ANTIBIOTIC PURIFICATION BY USING ZEOLITES ADSORBENT

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DECLARATION

I declare that this thesis entitled *"Antibiotic Purification by Using Zeolites 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 : ……

Name : Nur Munirah Binti Abd Wahab

Date : APRIL 2009

DEDICATION

To my beloved parents and siblings,

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First of all, I like to express my gratitude to Ilahi because giving me a good health condition during the period of finishing this project. Opportunities doing this project have taught me many new things. There is fun and sad time, but I believed that there are always people around me when I am in need and I would like to thank them from the bottom of my heart.

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ABSTRACT

Effective separation and purification of antibiotic has been an important issue in the pharmaceutical industries. A novel antibiotic adsorption has been developed in biotechnology to achieve high efficiency and economical separation processes. Application in separation and purification processes often used the ability of zeolites and other molecular sieves to exclude larger molecules to enter the pores and admit smaller ones. In this study, three types of zeolites which are Y, Beta and ZSM-5 have been used to study the effect of their performance on the antibiotic purification. The zeolite is used as an immobilized metal ion affinity stationary phase for antibiotic purification. The adsorption of Rifampicin antibiotic using zeolites was studied. Rifampicin adsorbance was analyzed by using UV/VIS Spectrophotometer. The zeolite Beta is recognized to have highest adsorption capacity compared to zeolite Y and ZSM-5. The adsorption capacity of Rifampicin depends on their types of structure, pore size of the zeolite, surface area as well as pore volume of the zeolite. The effect of pH on adsorption capacity was studied at four different pHs, namely 5, 7, 8, and 9. It is found that the adsorption capacity is the highest at pH 8 which is the nearest to the pKa of Rifampicin. Increase in pH lower than pKa value result in increasing adsorption capacity. But, increase in pH higher than pKa value results decreasing adsorption capacity. This is postulate due to the electrostatics repulsion between antibiotic molecules and the surface of adsorbent. Lastly, it can be concluded that the most efficient zeolite is Beta at pH 8. The adsorption isotherms data on Rifampicin are fitted to the Langmuir model.

ABSTRAK

Pengasingan dan penulenan antibiotik yang efektif telah menjadi isu yang penting dalam industri farmasi. Penjerapan antibiotik telah dibangunkan dalam industri bioteknologi untuk mencapai proses pengasingan yang efisien dan ekonomikal. Proses pengasingan dan penulenan antibiotik mengaplikasikan kebolehan zeolite dan penapis molekul yang lain untuk menghalang molekul yang lebih besar daripada memasuki liang-liang zeolite dan membenarkan molekul yang lebih kecil melaluinya. Dalam kajian ini, tiga jenis zeolite iaitu zeolite Y, Beta, dan ZSM-5 digunakan untuk mengkaji kesan aktiviti mereka ke atas antibiotik Rifampicin. Zeolite digunakan sebagai tarikan ion logam yang tidak bergerak dalam fasa pegun untuk proses penulenan antibiotik. Penjerapan antibiotik Rifampicin telah dikaji. Kadar penjerapan Rifampicin diuji menggunakan alat UV-VIS Spectrophotometer. Zeolite Beta telah dikenalpasti mempunyai nilai penjerapan yang paling tinggi berbanding zeolite Y, dan ZSM-5. Kapasiti penjerapan untuk Rifampicin bergantung kepada jenis struktur, saiz liang zeolite, luas permukaan, dan isipadu liang. Kesan pH ke atas kapasiti penjerapan telah dikaji bagi empat pH berbeza iaitu, 5, 7, 8, dan 9. Kapasiti penjerapan dikenalpasti paling tinggi pada pH 8, iaitu pada nilai yang paling hampir kepada nilai pKa Rifampicin. Peningkatan nilai pH di bawah nilai pKa akan menghasilkan kapasiti penjerapan yang turut meningkat. Tetapi, peningkatan nilai pH lebih tinggi daripada nilai pKa akan menghasilkan kapasiti penjerapan yang semakin menurun. Hal ini adalah disebabkan oleh daya tolakan elektrostatik antara Rifampicin molekul dan permukaan penjerap. Akhir sekali, zeolite Beta disimpulkan mempunyai efisiensi yang paling tinggi pada pH 8. Data isoterma penjerapan bagi Rifampicin adalah bertepatan dengan model Langmuir.

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LIST OF SYMBOLS

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

LIST OF ABBREVIATIONS

NMR	-	Nuclear Magnetic Resonance
Rif	-	Rifampicin
pH	-	Expressing acidity or alkalinity on a logarithmic scale
pKa	-	Acid Dissociation Constant
DNA	-	Deoxyribonucleic acid
RNA	-	Ribonucleic acid
MW	-	Molecular Weight
SG	-	Specific Gravity

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Purification is an important process in pharmaceutical production. Maximizing yield while maintaining required purity is paramount to reducing purification costs, but this goal is difficult to achieve when the product and its impurities are very similar. Crystallization is often used is this case, but results in a substantial loss of yield.

Antibiotics are substances that inhibit the growth of or destroy bacteria that cause infection. Antibiotics do not work against viral diseases such as the common cold or influenza. The word "antibiotics" comes from the Greek *anti*("against") and *bios*("life"). Antibiotics have been used since the 1930s to prevent or treat a wide variety of infections in plants, animals, and humans. Before that time, there were few effective ways of combating microbial infections (infections caused by microorganisms). Illnesses such as pneumonia, tuberculosis, and typhoid fever were essentially untreatable. Even minor infections could be deadly.

Zeolites are crystalline porous solids. They are tectosilicates consisting of corner-sharing AlO4 and SiO4 tetrahedra. Moreover, they are readily available, easy to obtain, stable, and inexpensive compared to other chromatographic carriers like sepharose. Zeolites have an unusual crystalline structure and a unique ability to change ions. A very large number of small channel are present in its structure. These channels have typical diameters of 0.5 to 0.7 nm, only slightly larger than the diameter of a water molecule. These channels are called microporosity. Beside this there are a number of larger pores,

the so-called mesoporosity. Positive ions are present in the channels, which can be exchanged for other ions.

This substitution of ions enables zeolites to selectively adsorb certain harmful or unwanted elements from soil, water and air. A classic example is the removal of calcium from hard water. Zeolites exchange sodium ions for calcium ions, which result in soft water. Zeolites also have strong affinity for certain harmful heavy metals such as lead, chromium, nickel and zinc. In the mesopores of zeolite suspended and colloidal particles can be trapped. In these pores dissolved organic molecules are adsorbed also.

There are numerous naturally occurring and synthetic zeolites, each with a unique structure. The pore sizes commercially available range from approximately 3 Å to approximately 8 Å. Some of the commercial materials are: A, beta, mordenite, Y, ZSM-5.

Adsorption is a process, similar to absorption, by which a substance in a gas or liquid becomes attached to a solid. The substance can be a pollutant, called an adsorbate, which is attracted to the surface of a special solid. Adsorption occurs naturally, but industrialists have perfected adsorption methods to clean up hazardous waste or purify drinking water.

Natural or organic methods of adsorption take place all the time. For example, the ocean adsorbs carbon dioxide in the atmosphere, which effects climate and atmospheric temperature. Early humans observed that if they charred a piece of bone all the way through, they could put the bone in food mixtures, like sugar water, and it would collect polluting particles that weren't edible, thereby purifying the food. Particles colored in our visible spectrum, as well as those with strong odors, are easiest to adsorb.

It's important to harness the power of adsorption in battling modern chemical hazards. Some solids are ideal for adsorption. They have a lot of surface area for their volume because they are pockmarked with micropores. Industrial and commercial uses for adsorption filters vary. For example, carbon makes cold drinking water taste better. A carbon filter can be heated to clean the surface of adsorbates and reused.

1.2 Problem Statement

Nowadays, there are many processes applied for antibiotic purification. Extraction and membrane separation film are mostly used in antibiotic purification. Unfortunately, both of the processes have their own weakness. The extraction for antibiotic need high cost and the purity of the antibiotic is lower than expected. As for membrane separation film, the membrane is easy to foul and need more maintenance. The maintenances also need high cost and it should have constant schedule of maintenance. When the membrane is fouling, the flux ratio is affected. So do the accuracy of the purification. This research is to find another best method for antibiotic purification, which is adsorption by using zeolite.

1.3 Objective of the Research

The objective of the research is to study the optimum condition for purification of antibiotic by using zeolite.

1.4 Scope of the Research

This research consist two of components:

i. The effect of types of zeolites used.
ii. The effect of zeolites on different pH of antibiotic solution

CHAPTER 2

LITERATURE REVIEW

2.1 Antibiotic

Antibiotics are substances that inhibit the growth of or destroy bacteria that cause infection. Antibiotics do not work against viral diseases such as the common cold or influenza. The word "antibiotics" comes from the Greek anti("against") and bios("life"). Antibiotics have been used since the 1930s to prevent or treat a wide variety of infections in plants, animals,and humans. Before that time, there were few effective ways of combating microbial infections (infections caused by microorganisms). Illnesses such as pneumonia, tuberculosis, and typhoid fever were essentially untreatable. Even minor infections could be deadly.

The years between 1928 and 1940 were the most productive in the discovery and development of antimicrobial drugs. In 1928 Sir Alexander Fleming, a Scottish physician, was working on ways to kill bacteria isolated from infected wounds. He observed that a mold growing in a laboratory culture was able to destroy that culture's bacteria. Since the mold that produced the bacteria-killingsubstance was a species of Penicillium, Fleming named the substance penicillin.

It is not known when the first antibiotic was used; folk medicine has used various molds to fight infections for centuries. In 1935 a German chemist named Gerhard Domagk discovered the first class of antibacterial agents, the sulfonamides.

Sulfanilamide (the parent compound of the sulfonamides) was originally part of a leather dye compound that was being screened for its potential ability to kill bacteria. It was found to be relatively nontoxic and when the dye was broken down in the body, it was converted to the compound sulfanilamide.

2.1.1 Side Effects of Antibiotics

Antibiotics can literally save lives and are effective in treating illnesses caused by bacterial infections. However, like all drugs, they have the potential to cause unwanted side effects. Many of these side effects are not dangerous, although they can make life miserable while the drug is being taken.

In general, antibiotics rarely cause serious side effects. The most common side effects from antibiotics are diarrhea, nausea, vomiting. Fungal infections of the mouth, digestive tract and vagina can also occur with antibiotics because they destroy the protective 'good' bacteria in the body (which help prevent overgrowth of any one organism), as well as the 'bad' ones, responsible for the infection being treated.

Some people are allergic to antibiotics, particularly penicillins. Allergic reactions cause swelling of the face, itching and a skin rash and, in severe cases, breathing difficulties. Allergic reactions require prompt treatment.

2.1.2 Types of Antibiotic

Although there are well over 100 antibiotics, the majority come from only a few types of drugs. These are the main classes of antibiotics.

- Penicillins such as penicillin and amoxicillin

- Cephalosporins such as cephalexin (Keflex)
- Macrolides such as erythromycin (E-Mycin), clarithromycin (Biaxin), and azithromycin (Zithromax)
- Fluoroquinolones such as ciprofloxacin (Cipro), levofloxacin (Levaquin), and ofloxacin (Floxin)
- Sulfonamides such as co-trimoxazole (Bactrim) and trimethoprim (Proloprim)
- Tetracyclines such as tetracycline (Sumycin, Panmycin) and doxycycline (Vibramycin)
- Aminoglycosides such as gentamicin (Garamycin) and tobramycin (Tobrex)

Most antibiotics have 2 names, the trade or brand name, created by the drug company that manufactures the drug, and a generic name, based on the antibiotic's chemical structure or chemical class. Trade names such as Keflex and Zithromax are capitalized. Generics such as cephalexin and azithromycin are not capitalized.

2.1.2.1 Macrolides

There are a couple of new relatives of erythromycin (azithromycin and clarithromycin) that work the same way, but kill more bugs and have slightly fewer side effects. The erythromycin-like antibiotics are also known as macrolides. Macrolides belong to the polyketide class of natural products. Macrolide antibiotics are used to treat respiratory tract infections, genital, gastrointestinal tract, soft tissue infections caused by susceptible strains of specific bacteria.

Macrolides bind with ribosomes from susceptible bacteria to prevent protein production. This action is mainly bacteriostatic, but can also be bactericidal in high concentrations. Macrolides cause very little allergy problems compared to the penicillins and cephalosporins, the biggest concern with these medicines is that they can irritate the stomach.

2.1.2.2 Penicillins

Penicillin was the first antibiotic discovered by Alexander Fleming in 1929. Penicillins are used to treat skin infections, dental infections, ear infections, respiratory tract infections, urinary tract infections, gonorrhea. Penicillins are sometimes combined with other ingredients called beta-lactamase inhibitors, which protect the penicillin from bacterial enzymes that may destroy it before it can do its work.

Penicillins are usually very safe. The greatest risk is an allergic reaction, which can be severe. People who have been allergic to cephalosporins are likely to be allergic to penicillins.Penicillins block the construction of bacteria cell walls, causing the walls to break down, and eventually killing the bacteria.

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2.2 Rifampicin

Rifampicin is a naturally made, non-peptide antibiotic. It is bactericidal, killing by disabling the protein expression system universally conserved by all bacteria. Specifically, rifampicin inhibits the RNA polymerase protein, which is responsible for binding to a strand of DNA as a template and using it to construct a strand of mRNA

Rifampicin inhibits RNA polymerase by bonding tightly in the RNA exit channel. Therefore, after transcription begins, the RNA transcript, trying to exit the RNAP through the exit channel, runs into the rifampicin sitting in the middle of the channel. This effectively halts transcription when the RNA transcript is merely two or three nucleotides in length. Below are the properties of rifampicin and the structure of rifampicin:

Table 2.1: Properties of Rifampicin

Molecular formula	C43H58N4O12
Molecular weight	823.0
Acid dissociation constant, pKa (in water)	7.9
Optical rotation, $[\alpha]_D^{25^0}$	+10.6°
Maximum absorbance	333nm
Melting point	183-188°C
Solubility in water	Slightly soluble
Appearance	Orange-brown to red-brown powder

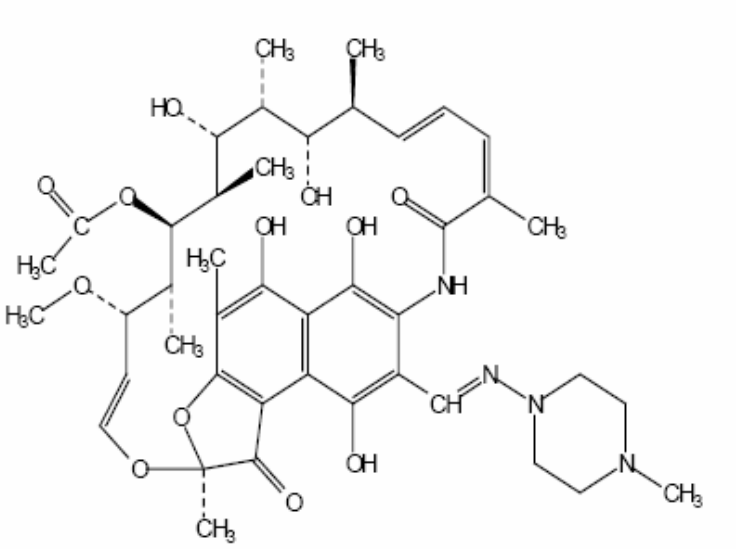

Figure 2.1: Molecular structure of Rifampicin

Despite this highly efficient method for killing bacteria, rifampicin is by no means a perfect antibiotic. The biggest problem arises from the fact that bacteria can