Study of Chitosan Extraction from Insects to Remove Metal Ions In Waste Water

by

Wan Rafsyam Wan Ab Rahim

Dissertation submitted in partial fulfillment of
the requirements for the
Bachelor of Engineering (Hons)
(Chemical Engineering)

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CERTIFICATION OF APPROVAL

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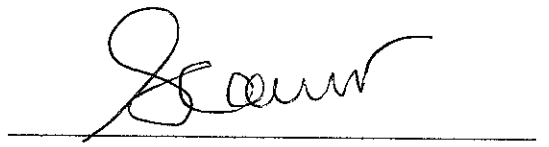
Approved by,



(PN. ASNA MOHD ZAIN)

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TRONOH, PERAK

May 2004

1. Chitosan
2. Metal ions
3. CE -- Theses

CERTIFICATION OF ORIGINALITY

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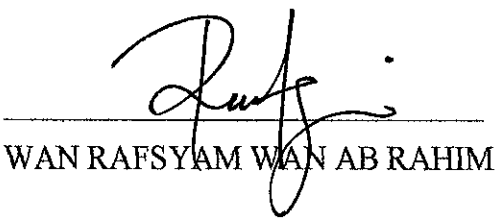

WAN RAFSYAM WAN AB RAHIM

ABSTRACT

Chitosan is a derivative of chitin and has numerous applications ranging from cosmetics to substrate material for membranes. This project is aimed to investigate the feasibility of producing a derived chitosan from insects by chemical extraction methods available in the chemical laboratory. Other objective is to use the chitosan in the waste water to remove the heavy metals ion. Common cockroaches (Homasilpha vicinia) are the specimen used in the experiment for chitin extraction.

The production of chitosan involves few method of extraction namely demineralization, deproteination, deacetylation, and acid extraction of chitin. Demineralization is the removal of minerals, primarily calcium carbonate ($CaCO_3$). Deproteination was conducted using diluted sodium hydroxide (NaOH) as treatments. Deacetylation then was done to obtain the chitosan using the concentrated NaOH and acetic acid.

As the chelating agent, for heavy metal ions of lead (Pb^{2+}), nickel (Ni^{2+}), copper (Cu^{2+}) and zinc (Zn^{2+}), chitosan can remove 95% and above of the heavy metal ions from the 50 ppm solution. Chitosan shows better result if compared to the chitin. At 50 ppm concentration solution, reduction of lead ions (Pb^{2+}) was at 100% with 0.20 g chitosan, 99.93% for nickel ions (Ni^{2+}) with 0.25 g chitosan, 97.30% for copper ions (Cu^{2+}) with 0.10 g chitosan and 97.62% for zinc ions (Zn^{2+}) with 0.25 g chitosan.

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CHAPTER 1
INTRODUCTION

1.1 Background of Study

Chitosan is a derivative of chitin and has numerous applications ranging from cosmetic to substrate material for membrane. The purpose of this project is divided into 2 parts:

1) To investigate the feasible method and parameters in extracting chitin and chitosan from insects.
2) To use the chitosan in waste water in order to remove heavy metal ions such as Lead, Copper and Zinc.

The production of chitosan involves demineralization, deproteination, deacetylation and acid extraction of chitin.

Chitosan is insoluble in water, organic solvents, alkali and mineral acids under most conditions. However, it is readily soluble in formic acid, acetic acid and other weak organic acids. Under prolonged heating and stirring, chitosan is soluble in concentrated mineral acids, especially if fine or precipitated chitosan is used. Only with nitric acid does chitosan form the insoluble chitosan nitrate.

Chitosan are effective flocculating agents for anionic wastes, such as proteinaceous particles in sewage effluent, brewery waste, etc. Its ability to chelate even trace levels of transition metals ions, especially poisonous heavy metals like mercury and cadmium, as well as many radionuclides like uranium, make it suitable for removing such ions from effluent and chemical wastes.

1.2 Problem Statement

The environmental pollution of such as from heavy metal can lead to human being's diseases becoming stranger and more incurable. Meanwhile one is easier to get ill when he is still young. All these problems make many scientists in the world seriously thinking about such problems and hope to find out the solutions. Chitosan probably can solve such problems.

Although much work has been done about the sorption of heavy metals on chitosan, mechanism of adsorption is not well established for lot of them. Chitosan can be prepared in different conditioning like gel beads, fibers or films. These preparations require solubilized chitosan and acetic acid which is largely used in this purpose.

Even though chitin is widely distributed in nature, it is never found in its pure form. Chitin in its natural state is tightly associated with proteins, lipids, pigments and calcium deposits. Thus, it needs to be purified before it is of any commercial use. Currently, the purification of chitin consists of two main steps (Khor, 2001):

1) Demineralisation: removal of minerals with dilute acid or chelating agents; and

2) Deproteination: protein separation with dilute alkali or proteolytic enzymes.

These steps can be carried out in any order. Following the demineralisation and deproteination steps, the product may be decolourised by removing the pigments present with acetone or hydrogen peroxide. However, this step is not essential as it depends on the specification of the required end product.

1.3 Objective and Scope of Study

The chitosan extraction from chitin is the first objective of the experiment and the second objective is to remove selected metal ions with the extracted chitosan. Chitosan extraction will involve the deacetylation of raw chitin. Raw chitin is treated with 50% NaOH (w/v). At concentrations below 45%, chitosan will not be formed. The product then is extracted with acetic acid solution.

The chitosan product will be used to remove metal ions content in the prepared synthetic waste water which acts as chelating agent. The concentrations of the ions in waste water after treatment will be observed and compared to the standard of EQA 1974. This step will be done to check whether the concentrations are following the rules and regulations stated by Environmental Quality Act (Sewage and Industrial Effluents) Regulations 1979.

The scope of the study related to the treatment of waste water in form of metal ions removal from the waste water. This new approach of study is closely related in finding the new different method of waste water treatment using biopolymer material. Conventionally, alum is used in the waste water treatment process.

The extraction, testing and analyzing of chitosan was done within 4-5 months which is about one semester study.

CHAPTER 2
LITERATURE REVIEW AND THEORY

2.1 Chitosan History

In the early 1960's, chitosan was investigated for its ability to bind with red blood cells and was considered for use as a hemostatic agent.

For the past three decades, chitosan has been used at water purification plants for detoxifying water. Chitosan is spread over the surface where it absorbs oils, greases and other potential toxins. The resulting surface film is then easily skimmed from the water.

As a dietary supplement, chitosan has been marketed for about 20 years in Europe and Japan as a "fat blocker".

Chitin was first described in 1811 by Braconnot, a professor of natural history in France. While conducting research on mushrooms, Braconnot isolated what was later to be called chitin. Some 22 years later, Odier authored an article on insects in which he noted that the same substance was present in the structure of insects as well as the structure of plants. Odier called this amazing substance chitin. The name chitin is derived from Greek meaning tunic or envelope. In 1843, Lassaigne demonstrated the presence of nitrogen in chitin.

In 1859 chitosan was discovered by Rouget. While experimenting with chitin, he observed that the compound could be manipulated via chemical and temperature treatments to become soluble. Ledderhose, in 1878 determined chitin to be made of glucosamine and acetic acid. It was not until 1894 that Hoppe-Seyler named the modified chitin, chitosan.

Early 20^{th} century research often involved sources of chitin including fungi and crab shells. The work of Rammelberg in the 1930's eventually led to the confirmation of the

identity of chitin from these sources. By hydrolyzing chitin in different manners, Purchase & Braum determined that chitin is a polysaccharide of glucosamine and by 1948, Matsusshima was able to obtain a patent for producing glucosamine from crab shells.

By 1950, the use of x-ray analysis had advanced the study of the occurrence of chitin in fungi. The advanced technology proved most reliable in determining the presence of chitin and cellulose in cell walls. In 1951 (140 years after Braconnot's initial observation), the first books on chitin were published.

2.2 From Waste to Niche Materials

According to Khor (2001), commencing in the 1950's, a more concerted effort began to appear in the scientific literature. A natural course in finding a solution to the abundant shellfish waste from seafood processing is the interaction between the industry and academy. One example is Tottori, Japan, famous for its snowcrabs and associated seafood processing facilities that generated such shellfish wastes. It is probably no coincidence that Professor Shigehiro Hirano of Tottori University (now retired) became an imminent pioneer in chitin research since the late 1960's. Eventually, this resulted in the increased understanding of the science and technology of chitin that culminated in the first milestone as it were, with Professor Muzzarelli's landmark book (Muzzarelli, 1977).

The driving force for much of the excitement surrounding chitin and chitosan are the potential applications that the materials can be used. Table 2.1 lists some examples of the known and potential applications for chitin, chitosan and their derivatives that have caught the imagination of scientist, raw material producers and manufacturers and users alike.

Table 2.1: Applications of Chitin, Chitosan and Their Derivatives (Goosen, Hirano, 1997)

Application Area	Specific Use
Water Treatment	• Coagulating / flocculating agents for polluted waste waters • Removal / recovery of metal ions from aqueous waste water
Agriculture	• Plant elicitor • Antimicrobial agents • Plant seed coating • Fertilizer
Textile and Paper	• Fibers for textile and woven fabrics • Paper and film
Biotechnology	• Chromatography packing • Enzyme immobilizing materials
Food / Health Supplements	• Natural thickeners • Food additives including pet food • Food processing (e.g. in sugar refining) • Filtration and clarification • Hypocholesterolemic agent (slimming agents)
Cosmetic	• Ingredients for hair and skin care (conditioners)
Biomedical	• Burns and wounds dressings for humans and animals • Biomaterial (e.g. absorbable sutures) • Anticoagulant or antithrombogenic materials (as sulfated-chitin derivatives) • Hemostatic agents (as chitosan) • Drug delivery, gene delivery

2.3 Chitin and Chitosan

Random House Unabridged Dictionary define chitin as a nitrogen-containing polysaccharide (pronounced KITE-in), related chemically to cellulose, which forms a semitransparent horny substance and is a principal constituent of the exoskeleton or outer covering of insects, crustaceans and arachnids.

Chitosan is the family of deacetylated chitins. Chitin, the insoluble polymer of poly N-acetyl-D-glucosamine (Fig 2.1), is the most abundant nitrogen-bearing organic compound found in nature, present in insect exoskeletons, crustacean shell, fungal cell wall, peritrophic membranes and the cocoons of insects. Chitin is converted to chitosan (poly D-glucosamine) by the removal of acetyl group in the deacetylation process (Fig 2.2).

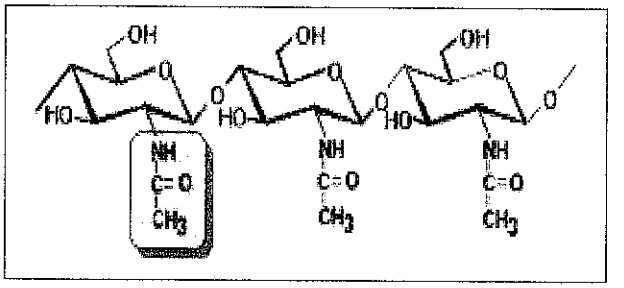

Figure 2.1: Structure of Chitin (poly N-acetyl-D-glucosamine)

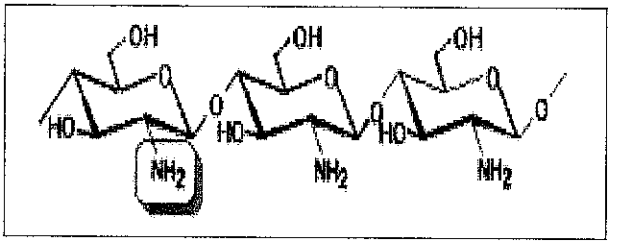

Figure 2.2: Structure of Chitosan (poly D-glucosamine)

Different sources of chitin have a different percentage of chitin content (Table 2.2). Chitin is easily hydrolyzed by acids, but is stable to dilute alkali; in warm concentrated alkali, it is oxidized by air. Chitin hydrolysates can be prepared by adding chitin to concentrated HCl.

Table 2.2: Variation in Chitin Content of Different Sources

Source	Percentage of Chitin (%)
Fungi	5-30
Worms	20-38
Squid	3-20
Scorpions	30
Spider	38
Cockroaches	35
Silkworms	44
Crabs	70

Chitosan, in general, have nitrogen content higher than 7% and a degree of acetylation lower than 0.40. The removal of acetyl group is a harsh treatment usually performed with concentrated NaOH. Protection from oxygen, with a nitrogen purge or by addition of sodium borohydride to the alkali solution, is necessary to avoid undesirable reactions such as depolymerization and generation of reactive species (Jolles, 1999).

Chitosan has a pKa about 6.2 and was partially soluble in dilute mineral acids such as HNO_3, HCl, H_3PO_4. This can be a problem in wastewater treatment process when chitosan is used for the recovery of metal ions from an acid media (Dambies, 2001).

Chitosan's Chemical Properties:

1) Linear polyamine (poly D-glucosamine)
2) Reactive amino groups
3) Chelates many transitional metal ions
4) Bacteriostatic and fungistatic effect
5) Protein separations

2.4 Bio-Stable and Biodegradable Chitosan

Khor (2001) says that chitosan are not normally water soluble (with the exception of low molecular weight components) at the body's pH of 7 but can be subject to effects of erosion caused by constant interaction with bodily fluids for example.

For a bio-stable role, chitosan will be expected to maintain its integrity throughout the period of use. The most credible method to make chitosan non-degradable is by chemical derivatization although strictly speaking, a material will degrade when used for an extended term. Chemical derivatization gives rise to structure not readily recognizable by enzymes and therefore chitosan is more degradation resistant.

Khor (2001) further defines that the implicit in the concept of a biodegradable biomaterial is the ability for the biomaterial to perform it's required for predetermined time period with the gradual dissipation of the biomaterial until ultimately, it is totally assimilated or disposed by the body. The term biodegradable normally refers to a material being susceptible and degraded by enzymes and other bio-based reactions when placed in the biological system. The term further implies that the deterioration of the material is controlled at a rate that is desirable for the material to perform its biomaterial role. However, biodegradable is not necessarily equivalent to bioerodible. Bioerodible

typically describes the situation where the material is being hydrolyzed or dissolved by aqueous media in the biological environment, suggesting physical erosion rather than biochemical action.

The biodegradability of chitosan into non-harmful residues has been a popular platform for projecting the biopolymer as a biomaterial extraordinaire.

2.5 Characterization of Chitosan Properties

In the utilization of chitosan, one major issue to-date has been lack of consistency in the properties of the biopolymer and the methods used to determine these properties. As stated before, chitosan is found as a copolymer of N-glucosamine. Therefore, the two most important determinants of chitosan's structural properties are the degree of acetylation (DA) or degree of deacetylation (DD) and molecular weight.

The degree of DA (or DD) identifies the biopolymer as chitin or chitosan, whereas the molecular weight determines the viscosity and rate of degradation. The residual protein, moisture content, ash content, lipid content, heavy metal content, impurity content and color are other properties most frequently reported. The characterization methods used to determine these properties also span a wide range, from economic methods for routine quality control to expensive instrumentation for obtaining sophisticated specialized information. The aim of having properly defined characterization methods for chitin and chitosan is to have a means to report reliably, the bulk biopolymer behavior and properties. This is necessary to set the appropriate and consistent expectations of the biopolymer properties.

Table 2.3 summarizes the established characterization methods for chitin and chitosan listed in the chitin handbook (Muzzarelli, 1997). Different solvents are required for chitin and chitosan. The Nuclear Magnetic Resonance (NMR) can also be used to probe

molecular structure. These methods to determine the various characteristics of chitin and chitosan are for research and general utility.

The quality control of chitosan production can now be readily substantiated as the methods required in determining properties such as the degree of acetylation and molecular weight are now well documented, refined and easily available. Therefore, a raw materials producer, manufacturer or end-user can list the desired properties sought and can verify them readily.

Table 2.3: Established characterization methods for chitin and chitosan from *Chitin Handbook* (Muzzarelli, 1997)

Properties	Characterization Method
Molecular weight	• Viscometry • Light scattering • High performance gel permeation chromatography
Degree of acetylation	• First derivative ultraviolet spectrophotometry • Infrared spectrophotometry • Enzymatic determination • Chromatographic determination • Nuclear Magnetic Resonance, NMR (^{13}C, ^{1}H, liquid-state and solid-state)
Degree of N-acetylation	• Metachromatic titration • Dye absorption • Infrared spectroscopy
Molecular structure	• Nuclear Magnetic Resonance, NMR (^{13}C, ^{1}H, liquid-state and solid-state)

2.6 Chitosan in Removal of Metal Ions in Waste Water

The high content of amino groups provide to chitosan very interesting chelating properties versus heavy metals. These can be cations like Pb^{2+} or Cu^{2+} or anions like MoO_4^{2-}.

Naturally abundant biosorbents such as chitosan has been applied successfully for the removal of traces of heavy metals (Lee, 2002). The effectiveness of using chitosan to remove lead and cadmium in drinking water has been demonstrated in previous studies carried out (Knorr, 1991). Chitosan from treated crab shells have also been used effectively to treat effluents from electroplating industry and for the removal of hexavalent chromium, where the removal was by sorption to the extent of 90%.

Chitosan is well known as a flocculants for suspended solids, this property making it ideal for the treatment of wastewater (Meyers, 2000). Hence, it is not surprising that one of the principle uses of chitosan is as a 'flocculating agent' for the clarification of industrial effluent, which includes sludge from breweries, sewage works and many more.

CHAPTER 3
METHODOLOGY

3.1 Extraction of Chitin from Insects

3.1.1 Preparation of Sample Insects

In the experiment, the sample insect used was common cockroaches (Homasilpha vicinia). About 50 raw cockroaches (100 g) were required in order to run this experiment. Before grinding them, the cockroaches were dried up under the sun for a few days to remove the unpleasant smell produced by the insect. Then, they were crushed into smaller pieces using pestle and mortar. The dried, smaller pieces will be ready to grind until powder form is obtained. The specimen was then grinded using a lab grinder to make the powder finer. This was also to ensure large surface area when it reacted with the chemicals, which were added later. The cockroaches' powder was then placed in the oven for 2 hours to make it more brittle and to prevent organic decomposition (Fig 3.1).

3.1.2 Sample Preparation Procedure

1) The shells were broken up into small pieces using a pestle and mortar.
2) The small pieces were then grinded into fine powder using the grinder.
3) The fine powder was dried in an oven at 120°C for 2 hours to dry the shells and to make them more brittle. This step also extended its shelf life, preventing organic decomposition.

Fig 3.1: Cockroaches' Powder Which Have Been Grinded

3.1.3 Extraction of Chitin using Method of Broussignac

For extraction of chitin, 2 steps were conducted in the process which was the demineralization and deproteination.

Demineralization is the removal of minerals, primarily calcium carbonate ($CaCO_3$). A mineral free chitin would be required for applications that have very low impurity tolerance. This process involves the decomposition of $CaCO_3$ to calcium salts, carbon dioxide (CO_2) and water (H_2O). Hydrochloric acid (HCl) is used because it produces calcium chloride ($CaCl_2$), but the large volumes of $CaCl_2$ solution must be disposed.

Deproteinization is a process where covalent chemical bonds have to be destroyed between the chitin-protein complexes. This is achieved with some difficulty especially if performed heterogeneously utilizing chemicals that will also depolymerize the biopolymer. Sodium hydroxide (NaOH) is the preferred reagent and typically 1.2 M NaOH solution is used with variations in the temperature and duration of treatment

parameters. The use of NaOH invariably results in partial deacetylation of chitin and hydrolysis of the biopolymer that lowers the molecular weight of chitin. The yield of chitin at this point is yet to be determined because of the current drying condition. The chitin at this point was composed of chitin together with an indeterminate amount of impurities (Fig 3.2).

The removal of these 2 major components, calcium carbonate and proteins gives raw chitin of reasonable quality. However, it must be noted that the quality of the final chitin obtained is first directly dependent on the starting raw material that needs to be consistent. The manner in which the sample are gathered, cleaned, dried and powdered will also affect the final quality as this determines the amount of impurities and accessibility of the sample to the chemicals. Raw chitin can be further processed depending on end-use requirements as in biomedical applications. It must be noted that in the recovery of any bioresource, the elimination of the last fraction of unwanted biological components is difficult and may not be necessary. There is insignificant economic benefit in doing so especially if it contributes little to increasing purity and degrades the biopolymer but may be warranted for biomedical applications.

3.1.4 Procedure in Extraction of Chitin

1) 20 cm^3 (20 ml) of 1.5 M of Hydrochloric acid (HCl) was added per gram of shell powder. This removed the calcium carbonate ($CaCO_3$) in the shell by neutralization. $CaCO_3$ constitutes the main bulk of the organic component of the shell. Only dilute acid was used to prevent hydrolysis of chitin.
2) The mixture was left for 2 days with constant stirring, to react to completion. Absence of effervescence was taken to mean all reaction had ceased. Most of the solution was decanted off at the end.
3) 1.2 M of Sodium hydroxide (NaOH), in similar proportion of HCl (20 ml per gram shell powder), was then added.
4) Heating at 90°C was carried out in a water bath for 1 hour. The mixture was then left to react for 1 day at room temperature.

5) Using a Buchner funnel, the powdery residue was collected, washed with distilled water and dried with acetone wash.

6) It was left overnight to let the weight stabilized.

Fig 3.2: Cockroaches' Powder, Chitin and Chitosan (from left to right)

3.2 Chitin to Chitosan

3.2.1 Deacetylation of Chitin to Chitosan using Method of Alimunar and Zainuddin

At alkali concentrations below 45%, chitosan will not be formed (Alimunar and Zainuddin, 1992) [9]. Higher concentrations of alkali correspond to a faster rate of deacetylation. 50 ml of concentrated solution, 50% w/v NaOH, was mixed with each gram of chitin.

50% w/v (weight-volume) NaOH is prepared by mixing 50 g NaOH in 100 mL distilled water. As the concentration is too high, the procedures need to be done carefully. The solution prepared was kept in the fume hood as the safety precaution step.
After left for complete deacetylation process, the solution was diluted before filtration. The previous step was done before filtration to prevent the concentrated solution from dehydrating the filter paper. The residue of the mixture was noticeably paler in color, as compared to the previous yield of chitin (Fig 3.2).

3.2.2 Procedure in Deacetylation of Chitin

1) The conversion of chitin to chitosan involved the deacetylation of the chitin using 50% (w/v) NaOH.
2) The solution was placed in water bath at 95°C for 3 hours.
3) 50 ml of the concentrated solution was mixed with each gram of chitin.
4) The mixture was left for 3 days.
5) The solution was then cooled down and diluted with distilled water until neutral pH.
6) The mixture then was extracted with 2% acetic acid, filtered and precipitated to obtain the purified chitosan.
7) The powdery residue was dried with acetone before it was collected.

3.2.3 Test for Chitosan

Purification of chitosan was attempted by dissolution in 1% ethanoic acid. The filtrate was collected leaving impurities behind as residue. Standard bench NaOH was added to precipitate the chitosan as a white gelatinous precipitate. The van Wisselingh test (Tracy, 1955) is the most accurate test for chitosan. When iodine-potassium iodide (I_2-KI) solution was added, the precipitate turned brownish red. The mixture was then acidified with H_2SO_4, upon with chitosan immediately turned blackish violet (Fig 3.3). By successfully testing for chitosan, we could indirectly infer that chitin was present in the processed powder at the end of deacetylation process. There is no simple, direct laboratory test for chitin.

3.2.4 Van Wisselingh Test

1) Chitosan was purified by dissolution in 1% ethanoic acid.
2) The filtrate was collected leaving impurities behind as residue.
3) 0.1 M sodium hydroxide (NaOH) was added to precipitate the chitosan as white gelatinous precipitate.
4) Iodine solution, 1% in potassium-iodide (I_2-KI) was added.
5) The mixture was then acidified with 0.01 M sulphuric acid (H_2SO_4).

Fig 3.3: Van Wisselingh Test on Chitosan

3.3 Chitosan Analysis

3.3.1 Atomic Absorption Spectrophotometer AA-6800 Shimadzu

The atomic absorption spectrometry uses absorption of light of intrinsic wavelengths by atoms. All atoms are classified into those having low energies and those having high energies. The state having low energies is called the ground state and the state having high energies is called the excited state.

When light of a certain intensity is given to many atoms in the ground state, part of the light is absorbed by atoms. The absorption rate is determined by the atomic density.

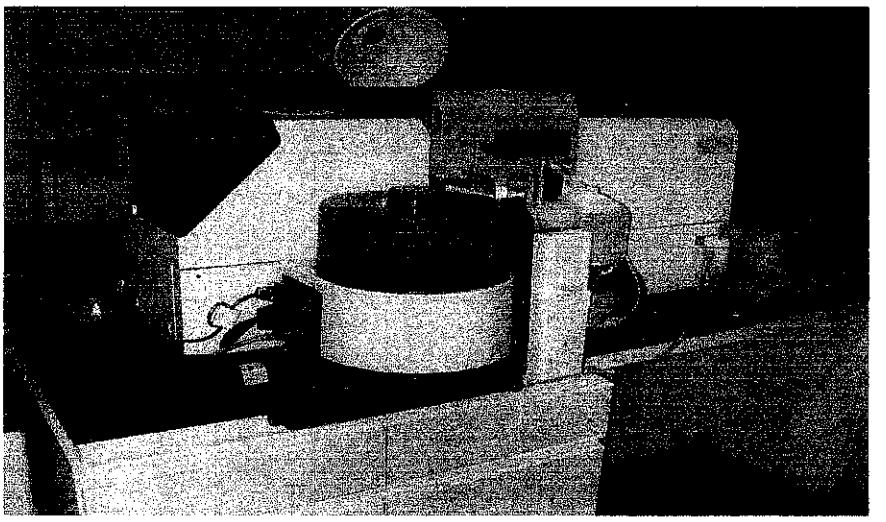

Figure 3.4: Atomic Absorption Spectrophotometer AA-6800 Shimadzu

3.3.2 Sample Preparation and Testing for Lead Ions (Pb^{2+})

1) 5 samples of 20 mL solution containing lead ions with 50 ppm concentration were prepared.
2) Different mass of chitosan was added into 5 samples which were 0.02 g, 0.04 g, 0.08 g, 0.16 g and 0.20 g.
3) The samples were placed in the shaker for about 2 hours for reaction.
4) Each solution from the samples was then tested for concentration using the Atomic Absorption Spectrophotometer (AAS).
5) A graph of mass of chitosan against the ions uptake was then plotted (Fig 3.5a).

3.3.3 Sample Preparation and Testing for Nickel Ions (Ni^{2+})

1) 5 samples of 50 mL solution containing nickel ions with 50 ppm concentration were prepared.
2) Different mass of chitosan was added into 5 samples which were 0.05 g, 0.10 g, 0.15 g, 0.20 g and 0.25 g.
3) The samples were placed in the shaker for about 2 hours for reaction.
4) Each solution from the samples was then tested for concentration using the Atomic Absorption Spectrophotometer (AAS).
5) A graph of mass of chitosan against the ions uptake was then plotted (Fig 3.5b).

3.3.4 Sample Preparation and Testing for Copper Ions (Cu^{2+})

1) 5 samples of 50 mL solution containing copper ions with 50 ppm concentration were prepared.
2) Different mass of chitosan was added into 5 samples which were 0.02 g, 0.04 g, 0.06 g, 0.08 g and 0.10 g.

3) The samples were placed in the shaker for about 2 hours for reaction.
4) Each solution from the samples was then tested for concentration using the Atomic Absorption Spectrophotometer (AAS).
5) A graph of mass of chitosan against the ions uptake was then plotted (Fig 3.5c).

3.3.5 Sample Preparation and Testing for Zinc Ions (Zn^{2+})

6) 5 samples of 50 mL solution containing zinc ions with 50 ppm concentration were prepared.
7) Different mass of chitosan was added into 5 samples which were 0.05 g, 0.10 g, 0.15 g, 0.20 g and 0.25 g.
8) The samples were placed in the shaker for about 2 hours for reaction.
9) Each solution from the samples was then tested for concentration using the Atomic Absorption Spectrophotometer (AAS).
10) A graph of mass of chitosan against the ions uptake was then plotted (Fig 3.5d)

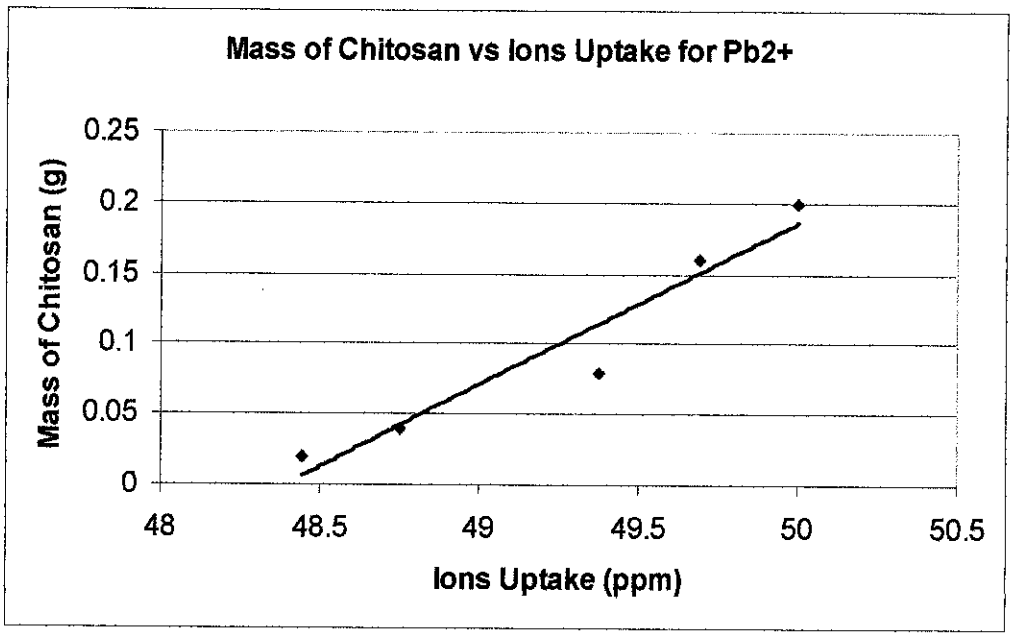

Fig 3.5a: Graph of Mass of Chitosan vs Ions Uptake for Pb^{2+}

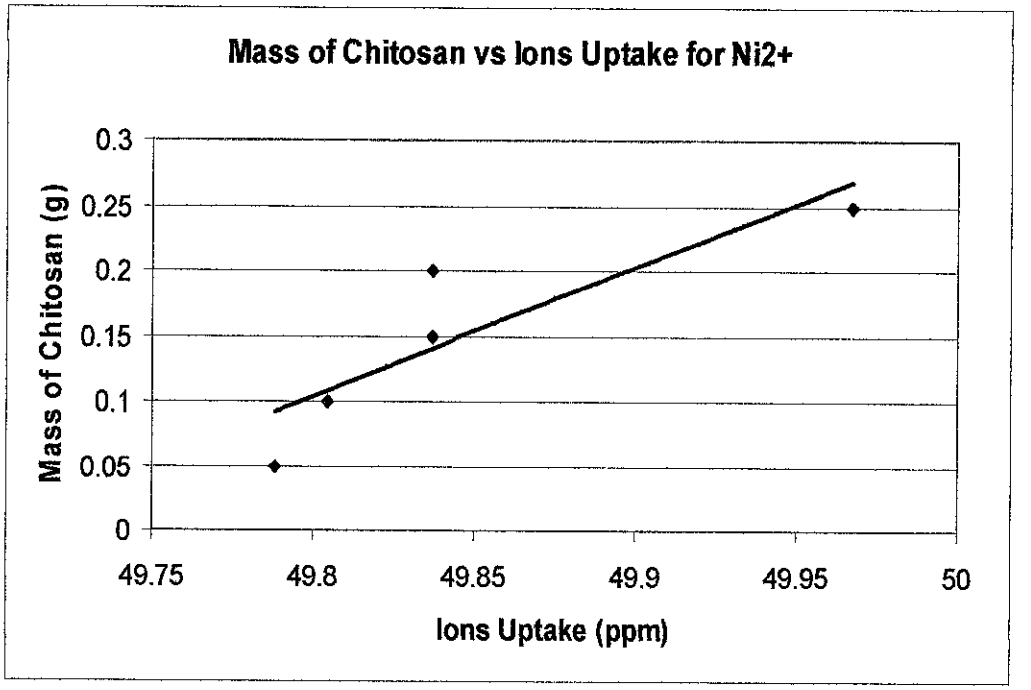

Fig 3.5b: Mass of Chitosan vs Ions Uptake for Ni^{2+}

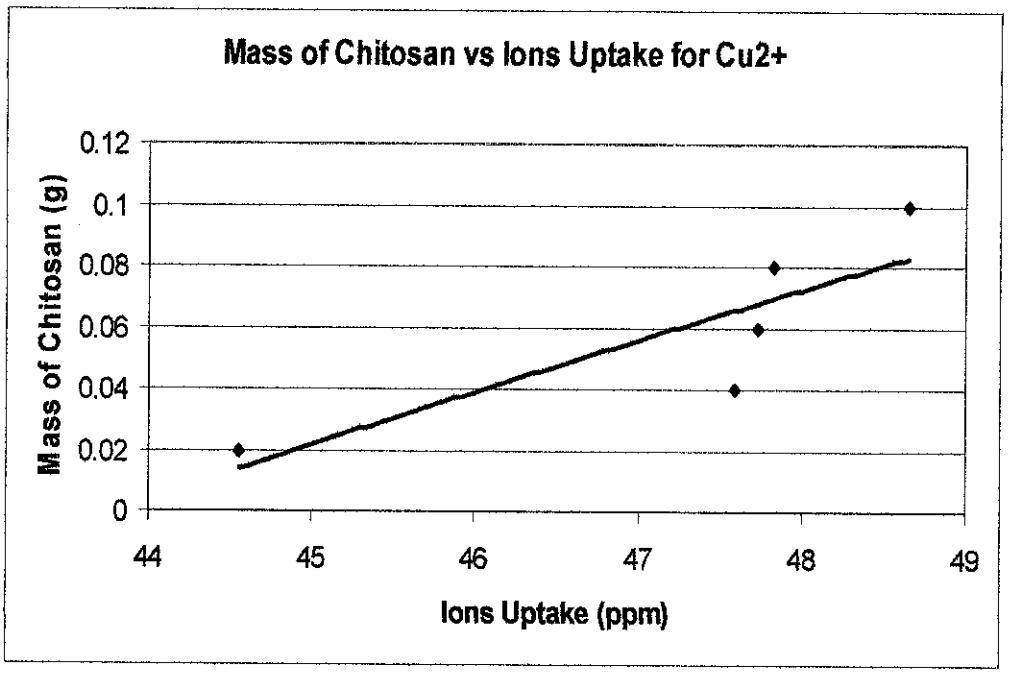

Fig 3.5c: Mass of Chitosan vs Ions Uptake for Cu^{2+}

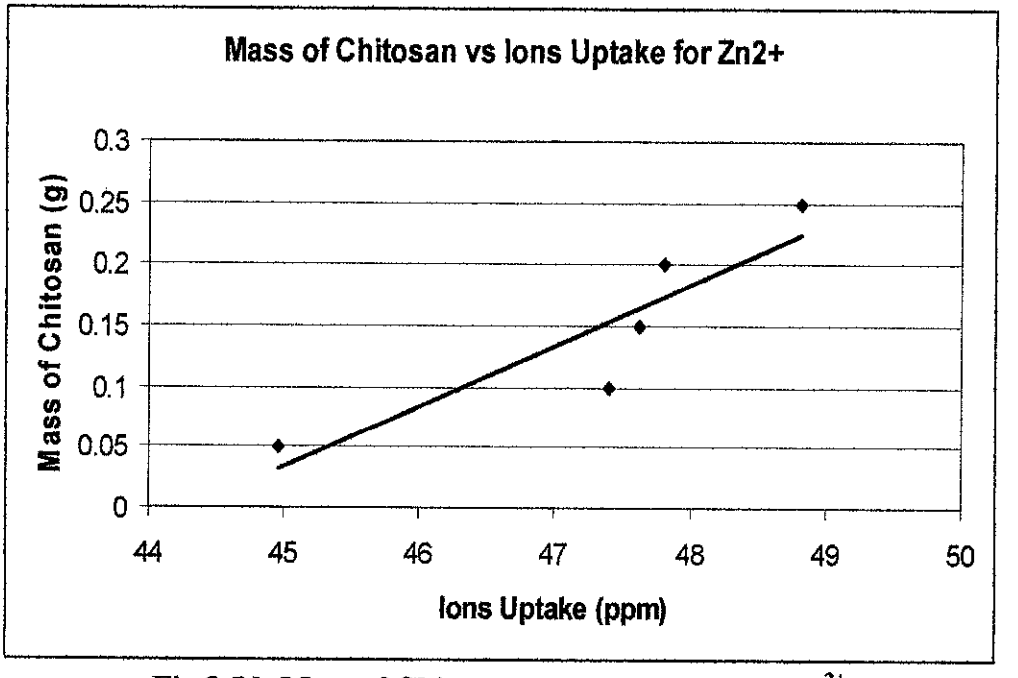

Fig 3.5d: Mass of Chitosan vs Ions Uptake for Zn^{2+}

CHAPTER 4
RESULTS AND DISCUSSION

4.1 RESULTS

The mass obtained during the lab experiment from the sample preparation until the production of chitin is represented in Table 4.1. The value was rounded to the nearest integer.

Table 4.1: Mass Obtained for Each Step

Sample Preparation	Demineralization	Deproteination	Deacetylation
Fine powder	1.5 M HCl	1.2 M NaOH	50% w/v NaOH
50 g	20 g	15 g	7 g

The yield of chitin from the extraction step (demineralization and deproteination) was 30% and was composed of chitin together with indeterminate amount of impurities.

The yield of chitosan from chitin was 46.67%, indicating that greatest loss in mass occurred in previous part. A difference percentage with the theoretical value which is 70% occurs due to the human error during the lab experiment.

Table 4.2 represents the raw data from the analysis of heavy metal ions uptake by the chitosan using the Atomic Absorption Spectrophotometer (AAS). Table 4.3 and 4.4 accompanying below are the analysis data from the raw data.

Table 4.2: The Raw Data from the Analysis of Using Chitosan in Heavy Metal Ions Removal

Type of Heavy Metal Ions	Sample (20 mL)	Amount of Chitosan, (g)	Initial Concentration, (ppm)	Final Concentration, (ppm)
Lead (Pb^{2+})	1	0.02	50	1.5556
	2	0.04	50	1.2444
	3	0.08	50	0.6222
	4	0.16	50	0.3111
	5	0.20	50	0.0000
Nickel (Ni^{2+})	1	0.05	50	0.2120
	2	0.10	50	0.1957
	3	0.15	50	0.1630
	4	0.20	50	0.1630
	5	0.25	50	0.0326
Copper (Cu^{2+})	1	0.02	50	5.4500
	2	0.04	50	2.4050
	3	0.06	50	2.2680
	4	0.08	50	2.1760
	5	0.10	50	1.3480
Zinc (Zn^{2+})	1	0.05	50	5.0379
	2	0.10	50	2.5948
	3	0.15	50	2.3794
	4	0.20	50	2.1897
	5	0.25	50	1.1880

Table 4.3 Percentage Reduction of Heavy Metal Ions

Sample	Lead Ions, Pb^{2+} (%)	Nickel Ions, Ni^{2+} (%)	Copper Ions, Cu^{2+} (%)	Zinc Ions, Zn^{2+} (%)
1	96.89	99.58	89.10	89.92
2	97.51	99.61	95.19	94.81
3	98.76	99.67	95.46	95.24
4	99.38	99.67	95.65	95.62
5	100.00	99.93	97.30	97.62
Maximum	100.00	99.93	97.30	97.62
Average	98.51	99.69	94.54	94.64

Table 4.4 Percentage Reduction of Heavy Metals Ions and Chitosan Used for 50 ppm Concentration

Heavy Metal Ions	Percentage Reduction (%)	Mass of Chitosan Used (g)
Lead Ions, Pb^{2+}	100.00	0.20
Nickel Ions, Ni^{2+}	99.93	0.25
Copper Ions, Cu^{2+}	97.30	0.10
Zinc Ions, Zn^{2+}	97.62	0.25

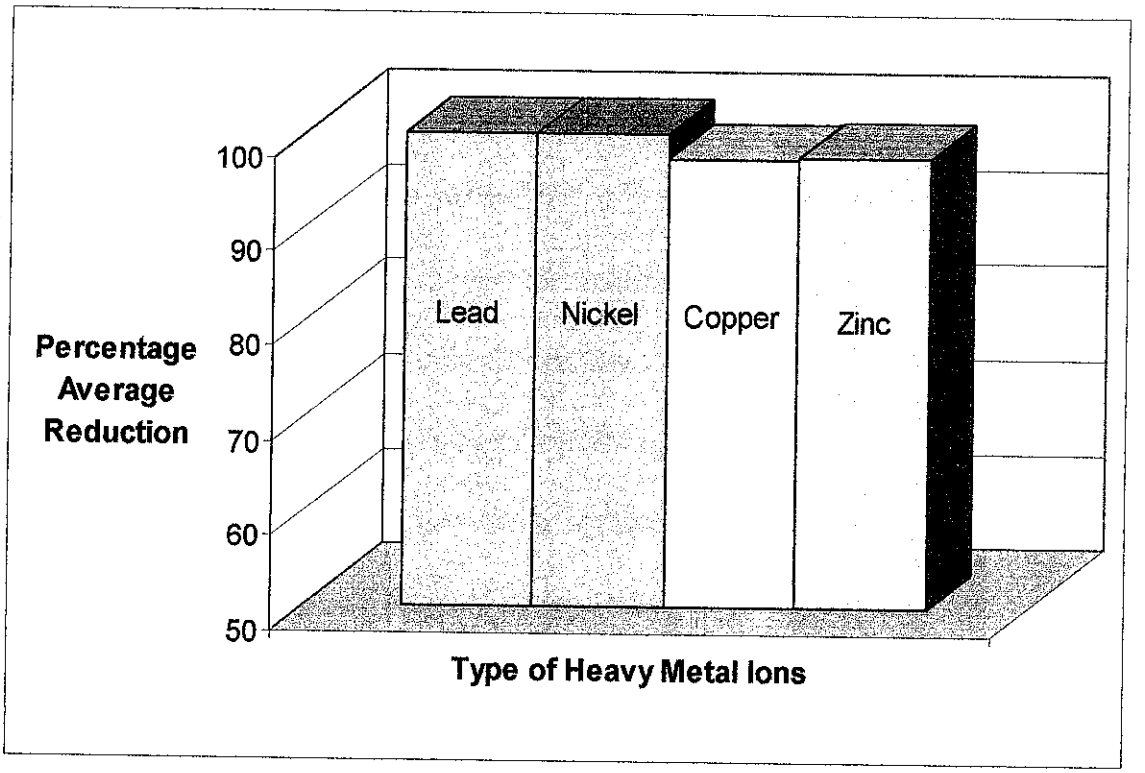

Fig 4.1 Bar Chart on Average Reduction of Heavy Metal Ions

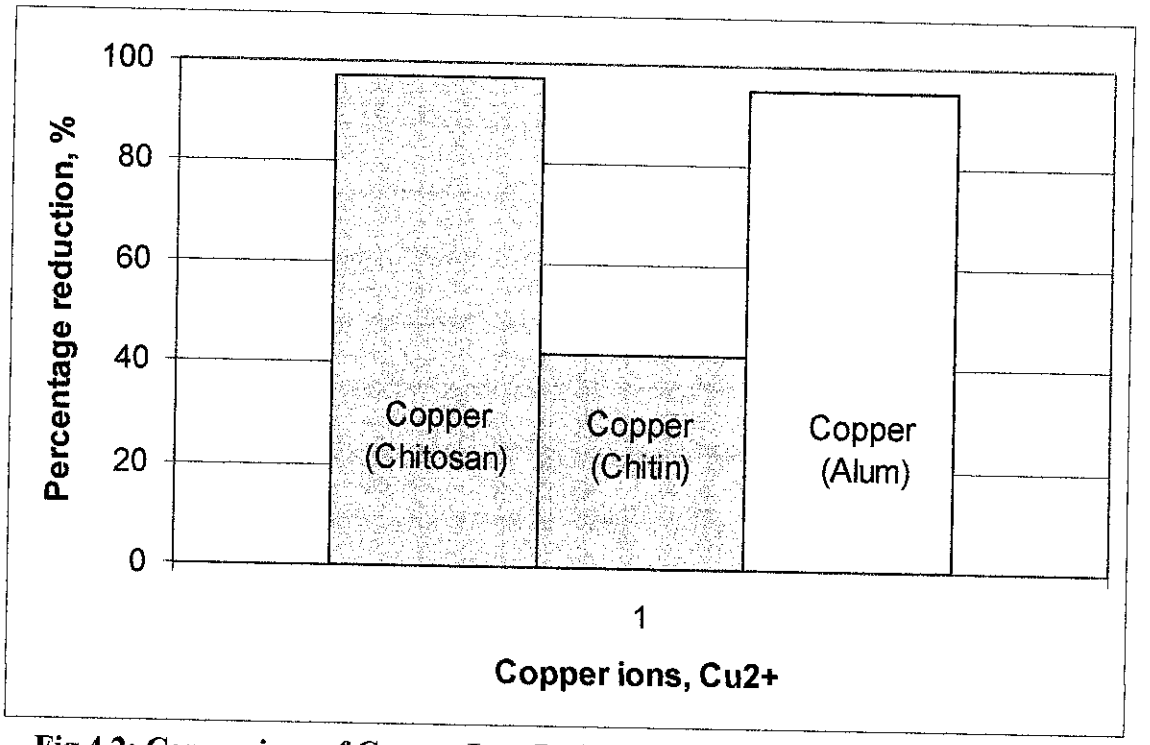

Fig 4.2: Comparison of Copper Ions Reduction by Chitosan, Chitin and Alum

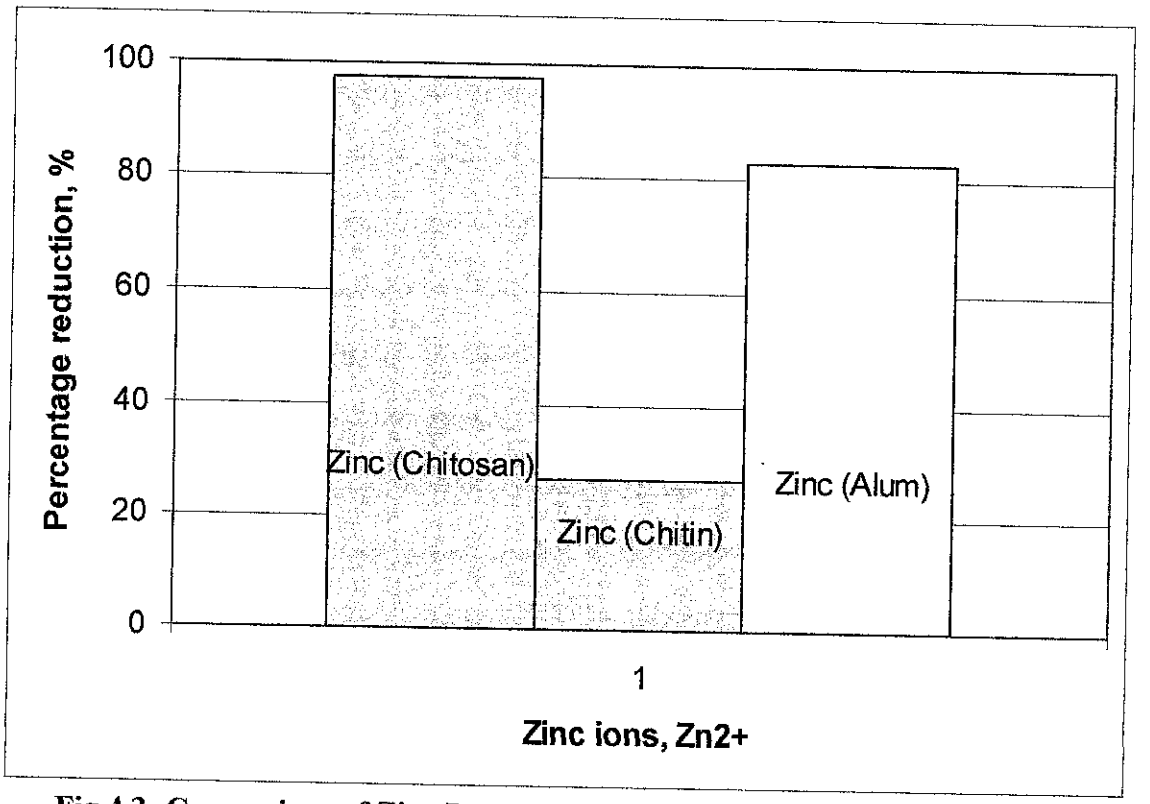

Fig 4.3: Comparison of Zinc Ions Reduction by Chitosan, Chitin and Alum

4.2 DISCUSSION

The rate of uptake of lead ions (Pb^{2+}), nickel ions (Ni^{2+}), copper ions (Cu^{2+}) and zinc ions (Zn^{2+}) by chitosan was tested using the AAS. Different grams of chitosan were added to each synthetic waste solution of 50 ppm concentration to find the optimum uptake. From the result in Table 4.3, chitosan was found to have removed 95% and above of all ions tested from 50 ppm solution after 2 hours.

In Fig 4.1, it shows that all the 4 heavy metal ions were successfully reduced by the chitosan. From the bar chart, the lead ions and nickel ions were nearly completely removed which are 98.51% and 99.69% respectively. Both heavy metal ions show better removal with the chitosan.

Compared with the previous experiment (Fig 4.2 and 4.3) using the copper ions (Cu^{2+}) and zinc ions (Zn^{2+}), chitosan is by far the better of the two in terms of chelation rate and maximum chelation efficiency. The maximum rate of uptake of copper by chitosan proved to be vastly superior to that of chitin.

Chitosan still proved to be a preferred biopolymer to be used in the removal of heavy metal ions in the waste water. The chelating efficiency can be improved by having more mass of chitosan used or a higher degree of purity of the chitosan.

Chitosan did not release any of the heavy metals ions back into the solution. This is an encouraging result, for it means that the process is virtually irreversible.

Khor (2001) stated that the main differentiation between chitosan from chitin is to consider their respective acetyl content. When the number of acetamido groups is more than 50% (more commonly 70 – 90%) the biopolymer is termed chitin. In chitin terminology, the number of acetamido groups is termed the degree of acetylation (DA). On the other hand, when the degree of deacetylation (DD) or the amino group is predominant, the biopolymer is termed chitosan. Muzzarelli (1977) stated that the instant differentiation between chitosan can be attained from their solubility and nitrogen content. Chitin is insoluble in aqueous acetic acid while vice versa for chitosan. The nitrogen content in purified sample is less than 7% for chitin and more than 7% for chitosan.

The disposal of the complexed chitosan-heavy metal ions after the treatment should not be a problem. If buried after use, no leaching into the soil will occur under normal conditions. However, what happens when the chitosan is biodegraded is still undetermined. Commercial firms might be willing to store them to release the ions by chemical means, especially if they are precious.

Present day industrial waste often contains high content heavy metal ions. Heavy metals are those following titanium in the periodic table. Due to the heavy metal content, industrial affluent, if left untreated, can have catastrophic effects on human health.

Therefore, it is necessary to minimize the heavy metal content in waste water before discharging it into the natural environment. Currently, the method commonly utilized requires the addition of lime (calcium hydroxide) to precipitate heavy metals out of solution as insoluble hydroxides.

However, to bring the levels of mercury, cadmium and lead, which are believed to be the most toxic metals at present, down to acceptable levels, further treatment is needed. Chitosan can take part in this process. Chitosan is an excellent chelating agent. Their affinity for heavy metal ions means that both biopolymers have great potential in the

field of water treatment. They are applicable for removing both trace and macro amounts of metal ions. Even radioactive metals, such as uranium, can also be easily chelated (Muzzarelli, 1973).

An additional advantage chitosan has over other means is that it is also a flocculating agent for solid particles suspended in water.

CHAPTER 5
CONCLUSION AND RECOMMENDATION

5.1 Conclusion

It is proved that the chitosan is superior compared to the chitin as the chelating agent. Percentage reduction of lead ions, nickel ions, copper ions and zinc ions was achieved at 95% and above in the experiment.

However, there are some concerns that need to be looked into in the production of chitin and chitosan. Chitin is often found bonded strongly to the proteins of the organic structure in which it is found. Therefore, there is an in evitable need to extract and purify the chitin. Extraction and purification from the exoskeletons of the insects was found to be quite troublesome. The method of Broussignac that was used only gave a yield of 30% via the laboratory experiment. Although this problem is partially compensated by the fact that the raw materials (in this case the common cockroaches) can be easily and cheaply required, more economical and feasible ways of producing chitin and chitosan are yet to be discovered.

For the 50 ppm concentration solution, reduction of lead ions (Pb^{2+}) is at 100% with 0.20 g chitosan, 99.93% for nickel ions (Ni^{2+}) with 0.25 g chitosan, 97.30% for copper ions (Cu^{2+}) with 0.10 g chitosan and 97.62% for zinc ions (Zn^{2+}) with 0.25 g chitosan.

5.2 Recommendation

A suggestion is to use enzymes to digest the shell instead hydrochloric acid (HCl) and sodium hydroxide (NaOH). Enzymes can be re-used repeatedly and although have a high unit cost, are far more efficient. Others sources of chitin can also be tested. One potential candidate is fungi which have cell walls composed primarily of chitin and most importantly they prolifiterate rapidly.

In discussion on chitosan, one major issue always arise has been the lack of consistency in the properties of the biopolymer and the methods used to determine these properties. Further studies need to be conducted to determine this biopolymer physical and chemical properties. For any particular manufacturer or researcher, it is important to note that one chosen characterization method applied across the board for all chitosan-based products will inject consistency in product evaluation, a requirement in the development of standard protocols.

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APPENDICES

Preparation of 50% weight-volume (w/v) NaOH

$$50\%(w/v)NaOH = \frac{50gNaOH}{100mLsolution}$$

1) 250 g sodium hydroxide (NaOH) was weighed with the mass balance.
2) The solute then was added to 500 mL volumetric flask and diluted with the distilled water.

Preparation of Heavy Metal Ions Solution

50 ppm of Lead Ions (Pb^{2+})

$$50ppm(Pb2+) = \frac{50mg}{L}(Pb2+)$$

1) 0.025 g of lead nitrate, $Pb(NO_3)_2$ was weighed with the micro mass balance.
2) The solute then was added to 500 mL volumetric flask and diluted with the distilled water.

50 ppm of Nickel Ions (Ni^{2+})

$$50ppm(Ni2+) = \frac{50mg}{L}(Ni2+)$$

1) 0.025 g of nickel sulfate ($NiSO_4$) was weighed with the micro mass balance.
2) The solute then was added to 500 mL volumetric flask and diluted with the distilled water.

50 ppm of Copper Ions (Cu^{2+})

$$50\,ppm(Cu2+) = \frac{50mg}{L}(Cu2+)$$

1) 0.025 g of nickel sulfate ($CuSO_4$) was weighed with the micro mass balance.
2) The solute then was added to 500 mL volumetric flask and diluted with the distilled water.

50 ppm of Zinc Ions (Zn^{2+})

$$50\,ppm(Zn2+) = \frac{50mg}{L}(Zn2+)$$

1) 0.025 g of zinc chloride ($ZnCl_2$) was weighed with the micro mass balance.
2) The solute then was added to 500 mL volumetric flask and diluted with the distilled water.

Van Wisselingh Test