ANTIBIOTIC PURIFICATION BY USING IMA ADSORBENTS

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I declare that this thesis entitle "*Antibiotic Purification by Using IMA Adsorbents"* is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and not concurrently submitted in candidature of any degree

Signature : ……

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Date : 30 APRIL 2009

Special Dedication of This Grateful Feeling to My…

Beloved father and mother;
Mr. Mohd Yusoff bin Hussin and Mrs. Rabainah binti Hashim

Loving brothers and sisters;
Shahiful, Shahrizal, Asrol, Zaidi, Zurianie,
Azmir, Huda, Azizah and Shalehuddin.

Supportive friends

For Their Love, Supports and Best Wishes

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ABSTRACT

The intensity to achieve highly efficient and economical separation process can be seen in developing of various methods in the recent year. While in purification of antibiotic there are many methods use such as using High performance liquid chromatography (HPLC) and Counter-current chromatography (CCC). The purpose of this research is to develop immobilized metal ion affinity zeolite by using solid state ion exchange method to investigate the effect of pH, types of adsorbent using different metal into rifampicin adsorption capacity. Adsorption of rifampicin using zeolite has a greatly potential due to ability to scale up easily, and highly selective. It was found that the highest adsorption capacity for rifampicin occur at pH 8 with Zr-HBeta as adsorbent. H-beta zeolite give highest adsorption capacity because it has higher diameter size, surface area and pore volume compare to Y zeolite. Increasing the surface area and pore volume will give better chances of rifampicin to adsorb into adsorbent. Meanwhile, pH 8 gives the highest adsorption capacity because it is closer with the pKa_2 value of rifampicin which is 7.9. While zirconium is the only transition metal containing both acidic and basic surface sites. So this will make it gives better adsorption capacity of rifampicin compare with ferum and nickel. The adsorption isotherm data of rifampicin was well correlated by the Langmuir model.

ABSTRAK

Keinginan yang tinggi untuk mencapai proses pemisahan yang ekonomi dan berkecekapan tinggi dapat dilihat melalui penghasilan pelbagai cara sejak kebelakangan ini. Terdapat pelbagai cara dalam penyulingan antibiotic seperti HPLC and CCC. Tujuan kajian ini adalah untuk menyediakan ion logam tarikan dimasukkan ke dalam zeolite menggunakan kaedah penukar ion berkeadaan pepejal untuk melihat kesan pH, jenis penjerap daripada jenis logam yang berlainan terhadap kapasiti penjerapan rifampicin. Penjerapan rifampicin menggunakan zeolite mempunyai potensi yang besar kerana mudah dioperasikan pada skala yang lebih besar, beroperasi secara berterusan dan mempunyai kememilihan yang tinggi. Kapasiti penjerapan tertinggi untuk rifampicin adalah pada pH 8 dengan mengunakan penjerap logam zirkonium. Zeolite H-beta memberikan kapasiti penjerapan tertinggi kerana ianya mempunyai saiz diameter, luas permukaan and isipadu pori yang lebih besar berbanding dengan zeolite Y. Pertambahan luas permukaan serta isipadu pori akan memberikan peluang yang lebih kepada rifampicin untuk menyerap ke dalam penjerap. Dalam pada itu, pH 8 memberikan kapasiti penjerapan tertinggi kerana ianya dekat dengan nilai pK_{a2} bagi rifampicin iaitu 7.9. Sementara itu, hanya zirkonium sahaja logam peralihan yang mengandungi sifat asid dan alkali bagi kedua-dua belah permukaan. Ini menjadikan zirkonium memberikan kapasiti penjerapan rifampicin lebih baik berbanding dengan ferum dan nikel. Data penjerapan rifampicin menunjukkan ianya menghampiri model Langmuir.

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LIST OF SYMBOLS

Al	Aluminium
C	Concentration mM
Fe	Ferum
Kd	Langmuir adsorption parameter
pH	Negative logarithmic molar concentration of hydrogen ion, -log[H+]
pKa	Acid dissociation constant
n	Freundlich constant
Na	Sodium
Ni	Nickel
q	Solute concentration in adsorbent mmol/g
qm	Langmuir isotherm parameter mmol/g
Si	Silica
Zr	Zirconium

LIST OF ABBREVIATIONS

CASMAC	Cascade-mode multi-affinity chromatography
CCC	Counter-current Chromatography
CEC	Cation Exchange Capacity
CNS	Central Nervous System
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
FTIR	Fourer Transform Infrared
H_3PO_4	Phosphoric acid
HSCCC	High Speed Counter-current Chromatography
IDA	Iminodiacetic acid
IMA	Immobilized metal ion affinity
IMAC	Immobilized metal ion affinity chromatography
IUPAC	International Union of Pure and Applied Chemistry
FDA	Food and Drug Administration
HPLC	High Performance Liquid Chromatography
K_2CO_3	Potassium carbonate
K_2HPO_4	Potassium Hydrogen Phosphate
KH_2PO_4	Potassium Dihydrogen Phosphate
$KHCO_3$	Potassium hydrogen carbonate
LEC	Ligand Exchange Chromatography
NTA	Nitrilotriacetic acid

RNA	Ribonucleic acid
UV-VIS	Ultra Violet Visible

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Antibiotics are organic substances produced by special microorganisms or other living systems. Generally, antibiotic are produced on an industrial scale using a fermentation process and capable at low concentration of inhibiting the growth of, or destroying another microorganism. Antibiotics have been isolated from numerous sources but mainly from bacteria (tetracyclines, bacitracin, polymyxin, chloramphenicol, and streptomycin) and fungi (cephalosporins, penicillins). Penicillin was the first antibiotic discovered by Sir Alexander Flemming in 1928. It is derive from the Penicillium mold and acts by destroying the cell wall of bacteria. The name penicillium was taken from the Latin penicillum meaning a painter's brush because the fronds of the fungus were thought to look like a painter's brush.

Antibiotics are the most important bioactive and chemotherapeutic compounds made by microbiological synthesis. They also include antimicrobial compounds present in higher plants and animals. They have proven their significance in varied fields like medicinal chemistry, agriculture and food industry.

Up to now about 40 000 antibiotics have been found and about 80 of them are in therapeutic use. They are isolated primarily from metabolic products of living cells. Various penicillins, cephalosporins and several other antibiotics are semi-synthetic ones, which mean one part of the molecule, i.e. 6-amino penicillanic acid is

prepared from say penicillin G or penicillin V, followed by synthetic introduction of an appropriate side chain.

Zeolites are crystalline, hydrated aluminosilicates of alkali and alkaline earth cations characterized by an ability to hydrate/dehydrate reversibly and to exchange some of their constituent cations with dissolved cations in solution, both without a major change in structure. The ion exchange property of these minerals has generated worldwide interest for use in diverse applications such as the treatment of nuclear, municipal, and industrial waste water. Although commercial applications of ion exchange processes have used mainly synthetic zeolites, the earliest studies of ion exchange phenomena were based on observations of natural materials, including natural zeolites.

Commercial adsorbents that display ultra porosity include activated carbons, activated clays, inorganic gels, such as silica gel and activated alumina, and the crystalline aluminosilicate zeolite. Activated carbons, activated alumina, and silica gel do not possess an ordered crystal structure and consequently their pores are non-uniform. The distribution of the pore diameters within the adsorbent particles may vary widely from 20 to several thousand Angstroms, as is the case for some activated carbons. Hence, all molecular species, with the possible exception of high molecular weight polymers, may enter the pores. Zeolite molecular sieves, on the other hand, have pores of uniform size (3–10Å) which are uniquely determined by the unit structure of the crystal. These pores will completely exclude molecules that are larger than their diameter. J. W. McBain (1932) originated the term “molecular sieves” to define porous solid materials that exhibit the property of acting as sieves on a molecular scale.

Synthetic adsorbents are widely used as polymeric media for recovery and separation of antibiotic or their intermediates, foods, etc. For example, they are used for separation of antibiotics such as penicillin, cephalosporin and their derivatives, because of their high adsorption capacity, mechanical strength and chemical stability suitable for industrial operations.

Column operations are commonly adopted for those applications. In this sense, the synthetic adsorbents are used as chromatographic separation media. Therefore, both pore and chemical characteristics of the synthetic adsorbents will affect the separation and adsorption capacity of target compounds.

1.2 Problem Statement

The development of an antibiotic is a long and costly proposal. It begins with basic research designed to identify organisms, which produce antibiotic compounds. During this phase, thousands of species are screened for any sign of antibacterial action. When one is found, the species is tested against a variety of known infectious bacteria. This is a complex procedure because thousands of antibiotic materials have already been discovered. Repeatedly, scientists find that their new antibiotics are not unique. If the material passes this phase, further testing can be done. This typically involves clinical testing to prove that the antibiotic works in animals and humans and is not harmful. If these tests are passed, the government agencies like the Food and Drug Administration (FDA) must then approve the antibiotic as a new drug. This whole process can take many years.

Normally production of an antibiotic depends on a fermentation process. During fermentation, amounts of the antibiotic-producing organism are grown and the organisms produce the antibiotic material, which can then be isolated for use as a drug. Development of antibiotics necessitates isolation and purification of a desired compound from a complicated matrix such as fermentation broth and crude extract. Analysis of antibiotics in formulated and unformulated samples demand a highly specific and rapid method as many antibiotics (e.g. β-lactams) also have serious stability problems.

There are many methods in separation of antibiotic. HPLC technology using sophisticated equipments and refined adsorbents highly facilitate the isolation of antibiotics; there are some drawbacks due to various complications arising from the

use of a solid support. Other method is Counter-current chromatography (CCC) is a unique form of liquid partition chromatography which utilizes a separation column free of solid support matrix. Because of this support-free system, the method provides an important advantage over other chromatographic methods by eliminating various complications including an adsorptive loss and deactivation of samples, contamination, etc.

Immobilized metal ion affinity chromatography (IMAC) is one of the most powerful separation methods available for protein fractionation. For antibiotic separation, this method is use wisely yet. Other thing is traditional stationary phase for IMAC are based on soft gel. But for this research, we will use some inorganic material adsorbent.

1.3 Objective of Study

The purpose of this research is to use zeolite (H-beta, Y) as an immobilized metal ion affinity stationary phase by using three different metal (zirconium, ferum and nickel) and rifampicin as an antibiotic solution for antibiotic separation.

1.4 Scopes of Study

In order to achieve objectives, the scopes for this research are:

1. To study the effect of different metal use in IMA
2. To study type of zeolite
3. To study the effect of pH
4. To study the effect of antibiotic concentration

CHAPTER 2

LITERATURE REVIEW

2.1 Antibiotics

Antibiotics are chemical compounds used to kill or inhibit the growth of infectious organisms. The antibiotic terms originally referred only to the organic compounds that produced by bacteria or molds that are toxic to other microorganisms. The term is now used freely to include synthetic and semi synthetic organic compounds. Antibiotic refers generally to antibacterial, however, because the term is loosely defined, it is preferable to specify compounds as being anti malarial, anti viral, or anti protozoa's. All antibiotics share the property of selective toxicity: They are more toxic to an invading organism than they are to an animal or human host. Penicillin is the most well-known antibiotic and has been used to fight many infectious diseases, including syphilis, gonorrhea, tetanus, and scarlet fever. Another antibiotic, streptomycin, has been used to combat tuberculosis.

Rifampicin (Figure 2.1) is the most important compound of rifamycin group inhibits the growth of most gram-positive and some -negative microorganisms by inhibiting their RNA synthesis. Rifampicin is a bactericidal antibiotic drug of the rifamycin group. It is a semi synthetic compound derived from *Amycolatopsis rifamycinica* (formerly known as *Amycolatopsis mediterranei* and *Streptomyces mediterranei*). It is an antibiotic used to treat infections, including tuberculosis (also known as TB). It can also be used to prevent infections in those who have been in contact with serious infections. Rifampicin has 2 pKa since it is a Zwitterion, pKa 1.7 related to 4-hydroxy and pKa 7.9 related to 3-piperazine nitrogen.

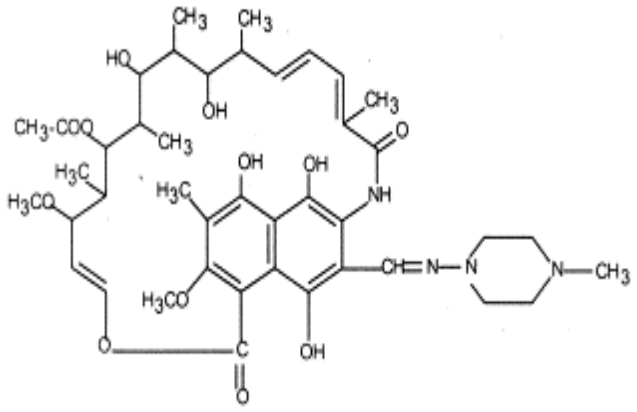

Figure 2.1: Chemical structure of rifampicin.

Penicillins can be divided into two groups, namely, (Albarellos et al., 2004) the natural penicillins, including benzyl penicillin (penicillin G) and its salts (sodium, potassium, benzathine and procaine), and penicillin V and (Albarellos et al., 2005) the semi-synthetic penicillins. This second group can be further divided into three sub-groups: (a) staphylococcal beta-lactamase-resistant penicillins or isoxazolilpenicillins (oxacillin, cloxacillin and dicloxacillin); (b) broad spectrum or aminobenzyl penicillins (ampicillin and amoxicillin), and (c) anti-pseudomonal penicillins (carbenicillin, ticarcillin, piperacillin).

Penicillin was the first microbial metabolite to distinguish between toxicity to the bacterial cell and toxicity to the mammalian host to permit its use in the systemic treatment of infections caused by gram-positive and -negative organisms in humans and animals. The basic structure of penicillin nucleus includes a β-lactam ring fused through nitrogen and adjacent tetrahedral carbon to a second heterocycle, which in natural penicillin is a five-membered thiazolidine ring that shown in figure 2.2. Semi-synthetic penicillins are produced starting from 6-aminopenicillanic acid, which are obtained from culture of *Penicillium chrysogenum*.

These molecule are more resistant to β-lactamase e.g. ampicillin, oxacillin etc.Penicillin and other β-lactams (cephalosporins) inhibit the synthesis of essential structural components of bacterial cell wall i.e. peptidoglycan which are absent in mammalian cells. Thus host cell metabolism remains unaffected and penicillins are regarded as one of the safest and most effective class of antibiotics being used for

bacterial infections. The analysis of degradation products in commercial penicillins has two-fold importance; firstly in pharmacokinetic studies it is desirable to distinguish between the drug and any degradation products, secondly allergic reactions attributed to penicillin may frequently because by such compounds. Accordingly it is essential to be able to detect the presence of these compounds in the pharmaceutical compounds.

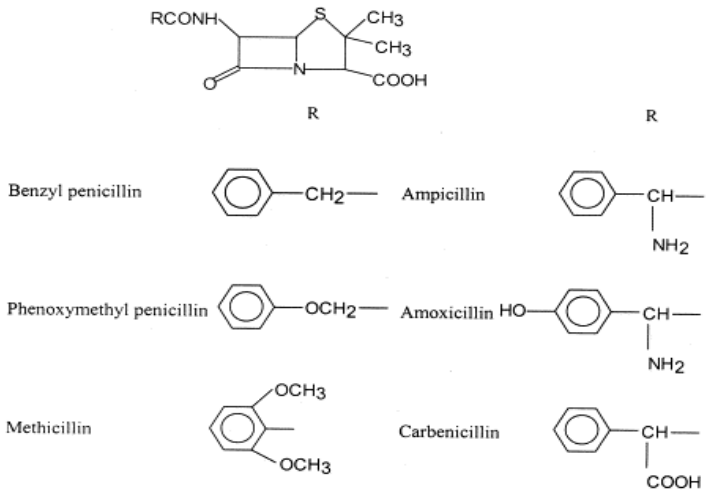

Figure 2.2: Chemical structure of some important penicillins.

Cephalosporins are β-lactam antibiotics, with the same fundamental structural requirements as penicillin that shown in figure 2.3. Heterocyclic ring fused to β-lactam ring is six membered (dihydrothiazine) in cephalosporins. The fused rings in β-lactams are not coplanar but folded along the C-N bond common to both rings; less markedly in cephalosporins than in penicillins. The first generation of cephalosporins are administered parenterally (cephalothin, cefazolin) or orally (cephalexin, cefadroxil). The second generation includes cefoxitin, cefotetan, cefamandole and cefuroxime. The third generation includes cefotaxime, ceftazidime, ceftizoxime, ceftriaxone and ceftiofur. Cefotaxime, ceftazidime, ceftizoxime and ceftriaxone consistently reach effective antibacterial concentrations in the central nervous system (CNS) in humans.(Albarellos et al., 2007).

Cephalosporins are used for the treatment of infections caused by most gram-positive and -negative bacteria, especially *Escherichia coli*, *Proteus mirabilis* and klebsiella. As discussed in penicillin only cephalosporin C is found in nature isolated

from cultures of fungi other i.e. semi-synthetic cephalosporins are derived from 7-aminocephalosporanic acid, product obtained from cephalosporin C hydrolysis. Literature suggests use of C18 column for chromatographic analysis of this class of antibiotics. Carbanepem, a newly synthesized β-lactam antibiotic, was analysed for its degradation products by multistage liquid chromatography-electrospray mass spectrometry.

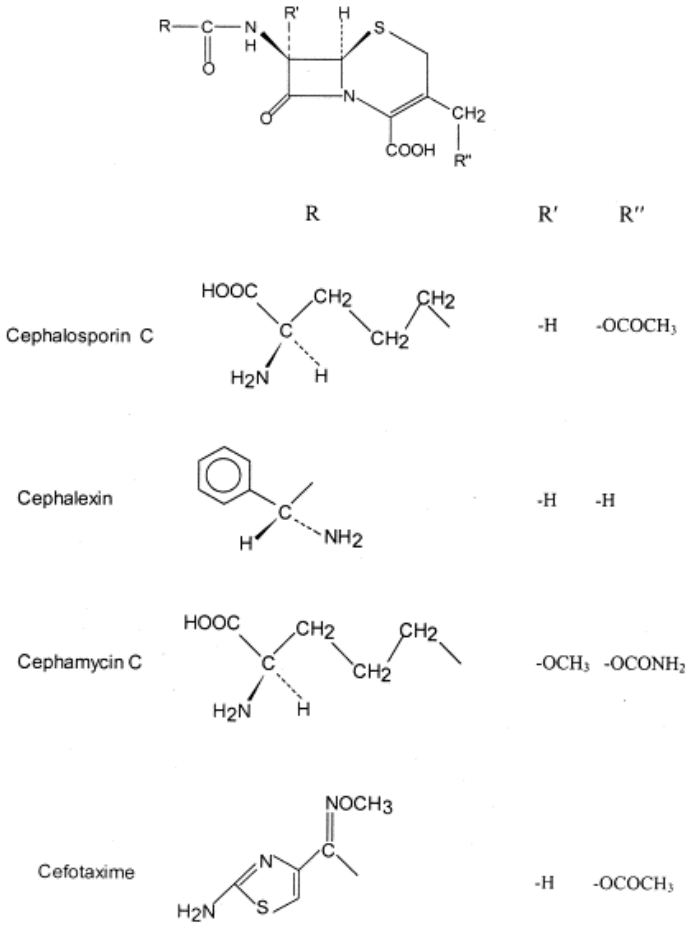

Figure 2.3: Chemical structure of cephalosporins.

2.1.1 Major principle and definition

While our scientific knowledge of antibiotics has only recently been developed, the practical application of antibiotics has existed for centuries. The first known use was by the Chinese about 2,500 years ago. During this time, they discovered that applying the moldy curd of soybeans to infections had certain therapeutic benefits. It was so effective that it became a standard treatment. Evidence