UNIVERSITI PUTRA MALAYSIA

PURIFICATION AND CHARACTERIZATION OF POLYCLONAL ANTI-HEPATITIS B CORE ANTIGEN IMMUNOGLOBULIN G FROM RABBIT SERUM

SHARIFAH MARIAM BT SAYED HITAM

IB 2016 7

PURIFICATION AND CHARACTERIZATION OF POLYCLONAL ANTI-HEPATITIS B CORE ANTIGEN IMMUNOGLOBULIN G FROM RABBIT SERUM

By

SHARIFAH MARIAM BT SAYED HITAM

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

June 2016

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

PURIFICATION AND CHARACTERIZATION OF POLYCLONAL ANTI-HEPATITIS B CORE ANTIGEN IMMUNOGLOBULIN G FROM RABBIT SERUM

By

SHARIFAH MARIAM BT SAYED HITAM

June 2016

Chairman : Professor Tan Wen Siang, PhD
Faculty : Institute of Bioscience

Antibodies are biologically active proteins produced by plasma cells in response to the presence of foreign substances. Antibodies such as immunoglobulin G (IgG) have been used extensively for diagnostic and therapeutic purposes. Polyclonal antibodies against hepatitis B core antigen raised in rabbits that react to diseases can be used as medication for human subjects. Protein A affinity chromatography, has been a standard method used to purify antibodies for the past few decades due to its high affinity to antibodies efficient product capture. However, this approach has a number of disadvantages, especially in large scale applications due to its low ligand stability and high production cost. Thus, a simple and economical method for antibody purification via precipitation and adsorption chromatography in combination with hydrophobic and ion exchange approaches, was investigated as an alternative to overcome this problem.The development of simple and short execution time as well as low cost techniques for antibody separation and purification by ammonium sulfate precipitation was examined in the current study. Salting out with different percentages of ammonium sulfate was introduced as an early step in this study to separate IgG from other serum impurities. Serum albumin is the main challenge in this purification process, as it is a major contaminant in the serum. From the precipitation study in a single purification system, 40% of ammonium sulfate saturation gave the best result in terms of the concentration of IgG recovered (7.8 mg/mL) and albumin removal (27.9 mg/mL). From the observations, concentrations of saturated ammonium sulfate (SAS) greater than 40% caused the precipitation of serum albumin together with the IgG, hence reducing its purity. At this ideal condition, the yield, purity and purification factor were 99%, 94% and 7.8, respectively. Besides, due to the incorporation of salt in this method, the final product showed high value of conductivity, which was 16 ± 1 mS/cm. Moreover, its high ionic strength could affect the productivity of the purified antibody as it would shield the active sites of the protein. Thus, SepFast^{TM} MM AH-1 which has a combination of anionic and hydrophobic groups was introduced to be incorporated with the salt precipitation method. The integrated system approach showed a promising result as it successfully reduced the ionic strength by up to 75%. Due to this, the need of desalting the final product was abolished. At the final polishing process, the yield and purity of the polyclonal IgG also showed a good improvement as it increased to 97% and 98%, relatively. In comparison to salt precipitation, the IgG recovered by the coupling method with mixed mode column was significantly more productive as it produced mostly monomeric immunoglobulin. Besides, a monomodal regularisation histogram revealed the particle size distribution of the polyclonal IgG recovered from

rabbit serum as being about 10.3 nm in diameter, which is equivalent to the reported size of IgG. The efficiency of the single-step purification approach based on prepacked SepFast^{TM} Supor DEAE column for the purification of the anti-HBcAg IgG from rabbit serum was also evaluated in this study. Furthermore, the optimal pH, which was determined as pH 8.0, offered the highest recovery of IgG of about 20% from the total proteins. This single step of purification showed excellent recovery with yield and purity at about 94% and 83%, which corresponded to 7.4 purification factor. The aggregation study by dynamic light scattering (DLS) revealed that the molecular size of the anti-HBcAg IgG from rabbit serum was around 10 nm, which is equivalent to the native IgG. In conclusion, this study shows that SepFast^{TM} MM AH-1 column has a great potential to be used in the polishing step as it is able to reduce the ionic strength of the sample solution and to separate the monomers from its aggregates. Besides, SepFast^{TM} Supor DEAE column chromatography shows potential application at industry scale to purify IgG with only a single step of application.

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

PENULENAN AND PENCIRIAN POLIKLONAL ANTI-HEPATITIS B TERAS ANTIGEN IMMUNOGLOBULIN G DARIPADA SERUM ARNAB

Oleh

SHARIFAH MARIAM BT SAYED HITAM

Jun 2016

Pengerusi : Profesor Tan Wen Siang, PhD
Fakulti : Institut Biosains

Antibodi merupakan protein biologi aktif yang dihasilkan oleh sel plasma sebagai tindak balas terhadap kehadiran bahan-bahan asing. Antibodi seperti imunoglobulin G (IgG) telah digunakan secara meluas untuk tujuan diagnostik dan terapeutik. Antibodi poliklonal terhadap antigen teras hepatitis B diperoleh daripada arnab yang mana bertindak balas bagi melawan jangkitan kuman dan penyakit merupakan sasaran untuk kajian ini. Kebiasaannya kromatografi afiniti protein A merupakan kaedah pengeluaran antibodi piawaian untuk beberapa dekad yang lalu kerana penghasilan produk yang sangat berkesan. Walau bagaimanapun, pendekatan ini telah menunjukkan beberapa kelemahan, terutamanya dalam aplikasi berskala besar kerana kestabilan ligan yang rendah dan protein A yang mahal. Justeru itu, satu kaedah penulenan antibodi yang mudah dan berkos rendah diperkenalkan melalui kaedah pemendakan dan penjerapan kromatografi yang bersifat dual iaitu gabungan hidrofobik dan pernukaran ion di perkenalkan untuk menangani masalah ini. Pembangunan kaedah yang mudah dan singkat serta teknik kos yang rendah untuk pemisahan dan penulenan antibodi dengan menggunakan kaedah pemendakan ammonium sulfat dikaji terlebih dahulu. Penggaraman keluar dengan peratusan ammonium sulfat yang berbeza merupakan langkah awal dalam kajian ini untuk memisahkan IgG daripada bendasing serum. Bagi sistem pemendakan tunggal ini, ketepuan 40% memberikan hasil yang paling optimum dari segi kepekatan IgG yang diperoleh semula (7.8 mg/mL) dan penyingkiran albumin (27.9 mg/mL). Daripada pemerhatian, peningkatan kepekatan ammonium sulfat tepu (SAS) dalam serum mengakibatkan albumin terdedah untuk termendak bersama-sama dengan IgG. Secara tidak langsung tindak balas ini menyebabkan ketulenan IgG berkurangan. Pada peratusan ketepuan ammonium sulfat yang optimum, perolehan semula, ketulenan, and penulenan faktor masing-masing adalah 99%, 94% dan 7.8. Kaedah ini menunjukkan nilai bacaan kekonduksian yang tinggi, iaitu 16 ± 1 mS/cm. Kesan kekuatan ion terkandung yang tinggi akan melemahkan produktiviti antibodi yang ditulenkan kerana ia boleh melindungi bahagian protein yang aktif. Justeru itu, penggunaan kaedah bersepadu seperti gabungan sistem kromatografi dengan pemendakan dilihat sebagai langkah yang boleh diambil bagi menangani masalah ini. Bagi tujuan ini penggunaan kromatografi mod campuran (SepFast^{TM} MM AH-1) yang bersifat dual fungsi iaitu mempunyai kumpulan anionik dan hidrofobik telah diperkenalkan bagi mengkaji keberkesanan sistem gabungan bersepadu ini. Hasil daripada penggabungan dua kaedah ini adalah memberangsangkan kerana ia berjaya mengurangkan kekuatan ionik sehingga 75%. Peratusan pengurangan yang tinggi ini menyebabkan kaedah penyahgaraman produk akhir tidak diperlukan dilaksanakan lagi. Proses penggilapan ini memberi peningkatan yang baik dari segi hasil perolehan dan

ketulenan poliklonal IgG iaitu meningkat kepada 97% dan 98% secara relatif. Berbanding dengan pemendakan garam tunggal, IgG yang diperoleh semula dengan kaedah gandingan dengan kromatografi mod campuran adalah jauh lebih produktif kerana ia menghasilkan imunoglobulin bersifat monomer secara dominan. Di samping itu, histogram regularisasi monomodal menunjukkan saiz taburan zarah poliklonal IgG yang diperoleh semula daripada serum arnab adalah 10.3 nm diameter di mana berpadanan dengan saiz IgG yang dilapor daripada kajian terdahulu. Pendekatan penulenan tunggal yang cekap dan cepat dengan menggunakan kromatografi SepFast™ Supor DEAE untuk penulenan anti-HBcAg IgG daripada serum arnab juga turut dikaji daripada penyelidikan ini. Hasil daripada kajian ini, pH optimum adalah pH 8.0 di mana ia memberikan perolehan tertinggi IgG iaitu kira-kira 20% daripada jumlah keseluruhan protein dalam serum. Penulenan langkah tunggal ini menunjukkan pemulihan yang sangat baik dengan hasil perolehan dan ketulenan pada kira-kira 94% dan 83%, dan ia berpadangan dengan faktor penulenan 7.4. Kajian pengagregatan oleh penyerakan cahaya dinamik (DLS) mendapati bahawa saiz molekul anti-HBcAg IgG daripada serum arnab adalah kira-kira 10 nm dan ia benar-benar sepadan dengan IgG asli. Kesimpulan daripada kajian ini menunjukan penggunaan SepFast™ MM AH-1 diperingkat penggilapan adalah amat berkesan memandangkan kebolehan kromatografi ini mengurangkan nilai kekuatan ionik dan memisahkan pencamtuman monomer-monomer daripada pengagregatan protein. Selain daripada itu, kajian menggunakan SepFast™ Supor DEAE dilihat berpotensi besar dalam aplikasi berskala industri bagi penulenan IgG dengan satu langkah sahaja.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude and appreciation to Professor Dr. Tan Wen Siang and Professor Dr. Tey Beng Ti for their guidance, encouragement, discussions and stimulating suggestions, which brought to the completion of this thesis. Their patience and dedication have inspired me to do my very best in completion of this study.

I would also like to sincerely thank my co-supervisors, Assoc. Prof. Dr. Janna Ong Abdullah, Professor Dr. Arbakariya Ariff for the advices and encouragement during the entire progress of project.

Acknowledgement also extended to Professor Dr. Mohd Azizan Mohd Noor and Dr. Ooi Chien Wei who kindly assisted and gave me invaluable suggestions and advices to complete this study.

I am truly indebted to my lab mates in the Department of Biotechnology and Biomolecular Sciences for their helpful collaboration and stimulating discussions. I learnt a lot from them.

I have furthermore to thank all staff in the Department of Biotechnology and Biomolecular Sciences, UPM and the Section of Bioengineering Technology MICET, Universiti Kuala Lumpur for their kind assistance.

Above ground, extremely huge thanks to my family for their endless love, moral support and encouragement, without them I would have never been able to complete this important step of my life.

I certify that a Thesis Examination Committee has met on 15 June 2016 to conduct the final examination of Sharifah Mariam Sayed Hitam on her thesis entitled "Development of "Purification Method and Characterization of Polyclonal Anti-Hepatitis B Core Antigen Immunoglobulin G from Rabbit Serum" in accordance with the Universities and University College Act 1971 and the Constitution of the University Putra Malaysia P.U. (A) 106 15 March 1998. The committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Name of Chairperson, PhD

(Chairman)

Name of Examiner 1, PhD

(Internal Examiner)

Name of Examiner 2, PhD

(Internal Examiner)

Name of External Examiner, PhD

(External Examiner)

ZULKARNAIN BIN ZAINAL, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 26 July 2016

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Tan Wen Siang, PhD
Professor
Faculty of Biotechnology and Biomolecular Science
Universiti Putra Malaysia
(Chairman)

Tey Beng Ti, PhD
Professor
Faculty of Engineering
Monash University
(Member)

Janna Ong Abdullah, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Science
Universiti Putra Malaysia
(Member)

Arbakariya Arif, PhD
Professor
Faculty of Biotechnology and Biomolecular Science
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: Sharifah Mariam bt Sayed Hitam GS 27366

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____	Signature: _____
Name of Chairman of Supervisory Committee : Professor Tan Wen Siang	Name of Member of Supervisory Committee : Professor Tey Beng Ti
Signature: _____	Signature: _____
Name of Member of Supervisory Committee : Assoc. Prof. Janna Ong Abdullah	Name of Member of Supervisory Committee : Professor Arbakariya Arif

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

α	alpha
β	beta
°C	degree centrigrade
µg	microgram
µl	microliter
Å	Ångstrom
AEC	anion exchange chromatography
BCIP	5-bromo-4-chloro-3-indoyl phos
BSA	bovine serum albumin
CA	caprylic acid
C-terminus	carboxy terminus
DEAE	diethylaminoethyl
DLS	dynamic light scattering
DNase	deoxyribonuclease
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
g	gram(s)
h	hour
HBcAg	Hepatitis B core antigen
HBeAg	Hepatitis B early antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCIC	hydrophobic charge-induction chromatography
HIC	hydrophobic interaction chromatography
IgG	immunoglobulin G
IPTG	isopropyl-β-thiogalactopyranoside
kDa	kilo Dalton
LB	Luria Bertani
M	molar
mA	milliampere
mg	milligram
min	minute

$MgCl_2$	magnesium chloride
MM	mixed mode
NaCl	sodium chloride
N-terminus	amino terminus
NBT	nitro blue tetrazolium
nm	nanometer
PAGE	polyacrylamide gel electrophoresis
p*I*	isoelectric point
rpm	revolution per minute
RT	room temperature
SDS	sodium dodecyl sulfate
TBS	tris-buffered saline
TEMED	tetramethyl ethylenediamine
v/v	volume by volume
w/v	weight by volume
x *g*	centrifugal force

CHAPTER 1

INTRODUCTION

Immunoglobulin (Ig) generated by the immune system of animals function as an antibody which responds to immunogens such as bacteria, viruses or cancer cells. Among all antibody isotopes, immunoglobulins G (IgGs) are the most abundant antibodies (80%) found in the blood of animals. Polyclonal antibodies, one of the therapeutic proteins present in the serum, are considered as more efficacious than monoclonal antibodies due to their ability to bind to multiple epitopes on an antigen (Newcombe *et al*., 2005). Thus, the production of polyclonal IgG against an antigen such as HBcAg can be useful in clinical diagnosis and therapeutic treatment for viral infection. Since a significant percentage (50% to 80%) of the total manufacturing cost of therapeutic antibodies are contributed by the downstream processing, the development of an effective and economical purification method for large scale IgGs production is vital to overcome these problems (Azevedo *et al*., 2009; Simaria *et al.*, 2012; Menegatti *et al*., 2013). Furthermore, escalating demands for increased protein titers, primarily for economic reasons, have shifted the bottleneck step from production to purification (Hober *et al*., 2007; Zhao *et al*., 2014). Hence, devising an efficient and economical purification strategy is a key challenge and one which is faced by the industry.

Hepatitis B is a liver-related disease caused by hepatitis B virus (HBV) infection. HBV is a human pathogen belongs to the family of *Hepadnaviridae that* was discovered by Blumberg and Alter in year 1965. The number of HBV carriers worldwide is estimated to be about 350 million (Lok and McMohan, 2009) and the annual mortality rate is approximately 1 to 2 million of these HBV carriers (Jung and Pape, 2002). About 200,000 to 300,000 of these HBV carriers die from cirrhosis and hepatocellular carcinoma, respectively (Perz *et al.,* 2006). HBV virion, is approximately 42 nm in diameter and consists of an inner protein core and an outer protein envelope. The HBV DNA is a partially double stranded DNA comprising 3,500 bases with one end attached to DNA polymerase that possesses reverse-transcription activity. The DNA was surrounded by a nucleocapsid composed of 22 kDa core protein (HBcAg). Enveloping the core nucleocapsid is the hepatitis B surface antigen (HBsAg) that is widely used in vaccination (Howard, 1986). There is a secreted pre-core protein in between HBsAg and HBcAg called the hepatitis B early antigen (HBeAg), as it is among the first to be detected in the serum of patient infected by the virus. All of these proteins activate the body immune system by producing antibodies against them, which makes diagnosis of the virus infection possible by enzyme immunoassay (Budkowska and Karwowska, 1982; Kimura *et al.,* 2003; Deng *et al*., 2008). HBcAg has emerged as an alternative target for treating chronic HB infection (Hacker *et al.,* 2003) and for vaccine development.

The development of techniques and methods for the separation and purification of macromolecules such as proteins has been an important prerequisite for many of the advancements made in bioscience and biotechnology. In the biopharmaceutical industry, chromatography is a vital and widely used separation and purification technology due to its high resolution. There are numerous ways in which an affinity-based method may be employed for the purification of recombinant proteins. The most common example of an affinity process is Protein A chromatography, which has been applied for over a decade in industrial and academic settings to capture and purify

antibodies (immunoglobulins) (Low *et al.,* 2007; Zhao *et al.,* 2014). However, the use of Protein A chromatography has several notable drawbacks including cost, risk of final product's contamination due to the leakage of Protein A from its matrices, and the requirement of low-pH elution buffer that can be deleterious to antibody activity. Leaching of Protein A is detectable after multiple use of the adsorbent. The leached Protein A from purified antibodies should be removed prior to human use as it may lead to adverse physiological effects (Cater-Franklin *et al.,* 2007). The use of Protein A in the production of large quantities of antibodies is not favorable due to its high cost and leakage after reuse for several times (Low *et al.,* 2007; Ghose *et al.,* 2007; Menegatti *et al.,* 2013). As antibody titers have increased, the need to develop an alternative method, which is economically effective is absolutely required.

Improvements in materials and the utilization of microprocessor-based instruments have made protein separations more predictable and controllable. Ion exchange chromatography is a useful tool for separating protein based on charge (Josic and Lim, 2001; Liu *et al.,* 2010). Separation by this technique is fairly selective, and the resins used are relatively inexpensive. Moreover, this method is ideal for reducing high molecular weight aggregates, charge-variants, residual DNA and host cell protein, leached Protein A and viral particles (Liu *et al.,* 2010). Anion exchange chromatography uses a positively-charged group (weakly basic such as diethyl, DEAE or dimethylamino ethyl, DMAE; or strong basic such as quaternary amino ethyl, Q or trimethylammonium ethyl, TMAE immobilized to the resin. It can be used either in the flow through mode or in the bind and elute mode, depending on the isoelectric point (p*I*) of the antibody and impurities to be removed (Liu *et al.,* 2010). Cation exchange chromatography uses a resin modified with negatively charged functional groups. They can be strong acidic ligands such as sulphopropyl, sulfoethyl and sulfoisobutyl groups or weak acidic ligands such as carboxyl group. Cation exchange chromatography has been applied for the purification processes of many antibodies with p*I* values ranging from neutral to basic. This negatively charged process can also provide the separation power to reduce antibody variants from the target antibody product such as deamidated products, oxidized species and N-terminal truncated forms, as well as high molecular weight species (Liu *et al.,* 2010). The application of DEAE anion exchanger in eliminating albumin from rabbit sera for polyclonal IgG recovery was reported by Wongchupan *et al.* 2011. The approach process was successfully removed the albumin and other contaminants. However there are some limitations occurred which are the loss of some IgGs and difficulty in scaling up due to the batch binding mode. Therefore, current study proposed the use of DEAE chromatography column that is more feasible for scaling up process.

The precipitation method can be a powerful tool to separate classes of proteins, which vary in size, charge, and surface area, among other characteristics (Mirica *et al*., 2012). Ammonium sulfate precipitation has been used as an early step in a purification protocol, and can be a highly effective method for separating proteins for quantitative recovery of proteins in a complex mixture. This method increases the surface tension of the solution, which effectively increases the hydrophobic effect, which then stabilizes the protein structure and allows the hydrophobic regions on the surfaces of different molecules to interact and eventually causes aggregation (Mirica *et al*., 2012). Longmire *et al.* (1971) and Perosa *et al.* (1990) reported the purification of human immunoglobulin by ammonium sulfate precipitation method. Since the mechanism of precipitation by ammonium sulfate would induce hydrophobic aggregation, this may lead to the reduction of antibody activity in final product (Page and Thorpe, 2002). As

far as this drawback is concerned, a method was developed in this study to combine the optimum salt induced precipitation with mixed-mode chromatography column.
Protein aggregation is a common problem in many biological system, experimental research, industrial and medical applications (Bondos and Bicknell, 2003; Al-Abdulla *et al.,* 2014). Proteins tend to aggregate under a variety or environmental conditions, and the extent of the aggregation is dependent on many factors, especially the conditions during the processing stages (Wang *et al*., 2010), which may lead to reduced or loss of biological activity (Bermudez and Forciniti, 2004). Dynamics light scattering (DLS) has become one of the common techniques to measure the extent of denaturation and the formation of aggregates (Ahrer *et al.,* 2003; Bermudez and Forciniti, 2004).

Recently, a manufacturer, Bio Toolomics Limited, recommended a strategy for antibody purification, which is by applying the polishing step to further remove the impurities. A mixed mode chromatography column with the combination of anionic and hydrophobic groups could be an approach to eliminate the impurities as well as to remove protein aggregates. Passing the clarified antibody feedstock through this column can polish the purity of the antibody and reduce the ionic strength in the protein solution. A coupled method of ammonium salt precipitation with this column could be an efficient way of overcoming the economy and physical limitations of Protein A affinity chromatography.

Hence, the main aim of this study was to develop a simple and cost effective method for purifying polyclonal antibody from animal sera. The objectives of this study were:

1. To identify the optimal percentage of saturated ammonium sulfate for precipitating polyclonal anti-HBcAg IgG from rabbit sera.
2. To examine the application of mixed-mode column as the polishing step to prevent aggregation of polyclonal anti-HBcAg IgG purified by precipitation.
3. To investigate the potential of DEAE anion exchanger as the adsorbent for negative chromatography for purifying polyclonal anti-HBcAg IgG from the sera.
4. To characterize the purified anti-HBcAg IgG by using dynamic light scattering and zetasizer analyses.

REFERENCES

Ahrer, K., Buchacher, A., Iberer, G., Josic, D. and Jungbauer, A. (2003). Analysis of aggregates of human immunoglobulin G using size-exclusion chromatography, static and dynamic light scattering. *Journal of Chromatography A*. 1009: 89-96.

Alavian, S.M., Ande,S.R., Coombs, K.M., Yeganeh, B., Davoodpour, P., Hashemi, M., Los, M. and Ghavami, S. (2011).Virus-triggered autophagy in viral hepatitis – possible novel strategies for drug development. *Journal of Viral Hepatitis*. 18: 821–830.

Alekseev, R.J. and Rebane, A.L. (2012). Serum Albumin: Structure, Functions, and Health Impact. Nova Science Publishers.

Azevedo, A.M., Rosa, P.A.J., Ferreira, I.F., Pisco, A.M.M.O., de Vries, J., Korporaal, R., Visser, T.J. and Aires-Barros, M.R. (2009). Affinity-enhanced purification of human antibodies by aqueous two-phase extraction. *Separation and Purification Technology*. 65: 31-39.

Bagchi, P. and Birnbaum, S.M. (1981). Effect of pH on the adsorption of immunoglobulin G on anionic poly(vinyltoluene) model latex particles. *Journal of Colloid and Interface Science*. 83: 460-478.

Beasley, R.P., Hwang, L.Y., Stevens, C.E., Lin, C.C., Hsieh, F.J. and Wang, K.Y. (1983). Efficacy of hepatitis B immune globulin for prevention of perinatal transmission of the hepatitis B virus carrier state. *Hepatology*. 3: 135-141.

Bermudez, O. and Forciniti, D. (2004). Aggregation and denaturation of antibodies: a capillary electrophoresis, dynamic light scattering, and aqueous two-phase partitioning study. *Journal of Chromatography B.* 807: 17-24.

Bilgicer, B., Thomas, S.W., Shaw, B.F., Kaufman, G.K., Krishnamurthy, V.M., Estroff, L.A., Yang, J. and Whitesides, G.M. (2009). A Non-Chromatographic Method for the Purification of a Bivalently Active Monoclonal IgG Antibody from Biological Fluids. *Journal American Chemical Society.* 131: 9361–9367.

BioToolomics (2008). Data sheet of SepFast MM AH-1, http://www.biotoolomics.com/ pdfiles/Datasheet SepFast MM AH-1.pdf

Blumberg, B.S. and Alter, H.J. (1965). A “new” antigen in leukemia sera. *JAMA* 191: 541-546.

Bon, L.C., Nicolai, T. and Durand, D. (1999). Growth and structure of aggregates of heat-denatured β-lactoglobulin. *International Journal of Food Science and Technology*. 34: 451–465.

Bondos, S.E., and Bicknell, A. (2003). Detection and prevention of protein aggregation before, during, and after purification. *Analytical Biochemistry*. 316: 223-31.

Borgstahl, G. E. O. (2007). How to use dynamic light scattering to improve the likelihood of growing macromolecular crystals. *Methods in Molecular Biology*. 363: 109-130.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*. 72: 248-254.

Budkowska, A. and Karwowska, S. (1982). Enzyme immunoassay for the detection of hepatitis B virus core antigen. *Journal of immunological Methods.* 51: 341-346.

Böttcher, B., Wynne, S.A. and Crowther, R.A. (1997). Determination of the fold of the core protein of hepatitis B virus by electron cryomicroscopy. *Nature*. 386: 88-91.

Calabozo, B., DUffort, O., Carpizo, J.A., Barber, D. and Polo, F. (2001). Monoclonal antibodies against the major allergen of Plantago lanceolata pollen 1 1: affinity chromatography purification of the allergen and development of an ELISA method for Pla 11 measuremant. *Allergy*. 5: 429-435.

Carter-Fraklin, J.N., Victa, C., Mc Donald, P. and Fahrner, R. (2007). Fragments of protein A eluted during protein A affinity chromatography. *Journal of Chromatography A*. 1163: 105-111.

Cheung, H., Chan, K. and Cheng, C. (2002). Production of polyclonal antibody against recombinant goldfish prolactin and demonstration of its usefulness in a non-competitive antigen-capture ELISA. *Comparative Biochemistry and Physiology. Part B. Biochemistry and Molecular Biology*. 131: 37-46.

Chi, E. Y., Krishnan, S., Randolph,T. W. and Carpenter J. F. (2003). Physical Stability of Proteins in Aqueous Solution: Mechanism and Driving Forces in Nonnative Protein Aggregation. *Pharmaceutical Research.* 20: 1325-1336.

Cohen, B.J. and Richmond, J.E. (1982). Electron microscopy of hepatitis B core antigen synthesized in *E. coli. Nature*. 296: 277-278.

Coligan, J.E., Dunn, B.M., Ploegh, H.L., Speicher, D.W. and Wingfield, P.T. *Current Protocols in Molecular Biology*. (Vol 1: Chapter 8). New York: Wiley.

Cooper, H.M. and Paterson,Y. (2002). Immunology. In F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidmen, J.A. Smith, K. Stuhl. Short protocols in molecular biology: a compendium of methods from current protocols in molecular biology (pp.11-21 – 11-24). New York:Wiley.

Cullen, D.C. and Lowe, C, R. (1994). AFM studies of protein adsorption. *Journal of Colloid and Interface Science.* 166: 102-108.

Deisenhofer, J. (1981). Crystallographic refinement and atomic models of human Fc fragment and its complex with fragment B of protein A from *Staphylococcus aureus*at 2.9-Å and 2.8-Å resolution. *Biochemistry*. 20: 2361–2370.

Deng, L.J., Xu, Y. and Huang, J., (2008) Developing a double-antigen sandwich ELISA for effective detection of human hepatitis B core antibody. *Comparative Immunology, Microbiology and Infectious Disease*. 31: 515-526.

Dyson, M.R., Murray, K. (1995). Selection of peptide inhibitors of interactions involved in complex protein assemblies: association of the core and surface antigens of hepatitis B virus. *Proceedings of the National Academy of Sciences. U S A*, 92: 2194-2198.

Engvall, E., Perlmann P. (1971). Enzyme-linked immunosorbent assay (ELISA) quantitative assay of immunoglobulin G. *Immunochemistry*. 8: 871-874.

Eursakun, S., Simsiriwong, P., Ratanabanangkoon, K. (2012). Studies on the fractionation of equine antivenom IgG by combinations of ammonium sulfate and caprylic acid. *Toxicon*. 60: 1022-1029.

Farrell, H. M., Jimenez-Flores, Jr. R., Bleck, G. T., Brown, E. M., Butler, J. E., Creamer, L. K., Hicks, C. L., Hollar, C. M., Ng-Kwai-Hang, K. F. and Swaisgood, H. E. (2004). Nomenclature of the Proteins of Cows' Milk—Sixth Revision. *Journal of Dairy Science.* 87:1641–1674.

Fasler, S., Skvaril, and Hans, U. L. (1988). Electrophoretic Properties of human IgG and its subclasses on sodium Dodecyl-Sulfate-Polyacrylamide gel electrophoresis and immunoblots. *Analytical Biochemistry.* 174: 593-600.

Goldstein, S.T., Zhou, F., Hadler, S.C., Bell, B.P., Mast, E.E., Margolis, H.S. (2005). A mathematical model to estimate global hepatitis B disease burden and vaccination impact. *International Journal of Epidemiology*. 34: 1329-1339.

Gsponer, J. and Vendruscolo, M. (2006). Theoretical approaches to protein aggregation. *Protein and Peptide Letters*. 13: 287-93.

Guo, G.H., Tan, D.M., Zhu P.A., Liu, F. (2009). Hepatitis B virus X protein promotes proliferation and upregulates TGF-β1 and CTGF in human hepatic stellate cell line, LX-2. *Hepatobiliary Pancreatic Diseases International*. 8: 59-64.

Hacker, H.J., Deres, K., Mildenberger, M. and Schröder C.H. (2003). Antivirals interacting with hepatitis B virus core protein and core mutations may misdirect capsid assembly in a similar fashion. *Biochemical Pharmacology*. 66: 2273-2279.

Hamada, H., Arakawa, T. and Shiraki, K. (2009). Effect of Additives on Protein Aggregation. *Current Pharmaceutical Biotechnology.*10: 400-407.

Hau, J. and Hendriksen, C. (2005). Refinement of polyclonal antibody production by combining oral immunization of chickens with harvest of antibodies from the egg yolk. *Institute for Laboratory Animal Research Journal*. 46: 294-299.

Helm, B., Kebo, D., Vercelli, D., Glovsky, M.M., Gould, H., Ishizaka, K, Geha R. and Ishizaka, T. (1989). Blocking of passive sensitization of human mast cells and basophil granulocytes with IgE antibodies by a recombinant human epsilon-chain fragment of 76 amino acids. *Proceedings of the National Academy of Sciences*. *U S A*. 86: 9465-9.

Hober, S., Nord, K. and Linhult, M. (2007). Protein A chromatography for antibody purification. *Journal of Chromatography B*. 848: 40-47.

Howard, C.R., Allan, J., Chen, S.E. and Steward, M.W. (1986) Progress toward a sysnthetic hepatitis B vaccine. *Protides of the Biological Fluids*. 34: 133-136.

James, K., Henney, C.S. and Stanworth D.R. (1964). Structural changes occurring in 7S gamma-globulins. *Nature*. 202: 563-566.

Jeng, K-S., Hu, C-P. and Chang, C. (1991). Differential formation of disulphide linkages in the core antigen of extracellular and intracellular hepatitis B virus core particles. *Journal of virology*. 65: 3924-3927.

Jøssang, T., Feder, J. and Rosenqvist, E. (1985). Heat Aggregation Kinetics of Human IgG. *Journal of Chemical Physics.* 82: 574-589.

Josic, D. and Lim, Y.P. (2001). Analytical and Preparative methods for Purification of Antibodies. *Food Technology and Biotechnology*. 39:215-226.

Jung, M.C. and Pape, G.R. (2002). Immunology of hepatitis B infection. *The Lancet Infectious Disease*. 2: 43-50.

Kimura, T., Rokuhara, A., Matsumoto, A., Yagi, S., Tanaka, E., Kiyosawa, K. and Maki, N., (2003). New enzyme immunoassay for detection of hepatitis B virus core antigen (HBcAg) and the rwlation between levels of HBcAg and HBV DNA. *Journal of Clinical Microbiology*. 41: 1901-1906.

Kira A. Markossian, K.A., Yudin, I.K. and Kurganov, B.I. (2009). Mechanism of Suppression of Protein Aggregation by α-Crystallin. *International Journal of Molecular Sciences.*10: 1314-1345.

Koshy, R. and Caselmann, W.H. (1998). Hepatitis B virus: molecular mechanisms in disease and novel strategies for therapy. London: Imperial College Press.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*. 227: 680-685.

Leenaars, M. and Hendriksen, C. (2005). Critical steps in the production of polyclonal and monoclonal antibodies: Evaluation and recommendations. *Institute for Laboratory Animal Research Journal*. 46: 269-79.

Leo, J.C. and Goldman, A. (2009).The immunoglobulin-binding Eib proteins from *Escherichia coli* are receptor for IgG Fc. *Molecular Immunology*. 46:1860-1866.

Leon, G., Lomonte, B. and Gutierrez, J.M. (2005). Anticomplementary activity of equine whole IgG antivenoms: comparison of three fractionation protocols. *Toxicon*. 45:123-128.

Lewis, D.J and Nail, S.L. (1997). The influence of ion exchange chromatography conditions on aggregation of equine IgG. *Process Biochemistry*. 32: 279-283.

Li, J., Han, W. and Yu, Y. (2013). Chromatography Method, Protein Engineering - Technology and Application, http://www.intechopen.com/books/protein-engineering-technology-and-application/chromatography-method. May 29.

Lindmark, R., Thoren-Tolling, K. and Sjoquist J. (1983). Binding of immunoglobulins to protein A and immunoglobulin levels in mammalian sera. *Journal of Immunological* Methods. 62: 1-13.

Lipman, N., Jackson, L., Trudel, L. and Weis-Garcia, F. (2005). Monoclonal versus polyclonal antibodies: Distinguishing, characteristics, application and information resources. *Institute for Laboratory Animal Research Journal*. 3: 258-268.

Liu, F. H., Ma, J., Winter, C and Bayer, R. (2010). Recovery and purification process development for monoclonal antibody production. *Landes Bioscience.* 25: 480-499.

Lok, A.S and Mc Mohan, B.J. (2009) Crohic hepatitis B: update 2009. *Hepatology*. 50: 661-662.

Longmire, R.L., Smith, R. S., Mc Millan, R. and Robert R.T.(1971) Quantitative of IgG by the ammonium sulfate technique-I. Interference of the primary interaction between ^{125}I Fc and rabbit anti-human Fc. *Immunochemistry*. 8; 141-151.

Low, D., O'Leary, R., and Pujar, N.S. (2007). Future of antibody purification. *Journal of Chromatography B*. 848: 48-63.

Marek, W., Muca, R., Wos, S., Piatkowski, W. and Antos, D. (2013). Isolation of monoclonal antibody from a Chinese hamster ovary supernatant. II: Dynamics of the integration separation on ion exchange and hydrophobic interaction chromatography media. *Journal Chromatography A.* 1305: 64-75.

McAleer, W.J., Buynak, E.B., Maigetter, R.Z., Wampler, D.E., Miller, W.J. and Hilleman, M.R. (1984). Human Hepatitis B vaccine from recombinant yeast. *Nature*. 307: 178-180.

McCue, T.J. (2014). Chapter Five- Use and application of hydrophobic interaction Chromatography for protein purification. *Method in Enzymology*. 541: 51-65.

McKinney, M.M. and Parkinson, A. (1987). A simple, non-chromatographic procedure to purify immunoglobulins from serum and ascites fluid. *Journal of Immunological Methods.* 96: 271-278.

Menegatti, S., Hussain, M., Naik, A.D., Carbonell, R.G., and Rao, B.M. (2013). mRNA display selection and solid-phase synthesis of Fc-binding cyclic peptide affinity ligands. *Biotechnology Bioengineering*. 110: 857-870.

Mirica, K.A., Lockett, M.R., Snyder, P.W., Shapiro, N.D., Mack,E.T., Nam, S. and Whitesides. G.M. (2012). Selective Precipitation and Purification of Monovalent Proteins UsingOligovalent Ligands and Ammonium Sulfate. *Bioconjugate Chemistry*. 23: 293−299.

Mohammad, S.F., Sharma, N. and Woodward, S.C. (1983). Disulfide linking of albumin to the hinge region of immunoglobulin G in normal human serum. *Biochimica et Biophysica Acta* . 28: 749-475.

Mohanty, J.G. and Elazhary, Y.(1989). Purification of IgG from serum with caprylic acid and ammonium sulfate precipitation is not superior to ammonium sulfate precipitation alone. *Comparative Immunology, Microbiology & Infectious Diseases*. 12: 153-160.

Morais, J.F., de Freitas, M.C.W., Yamaguchi, I.K., dos Santos, M.C. and da Silva, W.D. (1994). Snake antivenoms from hyperimmunized horses: comparison of the antivenom activity and biological properties of their whole IgG and F(ab')2 fragments. *Toxicon*. 32: 725–734.

Morris, A.M., Watzky, M.A. and Finke., R. G. (2009). Protein aggregation kinetics, mechanism, and curve-fitting: A review of the literature. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics.* 1794(3): 375–397.

Murray, K., Bruce, S.A., Hinnen, A., Wingfield, P., Van Erd, P.M., De Reus, A. and Schellekens, H. (1984). Hepatitis B virus antigens made in microbial cells immunise against viral infection. *The EMBO Journal*. 3: 645-650.

Nassal, M. and Schaller, H. (1993). Hepatitis B virus replication. *Trends Microbiol*ogy. 1: 221-228.

Newcombe, A.R., Cresswell, C., Davies, S.,Watson, K., Harris, G., O'Donovan,K. and Francis, K. (2005). Optimised affinity purification of polyclonal antibodies from hyper immunised ovine serum using a synthetic Protein A adsorbent, MAbsorbent® A2P. *Journal Chromatography B*. 814: 209-215.

Ng, M.Y., Tan, W.S., Abdullah, N., Ling, T.C. and Tey, B.T. (2006). Heat treatment of unclarified *Escherichia coli* homogenate improved the recovery efficiency of recombinant hepatitis B core antigen. *Journal of Virological Methods*. 137: 134-139.

Ostreiko, K.K., Tumanova, I.A. and Sykulev. (1987). Production and characterization of heat-aggregated IgG complexes with predetermined molecular masses: light-scattering study. *Immunology Letters* . 15: 311-316.

Pasek, M., Goto, T., Gilbert, W., Zink, B., Schaller, H., MacKay, P., Leadbetter, G. and Murray, K. (1979). Hepatitis B virus genes and their expression in *E. coli*. *Nature*. 282: 575-579.

Perosa, F., Carbone, R., Ferrone, S. and Dammacco, F. (1990). Purification of human immunoglobulins by sequential precipitation with caprylic acid and ammonium sulfate. *Journal of Immunological Methods*. 128: 9-16.

Perz, J.F., Armstrong, G.L., Farrington, L.A., Hutin, Y.J.F. and Bell, B.P. (2006). The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *Journal of Hepatology*. 45: 529-538.

Philo, J.S. and Arakawa, T. (2009). Mechanisms of protein aggregation. *Current Pharmaceutical Biotechnology* 10: 348-351.

Pollack, J.R. and Ganem, D. (1993). An RNA stem–loop structure directs hepatitis B virus genomic RNA encapsidation. *Journal of Virology*. 67: 3254–3263.

Pumpens, P. and Grens, E. (2001). HBV core particles as a carrier for B cell/T cell epitopes. *Intervirology*. 44: 98-114.

Qi, Y., Yan, Z. and Huang, J. (2001). Chromatography on DEAE ion-exchange and Protein G affinity columns in tandem for the separation and purification of proteins. *Journal of Biochemical and Biophysical Methods*. 49: 263-273.

Reik, L.M., Maines, S.L., Ryan, D.E., Levin, W., Bandiera, S. and Thomas, P.E. (1987). A simple, non-chromatographic purification procedure for monoclonal antibodies isolation of monoclonal antibodies against cytochrome P450 isozymes. *Journal of Immunological Methods*. 100: 123–130.

Rojas, G., Jimenez, J.M. and Gutierrez, J.M. (1994). Caprylic acid fractionation of hyperimmune horse plasma: description of a simple procedure for antivenom production. *Toxicon*. 32: 351–363.

Rosenqvist, E., Jøssang, T. and Feder, J. (1987). Thermal properties of human IgG. *Molecular Immunology*. 24:495-501.

Salvamani, S., Goh, Z.H., Ho,K.L, Tey,B.T. and Tan,W.S. (2013). Oligomerization state of the multimerization domain of Nipah virus phosphoprotein. *Process Biochemistry.* 48: 1476-1480.

Schwartz, W., Judd, D., Wysocki, M., Guerrier, L., Brick-Wilson, E. and Boschetti, E. (2001). Comparison of hydrophobic charge induction chromatography on protein A for harvest and purification of antibodies. *Journal Chromatography A*. 908: 251-263.

Shin, K., Hayasawa, H. and Lonnerdal, B. (2001). Purification and quantification of lactoperoxidase in human milk with use of immunoadsorbents with

antibodies against recombinant human lactoperoxidase. *American Journal of Clinical Nutrition.* 73: 984-989.

Simaria, A.S., Turner, R., and Farid, S.S. (2012). A multi-level meta-heuristic algorithm for the optimization of antibody purification process. *Biochemical Engineering Journal*. 69: 144-154.

Skvaril, S.F. and Lutz, H.U. (1988). Electrophoretic properties of human IgG and its subclasses on sodium dodecyl-sulfate-polyacrylamide gel electrophoresis and immunoblots. *Analytical Biochemistry*. 174: 593-600.

Smedley. R., Patterson, J., Miller, R., Massey, J., Wise, A., Maes, R., Wu, P., Kaneene, J. and Kiupel, M. (2007). Sensitivity and specificity of monoclonal and polyclonal immunohistochemical staining for West Nile virus in various organs from American crows (*Corvus brachyrhynchos*). *BMC Infectious Diseases.*7: 49.

Sommer, R., Tscheliessnig, A., Satzer, P., Schulz, H., Helk, B. and Jungbauer, A. (2015), Capture and intermediate purification of recombinant antibodies with combined precipitation methods. *Biochemical Engineering Journal*. 93: 200-211.

Sompayrac, L. (2008). How the immune system works, 3rd ed., Massachusetts: Blackwell. Pg: 24-35.

Stec, J., Bica, L. and Kuzak, J. (2004). Isolation and purification of polyclonal IgG antibodies from bovine serum by high performance liquid chromatography. *Bulletin of the Veterinary Institute in Pulawy.*48: 321-327.

Steinbuch, M. and Audran, R. (1969). The isolation of IgG from mammalian sera with the aid of caprylic acid. *Archives of Biochemistry and Biophysics*. 134: 279–284.

Swanson, J.A. and Kern, D.F. (1994). Characterization of pulmonary endothelial charge barrier. *American Journal of Physiology.* 266: 300-303.

Takahashi, K., Kishimoto, S., Ohori, K., Yoshizawa, H., Machida, A., Ohnuma, H., Tsuda, F., Munekata, E., Miyakawa, Y. and Mayumi, M. (1991). Molecular heterogeneity of e antigen polypeptides in sera from carriers of hepatitis B virus. *Journal Immunology*. 147: 3156-3160.

Tan, W.S. (2002). Inhibition of hepatitis B virus assembly with synthetic peptides derived from the viral surface and core antigens. *Journal of General and Applied Microbiology*. 48: 103-107.

Tan, W.S., Dyson, M.R. and Murray, K. (2003). Hepatitis B virus core antigen: enhancement of its production in *Escherichia coli*, and interaction of the core particles with the viral surface antigen. *Biological Chemistry*. 384: 363-371.

Tan, W.S., McNae, I.W., Ho, K.L. and Walkinshaw, M.D. (2007). Crystallization and X-ray analysis of the T = 4 particle of hepatitis B capsid protein with an

N-terminal extension. *Acta Crystallographica Section F: Structural Biology and Crystallization Communications.* 63: 642-647.

Tey, B.T., Yong, K.H., Ong, H.P., Ling, T.C., Ong, S.T., Tan, Y.P., Ariff, A. and Tan, W.S. (2004). Optimal conditions for hepatitis B core antigen production in shaked flask fermentation. *Biotechnology Bioprocess Engineering*. 9: 374-378.

Tishchenko, G.A., Bleha, M., Skvor, J. and Bostik, T. (1998). Effect of salt concentration gradient on separation of different types of specific immunoglobulins by ion-exchange chromatography on DEAE cellulose. *Journal Chromatography B: Biomedical Sciences and Applications*. 706: 157-166.

Tong, H.-F, Lin, D.-Q., Yuan, X.-M. and Yao, S.-J. (2012). Enhancing IgG purification from serum albumin containing feedstock with hydrophobic charge-induction chromatography. *Journal Chromatography A.* 1244: 116-122.

Towbin, H., Staehelin, T. and Gordon J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proceedings of the National Academy of Sciences.* U.S.A. 76: 4350-4354.

Tu, Z., Xu, Y., Fu, J., Huang, Z., Wang, Y., Liu, B., and Tao, Y. (2015). Preparation and characterization of novel IgG affinity resin coupling anti-Fc camelid single-domain antibodies. *Journal Chromatography B.* 983-984: 26-31.

Van der Vegt, W. Van der Mei, H. C. and Busscher, H.J. (1993). Interface free energies in protein solution droplets on FEB-Teflon by axisymmetric drop shape analysis by profile-IgG versus BSA. *Journal of Colloid and Interface Science*. 156: 129-136.

Verdoliva, A., Basile, G. and Fassina, G. (2000). Affinity purification of immunoglobolins from chicken eggs yolk using a new synthetic ligand. *Journal Chromatography B*. 9: 233-242.

Wang, W. (2005). Protein aggregation and its inhibition in biopharmaceutics. *International Journal of Pharmaceutical.* 289: 1-30.

Wang, W., Nema, S. and Teagarden, D. (2010). Protein aggregation--pathways and influencing factors. *International Journal of Pharmaceutical*. 10; 390:89-99.

Warkentin, P., Wälivaara, B., Lundström, I. and Tengvall, P. (1994). Different surface binding of albumin, immunoglobulin G and fibrinogen. *Biomaterials*.15: 786-795.

Weir, D.M and Stewart, J. (1993). *Immunology* (pp. 94-95). 7th Edition, Churchill Livingstone.

Wongchuphan, R., Tey, B.T., Tan, W.S., Subramanian, S.K., Taip, F.S. and Ling, T.C. (2011). Purification of rabbit polyclonal immunoglobulin G using anion exchangers. *Process Biochemistry*. 46: 101-107.

Wood, P. (2006). Understanding immunology, 2nd ed., Harlow: Pearson. Pg: 41-58.

Wynne, S.A., Crowther, R.A. and Leslie, A.G.W. (1999). The crystal structure of the human hepatitis B virus capsid. *Molecular Cell.* 3: 771-780.

Yang, H., Gurgel, P.V. and Carbonell, R.G. (2009). Purification of human immunoglobulin G via Fc-specific small peptide ligand affinity chromatography. *Journal Chromatography A*. 1216: 910-918.

Zaidi, S., Singh, K., Raisuddin, S., Jafri, A., Saxena, A., Choudhary, S. and Ray, P. (1995). Modulation of primary antibody response by protein A in tumor bearing mice. *Immunopharmacol. Immunotoxicol*. 17: 759-773.

Zhao, W.W., Liu, F.F., Shi, Q.H., Dong, X.Y. and Sun, Y. (2014). Biomimetic design of affinity peptide ligands for human IgG based on protein A-IgG complex. *Biochemical Engineering Journal.* 88: 1-11.