PROTEIN PURIFICATION USING ZEOLITE ADSORBENT

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I declare that this thesis entitled "*Protein Purification Using Zeolite Adsorbent"* is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature :

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Date : 30 APRIL 2009

To my beloved father and mother, Mr Faisal and Madam Nooreini

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IN THE NAME OF ALLAH THE MOST GRACIOUS AND THE MOST MERCIFUL

Praise to Allah, the Lord of the universe for His bounties bestowed upon us. Solawatus Salam to the Holy Prophet Muhammad S.A.W, the sole human inspiration worthy of imitation. I am grateful to Allah the Almighty, for gracing me with strength to complete this thesis.

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ABSTRACT

There has been much interest generated in the development of protein purification since many of modern biotechnology products can be produce from purified protein such as insulin, vaccines and food additives. The aim of this research is to study the most optimum condition to purify protein in order to produce a high purity protein with low cost by applying the principle adsorption process using zeolite adsorbent. Adsorption of Bovine Serum Albumin used as protein sample on Y, Beta and ZSM-5 zeolites at various pH values was carried out in sequence to investigate the effect of different pH value and different types of adsorbent on protein purification. Batch adsorption experiment was carried out by contacting the same amount of different zeolites synthesized with the Bovine Serum Albumin solution. Outcome of the experiment shows that maximum adsorption capacity occurred when Y zeolite was used as the adsorbent at pH 5 that is close to isoelectric point (pI) of Bovine Serum Albumin which is 4.7. This proves that adsorption capacity depends on the physicochemical properties of the zeolite as well as the pH of protein solution. The maximum adsorption on the zeolites tended to occur when the pH was at or just below the pI of the protein. At this pH, the protein sample is positively charged. Adsorption isotherms obtained on effect of different type of adsorbent at pH5 and effect of different pH on Y zeolite were found to be confirmed well to the ideal Langmuir model equation. In order to improve in the next study, other parameters such as protein size, temperature of the protein solution and protein concentration need to be considered as well.

ABSTRAK

Kini telah ramai yang menunjukkan minat di dalam penulenan protin apabila banyak produk bioteknologi yang moden dapat dihasilkan dari protin yang telah menjalani proses pemisahan seperti insulin, vaksin dan makanan tambahan. Tujuan kajian ini dijalankan adalah untuk mengenal pasti keadaan terbaik bagi penulenan protin dengan tujuan untuk menghasilkan protein yang mempunyai tahap ketulenan yang tinggi dengan kos pembiayaan yang rendah dengan cara mengaplikasikan prinsip proses penjerapan menggunakan zeolite. Penjerapan Bovine Serum Albumin yang digunakan sebagai sampel protin dijalankan ke atas zeolite Y, Beta dan ZSM-5 pada pelbagai nilai pH bertujuan untuk mengkaji kesan kepelbagaian nilai pH dan kesan penggunaan jenis zeolite yang berbeza ke atas proses penulenan protin. Kajian penjerapan secara 'batch' dijalankan dengan memasukkan jumlah zeolite yang seragam bersama-sama dengan larutan Bovine Serum Albumin. Keputusan daripada hasil kajian menunjukkan bahawa nilai maksimum penjerapan terjadi apabila zeolite Y digunakan pada pH 5, di mana pH tersebut merupakan pH yang paling hampir dengan nilai titik isoelektrik bagi Bovine Serum Albumin iaitu pada 4.7. Ini membuktikan bahawa kapasita penjerapan bergantung kepada sifat kimia-fizikal zeolite serta pH bagi larutan protin. Penjerapan maksima pada zeolite cenderung terjadi apabila nilai pH berada pada nilai titik isoelektrik atau nilai yang berhampiran dengan nilai titik isoelektrik bagi protin tersebut. Data keputusan yang diperoleh bagi penjerapan protin oleh zeolite didapati mematuhi konsep ideal Langmuir. Dalam rangka untuk mempertingkatkan data keputusan yang diperoleh untuk kajian selanjutnya, skop kajian lain seperti saiz protin, suhu bagi larutan protin dan kepekatan larutan protin perlu dipertimbangkan juga.

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LIST OF ABBREVIATIONS

BSA	-	Bovine Serum Albumin
DNA	-	Deoxyribonucleic acid
RNA	-	Ribonycleic acid
Da	-	Dalton
kDa	-	kiloDalton
SDS	-	Sodium Dodecyl Sulfate-polyacrylamide
NLLS	-	Non Linear least Squares
EF-AL	-	Extra Framework Aluminium
FAU	-	Faujasite
ELISA	-	Enzyme-Linked Immunosorbent Assay
pI	-	isoelectric point
RPM	-	revolutions per minute
Nm	-	nanometer
BEA	-	Beta
MFI	-	Pentasil

LIST OF SYMBOLS

G	-	Energy
H	-	Enthalpy
T	-	Temperature
x	-	Quantity adsorbed
m	-	Mass
P, P_0	-	Pressure
k,n	-	Empirical constant
θ	-	Theta
α	-	Alpha constant
q	-	Solute concentration
q_m	-	Langmuir isotherm parameter
C	-	Equilibrium concentration
K_d	-	Langmuir adsorption parameter
R^2	-	Regression value
V, V_m	-	Adsorbed gas quantity

CHAPTER 1

INTRODUCTION

1.1 Introduction

Proteins are large organic compounds made of amino acids formed in a linear chain. They are joined together by peptide bonds between the carboxyl and amino groups of adjacent amino acid residues. The study of proteins and their function is central to understanding both cells and organisms. There are a few reasons why proteins are important in biology which are; protein serve as a catalyst that maintain metabolic processes in the cell and also they serve as structural elements both within and outside the cell.

Various chromatographic methods such as ion exchange, affinity and hydrophobic interaction have been used for the separation of various kinds of proteins, but these resins have many difficulties. Some of them are very weak chemically and physically, for example, strong acid and alkali or high and low temperature will affect the process. Many proteins that are released and solubilized from biological structural matrices become very unstable and consequently, are essentially irrelevant from a biochemical perspective. Thus, a study had been conducted to prove those zeolites are able to adsorb protein on their surface with the intention to purify the protein (C.Hiroyuki, 2002).

Protein purification is the separation of a specific protein from contaminants in a manner that produces a useful end product. Effective separation and purification of proteins have been an important issue in the biomedical and pharmaceutical industries. Protein adsorption has been developed in biotechnology to achieve highly efficient with an 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. Micro porous molecular sieves, such as zeolite Y, ZSM-5 and Beta zeolite, have played important roles in acid catalysis because of their peculiar pore structures and strong intrinsic acidities.

It is challenging to develop simple, low cost, and scalable methods for large scale recombinant protein purification with a reasonable separation solution. Low cost protein purification methods are in high demand for mass production of low selling price enzymes that play an important role in the upcoming bio-economy (Jiong Hong, 2008). The surface adsorption of proteins on heterogeneous supports is important in a wide variety of medical and biochemical implant in a living body, the cell growth in a culture, or the functionality of a biosensor.

Zeolites are inorganic materials which have a highly ordered structure and can be synthesized with a nanocrystalline size. Zeolites also offer interesting characteristics, such as mechanical and chemical resistance as well as high surface area. On the other hand, zeolites are also known to be stale both in wet and dry conditions and well tolerated by microorganism, and therefore normally compatible with biochemical analyses.

Zeolites due to their low toxicity and high compatibility are considered new biomaterials for medical applications (Adalgisa Tavolaro, 2006). In this work, the protein adsorption on several type of zeolite at various pH were carried out using Bovine Serum Albumin (BSA) as model proteins. The interaction between protein and zeolite adsorbent will be studied using UV-VIS Spectrophotometer to monitor the purified protein solution. It is believes that this protein separation method can be scaled up easily because it is based on simple solid-liquid unit operations.

1.2 Problem Statement

A pure sample of protein is required to generate antibodies, conduct binding assays and study structure. Yet, the target protein must first be isolated; the debris, salts, and reagents washed away, the amount of protein quantified and also the sample concentrated.

At present, protein purification is a challenge because, in addition to the particular protein that is meant to be purified, the protein's cell contains several thousand other proteins along with nucleic acids (DNA and RNA), polysaccharides, lipids as well as small molecules. By purifying a protein, a specific protein will be separated from contaminants in a manner that it will produce a useful end product. However, a highly efficient method with a cost-effective way has yet to be developed. This study focuses particularly on the principle of protein adsorption using pure adsorbent particle in order to purify the protein sample.

1.3 Objective of Study

The objective of this research is to study the most optimum condition to purify protein in order to produce a high yield and high purity protein with low cost by applying the principle of adsorption process using zeolite adsorbent.

1.4 Scope of Study

In sequence to accomplish the objective, the following scopes have been identified:

1. Effect of different pH value on protein purification
2. Effect of different type of adsorbent on protein purification

For the first parameter, the effect of pH on the process of protein purification applying adsorption principle using zeolite adsorbent is being considered at four different pH's namely 3, 4, 5, and 7. The samples at various pH will be prepared and were continuously shaken at room temperature until it is in equilibrium condition. For the next parameter, the same experiment will be carried out by using different types of adsorbent which are zeolite Y, Beta and ZSM-5 in order to monitor the effect of different type of adsorbent used.

CHAPTER 2

LITERATURE REVIEW

2.1 Protein

The word protein was first mentioned in a letter sent by the Swedish chemist Jöns Jakob Berzelius to Gerhardus Johannes Mulder on July 10, 1838. 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. During that time, examples of protein 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.

Proteins are large organic compounds made of amino acids formed in a linear chain. They are joined together by peptide bonds between the carboxyl and amino groups of adjacent amino acid residues. Proteins are large biological molecules with molecular weight up to few million Daltons. For convenience, the protein weight is measured in thousands Daltons or kilo Daltons (kDa).

Proteins are made of amino acids linked into linear chains, called polypeptide chains. Amino acids links between each other by peptide bonds - this peptide bond is formed between the carboxyl and amino groups of neighbouring amino acids.

Proteins are formed by one or several polypeptide chains. The sequence of the polypeptide chain is defined by a gene with genetic code. There are only 20 standard amino acids are exists in living organism. Sometimes these amino acids are chemically modified in the protein after protein synthesis. In total the number of different proteins, which it is possible to produce from 20 amino acids is enormous. For example for 10 amino acid sequence it is possible to have 20^{10} different sequences, which is approximately equal to 10^{13} or 10 trillions of different structures. The study of proteins and their function is central to understanding both cells and organisms. There are a few reasons why proteins are important in biology which are; protein serve as a catalyst that maintain metabolic processes in the cell and also they serve as structural elements both within and outside the cell.

Protein structure is essential for correct function because it allows molecular recognition. For example, enzymes are proteins that catalyze biochemical reactions. The function of an enzyme relies on the structure of its active site, a cavity in the protein with a shape and size that enable it to fit the intended substrate very snugly. It also has the correct chemical properties to bind the substrate efficiently. The active site also contains certain amino acids that are involved in the chemical reaction catalyzed by the enzyme.

Although not all proteins are enzymes, but still, all in some way rely on molecular recognition in order to perform their functions. Transport proteins such as hemoglobin must recognize the molecules they carry, receptors on the cell surface must recognize particular signaling molecules, and transcription factors must recognize particular DNA sequences and antibodies must recognize specific antigens. The functional integrity of the cell depends critically on protein-protein interactions, particularly on the formation of multi-protein complexes.

Protein is the major component of living organisms thus it formed a wide variety of essential functions in cells. A number of products that are being produced by modern companies specified in biotechnologies use protein in their process. These proteins that are being used may be drugs such as insulin or they might be in the formed of molecular tools that allows researchers to use it as an enzymes.

Serum albumin is one of the most widely studied proteins and is the most abundant protein in plasma. Various researchers have studied the structure and properties of serum albumin and its interaction with other proteins in order to understand how serum albumin affects the functionality of foods in which they have been included as well as novel applications. The latter reason led to the study of the interaction between soluble wheat protein and bovine serum albumin.

2.1.1 Structure of Protein

Generally, proteins contain from a range of 50 to 1000 amino acid residues per polypeptide chain. A peptide bond is an amide bond formed by the reaction of an α-amino group (NH_2) of one amino acid with the carboxyl group (COOH) of another, as shown below in Figure 2.1

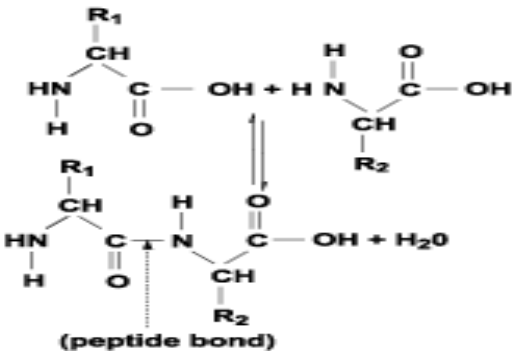


Figure 2.1: Peptide bond

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. Proteins can be informally divided into three main classes, which correlate with typical tertiary structures: globular proteins, fibrous proteins, and membrane proteins.

The structures build from 2 to 100 amino acids with molecular weight up to 10 kDa are usually called peptides. Longer polypeptide structures are classified as proteins. Some other classifications are appeal to the conformations stability of the amino acid chain. In this classification, peptides have many different conformations and can randomly change them, whereas proteins are structurally rigid with only one preferable conformation.

Based upon chemical composition, proteins are divided into two major classes: simple proteins, which are composed of only amino acids, and conjugated proteins, which are composed of amino acids and additional organic and inorganic groupings, certain of which are called prosthetic groups. Conjugated proteins include glycoproteins, which contain carbohydrates; lipoproteins, which contain lipids; and nucleoproteins, which contain nucleic acids.

The structure of proteins is a complex one which is divided into 4 parts. They are primary, secondary, tertiary and quaternary structures. For primary structure, this structure is responsible for the function of a protein. This structure is composed of various amino acids held together by peptide bonds. Proteins may have 1 or more polypeptide chains. Each polypeptide in a protein has amino acids linked with each other in a specific sequence and it is this sequence of amino acids that is said to be the primary structure of that protein. A protein containing a total of 100 amino acids residues is a very small protein, yet 20 different amino acids can be combined at one time in $(20)^{100}$ different ways.

The physical interaction of sequential amino-acid sub-units results in a so-called secondary structure, which often can either be a twisting of the polypeptide chain approximating a linear helix (α-configuration), or a zigzag pattern (β-configuration). Most globular proteins also undergo extensive folding of the chain into a complex three-dimensional geometry designated as tertiary structure. Many globular protein molecules are easily crystallized and have been examined by X-ray diffraction, a technique that allows the visualization of the precise three-dimensional positioning of atoms in relation to each other in a crystal.

The three dimensional structure of a protein is known as tertiary structure of a protein. This is a compact structure which means further folding of the secondary structure. It coils and folds in such a way that the hydrophobic side chains are held interior and the hydrophilic groups are held outside. This arrangement gives stability to the molecule. The tertiary structure is maintained by hydrogen bonds, disulfide bonds, ionic bonds and hydrophobic interactions. This structure brings distant amino acid side chains nearer. Two major molecular shapes are found which are fibrous and globular. The fibrous proteins such as silk collagen and a-keratins have large helical content and have rod like rigid shape and are insoluble in water.

In globular proteins such as hemoglobin the polypeptide chains consist partly of helical sections which are folded about the random cuts to give it a spherical shape. The primary, secondary and tertiary and quaternary levels of hemoglobin structure are given in figure at the next page.

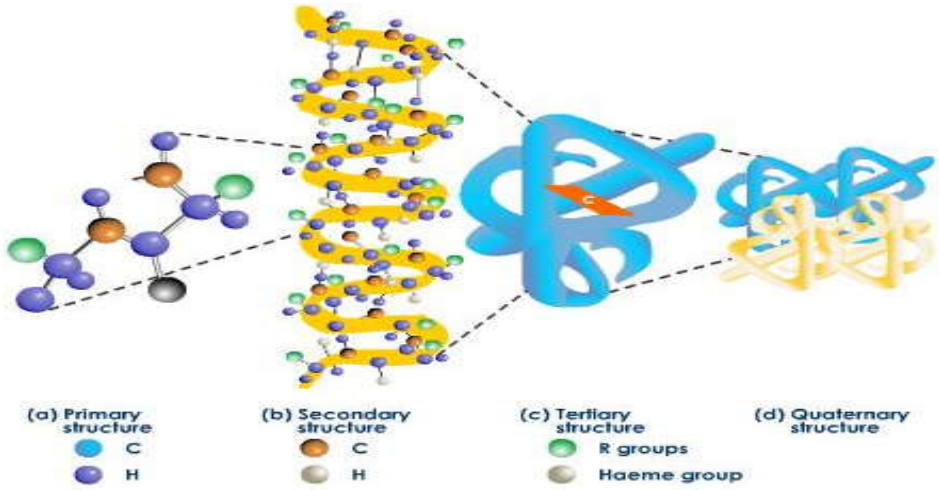


Figure 2.2: Primary, secondary, tertiary and quaternary structures of hemoglobin

2.1.2 Protein Biosynthesis

Protein biosynthesis is a process in which cells build proteins. The term is sometimes used to refer only to protein translation but more often it refers to a multi-step process, beginning with amino acid synthesis and transcription which are then used for translation. Protein biosynthesis, although very similar, differs between prokaryotes and eukaryotes. The processes by which proteins are synthesized biologically have become one of the central themes of molecular biology. The sequence of amino acid residues in a protein is controlled by the sequence of the DNA as expressed in messenger RNA at ribosomes.

Protein synthesis is the creation of proteins using DNA and RNA. Proteins can often be synthesized directly from genes by translating mRNA. When a protein is harmful and needs to be available on short notice or in large quantities, a protein precursor is produced. A pro-protein is an inactive protein containing one or more inhibitory peptides that can be activated when the inhibitory sequence is removed by proteolysis during posttranslational modification.

A pre-protein is a form that contains a signal sequence which is an N-terminal signal peptide that specifies its insertion into or through membranes that will targets them for secretion. The signal peptide is cleaved off in the endoplasmic reticulum. Prepro-proteins have both sequences (inhibitory and signal) still present.

For synthesis of protein, a succession of tRNA molecules charged with their appropriate amino acids have to be brought together with an mRNA molecule and matched up by base-pairing through their anti-codons with each of its successive codons. The amino acids then have to be linked together to extend the growing protein chain, and the tRNAs, relieved of their burdens, have to be released. This whole complex of processes is carried out by a giant multi-molecular machine, the ribosome, formed of two main chains of RNA, called ribosomal RNA (rRNA), and more than 50 different proteins. This molecular juggernaut latches onto the end of an mRNA molecule and then trundles along it, capturing loaded tRNA molecules and stitching together the amino acids they carry to form a new protein chain.

Short proteins can also be synthesized chemically by a family of methods known as peptide synthesis, which rely on organic synthesis techniques such as chemical ligation to produce peptides in high yield. Chemical synthesis allows for the introduction of non-natural amino acids into polypeptide chains, such as attachment of fluorescent probes to amino acid side chains. These methods are useful in laboratory biochemistry and cell biology, though generally not for commercial applications. Chemical synthesis is inefficient for polypeptides longer than about 300 amino acids, and the synthesized proteins may not readily assume their native tertiary structure. Most chemical synthesis methods proceed from C-terminus to N-terminus, opposite the biological reaction.

The events following biosynthesis include post-translational modification and protein folding. During and after synthesis, polypeptide chains often fold to assume, so called, native secondary and tertiary structures. This is known as protein folding. Many proteins undergo post-translational modification. This may include the

formation of disulfide bridges or attachment of any of a number of biochemical functional groups, such as acetate, phosphate, various lipids and carbohydrates. Enzymes may also remove one or more amino acids from the leading (amino) end of the polypeptide chain, leaving a protein consisting of two polypeptide chains connected by disulfide bonds.

2.1.3 Types of Protein

A protein molecule that consists of but a single polypeptide chain is said to be monomeric; proteins made up of more than one polypeptide chain, as many of the large ones are, are called oligomeric. Based upon chemical composition, proteins are divided into two major classes: simple proteins, which are composed of only amino acids, and conjugated proteins, which are composed of amino acids and additional organic and inorganic groupings, certain of which are called prosthetic groups. Conjugated proteins include glycoproteins, which contain carbohydrates; lipoproteins, which contain lipids; and nucleoproteins, which contain nucleic acids.

Classified by biological function, proteins include the enzymes, which are responsible for catalyzing the thousands of chemical reactions of the living cell; keratin, elastin, and collagen, which are important types of structural, or support, proteins; hemoglobin and other gas transport proteins; ovalbumin, casein, and other nutrient molecules; antibodies, which are molecules of the immune system, protein hormones, which regulate metabolism; and proteins that perform mechanical work, such as actin and myosin, the contractile muscle proteins.

Over time and diversity of organisms, a huge amount of proteins exist and perform a unique function in the body. Primarily, there are three types of protein, which are; fibrous proteins, globular proteins and conjugated proteins. Fibrous proteins are used for structural purposes in organisms. This is because fibrous

proteins are arranged in long strands and are insoluble in water. Examples of use include providing a barrier in the cell wall of plants and myosin in skeletal muscle.

Globular proteins are the polypeptide chains which are folded together into a knot like shape essential in the fact that are present in the following:

- Enzymes - Biological catalysts, enzymes are responsible speeding up reactions in an organism.
- Hormones - Hormones are chemical messengers responsible for initializing a response in organisms. Some hormones have a regulatory effect, explained in later chapters in the tutorial.
- Antibodies - Antibodies are used to defend the body against foreign agents such as bacteria, fungi and viruses.
- Structural Protein - Globular proteins form part of the cell membrane, which has a structural role as well as a role in transporting ions in and out the cell.

Conjugated proteins are essentially globular proteins that possess non-living substances, such as the haem found in hemoglobin, which possesses iron to a non-living substance. Therefore proteins play a vital role in many of organisms' biological processes and their organs.

2.1.4 Real-life application

Protein plays a foremost function in our daily life whereby these highly important compounds are applied everywhere in our life. Obviously, large percentage of our bodies' dry weight that is, the weight excluded the water composition, is build from protein. Our bones, for instance, are about one-fourth protein, and protein makes up a very high percentage of the material in our organs including the skin, glands, and bodily fluids.

Humans are certainly not the only organisms composed largely of protein. The entire animal world and the microbes that enter our bodies, likewise is constituted largely of protein. In addition, most of animal products, such as leather and wool, are nearly pure protein. In addition, protein such as hormones use for the treatment of certain conditions for example, insulin, which keeps people with diabetes alive. Found in every cell and tissue and composing the bulk of our bodies' structure, proteins are everywhere, promoting growth and repairing bone, muscles, tissues, blood, and organs.

Other form of protein is enzymes make possible a host of bodily processes, in part by serving as catalysts, or substances that speed up a chemical reaction without actually participating in, or being consumed by, that reaction. Enzymes enable complex, life-sustaining reactions in the human body reactions that would be too slow at ordinary body temperatures. They also are involved in fermentation, a process with applications in areas ranging from baking bread to reducing the toxic content of wastewater. Inside the body, enzymes and other proteins have roles in digesting foods and turning the nutrients into energy. They also move molecules around within our cells to serve an array of needs and allow healthful substances, such as oxygen, to pass through cell membranes while keeping harmful ones out. Proteins in the chemical known as chlorophyll facilitate an exceptionally important natural process, photosynthesis.

Proteins allow cells to detect and react to hormones and toxins in their surroundings, and as the chief ingredient in antibodies, which help us resist infection, they play a part in protecting our bodies against foreign invaders. The lack of specific proteins in the brain may be linked to such mysterious, terrifying conditions as Alzheimer and Creutzfeldt-Jakob diseases. Found in every cell and tissue and composing the bulk of our bodies' structure, proteins are everywhere, promoting growth and repairing bone, muscles, tissues, blood, and organs.

Similarly, proteins play a critical role in forensic science, or the application of medical and biological knowledge to criminal investigations. Fingerprints are an expression of our DNA, which is linked closely with the operation of proteins in our bodies. The presence of DNA in bodily fluids, such as blood, semen, sweat, and saliva, makes it possible to determine the identity of the individual who perpetrated a crime or of others who were present at the scene. The key is luminol, which reacts to hemoglobin in the blood, making it visible to investigators. This chemical, developed during the 1980s, has been used to put many a killer behind bars.

2.1.5 Purification of Protein

Protein purification is a series of processes intended to isolate a single type of protein from a complex mixture. Protein purification is vital for the characterization of the function, structure and interactions of the protein of interest. The various steps in the purification process may free the protein from a matrix that confines it, separate the protein and non-protein parts of the mixture, and finally separate the desired protein from all other proteins. Separation of one protein from all others is typically the most laborious aspect of protein purification. Separation steps exploit differences in protein size, physico-chemical properties and binding affinity.

Purification may be preparative or analytical. Preparative purifications aim to produce a relatively large quantity of purified proteins for subsequent use. Examples include the preparation of commercial products such as enzymes, nutritional proteins such as soy protein isolate, and certain biopharmaceuticals such as insulin. Analytical purification produces a relatively small amount of a protein for a variety of research or analytical purposes, including identification, quantification, and studies of the protein's structure, post-translational modifications and function. Among the first purified proteins were urease and Concanavalin A.

Centrifugation is a process that uses centrifugal force to separate mixtures of particles of varying masses or densities suspended in a liquid. By centrifugation technique, molecules of different protein values are usually separated by layering them on top of a dense solution and then running the centrifuge to force the molecules through the solution. The molecules values go farther because of either larger molecular weight or less frictional drag or both. When the different molecules have traveled different distances down the tube, the centrifugation is being stopped; the solution will be removed by puncturing the bottom of the tube or pumping the solution off the top.

An analytical purification generally utilizes three properties to separate proteins. First, proteins may be purified according to their isolectric points by running them through a pH graded gel or an ion exchange column. Second, proteins can be separated according to their size or molecular weight via size exclusion chromatography or by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Proteins are often purified by using 2D-PAGE and are then analysed by peptide mass fingerprinting to establish the protein identity. This is very useful for scientific purposes and the detection limits for protein are nowadays very low and nanogram amounts of protein are sufficient for their analysis.

The other technique, is by treating protein with SDS, a strong detergent, they are denatured. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), is a technique widely used in biochemistry, forensics, genetics and molecular biology to separate proteins according to their electrophoretic mobility which is a function of length of polypeptide chain or molecular weight as well as higher order protein folding, posttranslational modifications and other factors. The SDS gel electrophoresis of samples having identical charge to mass ratios results in fractionation by size and is probably the world's most widely used biochemical method. The bigger the macromolecule, the more SDS is bound, so that all macromolecules treated with SDS have the same ratio of charge to mass.

Affinity chromatography is used primarily for the separation and isolation of proteins. The technique relies upon the ability of proteins to recognize and bind to other molecules or ligands in a specific and reversible manner. The ligand is normally bound by the protein at a "specific" site often within a fold of the protein. Affinity chromatography uses an adsorbent comprising a porous support matrix to which the ligand is attached. The attachment or bonding is performed so that the immobilized ligand is still able to interact with the protein. If the ligand is relatively small, it is generally preferable to incorporate a spacer arm between ligand and matrix, allowing the immobilized ligand to interact with the protein. Figure 2.3 shows the steps in affinity chromatography technique:

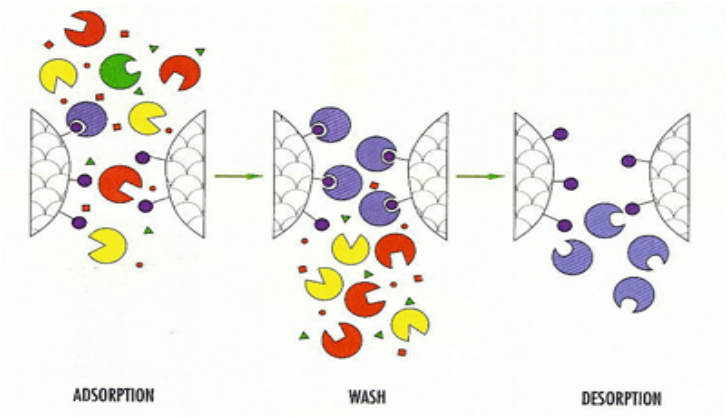


Figure 2.3: Affinity Chromatography Technique

An affinity separation is then performed by passing the impure protein over the adsorbent, incorporating the ligand, at which juncture the target protein is adsorbed while allowing contaminants such as other like proteins, lipids, carbohydrates, DNA, and pigments to pass through without hindrance. The adsorbent is normally contained within a chromatography column though the adsorption stage can be performed equally well by using the adsorbent as stirred slurry in batch binding mode.

A common technique involves engineering a sequence of 6 to 8 histidines into the C-terminal of the protein. The polyhistidine binds strongly to divalent metal ions such as nickel and cobalt. The protein can be passed through a column containing immobilized nickel ions, which binds the polyhistidine tag. All untagged proteins pass through the column. The protein can be eluted with imidazole, which competes with the polyhistidine tag for binding to the column, or by a decrease in pH which is typically to 4.5, which decreases the affinity of the tag for the resin. While this procedure is generally used for the purification of recombinant proteins with an engineered affinity tag, it can also be used for natural proteins with an inherent affinity for divalent cations.

2.2 Adsorption Process

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

Depending on the nature of attractive forces existing between the adsorbate and adsorbent, adsorption can be classified as:

i) Physical adsorption

ii) Chemical adsorption

In physical adsorption, the forces of attraction between the molecules of the adsorbate and the adsorbent are of the weak Van der Waals type. Since the forces of attraction are weak, the process of physisorption can be easily reversed by heating or decreasing the pressure of the adsorbate such as in the case of gases. The process is a dynamic one where an equilibrium state exists with molecules and the interaction between the adsorbate and adsorbent.

No chemical bonds are formed during physical adsorption which means attraction between the adsorbate and adsorbent exists by the formation of intermolecular electrostatic, such as London dispersion forces, or Van der Waals forces from induced dipole-dipole interactions, or may be dependent on the physical configuration of the adsorbent such as the porosity of activated carbons.

Dispersion forces are the result of rapid fluctuations in the electronic density of one adsorbent molecule inducing an electrical moment in a second atom (S.J Gregg,1982). If the adsorbate possesses a permanent dipole, or even a multipole, then additional interactions may occur, as charge distributions are induced in the adsorbent and interactions of these moments with any permanent field of the solid.

The process is a very general one and is analogous with that of condensation. Physisorption occurs to varying extents for all adsorbates, gases and vapours, with all adsorbing solids and the effect increases with decreasing temperature or increasing pressure. Physical adsorption is based on certain basic considerations and adsorption on a heterogeneous surface, that is a surface on which the sites are different, occurs at the sites of highest adsorption potential. The process of physical adsorption into the microporous structure of activated carbon follows the theory of Dubinin (M.M Dubinin, 1966)

The mechanism of adsorption is dependent upon the size of the admolecule in comparison with the pore width due to the energetic interactions between the chosen adsorbate and the pores. Admolecules initially adsorb into the pores with the highest energy, ignoring activated diffusion effects, then adsorption proceeds via filling of progressively larger, or decreasing energy, porosity. Some pores are capable of accommodating two or three admolecules and, therefore, may undergo co-operative adsorption effects by reducing the volume element thus increasing the energy and adsorptive potential of the pore.

The process of adsorption is always exothermic due to the increased ordering of the adsorbate on the adsorbent surface, reducing the entropy, as:

$$\Delta G = \Delta H - T\Delta S \qquad (2.1)$$

Thus the amount adsorbed should decrease with increasing temperature as a reduction in the thermal energy supplied to the process, by Le Chatelier's principle, favours the exothermic process of adsorption increasing the equilibrium uptake, except in the case of activated diffusion. It has been proposed by Lamond and Marsh, by the interpretation of data for physical adsorption of nitrogen on both polar and non-polar surfaces that physical adsorption is independent of the surface chemistry of the adsorbent.

In chemical adsorption which is Chemisorption, the forces of attraction between the adsorbate and the adsorbent are very strong; the molecules of adsorbate form chemical bonds with the molecules of the adsorbent present in the surface. Chemisorption involves the transfer of electrons between the adsorbent and the adsorbate with the formation of chemical bonds, by chemical reaction, between the two species causing adhesion of the adsorbate molecules. Chemical adsorption is far less common than physical adsorption and due to the chemical bonds formed regeneration of the adsorbent for subsequent re-use is often difficult or impossible.

Due to the fact that chemical bonds are formed during the adsorption process, desorption of the adsorbed phase may yield products which are chemically different to the original adsorbate. For example oxygen may chemically bond to the surface of a carbon, which upon desorption may evolve CO and CO_2 as products.

Table 2.1: Characteristics Associated with Physical/Chemical Adsorption

	Physical Adsorption	**Chemical Adsorption**
Heat of Adsorption (kJmol$^{1)}$	20- 40 c.f. heats of liquefaction	> 80 c.f. bulk-phase chemical reactions
Rate of Adsorption (at 273K)	Fast	Slow
Temperature Dependence of Uptake (with Increasing T)	Decreases	Increases
Desorption	Easy- by reduced pressure or increased temperature	Difficult - high temperature required to break bonds
Desorbed Species	Adsorbate unchanged	May be different to original adsorptive
Specificity	Non-specific	Very Specific
Monolayer Coverage	Mono or multilayer condition dependent	Monolayer

Adsorption is generally accompanied by release of energy, that is, most adsorption processes are exothermic in nature. Adsorption is a spontaneous process; therefore its free energy change is negative ($\Delta G<0$). However, the entropy change associated with adsorption is generally negative because the adsorbate molecules lose their translation freedom when they get attached to the surface of the adsorbent.

2.2.1 Introduction

Adsorption is a process in which atoms or molecules move from a bulk phase that is solid, liquid, or gas onto a solid or liquid surface. An example is purification by adsorption where impurities are filtered from liquids or gases by their adsorption onto the surface of a high-surface-area solid such as activated charcoal. Other examples include the segregation of surfactant molecules to the surface of a liquid, the bonding of reactant molecules to the solid surface of a heterogeneous catalyst, and the migration of ions to the surface of a charged electrode.

Adsorption is the basis of various emerging technologies that will be essential for addressing the problems of technologies that will be essential for addressing the problems of energy conservation and environmental protection.

Adsorption is to be distinguished from absorption, a process in which atoms or molecules move into the bulk of a porous material, such as the absorption of water by a sponge. Adsorption is a more general term that includes both adsorption and absorption and is a process in which molecules adsorbed on a surface are transferred back into a bulk phase. The term adsorption is most often used in the context of solid surfaces in contact with liquids and gases. Molecules that have been adsorbed onto solid surfaces are referred to generically as adsorbates, and the surface to which they are adsorbed as the substrate or adsorbent.

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.

2.2.2 Theory of Adsorption

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, shown in equation 2.2;

$$\frac{x}{m} = kP^{\frac{1}{n}} \qquad (2.2)$$

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.

There are three most widely used isotherm in terms of adsorption process which are;

- Langmuir Theory Adsorption
- Bet Theory Adsorption
- Freundlich Theory Adsorption

2.2.2.1 Langmuir Theory Adsorption

Theory of Langmuir Adsorption Isotherm in gas or solid and liquid or solid are based on the solutes retained by building up one or more solute layers on the surface of the adsorbent. The original Langmuir adsorption isotherm was for single layer adsorption and gives a curve that describes the fraction of the surface area of the adsorbent covered with solute, as a function of the concentration of the solute in the contacting liquid phase.

Langmuir isotherm is a semi-empirical isotherm derived from a proposed kinetic mechanism. It is based on four assumptions which are; the surface of the adsorbent is uniform, that is, all the adsorption sites are equivalent, adsorbed molecules do not interact, all adsorption occurs through the same mechanism and 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 Langmuir isotherm is a curve, convex to the solute concentration axis, and flattens out when the total surface is covered with solute. The isotherm for double layer adsorption is similar to single layer adsorption but the initial convex part of the curve is sharper. The adsorption isotherm only tends to linearity at very low concentrations of solute (at very low surface coverage) and so symmetrical peaks will only be achieved with very small samples. The Langmuir adsorption equation relates the coverage or adsorption of molecules on a solid surface to gas pressure or concentration of a medium above the solid surface at a fixed temperature.

The equation was developed by Irving Langmuir in 1916 is stated as shown in equation 2.3:

$$\theta = \frac{\alpha . P}{1 + \alpha . P} \qquad (2.3)$$

θ or theta is the percentage coverage of the surface, P is the gas pressure or concentration, α alpha is a constant. The constant α is the Langmuir adsorption constant and increases with an increase in the strength of adsorption and with a decrease in temperature.

According to basic adsorption theory, adsorption can be regarded as a reaction between adsorbate molecules, which is in this study, is Bovine Serum Albumin, and active sites of adsorbent which is the zeolite. Adsorption uptakes were accurately described by the Langmuir isotherm which is given by equation 2.4;

$$q = \frac{q_m C}{K_d + C} \qquad (2.4)$$

Whereby q is solute concentration in adsorbent, q_m is the Langmuir isotherm parameter, C is the equilibrium concentration and K_d is the Langmuir adsorption parameter. The transformed form of Langmuir equation is given by equation 4.2, which will be used to calculate the Langmuir parameter constants by fitting adsorption isotherm data obtained for the protein sample.

$$\frac{C}{q} = \frac{1}{q_m} C + \frac{K_d}{q_m} \qquad (2.5)$$

The Langmuir parameter constants were calculated by nonlinear regression analysis of adsorption isotherm data using Langmuir transformed equation 2.5, which give a good correlation coefficient ($R^2 > 0.99$).

Langmuir isotherm shows that at low pressures of the adsorbate, the extent of adsorption is linear and at high pressure the extent of adsorption is a constant. This form of isotherm is shown in Figure 2.4;

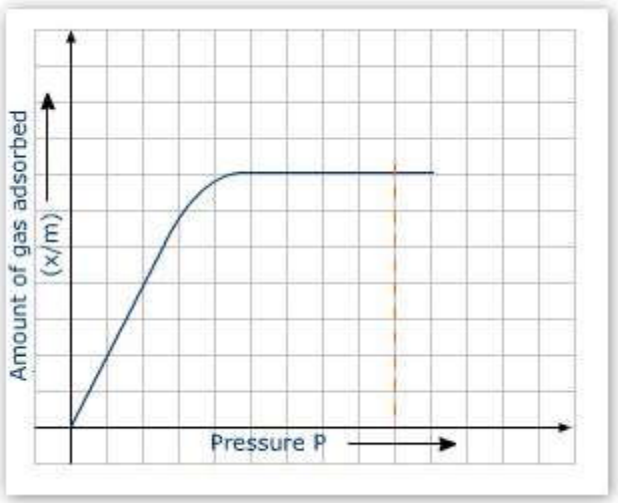


Figure 2.4 : Langmuir Adsorption Isotherm

Langmuir proposed a linear regression technique in 1918, and it should be referred to as the Langmuir linear regression when applied to adsorption isotherms. The Langmuir regression shown as equation 2.6 has very little sensitivity to data error. It has some bias toward fitting the data in the middle and high concentration range.

$$\frac{c}{\Gamma} = \frac{c}{\Gamma_{max}} + \frac{1}{K\Gamma_{max}} \qquad (2.6)$$

There are two kinds of nonlinear least squares (NLLS) regression techniques that can be used to fit the Langmuir equation to a data set. They differ only on how the goodness-of-fit is defined. In the v-NLLS regression method, the best goodness-of-fit is defined as the curve with the smallest vertical error between the fitted curve and the data. In the n-NLLS regression method, the best goodness-of-fit is defined as the curve with the smallest normal error between the fitted curve and the data. Using

the vertical error is the most common form of NLLS regression criteria. Definitions based on the normal error are less common. The normal error is the error of the datum point to the nearest point on the fitted curve. It is called the normal error because the trajectory is normal that is, perpendicular to the curve.

It is a common misconception to think that NLLS regression methods are free of bias. However, it is important to note that the v-NLLS regression method is biased toward the data in the low concentration range. This is because the Langmuir equation has a sharp rise at low concentration values, which results in a large vertical error if the regression does not fit this region of the graph well. Conversely, the n-NLLS regression method does not have any significant bias toward any region of the adsorption isotherm.

Whereas linear regressions are relatively easy to pursue with simple programs, such as excel or hand-held calculators, the nonlinear regressions are much more difficult to solve. The NLLS regressions are best pursued with any of various computer programs.

2.2.2.2 Bet Theory Adsorption

The most widely used isotherm dealing with multiplayer adsorption was derived by Stephen Brunauer, Paul Emmet and Edward Teller, is called the BET isotherm. 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 BET isotherm works best at low pressures where only a few complete monolayer of adsorbate may have formed since highly porous or irregular solids, such as alumina, cannot form regular layers. The BET equation can be mathematically represented by equation 2.7:

$$\frac{1}{v\left[(P_0/P)-1\right]} = \frac{c-1}{v_m c}\left(\frac{P}{P_0}\right) + \frac{1}{v_m c} \qquad (2.7)$$

P and P_0 in the BET equation are the equilibrium and the saturation pressure of adsorbates at the temperature of adsorption, v is the adsorbed gas quantity, and v_m is the monolayer adsorbed gas quantity. E_1 is the heat of adsorption for the first layer, and E_L is that for the second and higher layers and is equal to the heat of liquefaction. BET plot is shown in figure 2.2.3

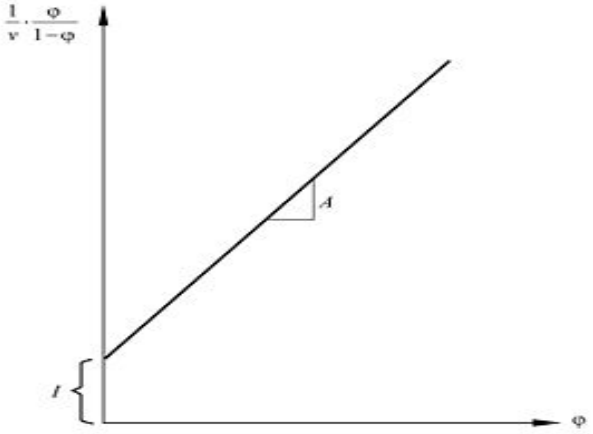


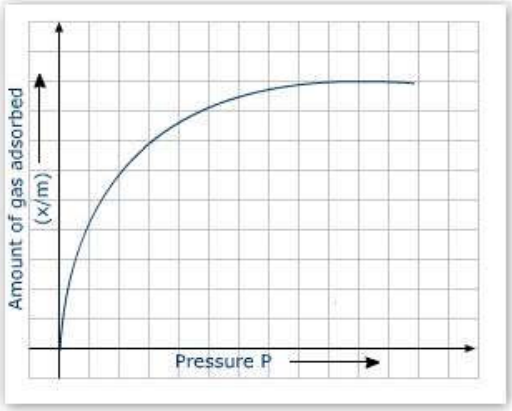
Figure 2.5: BET plot

2.2.2.3 Freundlich Theory Adsorption

The Freundlich Adsorption Isotherm is an adsorption isotherm, which is a curve that related the concentration of a solute on the surface of an adsorbent, to the concentration of the solute in the liquid with which it is in contact. Freundlich adsorption isotherm is described by equation 2.8 :

$$\frac{x}{m} = kP^{\frac{1}{n}} \quad (n > 1) \qquad (2.8)$$

where K and n are the parameters of the equation which depend on the nature of the gas, and P is the pressure. At equilibrium, pressure P_s, x/m reaches its maximum value, that is, no further adsorption takes place even if the pressure is increased. A saturation state has been achieved. The adsorption isotherm which conforms to Freundlich equation is shown in below figure 2.6:



The Freundlich isotherm curves in the opposite way and is exponential in form. It often represents an initial surface adsorption followed by a condensation effect resulting from extremely strong solute-solute interaction. In chromatography the Freundlich isotherm is not common, which is to say, most adsorption processes are best described by the Langmuir isotherm.

2.3 Adsorbent

Adsorbent can be defined as a substance, naturally in a porous state. It possesses a large surface area, in order to adsorb substances onto its surface using the principle of intermolecular forces, but in a certain condition with an extremely low concentration, the adsorption isotherm will be linear whereas at higher concentrations the adsorption isotherm may be Langmuir or Freundlich. It is proven that solutes can spread out among the adsorbent surface thus adsorbents are used as a stationary phases in gas-solid and liquid-solid chromatography. Adsorbents are also used for extraction purposes such as subtracting traces of organic materials from large volumes of solution. Typical adsorbents used in gas-solid chromatography are silica gel, alumina, carbon and bonded phase.

Nowadays, adsorbent product can be found in multi purpose applications such as in biochemistry and biotechnology product to improve the quality of everyday life. Adsorbents are used usually in the form of spherical pellets, rods, moldings, or monoliths with hydrodynamic diameters between 0.5 and 10 mm. They must have high abrasion resistance, high thermal stability and small pore diameters, which results in higher exposed surface area and hence high surface capacity for adsorption. The adsorbents must also have a distinct pore structure which enables fast transport of the gaseous vapors. Most industrial adsorbents fall into one of three classes:

- Oxygen-containing compounds – Are typically hydrophilic and polar, including materials such as silica gel and zeolites.
- Carbon-based compounds – Are typically hydrophobic and non-polar, including materials such as activated carbon and graphite.
- Polymer-based compounds - Are polar or non-polar functional groups in a porous polymer matrix.

Adsorbents that is oxygen containing compound such as silica gel is a chemically inert, nontoxic, polar and dimensionally stable which is less than 400 °C amorphous form of SiO_2 . It is prepared by the reaction between sodium silicate and sulfuric acid, which is followed by a series of after-treatment processes such as aging, pickling, etc. These after treatment methods results in various pore size distributions. Silica is used for drying of process air such as oxygen, natural gas and adsorption of heavy or polar hydrocarbons from natural gas.

For carbon based compound, activated carbon is a highly porous, amorphous solid consisting of micro-crystallites with a graphite lattice, usually prepared in small pellets or a powder. It is non-polar and cheap. One of its main drawbacks is that it is combustible. Activated carbon can be manufactured from carbonaceous material, including coal such as bituminous, sub-bituminous, and lignite, peat, wood, or nutshells. The manufacturing process consists of two phases, carbonization and activation. The carbonization process includes drying and then heating to separate by-products, including tars and other hydrocarbons, from the raw material, as well as to drive off any gases generated. The carbonization process is completed by heating the material at 400–600 °C in an oxygen-deficient atmosphere that cannot support combustion.

Activated carbon is used for adsorption of organic substances and non-polar adsorbates and it is also usually used for waste gas as well as waste water treatment. It is the most widely used adsorbent. Its usefulness derives mainly from its large micropore and mesopore volumes and the resulting high surface area.

Platinum and palladium, especially in finely divided form, are great adsorbents of hydrogen and other gases. They therefore serve as excellent catalysts of various chemical reactions. For example, platinum-supported catalysts are used in the refining of crude oil, reforming, and other processes for producing high-octane gasoline and aromatic compounds for the petrochemical industry. Also, the chemical industry uses platinum or a platinum-rhodium alloy to catalyze the partial oxidation

of ammonia to yield nitric oxid which is the raw material for fertilizers, explosives, and nitric acid.

At room temperature and atmospheric pressure, palladium can adsorb up to 900 times its own volume of hydrogen. Thus, palladium can be used to store substantial quantities of hydrogen safely. Finely divided palladium is a good catalyst for reactions such as hydrogenation which is addition of hydrogen atoms, dehydrogenation which is removal of hydrogen atoms, and petroleum cracking that is breaking of large, complex hydrocarbons to smaller, simpler ones. Since 1979, the automotive industry has been using palladium, platinum, and rhodium as catalysts in catalytic converters to treat automobile exhaust emissions.

Portal site mediated adsorption is a model for site-selective activated gas adsorption in metallic catalytic systems that contain a variety of adsorption sites. In such systems, low-coordination "edge and corner" defect-like sites can exhibit significantly lower adsorption enthalpies than high-coordination (basal plane) sites. As a result, these sites can serve as "portals" for very rapid adsorption on the rest of the surface. The phenomena relies on the common "spillover" effect, where certain adsorbed species exhibit high mobility on some surfaces. This model explains seemingly inconsistent observations of gas adsorption thermodynamics and kinetics in catalytic systems where surfaces can exist in a range of coordination structures, and it has been successfully applied to bimetallic catalytic systems where synergistic activity is observed.

The original model was developed by T.S. King and coworkers to describe hydrogen adsorption on silica-supported silver-ruthenium and copper-ruthenium bimetallic catalysts. The same group applied the model to carbon monoxide (CO) hydrogenation which is Fischer-Tropsch synthesis. C. Zupanc and coworkers subsequently confirmed the same model on magnesia-supported cesium-ruthenium bimetallic catalysts.

Zeolites are natural or synthetic crystalline aluminosilicates which have a repeating pore network and release water at high temperature. Zeolites are polar in nature. They are manufactured by hydrothermal synthesis of sodium aluminosilicate or another silica source in an autoclave followed by ion exchange with certain cations (Na^{+} , Li^{+} , Ca^{2+} , K^{+} , NH_4^{+}). The channel diameter of zeolite cages usually ranges from 2 to 9 Å (200 to 900 pm). The ion exchange process is followed by drying of the crystals, which can be pelletized with a binder to form macroporous pellets. Zeolites are applied in drying of process air, CO_2 removal from natural gas, CO removal from reforming gas, air separation, catalytic cracking, and catalytic synthesis and reforming.

Non-polar siliceous zeolites are synthesized from aluminum-free silica sources or by dealumination of aluminum-containing zeolites. The dealumination process is done by treating the zeolite with steam at elevated temperatures, typically greater than 500 °C (1000 °F). This high temperature heat treatment breaks the aluminum-oxygen bonds and the aluminum atom is expelled from the zeolite framework.

The carbonized particles are “activated” by exposing them to an oxidizing agent, usually steam or carbon dioxide at high temperature. This agent burns off the pore blocking structures created during the carbonization phase and so, they develop a porous, three-dimensional graphite lattice structure. The size of the pores developed during activation is a function of the time that they spend in this stage. Longer exposure times result in larger pore sizes. The most popular aqueous phase carbons are bituminous based because of their hardness, abrasion resistance, pore size distribution, and low cost, but their effectiveness needs to be tested in each application to determine the optimal product.

2.3.1 Zeolite

The classical definition of a zeolite is a crystalline, porous aluminosilicate. However, some relatively recent discoveries of materials virtually identical to the classical zeolite, but consisting of oxide structures with elements other than silicon and aluminum have stretched the definition. Most researchers now include virtually all types of porous oxide structures that have well-defined pore structures due to a high degree of crystalline in their definition of a zeolite.

In these crystalline materials we call zeolites, the metal atoms which is classically, silicon or aluminum are surrounded by four oxygen anions to form an approximate tetrahedron consisting of a metal cation at the center and oxygen anions at the four apexes. The tetrahedral metals are called T-atoms for short, and these tetrahedra then stack in beautiful, regular arrays such that channels form. The possible ways for the stacking to occur is virtually limitless, and hundreds of unique structures are known.

The zeolitic channels or pores are microscopically small, and in fact, have molecular size dimensions such that they are often termed "molecular sieves". The size and shape of the channels have extraordinary effects on the properties of these materials for adsorption processes, and this property leads to their use in separation processes. Molecules can be separated via shape and size effects related to their possible orientation in the pore, or by differences in strength of adsorption.

Since silicon typically exits in a 4+ oxidation state, the silicon-oxygen tetrahedra are electrically neutral. However, in zeolites, aluminum typically exists in the 3+ oxidation state so that aluminum-oxygen tetrahedra form centers that are electrically deficient one electron. Thus, zeolite frameworks are typically anionic, and charge compensating cations populate the pores to maintain electrical neutrality. These cations can participate in ion-exchange processes, and this yields some

important properties for zeolites. When charge compensating cations are "soft" cations such as sodium, zeolites are excellent water softeners because they can pick up the "hard" magnesium and calcium cations in water leaving behind the soft cations. When the zeolitic cations are protons, the zeolite becomes a strong solid acid. Such solid acids form the foundations of zeolite catalysis applications including the important fluidized bed cat-cracking refinery process. Other types of reactive metal cations can also populate the pores to form catalytic materials with unique properties. Thus, zeolites are also commonly used in catalytic operations and catalysis with zeolites is often called "shape-selective catalysis”

The open porous nature of the zeolite structure is one of its most important properties. Open pores and voids within the structure provide places for mobile cations and water to reside. A few examples of the open nature of the zeolite structure are shown in Figure 2.7 below;

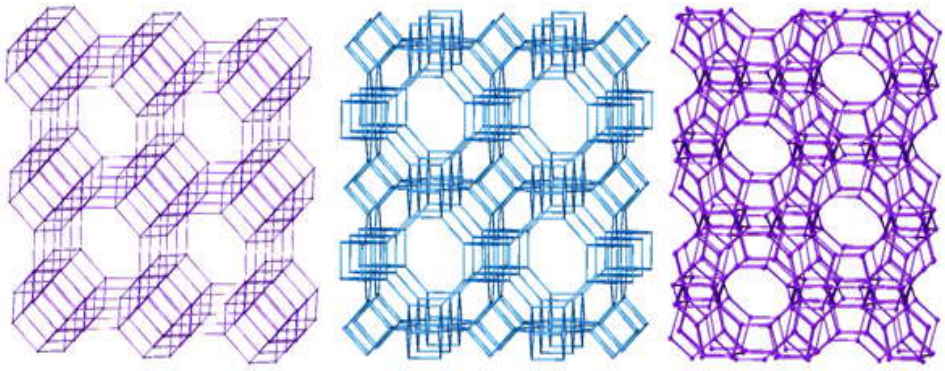


Figure 2.7 : Example of crystal structures of zeolites

There are three main uses of zeolites in industry which is catalysis, gas separation and ion exchange. Zeolites are extremely useful as catalysts for several important reactions involving organic molecules. The most important are cracking, isomerisation and hydrocarbon synthesis. Zeolites can promote a diverse range of catalytic reactions including acid-base and metal induced reactions.

Zeolites can also be acid catalysts and can be used as supports for active metals or reagents. Zeolites can be shape-selective catalysts either by transition state selectivity or by exclusion of competing reactants on the basis of molecular diameter. They have also been used as oxidation catalysts. The reactions can take place within the pores of the zeolite, which allows a greater degree of product control. The main industrial application areas are: petroleum refining, synfuels production, and petrochemical production. Synthetic zeolites are the most important catalysts in petrochemical refineries.

Zeolites are used to adsorb a variety of materials. This includes applications in drying, purification, and separation. They can remove water to very low partial pressures and are very effective desiccants, with a capacity of up to more than 25% of their weight in water. They can remove volatile organic chemicals from air streams, separate isomers and mixtures of gases. A widely used property of zeolites is that of gas separation. The porous structure of zeolites can be used to "sieve" molecules having certain dimensions and allow them to enter the pores. This property can be fine tuned by variating the structure by changing the size and number of cations around the pores. Other applications that can take place within the pore include polymerisation of semi conducting materials and conducting polymers to produce materials having unusual physical and electrical attributes.

Hydrated cations within the zeolite pores are bound loosely to the zeolite framework, and can readily exchange with other cations when in aqueous media. Applications of this can be seen in water softening devices, and the use of zeolites in detergents and soaps. The largest volume use for zeolites is in detergent formulations where they have replaced phosphates as water-softening agents. They do this by exchanging the sodium in the zeolite for the calcium and magnesium present in the water. It is even possible to remove radioactive ions from contaminated water.

2.3.2 Beta Zeolite

Beta zeolite is an old zeolite discovered before Mobil began the "ZSM" naming sequence. As the name implies, it was the second in an earlier sequence. The structure of Beta zeolite was only recently determined because the structure is very complex and interest was not high until the material became important for some dewaxing operations. Beta zeolite consists of an intergrowth of two distinct structures termed Polymorphs A and B. The polymorphs grow as two-dimensional sheets and the sheets randomly alternate between the two. Both polymorphs have a three dimensional network of 12-ring pores. The intergrowth of the polymorphs does not significantly affect the pores in two of the dimensions, but in the direction of the faulting, the pore becomes tortuous, but not blocked.

Beta zeolite has been used as an acid catalyst in organic chemical conversion such as alkylation and acylation. This catalyst is reported to have Brønsted acid sites in the micropores and on the external surface, and Lewis acid sites predominantly at the internal surface due to the local defects. Shockingly, Beta zeolite is rarely been used as a solid acid catalyst in organic transformation due to the lack of studies in this matter.

Beta zeolite, a 12-ring aperture (7.66.4 A) three-dimensional high-silica zeolite, currently receives much attention as a potential catalyst in numerous reactions. In addition to its Brønsted acidic properties it displays Lewis acidity as well. Interestingly, this Lewis acidity is believed not to be solely generated by extra frame work aluminum (EF-Al) species , but can also be displayed by frame work aluminum atoms in a non-tetrahedral environment.

Beta zeolite possesses a three-dimensional, 12-ring, interconnected channel system with pore diameters of 0.53 x 0.57 nm and 0.71 x 0.73 nm. Beta zeolite is one of industrial interest as a catalyst for fluid catalytic cracking, hydro-treating,

dewaxing, and alkylation, significant effort has been devoted to synthesize and modify it. Thus, beta membranes have potential for separations and catalytic membrane reactors. The micro-porous molecular structure of a Beta zeolite is shown in Figure 2.8;

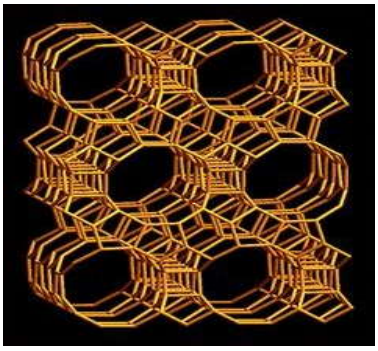


Figure 2.8 : The micro-porous molecular structure of a Beta zeolite

In a protic solvent such as hexanes, Beta zeolite acts as Lewis acid at the internal micropores surface, thus showing the shape-selectivity. In contrast, the large external surface of this catalyst may behave mainly as Brønsted acid in the presence of protic solvent such as methanol that was used in deprotection. Beta zeolite can also be recycled for several times without losing its efficiency (Ji-Eun Choi, 2001).

2.3.3 ZSM-5 Zeolite

ZSM-5 is an aluminosilicate zeolite mineral belonging to the pentasil family of zeolites. Its chemical formula is $Na_nAl_nSi_{96-n}O_{192}•16H_2O$. It is patented by Mobil Oil Company in 1975. This particular zeolite, is an aluminosilicate zeolite with a high silica and low aluminium content. Its structure is based on channels with intersecting tunnels. The aluminium sites are very acidic. The substitution of Al^{3+} place of the tetrahedral Si^{4+} silica requires the presence of an added positive charge. When this is H^+, the acidity of the zeolite is very high. The reaction and catalysis chemistry of the ZSM-5 is due to this acidity. The micro-porous molecular structure of a zeolite, ZSM-5 is shown in Figure 2.9:

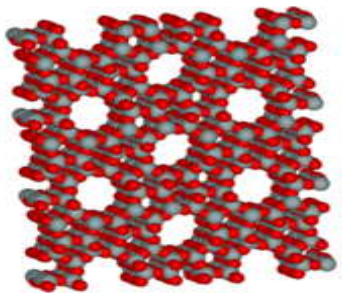


Figure 2.9 : The micro-porous molecular structure of a zeolite, ZSM-5

ZSM-5 is mainly used for hydrocarbon interconversion, meta-xylene to para-xylene, for example. ZSM-5 is a zeolite with a high silica to alumina ratio. The substitution of an aluminum ion (charge 3+) for a silicon ion (charge 4+) requires the additional presence of a proton. This additional proton gives the zeolite a high level of acidity, which causes its activity. ZSM-5 is a highly porous material and throughout its structure it has an intersecting two-dimensional pore structure.

ZSM-5 has two types of pores, both formed by 10-membered oxygen rings. The first of these pores is straight and elliptical in cross section, the second pores intersect the straight pores at right angles, in a zig-zag pattern and are circular in cross section shown in figure 2.10:

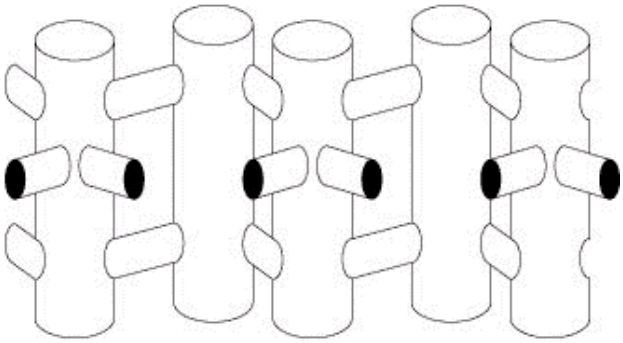


Figure 2.10 : Zig-zag pattern of ZSM-5 pores

This unique two-dimensional pore structure allows a molecule to move from one point in the catalyst to any where else in the particle. The large openings are the elliptical, straight pores in ZSM-5. An 8-oxygen ring zeolite will not produce molecules with 6 or more carbons, molecules of this size will not fit into the small pores of these zeolites. The large pores of a 12-oxygen ring zeolite produce large amounts of C-11 and C-12 compounds, which are undesirable products for gasoline.

ZSM-5 has a high silicon to aluminum ratio. Whenever an Al^{3+} cation replaces a Si^{4+} cation, an additional positive charge is required to 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.

ZSM-5 is used commercially as a catalyst in fluid cat-cracking (FCC) units in oil refineries to increase the motor octane of gasoline, increase the total LPG and increase the olefin content of the fraction. This is known as the secondary "cracking" of gasoil. (Materials, 2008. *Zeolite*).

2.3.4 Y Zeolite

Y zeolite is an industrially important catalytic material, and has been studied from various view-points. Y zeolite contains a framework system of supercages, which are connected by a three-dimensional array of large diameter channels and this array enables a much easier diffusion of reactants and products**.** The large pore opening and the three-dimensional channel system of the Y zeolite favor the formation of bulky reactions products.

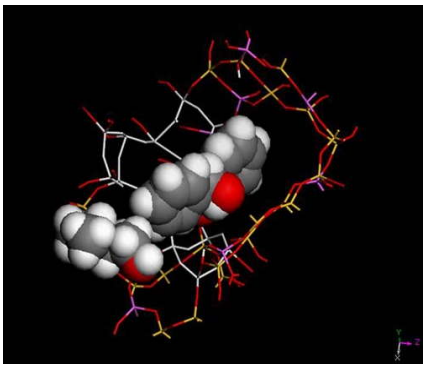


Figure 2.11 : The micro-porous molecular structure of a Y zeolite,

Y zeolite exhibits the FAU (faujasite) structure. It has a 3-dimensional pore structure with pores running perpendicular to each other in the x, y, and z planes similar to LTA, and is made of secondary building units 4, 6, and 6-6. The pore diameter is large at 7.4Å since the aperture is defined by a 12 member oxygen ring, and leads into a larger cavity of diameter 12Å. The cavity is surrounded by ten sodalite cages (truncated octahedra) connected on their hexagonal faces. The unit cell

is cubic (a = 24.7Å) with Fd-3m symmetry. Y zeolite has a void volume fraction of 0.48, with a Si/Al ratio of 2.43. It thermally decomposes at 793 °C

Y zeolite, like zeolite A, is synthesized in a gelling process. Sources of alumina (sodium aluminate) and silica (sodium silicate) are mixed in alkaline (NaOH) aqueous solution to give a gel. The gel is then usually heated to 70-300 ºC to crystallize the zeolite. The zeolite is present in Na^+ form and must be converted to acid form. To prevent disintegration of the structure from acid attack, it is first converted to the $NH4^+$ form before being converted to acidic form. If a hydrogenation metal such as platinum is needed, it is deposited via impregnation or ion exchange.

The most important use of Y zeolite is as a cracking catalyst. It is used in acidic form in petroleum refinery catalytic cracking units to increase the yield of gasoline and diesel fuel from crude oil feedstock by cracking heavy paraffins into gasoline grade napthas. Y zeolite has superseded zeolite X in this use because it is both more active and more stable at high temperatures due to the higher Si/Al ratio. It is also used in the hydrocracking units as a platinum/palladium support to increase aromatic content of reformulated refinery products.

Y zeolite has been the subject of tremendous interest of both the scientific and industrial world. They are used on a large industrial scale for a great variety of processes, from simple drying to complicated catalytic reactions. In the last decade, green chemistry has been recognized as a new approach to scientifically based environmental protection, and catalysis has manifested its role as a fundamental tool in pollution prevention. Y zeolites is also well known as environmentally friendly catalysts. Due to the diversification of the application for which these catalysts are employed, considerable research has been focused on the characterization of the active sites, a key property for applications in Y catalysis (Vera Dondour, 2005).

2.4 Bovine Serum Albumin

2.4.1 Introduction

Serum albumin is one of the most widely studied proteins and is the most abundant protein in plasma with a typical concentration of 5 g/100ml. Various researchers have studied the structure and properties of serum albumin and its interaction with other proteins in order to understand how serum albumin affects the functionality of foods in which they have been included as well as novel applications. The latter reason led to the study of the interaction between soluble wheat protein and bovine serum albumin.

Albumin is generally regarded as a serum albumin or plasma albumin. The word albumin is also used to describe a protein or a group of proteins defined by solubility in water for example the albumin fraction of wheat. Albumin is the most abundant protein in the circulatory system and contributes 80% to colloid osmotic blood pressure (Carter and Ho, 1994). It has now been determined that serum albumin is chiefly responsible for the maintenance of blood pH (Figge et al., 1991). In mammals albumin is synthesized initially as preproalbumin by the liver. After removal of the signal peptide, the resultant proalbumin is further processed by removal of the six-residue propeptide from the new N-terminus. The albumin released into circulation possesses a half-life of 19 days.

Bovine serum albumin, BSA, also known as "Fraction V", is a serum albumin protein that has numerous that do not need it for stabilization. BSA is used because of its stability, its lack of effect biochemical applications including ELISAs (Enzyme-Linked Immunosorbent Assay), blots, and immune-histochemistry. It is also used as a nutrient in cell and microbial culture. In restriction digests, BSA is used to stabilize some enzymes during digestion of DNA and to prevent adhesion of

the enzyme to reaction tubes and other vessels. This protein does not affect other enzymes 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.

The nickname "Fraction V" refers to albumin being the fifth fraction of the original Edwin Cohn purification methodology that made use of differential solubility characteristics of plasma proteins. By manipulating solvent concentrations, pH, salt levels, and temperature, Cohn was able to pull out successive "fractions" of blood plasma. The process was first commercialized with human albumin for medical use and later adopted for production of BSA.

Bovine serum albumin has been given little attention in respect to its role in the functional properties of whey protein concentrates, and makes up only about 5% of the protein in whey protein concentrates. Its primary biological function has been associated with its lipid binding properties (Fox and Flynn, 1992), but the mechanism of this role has not been clearly elucidated. It may play a role in mediating lipid oxidation, since BSA has been shown invitro to protect lipids against phenolic induced oxidation.

2.4.2 Characteristics of Bovine Serum Albumin

The substantial information on serum albumin has led to some contradictory results and discussions. Based largely on hydrodynamic experiments and low-angle X-ray scattering serum albumin was postulated to be an oblate ellipsoid with dimensions of 140 × 40Å shown in figure 2.12. Experiments have continued to support these dimensions compiled a diverse variety of data and constructed a model of albumin as having the shape of a cigar.

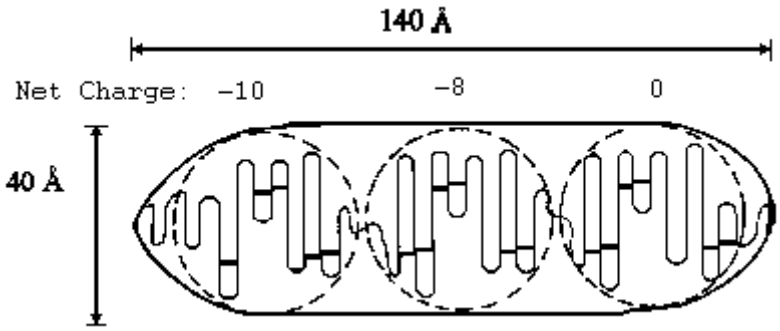


Figure 2.12: Classical preception of the structure of serum albumin

BSA molecule is made up of three homologous domains which are divided into nine loops by 17 disulphide bonds. The loops in each domain are made up of a sequence of large-small-large loops forming a triplet. Each domain in turn is the product of two subdomains. The primary structure of albumin is unusual among extracellular proteins in possessing a single sulfohydryl group.

The viscosity of a protein solution depends on its intrinsic characteristics, such as molecular mass, size, volume, shape, surface charge and ease of deformation. In addition, viscosity is influenced by environmental factors such as pH, temperature, ionic strength, ion type, shear conditions and heat treatment. Serum albumin has been reported to have intrinsic viscosity values of 3.7-4.2 ml/g. The viscosity of solutions

of BSA increased linearly with concentration up to 65 mg/ml and exponentially at higher concentrations.

CHAPTER 3

METHODOLOGY

3.1 Materials

3.1.1 General Chemical

For the adsorption experiment, Bovine Serum Albumin in a solid form was used as the protein sample with purity of 98-99%, purchased from Sigma was used without further purification. The zeolite Y, zeolite Beta, and zeolite ZSM-5, used was obtained from Zeolyst International Aqueous manufactured in United State. Aqueous phase were made up in freshly deionised water with resistivity 15 to 16 MΩ cm. All the buffer solutions used were prepared using phosphoric acid (H_3PO_4), potassium dihydrogen orthophosphate (KH_2PO_4), dipotassium hydrogen phosphate anhydrous (K_2HPO_4), potassium hydrogen carbonate ($KHCO_3$), as well as potassium carbonate anhydrous (K_2CO_3). Calculation was made in order to determine concentration of buffer which is 0.1 M. All the buffers were refrigerated in a freezer with the temperature below 5°C.

3.1.2 Pretreatment of Zeolite

The properties of the zeolites are determined by the chemical composition and the size and dimensionality of the channel system (Daniel Klint, 1997). Zeolites Y, Beta and ZSM-5, are the type of zeolites that are being used to study the effect of different types of adsorbents on the purification of Bovine Serum Albumin. By using a furnace, all the zeolites were calcined at temperature 300°C for 3 hours. The calcination process were done in order to remove weakly bonded acid, thereby minimizing leaching of acid from the product bound to the zeolite during prolonged operation under conditions of high relative humidity.

3.2 Preparation of Bovine Serum Albumin Solution

For the adsorption experiment, Bovine Serum Albumin (BSA) purchased from Sigma was used. The respectively protein model has the molecular weight of 65000g/mol. The isoelectric point (pI) value of the protein model in water at 25°C is 4.7 and the concentrations ranging from 0.01 mM to 0.03 mM were prepared for every pH range and types of zeolites. A certain amount of needed Bovine Serum Albumin is being dissolved into 100 mL of deionized water to obtain the desired concentration. The calculation was shown in the appendix.

3.3 Preparation of Buffer Solution

A buffer solution is one which resists changes in pH when small quantities of an acid or an alkali are added to it. Buffers that are going to be prepared depend on the pH of the Bovine Serum Albumin solution. All the buffer solutions were prepared by dissolving a needed amount of phosphoric acid (H_3PO_4), potassium

dihydrogen orthosphospate (KH_2PO_4), dipotassium hydrogen phosphate anhydrous (K_2HPO_4), potassium hydrogen carbonate ($KHCO_3$) and potassium carbonate anhydrous (K_2CO_3) in 100 mL deionized water to obtain a concentration of 0.1 M. Calculation was shown in the appendix. When all the buffer solutions with the concentration of 0.1 M have been prepared, each of their pH was determined using a pH meter. For pH solution ranged from 3 to 4, solution of phosphoric acid (H_3PO_4) was mixed with solution of potassium dihydrogen orthosphospate (KH_2PO_4), for pH solution ranged from 4 to 8, solution of potassium dihydrogen orthosphospate (KH_2PO_4) was mixed with solution of dipotassium hydrogen phosphate anhydrous (K_2HPO_4) , and for pH solution ranged from 8 to 10, solution of potassium hydrogen carbonate ($KHCO_3$) was mixed with solution of potassium carbonate anhydrous (K_2CO_3). All the buffer solutions used were prepared from original chemicals without further purification.

3.4 Experimental procedure

Batch experiments were conducted to study the effect of variable pH values and types of adsorbent onto Bovine Serum Albumin (BSA) adsorption. Calculations were made to estimate the amount of needed BSA with the concentrations of 0.01 mM, 0.015 mM, 0.02 mM, 0.025 mM and 0.03mM in 100 mL deionized water.

Initial adsorbance of Bovine Serum Albumin had to be checked first before verifying the value of adsorbance by zeolites. Batch adsorption experiment was carried out by contacting the same amount of BSA solution with 1 mL of buffer solution for pH 3, 4, 5 and 7. The buffer and protein solution were vigorously shaken at room temperature until equilibrium is reached before being analyzed.

In order to verify the value of adsorbance by zeolites, 10 mg of each type of zeolites was placed into test tubes containing a mixture of 2 mL of protein solution

with the intended concentration and 1 mL of buffer solution at various pH. The mixtures in the test tubes were shaken at room temperature and centrifuge before being analyzed.

Centrifugation provides a centripetal force that can be many hundreds or thousands of times the force of gravity, thus speeding up the process considerably. The principle use is the greater the number of revolutions per minute (RPM), the greater the force of gravity. After the centrifugation process was done, the suspended zeolites particles will settle to the bottom of the test tube. Centrifugation was made to avoid the interference from scattering particles in the UV-VIS analysis. The equilibrated samples were centrifuged for 10 minutes at 2000 rpm. Subsequently, the supernatants samples were transferred to new tubes, before it was ready to be analyzed. Centrifugation process was done using a centrifuger which is shown in Figure 3.1:

Figure 3.1: Laboratory Tabletop Centrifuge

3.5 Protein Adsorption Measurement

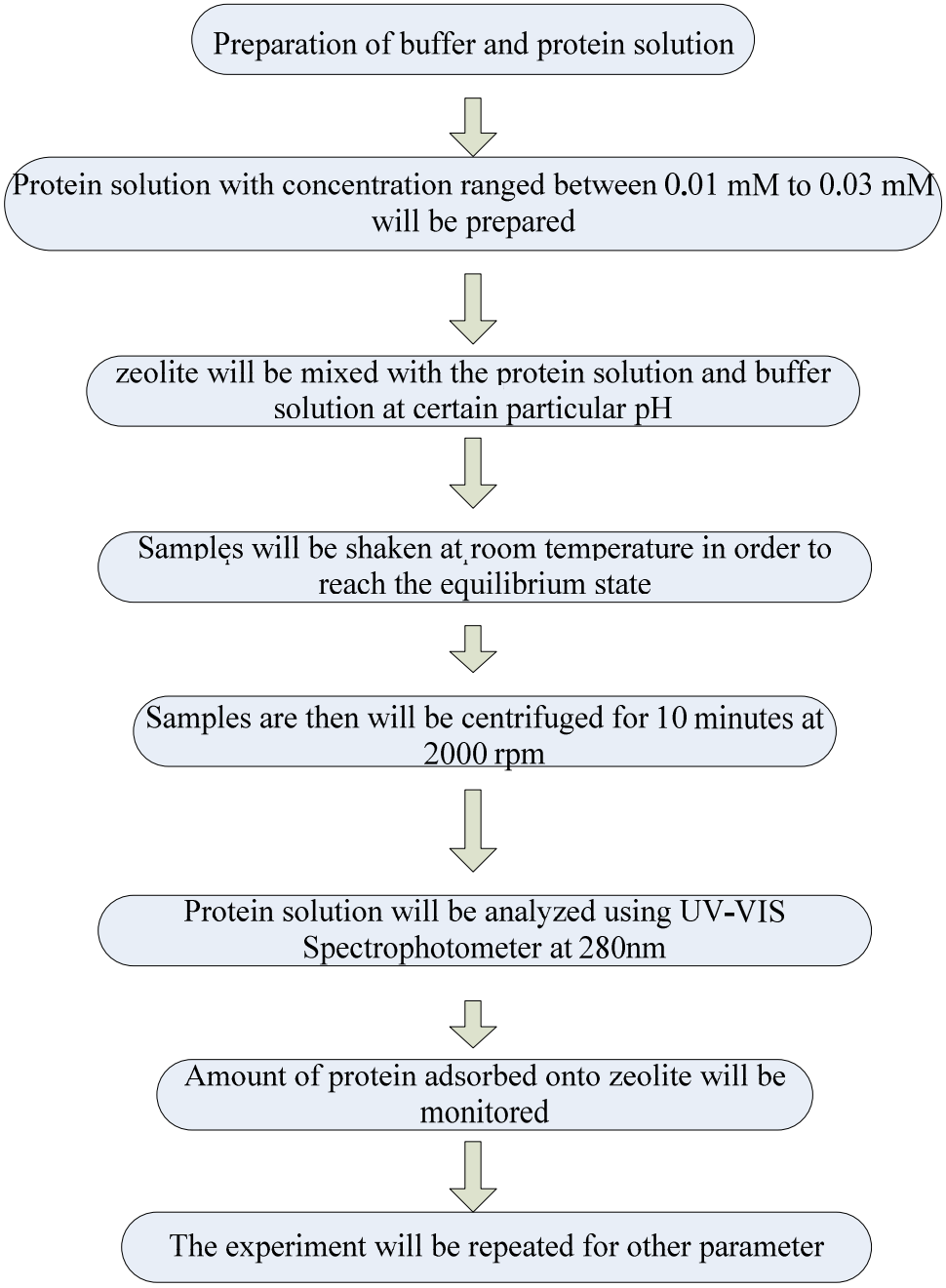
After centrifugation, a sample of supernatant was withdrawn, and the protein solution concentration was analyzed by Lambda 35 UV/VIS Spectrophotometer (PerkinElmer,Inc) at 280 nm as shown in Figure 3.2:

Figure 3.2: UV/VIS Spectrophotometer

The amount of protein adsorbed onto each zeolite was calculated based on mass balance. Experimental work was conducted at room temperature. The percentage of protein solution used in contact with zeolite crystals ranged from 0.4 to 0.04. The batch adsorption experiment was repeated for different type of adsorbent but with the same concentration used in the previous experiment. It is suggested that a blank run was performed for each experiment for good control in experimental conditions.

The separation efficiency was evaluated from the number and the separation degree of the eluting peaks, and the amount of each kind of protein adsorbed on the column could be estimated by the area or height of the corresponding eluting peaks.

3.6 Overall Process of Bovine Serum Albumin Adsorption Measurement



CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Introduction

Adsorption is one of the most uncomplicated methods to immobilize the proteins with the additional advantage of being an easy and inexpensive method. These methods profits from interactions between the surface of the support and the outer shell of biological molecules. Since the zeolite structures have microporous pores which are too small with respect to the kinetic diameter of Bovine Serum Albumin (BSA), the adsorption occurs solely on the external crystalline surface (A. Tovaro, 1999). The type of possible interactions and their strength between zeolites and proteins are quite complicated. They could consist in acid-base reactions between the amino groups of the protein and the surface hydroxyl groups of the zeolites or Van der Waals or electrostatic interactions. In general, proteins with hydrodynamic diameter larger than the pore diameter adsorb on the outer surface of the zeolites, while relatively small proteins interact with the microporous and show high loadings. It has been reported earlier that the protein size is the limiting factor for adsorption to occur.

4.2 Result of BSA Adsorption Capacity

In sequence to achieve the objective of this study, the adsorption capacity of BSA solution with concentration range from 0.01 mM to 0.03 mM on Y, Beta, and ZSM-5 zeolite with various pH values were being recorded and analyzed. For Y zeolite, high values of adsorption capacity were recorded. At pH 5 with BSA solution concentration of 0.03 mM, adsorption capacity was the highest which is 4.9718×10^{-3} mmol/g. Table below shows all the data for Y zeolite for pH 3, 4, 5 and 7 with the concentration range from 0.01 mM to 0.03 mM.

Table 4.1: Adsorption of BSA on Y zeolite for various pH

Adsorbent	pH	Concentration(mM)	Adsorbance	$qx10^3$(mmol/g)
Y	3	0.01	0.198	0.27086
		0.015	0.113	2.694
		0.02	0.318	1.7423
		0.025	0.367	2.2987
		0.03	0.529	1.9253
	4	0.01	0.095	1.049
		0.015	0.104	2.8829
		0.02	0.219	2.2979
		0.025	0.349	2.4476
		0.03	0.42	2.9258
	5	0.01	0.016	1.8552
		0.015	0.033	3.1777
		0.02	0.068	3.7913
		0.025	0.039	4.9381
		0.03	0.075	4.9718
	7	0.01	0.119	0.6713
		0.015	0.28	1.7335
		0.02	0.316	1.0548
		0.025	0.499	1.763
		0.03	0.507	2.0507

For Beta zeolite, the adsorption capacity for each pH that has been recorded was less than the adsorption capacity for Y zeolite. For Beta zeolite, pH 5 with the concentration of 0.02 mM has the highest amount of adsorption capacity that is 2.7794 x 10^{-3} mmol/g. As for the lowest adsorption capacity for Beta zeolite, pH 3 with the concentration of 0.01 mM has the minimal value that is 0.068 x 10^{-3} mmol/g. Table 4.2 below shows all the data for Beta zeolite for pH 3, 4, 5 and 7 with the concentration range from 0.01 mM to 0.03 mM.

Table 4.2: Adsorption of BSA on Beta zeolite for various pH

Adsorbent	pH	Concentration(mM)	Adsorbance	qx10^3(mmol/g)
Beta	3	0.01	0.226	0.0658
		0.015	0.321	1.1713
		0.02	0.48	0.5564
		0.025	0.52	1.1786
		0.03	0.639	1.1201
	4	0.01	0.105	0.9776
		0.015	0.149	2.5618
		0.02	0.381	1.1418
		0.025	0.631	0.4353
		0.03	0.644	1.3273
	5	0.01	0.102	1.275
		0.015	0.141	2.4488
		0.02	0.218	2.7794
		0.025	0.263	3.427
		0.03	0.524	1.9429
	7	0.01	0.194	0.118
		0.015	0.305	1.5491
		0.02	0.392	0.4942
		0.025	0.512	1.6671
		0.03	0.617	1.2393

For ZSM-5 zeolite, the adsorption capacity for each pH that has been recorded was less than the adsorption capacity for Y and Beta zeolite. For ZSM-5 zeolite, pH 5 with the concentration of 0.03 mM has the highest amount of adsorption capacity that is 1.8889 x 10^{-3} mmol/g. As for the lowest adsorption capacity for ZSM-5 zeolite, pH 7 with the concentration of 0.01 mM has the minimal value that is 0.0074 x 10^{-3} mmol/g. Table below shows all the data for ZSM-5 zeolite for pH 3, 4, 5 and 7 with the concentration range from 0.01 mM to 0.03 mM.

Table 4.3 : Adsorption of BSA on ZSM-5 zeolite for various pH

Adsorbent	pH	Concentration(mM)	Adsorbance	qx10^3(mmol/g)
ZSM-5	3	0.01	0.228	0.05124
		0.015	0.339	1.0395
		0.02	0.521	0.2562
		0.025	0.62	0.4466
		0.03	0.752	0.2928
	4	0.01	0.16	0.5852
		0.015	0.254	1.8125
		0.02	0.303	1.6984
		0.025	0.682	0.0714
		0.03	0.755	0.5352
	5	0.01	0.231	0.4048
		0.015	0.388	0.7825
		0.02	0.482	0.9984
		0.025	0.499	1.8349
		0.03	0.532	1.8889
	7	0.01	0.209	0.0074
		0.015	0.341	1.2835
		0.02	0.44	0.1402
		0.025	0.621	0.8631
		0.03	0.731	0.3983

4.3 Effect of different type of adsorbent on BSA purification

The adsorption of BSA was examined on Y, Beta and ZSM-5 zeolite in order to study the effect of different types of adsorbents. In sequence to exemplify the effect of different type of adsorbent on BSA adsorption uptakes, Figure 4.1 indicates the amount of adsorption of BSA for various pH. From the figure below, it can be clearly seen that H-Y zeolite has the highest amount of adsorption uptakes compared to Beta zeolite and ZSM-5 zeolite for every pH that has been inspected.

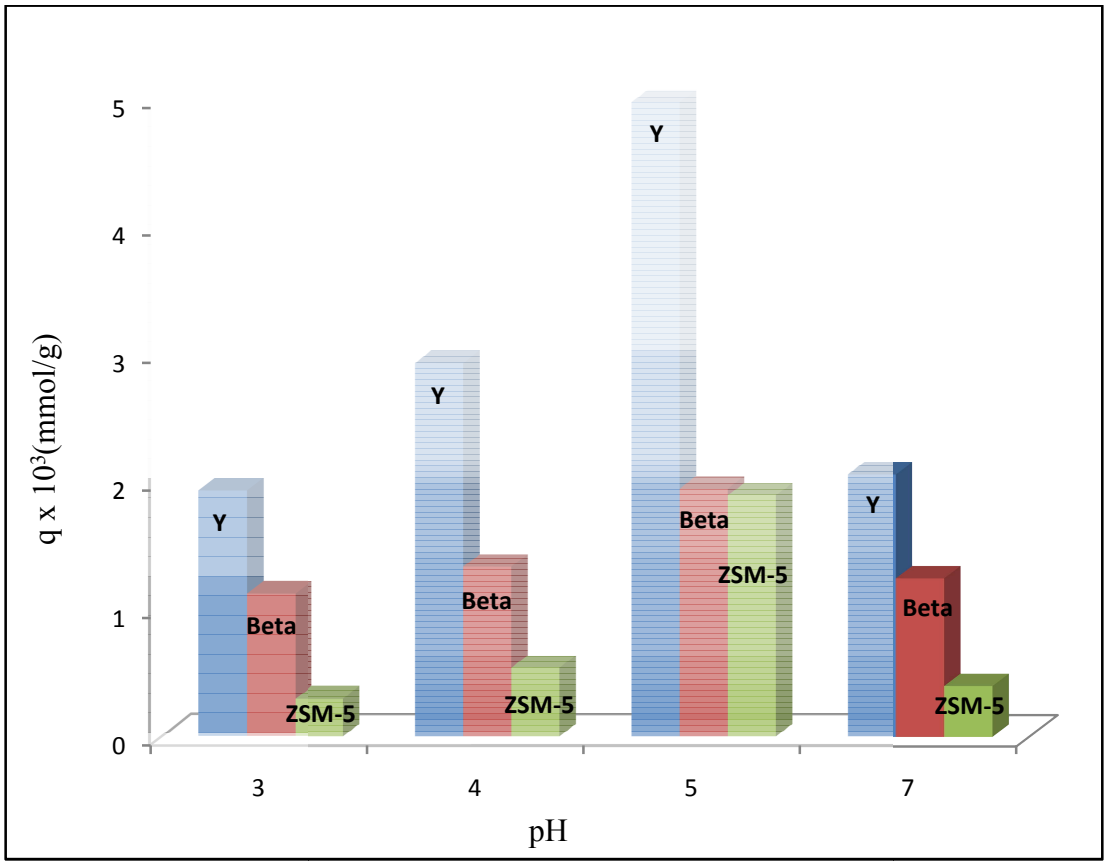


Figure 4.1: Effect of different type of adsorbent on BSA adsorption capacity

From the bar chart, it shows that the highest adsorption uptake is when Y zeolite is being used as adsorbent at pH 5 which recorded the value of 4.9718 x 10^{-3} mmol/g while for Beta zeolite, the adsorption uptake is 1.9429 x 10^{-3} mmol/g and for ZSM-5 zeolite is 1.8889 x 10^{-3} mmol/g. The lowest adsorption uptake is when ZSM-5 zeolite is being used as adsorbent at pH 3 which is 0.2928 x 10^{-3} mmol/g.

This result proves that adsorption capacity of Bovine Serum Albumin depends on the types of adsorbent used, which means the adsorption stoichiometries depends on the channel size of the zeolite as well as the zeolite structure. For each Y, Beta and ZSM-5 zeolite, adsorption capacity of Bovine Serum Albumin solution depends on their types of structure, pore size of the zeolite, surface area as well as pore volume of the zeolite. Table 4.4 shows the physicochemical properties of Y, Beta and ZSM-5 zeolite (A.Chica, 2005).

Table 4.4: Physicochemical properties of Y, Beta and ZSM-5 zeolite

Type of Zeolite	Zeolite Structure	Pore Size(nm)	Surface Area(m^2/g)	Pore Volume(cm^3/g)
Y	Faujasite(FAU)	0.74 x 0.74	680	0.302
Beta	Beta(BEA)	0.66 x 0.67	650	0.189
ZSM-5	Pentasil (MFI)	0.53 x 0.56	400	0.128

From the table shows above, it can be observed that Y zeolite has the largest pore size which is 0.74 x 0.74 nm thus automatically it will have the highest pore volume among all the zeolites. Zeolite Y also has the largest surface area which is 680 m^2/g compare to the surface area of Beta and ZSM-5 zeolite which is 650 m^2/g and 400 m^2/g respectively. The lowest adsorption capacity recorded by ZSM-5 zeolite is due to its minor pore size and lowest surface area, thus ZSM-5 zeolite will adsorbs BSA solution more selectively than Beta and Y zeolite since the smaller channels on ZSM-5 zeolite will restrict the size of adsorbed protein.

4.4 Effect of different pH value on BSA purification

In order for better understanding the role played by the pH value of the BSA solution, three different immobilization experiments were performed at pH 3, 4, 5 and 7. The pH dependence of BSA adsorption onto zeolite is shown in Figure 4.2. Experiments were carried out using BSA solutions at different pH values adjusted by using buffer solutions.

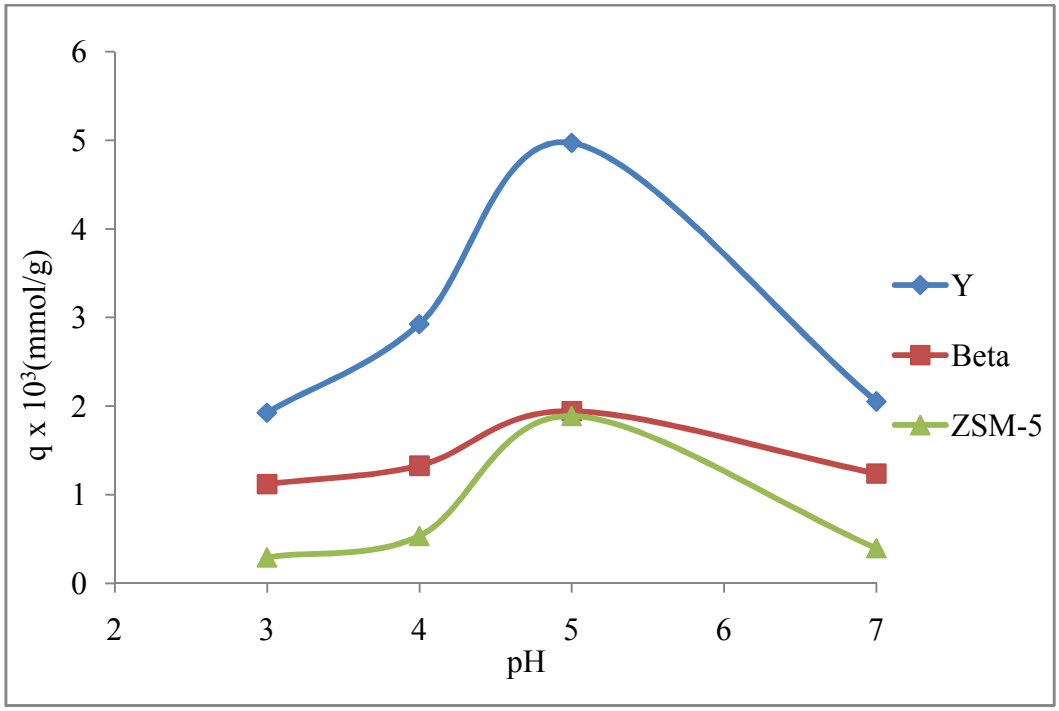


Figure 4.2: Effect of different pH value on BSA adsorption capacity

In a previous study of BSA adsorption onto the hydrophilic silica/water interface using neutron reflection, it has been examined that the concentration dependence of the surface excess of BSA at pH closes to its isoelectric point (pI) which is 4.7. Adsorption pH is the most important parameter influencing the binding capacity for the adsorption of proteins. The change of buffer pH altered the charge distribution, net charge of BSA molecule and charge group in the contact regions, conformational structure, and zeta potential of the surface of proteins.

From Figure 4.2, effect of different pH values on BSA adsorption capacity, the amount adsorbed for BSA solution adsorption uptakes increases as the pH of the solution increases for each zeolite, from pH 3 to pH 5, and reached a maximum adsorption at pH 5 but decrease gradually from pH 5 to pH 7. As deviating from the isoelectric point, the adsorbed amount of protein was decreased drastically. This result is in good agreement with the results obtained in previous studies reported adsorption isotherms with different adsorbents for different pH values.

The pH value that gave the highest adsorption uptakes, among those pH tested, was found to be at pH 5 which is the most close to the isoelectric point (pI) of BSA which is 4.7 when Y zeolite was used as the adsorbent. For each of the four different pH conditions, Y zeolite has the greater amount of adsorption uptakes compared to adsorption uptakes of Beta and ZSM-5 zeolite. At pH 5, the amount of adsorption uptakes for Y zeolite is 4.9718 x 10^{-3} mmol/g, for Beta zeolite is 1.9429 x 10^{-3} mmol/g and for ZSM-5 zeolite is 1.8889 x 10^{-3} mmol/g.

As for the lowest adsorption uptakes for BSA solution is when ZSM-5 zeolite was used as the adsorbent at pH 3 which recorded 0.2928 x 10^{-3} mmol/g of adsorption uptakes. For Y zeolite, the amount of adsorption uptakes at pH 3 is 1.9253 x 10^{-3} mmol/g while for Beta zeolite, the amount of adsorption uptakes at pH 3 is 1.1201 x 10^{-3} mmol/g. It is found that the adsorption of BSA is strongly dependent on pH which means pH has a significant effect on protein-surface interaction. The isoelectric point of BSA is 4.7, so when the pH value is closed to 4.7 to 5, protein sample is positively charged. In this case there is an electrostatic attraction between BSA and surface of the zeolite. The maximum adsorption on the zeolites tended to occur when the pH was at or just below the pI of the proteins. With increasing pH which is higher than pI the electrostatic repulsion between the protein and surface increases as both are negatively charged.

Additionally, repulsion between adsorbed BSA molecules also increases at higher surface coverage. These two effects combine to reduce the adsorption capacity

when the pH is increased above pI. Some proteins that adsorbed to the zeolites with high Si/Al ratios could bind to the zeolites at a pH above the pI value. BSA is known to be a soft protein with a low conformational stability meaning that BSA adsorbs onto negatively charged surfaces at pH values higher than the value of its isoelectric point.

Zeolites adsorbed biopolymers on their surface; this may be as a result of the following factors; which are; below pI, mainly the Coulombic attraction similar to ionexchange chromatography; at pI, probably hydrophobic interactions and the mesopore structure; and above pI, hydrophobic interactions and substitution of water at the Lewis acid sites of Al. When the Si/Al ratio is high, but Al level low, and in the presence of mesopores between the zeolite particles the adsorption was maximal at pI, thus this suggests that the adsortion is markedly dependent on the number of hydrophobic interaction points on the mesopores and their morphology.

4.5 Adsorption Isotherm of BSA

Protein adsorb on any surface with only a few exceptions. The fractional coverage is therefore strongly dependent on the bulk concentrations of the proteins. According to basic adsorption theory, adsorption can be regarded as a reaction between adsorbate molecules, which is in this study, is Bovine Serum Albumin, and active sites of adsorbent which is the zeolite. Adsorption uptakes were accurately described by the Langmuir isotherm which is given by equation 4.1;

$$q = \frac{q_m C}{K_d + C} \qquad (4.1)$$

Whereby q is solute concentration in adsorbent, q_m is the Langmuir isotherm parameter, C is the equilibrium concentration and K_d is the Langmuir adsorption parameter. The transformed form of Langmuir equation is given by equation 4.2, which will be used to calculate the Langmuir parameter constants by fitting adsorption isotherm data obtained for Bovine Serum Albumin.

$$\frac{C}{q} = \frac{1}{q_m} C + \frac{K_d}{q_m} \qquad (4.2)$$

The Langmuir parameter constants were calculated by nonlinear regression analysis of adsorption isotherm data using Langmuir transformed equation 4.2, which give a good correlation coefficient ($R^2 > 0.99$).

4.5.1 Adsorption Isotherm on Effect of Different Type of Adsorbent

The adsorption isotherms of Bovine Serum Albumin on Y, Beta and ZSM-5 zeolite at pH 5 are shown on Figure 4.3. From the figure, it can be clearly seen that the adsorption isotherms obtained on effect of different type of adsorbent at pH 5 were found to be confirm well to the ideal Langmuir model equation.

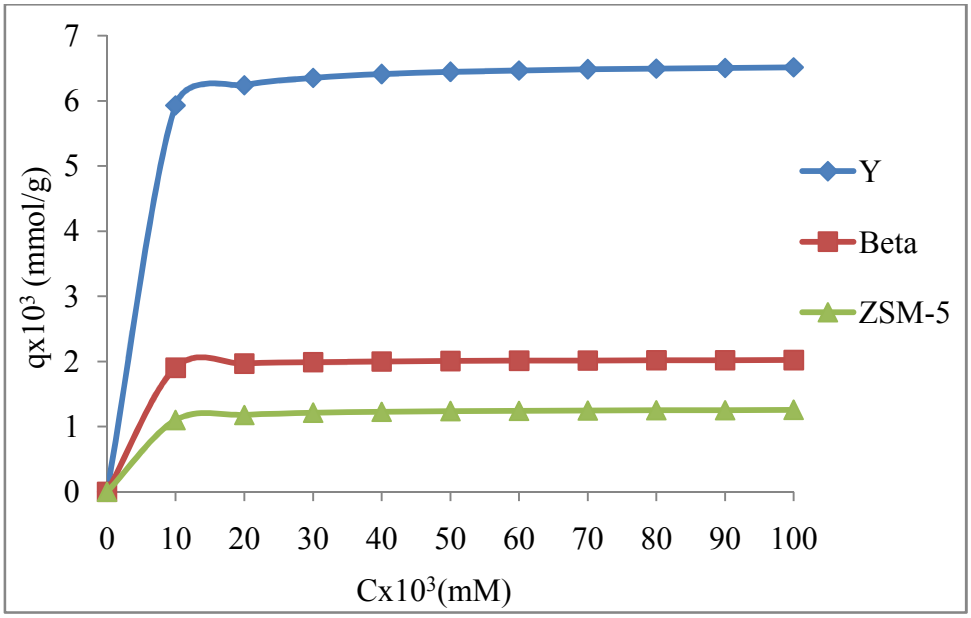


Figure 4.3: Adsorption isotherm on effect of different type of adsorbent at pH 5

Figure 4.3 shows that Y zeolite has the highest adsorption capacity which approaches the value of 6 x 10^{-3} mmol/g dry adsorbent. ZSM-5 zeolite gave the lowest adsorption capacity compared to Y and Beta zeolite. This result shows that adsorption capacity were essentially depends of the physicochemical properties of the zeolite such as the pore volume and the surface area of the zeolite. Since the adsorption isotherms fits well to the Langmuir model equation, we can conclude that the adsorption of BSA on different types of adsorbents is well matched with the Langmuir assumptions which are; adsorption cannot proceed beyond monolayer coverage, all surface sites are equivalent and can accommodate, at most, one adsorbed atom and the ability of a molecule to adsorb at a given site is independent of the occupation of neighboring sites.

4.5.2 Adsorption Isotherm on Effect of Different pH Value

The adsorption isotherms of Bovine Serum Albumin on Y zeolite at pH ranges from pH 3, 4, 5 and 7 are shown on Figure 4.4.

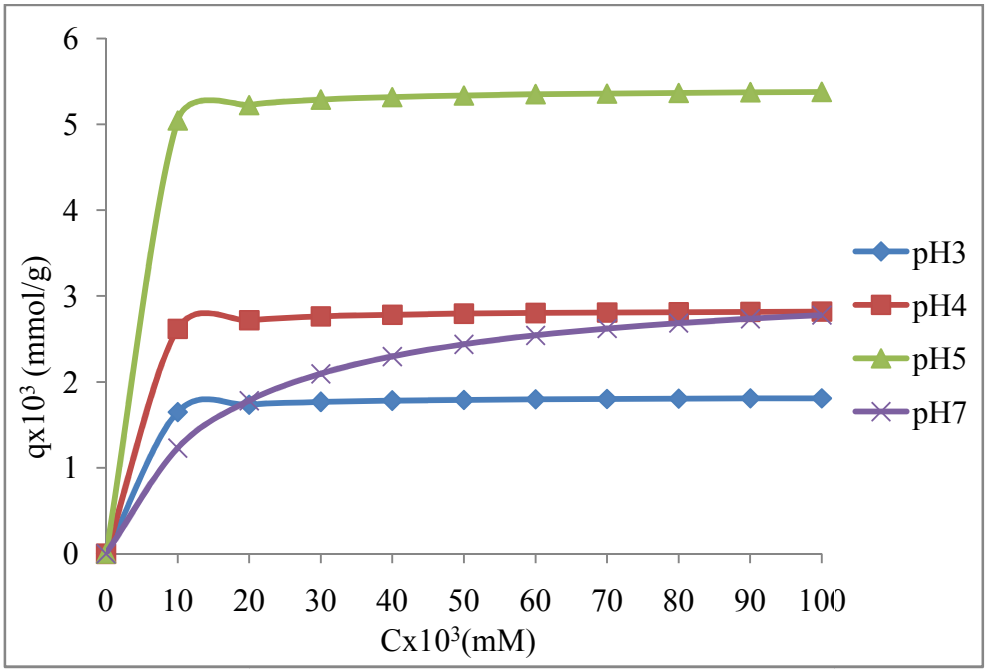


Figure 4.4: Adsorption isotherm on effect of different pH on Y zeolite

From the figure, it can be observed that at higher molar concentration, it seems that the adsorption uptake of BSA approaches a maximum around 5.3777×10^{-3} mmol/g for pH 5, meanwhile the adsorption for BSA increases at higher concentration for all pH. pH 3 gave the lowest adsorption capacity compared to other pH. This is postulated to be due to electrostatic repulsion between BSA and the surface of the adsorbent while a sharp initial rise of the isotherm in Figure 4.4, suggesting a high affinity between BSA and zeolite surface. It can be concluded that the adsorption of BSA on effect of different pH is well matched with the Langmuir assumptions which are; adsorption cannot proceed beyond monolayer coverage, all surface sites are equivalent and can accommodate, at most, one adsorbed atom and the ability of a molecule to adsorb at a given site is independent of the occupation of neighboring sites.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

In the course of this study, it is found that zeolite is able to adsorb protein such as Bovine serum albumin and might be suitable as a new and potential carrier for purification purposes since ion exchange, affinity, and hydrophobic interaction have been used for the separation of various kinds of protein but they seems to be weak chemically and physically. Increasing the value of pH higher than the value of isoelectric point (pI) will lead to the decrease in the adsorption capacity for Bovine Serum Albumin. This is postulated to be due to electrostatic repulsion between protein and the surface adsorbent. The adsorption isotherm data of protein is well fitted to the Langmuir model. The adsorption capacity is clearly a function of Bovine Serum Albumin solution pH and the physicochemical properties of the zeolites used as the adsorbent.

It is found that, zeolites adsorbed the Bovine Serum Albumin and that there were three physicochemical principles underlying the adsorption which are; below the isoelectric point of Bovine Serum Albumin that is 4.7, it was mainly Coulomb's attraction, similar to ion-exchange chromatography, the second principle is at the isoelectric point of every protein, it involved hydrophobic interaction which is a kind of Van der Waals attraction, together with the microporous structure of the zeolite and the third principle is when pH above the isoelectric point, it was the sum of Coulomb's repulsion and attraction, such as a hydrophobic interaction.

In this work, protein adsorption of Bovine Serum Albumin on Y, Beta and ZSM-5 zeolite was carried out and it is found that the highest adsorption capacity for Bovine Serum Albumin was on Y zeolite. Bovine Serum Albumin adsorption stoichiometries increased with the increasing zeolite pore volume and the surface area of the zeolite, suggesting that the extent of oligomerization is influenced by spatial constraints within channels (A.Chica, 2005). The maximal loading is clearly a function of solution pH. The amount adsorbed for Bovine Serum Albumin is maximal at pH5 which is close to the pI value which is 4.7. Bovine Serum Albumin is positively charged at pH below the isoelectric point and negatively charged at pH above the isoelectric point.

5.2 Recommendations

In this study, zeolite has proved to be both physically and chemically superior since it will not be affected by strong acid and alkali thus it will facilitate the process of protein purification. Selective adsorption due to the uniform pore size of the zeolite will guarantee an efficient separation of various proteins. These unique properties of zeolite molecular sieves should be investigated and discussed further to expand the application of zeolites to biochemistry and biotechnology industries.

It is noted that, types of the adsorbent zeolites as well as pH of the protein solution are important parameters influencing the adsorption process. In the future, other parameters such as protein size, temperature of the protein solution and protein concentration should be performed in the methods of experiment in order to accomplish a maximum degree of protein purification.

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APPENDICES

Preparation Bovine Serum Albumin (BSA) Solution

Concentration of 0.01 mM BSA in 100 mL deionized water

Molecular weight of BSA = 65000 g/mol

0.01 mM = 0.01 x 10^{-3} mol

$$\text{Mass} = \frac{\text{Final Concentration} - \text{Initial Concentration}}{\text{Molecular Weight}}$$

$$0.01 \times 10^{-3} = \frac{\text{Final Concentration}}{0.1\ \text{Liter}} \times \frac{1}{65000\ \text{g/mol}}$$

Grams needed = 0.01 x 10^{-3} x 0.1 x 65000

= 0.065 gram

0.065 gram of BSA is needed to prepare concentration 0.01mM BSA dissolved in 100 mL deionized water.

Concentration of 0.015 mM BSA in 100 mL deionized water

Molecular weight of BSA = 65000 g/mol

0.015 mM = 0.015 x 10^{-3} mol

$$\text{Mass} = \frac{\text{Final Concentration} - \text{Initial Concentration}}{\text{Molecular Weight}}$$

$$0.015 \times 10^{-3} = \frac{\text{Final Concentration}}{0.1\ \text{Liter}} \times \frac{1}{65000\ \text{g/mol}}$$

Grams needed = 0.015 x 10^{-3} x 0.1 x 65000

= 0.0975 gram

0.0975 gram of BSA is needed to prepare concentration 0.015mM BSA dissolved in 100ml deionized water.

Preparation of Buffer Solution

Buffer 0.1 M of H_3PO_4

JMR H_3PO_4 = 98 g/mol

Purity = 85%

Specific gravity = 1.71 g/ml

Solution(gram) = molarity x solution

= 0.1 x 0.1 x 98

= 0.98 g

$$\text{volume of solution} = \frac{\text{gram}}{\text{specific gravity}} \times \frac{1}{\text{purity}}$$

$$= \frac{0.98\text{ g}}{1.71\text{ g/ml}} \times \frac{1}{85}$$

$$= 0.6742\text{ mL}$$

0.6742 mL of H_3PO_4 is needed to prepare concentration 0.1 M H_3PO_4 buffer solution dissolved in 100 mL deionized water.

Buffer 0.1 M of KH_2PO_4

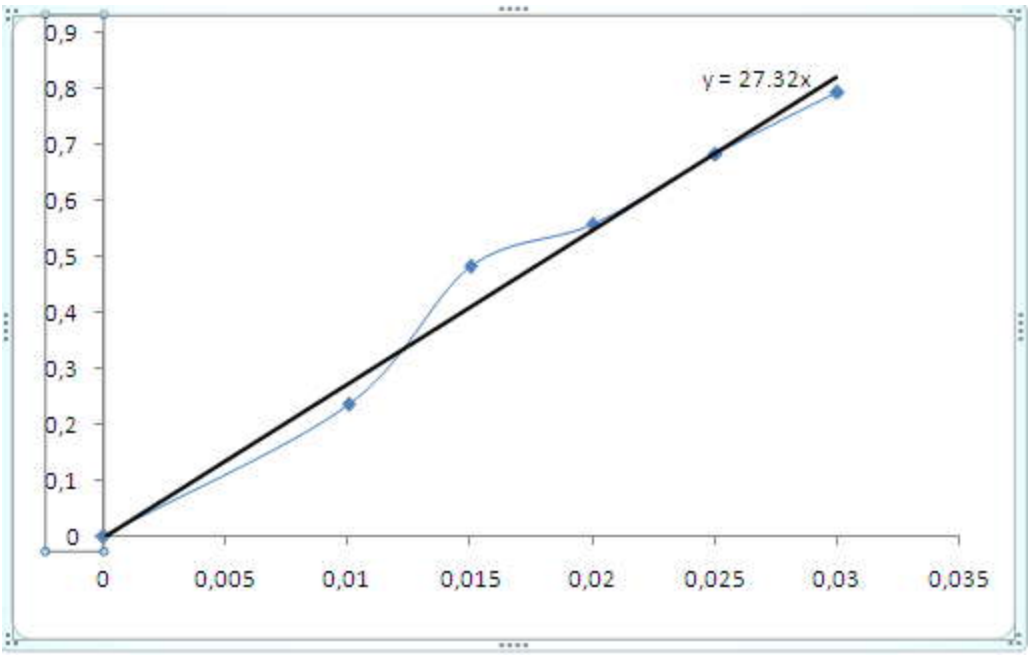
JMR of KH_2PO_4 = 136.09 g/mol

Grams needed = 0.1 x 0.1 x 136.09

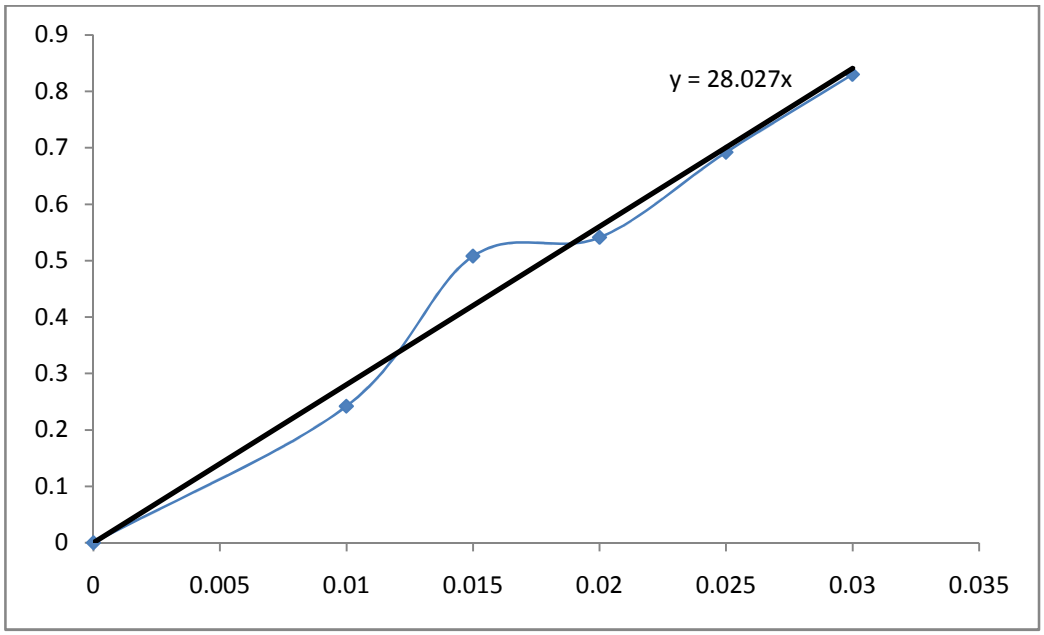
= 1.3609 g

1.3609 g of KH_2PO_4 is needed to prepare concentration 0.1 M buffer solution KH_2PO_4 dissolved in 100 mL deionized water.

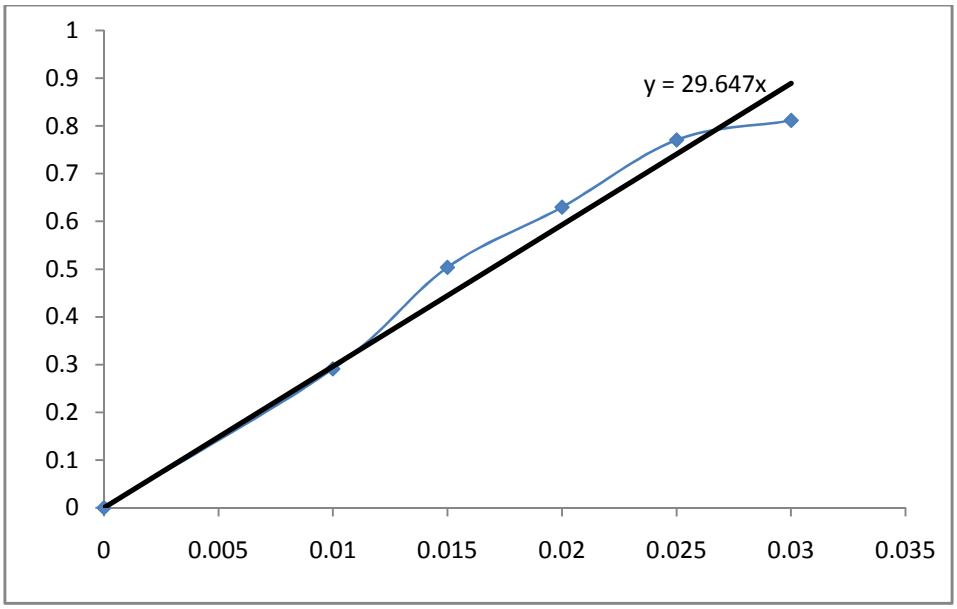
Graph for Initial Adsorbance of BSA at Various pH



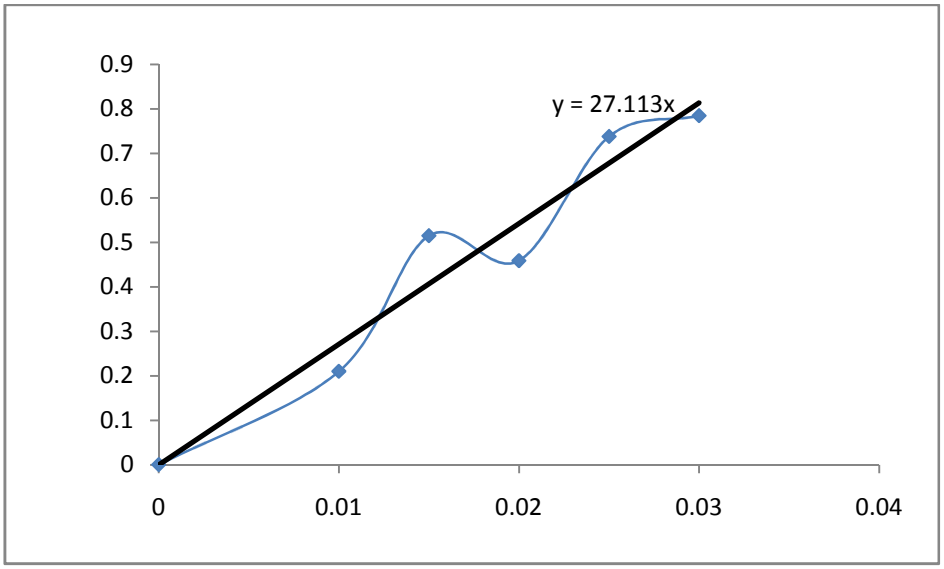
Graph of initial adsorbance of BSA at pH 3



Graph of initial adsorbance of BSA at pH 4



Graph of initial adsorbance of BSA at pH 5



Graph of initial adsorbance of BSA at pH 7

Data for Langmuir Adsorption Isotherm

Adsorption isotherm on effect of different type of adsorbent at pH 5

i) Y Zeolite

BSA concentration	adsorbance	initial	$qx10^{-3}$	$Cx10^{-3}$	C/q
0.01	0.016	0.291	1.8552	0.5397	0.2909
0.015	0.033	0.504	3.1777	1.1131	0.3503
0.02	0.068	0.63	3.7913	2.2937	0.605
0.025	0.039	0.771	4.9381	1.3155	0.2664
0.03	0.075	0.812	4.9718	2.5298	0.5088

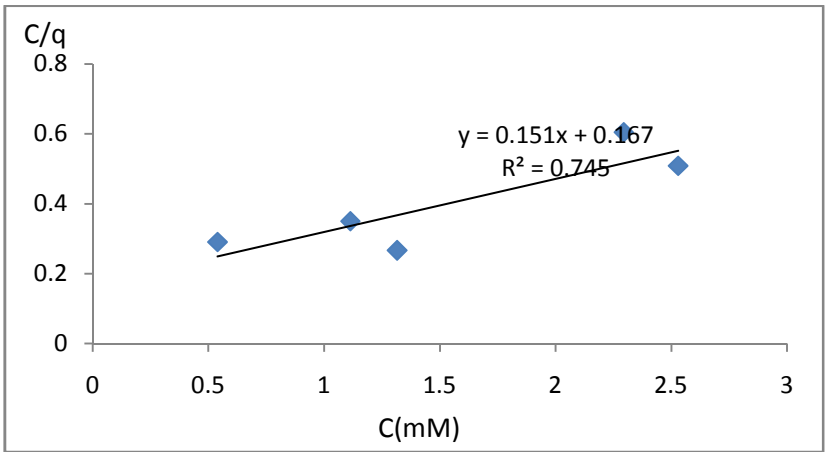
ii) Beta Zeolite

BSA concentration	adsorbance	initial	$qx10^{-3}$	$Cx10^{-3}$	C/q
0.01	0.102	0.291	1.275	3.4405	2.6984
0.015	0.141	0.504	2.4488	4.756	1.9422
0.02	0.218	0.63	2.7794	7.3532	2.6456
0.025	0.263	0.771	3.427	8.871	2.5886
0.03	0.524	0.812	1.9429	17.6746	9.097

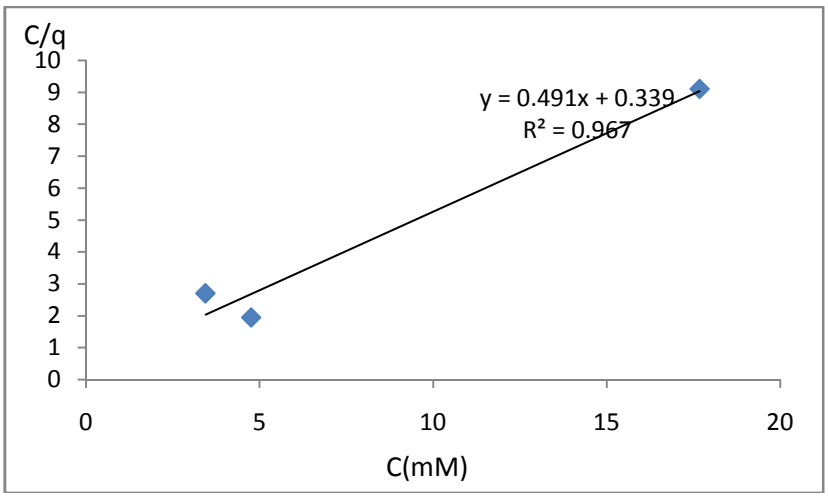
iii) ZSM-5 Zeolite

BSA concentration	adsorbance	initial	$qx10^{-3}$	$Cx10^{-3}$	C/q
0.01	0.231	0.291	0.4048	7.7917	19.2483
0.015	0.388	0.504	0.7825	13.0873	16.725
0.02	0.482	0.63	0.9984	16.258	16.2841
0.025	0.499	0.771	1.8349	16.8313	9.1729
0.03	0.532	0.812	1.8889	17.9444	9.503

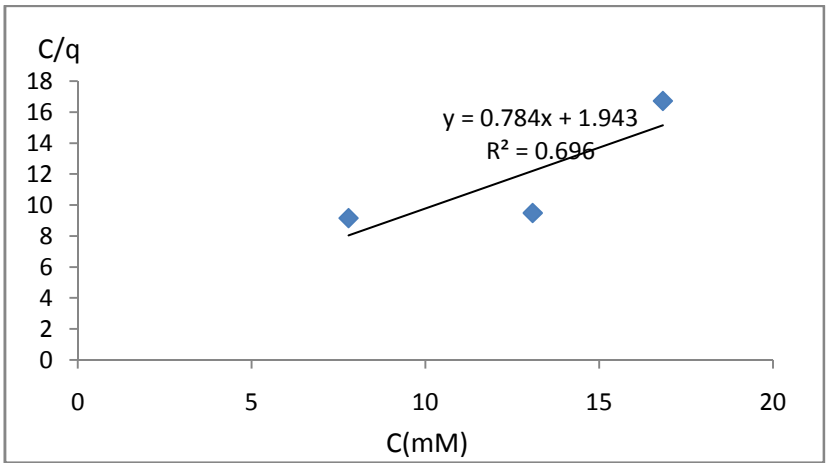
Adsorption Isotherm of BSA at pH 5 onto Y, Beta and ZSM-5 zeolite



Adsorption isotherm for Y zeolite at pH 5



Adsorption isotherm for Beta zeolite at pH 5



Adsorption isotherm for ZSM-5 zeolite at pH 5

Data for Langmuir isotherm of BSA at pH 5 onto Y, Beta and ZSM-5 zeolite

$$\square = \frac{\square\square\ \square}{\square + \square\square}$$

	Y	**Beta**	**ZSM-5**
$Cx10^{-3}$	$qx10^{-3}$	$qx10^{-3}$	$qx10^{-3}$
0	0	0	0
10	5.9291	1.9028	1.0969
20	6.2391	1.9663	1.1794
30	6.3498	1.9884	1.2097
40	6.4066	1.9997	1.2255
50	6.4412	2.0065	1.2351
60	6.4644	2.0111	1.2416
70	6.4811	2.0143	1.2463
80	6.4937	2.0168	1.25
90	6.5036	2.0187	1.2527
100	6.5115	2.0203	1.2549

Data for Langmuir Adsorption Isotherm

Adsorption isotherm on effect of various pH for Y zeolite

Adsorption of BSA on **Y zeolite** for various pH

i) pH 3

BSA concentration	Adsorbance	Initial	$qx10^{-3}$	$Cx10^{-3}$	C/q
0.01	0.198	0.235	0.27086	7.2474	26.757
0.015	0.113	0.481	2.694	4.1362	1.5353
0.02	0.318	0.556	1.7423	11.64	6.6808
0.025	0.367	0.681	2.2987	13.4333	5.8439
0.03	0.529	0.792	1.9253	19.3631	10.0572

ii) pH 4

BSA concentration	Adsorbance	Initial	$qx10^{-3}$	$Cx10^{-3}$	C/q
0.01	0.095	0.242	1.049	3.3896	3.2313
0.015	0.104	0.508	2.8829	3.7107	1.2871
0.02	0.219	0.541	2.2979	7.8139	3.4005
0.025	0.349	0.692	2.4476	12.4523	5.0876
0.03	0.42	0.83	2.9258	14.9856	5.1219

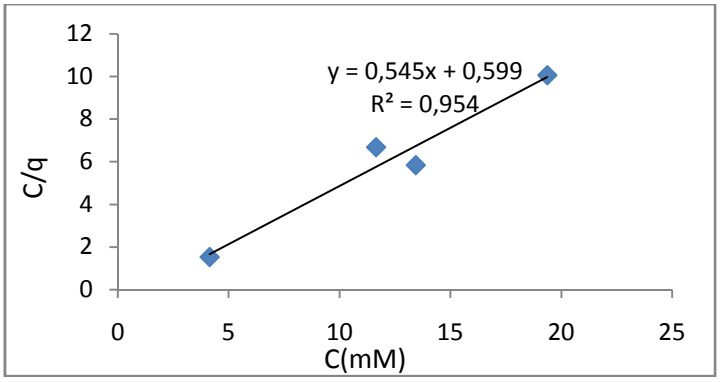
iii) pH 5

BSA concentration	Adsorbance	Initial	$qx10^{-3}$	$Cx10^{-3}$	C/q
0.01	0.016	0.291	1.8552	0.5397	0.2909
0.015	0.033	0.504	3.1777	1.1131	0.3503
0.02	0.068	0.63	3.7913	2.2937	0.605
0.025	0.039	0.771	4.9381	1.3155	0.2664
0.03	0.075	0.812	4.9718	2.5298	0.5088

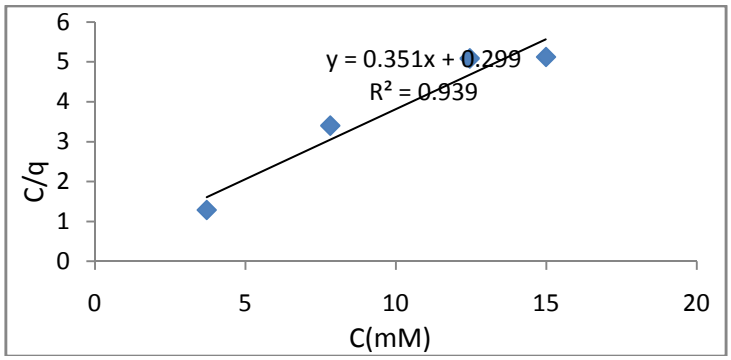
iii) pH 7

BSA concentration	Adsorbance	Initial	$qx10^{-3}$	$Cx10^{-3}$	C/q
0.01	0.119	0.21	0.6713	4.389	6.5381
0.015	0.28	0.515	1.7335	10.3272	5.9574
0.02	0.316	0.459	1.0548	11.6549	11.0494
0.025	0.499	0.738	1.763	18.4045	10.4393
0.03	0.507	0.785	2.0507	18.6995	9.1186

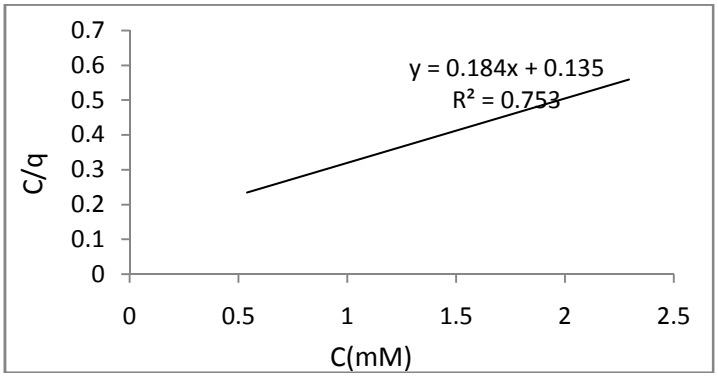
Adsorption Isotherm of BSA at various pH for Y zeolite



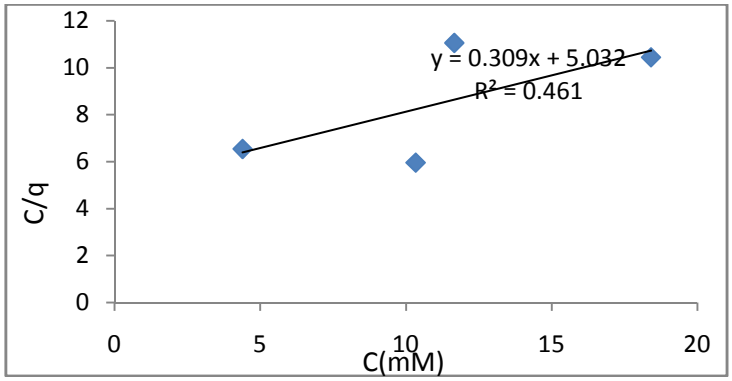
Adsorption Isotherm of BSA at pH 3 for Y zeolite



Adsorption Isotherm of BSA at pH 4 for Y zeolite



Adsorption Isotherm of BSA at pH 5 for Y zeolite



Adsorption Isotherm of BSA at pH 7 for Y zeolite

Data for Langmuir isotherm of BSA at various pH for Y zeolite

$$\theta = \frac{\text{[illegible]}}{\text{[illegible]}}$$

	pH3	**pH4**	**pH5**	**pH7**
$Cx10^{-3}$	$qx10^{-3}$	$qx10^{-3}$	$qx10^{-3}$	$qx10^{-3}$
0	0	0	0	0
10	1.6506	2.6209	5.0469	1.2306
20	1.7365	2.7209	5.2255	1.7825
30	1.7672	2.7656	5.2878	2.0958
40	1.7829	2.7848	5.3196	2.2978
50	1.7925	2.7965	5.3388	2.4388
60	1.7989	2.8043	5.3517	2.5428
70	1.8035	2.8099	5.3609	2.6227
80	1.807	2.8141	5.3679	2.686
90	1.8097	2.8174	5.3733	2.7374
100	1.8119	2.8201	5.3777	2.7799