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PURIFICATION OF HUMAN INTERFERON ALPHA-2b FROM *ESCHERICHIA COLI* USING AQUEOUS TWO-PHASE SYSTEMS

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PURIFICATION OF HUMAN INTERFERON ALPHA-2b FROM *ESCHERICHIA COLI* USING AQUEOUS TWO-PHASE SYSTEMS

By

LIN YU KIAT

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

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

PURIFICATION OF HUMAN INTERFERON ALPHA-2b FROM *ESCHERICHIA COLI* USING AQUEOUS TWO-PHASE SYSTEMS

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Chairman : Professor Arbakariya bin Ariff, PhD

Faculty : Institute of Bioscience

Interferon-alpha2b (IFN-α2b) has very high market value since it can be used in treating many diseases. Nevertheless, the development of an efficient and cost-effective purification process has been referred as the important part of IFN production since the biopharmaceutical product cost relies much on the purification process. Aqueous two-phase systems (ATPSs) require simply low investment cost and capable to integrate the numerous conventional downstream processes into a single step. Thus, this experimental project focused on the design of different ATPSs to reduce the steps in purification, thereby increasing the yield and reducing the cost and time of production.

Purification of intracellular human recombinant IFN-α2b from *Escherichia coli* (*E. coli)* using polyethylene glycol (PEG)/potassium phosphate ATPS was investigated. The influences of system parameters including PEG molecular weight, tie-line length (TLL), volume ratio (V_R), biomass loading, pH system and sodium chloride (NaCl) concentration (%w/w) were studied. The results showed that the optimum condition for purification of IFN-α2b was achieved at 4.0% (w/w) PEG-8000, 13.0% (w/w) potassium phosphate, 0.5% (w/w) NaCl, V_R of 0.2, pH 6.5 and 10% (w/w) crude load. A purification factor (P_{FT}) of 26.3 and 40.7% recovery yield were achieved in the optimized process.

Furthermore, an alcohol/salt-based ATPS for the purification of intracellular human recombinant IFN-α2b was studied. The influences of 9 biphasic systems comprising alcohol-based top phase (ethanol, 2-propanol and 1-propanol) and salt-based bottom phase (ammonium sulfate, potassium phosphate and sodium citrate) were investigated in IFN purification. The results showed that the optimum condition for purification of IFN-α2b was achieved in ATPS of 2-propanol 18% (w/w) with ammonium sulfate 22% (w/w) system in the presence of 1.0% (w/w) NaCl. The purified IFN-α2b recorded a P_{FT} of 16.24 with the yield of 74.64%.

The effectiveness of ATPS for the purification of IFN-α2b was proven in this study. These ATPS purification methods provided alternative methods for IFN-α2b purification. The P_{FT} result of PEG/ potassium phosphate ATPS is higher than the P_{FT} result recorded in alcohol/salts ATPS. Therefore, suggesting that the PEG/ potassium phosphate ATPS is the prominent method for IFN-α2b purification.

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

PENULENAN INTERFERON ALPHA-2b MANUSIA DARIPADA *ESCHERICHIA COLI* DENGAN SISTEM DUA-FASA AKUEUS

Oleh

LIN YU KIAT

December 2011

Pengerusi : Profesor Arbakariya bin Ariff, PhD

Fakulti : Institut Biosains

Interferon-alpha2b (IFN-α2b) mempunyai nilai pasaran sangat tinggi kerana ia boleh digunakan untuk mengubati banyak penyakit. Namun, pembangunan proses penulenan yang cekap dan kos rendah merupakan bahagian penting bagi pengeluaran produk IFN kerana kos pengeluaran produk biofarmaseutikal amat bergantung kepada proses penulenan. Penggunaan sistem dua-fasa akueus (SDFA) hanya memerlukan kos pelaburan yang rendah dan mampu mengintegrasikan pelbagai proses konvensional menjadi sebagai satu langkah. Maka, fokus projek ini ialah untuk mencipta pelbagai SDFA yang dapat mengurangkan langkah-langkah dalam penulenan, meningkatkan hasil dan mengurangkan kos.

Penulenan IFN-α2b intraseluler manusia rekombinan dari *E. coli* menguna SDFA polietilena glikol (PEG)-kalium fosfat telah dikaji. Pengaruhan parameter sistem termasuk berat molekul PEG, kepanjangan garis pakatan (KGP), nisbah isipadu, biojisim dimasuk, sistem pH dan konsentrasi natrium klorida (NaCl) (% b/b) telah diuji. Keputusan kajian menunjukkan bahawa keadaan optimum untuk penulenan IFN-α2b dicapai pada 4.0% (b/b) PEG-8000, 13.0% (b/b) fosfat kalium, 0.5% (b/b) NaCl, $0.2V_R$, dan 10% (b/b) biojisim dalam pH 6.5. Faktor penulenan (P_{FT}) sebanyak 26.3 dan 40.7% hasil pemulihan telah dicapai dalam keadaan optimum.

Selain itu, penulenan intraselular manusia rekombinan IFN-α2b oleh SDFA alkohol/garam telah dikaji. Penulenan IFN telah diuji dengan 9 sistem terdiri daripada fasa atas yang berasaskan alkohol (etanol, 2-propanol dan 1-propanol) dan fasa bawah yang berasaskan garam (amonium sulfat, fosfat kalium dan natrium sitrat). Keputusan kajian menunjukkan bahawa keadaan optimum untuk penulenan IFN-α2b dicapai dalam sistem SDFA yang terdiri daripada 18% (b/b) 2-propanol dengan 22% (b/b) amonium sulfat, dan 1.0% (b/b) NaCl. Keputusan P_{FT} sebanyak 16.24 dengan penghasilan setinggi 74.64% telah dicapai.

Keberkesanan SDFA untuk penulenan IFN-α2b telah terbukti dalam kajian ini. Kaedah-kaedah SDFA ini telah memberi kaedah alternatif penulenan IFN-α2b. Keputusan P_{FT} sistem SDFA PEG / kalium fosfat adalah lebih tinggi daripada keputusan yang dicatat dalam sistem SDFA alkohol/ garam. Maka, sistem SDFA PEG / kalium fosfat dipercayai cara yang menonjol untuk penulenan IFN-α2b dalam kajian ini.

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I certify that a Thesis Examination Committee has met on 16.12.2011 to conduct the final examination of Lin Yu Kiat on his thesis entitled "Purification of Human Interferon Alpha-2b from *Escherichia coli* using Aqueous Two-Phase Systems" 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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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 concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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LIN YU KIAT

Date:16 December 2011

TABLE OF CONTENTS