ADSORPTION CHARACTERISTICS OF PROTEIN ON ZEOLITE ADSORBENT

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ADSORPTION CHARACTERISTICS OF PROTEIN ON ZEOLITE ADSORBENT

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A dissertation submitted in partial fulfilment of the
requirements for the award of the degree of
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To beloved family ....

Ismail Maidin, Zakiah Seman, Mohammad Nazri Ismail,

Noor Maizia Ismail & Mohd Zakri ismail,

.... and dearest fiance,

Syukry Mohd Sidek

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ABSTRACT

Adsorption of proteins using zeolites has a great potential in the isolation step of protein industry. Over the years, many interests have been shown in the development of new adsorbents thus has helped the growth of protein separation using adsorption technique. In this research, the protein adsorption capacity of several zeolites is studied over a range of solution pHs. The adsorption of proteins, cytochrome c, α-chymotrypsin and bovine serum albumin on zeolites with various pore diameters, and pH was studied. The concentration of protein was measured using UV/VIS Spectrophotometer. The adsorption capacity for cytochrome c and α-chymotrypsin was found to be the highest at pH 9 and 6 respectively. Bovine serum albumin adsorption was highest at pH 3. Increase in pH above the pI value lead to the decrease in the adsorption capacity for α-chymotrypsin and bovine serum albumin. This is postulated to be due to electrostatic repulsion between proteins and the surface of zeolite. The adsorption of different proteins onto H-Beta zeolite, MCM-41, and ZSM-5 shows the effect of protein properties and zeolites to the adsorption capacity. The adsorption isotherm data of protein is approaching the Langmuir model. FTIR analysis was performed for the adsorbed protein to study the interaction between the protein and H-Beta zeolite surface. The observed decrease in the intensities of amide groups in protein structure is most likely a consequence of binding of protein onto H-Beta zeolite.

ABSTRAK

Penjerapan protein menggunakan zeolite mempunyai potensi yang baik untuk industri pemisahan protein. Perkembangan proses pemisahan protein menggunakan kaedah penjerapan meningkat dengan adanya pertumbuhan dalam penyediaan zeolite. Dalam penyelidikan ini, keupayaan penjerapan di kaji untuk beberapa jenis zeolite dalam larutan pH yang berbeza. Penjerapan protein, cytochrome c, α-chymotrypsin, dan bovine serum albumin ke atas zeolite pelbagai diameter dan pH telah dijalankan. Kepekatan protein telah diukur menggunakan UV/VIS Spectrophotometer. Keupayaan penyerapan yang tertinggi untuk cytochrome c telah di ketahui pada pH 9 dan α-chymotrypsin pada pH 6. Bovine serum albumin merekodkan keupayaan penjerapan tertinggi pada pH 3. Peningkatan nilai pH melebihi nilai tahap elektrostatik menyebabkan pengurangan penjerapan α-chymotrypsin dan bovine serum albumin. Ini adalah disebabkan oleh penolakan elektrostatik antara protein dan permukaan zeolite. Penjerapan protein ke dalam zeolite H-Beta, MCM-41, dan ZSM-5 menunjukkan kesan sifat-sifat protein dan zeolite ke atas keupayaan penjerapan. Data penjerapan protein menghampiri model Langmuir. Analisis FTIR di jalankan untuk mengkaji interaksi di antara protein dan permukaan zeolite H-Beta. Melalui pemerhatian, pengurangan intensiti kumpulan amida di dalam struktur protein berkemungkinan disebabkan oleh percantuman protein ke atas zeolite H-Beta.

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LIST OF SYMBOLS

A	Adsorbance
Al	Aluminum
BSA	Bovine serum albumin
C, c	Concentration, mM
C_e	Equilibrium concentration of the adsorbate in fluid phase, mM
CF	Calibration factor
d	Thickness of the sample
K	Characteristics constant
K_d	Langmuir adsorption parameter, l/mmol
n	Characteristics constant
pH	Negative logarithmic molar concentration of hydrogen ion, -log $[H^+]$
pI	Isoelectric point
PZC	Point zero charge
εύ	extinction coefficient
q, q_e	Solute concentration in adsorbent mmol/g dry adsorbent
q_m	Langmuir isotherm parameter, mmol/g dry adsorbent
FTIR	Fourier transform infrared spectroscopy
SEM	Scanning electron microscopy
Si	Silica
UV-VIS	Ultraviolet and visible regions
XRD	X-Ray diffraction

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CHAPTER I

INTRODUCTION

1.1 Background

Proteins are by far the most abundant biopolymers in living cells and have diverse biological functions such as biocatalyst, structural component, transport molecules, regulatory substances and protective molecules. Proteins exist in the form of enzymes, collagen, hemoglobin, serum albumin, hormones, and antibodies. Proteins also have a diverse array of applications. A large number of protein products are used as foods, food additives and as nutraceuticals which are mostly obtained from various microbial, plant and animal sources. Depending on their specific application, it needs to be processed to varying degrees. Nutraceuticals have greater purity requirements than do food additives and these in turn have to be processed to a greater extend than food.

Pharmaceuticals useful proteins are frequently referred to as therapeutic proteins. Most of the recent development in the area of protein bioseparation are centered on therapeutic proteins. Enzymes, which are biological catalysts, can be used in vitro for

industrial scale catalysis. These enzymes are referred to industrial enzymes and are produced in large quantities. Another major use of enzymes is in diagnostics. Enzymes are also used as components of detergent formulations and cosmetic products.

The rapid development of biological science and biotechnology requires more specific and efficient methods to enrich and separate biomolecules such as proteins, enzymes or nucleic acids from practically complicated systems (Ruedi and Cravatt, 2002). Protein bioseparation which refers to the recovery and purification of protein products from various biological feed streams is an important unit operation in the food, pharmaceutical, and biotechnological industry. Protein bioseparation is at present more important in the bioprocess industry than at any time before. The need of protein purification is driven by several factors including reduction in bulk, concentration enrichment, removal of specific impurities, prevention of catalyst other that the type desired, prevention of catalyst poisoning, recommended product specifications, enhancement of protein stability, and reduction of protein degradation.

High-value bioproducts such as proteins or peptides are usually fragile molecules. Therefore, they require highly specialized and mild processing conditions and may need to be separated from a complex mixture of molecules, including cell debris. This combination of factors makes separation difficult. At present, most separation schemes are scaled-up laboratory procedures and more research works are needed to improve their performance. Hence, before these bioprocesses can become commercially viable, nontraditional, lower-cost separation methods need to be developed. There are many techniques available in protein separation such as membrane filtration, liquid-liquid extraction, precipitation, and chromatography (Harrison, 1994). Different means of separation and purification of proteins are used due to the complexity of the molecules.

Over the years, many interests have been shown in the development of new adsorbents thus has helped the growth of protein separation using adsorption technique. Research is under way to develop extracting solvents, resins, and sorbents that are more selective and have a higher capacity than do current materials. High selectivity is often needed to separate these molecules from mixtures containing impurities with similar chemical and physical properties. Mesoporous molecular sieves of the M41S family, including MCM-41 and MCM-48, offer substantial promise as separations media due to their properties such as a highly regular structure, uniform pore sizes and high surface areas (Kisler et al., 2000).

Proteins adsorb to a variety of types of solid phases, usually in a selective manner (Scopes, 1987). Therefore, there is an inclination towards using the adsorption technique for separation of protein. Although adsorption of protein is the subject of many studies, only a few equilibrium studies are available. Interests in using zeolite based adsorbent for separation have been shown by isotherm studies of Cytochrome C (Vinu and Hartmann, 2004) and studies of adsorption of Pepsin, Glucose Oxidase, Myoglobin, Horsedish Peroxidase, Trypsin and Cytochrome C (Deere et al., 2002). Recently, study on the relation between Al content and performance of sorbents for bovine serum albumin adsorption was also investigated (Ji et al., 2004). For adsorption on mesoporous molecular sieves, there is also study made on Trypsin, Lysozyme, and Riboflavin (Kisler et al., 2000).

Recently, extensive research has been focusing on commercial proteins. Adsorption techniques, especially when adopted in column chromatography, frequently result in purification steps that give the greatest increase in protein purity. However, detailed study is required as the design of adsorption processes is complicated by nonlinear equlibria and by strong solute interactions. More laboratory work has to be conducted to increase the adsorption capacity and deals with the complexity of the solids. The discovery of mesoporous molecular sieves has expanded the available pore

sizes of zeolites, thus opening new possibilities in the immobilization of large molecules such as enzymes and vitamins. The extent of adsorption after a limited contact time depended on the relative sizes of the solutes and the pores, supporting the promise of these materials for size selective separations. However, development of these materials for separations has been limited, particularly for processes exploiting their potential for size-based selectivity. Size selectivity should be enhanced as the pore size approaches the adsorbing solute size, but pore blockages and hindered diffusion may limit the adsorption kinetics and capacity. These limitations maybe reduced by enlarging the pore size relative to that of the solute, though this may also involve sacrificing selectivity.

Based on the limitations mentioned above, it is clear that little work has been carried out to explore the potential of adsorption of proteins. Therefore, more research is required to help increase the knowledge in protein separation since there is a promising future installed for it.

1.2 Objectives and Scopes of Study

The objective of this study is to investigate the adsorption characteristics of proteins on zeolite adsorbent. Apart from that, the study is also keen to develop an equilibrium model of the proteins. Parameters such as pH solution, protein type, concentration and different pore size of zeolites are varied to study the effect to the adsorption characteristics. Also, Fourier Transform Infrared (FTIR) Spectroscopy measurement technique was used to study the interaction between the proteins and zeolites surface due to adsorption.

1.3 Report Outline

This report consists of five chapters which describes the research in detail and sequential manner. Chapter I illustrates the problems faced by the protein industry in separation process as well as the objectives of the study. Chapter II contains the literature review of protein industry, bioseparation as well as zeolite for better understanding on the fundamental of the adsorption process. Chapter III discussed all the materials and methods used throughout the study meanwhile the results are included in Chapter IV. Last but not least, Chapter V summarized the findings of the study and some useful recommendations for further study.

1.4 Summary

An ideal protein bioseparation process should combine high productivity with high selectivity of separation and is feasible at mild operating conditions. Therefore, there is a great potential of using adsorption method in protein separation. Thorough study on the protein separation using adsorbent is required in order to know the adsorption performance. 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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