

**ANTIBIOTIC PURIFICATION BY USING METAL ION AFFINITY
ZEOLITE ADSORBENT**

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ABSTRACT

Antibiotics are chemical substances that can inhibit the growth of, and even destroy, harmful microorganisms. They are derived from special microorganisms or other living systems, and are produced on an industrial scale using a fermentation process. For a new antibiotic, manufacturers must be able to easily isolate it. For the isolation process, choosing a suitable adsorbent such as zeolite will result in high yield and a better quality of antibiotic product. Impurities from the fermentation process need to be isolated from the antibiotic. The purpose of this research is to use zeolite as an immobilized metal ion affinity stationary phase for antibiotic purification and to study the best parameter in purification process. In this research, zeolite was used as an immobilized metal ion affinity stationary phase for antibiotic purification. The method used in this research to purify the antibiotic was by using adsorption process. The antibiotic used in this research is *Rifampicin*. Zeolite H-Y and zeolite H-Beta that immobilized with metal ion was used as an adsorbent. Three parameters have been studied to elucidate the best condition for purification process, that is effect of contact time, effect of different pH and effect of different adsorbent. The samples were analyzed using UV-VIS Spectrometer. The results show that the optimum contact time of adsorption of rifampicin is at 7 minutes. At this time, the zeolite has reached its limit to adsorb impurities. The maximum rifampicin adsorption is at pH 7. Effect of pH is related to pKa of the antibiotic. The third parameter studied shows that the best adsorbent is zeolite that immobilized with metal nickel. From the data and supported theories by various authors, it can be concluded that the different conditions in adsorption process have a significant effect on antibiotic purification.

ABSTRAK

Antibiotik adalah bahan kimia yang membantutkan pertumbuhan mikroorganisma berbahaya. Ia berasal daripada mikroorganisma khusus atau hidupan lain dan dihasilkan diindustri menggunakan fermentasi. Untuk antibiotik baru, pembuat mesti mampu memisahkan dengan mudah. Untuk proses pengasingan, pemilihan penjerap yang sesuai seperti zeolite akan memberikan hasil yang tinggi dan kualiti yg lebih baik. Hasil sampingan daripada proses fermentasi perlu diasingakan daripada antibiotic. Tujuan penyelidikan ini adalah untuk menggunakan zeolite sebagai IMA untuk penulenan antibiotic dan mengkaji keadaan yang terbaik dalam proses penulenan. Kaedah yang digunakan dalam penyelidikan ini adalah penulenan antibiotic menggunakan proses penjerapan. Antibiotik yang digunakan adalah Rifampicin. Penjerap yang digunakan adalah zeolite H-Y dan H-Beta yang telah diubah dengan oksida logam. Tiga parameter yg menjelaskan kondisi yang terbaik dalam proses penulenan ialah kesan perbezaan masa sentuhan, kesan perbezaan pH dan kesan perbezaan penjerap. Keputusan daripada eksperimet menunjukan masa sentuhan yang memberikan hasil yang optimum adalah 7 minit. Ini adalah kerana, pada minit ke 7, penjerap telah mencapai batasan untuk menjerap. Penjerapan antibiotic yang tertinggi adalah pada pH 7. Kesan pH dalam proses ini berkait rapan dengan nilai pKa antibiotic itu sendiri. Parameter yang ketiga menunjukkan zeolite yang diubah suai dengan nikel memberikan nilai penjerapan antibiotic yang tertinggi. Daripada data yang diperolehi dan disokong oleh beberapa penulis, kesimpulan yang boleh dibuat adalah penyelidikan terhadap pelbagai parameter member kesan yang penting dalam penulenan antibiotic.

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LIST OF SYMBOLS

%	Percent
$^{\circ}\text{C}$	Degree Celsius
h	Hour
t	Tonne
rpm	Revolution per minute
mm	Millimeter
kg	Kilogram
ppm	part per million
mg	Milligram
ml	Milliliter
Min	Minute
Kg/M^3	Kilogram per Meter ³

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CHAPTER 1

INTRODUCTION

1.1 Background of the study

Antibiotics are chemical substances that can inhibit the growth of, and even destroy, harmful microorganisms. They are derived from special microorganisms or other living systems, and are produced on an industrial scale using a fermentation process. Although the principles of antibiotic action were not discovered until the twentieth century, the first known use of antibiotics was by the Chinese over 2,500 years ago. Today, over 10,000 antibiotic substances have been reported. Currently, antibiotics represent a multibillion dollar industry that continues to grow each year (Nussbaum, 2006).

Antibiotics are used in many forms each of which imposes somewhat different manufacturing requirements. For bacterial infections on the skin surface, eye, or ear, an antibiotic may be applied as an ointment or cream. If the infection is internal, the antibiotic can be swallowed or injected directly into the body. In these cases, the antibiotic is delivered throughout the body by absorption into the bloodstream (Kirk, 1992).

Antibiotics differ chemically so it is understandable that they also differ in the types of infections they cure and the ways in which they cure them. Certain antibiotics destroy bacteria by affecting the structure of their cells. This can occur in one of two ways. First, the antibiotic can weaken the cell walls of the infectious bacteria, which causes them to burst. Second, antibiotics can cause the contents of

the bacterial cells to leak out by damaging the cell membranes. Another way in which antibiotics function is by interfering with the bacteria's metabolism. Some antibiotics such as tetracycline and erythromycin interfere with protein synthesis. Antibiotics like rifampin inhibit nucleic acid biosynthesis. Still other antibiotics, such as sulfonamide or trimethoprim have a general blocking effect on cell metabolism (Crueger, 1989).

The commercial 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. If the results are promising, the organism is grown on a large scale so the compound responsible for the antibiotic effect can be isolated. This is a complex procedure because thousands of antibiotic materials have already been discovered. Often, 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 Food and Drug Administration (FDA) must then approve the antibiotic as a new drug. This whole process can take many years.

The large-scale production of an antibiotic depends on a fermentation process. During fermentation, large amounts of the antibiotic-producing organism are grown. During fermentation, the organisms produce the antibiotic material, which can then be isolated for use as a drug. For a new antibiotic to be economically feasible, manufacturers must be able to get a high yield of drug from the fermentation process, and be able to easily isolate it. Extensive research is usually required before a new antibiotic can be commercially scaled up.

For isolation process it depends on types of antibiotic. There are two types of antibiotic, antibiotic compounds that are water soluble and an oil-soluble antibiotic such as penicillin. For antibiotic that are water soluble, an ion-exchange method may be used for purification. In this method, the compound is first separated from the waste organic materials in the broth and then sent through equipment, which

separates the other water-soluble compounds from the desired one. For antibiotic that are an oil-soluble, a solvent extraction method is used. In this method, the broth is treated with organic solvents such as butyl acetate or methyl isobutyl ketone, which can specifically dissolve the antibiotic. The dissolved antibiotic is then recovered using various organic chemical means (Crueger, 1989). For this study, the isolation process to purify the antibiotic from other substance will be the focus. The method that will be used to separate the antibiotic is separation process using various types of adsorbent. Basically the separation process is used to transform a mixture of substances into two or more distinct products. The separated products could differ in chemical properties or some physical property, such as size, or crystal modification or other separation into different components.

Barring a few exceptions, almost every element or compound is found naturally in an impure state such as a mixture of two or more substances. Many times the need to separate it into its individual components arises. Separation applications in the field of chemical engineering are very important.

Adsorbent that used in this study is zeolites H-beta and H-Y. This adsorbent is mixed with other metal. Zeolites are used to separate molecules (only molecules of certain sizes and shapes can pass through), as traps for molecules so they can be analyzed.

There are some factors that can be considered during the separation process. The pH of the solution can affect the separation process. The pH of the solution can be controlled by adding a buffer solution into the process. Another factor that can be considered during the experiment is time for the process. The manipulated variable in this research is types of adsorbent, pH of the solution and time for the process.

1.2 Problem statement

The current problem in commercially scale up a new antibiotic is the manufacturers must be able to get a high yield of drug from the fermentation process, and be able to easily isolate it in order for a new antibiotic to be economically feasible.

For the isolation process, choosing a suitable adsorbent such as zeolite will result in high yield and a better quality of antibiotic product. Impurities from the fermentation process need to be isolate from the antibiotic. This means that this research will concern on antibiotic purification.

In this research, a various types of adsorbent will be used to solve the problem and certain parameter such as pH and time for the separation will be manipulated in order to get a high yield and a better quality of antibiotic. The ideal or suitable condition (*Temperature & time*) for better antibiotic yield production will be determined. The antibiotic yield extracted will be the dependent variables in this research.

1.3 Objectives

The purpose of this research is to use zeolite as an immobilized metal ion affinity stationary phase for antibiotic purification and to study the best condition in purification process.

1.4 Scope of research

The scopes in this research work are:

1. To study the effect of different adsorbent on antibiotic separation.
2. To study the effect of different pH on antibiotic separation.
3. To study the effect of different separation time.

CHAPTER 2

LITERATURE REVIEW

2.1 Antibiotic

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 suggests that other cultures used antibiotic-type substances as therapeutic agents. The Sudanese-Nubian civilization used a type of tetracycline antibiotic as early as 350 A.D. In Europe during the Middle Ages, crude plant extracts and cheese curds were also used to fight infection (Waksman, 1947).

The development of modern antibiotics depends on a few key individuals who demonstrated to the world that materials derived from microorganisms could be used to cure infectious diseases. One of the first pioneers in this field was Louis Pasteur. In 1877, he and an associate discovered that the growth of disease-causing anthrax bacteria could be inhibited by a saprophytic bacterium. They showed that large amounts of anthrax bacilli could be given to animals with no adverse effects as long as the saprophytic bacilli were also given. Over the next few years, other observations supported the fact that some bacterially derived materials could prevent the growth of disease-causing bacteria (Stinson & Stephan, 1996).

In 1928, Alexander Fleming made one of the most important contributions to the field of antibiotics. In an experiment, he found that a strain of green *Penicillium* mold inhibited the growth of bacteria on an agar plate. This led to the development of the first modern era antibiotic, penicillin. A few years later in 1932, a paper was published which suggested a method for treating infected wounds using a penicillin preparation. Although these early samples of penicillin were functional, they were not reliable and further refinements were needed. These improvements came in the early 1940s when Howard Florey and associates discovered a new strain of *Penicillium*, which produced high yields of penicillin. This allowed large-scale production of penicillin, which helped launch the modern antibiotics industry (Crueger, 1986).

After the discovery of penicillin, other antibiotics were sought. In 1939, work began on the isolation of potential antibiotic products from the soil bacteria streptomycetes. It was around this time that the term antibiotic was introduced. Selman Waxman and associates discovered streptomycin in 1944. Subsequent studies resulted in the discovery of a host of new, different antibiotics including actinomycin, streptothricin, and neomycin all produced by *Streptomyces*. Other antibiotics that have been discovered since include bacitracin, polymyxin, viomycin, chloramphenicol and tetracyclines. Since the 1970s, most new antibiotics have been synthetic modifications of naturally occurring antibiotics (Crueger, 1986).

2.1.1 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 (William & Carol, 1978).

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 (William & Carol, 1978).

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 easily acquire strong resistance or even immunity to rifampicin through a variety of mutations, most of them a single amino acid substitution. The problem is an interesting paradox, since the reason rifampicin works so well is that it is a rigid molecule, and sits tightly in the pocket where it binds, allowing the bonds to be very strong (Jianfang et al., 2007). However, this also means that if an amino acid on the edge of the channel with a small sidechain is replaced with an amino acid with a large sidechain, rifampicin may not be able to bind, simply because it cannot fit in the space. This occurs in three primary positions, one of which occurs 9% of the time, another of which occurs 36% of the time, and the last of which occurs an alarming 41% of the time.

Because bacteria gains immunity to rifampicin in a relatively short amount of time, it used only in very special circumstances in an effort to keep bacteria vulnerable to rif, hopefully making it effective when it does need to be used. The most common usage of rifampicin is as part of a tuberculosis therapy program. Tuberculosis (TB) is an extremely contagious, extremely lethal bacterial disease, which often lurks in the lungs until the host has a weakened immune system, at which time it manifests itself, resulting in symptoms which are debilitating at best. In fact, TB remains the leading cause of preventable death by a pathogen in the world. However, unless the bacteria are immune already, rifampicin is very effective, cutting the therapy time from two years down to six months. The problem is that with cases in the US declining to the point of nonexistence, pharmaceutical companies no longer see it as a source of profit, and all but three have ceased making it, even though TB is still common among people in developing countries, who are unable to pay for the treatment. In fact, the three that are still making it are only

doing so because they are being forced to do so by the World Health Organization (Coulson & Christopher, 1994).

2.2 Zeolites

Zeolites are microporous crystalline solids with well-defined structures. Generally they contain silicon, aluminium and oxygen in their framework and cations, water and/or other molecules within their pores (Scott et al., 2003). Many occur naturally as minerals, and are extensively mined in many parts of the world. Others are synthetic, and are made commercially for specific uses, or produced by research scientists trying to understand more about their chemistry.

Because of their unique porous properties, zeolites are used in a variety of applications with a global market of several million tonnes per annum. In the western world, major uses are in petrochemical cracking, ion-exchange (water softening and purification), and in the separation and removal of gases and solvents (British zeolite association, 2001). Other applications are in agriculture, animal husbandry and construction. They are often also referred to as *molecular sieves*.

2.2.1 Framework Structure

A defining feature of zeolites is that their frameworks are made up of 4-connected networks of atoms. One way of thinking about this is in terms of tetrahedra, with a silicon atom in the middle and oxygen atoms at the corners. These tetrahedra can then link together by their corners (see illustration) to form a rich variety of beautiful structures. The framework structure may contain linked cages, cavities or channels, which are of the right size to allow small molecules to enter as shown in Figure 2.1.

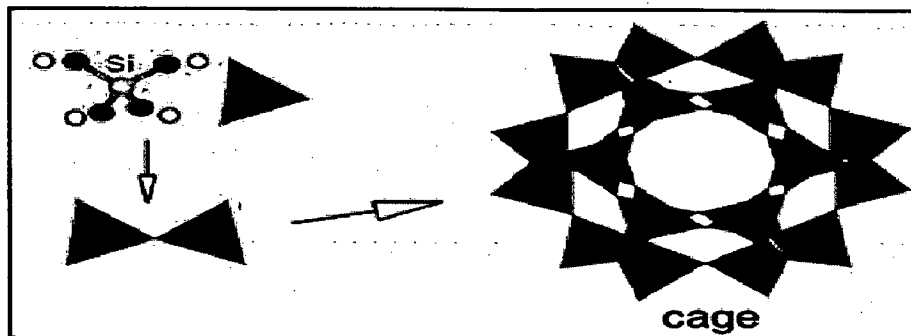


Figure 2.1: Zeolite Structure (British zeolite association, 2001)

In all, over 130 different framework structures are now known. In addition to having silicon or aluminium as the tetrahedral atom, other compositions have also been synthesised, including the growing category of microporous aluminophosphates, known as ALPOs.

2.2.2 Catalysis

Zeolites have the ability to act as catalysts for chemical reactions which take place within the internal cavities. An important class of reactions is that catalysed by hydrogen-exchanged zeolites, whose framework-bound protons give rise to very high acidity. This is exploited in many organic reactions, including crude oil cracking, isomerisation and fuel synthesis. Zeolites can also serve as oxidation or reduction catalysts, often after metals have been introduced into the framework (Bell, 2001). Examples are the use of titanium ZSM-5 in the production of caprolactam, and copper zeolites in NO_x decomposition.

Underpinning all these types of reaction is the unique microporous nature of zeolites, where the shape and size of a particular pore system exerts a steric influence on the reaction, controlling the access of reactants and products. Thus zeolites are often said to act as shape-selective catalysts. Increasingly, attention has focused on fine-tuning the properties of zeolite catalysts in order to carry out very specific syntheses of high-value chemicals e.g. pharmaceuticals and cosmetics (Bell, 2001).

2.2.3 Adsorption and Separation

The shape-selective properties of zeolites are also the basis for their use in molecular adsorption. The ability preferentially to adsorb certain molecules, while excluding others, has opened up a wide range of molecular sieving applications. Sometimes it is simply a matter of the size and shape of pores controlling access into the zeolite. In other cases different types of molecule enter the zeolite, but some diffuse through the channels more quickly, leaving others stuck behind, as in the purification of *para*-xylene by silicalite. Figure 2.2 shows the shape of *para*-xylene. Some of the molecule enter this shape leaves by diffusion leaving other stuck behind.

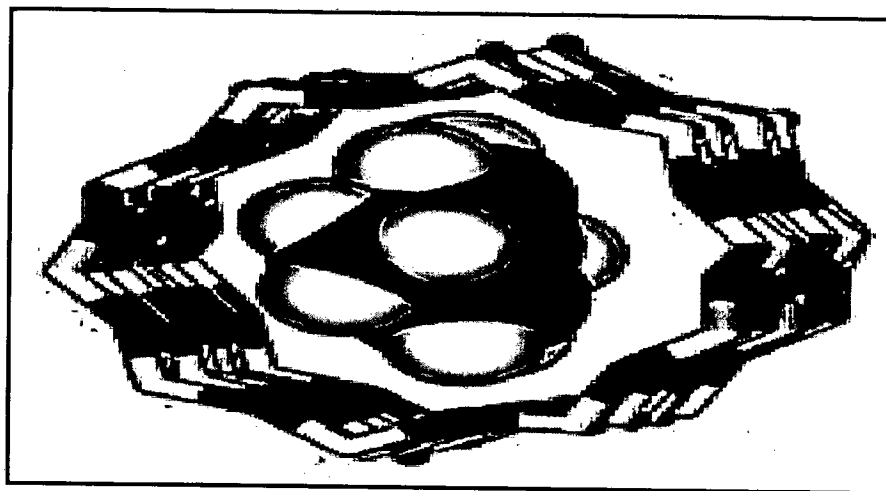


Figure 2.2: The shape of *para*-xylene (British zeolite association, 2001).

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

2.2.4 Ion Exchange

The loosely-bound nature of extra-framework metal ions (such as in zeolite NaA, right) means that they are often readily exchanged for other types of metal when in aqueous solution. This is exploited in a major way in water softening, where alkali metals such as sodium or potassium prefer to exchange out of the zeolite, being replaced by the "hard" calcium and magnesium ions from the water. Many commercial washing powders thus contain substantial amounts of zeolite. Commercial waste water containing heavy metals and nuclear effluents containing radioactive isotopes can also be cleaned up using such zeolites. Figure 2.3 shows that the zeolite A contained alkali metal. The alkali metal prefer to exchange out of the zeolite.

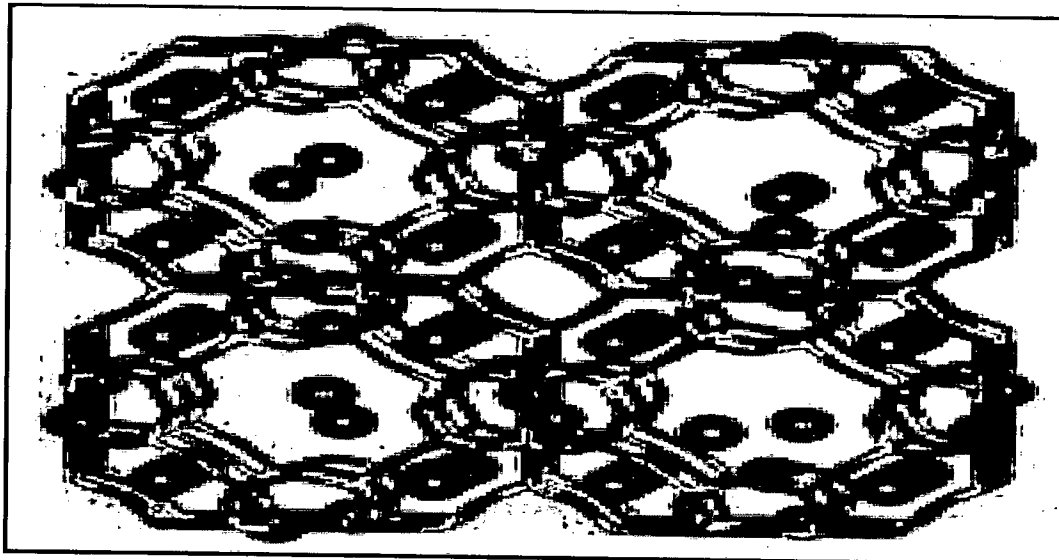


Figure 2.3: Sodium Zeolite A, used as a water softener in detergent powder (British zeolite association, 2001)

2.3 BUFFER

A buffer solution is one which resists changes in pH when small quantities of an acid or an alkali are added to it.

2.3.1 Acidic buffer solutions

An acidic buffer solution is simply one which has a pH less than 7. Acidic buffer solutions are commonly made from a weak acid and one of its salts - often a sodium salt. A common example would be a mixture of ethanoic acid and sodium ethanoate in solution. In this case, if the solution contained equal molar concentrations of both the acid and the salt, it would have a pH of 4.76. It wouldn't matter what the concentrations were, as long as they were the same. The pH of the buffer solution can be by changing the ratio of acid to salt, or by choosing a different acid and one of its salts (Hulanicki, 1987). Ethanoic acid is a weak acid, and the position of this equilibrium will be well to the left:



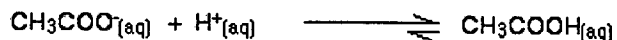
Adding sodium ethanoate to this adds lots of extra ethanoate ions. According to Le Chatelier's Principle, that will tip the position of the equilibrium even further to the left.

The solution will therefore contain these important things (Denny et al., 2000):

- lots of un-ionised ethanoic acid;
- lots of ethanoate ions from the sodium ethanoate;
- Enough hydrogen ions to make the solution acidic.

Adding an acid to this buffer solution

The buffer solution must remove most of the new hydrogen ions otherwise the pH would drop markedly. Hydrogen ions combine with the ethanoate ions to make ethanoic acid. Although the reaction is reversible, since the ethanoic acid is a weak acid, most of the new hydrogen ions are removed in this way.



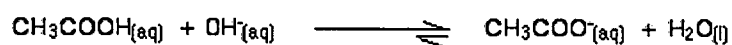
- Since most of the new hydrogen ions are removed, the pH won't change very much - but because of the equilibria involved, it *will* fall a little bit.

Adding an alkali to this buffer solution

Alkaline solutions contain hydroxide ions and the buffer solution removes most of these. This time the situation is a bit more complicated because there are *two* processes which can remove hydroxide ions.

Removal by reacting with ethanoic acid

The most likely acidic substance which a hydroxide ion is going to collide with is an ethanoic acid molecule. They will react to form ethanoate ions and water.



Because most of the new hydroxide ions are removed, the pH doesn't increase very much.

Removal of the hydroxide ions by reacting with hydrogen ions



Hydroxide ions can combine with these to make water. As soon as this happens, the equilibrium tips to replace them. This keeps on happening until most of the hydroxide ions are removed.

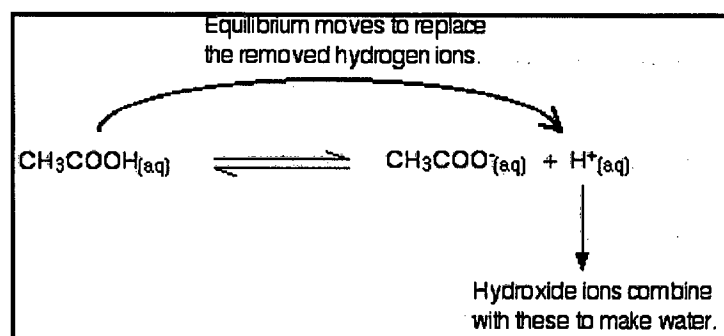
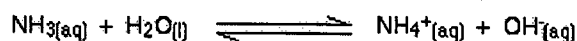


Figure 2.4: Reaction by acidic buffer solution

2.3.2 Alkaline buffer solutions

An alkaline buffer solution has a pH greater than 7. Alkaline buffer solutions are commonly made from a weak base and one of its salts. A frequently used example is a mixture of ammonia solution and ammonium chloride solution. If these were mixed in equal molar proportions, the solution would have a pH of 9.25. A buffer solution has to contain things which will remove any hydrogen ions or hydroxide ions - otherwise the pH will change. Acidic and alkaline buffer solutions achieve this in different ways (Hulanicki, 1987). Ammonia is a weak base, and the position of this equilibrium will be well to the left:



Adding ammonium chloride to this adds lots of extra ammonium ions. According to Le Chatelier's Principle, that will tip the position of the equilibrium even further to the left. The solution will therefore contain these important things:

- lots of unreacted ammonia;
- lots of ammonium ions from the ammonium chloride;
- enough hydroxide ions to make the solution alkaline.

Adding an acid to this buffer solution

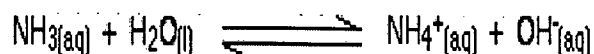
There are *two* processes which can remove the hydrogen ions.

Removal by reacting with ammonia The most likely basic substance which a hydrogen ion is going to collide with is an ammonia molecule. They will react to form ammonium ions.



Most, but not all, of the hydrogen ions will be removed. The ammonium ion is weakly acidic, and so some of the hydrogen ions will be released again.

Removal of the hydrogen ions by reacting with hydroxide ions



Hydrogen ions can combine with these hydroxide ions to make water. As soon as this happens, the equilibrium tips to replace the hydroxide ions. This keeps on happening until most of the hydrogen ions are removed.

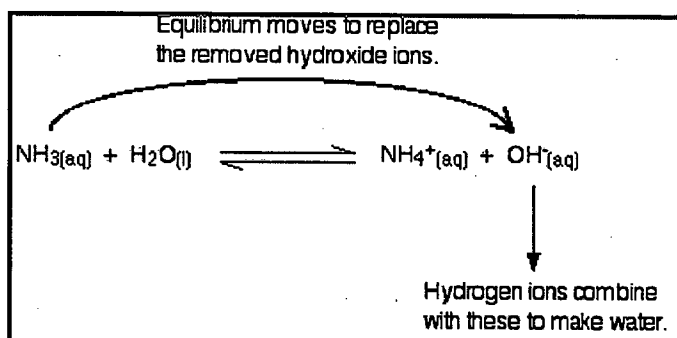


Figure 2.5: Reaction by alkali buffer solution

Adding an alkali to this buffer solution

The hydroxide ions from the alkali are removed by a simple reaction with ammonium ions.



Because the ammonia formed is a weak base, it can react with the water - and so the reaction is slightly reversible. That means that, again, most (but not all) of the the hydroxide ions are removed from the solution.

2.4. Adsorption process

Adsorption is the accumulation of atoms or molecules on the surface of a material. This process creates a film of the adsorbate (the molecules or atoms being accumulated) on the adsorbent's surface. 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.

In simple terms, adsorption is "the collection of a substance onto the surface of adsorbent solids." It is a removal process where certain particles are bound to an adsorbent particle surface by either chemical or physical attraction. Adsorption is often confused with Absorption, where the substance being collected or removed actually penetrates into the other substance (Reynolds & Richards, 1996).

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 (Cussler, 1997).

Similar to surface tension, adsorption is a consequence of surface energy. In a bulk material, all the bonding requirements (be they ionic, covalent, or metallic) of the constituent atoms of the material are filled by other atoms in the material. However, atoms on the surface of the adsorbent are not wholly surrounded by other adsorbent atoms and therefore can attract adsorbates. The exact nature of the bonding depends on the details of the species involved, but the adsorption process is generally classified as physisorption (characteristic of weak van der Waals forces) or chemisorption (characteristic of covalent bonding) (Pruski et al., 1999).