PREPARATION AND CHARACTERIZATION OF A HIGHLY SPECIFIC AFFINITY ULTRAFILTRATION MEMBRANE FOR TRYPSIN SEPARATION

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DOCTOR OF PHILOSOPHY
UNIVERSITI MALAYSIA TERENGGANU
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SOFIAH HAMZAH

Thesis Submitted in Fulfillment of the Requirement for the Doctor of Philosophy in the Faculty of Science and Technology Universiti Malaysia Terengganu

November 2012

This thesis is dedicated to

My husband (Mohd Shukri Samsuddin)

My Parents (Hamzah Abdullah and Rokiah Abdullah)

My Son (Muhammad Amirul Irsyad & Muhammad Amir Irfan)

My family

And

All those noble and sublime personalities whose serenity, courage and wisdom led me to
the Path of Guidance

Abstract of this thesis presented to the Senate of Universiti Malaysia Terengganu in fulfillment of the requirement for the Doctor of Philosophy

PREPARATION AND CHARACTERIZATION OF A HIGHLY SPECIFIC AFFINITY ULTRAFILTRATION MEMBRANE FOR TRYPSIN SEPARATION

SOFIAH BINTI HAMZAH

November 2012

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This study aimed to develop a highly specific affinity ultrafiltration membrane for trypsin separation. The research has been divided into three major stages; (1) Fabrication of basic membrane for affinity support, (2) Membrane surface modification using chitosan and (3) Further modification of the Chitosan/PSf membrane for affinity purpose. For the first stage, three types of ultrafiltration membrane with different polymer concentration (15 wt. % [PSf 15], 17 wt. % [PSf 17] and 19 wt. % [PSf 19] polysulfone) were prepared via a simple dry/wet phase inversion technique. The fabricated membranes were characterized in term of permeability coefficient,membrane thickness and porosity, membrane morphology, molecular weight cut-off (MWCO) and membrane surface charge. The separation performances of these membranes were evaluated using single solution of trypsin enzyme. PSf 15 has been chosen for basic membrane for affinity matrix since all its characteristics fulfilled the requirement of the

development of affinity membrane. In the second stage of this study, PSf 15 has been modified using chitosan solution with different dip times (30, 60, 90 and 120 minutes) in order to improve the surface hydrophilicity. Membranes with a 60-min dip time provided an optimum trypsin transmission (about 91.8%). Such membranes have a high permeability coefficient (71 $L.m^{-2} h^{-1}$) and membrane porosity (about 89.6%). The hydrophilicity of this modified membrane was also improved by 50% compared with the native membrane, and its flux recovery was about 89.8%. The successful assembly of chitosan onto the membrane's surface was ascertained by ATR-FTIR and X-ray diffractometry (XRD). Its morphology showed significant different from that of native membrane and this hybrid membrane can also be a good matrix for affinity membrane. Further modification of chitosan/PSf membrane was performed in the final stage of research. Choice of chemistry in coupling ligands to support materials were investigated using different amount of activator (glutaraldehyde) and ligand concentration. In order to determine the optimum condition for maximum adsorption capacity of trypsin, adsorption studies were performed at different pH (5, 7, 8,10, 12) , ionic strength (0.01, 0.05, 0.1, 0.3 and 0.5M) and initial trypsin concentration (0.1, 0.3, 0.5, 0.7 and 0.9 mg/ml). Affinity ultrafiltration experiment was operated using the best developed affinity membrane (membrane activated with 3:7 ratio of GTA:buffer and immobilized with 0.7 mg/ml ligand) and optimum condition of feed solution (0.9 mg/ml trypsin in 0.1 M phosphate buffer, pH 7). Optimum adsorption obtained using 0.9 mg/ml initial trypsin solution (pH 7 and 0.1M ionic strength). While the best desorption of this enzyme (92% recovery) occurred when the elution process was performed using 0.01 M Tris-HCl buffer (pH 8) with the addition of 0.05M potassium chloride. Eventually, the

outcomes of this research are expected to be a stepping stone to design and optimize the

enzyme purification system which can be a great achievement in the development of

biotechnology field in the future.

PENYEDIAAN DAN PENCIRIAN MEMBRAN AFINITI PENURAS ULTRA SPESIFIK UNTUK PEMISAHAN TRIPSIN

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Kajian ini bertujuan untuk menghasilkan membran afiniti yang sangat khusus untuk pemisahan trypsin. Kajian ini yang telah dibahagikan kepada tiga peringkat utama; (1) Fabrikasi membran asas untuk membran afiniti , (2) membran modifikasi menggunakan kitosan dan (3) modifikasi lanjutan membran kitosan/ polisulfona untuk menghasilkan membran afiniti. Untuk peringkat pertama, tiga jenis membran ultraturasan dengan kepekatan polimer yang berbeza (15 wt.% [PSf 15], 17 wt% [PSf 17] dan 19 wt% [PSf 19] polysulfona) telah dihasilkan melalui kaedah fasa balikan kering/basah. Membran yang yang telah difabrikasi dicirikan dari segi pengukuran kebolehtelapan, morfologi membran, nilai sekatan jisim molekul (MWCO) dan cas permukaan membran. Prestasi ketelapan membran-membran ini telah dinilai dengan menggunakan larutan enzim tripsin. PSf 15 telah dipilih sebagai membran asas untuk matrik afiniti kerana semua ciri-ciri membran ini menepati keperluan untuk menghasilkan membran afiniti. Dalam peringkat kedua kajian ini, PSf 15 telah diubah suai dengan menggunakan larutan

kitosan dengan masa rendaman yang berbeza (30, 60, 90 dan 120 minit) untuk meningkatkan sifat hidrofilik permukaan membran. Membran dengan masa 60 minit berendam menghasilkan transmisi tripsin yang optimum (kira-kira 91.8%). Membran tersebut mempunyai pekali kebolehtelapan (71 LM^{-2} h^{-1}) dan keliangan (kira-kira 89.6%) yang tinggi. Sifat hidrofilik membran yang dimodifikasi ini juga meningkat sebanyak 50% berbanding dengan membran asalnya, dan pemulihan fluks adalah kira-kira 89.8%. Kewujudan kitosan ke permukaan membran telah ditentukan oleh ATR-FTIR dan teknik pembelauan sinar-X (XRD). Morfologi membran ini menunjukkan perbezaan ketara daripada membran asalnya dan membran hibrid ini juga boleh menjadi matriks baik untuk membran afiniti. Lanjutan modifikasi membran kitosan /PSf telah dilakukan di peringkat akhir penyelidikan untuk menghasilkan membran afiniti. Pemilihan secara kimia dalam menggandingkan ligan dan membran sokongan telah dikaji dengan menggunakan jumlah pengaktif yang berbeza (glutaraldehid), dan kepekatan ligan yang berbeza. Untuk menentukan keadaan optimum bagi kapasiti maksimum penjerapan tripsin, kajian penjerapan telah dijalankan pada pH yang berlainan (5, 7, 8,10, 12), kekuatan ionik (0.01, 0.05, 0.1, 0.3 dan 0.5M) dan kepekatan awal trypsin (0.1, 0.3, 0.5, 0.7 dan 0.9 mg / ml). Eksperimen affiniti ultraturasan telah dikendalikan menggunakan membran afiniti terbaik (membran yang diaktifkan dengan nisbah 03:07 GTA: larutan dan kepekatan ligan 0.7 mg / ml) dan kondisi larutan tripsin yang optimum (0.9 mg/ml tripsin; dilarutkan di dalam 0.1 M larutan fosfat, pH 7). Optimum penjerapan diperolehi dengan menggunakan 0.9 mg / ml larutan awal tripsin (pH 7 dan kekuatan ionik 0.1M). Manakala yang penyahjerapan terbaik enzim ini (92% pemulihan) berlaku apabila proses elusi telah dilakukan dengan menggunakan

larutan mampan 0.01 M Tris-HCl (pH 8) dengan penambahan kalium klorida 0.05M. Hasil daripada kajian ini diharapkan mampu untuk menjadi batu loncatan untuk merekabentuk dan mengoptima sistem penulinan protein yang boleh menjadi pencapaian yang baik untuk membangunkan bidang bioteknologi pada masa hadapan.