

### **UNIVERSITI PUTRA MALAYSIA**

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

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



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

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C

....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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NORAZRINA PAKIMAN July 2012

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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 highthroughput 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 96well 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  $\pm 2.58\%$  with an average aggregation index of 5.29  $\pm 0.42$ . 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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#### 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

			Page
DEDI ABST ABST ACKI APPF DECI LIST LIST LIST LIST	ICATIO TRACT TRAK NOWL ROVAI LARAT OF TA OF FI OF AI	ON C LEDGEMENTS L FION ABLES GURES BBREVIATIONS PPENDICES	ii iii v vii viii x xiv xvi xvi xviii xxi
CHA	PTER		
1.	INTR	RODUCTION	
	1.1	Overall Review and Problem Statement	1
	1.2	Objectives of Study	3
	1.3	Overall Scope of Work	4
2.		CRATURE REVIEW	5
	2.1	2 1 1 Structure and Classes of Monoclonal Antibodies	5
		2.1.2 Genetically Engineered Monoclonal Antibodies	8
	22	Application of Monoclonal Antibody in Pharmaceutical	0
	2.2	Industry	10
	2.3	Platform Process for Purification of Monoclonal Antibody	13
	2.4	Introduction to Protein-A Affinity Chromatography	18
		2.4.1 History and Development of Protein-A Affinity	
		Chromatography	18
		2.4.2 Protein-A Ligand and Support Matrices	21
		2.4.3 Mechanism of Protein-A – Antibody Interaction	26
	2.5	Optimization of Protein-A Affinity Chromatography	28
		2.5.1 Protein-A Media Screening	29
		2.5.2 Optimization of Binding Step	33
		2.5.3 Optimization of Intermediate Wash Step	35
		2.5.4 Optimization of Elution Step	36
	2.6	Approaches to Chromatography Process Optimization	38
		2.6.1 High-throughput (HT) Chromatography in 96 well	
		Filter Plate	39
		2.6.2 Workflow of Chromatography Operation in 96 well	
		Filter Plate	42
		2.6.3 Design of Experiment (DOE) Approach	44

3.	MATERIALS AND METHODOLOGY			
	3.1	Materials	47	
		3.1.1 Chemicals	47	
		3.1.2 Humanized Monoclonal Antibody	47	
		3.1.3 Protein-A Affinity Resins	48	
		3.1.4 96-well Filter Plate	48	
		3.1.5 Statistical Software	48	
	3.2	Overall Scope of Work	49	
	3.3	Preliminary Work	50	
		3.3.1 Comparison of Protein-A Affinity Resins	50	
		3.3.2 Overview and Improvement of High Throughput		
		Chromatography Operation	54	
	3.4	Preparation for Overall Experiments	56	
		3.4.1 Design of Experiments for Binding and Elution		
		Condition Optimization	56	
		3.4.2 Binding and Elution Buffer Preparation	58	
		3.4.3 Antibody Sample Preparation	61	
		3.4.4 Filter Plate Preparation	62	
		3.4.5 Column Packing and Efficiency Test	62	
	3.5	High- Throughput Optimization of Binding Conditions	63	
	3.6	High- Throughput Optimization of Elution Conditions	65	
	3.7	Adsorption Isotherm Study	67	
	3.8	Dynamic Binding Capacity Study	70	
	3.9	Verification Run at Optimized Conditions	71	
	3.10	Analytical Testing	73	
		3.10.1 Antibody Concentration by Enzyme Linked		
		Immunosorbent Assay (ELISA)	73	
		3.10.2 Host Cell Protein Concentration by Enzyme		
		Linked Immunosorbent Assay (ELISA)	74	
		3.10.3 Protein Concentration by Absorbance Method	75	
		3.10.4 Aggregation Index	76	
		3.10.5 Sodium-dodecyl Sulfate Polyacrylamide Gel		
		Electrophoresis (SDS-PAGE)	77	
4.	RESU	ULT AND DISCUSSION		
	4.1	High- Throughput Optimization of Binding Conditions	79	
		4.1.1 Antibody Binding Analysis	81	
		4.1.2 Optimization Criteria and Ranges	86	
	4.2	High- Throughput Optimization of Elution Condition	88	
		4.2.1 Recovery Analysis	91	
		4.2.2 Aggregation Index Analysis	96	
		4.2.3 Optimization Criteria and Ranges	100	
	4.3	Adsorption Isotherm Study	102	
	4.4	Dynamic Binding Capacity Study	106	
		4.4.1 Breakthrough Capacity and Loading Capacity	106	
		Determination		

	4.5	<ul> <li>4.4.2 Case Study for Loading Flow Rate Optimization</li> <li>Verification Run at Optimized Conditions</li> <li>4.5.1 Purification Performance Data</li> <li>4.5.2 Purity and Host Cell Protein Content</li> </ul>	108 111 111 115
5.	CON	CLUSION AND RECOMMENDATION	
	5.1 5.2	Summary and Conclusion Recommendations for Future Work	120 122
REFI	ERENC	CES	124
APPI	ENDIC	ES DE STUDENT	129 144
LIST	OF PU	JBLICATIONS	144