FABRICATION OF CHROMATOGRAPHIC MEMBRANE VIA SURFACE-GRAFTED MEMBRANE FOR PROTEIN SEPARATION

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ABSTRACT

Chromatography process was used extensively for protein separation in biotechnology industry. Chromatography resin bead was normally packed in cylindered column during the operation. However there were limitation on using packed bed chromatography such as high pressure drop, low processing rate and slow binding. Membrane chromatography has ability to overcome the limitation of the conventional packed bed chromatography for protein separation. Membrane chromatography used an adsorptive membrane that carried specific chromatography functionality. In the current study, commercial polyamide (PA) microfiltration membrane was modified with acrylic acid monomer to prepare a membrane chromatography. Two modification methods were compared which are ultra violet (UV) photo grafting and chemical grafting via redox reaction. Modification parameters were studied using One Factor at Time (OFAT) which are initiator concentration (1-50 mM), monomer concentration (0.2-5 M) and grafting time (5-60 minutes). The best grafting technique which gave the highest protein binding capacity was achieved by using UV photo grafting. The highest lysozyme (LZY) binding capacity achieved was 0.175 mg LZY/cm^2 membrane for the membrane prepared via UV photo grafting using 10 mM of benzophenone (BP) photo-initiator, 0.1 M of acrylic acid (AA) and 15 minutes of reaction time. The best conditions of UV photo grafting modification obtained from OFAT study were used in Response Surface Methodology (RSM) for optimization of protein binding. Based on the analysis of variance (ANOVA), the quadratic model was chosen with the R squared of the model was 0.9468. The optimum value for each parameter studied in RSM was 20.71 mM of BP, 0.29 M of AA and 18.19 min of grafting time. The optimized membrane chromatography was tested and characterized in term of degree of grafting, contact angle measurement, Scanning Electron Microscope (SEM), Fourier Transform Infra-Red (FTIR), pure water flux and permeability test. The protein binding capacity at optimized condition was 0.180 mg LZY/cm^2. This value is less than 4% of the value predicted by the model. This quadratic model can be used successfully to produce the membrane chromatography from PA membrane through UV grafting for LZY binding.

ABSTRAK

Proses kromatografi telah digunakan secara meluas untuk pemisahan protein dalam industry bioteknologi. Resin kromatografi turus terpadat beroperasi di dalam kolum silinder. Manik resin kromatografi biasanya dipadatkan di dalam turus silinder semasa beroperasi. Walau bagaimanapun terdapat had dalam penggunaan kromatografi turus terpadat seperti kejatuhan tekanan yang tinggi, kadar pemprosesan yang rendah dan pengikatan yang lambat. Membran kromatografi berkebolehan untuk menggatasi pengehadan kromatografi turus terpadat untuk pemisahan protein. Membran kromatografi adalah membran lekatan yang mempunyai fungsi kromatografi yang khusus. Dalam kajian semasa, membran penuras mikro komersial poliamida (PA) telah diubahsuai dengan monomer asid akrilik untuk menghasilkan membran kromatografi. Dua kaedah pengubahsuaian telah dibandingkan iaitu pencantuman ultraungu dan pencantuman kimia melalui tindak balas redoks. Beberapa parameter pengubahsuaian telah dikaji melalui kaedah satu faktor pada satu masa (OFAT) iaitu kepekatan pemula (1-50 mM), kepekatan monomer (0.2-5 M) dan masa cantuman (5-60 minit). Teknik pencantuman terbaik yang memberikan kapasiti lekatan protein yang paling tinggi dicapai oleh kaedah pencantuman ultraungu. Kapasiti lekatan lysozyme tertinggi (LZY) dicapai adalah 0.175 mg LZY / cm^2 membran untuk membran yang disediakan melalui pencantuman ultraungu menggunakan 10 mM benzophenone (BP), 0.1 M asid akrilik (AA) dan 15 minit masa tindak balas . Kondisi yang terbaik dalam pencantuman ultraungu yang diperoleh daripada kajian OFAT telah digunakan dalam kaedah sambutan permukaan (RSM) untuk pengoptimuman jumlah protein yang melekat. Berdasarkan analisis varians (ANOVA), model kuadratik telah dipilih dengan nilai kuasa dua *R* adalah 0.9468. Nilai optimum bagi setiap parameter yang dikaji dalam RSM adalah 20.71 mM BP, 0.29 M AA dan 18.19 minit masa cantuman. Membran kromatografi optimum telah diuji dan dicirikan dari segi darjah pencantuman, pengukuran sudut sentuh, mikroskop imbasan elektron (SEM), spektroskopi inframerah transformasi fourier (FTIR) dan fluks air tulen Kapasiti lekatan protein pada keadaan optimum adalah 0.180 mg LZY / cm^2. Nilai ini adalah kurang daripada 4% daripada nilai yang diramalkan oleh model. Model kuadratik ini boleh digunakan dengan jayanya untuk menghasilkan kromatografi membran daripada membran PA melalui pencantuman ultraungu untuk lekatan LZY.

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LIST OF SYMBOLS

%	Percent
~	Approximately
>	More than
µm	Micrometer
A	Monomer concentration
A	Area
B	Exposure time
C	Initiator concentration
C_f	Final concentration
C_o	Initial concentration
DG	Degree of grafting
F	Fisher test
J	Water flux
M	Molar ratio
p	Probability
R	Chemical group
R^2	Coefficients
t	Time
V	Volume
W_g	Mass of grafted membrane
W_o	Mass of original membrane
wt	Weight
x	Variables
y	Response

α	Alpha
γ	Gamma
λ	Wavelength

LIST OF ABBREVIATIONS

AA	Acrylic acid
AAG	2-acrylamidoglycolic acid
AETMA	[2-(acryloyloxy) ethyl] trimethyl ammonium chloride
AMPS	2-acrylamido-2-methyl-1-propanesulfonic acid
ANOVA	Analysis of variance
Arg	Arginine
BP	Benzophenone
BSA	Bovine serum albumin
CCD	Central Composite Design
CHA	Coralline hydroxyapatite
DAMP	1, 5- diamino-2-methylpentane
DMAA	Dimethyl acrylamide
DMAEMA	N,N-Dimethylaminoethyl Methacrylate
DNA	Deoxyribonucleic acid
EDA	Ethylenediamine
EVAL	Ethylene vinyl alcohol
FT-IR	Fourier Transform spectroscopy
GMA	Glycidyl methacrylate
HAP	Hydroxyapatite
HEMA	2-hydroxyethyl methacrylate
HIC	Hydrophobic interaction chromatography
His	Histidine
IgA	Immunoglobulin A

IgG	Immunoglobulin G
IgM	Immunoglobulin M
ITs	Immunotoxins
Lsy	Lysine
LZY	Lysozyme
MA	Methacrylic acid
mAb	Monoclonal antibody
MBAA	Methylene bisacrylamide
MF	Microfiltration
mM	Milimolar
MMM	Mixed matrix membrane
MOETMA	[2-(methacryloyloxy)ethyl]- trimethylammonium
MPC	2-methacryloyloxyethyl phosphorycholine
MWCNT	Multiwalled carbon nanotubes
n/a	Not available
NF	Nanofiltration
nm	Nano meter
NSBC	N-O-sulfonic acid benzyl chitosan
NVP	N-2-vinyl pyrolidinone
OFAT	One factor at a time
PA	Polyamide
PAES	Arylene ether sulfone
PEGMA	Poly(ethylene glycol) methyl ether methacrylate
PEK-C	Polyether ketone
PES	Polyethersulfone

PET	Polyethylene terephthalate
Phe	Phenylalanine
pI	Isoelectric point
PMAA	Polymethacrylate acid
PNIPAM	Poly(N-isopropylacrylamide)
PSA	Pressure-sensitive adhesive
PVA	Poly vinyl alcohol
PVDF	Polyvinylidene fluoride
RNA	Ribonucleic acid
RSM	Response Surface methodology
SEC	Size exclusion chromatography
SEM	Scanning Electron Microscope
S-PAES	Sulfonated poly(arylene ether sulfone)
SPAESSS	Sulfonated poly (arylene ether sulfide sulfoxide sulfone)
SPB	Sodium perborate
SPM	3-sulfopropyl methacrylate
SPS	Sulfonated-polysulfone
TEOS	Tetraethyl orthosilicate
TFC	Thin film composite
Trp	Tryptophan
UF	Ultrafiltration
UV	Ultra Violet
VSA	Vinyl sulfonic acid

CHAPTER 1

INTRODUCTION

1.1 RESEARCH BACKGROUND

Increasing growth in biotechnology industries such as pharmaceutical and food industries requires reliable and efficient methods to purify the commercial-scale quantities of proteins (Przybycien et al., 2004). Various type of separation can be used to capture the desirable protein of interest. The most common technique is by using packed bed chromatography. In packed bed chromatography, resin bead with specific functionality based on different charged group, hydrophobicity or affinity, was packed in cylindrical column. The chromatography media inside the column will interact specifically with the protein during the feed flow through the column. However, the conventional packed bed chromatography shows several weakness such as high pressure drop, limited diffusional of solute to the binding site and low flow rate capability (Ghosh, 2002; Klein, 2000; Zeng and Ruckenstein, 1999).

Membrane chromatography can be used to overcome the limitation of the conventional packed bed chromatography. Membrane chromatography consists of an adsorptive membrane that carried similar chromatography functionality as the resin bead. The pressure drop across the membrane chromatography is very low since it only used very thin membrane as the chromatography media. Due to this pressure drop, it is possible to operate the membrane chromatography system using high flowrate. The binding of the protein in membrane chromatography is through the convective flow which is very fast and can prevent the protein from denaturation (Borneman et al., 2010; Zhong et al., 2011; Guo and Ruckenstein, 2003).

Membrane chromatography is functioned similar to the normal chromatography resin bead which is depend on the type of functionality attach to the membrane. The production of chromatographic membranes involve a chemical modification of the microfiltration membrane with specific type of functional groups to produce different types of chromatography interactions inside the membrane (Saufi and Fee, 2009). Different types of interaction are also possible for membrane chromatography such as anion exchange, cation exchange, hydrophobic interaction and affinity interaction. Three steps are usually involved in the preparation of adsorptive membranes chromatography: (1) preparation of the base membrane, (2) chemical activation of the base membrane and (3) coupling of ligands to the activated membrane (Zeng and Ruckenstein, 1999).

Common method to activate the polymer base material was through grafting technique, which introducing various type of functional group on the membrane surface. Grafting was a promising method to modify polymers by attaching the specific monomer onto the polymer chain by covalent bond. Several type of grafing technique has been discovered such as UV grafting, chemical grafting, photochemical grafting, plasma radiation induced grafting and enzymatic grafting. UV grafting is one of the common methods used for modification of ultrafiltration (UF) membrane for fouling control. UV grafting is simple, useful and versatile in improving the surface properties of the polymers (Deng et al., 2009). The membrane was exposed to the UV light at certain wavelength and intensity in specified time of radiation. The monomer is then grown continuously on the radical groups generated during the UV grafting process.

Commercial polyethersulfone (PES) UF membrane was grafted by Taniguchi and Belfort (2004) at 300 nm wavelength with various types of monomer such as N-2-vinyl pyrolidinone (NVP), 2-hydroxyethyl methacrylate (HEMA), acrylic acid (AA), 2-acrylamidoglycolic acid (AAG), 3-sulfopropyl methacrylate (SPM) and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) in order to produce membrane that has low protein fouling. Abuhabib et al. (2012) combined two monomers, which are acrylic acid and ethylenediamine dihydrochloride during grafting to improve the fouling resistance of PES nanofiltration (NF) membrane for brackish water desalination. Mansourpanah and Momeni Habili (2013) studied the effect of UV-irradiation time and AA

concentration on the performance and morphology of modified polyamide thin film composite (TFC) membranes.

Another approach for monomer grafting is through chemical grafting via redox reaction. The concept is quite similar to UV photo-grafting which produced free radical on the membrane surface for monomer attachment, but it is assists by redox initiator pair. Different redox initial pairs were used during grafting such as potassium persulfate ($K_2S_2O_8$)– potassium thiosulfate ($K_2S_2O_3$) (Van der Bruggen, 2009), $K_2S_2O_8$– sodium sulfite (Na_2SO_3) (Wang et al., 2007), $K_2S_2O_8$–$Na_2S_2O_5$ (Belfer et al., 1998a) and potassium methabisulfite ($K_2S_2O_5$)–$Na_2S_2O_8$ (Bernstein et al., 2010).

Much of the membrane modification in the previous works, mainly aim for producing fouling resistant membrane by introducing new functionality of the membrane. This new functional group can also be a charged group similar to the charge group required for the binding in the chromatography process. Therefore, in the current study, membrane with negatively charged functional group was produced for binding to the protein through ionic interaction for preparing membrane chromatography. In the first part of the study, polyamide membrane was grafted with acrylic acid monomer using two modification approaches which are chemical grafting using redox initiator and UV photo-grafting. The effects of modification parameters on the protein binding capacity were studied such as initiator concentration, monomer concentrations and time of reaction. The best conditions obtained from the first part of study were used for optimization using Response Surface Methodology (RSM) for the membrane chromatography prepared from UV photo grafting modification.

1.2 PROBLEM STATEMENT

The overall cost of bioproduct depends strongly on the efficiency of the downstream processing for protein separation and purification. The requirement of effective strategy and high quality product demands the improvement of the conventional chromatography system. The technical limitation by packed bed chromatography could be resolved using membrane chromatography especially regarding to the mass transfer since the convection with membrane is faster than diffusion through the resin bead. Thus, the time consumption of the overall purification process will be shorten and the operational cost will be reduced.

Membrane chromatography of various interactions such as affinity, anion/cation exchange and hydrophobic interaction was actively being produced for protein separation. In the previous study, different types of grafting were applied to functionalize the UF membrane for producing fouling resistant membrane. UF membrane based on ionic charged group is capable to minimize the membrane fouling by repelling the accumulation of the charged pollutant onto the membrane surface. Due to the successful used of grafting technique on modification of these UF membrane, it has brought the great interest to the researcher to modify polymeric membrane becoming membrane chromatography for protein separation.

Although both UF and membrane chromatography system used the charged membrane, but the chromatography system operated differently with the UF filtration. In chromatography system, it is targeted to bind as much as protein onto the membrane during the binding step and this protein can be recovered later in elution step. Therefore it is very critical to investigate on how the preparation of charged membrane for used in membrane chromatography system effects the performance of the membrane for protein binding. This study was aim to compare the performance of membrane chromatography prepared using UV photo grafting and chemical grafting via redox initiation as these two grafting methods were used extensively in preparing charged UF membrane.

Various grafting parameters such as initiator type and concentration, monomer type and concentration and grafting time affect the performance of the membrane chromatography. It is crucial to study the effects of these parameters toward the performance of the membrane chromatography system. In addition optimization of process parameters is important in order to produce high performance membrane chromatography for protein binding. One Factor at Time (OFAT) and Response Surface Methodology (RSM) were used during the optimization step of the UV photo grafting technique in preparing membrane chromatographic membrane. The application of RSM can determine the optimal condition for the process and understand the interaction between the parameters involved.

1.3 OBJECTIVE OF THE RESEARCH

The objectives of this study are:

i) To fabricate negatively charged membrane chromatography from the commercial polyamide (PA) microfiltration (MF) using acrylic acid monomer.
ii) To compare the preparation of the membrane chromatography using UV photo grafting and chemical grafting
iii) To study the effect of grafting parameters which are initiators concentration, acrylic acid monomer concentration and grafting time.
iv) To optimize the surface grafted PA membrane by Response Surface Methodology (RSM).

1.4 SCOPES OF THE RESEARCH

In order to achieve the research objective, the following scopes of study have been outlined:

i. The protein binding capacity of cation exchanger membrane chromatography prepared using two modification methods which are UV photo grafting and chemical grafting via redox reaction was compared.
ii. The effect of grafting parameters which are initiators concentration (0.5-50 mM), acrylic acid monomer concentration (0.2-5 M) and reaction time (3-60 minutes) on the performance of the membrane chromatography was investigated.
iii. The preparation of membrane chromatography via UV photo grafting was optimized using Response Surface Methodology approach.
iv. The performance of membrane chromatography was evaluated using lysozyme (LZY) binding capacity.
v. The modified membrane was optimized using Response Surface Methodology (RSM).
vi. The membrane was characterized in term of degree of grafting, contact angle measurement, Scanning Electron Microscope (SEM), Fourier Transform Infra-Red (FTIR) and pure water flux test.

1.5 THESIS OVERVIEW

This thesis consists of 5 chapters. A brief overview of each chapter is described in the following paragraph.

Chapter 1 presents the background of the research and followed by the research objective and the scopes of the research. In Chapter 2, the literature review related to the protein separation, bioseparation technique (precipitation and centrifugation, membrane filtration, chromatography and membrane chromatography), preparation of membrane chromatography by chemical grafting, UV photo grafting, mixed matrix membrane chromatography and blending were present.

Chapter 3 outlined the material, apparatus and methods used in this study. The experiments were start with screening of modification route, which consist of chemical and UV photo grafting. In the second part, UV photo grafting was optimized by Response Surface Methodology (RSM) involving Central Composite Design (CCD).

The performance and characteristic of modified membrane chromatography was presented in Chapter 4. The discussions of the OFAT, optimization and membrane characterization were explained, compared and supported with the relevant justification and assumption.

Finally, conclusion and recommendations for the future works are outlined in the Chapter 5. The conclusion was made based on the research finding and the recommendations were suggested to improve the research in the future work.

CHAPTER 2

LITERATURE REVIEW

2.1 PROTEIN SEPARATION

The integration of biotechnology and chemical engineering in bioindustries can give a significant benefit in developing new products that can satisfy the human needs and demands. However the separation process has become a critical step in order to produce acceptable purified biological active compound. Reliable and efficient separation technique is required to purify commercial-scale quantities of proteins for use in pharmaceutical and food industries (Przybycien et al., 2004). Downstream processing is the term referred to the processing step beyond the bio reaction or also known as bioseparation. A series of separation processes involve in bioseparation in order to isolate a single type of protein from a complex mixture. The purification process needs at least two or more stages to achieve the final purity required for the particular application (Asenjo, 1990).

Several reasons for protein purification is due to the requirement of high purity; concentration enrichment; removal of specific impurities (e.g. toxins from therapeutic products); prevention of catalysis other than the type desired; prevention of catalysis poisoning; recommended product specifications; enhancement protein stability and reduction of protein denaturation (Saxena et al., 2009). Another important reason to separate protein is in food allergen determination as 80% of allergic reactions in children are due to milk, peanut and egg (Taylor et al., 2002). As an example lysozyme is one of major allergens found in egg white (Holen and Elsayed, 1990). Therefore, there is an urgent need for the development and approaches for the removal and prevention of protein allergy in food products (Mine, 2008). Protein separation is also

vital for the characterization of the function, structure and interactions of the protein of interest.

The methods and technique choose in bioseperation is depends on the purpose for which the protein is needed. For example, crude proteins extract or enzymes are normally required in most households detergents, disheswasher or agricultural products insect and pest repellent. However, if the protein is aimed for therapeutic or food application, it must be extremely pure. Hence, the purification must then be done in several subsequent steps. Pharmaceutical product normally require very high purity (>95%) products and therefore, are the most challenging task in bioseparation (Forciniti, 2008).

2.2 BIOSEPARATION TECHNIQUE

Proteins are macromolecules consisting of one or more polypeptides chain. Each polypeptide consists of a chain of amino acids linked together by peptide (amide) bond as shown in Figure 2.1. These amino acids contain various chemical groups attached to it side chain (represent by R in Figure 2.1). This functional side group can be positive, negative or neutral charged, hydrophobic or hydrophilic characteristic. Protein carry both positively and negatively charged groups and are called “amphoteric”. The charge carried is dependent upon the pH, with the pH value at which have zero net charge termed the *isoelectric point,* (pI). At a pH below the pI the net charge of the protein is positive, and the protein will bind to a cation exchanger. Meanwhile, at a pH above the pI the net charge of the protein is negative and the protein will bind to an anion exchanger (Wheelwright, 1993). Any influence on polypeptides chain such as certain chemical and heat may disrupt the native conformation of the protein and lead to the denaturation of the protein. Protein denaturation will results in loss of protein functional activity (Walsh, 2002).