PROTEIN PURIFICATION BY USING IMMOBILIZED METAL ION AFFINITY (IMA) ADSORBENT

FIDELIA SAWAI ANAK MICHAEL MULOK

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Faculty of Chemical and Natural Resources Engineering
Universiti Malaysia Pahang

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TABLE OF CONTENTS

ABSTRACT

Effective separation and purification of proteins has been an important issue in the biomedical and pharmaceutical industries. A novel protein adsorption has been developed in biotechnology to achieve highly efficient and economical separation processes. Application in separation and purification processes often used the ability of zeolites and other molecular sieves to exclude molecules too large to enter the pores and admit smaller ones. In this study, two zeolites which are H-Y and H-Beta have been modified by adding a type of metal into each zeolite to enhance the performance of the zeolites. Three types of metals were used. They were nickel oxide, ferum oxide and zirconium oxide. The zeolite is used as an immobilized metal ion affinity stationary phase for protein purification. The adsorption of Bovine Serum Albumin (BSA) protein using modified zeolites was studied. The effect of pH on adsorption capacity was studied at three different pHs, namely 3, 5 and 8. It is found that the adsorption capacity is the highest at pH 5 which is the nearest to the pI of BSA. Increase in pH higher than the pI leads to the decrease in the adsorption capacity. This is caused by electrostatics repulsion between protein and the surface of adsorbent. Bovine serum albumin concentration was analyzed by UV/VIS Spectrophotometer. It is obvious that as the concentration is higher, the adsorption of Bovine Serum Albumin (BSA) protein is also higher. This is because as the sample is more concentrated, it contained more protein so the adsorption will also be increased. It can be concluded that the most efficient zeolite is H-Beta combined with zirconium oxide. Ismail *et al.* (2005) has said that the molecular sieve H-Beta zeolite has been explored for its ability to adsorb proteins from aqueous solution in batch experiment. Zirconium oxide is the most efficient metal compared to nickel oxide and ferum oxide. The adsorption isotherms are confirmed to be ideal to the Langmuir model.

ABSTRAK

Pengasingan dan penulenan protein yang efektif telah menjadi isu yang penting dalam industri bioperubatan dan farmasi. Penjerapan protein telah dibangunkan dalam industri bioteknologi untuk mencapai proses pengasingan yang amat efisien dan ekonomikal. Proses pengasingan dan penulenan protein mengaplikasikan kebolehan zeolite dan penapis molekul yang lain untuk menghalang molekul yang terlalu besar daripada memasuki liang-liang pada zeolite dan membenarkan molekul yang lebih kecil melaluinya. Dalam kaji selidik ini, dua jenis zeolite iaitu H-Beta dan HY telah digunakan. Zeolite-zeolite ini telah diubahsuai dengan mencampurkan sejenis logam ke dalam setiap satu zeolite supaya fungsi zeolite dapat dipertingkatkan. Tiga jenis logam telah dipilih iaitu nikel oksida, ferum oksida dan zirkonium oksida. Untuk penulenan protein, zeolite digunakan sebagai tarikan ion logam yang tidak bergerak dalam fasa pegun Kaji selidik ini telah dijalankan dengan menggunakan zeolite yang telah diubahsuai. Nilai pH yang berbeza iaitu pH3, pH5 dan pH8 telah ditetapkan bagi mengkaji kesan pH terhadap kapasiti penjerapan. Penjerapan berlaku paling tinggi pada pH5, iaitu pH yang berdekatan dengan pI protein BSA. Jika pH lebih tinggi dari pI protein, kapasiti penjerapan menjadi lebih rendah. Ini disebabkan oleh daya tolakan elektrostatik antara protein dan permukaan jerapan. Kepekatan protein BSA telah dianalisis menggunakan UV/VIS Spectrophotometer. Jika kepekatan semakin tinggi, penjerapan protein juga semakin tinggi. Ini adalah kerana lebih tinggi kepekatan, ia mengandungi lebih banyak protein jadi penjerapan menjadi lebih tinggi. Zeolite yang paling efisien ialah H-Beta bercampur dengan zirkonium oksida. Ismail *et al.* melaporkan bahawa penapis molekul seperti H-Beta telah dikaji kebolehannya untuk menjerap protein daripada larutan cecair dalam eksperimen sekumpulan. Zikronium oksida adalah logam paling efisien berbanding logam nikel dan ferum. Isoterma penjerapan bagi protein BSA ini adalah ideal dengan model Langmuir.

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

x	-	Quantity adsorbed
m	-	Mass of the adsorbent
P	-	Pressure of adsorbate
k,n	-	Empirical constants
A	-	Gas molecule
S	-	Adsorption site
θ	-	Fraction of the adsorption sites occupied
v_{mon}	-	STP volume of adsorbate
v	-	Volume
θ_E	-	Fraction of empty sites
i	-	Each one of the gases that adsorb
T	-	Temperature
ΔH	-	Entropy change
c	-	Equilibrium constant

LIST OF ABBREVIATIONS

NMR	-	Nuclear Magnetic Resonance
BSA	-	Bovine Serum Albumin
pH	-	Expressing acidity or alkalinity on a logarithmic scale
pI	-	Isoelectric point
DNA	-	Deoxyribonucleic acid
RNA	-	Ribonucleic acid
IMAC	-	Immobilized Metal Ion Affinity Chromatography
HPLC	-	High-performance liquid chromatography

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Proteins are large organic compounds made of amino acids arranged in a linear chain and joined together by peptide bonds between the carboxyl and amino groups of adjacent amino acid residues. The sequence of amino acids in a protein is defined by a gene and encoded in the genetic code. Although this genetic code specifies 20 "standard" amino acids plus selenocysteine and - in certain archaea - pyrrolysine, the residues in a protein are sometimes chemically altered in post-translational modification: either before the protein can function in the cell, or as part of control mechanisms.

Proteins were recognized as a distinct class of biological molecules in the eighteenth century by Antoine Fourcroy and others, distinguished by the molecules' ability to coagulate or flocculate under treatments with heat or acid. For examples at the time included albumin from egg whites, blood, serum albumin, fibrin, and wheat gluten. Dutch chemist Gerhardus Johannes Mulder carried out elemental analysis of common proteins and found that nearly all proteins had the same empirical formula. The term protein to describe these molecules was proposed in 1838 by Mulder's associate Jöns Jakob Berzelius. Mulder went on to identify the products of protein degradation such as the amino acid leucine for which he found a molecular weight of 131 Da.

The difficulty in purifying proteins in large quantities made them very difficult for early protein biochemists to study. Hence, early studies focused on proteins that

could be purified in large quantities, e.g., those of blood, egg white, various toxins, and digestive/metabolic enzymes obtained from slaughterhouses.

Effective separation and purification of proteins has been an important issue in the biomedical and pharmaceutical industries. A novel protein adsorption has been developed in biotechnology to achieve highly efficient and economical separation processes. In many cases, proteins which have similar physical and chemical properties need to be separated, and thus highly selective adsorbents are desired. Microporous molecular sieves, such as zeolite Y, ZSM-5 and zeolite Beta, have played important roles in acid catalysis because of their peculiar pore structures and strong intrinsic acidities (Ismail *et al.*, 2005).

Application in separation and purification processes often used the ability of zeolites and other molecular sieves to exclude molecules too large to enter the pores and admit smaller ones. Similarly, shape-selective catalysis takes advantage of the ability of the pores to favor the admission of smaller reactant molecules, the release of the smaller reaction products molecules, or restriction of the size of transition-state complexes inside the micropores of the zeolite (Shermon, 1999).

Protein separation can be done in a number of ways. For example, using membrane chromatography, glass fiber membrane modified with short-chain organosilicon derivatives, using solution by cellulose acetate (AC)/ polycarbonate (PC) blend ultrafiltration membranes and by using immobilized metal ion affinity chromatography (IMAC).

In this study, immobilized metal ion affinity adsorbent will be used. The zeolites will be modified by inserting three different types of metal, one at the time into two different zeolites. The study will be about which one of these metals can work efficiently with each zeolite to separate the chosen protein.

Immobilized metal ion affinity chromatography (IMAC) has shown promise of isolating desired proteins from a mixture based on their difference of affinity for chelated metal ions. With its technological superiority, such as large adsorption capacity, mild separation condition, simple ligands and wide applications, IMAC has become powerful tool for biotechnological products separation, such as proteins,

amino acids and gene products. In spite of many sophisticated applications for IMAC, the theoretical analysis of immobilized metal chromatography has remained insufficient (Sun XD *et al*., 2000).

1.2 Problem Statement

The efficiency needed for protein separation in biochemical, biomedical and pharmaceutical industries has always been a concern. Many developments in biotechnology have been made in order to accomplish highly effective and economical processes. Therefore highly selective adsorbents are needed.

There is no 100% efficient ways to separate protein. When using adsorption process, one of the types of adsorbents that are used is zeolite. Pure zeolites are still not efficient enough to separate protein. Therefore, in this study, two zeolites which are H-Y and H-Beta will be modified by adding a type of metal into each zeolite to enhance the performance of the zeolites. Three types of metals will be used. They are nickel, ferum and zirconium.

1.3 Objective of the Project

Objective of the study is to use zeolite as an immobilized metal ion affinity stationary phase for protein purification.

1.4 Scope of Research Work

The scope depends on the parameters that are used in the experimental process. One of the parameters is the pH of solution which can affect the adsorption capacity. Increase in pH higher than the protein pI lead to the decrease in the adsorption

capacity for the protein. This is because of electrostatic repulsion between protein and the surface of adsorbent. Other parameters are types of metals and types of zeolites.

CHAPTER 2

LITERATURE REVIEW

2.1 Proteins

Proteins are macromolecules. They are constructed from one or more unbranched chains of amino acids; that is, they are polymers. A typical protein contains 200–300 amino acids but some are much smaller and some much larger.

Proteins have high molar, ranging from about 5000g to $1x10^7$g, and yet the percent composition by mass of the elements in proteins is remarkably constant: 50-55% of carbon; 7% of hydrogen; 23% of oxygen; 16% of nitrogen; and 1% of sulfur (Chang, 2003).

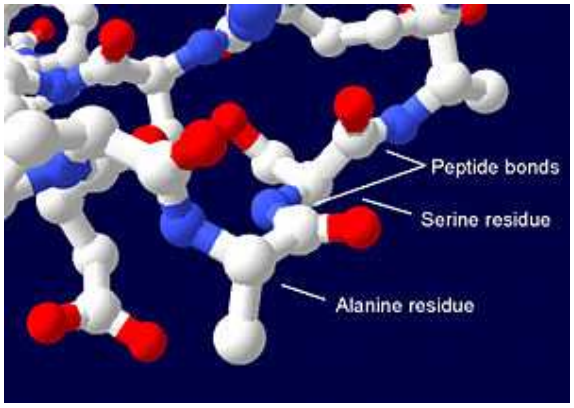

Figure 2.1: Section of a protein structure showing serine and alanine residues linked together by peptide bonds. Carbons are shown in white and hydrogens are omitted for clarity.

All amino acids possess common structural features, including an α carbon to which an amino group, a carboxyl group, and a variable side chain are bonded. Only proline differs from this basic structure as it contains an unusual ring to the N-end amine group, which forces the CO–NH amide moiety into a fixed conformation. The side chains of the standard amino acids, detailed in the list of standard amino acids, have different chemical properties that produce three-dimensional protein structure and are therefore critical to protein function. The amino acids in a polypeptide chain are linked by peptide bonds formed in a dehydration reaction. Once linked in the protein chain, an individual amino acid is called a residue, and the linked series of carbon, nitrogen, and oxygen atoms are known as the main chain or protein backbone. The peptide bond has two resonance forms that contribute some double-bond character and inhibit rotation around its axis, so that the alpha carbons are roughly coplanar. The other two dihedral angles in the peptide bond determine the local shape assumed by the protein backbone.

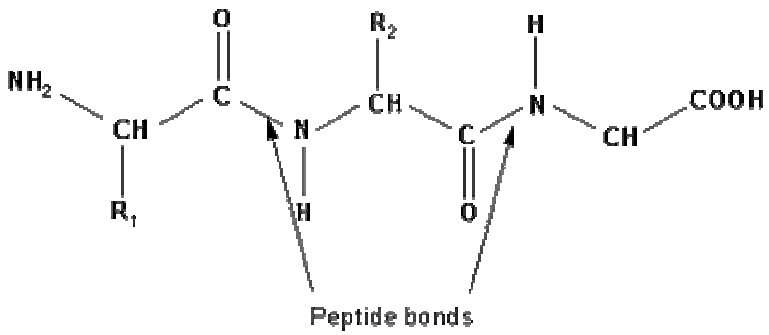

Figure 2.2: The Peptide Bond

Most proteins fold into unique 3-dimensional structures. The shape into which a protein naturally folds is known as its native state. Although many proteins can fold unassisted, simply through the chemical properties of their amino acids, others require the aid of molecular chaperones to fold into their native states.

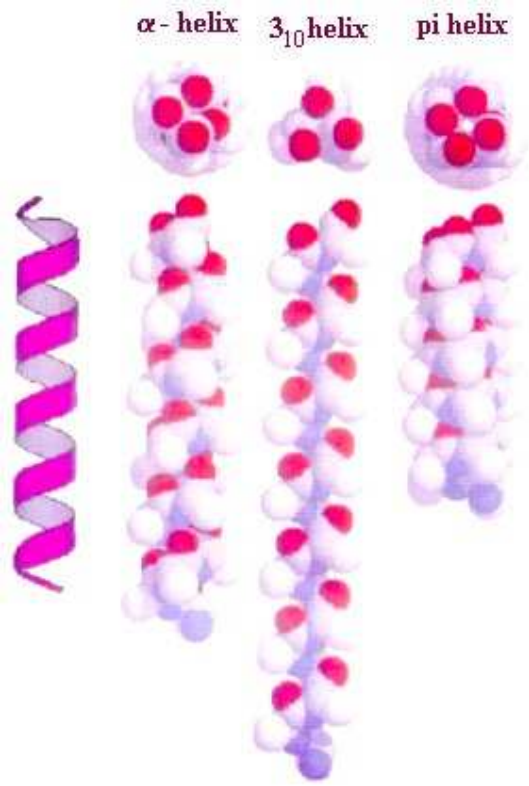

Figure 2.3: Image of alpha helix, 3_{10} helix and pi-helix

Biochemists often refer to four distinct aspects of a protein's structure: Primary structure: the amino acid sequence; Secondary structure: regularly repeating local structures stabilized by hydrogen bonds. The most common examples are the alpha helix and beta sheet. Because secondary structures are local, many regions of different secondary structure can be present in the same protein molecule; Tertiary structure: the overall shape of a single protein molecule; the spatial relationship of the secondary structures to one another. Tertiary structure is generally stabilized by nonlocal interactions, most commonly the formation of a hydrophobic core, but also through salt bridges, hydrogen bonds, disulfide bonds, and even post-translational modifications. The term "tertiary structure" is often used as synonymous with the term fold; Quaternary structure: the shape or structure that results from the interaction of more than one protein molecule, usually called protein subunits in this context, which function as part of the larger assembly or protein complex.

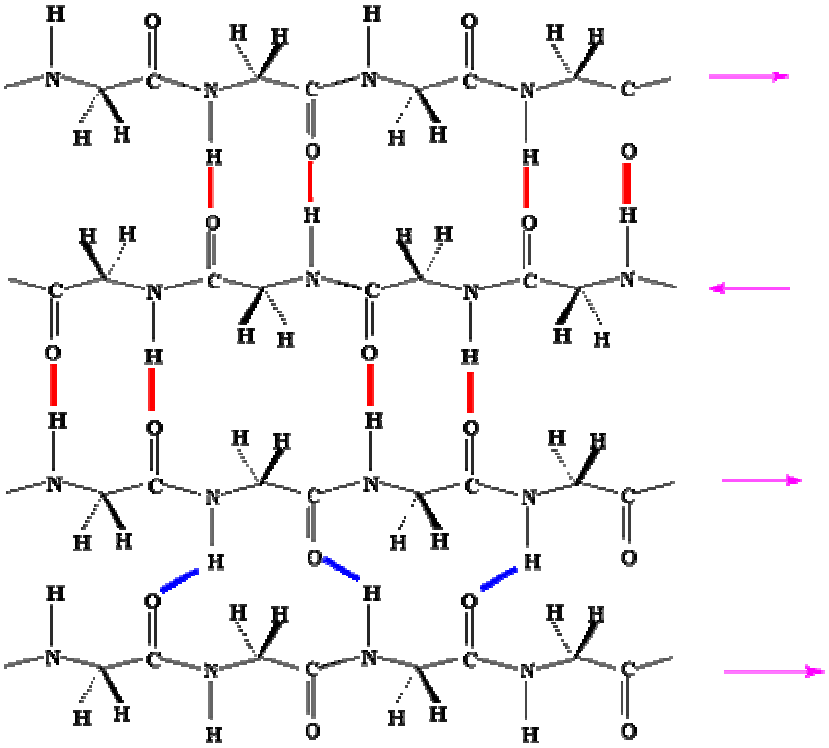

Figure 2:4: Hydrogen bond patterns in beta-sheets

Table 2.1: Parameters of regular secondary structures

Structure	Φ	ψ	n	p(Å)	A	H-bond(CO,HN)
Right-handed alpha helix [3.6_{13}helix]	-57	-47	3.6	5.4	13	i,i+2
3_{10}-helix	-74	-4	3.0	6.0	10	i,i+3
pi-helix	-57	-70	4.4	5.0	16	i,i+4
Parallel beta strand	-119	113	2.0	6.4		
Antiparallel beta strand	-139	135	2.0	6.8		

Notes: n is the number of residues per helical turn.

p is the helical pitch.

A is the atoms in H-bonded loop.

Proteins can be informally divided into three main classes, which correlate with typical tertiary structures. Firstly, fibrous proteins are composed of long linear

polypeptide chains that are bundled together to form rods or sheets. These proteins are insoluble in water and serve structural roles, giving strength and protection to tissues and cells. Secondly, globular proteins are coiled into compact shapes with hydrophilic outer surfaces that make them water soluble. Enzymes and transport proteins are globular to make them soluble in the blood and other aqueous environments in cells (Smith, 2006); and thirdly membrane proteins often serve as receptors or provide channels for polar or charged molecules to pass through the cell membrane.

Discovering the tertiary structure of a protein, or the quaternary structure of its complexes, can provide important clues about how the protein performs its function. Common experimental methods of structure determination include X-ray crystallography and NMR spectroscopy, both of which can produce information at atomic resolution. Cryoelectron microscopy is used to produce lower-resolution structural information about very large protein complexes, including assembled viruses; a variant known as electron crystallography can also produce high-resolution information in some cases, especially for two-dimensional crystals of membrane proteins. Solved structures are usually deposited in the Protein Data Bank (PDB), a freely available resource from which structural data about thousands of proteins can be obtained in the form of Cartesian coordinates for each atom in the protein.

Many more gene sequences are known than protein structures. Further, the set of solved structures is biased toward proteins that can be easily subjected to the conditions required in X-ray crystallography, one of the major structure determination methods. In particular, globular proteins are comparatively easy to crystallize in preparation for X-ray crystallography. Membrane proteins, by contrast, are difficult to crystallize and are underrepresented in the PDB. Structural genomics initiatives have attempted to remedy these deficiencies by systematically solving representative structures of major fold classes. Protein structure prediction methods attempt to provide a means of generating a plausible structure for proteins whose structures have not been experimentally determined.

Proteins are not entirely rigid molecules. In addition to these levels of structure, proteins may shift between several related structures while they perform their biological function. In the context of these functional rearrangements, these

tertiary or quaternary structures are usually referred to as "conformations", and transitions between them are called *conformational changes.* Such changes are often induced by the binding of a substrate molecule to an enzyme's active site, or the physical region of the protein that participates in chemical catalysis. In solution all proteins also undergo variation in structure through thermal vibration and the collision with other molecules, see the animation on the right.

The best-known role of proteins in the cell is their duty as enzymes, which catalyze chemical reactions. Enzymes are usually highly specific catalysts that accelerate only one or a few chemical reactions. Enzymes carry out most of the reactions involved in metabolism and catabolism, as well as DNA replication, DNA repair, and RNA synthesis. Some enzymes act on other proteins to add or remove chemical groups in a process known as post-translational modification. About 4,000 reactions are known to be catalyzed by enzymes. The rate acceleration conferred by enzymatic catalysis is often enormous - as much as 10^{17}-fold increase in rate over the uncatalyzed reaction in the case of orotate decarboxylase (78 million years without the enzyme, 18 milliseconds with the enzyme).

The molecules bound and acted upon by enzymes are known as substrates. Although enzymes can consist of hundreds of amino acids, it is usually only a small fraction of the residues that come in contact with the substrate, and an even smaller fraction - 3-4 residues on average - that are directly involved in catalysis. The region of the enzyme that binds the substrate and contains the catalytic residues is known as the active site. This was first suggested by Emil Fischer in 1894 that both the enzyme and the substrate must be geometrically compatible for them to bind and perform a certain task. This is referred to as the Lock and Key Theory.

Proteins also have structural or mechanical functions, such as actin and myosin in muscle and the proteins in the cytoskeleton, which form a system of scaffolding that maintains cell shape. Other proteins are important in cell signaling, immune responses, cell adhesion, and the cell cycle.

Proteins are also necessary in animals' diets, since animals cannot synthesize all the amino acids they need and must obtain essential amino acids from food.

Through the process of digestion, animals break down ingested protein into free amino acids that are then used in metabolism.

Type of protein that is used in this project is Bovine Serum Albumin (BSA). It is a serum albumin protein that has numerous biochemical applications including Enzyme-Linked Immunosorbent Assay (ELISAs), blots, and immunohistochemistry. Alternative uses are as a nutrient in cell and microbial culture. In restriction digests, BSA is used to stabilise some enzymes during digestion of deoxyribonucleic acid (DNA) and to avoid adhesion of enzyme to reaction tubes and other vessels. It does not affect other enzymes that do not need it for stabilisation. BSA is used because of its stability, its lack of effect in many biochemical reactions, and its low cost since large quantities of it can be readily purified from bovine blood, a byproduct of the cattle industry.

2.1.1 Bovine Serum Albumin (BSA)

2.1.1.1 Structure

The molecular weight of BSA has frequently been cited as 66,120 or 66,267, but it was revised in 1990 to 66,430. All three values are based on amino acid sequence information available at the time of publication.

BSA is a single polypeptide chain consisting of about 583 amino acid residues and no carbohydrates. At pH 5-7 it contains 17 intrachain disulfide bridges and 1 sulfhydryl group.

2.1.1.2 Physical Properties

The pI in Water at 25^0C is 5.2 and the extinction coefficient is 0.667 ml mg^{-1} cm^{-1}.The pH of 1% Solution is 5.2 to 7.Optical rotation for $[\alpha]_{259}$ is 61°and for $[\alpha]_{264}$ is

63°. The stokes radius (r_s) is 3.48 nm. The Sedimentation constant, $S_{20,W}$ X 10^{13} is 4.5 (monomer), 6.7 (dimer). The Diffusion constant, $D_{20,W}$ X 10^{7} is 5.9. The partial specific volume, V_{20} is 0.733. The intrinsic viscosity, η is 0.0413. The frictional ratio, f/f_0 is 1.30. Overall dimensions, Å is 40 X 140. The refractive index increment (578 nm) X 10^{-3} is 1.90. The optical absorbance, $A^{1\ gm/L}$ at 279nm is 0.667. The mean residue rotation, $[m']_{233}$ is 8443 and the Mean residue ellipticity is 21.1 for $[\theta]_{209}$ nm and 20.1 for $[\theta]_{222}$ nm. The estimated α-helix, in percentage (%) is 54 and estimated β-form, in percentage (%) is 18.

2.1.1.3 Solubility/Solution Stability

Albumins are readily soluble in water and can only be precipitated by high concentrations of neutral salts such as ammonium sulfate. The solution stability of BSA is very good (especially if the solutions are stored as frozen aliquots). In fact, albumins are frequently used as stabilizers for other solubilized proteins (e.g., labile enzymes). However, albumin is readily coagulated by heat. When heated to 50°C or above, albumin quite rapidly forms hydrophobic aggregates which do not revert to monomers upon cooling. At somewhat lower temperatures aggregation is also expected to occur, but at relatively slower rates.

2.1.1.4 Product description/usage

Albumins are a group of acidic proteins which occur plentifully in the body fluids and tissues of mammals and in some plant seeds. Unlike globulins, albumins have comparatively low molecular weights, are soluble in water, are easily crystallized, and contain an excess of acidic amino acids. Serum and plasma albumin is carbohydrate-free and comprises 55-62% of the protein present.

Albumin binds water, Ca^{2+}, Na^{+}, and K+. Due to a hydrophobic cleft, albumin binds fatty acids, bilirubin, hormones and drugs. The main biological function of

albumin is to regulate the colloidal osmotic pressure of blood. Human and bovine albumins contain 16% nitrogen and are often used as standards in protein calibration studies. Albumin is used to solubilize lipids, and is also used as a blocking agent in Western blots or ELISA applications. Globulin free albumins are suitable for use in applications where no other proteins should be present (e.g., electrophoresis).

2.2 Zeolites

Zeolites are three-dimensional (similar to honeycomb), microporous, crystalline solids with well-defined structures that contain aluminium, silicon and oxygen in their regular framework.

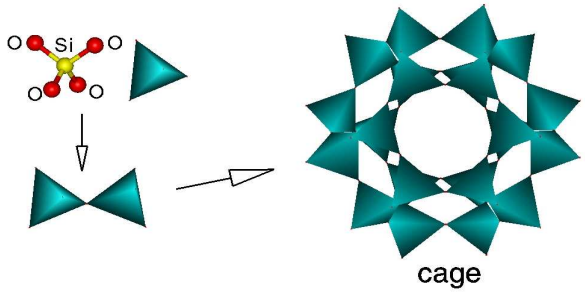

Figure 2.5: Framework structure of a zeolite

Zeolites have the ability to act as catalysts for chemical reactions which take place within the internal cavities. An important class of reactions is that catalysed by hydrogen-exchanged zeolites, whose framework-bound protons give rise to very high acidity. This is exploited in many organic reactions, including crude oil cracking, isomerisation and fuel synthesis. Zeolites can also serve as oxidation or reduction catalysts, often after metals have been introduced into the framework. Examples are the use of titanium ZSM-5 in the production of caprolactam, and copper zeolites in NOx decomposition.

Underpinning all these types of reaction is the unique microporous nature of zeolites, where the shape and size of a particular pore system exerts a steric influence on the reaction, controlling the access of reactants and products. Thus zeolites are

often said to act as shape-selective catalysts. Increasingly, attention has focused on fine-tuning the properties of zeolite catalysts in order to carry out very specific syntheses of high-value chemicals e.g. pharmaceuticals and cosmetics.

The shape-selective properties of zeolites are also the basis for their use in molecular adsorption. The ability preferentially to adsorb certain molecules, while excluding others, has opened up a wide range of molecular sieving applications. Sometimes it is simply a matter of the size and shape of pores controlling access into the zeolite. In other cases different types of molecule enter the zeolite, but some diffuse through the channels more quickly, leaving others stuck behind, as in the purification of para-xylene by silicalite.

Cation-containing zeolites are extensively used as desiccants due to their high affinity for water, and also find application in gas separation, where molecules are differentiated on the basis of their electrostatic interactions with the metal ions. Conversely, hydrophobic silica zeolites preferentially absorb organic solvents. Zeolites can thus separate molecules based on differences of size, shape and polarity.

The loosely-bound nature of extra-framework metal ions (such as in zeolite NaA) means that they are often readily exchanged for other types of metal when in aqueous solution. This is exploited in a major way in water softening, where alkali metals such as sodium or potassium prefer to exchange out of the zeolite, being replaced by the "hard" calcium and magnesium ions from the water. Many commercial washing powders thus contain substantial amounts of zeolite. Commercial waste water containing heavy metals and nuclear effluents containing radioactive isotopes can also be cleaned up using such zeolites.

Zeolites contribute to a cleaner, safer environment in a great number of ways. In fact nearly every application of zeolites has been driven by environmental concerns, or plays a significant role in reducing toxic waste and energy consumption.

In powder detergents, zeolites replaced harmful phosphate builders, now banned in many parts of the world because of water pollution risks. Catalysts, by definition, make a chemical process more efficient, thus saving energy and indirectly

reducing pollution. Moreover, processes can be carried out in fewer steps, minimizing unnecessary waste and by-products. As solid acids, zeolites reduce the need for corrosive liquid acids, and as redox catalysts and sorbents, they can remove atmospheric pollutants, such as engine exhaust gases and ozone-depleting CFCs. Zeolites can also be used to separate harmful organics from water, and in removing heavy metal ions, including those produced by nuclear fission, from water.

Zeolites are divided into two types. There are natural and synthesized zeolites. Most synthesized zeolites are used commercially.

The biggest differences between natural and synthetic zeolites are firstly synthetics are manufactured from energy consuming chemicals and naturals are processed from natural ore bodies. Secondly, synthetic zeolites have a silica to alumina ratio of 1 to 1 and clinoptotilite (clino) zeolites have a 5 to 1 ratio; and lastly, clino natural zeolites do not break down in a mildly acid environment, where synthetic zeolites do. The natural zeolite structure has more acid resistant silica to hold its structure together. The clino natural zeolite is broadly accepted for use in the agricultural industry as a soil amendment and as a feed additive.

2.2.1 Natural zeolites

Natural zeolites form where volcanic rocks and ash layers react with alkaline groundwater. Zeolites also crystallised in post-depositional environments over periods ranging from thousands to millions of years in shallow marine basins. Naturally occurring zeolites are rarely pure and are contaminated to varying degrees by other minerals, metals, quartz or other zeolites. As a result, naturally occurring zeolites are excluded from many important commercial applications where uniformity and purity are essential.

2.2.1.1 Types of Natural Zeolites

There are about 48 naturally occurring zeolites known. One of the natural zeolites is known as **clinoptilolite**. Clinoptilolite is a natural zeolite comprising a microporous arrangement of silica and alumina tetrahedra. It has the complex formula: $(Na,K,Ca)_{2-3}Al_3(Al,Si)_2Si_{13}O_{36}\cdot 12(H_2O)$. It forms as white to reddish tabular monoclinic tectosilicate crystals with a Mohr hardness of 3.5 to 4 and a specific gravity of 2.1 to 2.2. It commonly occurs as a devitrification product of volcanic glass shards in tuff and as vesicle fillings in basalts, andesites and rhyolites. It was described in 1969 from an occurrence in Owl Canyon, San Bernardino County, California.

Use of clinoptilolite in industry and academia focuses on its ion exchange properties having a strong exchange affinity for ammonia (NH_4^+). A typical example of this is in its use as an enzyme based urea sensor. It is also used as fertiliser.

The other type is called **chabazite**. Chabazite is a tectosilicate mineral of the zeolite group with formula: $(Ca,Na_2,K_2,Mg)Al_2Si_4O_{12}\cdot 6H_2O$. Recognized varieties include Chabazite-Ca, Chabazite-K, Chabazite-Na, and Chabazite-Sr depending on the prominence of the indicated cation.

Figure 2.6: Orange chabazite rhombs with white heulandite from Nova Scotia

Chabazite crystallizes in the trigonal crystal system with typically rhombohedral shaped crystals that are pseudo-cubic. The crystals are typically twinned, and both contact twinning and penetration twinning may be observed. They may be colorless, white, orange, brown, pink, green, or yellow. The hardness ranges from 3 to 5 and the specific gravity from 2.0 to 2.2. The luster is vitreous.

It was named chabasie in 1792 by Bosc d'Antic and later changed to the current spelling.

Chabazite occurs most commonly in voids and amygdules in basaltic rocks.

Chabazite is found in India, Iceland, the Faroe Islands, the Giants Causeway in Northern Ireland, Bohemia, Italy, Germany, along the Bay of Fundy in Nova Scotia, Oregon, Arizona, and New Jersey.

Philipsite is another kind of natural zeolite. It is a mineral of the zeolite group; a hydrated potassium, calcium and aluminium silicate, approximating to $(Ca,Na_2,K_2)_3Al_6Si_{10}O_{32}\cdot 12H_2O$. (Also with sodium replaced by calcium: $KCaAl_3Si_5O_{16}\cdot 6H_2O$[1]). The crystals are monoclinic, but only complex cruciform twins are known, these being exactly like twins of harmotome. Crystals of phillipsite are, however, usually smaller and more transparent and glassy than those of harmotome. Spherical groups with a radially fibrous structure and bristled with crystals on the surface are not uncommon. The Mohs hardness is 4.5, and the specific gravity is 2.2. The species was established by A. Levy in 1825 and named after William Phillips. French authors use the name christianite (after Christian VIII of Denmark), given by A. Des Cloizeaux in 1847.

Figure 2.7: Philipsite (unknown scale)

Phillipsite is a mineral of secondary origin, and occurs with other zeolites in the amygdaloidal cavities of mafic volcanic rocks: for example in the basalt of the Giants Causeway in County Antrim, and near Melbourne in Victoria; and in Lencitite near Rome. Small crystals of recent formation have been observed in the masonry of the hot baths at Plombires and Bourbonneles-Bains, in France. Minute spherical aggregates embedded in red clay were dredged by the Challenger from the bottom of the Central Pacific, where they had been formed by the decomposition of lava.

The last example is **mordenite**. This zeolite is a rare zeolite mineral with the chemical formula, $(Ca,Na_2,K_2)Al_2Si_{10}O_{24}\cdot 7H_2O$.

Figure 2.8: Mordenite crystals from India

It was first described in 1864 by Henry How. He named it after the small community of Morden, Nova Scotia, Canada, along the Bay of Fundy, where it was first found.

Mordenite is orthorhombic. It crystallizes in the form of fibrous aggregates, masses, snd vertically striated prismatic crystals. It may be colorless, white, or faintly yellow or pink. It has Mohs hardness of 5 and a density of 2.1. When it forms well developed crystals they are hairlike; very long, thin, and delicate.

The mineral is found in volcanic rock such as rhyolite, andesite, and basalt. It is associated with other zeolites such as stilbite and heulandite. Good examples have been found in Iceland, India, Italy, Oregon, Washington, and Idaho

2.2.2 Synthesized zeolites

Synthetic zeolites are formed by a process of slow crystallisation of a silica-alumina gel in the presence of alkalis and organic templates. One of the vital process used to create zeolite syntheses is sol-gel processing. The product properties depend on reaction mixture composition, pH of the system, operating temperature, pre-reaction ‘seeding’ time, reaction time and templates used. In sol-gel process, other elements for example metals and metal oxides can be easily incorporated.

2.2.2.1 Types of Synthesized Zeolites

There are about 150 zeolite types have been synthesized. One of them is **ZSM-5** zeolite. ZSM-5 (structure type MFI) is an aluminosilicate zeolite mineral belonging to the pentasil family of zeolites. Its chemical formula is $Na_nAl_nSi_{96-n}O_{192}\cdot 16H_2O$ ($0<n<27$). Patented by Mobil Oil Company in 1975, it is widely used in the petroleum industry as a heterogeneous catalyst for hydrocarbon isomerization reactions.

ZSM-5 is composed of several pentasil units linked together by oxygen bridges to form pentasil chains. A pentasil unit consists of eight five-membered rings. In these rings, the vertices are Al or Si and an O is assumed to be bonded between the vertices.

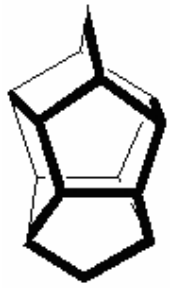

Figure 2.9: Pentasil Unit

The pentasil chains are interconnected by oxygen bridges to form corrugated sheets with 10-ring holes. Like the pentasil units, each 10-ring hole has Al or Si as vertices with an O assumed to be bonded between each vertex. Each corrugated sheet is connected by oxygen bridges to form a structure with “straight 10-ring channels running parallel to the corrugations and sinusoidal 10-ring channels perpendicular to the sheets.” Adjacent layers of the sheets are related by an inversion point. The estimated pore size of the channel running parallel with the corrugations is 5.4 – 5.6 Å.

ZSM-5 is a synthetic zeolite, closely related to ZSM-11. There are many ways to synthesize ZSM-5, a common method is as follows

$SiO_2 + NaAlO_2 + NaOH + N(CH_2CH_2CH_3)_4Br + H_2O \rightarrow$ ZSM-5 + analcime + alpha-quartz

ZSM-5 is typically prepared at high temperature and high pressure in a Teflon coated autoclave and can be prepared using varying ratios of SiO_2 and Al containing compounds.

ZSM-5 has high silicon to aluminum ratio. Whenever an Al^{3+} cation replaces a Si^{4+} cation, an additional positive charge is required the keep the material charge-neutral. With proton (H^+) as the cation, the material becomes very acidic. Thus the acidity is proportional to the Al content. The very regular 3-D structure and the acidity of ZSM-5 can be utilized for acid-catalyzed reactions such as hydrocarbon isomerization and the alkylation of hydrocarbons. One such reaction is the isomerization of meta-xylene to para-xylene. Within the pores of the ZSM-5 zeolite, para-xylene has a much higher diffusion coefficient than meta-xylene. When the isomerization reaction is allowed to occur within the pores of ZSM-5, para-xylene is able to traverse along the pores of the zeolite, diffusing out of the catalyst very quickly. This size-selectivity allows the isomerization reaction to occur quickly in high yield.

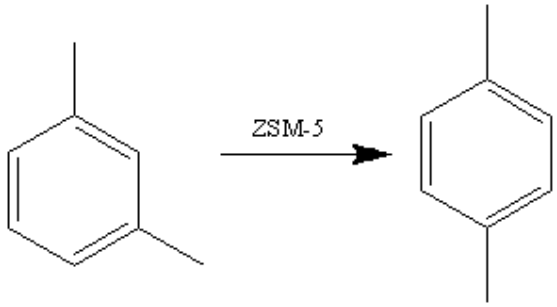

Figure 2.10: Isomerization of ZSM-5

The next example is **ZSM-11**. ZSM-11 is synthesized using the conventional way; it contains at least one of quaternary cations of Group VA as well as substantial amount of sodium ions. ZSM-11 has very similar aluminium contents and particle sizes to ZSM-5. ZSM-11 produces larger amounts of C_g-aromatics and C_{6+}-aliphatics compared to ZSM-5. There is more alkylation activity in the p-xylene-methanol reaction in ZSM-11 than in ZSM-5.

The third example of synthesized zeolite is **SSZ-13**. SSZ-13 (structure type CHA) is an aluminosilicate zeolite mineral belonging to the ABC-6 family of zeolites.

Its chemical formula is $RN_aNa_bAl_{2.4}Si_{33.6}O_{72}•wH_2O$ (1.4<a<27)(0.7<b<4.3)(1<w<7), where RN is N,N,N-1-trimethyladamantammonium. The material was patented by Chevron research Company in 1985, and has a potential use as a heterogeneous catalyst for the methanol-to-olefins (MTO) process.

CHA can be synthesized from the following method. The material is synthesized from the following batch composition:

$$10\ Na_2 : 2.5\ Al_2O_3 : 100\ SiO_2 : 4400\ H_2O : 20\ RN\text{-}OH.$$

2 g 1N NaOH, 2, 78 g 0.72 M N,N,N-1-trimethyladamantammoniumhydroxide and 3.22 g deionized water are mixed. 0,1 g Al_2O_3 is added to the solution and mixed with 0.6 g fumed silica). The resulting viscous gel is aged for two hours and heated for 4 days at 160^oC in a Teflon lined steel autoclave.

SSZ-13 is a high silica zeolite with the CHA topology. Materials with this topology are of industrial interest, as potential catalysts for application in the methanol-to-olefins (MTO) reaction.

Final example of synthesized zeolite is **Faujasite**. It is a mineral from the family of zeolites. It occurs in natural form and is as well synthesized industrially.

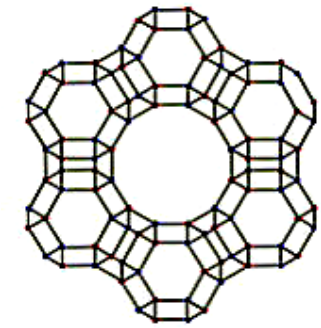

Figure 2.11: Structure of Faujasite

The faujasite framework consists of sodalite cages which are connected through hexagonal prisms. The pores are arranged perpendicular to each other. The pore, which is formed by a 12-membered ring, has a relatively large diameter of 7.4 Å. The inner cavity has a diameter of 12 Å and is surrounded by 10 sodalite cages. The

unit cell is cubic with a length of 24.7 Å. Zeolite Y has a void fraction of 48 % and a Si/Al ratio of 2.43. It thermally decomposes at 793 ℃.

Faujasite is synthesized as other zeolites from alumina sources such as sodium aluminate and silica sources such as sodium silicate. Other alumosilicates such as kaolin are used as well. The ingredients are dissolved in a basic environment such as sodium hydroxide aqueous solution and crystallized at 70 to 300°C (usually at 100°C). After crystallization the faujasite is in it is sodium form and must be ion exchanged with ammonium to improve stability. The ammonium ion is removed later by calcination which renders the zeolite in its acid form.

2.2.3 Immobilized Metal Ion Affinity Chromatography (IMAC)

Immobilized Metal Ion Chromatography (IMAC) is a type of high-performance liquid chromatography (HPLC). This chromatography is the most resourceful and widely-used type of elution chromatography. The method is applied by chemists for separating and determining species in a variety of organic, inorganic, and biological materials.

Immobilized metal ion/Metal Chelate affinity chromatography is separation technique that is based on coordinate covalent binding between proteins and metal ions. Proteins have a wide variety of amino acids composition which, in effect, generates a range of different affinities towards metal ions. However, not many naturally occurring proteins have affinity for metal ions, so the technique is mainly used to purify recombinant proteins. For example proteins can be engineered to contain a poly-histidine tail (histidine can generally act as a ligand towards divalent metal cations). If the stationary phase is immobilized with divalent metal cations, a mixture of proteins can be separated based on their ability to interact with the metal ions. Those proteins containing a higher number of histidine residues would be able to bind to the column more tightly than those with fewer histidine residues.

Several different types of immobilized metal ion column have been developed to separate various proteins (e.g. Fe, Co, Cd, Ni, or Zn). Protein separation in IMAC

generally depends on the strength of the metal ion-protein bond. Thus, choosing the type of immobilized ion is crucial to the success protein separation. By far the most widely-used technique is to use an immobilized nickel column, and to engineer poly-histidine tags of six or more residues onto the recombinant proteins of interest. One thing to keep in mind is that the binding between metal ion and protein must be reversible, allowing elution of bounded protein at later steps. Three different elution strategies can be applied to IMAC competitive elution, stripping elution and pH Adjustment.

2.3 Adsorption

2.3.1 Adsorption Process

Adsorption involves, in general, the accumulation of solute molecules at an interface (including gas-liquid, as in foam fractionation separation; and liquid-liquid). Here we considered only gas-solid and liquid-solid interfaces, with solute from the fluid attaching selectively to the solid. The accumulation per area is small; thus, highly porous solids with very large internal area per unit volume are preferred. The surfaces are usually irregular, and the bonding energies (primarily from Van der Waals forces, as in vapor condensation) vary widely from one site to another. However, with “molecular sieves”, the adsorptive surfaces are provided by channels or cavities within a macrocrystal structure; the sieves exhibit high uniformity of adsorbent surface with a practically constant binding energy (Perry *et al.,* 1984).

Adsorption is a process that occurs when a gas or liquid solute accumulates on the surface of a solid or a liquid (adsorbent), forming a film of molecules or atoms (the adsorbate). It is different from absorption, in which a substance diffuses into a liquid or solid to form a solution. The term *sorption* encompasses both processes, while desorption is the reverse process.

Adsorption is present in many natural physical, biological, and chemical systems, and is widely used in industrial applications such as activated charcoal, synthetic resins, and water purification. Adsorption, ion exchange, and

chromatography are sorption processes in which certain adsorbates are selectively transferred from the fluid phase to the surface of insoluble, rigid particles suspended in a vessel or packed in a column.

Similar to surface tension, adsorption is a consequence of surface energy. In a bulk material, all the bonding requirements (be they ionic, covalent, or metallic) of the constituent atoms of the material are filled by other atoms in the material. However, atoms on the surface of the adsorbent are not wholly surrounded by other adsorbent atoms and therefore can attract adsorbates. The exact nature of the bonding depends on the details of the species involved, but the adsorption process is generally classified as physisorption (characteristic of weak van der Waals forces) or chemisorption (characteristic of covalent bonding).

In adsorption processes one or more components of a gas or liquid stream are adsorbed on the surface of a solid adsorbent and a separation is accomplished. In commercial processes, the adsorbent is usually in the form of small particles in a fixed bed. The fluid is passed through the bed and the solid particles adsorb components from the fluid. When the bed is almost saturated, the flow in this bed is stopped and the bed is regenerated thermally or by other methods so that desorption occurs. The adsorbed material (adsorbate) is thereby recovered and the solid adsorbent is ready for another cycle of adsorption (Geankoplis, 2003).

The overall adsorption process consists of a series of steps in series. When the fluid is flowing past the particle in a fixed bed, the solute first diffuses from the bulk fluid to the gross exterior surface of the particle. Then the solute diffuses inside the pores to the surface of the pore. Finally, the solute is adsorbed on the surface. Hence, the overall adsorption process is a series of steps (Geankoplis, 2003).

2.3.2 Isotherms

Adsorption is usually described through isotherms, that is, the amount of adsorbate on the adsorbent as a function of its pressure (if gas) or concentration (if liquid) at constant temperature. The quantity adsorbed is nearly always normalized by the mass of the adsorbent to allow comparison of different materials.

The first mathematical fit to an isotherm was published by Freundlich and Küster (1894) and is a purely empirical formula for gaseous adsorbates,

$$\frac{x}{m} = kP^{\frac{1}{n}}$$

where x is the quantity adsorbed, m is the mass of the adsorbent, P is the pressure of adsorbate and k and n are empirical constants for each adsorbent-adsorbate pair at a given temperature. The function has an asymptotic maximum as pressure increases without bound. As the temperature increases, the constants k and n change to reflect the empirical observation that the quantity adsorbed rises more slowly and higher pressures are required to saturate the surface.

2.3.2.1 Langmuir

In 1916, Irving Langmuir published a new model isotherm for gases adsorbed on solids, which retained his name. It is a semi-empirical isotherm derived from a proposed kinetic mechanism. It is based on four assumptions:

1. The surface of the adsorbent is uniform, that is, all the adsorption sites are equivalent.
2. Adsorbed molecules do not interact.
3. All adsorption occurs through the same mechanism.
4. At the maximum adsorption, only a monolayer is formed: molecules of adsorbate do not deposit on other, already adsorbed, molecules of adsorbate, only on the free surface of the adsorbent.

These four assumptions are seldom all true: there are always imperfections on the surface, adsorbed molecules are not necessarily inert, and the mechanism is clearly not the same for the very first molecules to adsorb as for the last. The fourth condition is the most troublesome, as frequently more molecules will adsorb on the monolayer; this problem is addressed by the BET isotherm for relatively flat (non-microporous) surfaces. The Langmuir isotherm is nonetheless the first choice for most models of

adsorption, and has many applications in surface kinetics (usually called Langmuir-Hinshelwood kinetics) and thermodynamics.

Langmuir suggested that adsorption takes place through this mechanism: $A_g + S \rightleftharpoons AS$, where A is a gas molecule and S is an adsorption site. The direct and inverse rate constants are k and k_{-1}. If we define surface coverage, θ, as the fraction of the adsorption sites occupied, in the equilibrium we have

$$K = \frac{k}{k_{-1}} = \frac{\theta}{(1-\theta)P} \qquad \text{or} \qquad \theta = \frac{KP}{1 + KP}$$

where *P* is the partial pressure (gas) or the molar concentration of the solution (liquid). For very low pressures $\theta \approx KP$ and for high pressures $\theta \approx 1$.

θ is difficult to measure experimentally; usually, the adsorbate is a gas and the quantity adsorbed is given in moles, grams, or gas volumes at standard temperature and pressure (STP) per gram of adsorbent. If we call v_{mon} the STP volume of adsorbate required to form a monolayer on the adsorbent (per gram of adsorbent), $\theta = \upsilon / \upsilon_{mon}$ and we obtain an expression for a straight line:

$$\frac{1}{\upsilon} = \frac{1}{K\upsilon_{mon}}\frac{1}{P} + \frac{1}{\upsilon_{mon}}$$

Through its slope and *y*-intercept we can obtain v_{mon} and *K*, which are constants for each adsorbent/adsorbate pair at a given temperature. v_{mon} is related to the number of adsorption sites through the ideal gas law. If we assume that the number of sites is just the whole area of the solid divided into the cross section of the adsorbate molecules, we can easily calculate the surface area of the adsorbent. The surface area of an adsorbent depends on its structure; the more pores it has, the greater the area, which has a big influence on reactions on surfaces.

If more than one gas adsorbs on the surface, we define $\boldsymbol{\theta_E}$ as the fraction of empty sites and we have

$$\theta_E = \frac{1}{1 + \sum_{i=1}^{n} K_i P_i}$$

and

$$\theta_j = \frac{K_j P_j}{1 + \sum_{i=1}^{n} K_i P_i}$$

where *i* is each one of the gases that adsorb.

2.3.2.2 BET

Often molecules do form multilayers, that is, some are adsorbed on already adsorbed molecules and the Langmuir isotherm is not valid. In 1938 Stephan **B**runauer, Paul **E**mmett, and Edward **T**eller developed a model isotherm that takes that possibility into account. Their theory is called BET Theory, after the initials in their last names. They modified Langmuir's mechanism as follows:

$A_{(g)} + S \rightleftharpoons AS$

$A_{(g)} + AS \rightleftharpoons A_2S$

$A_{(g)} + A_2S \rightleftharpoons A_3S$ and so on

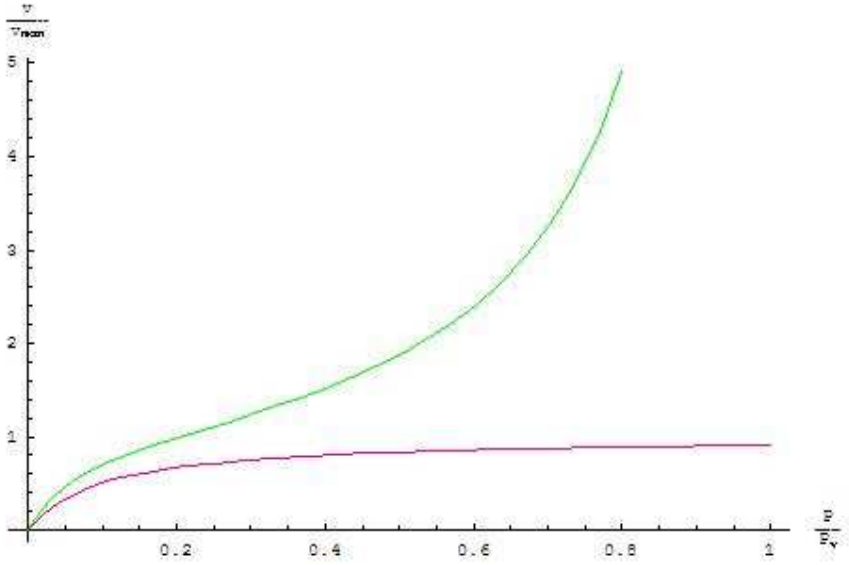

Figure 2.12: Langmuir isotherm (red) and BET isotherm (green)

The derivation of the formula is more complicated than Langmuir's. We obtain:

$$\frac{x}{v(1-x)} = \frac{1}{v_{mon}c} + \frac{x(c-1)}{v_{mon}c}$$

x is the pressure divided by the vapor pressure for the adsorbate at that temperature (usually denoted P / P^0), v is the STP volume of adsorbed adsorbate, v_{mon} is the STP volume of the amount of adsorbate required to form a monolayer and c is the equilibrium constant K we used in Langmuir isotherm multiplied by the vapor pressure of the adsorbate. The key assumption used in deriving the BET equation that the successive heats of adsorption for all layers except the first are equal to the heat of condensation of the adsorbate.

The Langmuir isotherm is usually better for chemisorption and the BET isotherm works better for physisorption for non-microporous surfaces.

2.3.2.3 Adsorption Enthalpy

Adsorption constants are equilibrium constants; therefore they obey Van't Hoff's equation:

$$\left(\frac{\partial InK}{\partial \frac{1}{T}} \right)_{\theta} = -\frac{\Delta H}{R}$$

As can be seen in the formula, the variation of K must be isosteric, that is, at constant coverage. If we start from the BET isotherm and assume that the entropy change is the same for liquefaction and adsorption we obtain $\Delta H_{ads} = \Delta H_{liq} - RT\ln c$, that is to say, adsorption is more exothermic than liquefaction.

CHAPTER 3

METHODOLOGY

3.1 Materials

For adsorption experiments, Bovine Serum Albumin (BSA) is purchased as a sample of protein. H-Y and H-Beta zeolite are used. The metals used are nickel oxide, ferum oxide and zirconium oxide. Aqueous phase are made up in freshly deionized water. Buffer are prepared by using phosphoric acid (H_3PO_4), potassium dihydrogen orthophosphate (KH_2PO_4) and di-potassium hydrogen phosphate anhydrous (K_2HPO_4). All the buffer solutions used are prepared from original chemicals without further purification.

3.2 Preparation of Immobilized Metal Ion Affinity Adsorbent

In preparing the adsorbent, solid-state ion exchange principle is used. H-Y zeolite is mixed with nickel oxide (10% of mixture, by weight percent). The mixture later is grounded using a mortar. Then it will be calcined in a furnace at 873K for 24 hours. This is done to remove impurities. The preparation is repeated for H-Y mix with ferum oxide; H-Y mix with zirconium oxide; H-Beta mix with nickel oxide; H-Beta mix with ferum oxide; and H-Beta mix with zirconium oxide.

3.3 Solution Preparation

3.3.1 Protein Solution Preparation

Bovine serum albumin (BSA) solutions with concentrations ranging from 0.01 mM to 0.03 mM are prepared by dissolving different amounts of bovine serum albumin into deionized water.

3.3.2 Buffer Preparation

In preparing the buffer solutions, phosphoric acid (H_3PO_4) is mixed with potassium dihydrogen orthophosphate (KH_2PO_4) with pH ranging from pH 3.0-4.0. Potassium dihydrogen orthophosphate (KH_2PO_4) is mixed with di-potassium hydrogen phosphate anhydrous (K_2HPO_4) with pH ranging from pH4.0-8.0.

3.4 Experimental Procedures

10 mg of modified zeolite is placed in test tubes containing a mixture of 2mL of Bovine serum albumin (BSA) solution and 1mL of buffer solution at various pH. The samples are continuously shaken at 293K until equilibrium is reached. Samples will be centrifuged for about 10 minutes at 2000 rpm. Centrifugation is made to avoid the interference from scattering particles in the UV-VIS analysis.

Figure 3.1: Refrigerated Centrifuge

3.5 Analytical Procedure

After centrifugation, a sample of supernatant is withdrawn, and the bovine serum albumin concentration is analyzed by UV/VIS Spectrophotometer at 280nm. The amount of protein adsorbed onto each zeolite is calculated based on mass balance.

Figure 3.2: UV/VIS Spectrophotometer

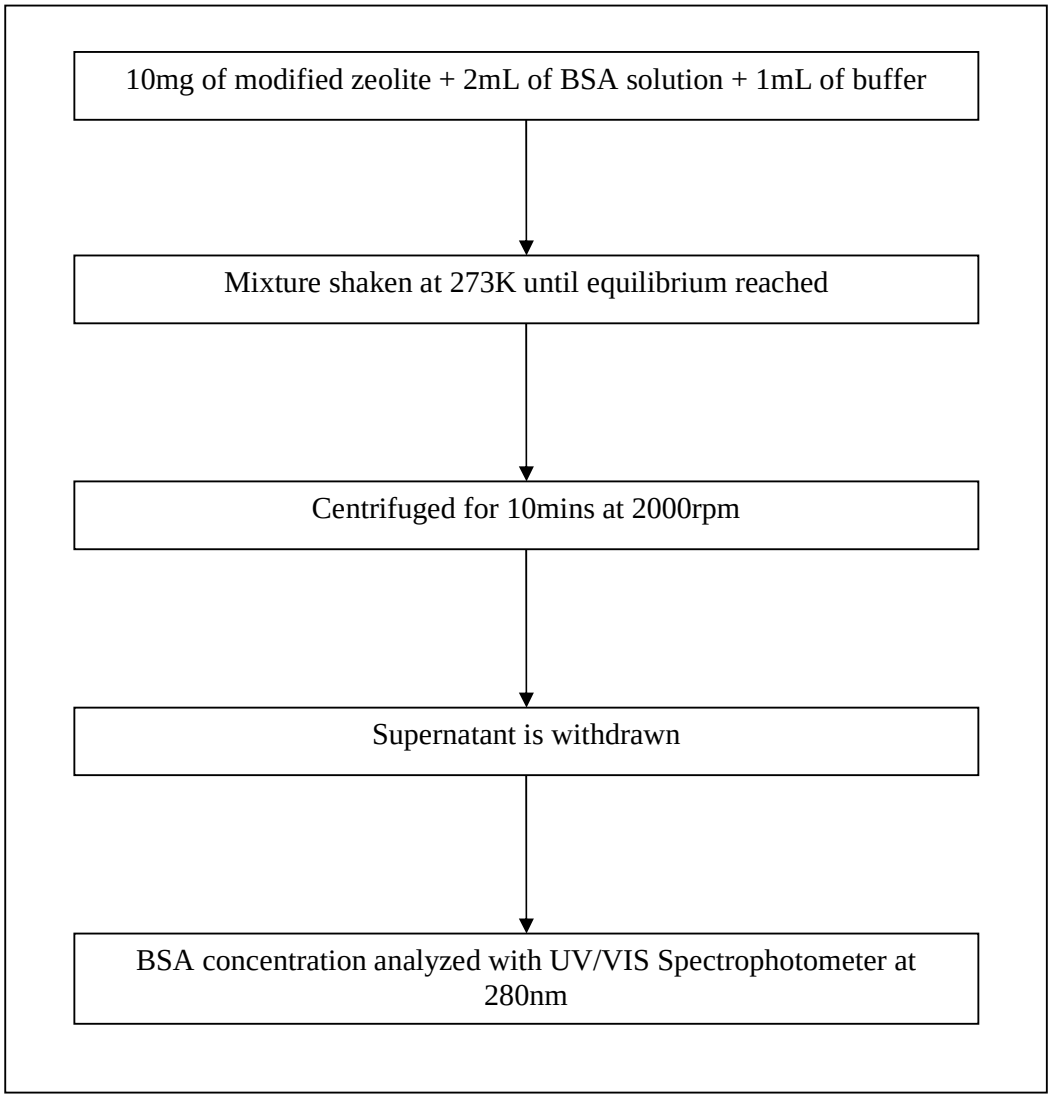

Figure 3.3: Summary of experimental procedures.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Q of Bovine Serum Albumin (BSA) protein solution

The tables below show the values of q for six different adsorbents. As the concentration increases, the values of q also increase.

Table 4.1: The values of q for Beta-Zr

Adsorbent	pH	Concentration (mM)	Adsorbance	q x 10^3 (mmol/g)
Beta-Zr	3	0.010	0.170	0.84
		0.015	0.281	1.05
		0.020	0.965	1.42
		0.025	1.006	1.38
		0.030	1.159	2.40
	5	0.010	0.098	1.38
		0.015	0.153	1.95
		0.020	0.254	2.60
		0.025	0.308	2.31
		0.030	0.454	4.06
	8	0.010	0.172	1.25
		0.015	0.301	0.88
		0.020	0.570	1.05
		0.025	0.721	1.16
		0.030	0.789	1.44

Table 4.2: The values of q for Beta-Ni

Adsorbent	pH	Concentration (mM)	Adsorbance	q x 10^3 (mmol/g)
Beta-Ni	3	0.010	0.275	0.48
		0.015	0.367	0.75
		0.020	0.760	2.12
		0.025	0.778	2.16
		0.030	1.159	2.40
	5	0.010	0.102	1.35
		0.015	0.210	1.61
		0.020	0.245	2.65
		0.025	0.254	2.67
		0.030	0.490	3.85
	8	0.010	0.345	0.29
		0.015	0.378	0.46
		0.020	0.595	0.91
		0.025	0.748	1.01
		0.030	0.812	1.32

Table 4.3: The values of q for Beta-Fe

Adsorbent	pH	Concentration (mM)	Adsorbance	q x 10^3 (mmol/g)
Beta-Fe	3	0.010	0.290	0.43
		0.015	0.380	0.71
		0.020	0.780	2.05
		0.025	0.790	2.12
		0.030	1.230	2.15
	5	0.010	0.167	1.25
		0.015	0.223	1.53
		0.020	0.253	2.60
		0.025	0.271	2.64
		0.030	0.550	3.50
	8	0.010	0.350	0.26
		0.015	0.389	0.40
		0.020	0.598	0.90
		0.025	0.760	0.94
		0.030	0.854	1.08

Table 4.4: The values of q for HY-Zr

Adsorbent	pH	Concentration (mM)	Adsorbance	q x 10^3 (mmol/g)
HY-Zr	3	0.010	0.257	0.53
		0.015	0.357	0.79
		0.020	0.720	2.26
		0.025	0.748	2.27
		0.030	1.179	2.33
	5	0.010	0.085	1.45
		0.015	0.136	2.05
		0.020	0.264	2.54
		0.025	0.315	2.28
		0.030	0.502	3.78
	8	0.010	0.341	0.31
		0.015	0.378	0.46
		0.020	0.565	1.08
		0.025	0.732	1.10
		0.030	0.800	1.38

Table 4.5: The values of q for HY-Ni

Adsorbent	pH	Concentration (mM)	Adsorbance	q x 10^3 (mmol/g)
HY-Ni	3	0.010	0.289	0.43
		0.015	0.370	0.74
		0.020	0.755	2.14
		0.025	0.798	2.10
		0.030	1.222	2.18
	5	0.010	0.191	0.83
		0.015	0.232	1.48
		0.020	0.255	2.59
		0.025	0.258	2.61
		0.030	0.510	3.74
	8	0.010	0.356	0.23
		0.015	0.388	0.40
		0.020	0.608	0.84
		0.025	0.758	0.95
		0.030	0.831	1.21

Table 4.6: The values of q for HY-Fe

Adsorbent	pH	Concentration (mM)	Adsorbance	q x 10^3 (mmol/g)
HY-Fe	3	0.010	0.300	0.39
		0.015	0.390	0.67
		0.020	0.790	2.02
		0.025	0.814	2.04
		0.030	1.245	2.10
	5	0.010	0.226	0.62
		0.015	0.240	1.43
		0.020	0.267	2.52
		0.025	0.260	2.60
		0.030	0.560	3.44
	8	0.010	0.361	0.20
		0.015	0.398	0.35
		0.020	0.620	0.78
		0.025	0.775	0.86
		0.030	0.868	1.00

4.2 Discussion

4.2.1 Effect of adsorbents

The graph below shows the effect of six different adsorbents at various concentrations which occurred at pH 5. The analysis is done to compare which adsorbent is the most effective to adsorb the protein.

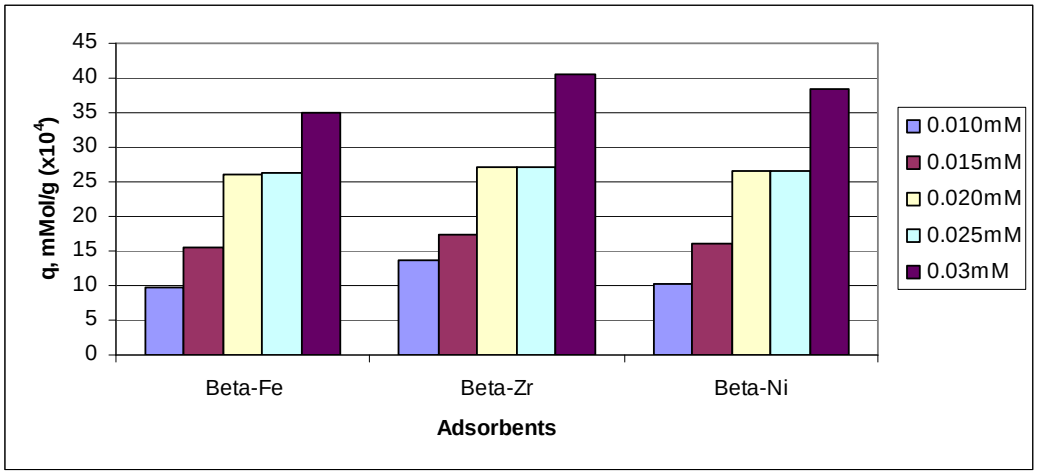

Figure 4.1: Effect of adsorbent H-Beta on the adsorption of BSA protein at pH 5

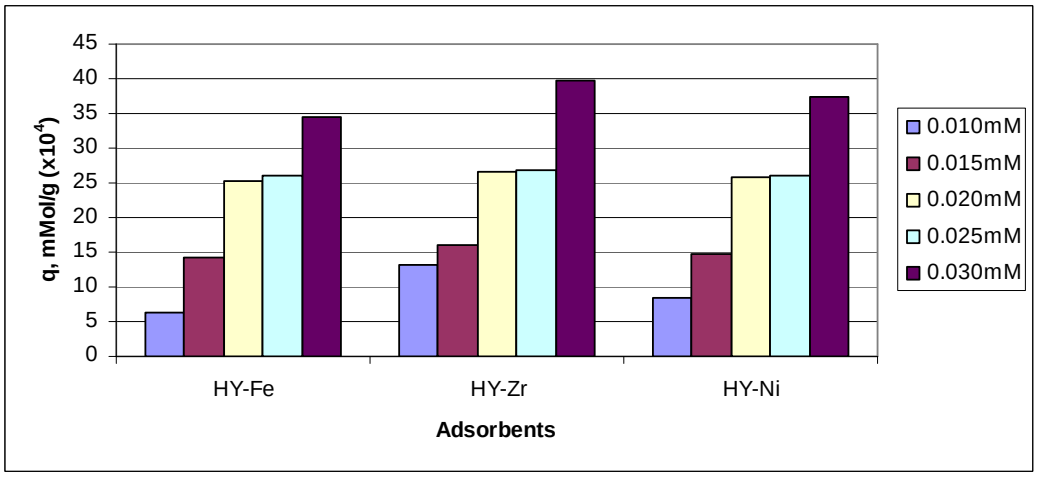

Figure 4.2: Effect of adsorbent H-Y on the adsorption of BSA protein at pH 5

From the chart above, it can be concluded that the most efficient zeolite is H-Beta combined with zirconium oxide. Ismail *et al.* (2005) has said that the molecular

sieve H-Beta zeolite has been explored for its ability to adsorb proteins from aqueous solution in batch experiment.

Zeolite H-Y mixed with zirconium oxide has the second best performance compared to the others. H-Y mixed with ferum oxide has the lowest adsorption capacity. Zeolite H-Beta and zeolite H-Y mixed with nickel oxide have a moderate efficiency.

H-Beta is better because of its bigger surface area compared to Y. The bigger the surface area, the higher the adsorption capacity will be.

Table 4.7 shows the physicochemical properties of Beta and Y. Beta has smaller crystal size compared to Y but has higher surface area than Y.

Table 4.7: Physicochemical properties of Beta and Y

Adsorbent	Adsorbent Structure	Crystal size (μm)	Surface Area (m^2/g)	Pore volume (cm^3/g)
Beta	Beta (BEA)	0.5	745	0.43
Y	Faujasite (FAU)	1.0	615	0.67

4.2.2 Effect of pH

The graphs below show the effect of pH on the adsorption of BSA protein at constant temperature, 293K. The various pH, pH3, pH5 and pH8 are selected to determine the optimum adsorption capacity of the protein.

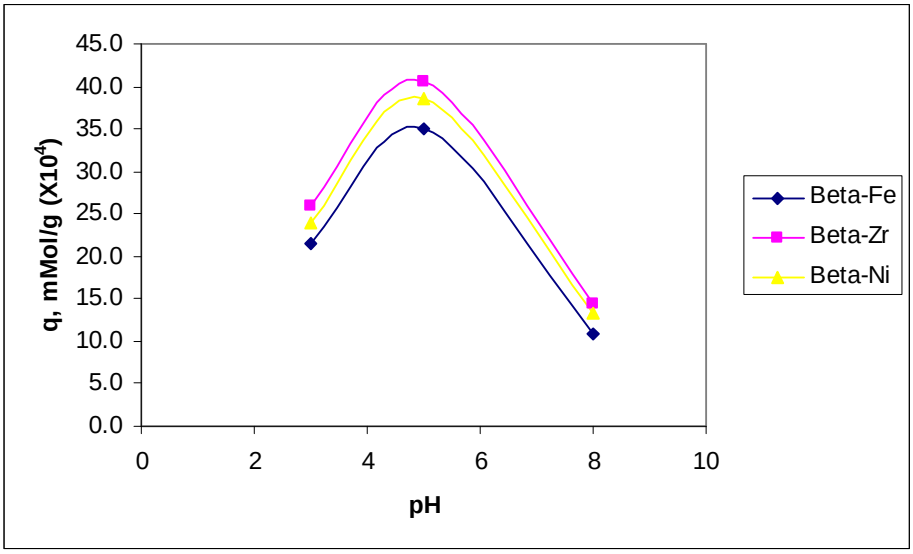

Figure 4.3: Effect o f pH on 0.03mM BSA solution using adsorbent H-Beta.

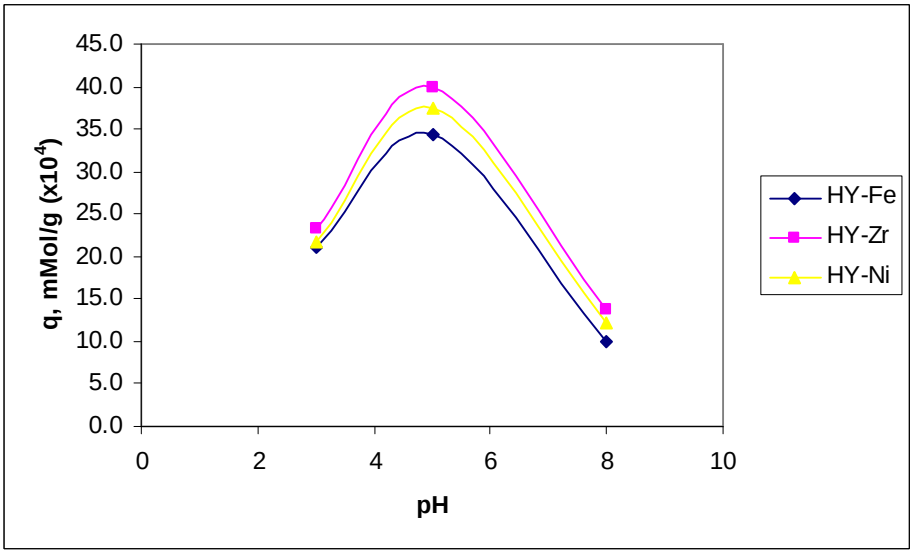

Figure 4.4: Effect of pH on 0.03mM BSA solution using adsorbent H-Y.

The chart above has shown that the maximum adsorption occurred at pH 5. The maximum adsorption occurred near isoelectric point of bovine serum albumin at pH 5.2. At pH 8, which is alkaline has shown that the adsorption of protein is very poor compared to at pH 3 and pH 5.

Increase in pH exceeding the protein pI value decrease the adsorption capacity due to electrostatic repulsive forces (Ismail *et al*., 2005). The adsorption increases with decreasing pH, although the effect is less marked at lower protein concentrations (Ellingsen, 1991).

The solution pH controls the overall charge of the BSA molecules, which increases as the pH deviates away from the isoelectric point (pH 5.2). The electrostatic repulsion resulting from the BSA charged functional groups influences the molecule conformation, especially at low ionic strength (Fullerton *et al*., 2006). As a result of conformational changes with pH, BSA molecules unfold and form different isomerization. For example, at low pH, BSA molecules have a tendency to aggregate due to dimerization (Brahma *et al*., 2005). Although BSA molecules are denatured at very low pH (pH 2), the molecules still behave like polymers and have the ability to adsorb to surfaces (Pincet *et al.*, 1994).

4.2.3 Effect of concentration

The bar chart below shows the effect of concentration on the adsorption of BSA protein solution. There are five different concentrations, 0.01mM, 0.015mM, 0.02mM, 0.025mM and 0.03mM. Various concentrations are used to analyse which concentration has the most adsorbance value.

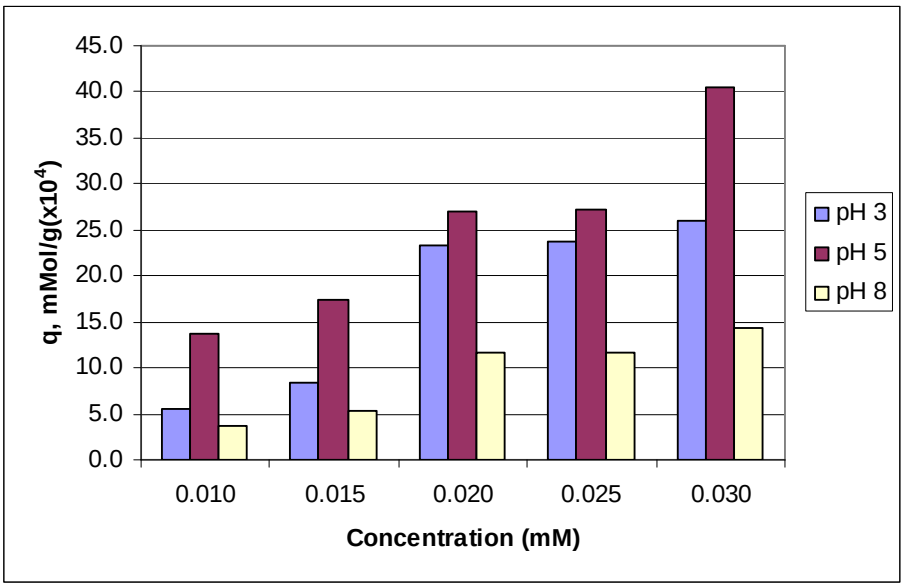

Figure 4.5: Effect of concentration on the adsorption of BSA by using Beta-Zr zeolite.

From the bar chart above, it is obvious that as the concentration is higher, the adsorption of Bovine Serum Albumin (BSA) protein is also higher.

Increasing the adsorbate concentration to 0.03mM increased the amount of the proteins adsorbed significantly.

At high concentration, the surface is covered by a thicker boundary layer due to very little time for the molecules to change conformation before the surface is covered (Omoniyi et al., 2007).

4.2.4 Isotherms

Adsorption is usually described through isotherms, that is, the amount of adsorbate on the adsorbent as a function of its pressure (if gas) or concentration (if liquid) at constant temperature. The quantity adsorbed is nearly always normalized by the mass of the adsorbent to allow comparison of different materials.

The Langmuir model is used to examine the isotherm adsorption data. A linear form of the Langmuir equation is used to find the dissociation coefficient, kd and maximum bovine serum albumin (BSA) protein binding capacity, q_m as well as the correlation coefficient. It is found that the Langmuir type expression fits the equilibrium isotherm data.

4.2.4.1 Effect of pH

The graph below shows the adsorption isotherm of different pH using adsorbent Beta-Zr. There are three different pH used, pH 3, pH 5 and pH 8.

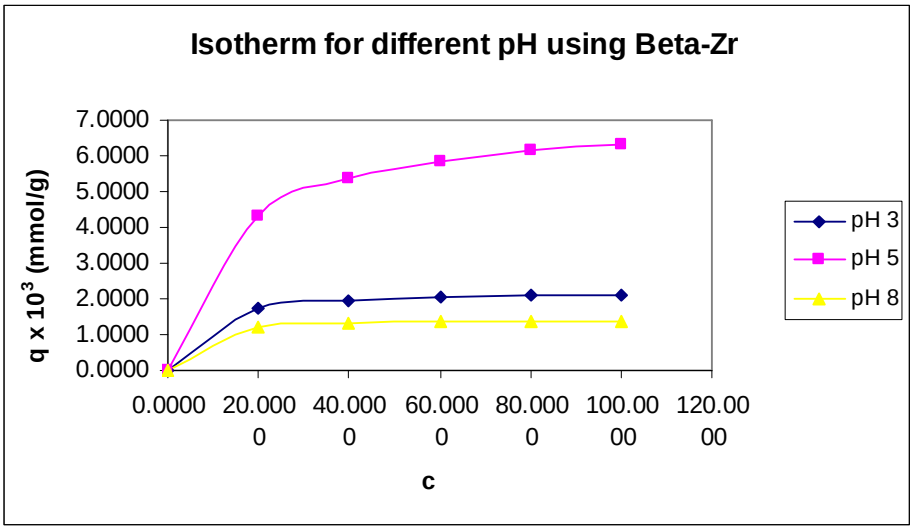

Figure 4.6: Isotherm for different pHs using Beta-Zr

The table above shows the adsorption isotherms of BSA protein at various pH. pH 5 has the highest adsorption isotherm compared to pH 3 and pH 8. The isotherm is

suggesting that the strongest interaction with the molecular sieve occurred at pH 5 which is nearest to the protein pI value which is pH 5.2.

4.2.4.2 Effect of different metal ions

The graph below shows the adsorption isotherm of different metal ions using adsorbent Beta at pH 5. The metals used are nickel oxide, ferum oxide and zirconium oxide.

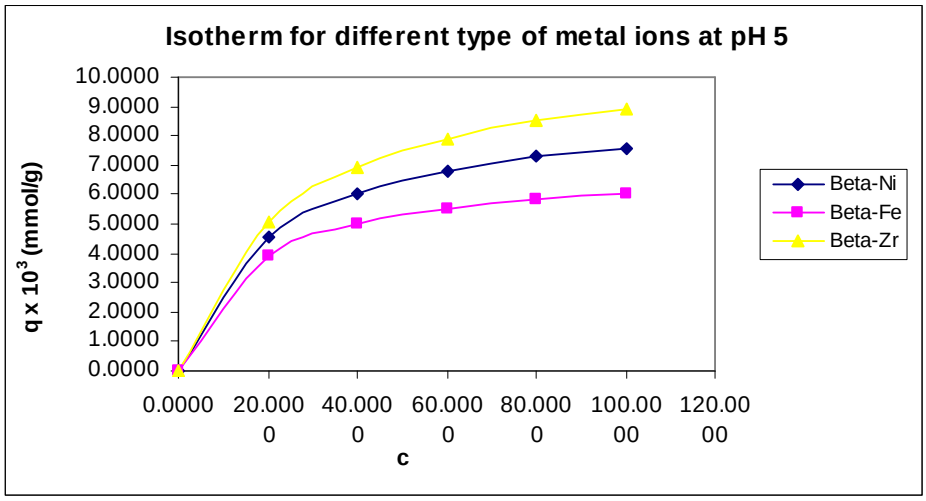

Figure 4.7: Isotherm for different metal ions using adsorbent Beta at pH 5.

From the graph above, it is obvious that the isotherm is well ideal with the Langmuir equation. The highest adsorption isotherm is Beta-Zr followed by Beta-Ni and Beta-Fe.

Zirconium oxide is the most efficient metal compared to nickel oxide and ferum oxide. Feng *et al.* (2007) said conventional IMAC adsorbents with iminodiacetic acid as the chelating group to immobilize Fe^{3+} lack enough specificity for efficient phosphoproteome analysis. He and his team also reported IMAC adsorbent through Zr^{4+} chelation to the phosphonate-modified poly(glycidyl methacrylate-co-ethylene dimethacrylate) polymer beads has a high specificity by effectively enriching phosphopeptides from the digest mixture of phosphoprotein (α- or ß-casein) and bovine serum albumin with molar ratio at 1:100.

4.2.4.3 Effect of adsorbents

The graph below shows the adsorption isotherm for different adsorbents at pH 5 using Beta and HY.

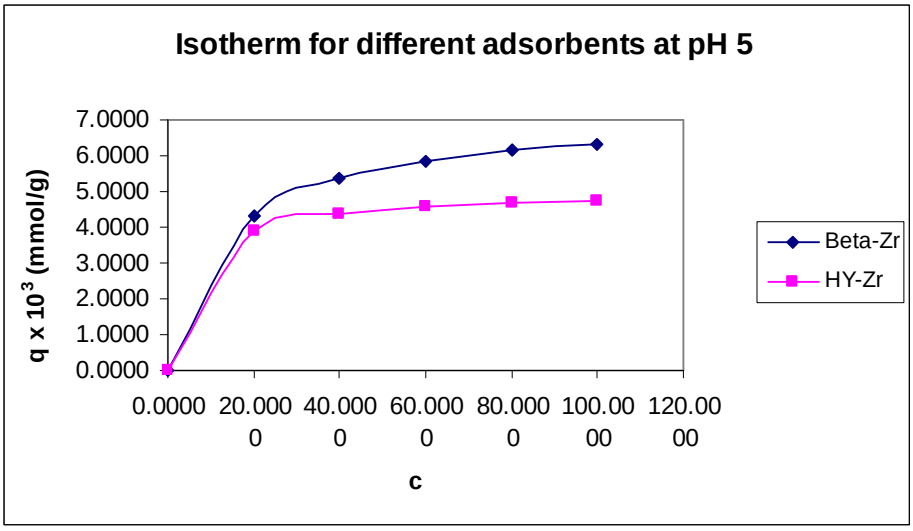

Figure 4.8: Isotherm for different adsorbents at pH 5

From the table it is known that H-Beta zeolite has a higher adsorption isotherm compared to HY-Zr. H-Beta zeolite also has a bigger surface area compared to HY. The bigger the surface area, the higher the adsorption capacity will be. Therefore, it is able to allow more BSA to penetrate through it.

CHAPTER 5

CONCLUSION

5.0 Conclusion

Microporous molecular sieves, such as zeolite Y, ZSM-5 and zeolite Beta, have played important roles in acid catalysis because of their peculiar pore structures and strong intrinsic acidities (Ismail *et al.*, 2005). During this research, it was discovered that the most efficient and effective method of protein purification is by combining zeolite H-Beta with zirconium oxide. Ismail *et al.* (2005) has said that the molecular sieve H-Beta zeolite has been explored for its ability to adsorb proteins from aqueous solution in batch experiment.

Protein size, isoelectric point and also protein surface chemistry are important parameters influencing the adsorption process. The maximum adsorption occurred at pH 5 because it is closer to the pI of the protein. Increase in pH higher than the protein pI lead to the decrease in the adsorption capacity for the protein. The adsorption increases with decreasing pH, although the effect is less marked at lower protein concentrations (Ellingsen, 1991).

The solution pH controls the overall charge of the BSA molecules, which increases as the pH deviates away from the isoelectric point (pH 5.2). The electrostatic repulsion resulting from the BSA charged functional groups influences the molecule conformation, especially at low ionic strength (Fullerton *et al*., 2006). As a result of conformational changes with pH, BSA molecules unfold and form different

isomerization. For example, at low pH, BSA molecules have a tendency to aggregate due to dimerization (Brahma *et al*., 2005). Although BSA molecules are denatured at very low pH (pH 2), the molecules still behave like polymers and have the ability to adsorb to surfaces (Pincet *et al.*, 1994).

As the concentration is higher, the adsorption of Bovine Serum Albumin (BSA) protein is also higher. This is because as the sample is more concentrated, it contained more protein so the adsorption will also be increased. At high concentration, the surface is covered by a thicker boundary layer due to very little time for the molecules to change conformation before the surface is covered (Omoniyi et al., 2007).

The Langmuir model is used to examine the isotherm adsorption data. A linear form of the Langmuir equation is used to find the dissociation coefficient, kd and maximum bovine serum albumin (BSA) protein binding capacity, q_m as well as the correlation coefficient. It is found that the Langmuir type expression fits the equilibrium isotherm data.

The shape-selective properties of zeolites are also the basis for their use in molecular adsorption. The ability preferentially to adsorb certain molecules, while excluding others, has opened up a wide range of molecular sieving applications. Sometimes it is simply a matter of the size and shape of pores controlling access into the zeolite.

5.1 Recommendations

Before the calcination is done, it is best to flatten the zeolites mixed with metal ions evenly in the crucible before placing it in the furnace. This is done so that the calcination occurs all around the mixer. Therefore, maximum removal of impurities will happen. When all the impurities are removed, the adsorption capacity will be maximized.

Secondly, after the centrifugation procedure, avoid shaking the centrifuged solution to prevent the purified solution to remix with zeolite. When the purified protein is accidentally remixed, it will influence the UV-VIS Spectrophotometer analysis.

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