ADSORPTION OF BOVINE SERUM ALBUMIN ONTO IMMOBILIZED COBALT MICROPOROUS AND MESOPOROUS SILICA ADSORBENTS

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Dedicated to all my family especially my husband an my litle son,lecturers and fellow friends...
For all your supports and prayers...

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ABSTRACT

The adsorptions of Bovine Serum Albumin (BSA) onto mesoporous and microporous silica adsorbents were done. The cobalt (Co) metal cation had been immobilized into mesoporous silica adsorbents (MCM-41 and SBA-15) and microporous silica adsorbents (Beta and ZSM-5). These absorbents have been characterized with x-ray diffraction (XRD) and nitrogen gas by Burreut, Emmer and Teller (BET) method. Adsorption of BSA onto these adsorbents has been studied with 5 different pH buffer solutions which were 2, 4, 5, 9 and 11. The results varied with the different pH used. The highest BSA adsorption for these adsorbents was at pH 4 which was close to isoelectric point (pI) for BSA. This is due to the zero net charge of BSA and thus no electrostatic repulsion or attraction between the amino acid. Result also showed that, the best adsorbent was Co-SBA-15 with the concentration of BSA at 0.005 mmol and pH 4 which produced 5.59×10^{-4} mmol per gram of dry adsorbent. When a comparison was made between mesoporous (MCM-41 and SBA-15) and microporous (Beta and ZSM-5) silica adsorbents, mesoporous silica adsorbents produces good adsorption capacity compared to microporous silica adsorbent. The cobalt cation showed its suitability with the adsorbents and produced good adsorption processes. In addition, when the adsorption was assisted with an ultrasound, the adsorption process become much better as can be seen from the results for adsorption using Co-MCM-41 compared to the conventional stirring. From the Fourier transform infrared (FTIR) study, the binding of protein and adsorbent can be explained by the interaction between the molecule of the protein and surface of the adsorbent.

ABSTRAK

Penjerapan Bovin Serum Albumin (BSA) oleh penjerap berliang meso dan penjerap berliang mikro telah dijalankan. Logam kobalt kation (Co) telah digerakkan ke dalam penjerap berliang meso (MCM-41 dan SBA-15) dan penjerap berliang mikro (Beta dan ZSM-5). Penjerap-penjerap ini telah digambarkan melalui penggunaan kaedah belauan sinar-x (XRD) dan gas nitrogen oleh Burreut, Emmer dan Teller (BET). Penjerapan BSA telah dijalankan pada lima jenis pH yang berbeza iaitu 2.0, 4.0, 5.0, 9.0 dan 11.0. Kajian menunjukkan kadar penjerapan untuk penjerap-penjerap berkadar terus dengan nilai pH. Kadar penjerapan BSA yang tertinggi berlaku pada pH 4 di mana pH ini adalah hampir kepada nilai titik isoelektrik (pI) untuk BSA. Ini adalah kerana pada nilai pI, BSA berada dalam keadaan cas yang kosong, jadi tiada daya tarikan elektrostatik atau daya tarikan di antara asid amino. Keputusan juga menunjukkan penjerap yang paling baik adalah Co-SBA-15 dengan kepekatan BSA pada 0.005 mmol pada pH 4 yang digunakan di mana memberi hasil yang baik iaitu 5.59×10^{-4} mmol per gram penjerap. Apabila perbandingan dibuat di antara penjerap berliang meso (MCM-41 dan SBA-15) dan penjerap berliang mikro (Beta dan ZSM-5), penjerap berliang meso memberi keputusan nilai jerapan yang tinggi berbanding penjerap berliang mikro. Pengenalan kation logam iaitu kation kobalt menunjukkan kesesuaiannya dengan penjerap dan memberikan hasil yang baik untuk proses penjerapan. Apabila ultrasonik digunakan dalam proses penjerapan, keputusan menunjukkan hasil penjerapan adalah memuaskan jika dibandingkan penjerapan dengan menggunakan pengadukan biasa di mana Co-MCM-41 memberikan hasil yang baik. Daripada kajian inframerah transformasi Fourier (FTIR), ikatan antara protein dan penjerap dapat dihuraikan melalui interaksi antara protin dan permukaan penjerap.

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LIST OF SYMBOLS

Å	-	Amstrong
C	-	Equilibrium concentration
° C	-	Degree of celcius
h	-	Hours
K_d	-	Langmuir adsorption parameter
Nm	-	Nanometer
q	-	Solute concentration in adsorbent (adsorption capacity)
q_m	-	Langmuir isotherm parameter
wt %	-	Weight percent

LIST OF ABBREVIATIONS

AlO_4	-	Aluminium tetra oxide
Al^{3+}	-	Ion aluminium
BSA	-	Bovine serum albumin
Co^{2+}	-	Ion cobalt
$CoNO_3$	-	Cobalt nitrate
Cu^{2+}	-	Ion copper
CTABr	-	Cetyltrymethylammonium bromide
DNA	-	Deoxyribonucleic acid
Fe^{2+}	-	Ion ferum
FESEM	-	Field emission scanning electron microscopy
FTIR	-	Fourier transform infrared
HCL	-	Hydrochloric acid
H_3PO_4	-	Phosphoric acid
IDA	-	Iminodiacetic acid
IEP	-	Isoelectric point
IMAC	-	Immobilized metal affinity chromatography
KH_2PO_4	-	Potassium phosphate
$KHCO_3$	-	Potassium hydrogen carbonate
K_2HPO_4	-	Di-potassium hydrogen phosphate anhydrous
K_2CO_3	-	Potassium carbonate anhydrous
NH_4OH	-	Ammonia

Ni^{2+}	-	Ion nickel
SiO_4	-	Silicon tetra oxide
TEOS	-	Tetraethyl orthosilicate
TO_4	-	Titanium tetra oxide
UV-VIS	-	Ultraviolet visible spectroscopy
XRD	-	X-ray diffraction
Zn^{2+}	-	Ion zinc

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CHAPTER 1

INTRODUCTION

1.1 Background

Protein are widely used in many sectors especially in pharmaceutical due to its structure that are larger and more complex than the traditional pharmaceutical product such as antibiotic, antianxiety and chemotherapeutic agents (Tavolaro *et al*. 2007). Because of their size, proteins are more flexible than classical pharmaceutical entities. This flexibility allows different proteins to fold into three-dimensionless structures as they are biosynthesized within the cell (Tavolaro *et al*. 2007). According to Mohd-Setapar *et al*. (2008), the separation of vitamins and proteins are important in the food and pharmaceutical industries. High selectivity is often needed to separate these molecules from mixtures containing impurities with similar chemical and physical properties. Zhou *et al*. (2012) concluded in their research regarding the possible non-covalent interactions such as electrostatic interactions, hydrogen bonding and hydrophobic interactions between the chiral amino acids and the natural proteins could affect the adsorption amount and binding state of proteins, which will result in different cell behaviors.

Abudiab *et al*. (1998) reported that, immobilized metal affinity chromatography (IMAC) has become a widespread analytical and preparative separation method for protein, peptides, nucleic acids, hormones and enzymes. Adsorption of protein using immobilized metal cation affinity using microporous and mesoporous silica adsorbents is a new method in purification of protein. The used of immobilized metal cation affinity is to enhanced the effectiveness of protein adsorption. According to Abudiab *et al*. (1998) immobilized metal affinity chromatography (IMAC) is widely used in the separation of biological materials on analytical, laboratory-scale and pilot-scale. The advantages of IMAC include the stability of the metal chelates over a wide range of solvent condition and temperature, the high metal loading that result in high protein loading capacities and the ease of product elution and ligand regeneration (Arnold, 1991). Abudiab *et al*. (1998) mentioned that the advantages of IMAC are consisting of its simplicity, universality, stability and cheapness.

Various chromatographic methods are used such as ion exchange, hydrophobic interaction and so on have been used for the separation of various kind of protein but weak and have many problems. The traditional stationary phase for IMAC are based on soft gel matrices such as agarose or cross-link dextran which are deemed as biologically compatible and highly active sorbents (Hemdam and Porath, 1985a; Hemdam and Porath, 1985b). However, the serious drawbacks of weak mechanical strength of this kind of materials limit its application to some degree especially under high pressure (Gaberc-Porekar and Menart, 2001). Some inorganic adsorbent such as silica based materials such as microporous zeolites, mesoporous molecular sieve silicas of the M41S family including MCM-41 and MCM-48, and SBA-15 might able to overcome this limitation due to their excellent mechanical properties as well as their modifiability (Kisler *et al*. 2001).

Bioproduct are typically sensitive to their environment. For the recovery of the products, many techniques can be used. Among these, adsorption is a major important. The applications of adsorption in food industry require protein to be supplied at high purities by processes such as ion exchange chromatography from natural or synthetic

sources. On the other hand, the applications of molecular sieves for this separation have been limited by the available pore size (< 1.5 nm) (Kisler *et al*. 2001). With the development of synthetic mesoporous molecular sieves the available pore size range has been extended, providing pore large enough to allow access of a number of biological molecules. These materials have unique properties desirable for adsorption including a highly regular structure, uniform pore sizes and high surface area (Agrawal *et al*. 2003).

Vinu and Hartman (2004) stressed that proteins released and solubilized from biological structural matrix usually become very unstable and consequently, are essentially irrelevant from biochemical perspectives. The biochemical solvent is water, and high pressure can maintain this solvent in liquid state at temperature below 0°C. Unstable proteins may survive under such conditions. They found that microporous zeolite and zeotype materials with uniform and molecular size porous are widely used; it cannot adsorb large biomoleculars such as protein due to limitation of micropore size (Vinu and Hartman, 2004).

The used of metal ion affinity adsorbent has opened a new dimension in protein separation. It is a collective term that is proposed to include all kinds of adsorptions whereby metal atoms or ions immobilized on a polymer cause or dominate the interaction at the sorption sites. Besides that, it also stable and inexpensive (Arnold, 1991). In this study, there is using immobilized metal ion affinity has been used. View to the respect efficiency, IMAC compares well with biospecific affinity chromatography, and the immobilized metal ion ligand complexes are more likely to withstand wear and tear than are antibodies or enzymes (Porath *et al*. 1975). It can perform under very mild and non-denaturing conditions. IMAC is suitable for preparative group fractionation of complex extracts and biofluids. It also can be used in high performance mode.

The exploiting the promising features of microporous zeolites and mesoporous molecular sieve silicas for separating biological molecules, including the ability to tailor their pore size in the range appropriate for size exclusion and the ability to immobilized

metal ion affinity ligand in their structural framework could overcome some drawbacks posed by other types of adsorbent, and enhance protein adsorption capacity and selectivity.

1.2 Problem Statement

The method of bioseparation nowadays have their own limitations such as poor yield and selectivity, high cost and many more. So there must be a way to increase the yield and improve the bioseparation processes. By modifying or improving the conventional method of bio separation this limitation can be overcome. Here the protein adsorption using adsorbents will be improved by using immobilized metal cation affinity. By referring to Mustafa *et al*. 2008, they have done the research on protein adsorption using mesoporous silica adsorbent only, so from this study the differences of using among two types of adsorbents which are mesoporous and microporous silica adsorbents will be studied.

1.3 Objectives of Study

- To study the effect of structural, physical and chemical properties on protein adsorption characteristics on microporous zeolites (Beta, H-Beta and ZSM-5) and mesoporous molecular sieve silicas (MCM-41 and SBA-15) as an immobilized metal ion affinity stationary phase for protein separation

- To study the adsorption capacity and selectivity and it differences among metal modified microporous and mesoporous silica adsorbents

- To study the effect of pH on the adsorption characteristic, size of pores and protein binding capacities

- To study the effect of ultrasonic during the adsorption processes as compared to the conventional used using mixing method.

1.3 Scopes of the Study

The aim of the study is to use microporos zeolites (H-Beta and ZSM-5) and mesoporous molecular sieve silicas (MCM-41 and SBA-15) as an immobilized metal ion affinity stationary phase for protein separation. These materials were synthesized and modified in order to study the effect of their structural, physical and chemical properties on protein adsorption characteristics. In addition, in order to enhance protein adsorption capacity and selectivity modification by immobilization by chelated metal ion which is Co^{2+} into microporous zeolite and mesoporous molecular sieve silicas by using impregnation method were studied. The protein adsorption capacity and selectivity of the synthesized and modified as well as immobilized metal ion affinity micoporous zeolites and mesoporous molecular sieve silicas were studied using bovine serum albumin (BSA) as model proteins in the forms of either as synthetic pure solution. Besides that the pH of the protein solution is varied to the five different pH which is pH 2, pH 4, pH 5, pH 9 and pH 11 in order to the study on the effect of pH on the adsorption processes. The concentration of the solution was varied to five concentrations ranging from 0.001 mmol up to 0.005 mmol. The adsorption processes will be compared using ultrasonic as assisted adsorption as using conventional stirring.

The structural, physical and chemical properties of commercial, synthesized and modified microporous zeolite and mesoporous molecular sieve silicas will be determined using XRD, BET nitrogen analyzer, UV-VIS and FTIR. The protein concentration will be determined using UV-VIS spectroscopy. The nature of interaction between protein

surface and the surface of synthesized and modified microporous zeolites and mesoporous molecular sieve silicas will be determine using XRD, BET nitrogen analyzer and FTIR.

1.4 Summary

Adsorption performance and selectivity of BSA on mesoporous and microporous silica adsorbents using immobilized metal cation affinity chromatography will be discuss detail on the next chapter. Chapter 2 will discussed about the literature review about the study. Next is chapter 3 which is telling us about the methodology used in this study. Then chapter 4 is discussing about the result obtained from the experimental work and last chapter is chapter 5 which mentioned the overall conclusion for this study. It is expected that in the future bioseparation industry especially that involving protein separation will benefit more from this separation method.

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