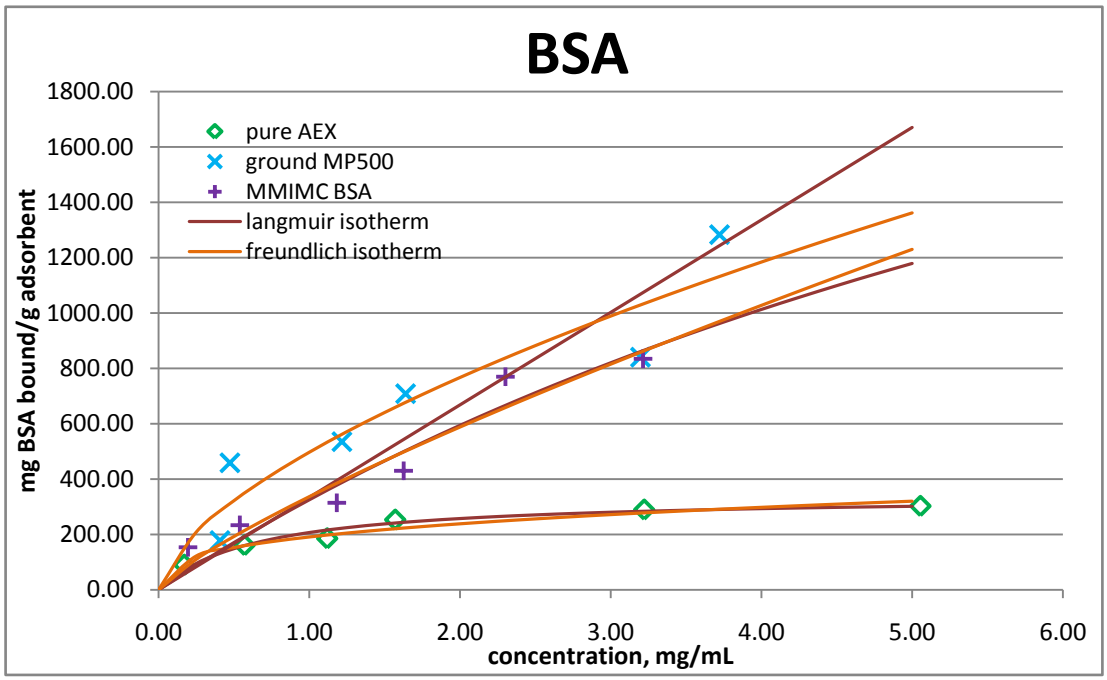


Figure 4.5: Static binding capacities of pure AEX, ground MP500 resin and a Mixed-Mode Interaction Membrane Chromatography (MMIMC) for Bovine Serum Albumin (BSA).

The binding capacity for LZY and BSA that fitted with Langmuir and Freundlich Isotherm were shown in Figure 4.4 and Figure 4.5. The data were fitted to both Langmuir and Freundlich adsorption isotherm. For the analysis data, the data is considered fit Langmuir adsorption Isotherm (Eq 3.4) using least square regression (Table 4.1). A Langmuir isotherm did provide good fit to the data follow to previous research (Avramescu et al., 2003; and Saiful et al. 2006). The data were then normalized to resin content in the adsorbent which are MMM or MMIMC.

The highest binding capacity for LZY is by using ground IR120 as adsorbent then followed by pure CEX and MMIMC. It contrary for BSA bounded. The highest binding capacity obtained by using ground MP500 as adsorbent then followed by MMIMC and pure CEX.