



UNIVERSITI PUTRA MALAYSIA

***DEVELOPMENT OF DYE AFFINITY ADSORBENTS FOR RECOVERY OF
POLYCLONAL ANTI-HEPATITIS B CORE ANTIGEN IMMUNOGLOBULIN G***

RATTANA WONGCHUPHAN

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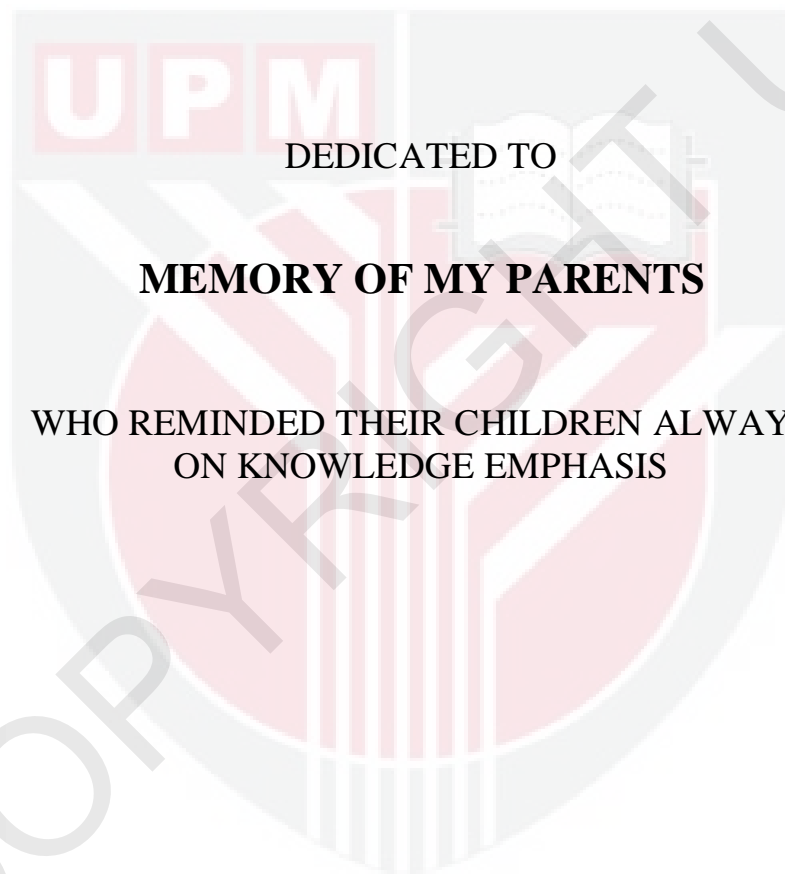
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By

RATTANA WONGCHUPHAN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

October 2010



DEDICATED TO

MEMORY OF MY PARENTS

WHO REMINDED THEIR CHILDREN ALWAYS
ON KNOWLEDGE EMPHASIS

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment
of the requirement for the degree of Doctor of Philosophy

**DEVELOPMENT OF DYE AFFINITY ADSORBENTS FOR RECOVERY OF
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October 2010

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Antibodies such as immunoglobulin G (IgG) have been used extensively for therapeutic and diagnostic purposes. Protein A affinity chromatography which is highly specific towards IgG is a standard method to purify it. However, using expensive and unstable protein A in large-scale production has increased the antibody production cost accordingly. Affinity dye-ligands which are widely used for protein purification has demonstrated their high binding capacity as 40 mg/mL comparable to protein A. Moreover, their widespread availability, ease and speed of preparation, chemical stability, and ease of storage, render them an attractive alternative choice. Especially, their economy is also a major consideration in replacement of expensive protein A. Thus, the development of selective recovery of polyclonal anti-hepatitis B core antigen immunoglobulin G (anti-HBcAg IgG) from rabbit sera has been investigated.

Four different reactive dye-ligands; Cibacron Blue 3GA (CB), Reactive Brown 10 (RB 10), Reactive Red 120 (RR 120) and Reactive Green 5 (RG 5) were covalently

attached on the Streamline quartz base matrix via triazine linkage under alkali condition. Essentially at start, IgG antibody's binding capacity screening of these immobilized dyes was required. Similar amount of dye-ligands attached on the bare matrix, determined by mass balance method was attributed relatively in comparison of adsorption capacities for different dye-ligands. From the simulating adsorption study in single protein system, the immobilized RG 5 was chosen as its capacity for fewer albumins and more IgG adsorbed at pH 7.0, compared to other immobilized dye-ligands possessing similar ligand density. The content of RG 5 immobilized on the matrix was 17.4 $\mu\text{mol/mL}$ adsorbent. About 64% of rabbit IgG was bound on the immobilized RG 5 at pH 7.0 in binary protein binding system with similar ratio of both albumin and rabbit IgG. The maximum adsorption capacity (q_m) of RG-5 immobilized adsorbent for rabbit IgG was 49.0 mg/mL adsorbent and the dissociation constant (K_d) value was found to be 3.33×10^{-6} M. The phenomenon of reversible IgG adsorption on the adsorbent appeared to follow the Langmuir-Freundlich isotherm model. Serum from the immunized rabbits against hepatitis B core antigen (HBcAg) was used as a feedstock containing polyclonal anti-HBcAg IgG solely for batch antibody purification study. Highly abundant albumin and other serum proteins which constitute about 80% of total serum protein are a major interference in dye-ligand affinity chromatographic studies. This leads to the strategy of removing contaminant proteins before subjecting to dye-ligand immobilized system. Anion exchange adsorbents like the Streamline DEAE and Streamline Q XL were introduced as their high capacity available for albumin. Although both anion exchangers were capable of removing most of albumin and other contaminants greater than 90%, the loss of IgG was higher in the presence of Q XL. As a result, the removal of albumin was accomplished in high efficiency via a strong adsorption on

DEAE under optimized conditions as followed: 0.5 mg/mL initial protein concentration, pH 8.0; 0.25 mL settled bed volume of Streamline DEAE. Consequently, 80% of polyclonal anti-HBcAg IgG was recovered. A two step procedure using Streamline DEAE anion exchanger and RG-5 immobilized adsorbent was performed for removing albumin and capturing IgG, respectively, under the optimized conditions. After antibody adsorption, bound IgG was eluted in elution medium, pH 8.0 containing 1.0 M NaCl, resulting about 53% IgG recovered with 86% purity and a purification factor of 6.

As exhibited in the current study, DEAE anion exchanger is credited for high efficacy to remove most contaminant proteins from rabbit serum. The purified antibodies can be a useful reagent in diagnosis of chronically infected hepatitis B carriers. Moreover, synthetic dye-ligands can be a potential alternative possessing a tendency of binding to biomolecules for several biological purposes.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PEMBANGUNAN DYE AFFINITY BAHAN PENJERAP UNTUK
POLIKLONAL ANTI-HEPATITIS B ANTIGEN CORE
IMMUNOGLOBULIN G**

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Antibodi seperti imunoglobulin G (IgG) secara umumnya telah banyak digunakan dalam tujuan rawatan dan diagnostik. Kromatografi afiniti Protein A yang sangat khusus terhadap IgG adalah kaedah piawai untuk penulenan antibodi. Walaubagaimanapun, penggunaan protein A yang mahal dan tidak stabil dalam kapasiti pengeluaran yang tinggi telah meningkatkan kos pengeluaran antibodi dengan setara. Ligand bahan penjerap yang digunakan secara berleluasa untuk tujuan penulenan protein telah menunjukkan kapasiti pengikatan yang tinggi iaitu 40 mg/mL setanding dengan protein A. Selain itu, ketersediaannya yang tersebar luas, mudah dan kepantasan persediaannya, kestabilan kimia, dan kemudahan penyimpanan, menjadikan protein ini suatu pilihan yang menarik. Paling utama factor ekonominya juga merupakan suatu pertimbangan yang utama sebagai pengganti untuk protein A yang mahal. Dengan demikian, perkembangan penemuan teras poliklonal anti-antigen hepatitis B imunoglobulin G yang terpilih dari sera arnab telah diselidiki.

Terdapat empat reaktif dye-ligan yang berbeza; iaitu Cibacron Biru 3GA (CB), reaktif Brown 10 (RB 10), reaktif Red 120 (RR 120) dan reaktif Green 5 (RG 5) adalah terikat secara kovalen pada dasar Streamline berasaskan matriks dan digunakan sebagai penjerap pegun untuk penjerapan protein melalui sambungan triazine dalam keadaan alkali. Pada permulaannya, penyaringan kapasiti pengikatan antibodi IgG dye pegun ini adalah diperlukan. Jumlah dye ligan yang sama juga terdapat pada matriks di mana ia ditentukan dengan kaedah keseimbangan jisim relatif berbanding kapasiti jerapan untuk dye ligan. Dari kajian simulasi dalam sistem jerapan protein tunggal, RG 5 yang pegun untuk dipilih kapasitinya untuk albumin sedikit dan lebih IgG diserap pada pH 7.0, dibandingkan dengan ligan dye ligan pegun mempunyai ketumpatan yang sama. Kandungan RG 5 pegun ialah pada 17.4 $\mu\text{mol/mL}$ penjerapan. Sebanyak 64% daripada IgG arnab terikat dalam sistem yang mengikat RG 5 pegun pada pH 7.0 dalam protein sistem binari dengan nisbah yang sama dari kedua-dua albumin dan IgG arnab. Penjerapan yang maksimum bagi (q_m) RG 5 pegun untuk IgG arnab adalah 49.0 mg/mL dan nilai pemalar perceraian (K_d) itu dijumpai dalam 3.33×10^{-6} M. Fenomena pembalikan penjerapan IgG pada bahan penjerap muncul berpandukan model isotherm Langmuir-Freundlich. Sera daripada arnab terimun terhadap antigen core hepatitis B (HBcAg) digunakan sebagai bahan asas yang mengandungi poliklonal anti-HBcAg IgG untuk kajian berkelompok penukaran antibodi. Kelimpahan albumin dan serum protein yang tinggi dimana terdiri daripada 80% jumlah serum protein merupakan gangguan utama dalam kajian afiniti dye ligan kromatografi. Ini menyebabkan kepada strategi penyingkiran bahan pencemar protein sebelum tumpuan terhadap sistem dye ligan pegun. Pertukaran penjerap anion DEAE Streamline dan Q XL Streamline diperkenalkan sebagai berkapasiti tinggi yang sedia ada untuk albumin. Walaupun kedua-dua penukar anion

mampu menghilangkan sebahagian besar daripada albumin dan pencemar lain lebih dari 90%, kehilangan IgG adalah lebih tinggi pada Q XL. Akibatnya, pembuangan albumin tercapai pada kecekapan yang tinggi melalui perjerapan yang kuat pada DEAE Streamline dalam keadaan dioptimumkan sebagai berikut: 0.5 mg/mL kepekatan serum protein, pH 8.0; 0.25 mL untuk DEAE Streamline. Akibatnya, 80% daripada IgG anti-HBcAg poliklonal telah ditemui. Terdapat dua langkah untuk menggunakan penukar anion DEAE Streamline dan RG 5 pegun adsorben dilakukan untuk menyingkirkan albumin dan mendapatkan IgG, masing-masing, pada keadaan optimum. Selepas penjerapan antibodi, batas IgG terilusi terikat dalam medium elusi, pH 8.0 yang mengandungi 1.0 M NaCl, IgG sebanyak 53% ditemui dengan ketulenan sebanyak 86% dan penulenan faktor sebanyak 6.

Menurut kajian terkini, penukaran anion DEAE dikreditkan untuk keberkesanan yang tinggi bagi menghapuskan sebahagian besar bahan pencemar protein pencemaran dari pada serum arnab. Antibodi yang ditulenan boleh menjadi alat yang berguna dalam diagnosis bagi pembawa jangkitan kronik hepatitis B. Tambahan pula, pewarna sintetik-ligan dapat menjadi sumber alternatif yang berpotensi untuk mengikat biomolekul bagi beberapa tujuan biologi.

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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 Doctor of Philosophy. 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 concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

RATTANA WONCHUPHAN

Date: 7 October 2010

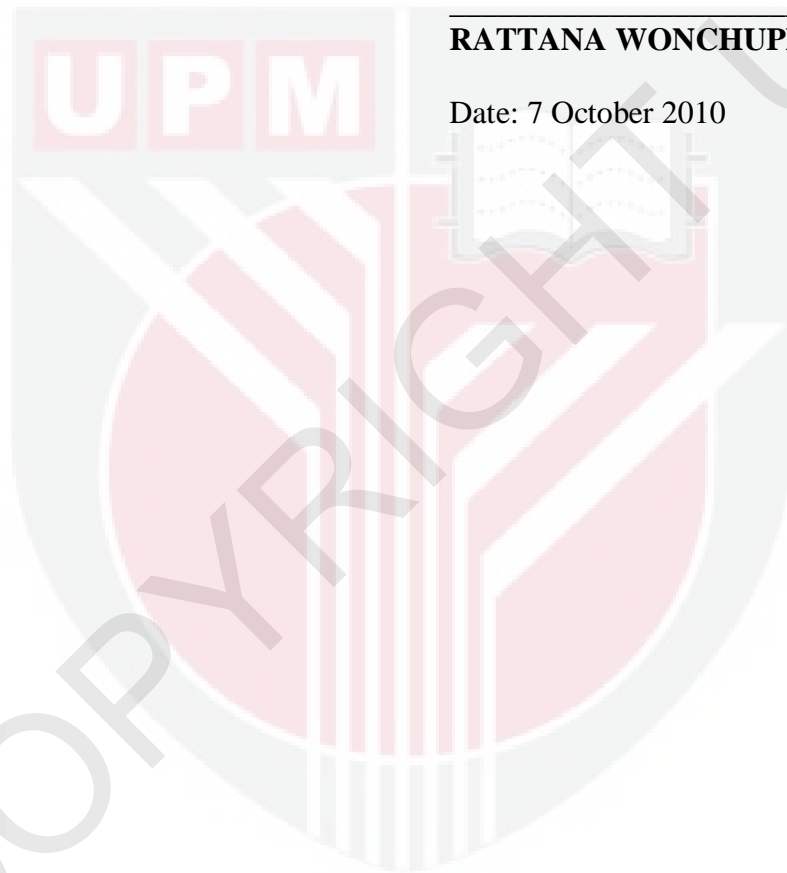


TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	ix
APPROVAL	x
DECLARATION	xii
LIST OF TABLES	xvi
LIST OF FIGURES	xviii
LIST OF ABBRAVIATIONS	xxi
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	6
2.1 Hepatitis B virus	6
2.2 Immunoglobulins	9
2.2.1 Classes of immunoglobulins	10
2.2.2 Antibody purification methods	12
2.3 Albumin	16
2.4 Affinity chromatography	17
2.5 Affinity dye-ligands	20
2.5.1 Classification of dyes based on simple structures	24
2.5.2 Interaction between dye-ligands and proteins	27
2.5.3 Cibacron Blue 3GA	27
2.5.4 Reactive Green 5	32
2.5.5 Reactive Red 120	33
2.5.6 Reactive Brown 10	35
2.6 Ion exchange chromatography	36
2.6.1 Weak anion exchanger	37
2.6.2 Strong anion exchanger	39
2.7 Concluding remarks	40
3 GENERAL MATERIALS AND METHODS	42
3.1 Preparation of HBcAg	42
3.1.1 Cultivation of <i>E. coli</i>	42
3.1.2 Enzymatic cell disruption	42
3.1.3 Ultrasonic cell disruption	43
3.1.4 Sucrose gradient centrifugation	43
3.2 Preparation of polyclonal anti-HBcAg IgG	44
3.3 Dye immobilization	47
3.4 Protein assays	48
3.4.1 The Bradford assay	48
3.4.2 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)	48
3.4.3 Band intensity analysis	49

3.4.4	Enzyme-linked immunosorbent assay (ELISA)	49
3.5	Calculations	50
3.6	Experimental flow	51
4	APPLICATION OF DYE-LIGANDS IN CONTAMINANT PROTEIN ADSORPTION	52
4.1	Introduction	52
4.2	Materials and methods	53
4.2.1	Materials	53
4.2.2	Dye-ligand immobilization	53
4.2.3	Determination of dye-ligand density	54
4.2.4	Determination of rate of mass transfer of BSA	56
4.2.5	BSA adsorption under various pH conditions	56
4.3	Results and discussion	57
4.3.1	Dye-ligand density determination	57
4.3.2	Protein uptake rate	59
4.3.3	Effect of pH on BSA adsorption	61
4.4	Conclusion	63
5	ADSORPTION STUDY OF IMMOBILIZED DYE-LIGANDS TOWARDS IMMUNOGLOBULIN G	65
5.1	Introduction	65
5.2	Materials and methods	66
5.2.1	Materials	66
5.2.2	Preparation of dye-ligand immobilized adsorbents	67
5.2.3	Determination of rate of mass transfer of rabbit IgG	67
5.2.4	Comparison of adsorption capacities of affinity dye-ligands	68
5.2.5	Protein adsorption procedure	69
5.2.6	Analysis methods	70
5.3	Results and discussion	70
5.3.1	Comparison of affinity dye-ligands in adsorption capacity	70
5.3.2	Effect of pH	71
5.3.3	Effect of temperature	77
5.3.4	Effect of ionic strength	78
5.3.5	Effect of initial protein concentration	80
5.3.6	Protein adsorption in batch binary system	84
5.4	Conclusion	87
6	REMOVAL OF CONTAMINANT PROTEIN USING ANION EXCHANGERS	89
6.1	Introduction	89
6.2	Materials and methods	91
6.2.1	Materials	91
6.2.2	Purification of HBcAg	91
6.2.3	Determination of HBcAg antigenicity	92
6.2.4	Preparation of rabbit serum	92
6.2.5	Intensity-based quantification of albumin depletion	93
6.2.6	Western blot analysis	93

6.2.7	Quantitation of specific antibodies	94
6.2.8	Effect of pH on serum albumin elimination	95
6.2.9	Effect of initial protein concentration on albumin elimination	95
6.3	Results and discussion	96
6.3.1	HBcAg purification	96
6.3.2	Antigenicity of purified HBcAg	99
6.3.3	Production of polyclonal anti-HBcAg antibodies	100
6.3.4	Effect of pH on albumin elimination	101
6.3.5	Effect of initial protein concentration on albumin elimination	107
6.3.6	Capacity of Streamline Q XL for serum albumin elimination	110
6.4	Conclusion	113
7	TWO-STEP ADSORPTION OF SERUM PROTEINS USING ANION EXCHANGER AND DYE-LIGAND IMMOBILIZED ADSORBENT	114
7.1	Introduction	114
7.2	Materials and methods	115
7.2.1	Materials	115
7.2.2	Rabbit sera	115
7.2.3	Protein analysis methods	116
7.2.4	Serum protein adsorption on RG-5 immobilized adsorbent	116
7.2.5	Two-step purification process	117
7.3	Results and discussion	118
7.3.1	Adsorption of serum proteins on RG-5 immobilized adsorbent	118
7.3.2	Purification of antibodies using two-step process	121
7.4	Conclusion	124
8	OVERALL SUMMARY AND FUTURE PERSPECTIVES	125
	REFERENCES	128
	APPENDICES	145
	BIODATA OF STUDENT	160