UNIVERSITI PUTRA MALAYSIA

HIGH THROUGHPUT OPTIMIZATION OF PROTEIN-A AFFINITY CHROMATOGRAPHY FOR CAPTURE OF HUMANIZED MONOCLONAL ANTIBODY

NORAZRINA PAKIMAN

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by

NORAZRINA PAKIMAN

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

July 2012

....THANK YOU ALLAH.....

"'for the love of my life, Asyraaf and Ammar"

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

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Chairman : Associate Professor Norhafizah Abdullah, PhD

Faculty : Faculty of Engineering

Monoclonal antibody (mAb) is a specific protein molecule that is widely used in biopharmaceutical industry for therapeutic and diagnostic application. The purification of the mAb products typically involved Protein-A affinity chromatography as the first capture step driven by the very specific binding of the mAb to Protein-A ligand. Optimization of the Protein-A affinity step is required to improve the performance and economics of the purification step, particularly to improve binding capacity, recovery of the product and to minimize formation of product aggregates. A typical approach for performing chromatography optimization in column operation is somehow time consuming and require significant amount of mAb sample. In this study, a high-throughput chromatography operation in 96-well filter plate was explored to perform the chromatography optimization. Conditions for binding and elution were optimized by

looking at different combination of pH and salt concentration for binding and different buffer type, concentration and pH for elution. Based on Design of Experiment (DOE) analysis, a 20 mM sodium phosphate buffer with addition of 180 mM sodium chloride at pH 7.4 was selected based on optimum binding. For elution, a 100 mM Glycine buffer at pH 3.2 was optimized in order to maximize recovery and minimizing formation of aggregates. Evaluation of binding capacity under static condition in the 96-well filter plate and dynamic binding in column operation were also performed. The maximum equilibrium capacity was determined at 59.6 mg IgG/ml resin while the 10% breakthrough capacity was observed at 53.3 mg IgG/ml resin and 46.4 mg IgG/ml resin at 150 cm/hr and 300 cm/hr flow rate respectively. The loading capacity was then calculated and a case study for purification of 100 L mAb sample was performed to evaluate the volumetric production rate at different flow rate. Based on the case study, flow rate of 300 cm/hr with 35 mg IgG/ml resin loading capacity was selected. Verification runs at the optimized conditions were performed in plate and column chromatography approach, and the performance of the purification was compared. Based on the results, the average recovery was achieved at 96.63 ±2.58% with an average aggregation index of 5.29 ±0.42. These results are very much comparable with the predicted data from DOE analysis. The impurities profile of the runs was also assessed where the results shows comparable profile for both product purified in column and plate chromatography operation.

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

PENGOPTIMUMAN PROSES KROMATOGRAFI AFINITI PROTIN-A BAGI PENULENAN PERTAMA ANTIBODI MONOKLONAL BERCIRI MANUSIA

Oleh

NORAZRINA PAKIMAN

Julai 2012

Pengerusi : Profesor Madya Norhafizah Abdullah, PhD

Fakulti : Fakulti Kejuruteraan

Antibodi monoklonal (mAb) adalah sejenis melekul protin yang spesifik dan digunakan secara meluas dalam industri biofarmasi untuk aplikasi perubatan dan diagnostik. Penulenan produk-produk mAb secara tipikalnya melibatkan proses kromatografi afiniti menggunakan Protin-A sebagai langkah penulenan pertama, disebabkan oleh ikatan yang sangat spesifik diantara Protin-A dan mAb molekul. Pengoptimuman proses kromatografi Protin-A perlu dijalankan untuk meningkatkan prestasi dan faktor ekonomi langkah ini, khususnya untuk meningkatkan kapasiti ikatan, perolehan semula produk dan meminimumkan pembentukan gumpalan produk. Kaedah yang selalunya dilakukan di dalam turus kromatografi bagi pengoptimuman proses adalah memakan masa dan memerlukan jumlah sampel mAb yang banyak. Dalam kajian ini, kaedah operasi kromatografi berpenghasilan tinggi menggunakan plat bertapis 96 lubang diteroka untuk menjalankan proses pengoptimumam kromatografi. Dengan mengkaji beberapa

kombinasi untuk pH dan kepekatan garam semasa proses ikatan serta jenis dan kepekatan larutan penimbal dan pH semasa pengeluaran produk, keadaan kromatografi ini dioptimumkan. Berdasarkan analisa eksperimen, larutan penimbal 20 mM natrium fosfat dengan penambahan 180 mM garam natrium klorida pada pH 7.4 dipilih sebagai keadaan optimum bagi proses ikatan. Untuk pengeluaran produk, larutan penimbal 100 mM Glisin pada pH 3.2 adalah paling optimum untuk memaksimumkan perolehan semula produk dan meminimumkan pembentukan gumpalan produk. Penilaian bagi kapasiti ikatan dibawah keadaan statik di dalam plat 96 lubang dan kapasiti dibawah keadaan dinamik di dalam turus juga dijalankan. Kapasiti maksimum diperolehi pada 59.6 mg IgG/ml resin, manakala kapasiti pada 10% pembolosan yang diperolehi adalah 53.3 mg IgG/ml resin pada kadar aliran 150 cm/sejam dan 46.4 mg IgG/ml resin pada kadar aliran 300 cm/sejam. Kapasiti muatan turus kemudian dikira dan kajian kes terhadap penulenan 100 L sampel mAb dijalankan untuk menilai kadar isipadu pengeluaran pada kadar aliran yang berbeza. Bedasarkan kajian kes, kadar aliran pada 300 cm/sejam dengan kapasiti muatan 35 mg IgG/ml resin adalah dipilih. Proses pengesahan pada keadaan optimum akhirnya dijalankan menggunakan operasi kromatografi plat dan turus, dan prestasi kedua kaedah ini dibandingkan. Berdasarkan keputusan, purata perolehan semula produk dicatatkan pada 96.63 ±2.58% dan purata indeks penggumpalan produk dicatatkan pada 5.29 ±0.42. Keputusan ini adalah hampir dibandingkan dengan keputusan yang diramalkan oleh analisa DOE semana pengoptimuman proses. Profil bendasing juga dikaji dan keputusan menunjukkan profil yang sama bagi operasi kromatografi di dalam turus dan plat.

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Not forgetting my fellow friends, colleagues and administrative staff of Inno Biologics, Faculty of Engineering and School of Graduate Studies, and all those who have been supporting me directly or indirectly in the completion of my Master Degree program, thank you for being part of this journey.

I certify that a Thesis Examination Committee has met on 10 July 2012 to conduct the final examination of Norazrina Pakiman on her thesis entitled "High Throughput Optimization of Protein-A Affinity Chromatography for Capture of Humanized Monoclonal Antibody" in accordance with the Universities and University College 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 the fulfillment 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 hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledge. I also declare that it has not been previously and is not concurrently submitted for any other degree at Universiti Putra Malaysia or other institution.

NORAZRINA PAKIMAN

Date: 10 July 2012

TABLE OF CONTENTS