UNIVERSITI PUTRA MALAYSIA

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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May 2013

Chairman: Prof. Arbakariya Ariff, PhD

Faculty: Institute of Bioscience

Epidermal growth factor (EGF) is a single chain polypeptide that exhibit generous bioactivities effect on proliferation of cells in cosmetics and pharmaceutical industries. It is well known that biopharmaceutical production cost relies very much on purification steps. Due to therapeutic potential and high market value of EGF, development of an efficient and cost-effective production process is required. Integrative chromatographic technique, Expanded Bed Adsorption (EBA), may be used to solve the separation problems related to the effect of particulates in the recovery process. Simple and rapid quantification method of the target protein needs to be developed for process monitoring and control during recovery and purification processes.

Performance of EGF recovery and purification with the presence of two different cell types, *Escherichia coli (E. coli)* homogenate (treated with osmotic shock) and *Pichia*

pastoris (P. pastoris) intact cells, were demonstrated using EBA. Optimum adsorption and elution experiments were performed in a batch mode. Biomass-adsorbent interaction and stability of bed formation were evaluated using Cell Transmission Index (CTI) and zeta potential. In order to monitor the purification performances, surface plasmon resonance (SPR) was used for the quantification of EGF during recovery and purification processes. An analysis method using SPR was developed by assessing the best choice of suitable biomolecular recognition for the quantification of EGF. Two types of EGF antibody, monoclonal (mAb) and polyclonal (pAb), were immobilized on the surface of chip and validated for its characteristics including specificity, range, intermediate precision and repeatability, recovery, dilution paradox and stability. Kinetics and affinity constants of antibodies-EGF binding were also evaluated using 1:1 Langmuir interaction model (global fitting).

Polyclonal antibody (pAb) is more suitable to be used as a stable ligand to quantify EGF continuously from the consideration of kinetics, binding rate and shelf life assessment. pAb has a better affinity (K_D = 7.39 x 10^{-9} M) than monoclonal antibody (mAb) (K_D = 9.54 x 10^{-10} M). From the kinetics evaluation, it was found that pAb has a faster reaction rate during sample injection, slower dissociation rate during buffer injection and higher level of saturation state than monoclonal antibody (mAb). Besides, pAb has a longer shelf life and greater cycle can be run. For purification of EGF, maximum binding of target protein can be achieved at pH 4.5 with less biomass adhesion. Recovery of EGF from *E. coli* homogenate gave higher grade purification as compared to *P. pastoris* intact cells using the preferred elution agents. A purification factor of 2.5 was achieved after increasing the elution

efficiency using lower ionic strength of elute. Since 100% Cell Transmission Index (CTI) was achieved, interaction of biomass-adsorbent could be considered as not an obstacle. Repulsion between negative mixed mode adsorbent with negatively charge surface of biomass at particular condition is expected due to electrostatic charge interaction.

Results from this study have demonstrated that EBA and SPR can be used as an attractive alternative for efficient and cost-productive procedure in the purification of EGF from fermentation broth with less biomass adhesion. With this approach, high overall product yield with reduced process time and cost are expected in the large scale industrial process for direct recovery and purification of recombinant protein from fermentation broths.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PEMULIHAN DAN PENULENAN FAKTOR PERTUMBUHAN EPIDERMAL DARIPADA MEDIA PENAPAIAN MENGGUNAKAN SISTEM LAPISAN TERKEMBANG

Oleh

IRA AMIRA ROSTI

Mei 2013

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Fakulti: Institut Biosains

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Faktor pertumbuhan epidermal adalah satu rantai polipeptida yang mempamerkan kesan bioaktiviti pada proliferasi sel-sel dalam industri kosmetik dan farmaseutikal. Memang diketahui bahawa kos pengeluaran biofarmaseutikal sangat bergantung kepada langkah-langkah penulenan. Disebabkan potensi terapeutik dan nilai pasarannya yang tinggi, pembentukan proses pengeluaran yang cekap dan kos efektif diperlukan. Teknik kromatografi integratif, sistem lapisan terkembang boleh digunakan untuk menyelesaikan masalah pemisahan yang berkaitan dengan kesan zarah dalam proses pemulihan. Kaedah kuantifikasi protein yang ringkas dan cepat perlu dibinakan untuk proses pemantauan dan kawalan semasa proses pemulihan dan penulenan.

Prestasi pemulihan dan penulenan EGF dengan kehadiran dua jenis sel; homogenat *Escherichia coli (E. coli)* (yang dirawat dengan kejutan osmosis) dan sel utuh *Pichia*

pastoris (P. pastoris) telah dinyatakan dengan menggunakan EBA. Eksperimen jerapan dan elusi optimum telah dijalankan dalam mod kelompok. Interaksi biojisim-penjerap dan kestabilan pembentukan lapisan telah dinilai menggunakan sel penghantaran index dan potensi zeta. Dalam usaha untuk memantau prestasi penulenan, SPR telah digunakan untuk mengkuantitikan jumlah EGF semasa proses pemulihan dan penulenan. Satu kaedah analisis menggunakan SPR telah dibangunkan dengan menilai pilihan biomolekul pengecaman terbaik yang bersesuai untuk pengkuantitian EGF. Dua jenis EGF antibodi; monoklon dan poliklon telah dipegunkan di atas permukaan cip dan disahkan untuk ciri-ciri termasuk kekhususan, julat, ketepatan perantaraan dan ulangan, perolehan, pencairan paradoks dan kestabilan. Kinetik dan pemalar afiniti ikatan antibodi-EGF telah dinilai menggunakan 1:1 Langmuir interaksi model (pemadanan global).

Antibodi poliklon (pAb) adalah lebih sesuai digunakan sebagai ligan yang stabil untuk pengkuantitian EGF berterusan daripada pertimbangan kinetik, kadar ikatan dan penilaian jangka hayat. pAb mempunyai afiniti yang lebih baik (K_D = 7.39 x 10^{-9} M) daripada antibodi monoklon (mAb) (K_D = 9.54 x 10^{-10} M). Lanjutan penilaian pemalar kinetik menunjukkan bahawa pAb mempunyai kadar tindak balas yang lebih cepat semasa suntikan sampel, kadar ceraian yang lebih perlahan semasa suntikan penimbal dan peringkat ketepuan yang lebih tinggi daripada antibodi monoklon (mAb). Selain itu, pAb mempunyai jangka hayat yang lebih panjang dan lebih banyak kitaran boleh dijalankan. Untuk penulenan EGF, ikatan maksimum protein sasaran boleh dicapai pada pH 4.5 dengan kurang lekatan biojisim. Pemulihan EGF daripada homogenat *E. coli* memberi gred penulenan yang lebih tinggi berbanding dengan sel utuh *P. pastoris* dengan menggunakan ejen elusi terbaik. Faktor

penulenan 2.5 telah dicapai selepas kecekapan elusi ditingkatkan dengan menggunakan elusi yang mempunyai kekuatan ionik yang lebih rendah. Interaksi biomass-penjerap tidak menjadi halangan sementelah 100% sel penghantaran indeks telah berjaya dicapai. Penolakan antara bahan penjerap mod campuran yang negatif dengan permukaan caj negatif biojisim pada keadaan tertentu adalah dijangka disebabkan oleh interaksi cas elektrostatik.

Keputusan daripada kajian ini telah menunjukkan bahawa penggunaan kaedah pendekatan ekonomi EBA dan SPR boleh digunakan sebagai alternatif yang menarik untuk mempunyai prosedur yang cekap dan produktif dari segi kos dalam proses penulenan dengan kurang lekatan biojisim. Dengan pendekatan ini, keseluruhan hasil produk dapat dipertingkatkan dengan mengambil masa yang kurang dan kos yang lebih rendah adalah dijangka dalam pengeluaran skala perindustrian yang besar untuk pemulihan secara langsung dan penulenan protein rekombinan daripada media penapaian.

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I certify that a Thesis Examination Committee has met on 14 May 2013 to conduct the final examination of Ira Amira Rosti on her thesis entitled "Recovery and Purification of Epidermal Growth Factor from Fermentation Broth using Expanded Bed Chromatography" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

IRA AMIRA ROSTI

Date: 14 May 2013

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