UNIVERSITI PUTRA MALAYSIA

SIMPLE SURFACE PLASMON RESONANCE BIO-SENSOR FOR THE QUANTIFICATION OF RECOMBINANT HUMAN EPIDERMAL GROWTH FACTOR (rhEGF) IN EXPANDED BED RECOVERY

FADZLIE WONG FAIZAL WONG

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By

FADZLIE WONG FAIZAL WONG

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

September 2010

With the name of ALLAH The Almighty,

For the sake of learning and knowledge……..

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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FADZLIE WONG BIN FAIZAL WONG

September 2010

Chairman: Associate Professor Ling Tau Chuan, PhD

Institute: Faculty of Engineering

The conventional quantification assays used to monitor the performance of protein downstream processing are typically not user friendly as it is laborious, time consuming and costly. At the same time, the emergence of multi-modal adsorbent, Streamline Direct HST (1.8 g/cm^3) to handle high ionic strength feedstock can potentially improve the recovery of protein in expanded bed adsorption (EBA). Hence, the objectives of the study are to develop a reliable quantification assay for recombinant human epidermal growth factor (rhEGF) by Surface Plasmon Resonance and to examine its application for the monitoring of rhEGF recovery by HST adsorbent in EBA. It was developed based on the BIAcore 3000 instrument with anti-rhEGF antibody immobilized on the CM5 chip. The performances of the assay were: assay linearity (25 to 250 ng/mL), accuracy (within 10% recovery of target), precision (below 3.4% CV), intra- and inter-assay precision (less than 20% CV) and quantification limit (8.0 ng/mL). In EBA study, the optimum binding condition of rhEGF onto Streamline HST was at pH 4 in acetate buffer without the NaCl or at high (1.8 M) NaCl concentration as determined from the batch binding experiment. The

electrostatic interaction, hydrophobic interaction and the hydrogen bonds are responsible for this. As for the elution, potassium buffer with pH 12 was used exploiting the effect of electrostatic repulsion. The effect of cells (*Escherichia coli* and *Pastoris pastoris*) on the performance of HST adsorbent in capturing rhEGF was also being examined by fitting the adsorption into the Langmuir isotherm. The thermodynamic parameters: maximum binding capacity (q_m) and equilibrium dissociation constant (K_d) are little affected as the concentration of these biomass increased from 0 to 4.73 % w/v. The result showed that the adsorption level was not a strong function of the biomass concentration. Next, the feasibility of using the HST adsorbent to capture rhEGF in EBA was finally tested using the single component sample (only rhEGF) with the presence of biomass (4.73 % w/v *E. coli* or *P. pastoris*). Fastline 10 column (Upfront, Denmark) was used to handle 20 mL of sample volume with adsorbent settled bed height of 2 cm (1.6 mL). The results demonstrated that a consistent yield can be obtained even with the presence of cells (97.38% for control, 96.94% for *P. pastoris* and 96.43% for *E. coli*) and a stable bed can be achieved, suggesting that no interference of adsorption by biomass occurred. In conclusion, the major contributions of the study are the development of reliable SPR quantification assay and the EBA process template that is useful for rhEGF purification from real feedstock.

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

BIO-PENDERIA ALUNAN PLASMON PERMUKAAN MUDAH BAGI PENJUMLAHAN REKOMBINAN FAKTOR PERTUMBUHAN EPIDERMAL MANUSIA (rhEGF) DALAM PEROLEHAN LAPISAN TERKEMBANG

By

FADZLIE WONG BIN FAIZAL WONG

September 2010

Pengerusi: Profesor Madya Ling Tau Chuan, PhD

Institut: Fakulti Kejuruteraan

Cerakin pengukuran konvensional yang digunakan untuk memantau prestasi pemprosesan hiliran protein biasanya tidak mesra pengguna disebabkan ianya memerihkan, memakan masa dan mahal. Di samping itu, kemunculan penjerap multi-modal, *Streamline Direct* HST (1.8 g/cm^3) untuk menangani stok suapan berkekuatan ionik tinggi berpotensi untuk memajukan proses pemulihan protin di dalam *expanded bed adsorption* (EBA). Maka, objektif kajian ini adalah untuk membangunkan satu cerakin pengukuran bagi rekombinan faktor pertumbuhan epidermal (rhEGF) yang boleh dipercayai dan untuk diaplikasikan dalam pemantauan proses pemulihan rhEGF dengan penjerap HST di dalam EBA. Ia adalah berdasarkan instrumen BIAcore 3000 dengan antibodi anti-rhEGF yang disekatgerakkan pada cip CM5. Prestasi cerakin tersebut adalah: julat linear (25 to 250 ng/mL), ketepatan (dalam 10% perolehan target), kejituan (bawah 3.4% CV), kejituan intra- dan inter-assay (kurang daripada 20% CV) dan had penentuan (8.0 ng/mL). Dalam kajian EBA, keadaan pengikatan yang optimum untuk rhEGF pada penjerap HST adalah pada pH 4 dalam penimbal

asetat dengan tiada NaCl atau dengan kepekatan NaCl yang tinggi (1.8 M) seperti yang telah ditentukan oleh eksperimen pengikatan kelompok. Interaksi elektrostatik, hidrofobik dan ikatan hidrogen bertanggungjawab untuk ini. Bagi proses elusi, penimbal potassium dengan pH 12 digunakan dengan mengekspoitasi kesan penolakan elektrostatik. Kesan kehadiran sel (*Escherichia coli* and *Pichia pastoris*) ke atas prestasi penjerap HST dalam mengikat rhEGF juga diperiksa dengan menyesuaikan proses penjerapan dengan *Langmuir isotherm*. Parameter-parameter termodinamik: kapasiti pengikatan maksimum (q_m) dan pemalar penceraian keseimbangan (K_d) terjejas sedikit apabila kepekatan bagi sel-sel tersebut ditingkatkan daripada 0 hingga 4.73 % w/v. Keputusan tersebut menunjukkan bahawa tahap penjerapan tidak bergantung kepada kepekatan sel. Kemudian, kebolehan untuk menggunakan penjerap HST dalam mengikat rhEGF di dalam EBA akhirnya diuji dengan menggunakan sampel satu komponen (hanya rhEGF) dengan kehadiran sel (4.73 % w/v *E. coli* or *P. pastoris*). Turus Fastline 10 (Upfront, Denmark) digunakan untuk menangani 20 mL isipadu sampel dengan 2 cm (1.6 mL) ketinggian lapisan termendak. Keputusan tersebut menunjukkan bahawa hasil yang konsisten boleh diperolehi walaupun dengan kehadiran sel (97.38% bagi kawalan, 96.94% bagi *P. pastoris* dan 96.43% bagi *E. coli*) dan lapisan yang stabil boleh dicapai, menunjukkan bahawa tiada gangguan penjerapan oleh biomas yang berlaku. Kesimpulannya, sumbangan utama kajian ini adalah pembangunan cerakin pengukuran SPR yang boleh dipercayai dan templat proses EBA yang berguna untuk penulenan rhEGF daripada stok suapan sebenar.

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I certify that an Examination Committee has met on 2nd September 2010 to conduct the final examination of Fadzlie Wong Faizal Wong on his Master of Science thesis entitled "A simple surface plasmon resonance bio-sensor for the quantification of recombinant human epidermal growth factor (rhegf) in expanded bed recovery" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to 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 been previously, and is not currently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

FADZLIE WONG FAIZAL WONG

Date: 2 September 2010

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