ADSORPTION OF COPPER(II) AND ZINC(II) IN 0.01 M HYDROCHLORIC ACID SOLUTION BY CHITOSANS

PONGDEJ EKWANIJCHA

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Chitosan or deacetylated chitin, is obtained from crab or shrimp shell. Recently, chitosan capsules have been produced and sold as a dietary supplement for weight control. Chitosan is taken to reduce the absorption of fats and cholesterol. However, chitosan and some derivatives of chitosan have been reported as having capacities to adsorb metal ions, including ions of essential minerals such as copper(II) and zinc(II) ions.

In this work, three chitosan samples, denoted as chitosan S, chitosan L and chitosan M, respectively, were studied for adsorption of Cu(II) and Zn(II) in 0.01 M HCl, the pH of gastric juice. Chitosan S is a sample containing no fillers, approximately 98%(w/w). Chitosan L and chitosan M contained fillers, such as ascorbic acid (5%w/w) and carbohydrate (32.6%w/w), respectively. Adsorption capacities of Cu(II) and Zn(II) ions on chitosans were measured after two hours in contact with solutions. The capacities were calculated based on Langmuir adsorption isotherm. The order of capacities for Cu(II) ion were: chitosan S (59.8 ± 3.0 mg/g) > chitosan L (52.2 ± 3.2 mg/g) > chitosan M (24.9 ± 1.4 mg/g). The order of capacities for Zn(II) ion were: chitosan S (20.3 ± 2.8 mg/g) ≈ chitosan L (19.8 ± 1.6 mg/g) > chitosan M (13.6 ± 1.1 mg/g). The adsorption capacities of Cu(II) ion for all chitosans were greater than Zn(II) ion. The adsorption of both metal ions on chitosans may take place via chemisorption at the $-NH_2$ group.

Effect of competing cation on metal adsorption in 0.01 M HCl were studied in binary system of Cu(II) and Zn(II). The amount of metal of interest and chitosan were chosen to be equal to a meal intaking, 1.34 mg Cu/g chitosan and 6.63 mg Zn/g chitosan. The adsorption of Cu(II) ion for all the chitosans did not change as the concentration of added Zn(II) ion increased. However, the adsorption of Zn(II) ion decreased as Cu(II) ion was added to the solution.

From the capacity data obtained in this work, chitosan L and chitosan M which are used as meal supplement, may inhibit gastric absorption of Cu(II) and Zn(II) ions. However, the experiments were not carried out in the exact condition of the stomach condition. An in vivo study should be carried out to confirm this.

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พงษ์เดช เอกวนิชชา: การดูดซับทองแดงและสังกะสีของไคโตแซนในสารละลายกรดไฮโดรคลอริก 0.01 โมลาร์ (ADSORPTION OF COPPER(II) AND ZINC(II) IN 0.01 M HYDROCHLORIC ACID SOLUTION BY CHITOSANS). คณะกรรมการควบคุมวิทยานิพนธ์: ดวงใจ นาคะปรีชา, Ph. D., ประพิณ วิไลรัตน์, Ph.D. 125 หน้า. ISBN 974-664-571-4

ไคโตแซนคือไคตินที่ถูกกำจัดหมู่อะซีติลซึ่งได้มาจากกระดองปูและเปลือกกุ้ง ในปัจจุบันนี้มีการผลิตไคโตแซนในรูปของแคปซูลเพื่อใช้เป็นผลิตภัณฑ์ในการควบคุมน้ำหนัก เนื่องจากไคโตแซนช่วยลดการดูดซึมไขมันและคลอเรสเตอรอลเข้าสู่ร่างกายได้ อย่างไรก็ตามไคโตแซนและอนุพันธ์บางชนิดยังสามารถดูดซับไอออนของโลหะต่างๆได้รวมทั้งไอออนของโลหะที่จำเป็นต่อร่างกายได้แก่ ไอออนของโลหะทองแดง (Cu(II)) และสังกะสี (Zn(II)) เป็นต้น

งานวิจัยนี้ได้ศึกษาความสามารถในการดูดซับ Cu(II) และ Zn(II) โดยไคโตแซนสามชนิดคือ ไคโตแซนเอส ไคโตแซนเอล และไคโตแซนเอ็ม โดยทำการทดลองในสารละลายกรดไฮโดรคลอริก 0.01 M เพื่อจำลองสภาพความเป็นกรดของน้ำย่อยจากกระเพาะอาหารซึ่งระหว่างไคโตแซนทั้งสามชนิดนี้ ไคโตแซนเอส เป็นตัวอย่างที่มีความบริสุทธิ์สูง (ประมาณร้อยละ 98 โดยน้ำหนัก) เนื่องจากไม่มีตัวเติมเต็มเป็นส่วนประกอบ ขณะที่ไคโตแซนเอล และไคโตแซนเอ็มมีตัวเติมเต็มคือกรดแอสคอร์บิค (ร้อยละ 5 โดยน้ำหนัก) และคาร์โบไฮเดรต (ร้อยละ 32.6 โดยน้ำหนัก) เป็นส่วนประกอบตามลำดับ ได้ทำการหาค่าความจุในการดูดซับของไคโตแซนต่อ Cu(II) และ Zn(II) ในระยะเวลาสองชั่วโมง ซึ่งอ้างอิงตามหลักการของ Langmuir adsorption isotherm จากการศึกษาพบว่า ลำดับความสามารถในการดูดซับ Cu(II) เป็นดังนี้คือ ไคโตแซนเอส (59.8 ± 3.0 mg/g) > ไคโตแซนเอล (52.2 ± 3.2 mg/g) > ไคโตแซนเอ็ม (24.9 ± 1.4 mg/g) ส่วนลำดับความสามารถในการดูดซับ Zn(II) คือ ไคโตแซนเอส (20.3 ± 2.8 mg/g)ใกล้เคียงกับไคโตแซนเอล (19.8 ± 1.6 mg/g) แต่มากกว่าไคโตแซนเอ็ม (13.6 ± 1.1 mg/g) ทั้งนี้พบว่าค่าความจุในการดูดซับของ Cu(II) มากกว่าของ Zn(II) เสมอไม่ว่าจะศึกษาในไคโตแซนชนิดใด สำหรับกลไกในการดูดซับไอออนของโลหะทั้งสอง (M^{2+}) จัดเป็นกระบวนการดูดซับทางเคมีซึ่งเกิดขึ้นที่หมู่เอมีน ($-NH_2$) บนไคโตแซน

งานวิจัยนี้ยังได้ศึกษาถึงอิทธิพลของไอออนตัวรบกวนที่อาจมีผลต่อการดูดซับของไอออนของโลหะที่สนใจในระบบที่มีทั้ง Cu(II) และ Zn(II) อยู่ในสารละลายกรดไฮโดรคลอริก 0.01 M ปริมาณของโลหะที่สนใจและไคโตแซนจะเท่ากับที่ร่างกายได้รับต่อการบริโภคหนึ่งมื้อ กล่าวคือ1.34 mg Cu/g chitosan และ 6.63 Zn/g chitosan พบว่าการดูดซับของ Cu(II) บนไคโตแซนทุกชนิดไม่เปลี่ยนแปลงไปเมื่อความเข้มข้นของ Zn(II) ที่เติมลงไปเพิ่มขึ้น อย่างไรก็ตามการดูดซับของ Zn(II) จะลดลงเมื่อความเข้มข้นของ Cu(II) ถูกเติมเข้าไปในระบบเพิ่มขึ้น

จากค่าความจุของการดูดซับ Cu(II) และ Zn(II) โดยไคโตแซนเอลและไคโตแซนเอ็มพบว่า ไคโตแซนทั้งสองชนิดอาจจะยับยั้งการดูดซึม Cu(II) และ Zn(II) ได้เมื่อบริโภคไคโตแซนทั้งสองชนิดเพื่อควบคุมน้ำหนักในแต่ละมื้อ อย่างไรก็ตามในงานวิจัยนี้ไม่ได้ศึกษาในสภาวะกระเพาะอาหารจริง จึงควรจะทำการทดลองในระบบ in vivo เพื่อยืนยันผลที่ได้นี้อีกครั้ง

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LIST OF ABBREVIATIONS

mL	Milliliter
mg L^{-1}	Milligram per liter
mm	Millimeter
μm	Micrometer (micron)
nm	Nanometer
^{o}C	Temperature in degree of Celsius
M	Molar
FAAS	Flame Atomic Absorption Spectrometry
ETAAS	Electrothermal Atomic Absorption Spectrometry
GPC	Gel Permeation Chromatography
NMR	Nuclear Magnetic Resonance
1DUVS	First Derivative Ultraviolet Spectrophotometry
FT-IR	Fourier Transform Infrared Spectrometry
ZCP	Zero crossing point
Ci	Initial concentration of metal ion
Ce	Residual concentration of metal ion
Qe	mg of metal ion adsorbed per gram chitosan
GlcNAc	*N*-acetyl-*D*-glucosamine
GlcN	*D*-glucosamine

CHAPTER I

INTRODUCTION

1.1 Importance of copper and zinc in human body

1.1.1 General introduction [1]

Chemical analysis shows that the human body is made up of specific chemical elements. Four of these elements: oxygen, carbon, hydrogen, and nitrogen make up 96 percent of body weight. All the remaining elements, called *mineral elements* or just *minerals*, represent only four percent of body weight. Nevertheless, these elements are essential for good health.

A mineral is an inorganic (nonliving) element that is necessary for the body to build tissues, regulate body fluids, or assist in various body functions. Minerals are found in all body tissues. They can not provide energy by themselves, but in their role as body regulators, they contribute toward the production of energy within the body.

Amongst other minerals reportedly important to human body copper and zinc are always included.

1.1.2 Copper [2].

1.1.2.1 Occurrence in the body

Generally, copper and iron are metabolized in the body in much the same way, and share some functions.

The adult human body contains from 100 to 150 mg of copper, distributed mainly in muscle, bone, the liver, heart, kidneys, and central nervous system. A small quantity is bound to plasma protein. The serum values are highly variable but range from 130 to 230 μg per 100 mL. In the serum, about 5% of the copper is bound with an α-globulin, as the copper-binding protein *ceruloplasmin*.

1.1.2.2 Metabolic functions of copper

Copper is associated with iron in several important metabolic functions:

- Copper, like iron, is involved in the cytochrome oxidation system of tissue cells for energy production, as well as being a constituent of several other oxidative enzymes for amino acids.
- Copper is essential together with iron, in the formation of hemoglobin. A copper-containing protein, *erythrocuprein*, is in red blood cells.
- Copper seems to promote absorption of iron from the gastrointestinal tract. Copper also appears to

involved in transporting iron from the tissues into the plasma.

In addition to these iron-related functions, copper is involved in two other areas of metabolism: (a) bone formation, and (b) brain tissue formation and maintenance of myelin in the nervous system.

1.1.2.3 Requirement and food sources

Balance studies indicate that adults require about 2.5 mg of copper daily. Infants and children required about 0.05 mg per kilogram of body weight.

Copper is widely distributed in natural foods include meat, shellfish, nuts, seeds, legumes, and whole grains. The average daily diet contains from 2.5 to 5.0 mg. Chitchumroonchokchai C. [3] had found that the daily intake of copper in Thai nutrition is 0.32 to 4.01 mg Cu per day.

1.1.2.4 Clinical application

Deficiency of copper can result in anemia and bone disease. An excess accumulation of copper occurs in a rare inherited condition known as Wilson's disease, that is characterized by degenerative changes in brain tissue (basal ganglia) and in the liver. Large amounts of copper are absorbed and storage is increased in the liver, kidneys, and cornea. A copper-chelate, penicillamine, is used to bind the excess copper and cause it to be excreted.

1.1.3 Zinc [2]

1.1.3.1 Occurrence in the body

Zinc occurs in the human body in amounts larger than those of other trace elements except iron. The body's total zinc content, from 1.3 to 2.3 g, is distributed in many tissues including the pancreas, liver, kidney, lung, muscles, bones, and eye (cornea, iris, retina, and lens), endocrine glands, prostate secretions, and spermatozoa. The plasma zinc level is about 120 μg per 100 mL.

1.1.3.2 Metabolic of zinc

Enzyme constituent: Zinc functions mainly as an essential constituent of carbonic anhydrase, carboxypeptidase, and lactic dehydrogenase.

Zinc is an integral part of carbonic anhydrase, which act as a carbon dioxide carrier, especially in red blood cells. It takes up carbon dioxide from cells, combines it with water to form carbonic acid (H_2CO_3), and then releases carbon dioxide from the capillaries into the alveoli of the lung. This enzyme also functions in the renal tubule cells in the maintenance of acid-base balance, in mucosal cells, and in glands of the body.

Zinc is a cofactor of the protein-splitting enzyme, *carboxypeptidase*, which removes the carboxyl group (COOH) from peptides to produce amino acids. Zinc, therefore, has key role in protein digestion.

Zinc is a part of lactic *dehydrogenase*. This enzyme is essential for the interconversion of pyruvic acid and lactic acid in the glycolytic pathway for glucose oxidation. Thus, zinc also plays a part in carbohydrate digestion.

Two additional roles of zinc in metabolism are important but their significance is less well understood.

Insulin: Zinc combines readily with insulin in the pancreas, zinc-insulin serves perhaps as the storage form of this hormone. The diabetic pancreas contains about half the normal amount of zinc.

Leukocytes: A considerable quantity of zinc bound to protein is present in leukocytes although its function in white cells is unknown. The leukocytes of patients with leukemia contain about 10% less zinc than normal.

1.1.3.3 Requirement and food sources of zinc

No specific quantitative requirement for zinc has been established in man. Balance studies indicate that a daily intake of 0.3 mg per kilogram of body weight is adequate. Since the usual intake on average is from 10 to 15 mg, deficiency is unlikely. Chitchumroonchokchai C. [3] had found that the daily intake of zinc in Thai nutrition is 3.79 to 19.90 mg Zn per day.

Zinc is easily obtained in widespread natural sources. The best sources of zinc are seafood, especially oysters, meat, and eggs.

1.1.3.4 Clinical application

In some populations where dietary intake of zinc is low, retarded physical growth (dwarfism) and retarded sexual maturation, can take place

especially in males. Zinc deficiency causes poor wound healing in patients. Also there is a possible relation to liver disease with zinc deficiency.

1.2 Chitosan

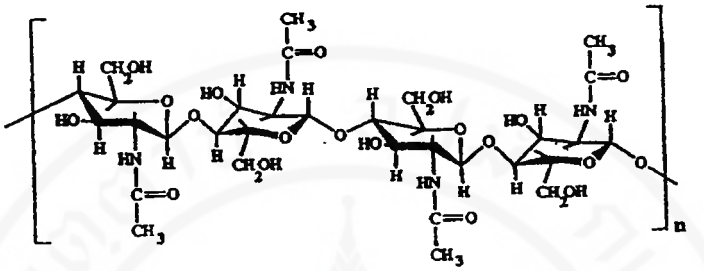
1.2.1 General introduction

Natural, non-toxic, biopolymers chitin and chitosan are now widely produced commercially from crab and shrimp waste shells. During the past few decades, chitin and chitosan have attracted significant interest in view of varied proposed novel applications. Use of these two functional polymers, especially chitosan, is denoted over a broad range of scientific areas, including use in biomedical, food, and various chemical industries [4].

Chitin (poly-β(1→ 4)-N-acetyl-D-glucosamine), has been regarded as a potential marine resource because it is a useful aminopolysaccharide analogous to cellulose structurally and naturally abundant, especially in the cuticle of the marine crustacean such as crab and shrimp.

Chitosan (poly-(1→4)-2-amino-2-deoxy-β-D-glucan), is a natural polysaccharide which is obtained by chitin hydrolysis in an alkaline medium. Usually, the deacetylation of chitin is carried out in sodium hydroxide or potassium hydroxide at high temperature and heterogeneous condition [5]. The molecular structure of chitin and chitosan are shown in Figure 1.1

a) Chitin



b) Chitosan

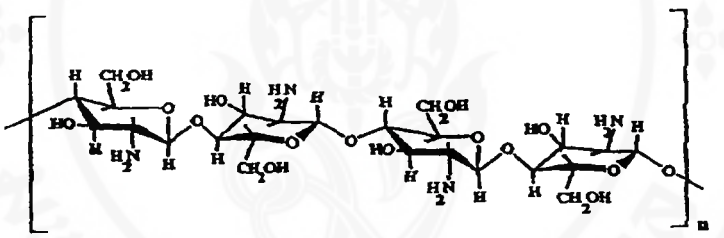


Figure 1.1 Molecular structure of chitin and chitosan.

Chitosan was first discovered by Rouget in 1859 when he boiled chitin in a concentrated potassium hydroxide solution [5]. This resulted in the deacetylation of chitin. Chitosan was produced industrially for the first time in the world in 1971. Advantages of chitosan include availability, low cost, high biocompatibility, biodegradability, ease of chemical modification and its also nontoxic [6].

1.2.2 Production of chitosan

Both chitin and chitosan are processed industrially in large scale from crustacean outer shell waste especially crab and shrimp shells. The process of chitin from crustacean shell waste consists of two main steps. The first step is protein separation and the second step is calcium carbonate separation [5]. Proteins are removed from ground shell by treating with a dilute aqueous sodium hydroxide solution. The residual material such as calcium carbonate is extracted with dilute with aqueous hydrochloric solution.

Deacetylation of chitin to chitosan is performed by treatment with hot concentrated sodium hydroxide solution. Figure 1.2 outlines a simplified flow diagram of chitin and chitosan manufacturing process [5].

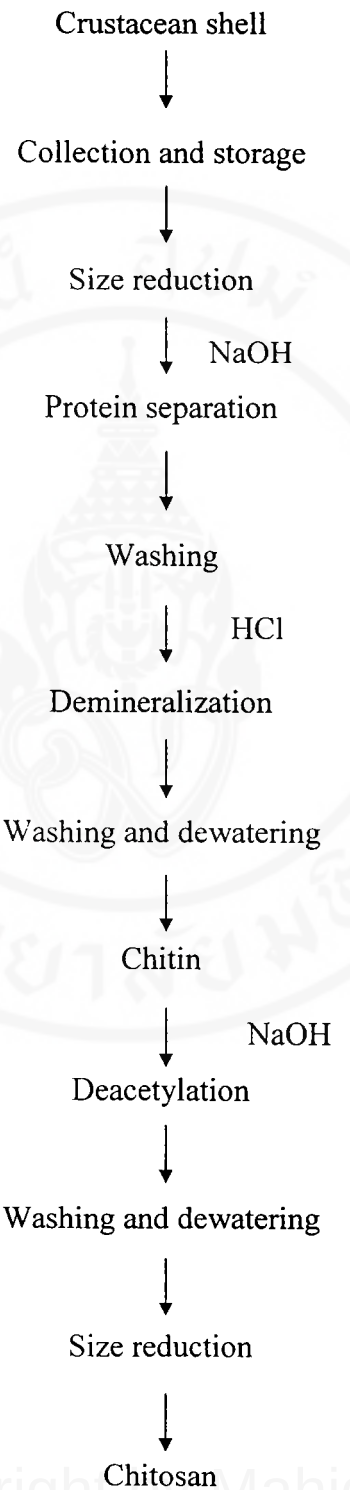


Figure 1.2 Simplified flow diagram of chitin and chitosan processing.

1.2.3 Properties of chitosan

1.2.3.1 Degree of deacetylation

Chitosan is less easily defined in terms of its exact chemical composition. It is a general term for a series of deacetylated chitins that have been sufficiently deacetylated to allow their solution in dilute aqueous acidic solution [5]. The difference between chitin and chitosan lies in the degree of deacetylation. General, the reaction of deacetylating chitin in an alkaline solution can not reach completion even under harsh treatment. The degree of deacetylation usually ranges from 70% to 95 %, depend on the method used. Most publications use the term chitosan when the degree of deacetylation is more than 70 %.

The degree of deacetylation is one of the more important chemical characteristics of chitosan. This determines the content of free amino groups in the polysaccharide. Many methods have been used to determine the degree of deacetylation of chitosan. These include infrared spectrometry (IR) [7-9], near infrared spectrometry [10], UV-spectrophotometry [11], first derivative ultraviolet spectrophotometry (1DUVS) [12], colloidal titration [13], linear potentiometric titration (LPT) [14], enzymatic determination [15], ^{1}H NMR[16], solid-state ^{13}C NMR [17], and ninhydrin test [18].

1.2.3.2 Molecular weight

The molecular weight of native is usually larger than one million while commercial chitosan products fall between 100,000 and 1,200,000. There are many methods for preparing low molecular weight of chitosan. For

example, obtained a colorless sample with an average molecular weight of only 60,000 by treating chitosan with 0.05 % ClO_2 [6].

Several methods such as gel permeation chromatography (GPC) [19], light scattering [20], and viscosity [21] can be used to determine the molecular weight of chitosan.

12.3.3 Solvent and solution properties

Chitosan is insoluble in water, alkali, and organic solvents, but soluble in most solutions of organic acid when the pH of the solution less than 6. The preparation of chitosan is usually designed to ensure that a product is completely soluble in organic acid [22]. Acetic acid and formic acid are two of the most widely used acids for dissolving chitosan. The solubility of chitosan with organic acid as solvent comes from the protonation of amino groups on chitosan as shown in Figure 1.3, leading to soluble quaternary ammonium salt.

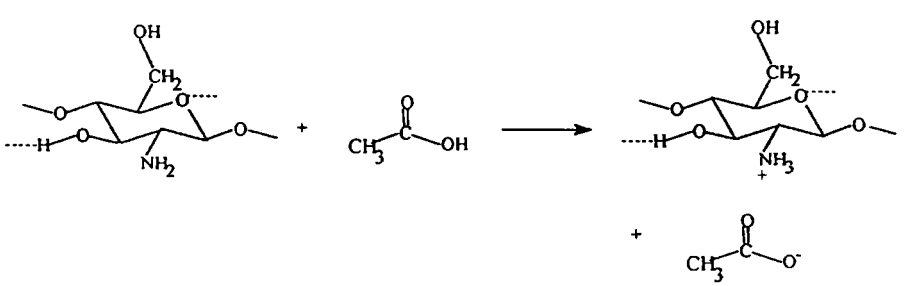


Figure 1.3 The solubility of chitosan in acidic acid

Some dilute inorganic acids such as nitric acid, perchloric acid, phosphoric acid, and hydrochloric acid can also be used to prepare chitosan solution, but only after prolonged stirring and warming.

1.2.3.4 Viscosity

The viscosity of chitosan in solution is influenced by many factors, such as the degree of polymer deacetylation, molecular weight, concentration, ionic strength, pH, and temperature [6]. In general, as the temperature rises, the viscosity of the polymer solution decreases. However, a pH change in the polymer solution may give different results depend on the type of acids employed. With acetic acid, the viscosity of chitosan tends to increase with decreasing pH while with hydrochloric acid, the viscosity decreases when the pH is lowered.

1.2.3.5 Coagulation ability and adsorption of metal ions

Chitosan is a good coagulating agent and flocculant due to the high density of amino groups, which can interact with negatively charged substances proteins, solids, dyes, and polymers. However, chitosan behaves quite differently with respect to transition metals such as iron, copper, cobalt, nickel [23], and zinc [24], including high toxic metal such as mercury [25] and lead [26]. The nitrogen in the amino group of the chitosan molecule acts as an electron donor and is presumably responsible for selective chelating with metal ions. The free amino group in chitosan was considered to be much more effective for binding metal ions than the acetyl groups in chitin [27]. This leads to consider that the higher free amino group content of chitosan should give higher metal ion adsorption rates. However, the adsorption

ability of chitosan is dependent on many other factors, such as crystallinity, deacetylation, and affinity for water [6].

1.2.4 Applications of chitosan

The industrial production and use of chitosan has been steadily increasing since the 1970s. At that time, the major applications of chitosan were centered on sludge dewatering, food processing, and metal ions chelation. The present trend, industrial applications, however, is toward producing high value products, such as cosmetics, drug carrier, feed additive, semipermeable membranes, and phamaceutics.

1.2.4.1 Water treatment

One of the easiliest applications of chitosan was for chelating metal from wastewater. Chitosan was a powerful chelating agent because of its high amino group content [5]. The nitrogen in amino group of chitosan molecule acts as an electron donor and is presumably responsible for chelating with metal ions such as copper, zinc, lead, mercury, and uranium [28].

1.2.4.2 Agriculture

Chitosan has many potential applications in agriculture because the polymer is essentially occurring and biodegradable; therefore, it should not cause pollution problems. One application that is widely employed at present is seed coating [29]. Chitosan treatment in coating seed had many beneficial, such as inhibition of

fungal pathogens in the vicinity of the seeds and enhancement of plant-resistant responses against diseases. Due to the increase in crop yield, this method has been accepted for wheat coating in eleven states in the United State.

1.2.4.3 Medical uses

Chitosan is being evaluated in number of medical applications such as wound dressing, artificial skin, accelerating bone formation [6], and delivering lower concentrations of drugs in the body and reducing side effects [30,31]. Chitosan is a good additive in the preparation of biomaterials such as employed contact lenses that are required to be optically clear, safe, wettable, and gas permeable. Several techniques for preparing contact lenses have been reported resulting in chitosan lenses and blue chitosan lenses [32].

1.2.4.4 Cosmetics

Applications of chitosan in cosmetic were reviewed by many reports [6,33]. It is being used as moisturizers, cleaners, bath lotion for increasing skin softness, hair and skin fixatives, and hair conditioners [33].

1.2.4.5 Food applications

Owing to the high chelating and coagulation ability of chitosan, the polymer has been widely utilized in the food industry. It is an effective coagulation agent flocculant especially in aiding the coagulation of protein from food process wastes [34]. In the case of beverages, chitosan was used to remove dyes from orange juice [35], solids, β-carotene, and acid substances from apple and carrot juice [36,37].

Chitosan was also used to extend the preservation time of foods because of its antibiotic properties [6].

1.2.4.6 Application of chitosan as dietary supplement

Nowadays chitosan capsules are produced and sold as dietary supplements for adsorption of fats and cholesterol. Chitosan is supposed to decrease adsorption of them in the stomach which is acidic condition before they have a chance to metabolized.

It has been reported in animal and clinical studies that chitosan ingestion effectively lowers serum cholesterol. In animal study [38], when chitosan was fed to male rats on a high cholesterol diet, the rise in plasma cholesterol and triglyceride were prevented. This cholesterol lowing effect was substantiated by that chitosan supplement (2-5%) to male rats fed a high cholesterol diet 20 days results in a significant reduction (25-30%) of plasma cholesterol concentration without influencing the amount of food intake or growth of the animals.

In clinical study [39], chitosan at a dose of 3-6 gram per day ingested as biscuits by eight adult healthy males for two weeks. The results induced a significant decrease in the total serum cholesterol (188 mg/dl to 177 mg/dl) and increase in serum HDL-cholesterol (51 mg/dl to 56 mg/dl).

1.3 Risk of using chitosan as dietary supplement

1.3.1 Growth retardation

When medium size chitosan particles were administrated to male rats as a 10% supplement in the diet, growth of the animal was retarded [38]. Moreover, ingestion of fine chitosan particles caused growth retardation at a lower dose of 2% supplement. Chitosan at a dose of 5 mg administered to mice induced a significant decrease in their body weights, associated with inactivity of the animals in the fifth week [40]. Administration of a casein diet with 5% chitosan supplement caused a significant decrease in their body weights. These results suggest that long-term oral intake of chitosan especially in high amounts may have deleterious effect on growth.

In young male albino rats fed a semipurified diet supplemented with 5% chitosan for 31 days, the food intake and body weight gain were lower than control and groups receiving other dietary fiber supplements including chitin [41]. These results suggest that among the dietary fibers tested, chitosan has most pronounced effect on the nutritional status of animals.

1.3.2 Fat-soluble vitamins and minerals

An important undesirable biochemical effect of chitosan treatment is the reduction in the absorption of minerals and fat-soluble vitamins (A, D, E, and K). In addition to the entrapment of lipids and cholesterol, chitosan gels in the intestines also bind fat-soluble vitamins and minerals. Chitosan administered with sodium ascorbate to male rats fed a high fat diet for two weeks results in a significant decrease in mineral adsorption associated with a reduction in bone mineral content [42]. An associated finding is a marked decrease in serum vitamin E level. Calcium absorption is also reduced due to the entrapment by the chitosan gels of calcium and probably of vitamin D. High calcium supplement can prevent the decrease in the bone mineral content observed in chitosan-treated animals. Dietary chitosan may also effect calcium metabolism in animals [43]. This hypothesis was significantly based on the finding that whole-body retention of ^{47}Ca by rats on a 5% chitosan diet was significantly decreased due to accelerated urinary excretion of the radiolabeled Ca.

Short-term ingestion of chitin or chitosan appears not affect zinc absorption. Zinc absorption in young male rats fed a semipurified diet 5% chitin or chitosan supplement for 31 days remained unchanged [41]. Zinc content in the tibia and femur of the dietary fiber-fed animals tends to be lower than that of the control group, but the differences are not statically significant. Because of this apparent lowering content, zinc absorption and its bone content should be determined after prolonged ingestion.

1.4 Adsorption isotherm and adsorption capacity

The process of adsorption involves separation of a substance from one phase accompanied by its accumulation or concentration at the surface of another. The adsorbing phase is the adsorbent, and the material concentrated or adsorbed at the surface of that phase is the adsorbate.

The adsorption of a substance from one phase to the surface of another in a specific system leads to a thermodynamically defined distribution of that substance between the phases when the system reaches equilibrium; that is, when no further net adsorption occurs. The common manner in which to depicte this distribution is to express the amount of substance adsorbed per unit weight of adsorbent, Qe, as a function of the residual equilibrium concentration, Ce, of substance remaining in the solution phase. An expression of this type, termed an adsorption isotherm, defines the functional equilibrium distribution of adsorption with concentration of adsorbate in solution at constant temperature. Commonly the amount of adsorbed material per unit weight of adsorbent increases with increasing concentration, but not direct proportion [44].

Experimental isotherm are useful for describing adsorption capacity to facilitate evaluation of feasibility of this process for a given application, for selection of the most appropriate adsorbent, and for preliminary determination of adsorbent dosage requirements. Moreover, the isotherm plays a crucial functional role in predictive modeling procedures for analysis and design of adsorption systems. Langmuir model has been commonly described adsorption isotherm relationships.

Langmuir I. proposed a theory to describe the adsorption of gas molecules onto metal surfaces in 1918 [45]. The Langmuir isotherm model has been successfully applied to many adsorption processes, including the adsorption of aqueous on solid phase. A basic assumption of the Langmuir theory is the adsorption takes place at specific sites within the adsorbent. It is then assumed that once adsorbate molecule occupies a site, no further adsorption can take place at that site. Theoretically, therefore, a saturation value is reached beyond which no further adsorption can take place. The saturation at monolayer can then be represented by the expression:

$$Q_e = \frac{X_m K C_e}{1 + K C_e} \qquad (1.1)$$

where, C_e is the equilibrium concentration (mg L^{-1}) and Q_e is the amount of metal adsorbed at equilibrium (mg g^{-1}). X_m is the maximum amount of metal adsorbed per gram of adsorbent or the adsorption capacity. K (mg^{-1} L) is a constant related to the energy of adsorption (K = rate constant of adsorption/rate constant of desorption).

Equation 1.1 can be transformed into a linear form equation 1.2.

$$\frac{C_e}{Q_e} = \frac{1}{X_m K} + \frac{C_e}{X_m} \qquad (1.2)$$

X_m and 1/K can be determined from the slopes and intercepts of the linear plots of C_e/Q_e versus C_e.

Besides the method of transformation of non-linear equation to linear, there have been some computer programs for non-linear fitting. Those could approximately be used to determine the adsorption capacity (X_m) and the K constant.

In this work, the adsorption capacities of Cu(II) and Zn(II) in 0.01 M HCl by chitosans can be determined by Langmuir isotherm. As described in Section 1.2.3.5, chitosan is a well-known adsorbent for adsorption of transition metals such as Fe^{2+}, Co^{2+}, Ni^{2+}, Hg^{2+}, and Pb^{2+} including Cu^{2+} and Zn^{2+}. The amine group ($-NH_2$) on chitosan can serve as coordination site for metal adsorption. The adsorption of Cu(II) and Zn(II) on chitosan of adsorption at the monolayer at equilibrium can be represented by Langmuir adsorption isotherm (equation 1.2). In this work, the adsorption capacities of Cu(II) and Zn(II) on chitosans were obtained by using ENZFITTER program.

1.5 Aim of this work

Nowadays chitosan capsules are produced and sold as dietary supplement for weight control. Chitosan is taken to decrease the absorption of fats and cholesterol before they are absorbed into the body. However, chitosan and some derivatives have also been reported of their capacities in adsorption of metal ions including the ions of essential minerals such as Cu(II) and Zn(II). Therefore there might be some possibilities that people who usually use chitosan for weight control may be lack of these two essential minerals as well as other minerals.

In this work, adsorption capacities of Cu(II) and Zn(II) in 0.01 M hydrochloric acid solution by three chitosans, namely chitosan S, chitosan L, and chitosan M, were studied. Adsorption of Cu(II) and Zn(II) on these chitosans in binary system were also studied.

The information of adsorption capacity of chitosan supplement will be used to predict the possibility of interference of chitosan in *gastric absorption* of copper and zinc ion in gastric condition.

CHAPTER II

EXPERIMENTAL

Three chitosan samples were used in these studies. Chitosan S was obtained from shrimp shells and was supplied by a local producer in Thailand. Chitosan L and chitosan M are commercial chitosans used as dietary supplements. They were obtained from a local supermarket. In this chapter, a description of procedures and experimental techniques are presented. General procedures of preparation of reagents and method of characterization of chitosan are given.

2.1 Instrumentation

2.1.1 Flame atomic absorption spectrometer (FAAS)

All the FAAS measurements of this study were obtained using a Perkin-Elmer atomic absorption spectrometer Model 3100, (Norwalk, CT, USA) with Deuterium-lamp background correction system. The operating conditions for copper and zinc determination are presented in Table 2.1

Table 2.1 Operating conditions of FAAS for determination of Cu and Zn

Element	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Acetylene : Air
Cu	324.8	0.7	5	2 : 4
Zn	213.9	0.7	6	2 : 4

2.1.2 Electrothermal atomic absorption spectrometer (ETAAS)

ETAAS measurements in this study were used for determination of Cu(II) concentrations. The measurements were obtained using a Perkin-Elmer atomic absorption spectrometer Model 3100 equipped with a deuterium-arc background corrector and HGA-600 heated graphite atomizer. The sample was introduced by AS-60 autosampler. The cooling system HGA was also used to cool down the temperature of the atomizer. Pyrolytic graphite- coated graphite tubes were use throughout. The radiation source was a copper hollow cathode lamp operated at 5 mA, and the 324.8 nm wavelength was monitored. The spectral bandwidth used was 0.7 nm. A Compaq 3/25S computer with an EPSON LQ-860$^+$ printer were used in the system control and the data analysis.

2.1.3 UV-Vis spectrophotometer

Two UV-Vis spectrophotometers were used in this work. A Jasco UVIDEC-650 (Japan) was used for determination of degree of deacetylation of chitosan by first derivative ultraviolet spectrophotometry (1DVUS) technique.

Another spectrophotometer was a Jenway, Model 6405 (England) which was used for determination of degree of deacetylation of chitosan by ninhydrin test.

2.1.4 Shaking incubator

A gyratory shaker, Model G-25 (New Jersey, USA), was used as shaking incubator. Temperature for incubation can be controlled at 37 ± 2 °C.

2.1.5 Fourier transform infrared (FT-IR) spectrometer

A Perkin-Elmer FT-IR spectrometer Model system 2000 was used for determination of degree of deacetylation of chitosan. FT-IR spectra in the region 4,000 to 370 cm^{-1} were typically an average of 16 scans with 1 cm^{-1} resolution.

2.1.6 Other equipment

a) Analytical balance

A Precisa 40 SM-200A balance (Zurich, Switzerland) with ± 0.1 mg precision was used.

b) Centrifuge

Hettich Universal II centrifuge, Model D 7200 (Germany), was used to separate chitosan from the supernatant after the incubation.

c) Sieves

Endoecotts Ltd. (London, England), test sieves with the apertures of 45, 125, 300, and 425 μm were employed to separate chitosan sample into groups of particle size range.

d) pH measurement

Fisher Scientific Model 955 pH meter with a combination glass electrode (USA) was used for all pH measurements. Commercial standard buffers, pH 4.01 and 7.00 from E. Merck (Darmstadt, Germany) were employed for the instrument calibration.

2.2 Chemical and reagents

2.2.1 Chemicals

All solutions were prepared using deionized distilled water obtained from a Milli-Q system (Milliford, Massachusetts, USA). All chemicals and reagents were analytical grade and were obtained from various sources. The list of manufacturers is in Table 2.2

Table 2.2 List of chemicals

Chemicals	Formula	Supplier
Cupric chloride dihydrate	$CuCl_2\,2H_2O$	Fluka (Buchs, Switzerland)
Zinc chloride	$ZnCl_2$	Merck (Darmstadt, Germany)
Silver nitrate	$AgNO_3$	Carlo Elba (Val de Reuil, France)
Hydrochloric acid (37%)	HCl	Merck (Darmstadt, Germany)
Nitric acid (70.5%)	HNO_3	J. T. Baker (Philipsburg, USA)
Acetic acid (99.9%)	CH_3COOH	J. T. Baker (Philipsburg, USA)
Ninhydrin	$C_9H_6O_4$	Merck (Darmstadt, Germany)
N-acetyl-*D*-glucosamine	$C_8H_{15}NO_6$	Sigma-Aldrich (Steinheim, Germany)
D-glucosamine hydrochloride	$C_6H_{14}ClNO_5$	Merck (Darmstadt, Germany)
Stannous (II) chloride	$SnCl_2\,2H_2O$	Merck (Darmstadt, Germany)
Ethylene glycol monomethyl ether	$C_3H_8O_2$	Merck (Darmstadt, Germany)

2.2.2 Preparation of standard solutions and reagents

2.2.2.1 Hydrochloric acid solution (1.0 M)

15.6 mL of concentrated hydrochloric acid (37%) was dissolved in deionized distilled water and was diluted to 250 mL to make 1.0 M HCl. This solution was diluted to the concentration of 0.01 M hydrochloric acid which was used for all adsorption experiments.

2.2.2.2 Copper chloride solution (10,000 mgL^{-1})

13.42 g of $CuCl_2 \cdot 2H_2O$ was weighed and dissolved with 0.01 M HCl in a volumetric flask to 500.0 mL. This primary stock solution of copper (II) chloride was used in the preparation of standard copper(II) solutions throughout the work. Appropriate dilution was carried out using the same acid, that is 0.01 M HCl.

2.2.2.3 Zinc chloride solution (10,000 mgL^{-1})

10.43 g of $ZnCl_2$ was weighed and dissolved with 0.01 M HCl in a volumetric flask to 500.0 mL. This primary stock solution of zinc(II) chloride was used in the preparation of standard zinc(II) solutions throughout the work. Appropriate dilution was carried out using the same acid, that is 0.01 M HCl.

2.2.2.4 Ninhydrin reagent

2.0 g of ninhydrin was accurately weighed and dissolved in 50.0 mL of ethylene glycol monomethyl ether, then 25.0 mL of 4.0 M sodium acetate buffer (pH 5.5) and 0.08 g of stannous (II) chloride dihydrate were added to the solution, respectively. This solution was made up to 100.0 mL with deionized distilled water in volumetric flask. Only freshly prepared ninhydrin reagent should be used for determination of degree of deacetylation of chitosan.

2.3 Measurement of some characteristic properties of chitosans

2.3.1 Composition of chitosan samples

Three chitosan samples were used in these studies. A sample named as chitosan S was obtained from shrimp shells and was supplied by a local producer in Thailand. The manufacturer has claimed for the sample being a pure and non-derivatized form.

The other two samples, which are chitosan L and chitosan M, are commercially available in supermarket. These samples were used as dietary supplements. Chemical composition of chitosan L and chitosan M on the package labels are summarized in Table 2.3.

Table 2.3 Chemical composition of chitosan L and chitosan M.

Sample	Chemical composition (%w/w)				
	Chitosan	Fillers			
		Ascorbic acid	Carbohydrate	Fiber	Unknown
Chitosan L	67.5	5.0	-	-	27.5
Chitosan M	49.0	-	32.6	18.4	-

According to the supplier's labels, chitosan L was obtained from crab shells and chitosan M was obtained from crab and shrimp shells.

2.3.2 Particle size distribution

Particle size distribution of chitosans was obtained by sieving the chitosan samples through Endicott, sieves with the apertures range from a 45 to 425 μm. A Retsch 5657 shaker was used for this size separation. Percentages of size distribution were calculated based on the weight collected for each size range. The results are shown in Figure 3.1, Section 3.1.1.

2.3.3 Molecular weight

Molecular weights of chitosans were determined by gel permeation chromatograph (GPC), Model PL-110 (Polymer Laboratory Ltd.), using an Ultralinear hydrogel column with a reflective index (RI) detector. An aqueous solution consisted of 0.50 M acetic acid and 0.50 M sodium acetate (1:1 v/v) was used as the eluent.

Three chitosan samples, chitosan S, chitosan L and chitosan M were dissolved in the above eluent and filtered through 0.45 μm Nylon filter. Pullulan standards (polysaccharide) range of $5.80 \times 10^3 - 1.66 \times 10^6$ Dalton were used for the calibration in this work. The operating conditions using injection volume 100 μL, flow rate 0.6 mL min^{-1}, and temperature was set at 30 °C.

The experiment was carried out by an official operator at the National Metal and Materials Technology Center (MTEC). The results are tabulated in Table 3.1, Section 3.1.2.

2.3.4 Degree of deacetylation

Four techniques were used to determine the degree of deacetylation of chitosan samples: ninhydrin test, first derivative ultraviolet spectrophotometry (1DUVS), IR, and solid-state ^{13}C NMR. Degree of deacetylation is presented as the percentage of deacetylation. The calculation could be base on a mole basis or base on the measure of signal.

2.3.4.1 By ninhydrin test

Ninhydrin test is the method which estimates the amount of chitosan by direct detection of the $-NH_2$ group on the glycoside repeat unit of chitosan. This method was proposed by Curotto E. et al. [18].

Standard solutions were prepared by pipetting 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mL of *D*-glucosamine solution (100 mgL^{-1} in 2 % acetic acid) into separate volumetric flasks. Acetic/acetate buffer (1.5 mL, pH 5.5, 4.0 M) and 6.0 mL of ninhydrin reagent were added to each volumetric flask, respectively. Finally, the volume was adjusted to 10.0 mL with 2 % acetic acid. The solution was transferred to test tube and heated in a boiling water bath for 10 minutes before recording the UV absorbance at 570 nm of each solution. The calibration curve was obtained by plotting the absorbance against the concentration of standard solutions, as shown in Figure 3.3, Section 3.1.3.1

Three chitosan samples that were washed by deionized distilled water as described in Appendix II were accurately weighed approximately 0.01 g and added with acetic acid (2% v/v) 20 mL. The mixture was heated until chitosan sample was dissolved. The chitosan solution was cooled down and made up to 100.0 mL with

2% (v/v) acetic acid in volumetric flask. The concentration of chitosan solutions was 100 mgL^{-1}.

Sample solutions consisted of 2.0 mL of chitosan solution (100 mgL^{-1} in 2 % acetic acid) to which was added the buffer, ninhydrin reagent, and topped up to 10.0 mL with 2% (v/v) acetic acid. The solution was transferred to test tube and heated as discussed above. The absorbance value at 570 nm was recorded. The amount of chitosan in the sample was used to estimate the degree of deacetylation by calculation based upon the equation 3.1 (derivation of this equation is in Appendix III), Section 3.1.3.1. Triplicate was performed for each sample.

2.3.4.2 By first derivative ultraviolet spectrophotometry (1DUVS)

The degree of acetylation of chitosan can be determined in solution of chitosan in acetic acid by first derivative ultraviolet spectrophotometry at a wavelength where zero crossing point (ZCP) taken place. At this wavelength, the *N*-acetyl-*D*-glucosamine (GlcNAc) absorbance readings are linearly dependent on concentration and are not influenced by the presence of acetic acid (0.1 to 0.3 M). This method was first proposed by Muzzarelli R.A.A. et al. in 1985 [12].

In this experiment, absorption spectra (zero order and first derivative) were obtained by using a Jasco UVIDEC-650 UV-Vis spectrophotometer (Japan). The zero crossing point (ZCP) was determined by superimposing the first derivative spectra of 0.01, 0.02 and 0.03 M of acetic acid solution.

I. Calibration curve

Solutions of 5.0-40.0 mg L^{-1} *N*-acetyl-*D*-glucosamine in 0.01 M acetic acid solution, or so-called GlcNAc, were prepared. These solutions were scanned using to spectrophotometer to obtain their first derivative spectra. The vertical distance from ZCP to each GlcNAc solution spectrum, H_1, was measured (mm). A linear calibration curve was obtained by plotting the H_1 values against the corresponding GlcNAc concentrations as shown in Figure 3.5, Section 3.1.3.2.

II. Correction of the effect of D-glucosamine on H values

As proposed by Muzzarelli R.A.A. et al. [12], the presence of *D*-glucosamine (GlcN) may give rise to a large *H* value for GlcNAc than expected. Therefore, a reference curve for correcting this discrepancy was necessary. A fixed concentration of 10.0 mgL^{-1} GlcNAc in 0.01M acetic acid solution was prepared and various concentrations of GlcN from 0 to 200 mg L^{-1} was added to the GlcNAc solution to give a series of different percentages of GlcNAc solutions (w/w). The percentages of GlcNAc were 100, 50, 33, 28, 25, 20, 16.7, 12.5, 10, 9.1, 6.3, and 4.8 %(w/w). The *H* values of the pure GlcNAc solution, H_1, and the *H* values of the solutions of different percentages of GlcNAc, H_2, were measured. The reference curve was obtained by plotting H_1/H_2 against the corresponding GlcNAc percentage as shown in Figure 3.6, Section 3.1.3.2.

III. Determination of the degree of deacetylation of chitosan samples.

Three chitosan samples that were washed as described in Appendix II, were accurately weighed approximately 0.01 g. Aliquots of 10.0 mL of 0.10 M acetic acid was added to the chitosan samples. The mixture was heated until chitosan sample dissolved, then the chitosan solutions were cool down and made up to 100.0 mL with deionized distilled water. Triplicates were performed for each sample. The *H* values of the chitosan samples, H_2, were measured and the contribution due to GlcNAc was obtained from the calibration curve. The degree of deacetylation of the samples were determined by the equation 3.4 (derivation of this equation is in Appendix III), Section 3.1.3.2.

2.3.4.3 By FT-IR spectroscopic technique

Domszy J.G. et al. [7] proposed the determination of the degree of deacetylation of chitosan by infrared spectroscopic technique.

The chitosan samples were previously washed as described in Appendix II, Approximately 2 mg of chitosan was milled with 150 mg of KBr in agate motar until the mixture was fine. The mixture was placed between the bottom die and the top die together with adjustment of the surface until smooth. The mixture was compressed with hydraulic pressure about 7 tons for 10 minutes. The disc was placed in the disc holder for further analysis by FT-IR spectrometry. Duplicate experiments were carried out for each chitosan.

The percentage of free amine groups was determined by a linear relationship between the amide I band at 1655 cm^{-1} and hydroxyl band (internal standard) at 3450 cm^{-1} as shown in the equation 3.5, Section 3.1.3.3.

The absorption band at 3450 cm^{-1} was determined from 1902 to 3837 cm^{-1} as an internal standard. The absorbance of the amide I band at 1655 cm^{-1} was calculated from 1277 to 1902 cm^{-1}. The ratio was 1.33, using the baselines indicated in Figure 2.1 of fully *N*-acetylated chitosan (A_{1655}) and the area within 1902 to 3837 cm^{-1} of the hydroxyl band (A_{3450}) [7].

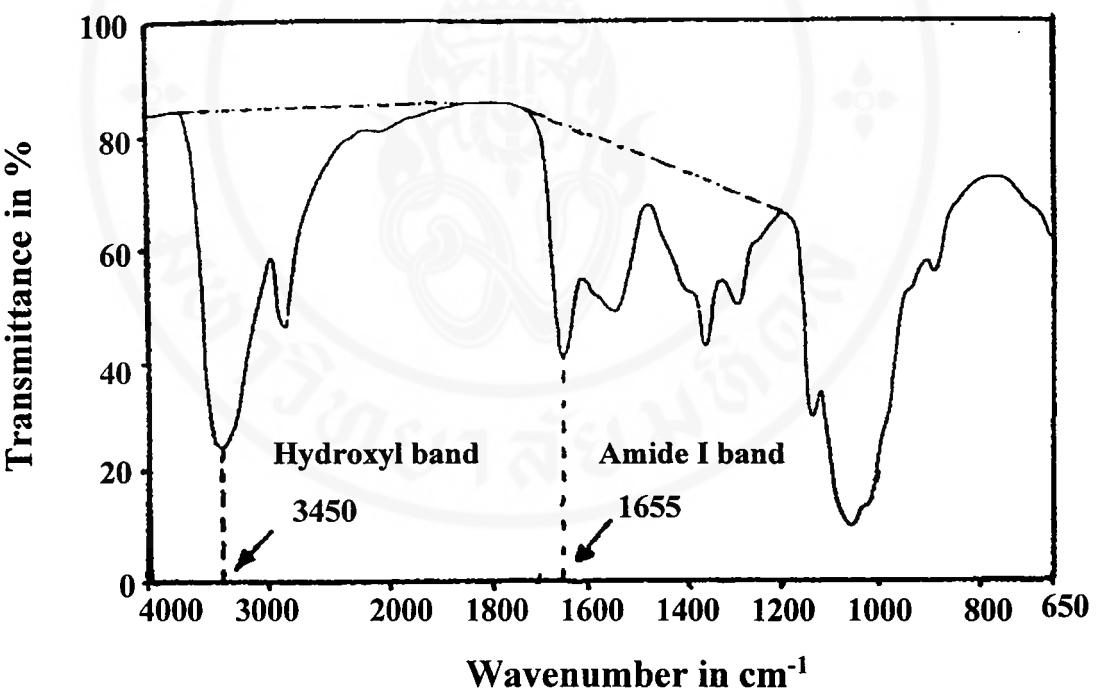


Figure 2.1 IR spectrum of partially *N*-acetylated chitosan showing the baselines used in determining the areas of the absorption bands A_{1655} and A_{3450} for the 1655 cm^{-1} and 3450 cm^{-1} bands.

2.3.4.4 By solid-state ^{13}C NMR technique

Solid-state ^{13}C NMR spectra were recorded at 300 MHz on a Bruker NMR spectrometer (Germany). The experiment was carried out by an official operator at the National Metal and Materials Technology Center (MTEC). Approximately 50 milligrams of solid chitosan samples, that were washed as described in Appendix II, were inserted into a 5 mm rotor before measuring the carbon resonances from 0 to 200 ppm chemical shift.

The degree of deacetylation of chitosan was calculated from solid-state ^{13}C NMR [17] data by comparing the area of the CH_3 resonance to the resonance of the glucose carbons as shown in Figure 2.2. Integration 1 corresponds to the glucose carbon atoms where integration 2 corresponds to the methyl group, which is proportional to the acetyl content. The percentage of deacetylation of chitosan was calculated using the equation 3.6, Section 3.1.3.4

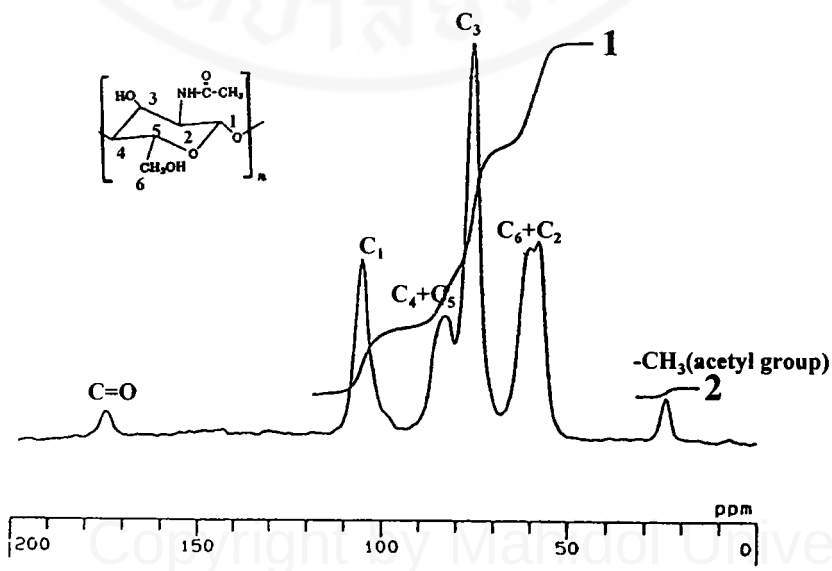


Figure 2.2 Solid-state ^{13}C NMR spectrum of partially *N*-acetylated chitosan.

2.4 Kinetic studies

Kinetic of adsorptions of Cu(II) and Zn (II) were studied on three chitosans. The purpose of the kinetic experiments was to determine the time required for each adsorption system to reach equilibrium.

To investigate the adsorption kinetics of Cu(II) on three chitosans, a fixed concentration of 100 mgL^{-1} of Cu(II) was used throughout this study. For the kinetics study of adsorption of Zn(II) on the same samples, a fixed concentration of 200 mgL^{-1} of Zn(II) were used.

The kinetic experiments were carried out in 125 mL stoppered conical flasks, each containing 0.1 g of accurately weighed chitosan and 25.0 mL of the metal solution prepared in 0.01 M HCl. The mixtures were incubated at 37 °C using shaking incubator. At various intervals within four hours period, the mixtures were centrifuged in PTFE tubes. The supernatant was then separated form chitosans and analyzed for residual metal concentration using standard addition FAAS method.

Removal of a metal ion (M^{2+}) or the adsorptivity was calculated based on milligram of the metal adsorbed per gram chitosan. The pH of the supernatant was measured using a glass electrode and a pH meter.

2.5 Isotherm Studies

Each isotherm was obtained by addition of an accurately weighed 0.1 g of chitosan to a 125 mL stoppered conical flask, containing 25.0 mL of either Cu(II) or Zn(II) in 0.01 M HCl solutions. Initial metal concentrations (Ci) were varied. Ci of Cu(II) was approximately from 0.5 to 1200 mgL^{-1}. Ci of Zn(II) was approximately from 5 to 800 mgL^{-1}.

The adsorbent and metal solution in each flask was incubated at 37 °C for two hours using shaking incubator. This contact time is similar to the time spent for food under gastric condition. After two hours of contact, the supernatants were separated from chitosans by centrifugation at 5,000 rounds per minute for 10 minutes. Residual metal concentration in the supernatant (Ce) was determined by standard addition FAAS method. The residual concentration of Cu(II) were sometimes determined by ETAAS when the concentrations are lower than the detection limit of the FAAS method. From the residual concentration, values of adsorptivity (milligram of metal ion adsorbed per gram chitosan) or Qe could be calculated.

Isotherm of adsorption of Cu(II) and Zn(II) on chitosans were constructed by plotting the Qe values against Ce values. Figure 3.10 and Figure 3.11, Section 3.3.1 were the plots obtained.

2.6 Adsorption of copper(II) and zinc(II) ions on chitosans in binary system

Effect of competing cation on metal adsorption in 0.01 M HCl solution was studied in a binary system of Cu(II) and Zn(II). Adsorption of a metal on chitosans was measured when the other metal was added to the system.

Determination of adsorptivities of Cu(II) and Zn(II) were carried out in similar ways to the experiment described for isotherm studies in Section 2.5. When Zn(II) was considered as the competing cation in the binary system, the concentration of Cu(II) in equilibrating solutions was fixed constant, whereas Zn(II) concentration was varied. Similarly, the concentration of Zn(II) was fixed constant, when Cu(II) considered as the competing cation was varied.

Chitchumroonchokchai C. [3] had studied daily intake of copper and zinc in Thai nutrition. The value of daily intake was used for estimation of milligram metal intake per meal. The maximum intakes of copper and zinc, when calculated per meal, are approximately 1.34 mg Cu/meal and 6.63 mg Zn/meal, respectively. From this information, the adsorption experiment was designed for the binary system to contain different numbers of mole of the competing metal from 0 to 10 or 20 while the number of mole of a metal of interest was fixed constant at 1. The fixed amounts of metals of interest were designed to be equal to the meal intakes of Cu(II) and Zn(II) at 1.34 mg Cu (or 0.021 mmol Cu) and at 6.63 mg Zn (or 0.101 mmol Zn). Supposing one gram of chitosan (one gram is the recommended meal dose for chitosan L and chitosan M) is taken each meal, the following intake loads should be considered: 1.34

mg Cu/g and 6.63 mg Zn/g. Hence, in to the experimental design, the values of intake load were fixed whereas the concentration of the competing species were varied to give different loading of the competing cation on one gram chitosan (details have shown in Appendix VIII). The results are shown in section 3.4.

CHAPTER III

RESULTS AND DISCUSSION

3.1 Characterization of chitosans

Three chitosan samples were used in this work, namely chitosan S, chitosan L and chitosan M. Chitosan S is made from shrimp shells and was supplied by a local factory in Thailand. Chitosan L and chitosan M were obtained from a local supermarket. These two samples are sold as dietary supplements for reducing gastric adsorption of fat and cholesterol. Amongst the three, chitosan S is claimed by the manufacturer for being a pure and a non-derivatized sample. Chitosan L and chitosan M contain some fillers such as ascorbic acid and starch, respectively (see Table 2.3, Section 2.3.1 for details). As well as measuring the adsorption capacities of these samples for Cu(II) and Zn(II), the following characteristic properties of chitosan samples were also determined.

3.1.1 Particle size distribution

Particle size distributions of three chitosans were studied as described in Section 2.3.2. The size distributions are shown in Figure 3.1. According to the operation, the samples were sieved as received. Thus the results represent the distribution of size range of the product, including chitosan and fillers. Figure 3.1 indicates that chitosan S consists mainly of the size greater than 425 μm. Particle size

of chitosan L lies mainly in 125-300 μm. Chitosan M has a wide range of size distribution over the standard sieves used from < 45 μm to 300 μm.

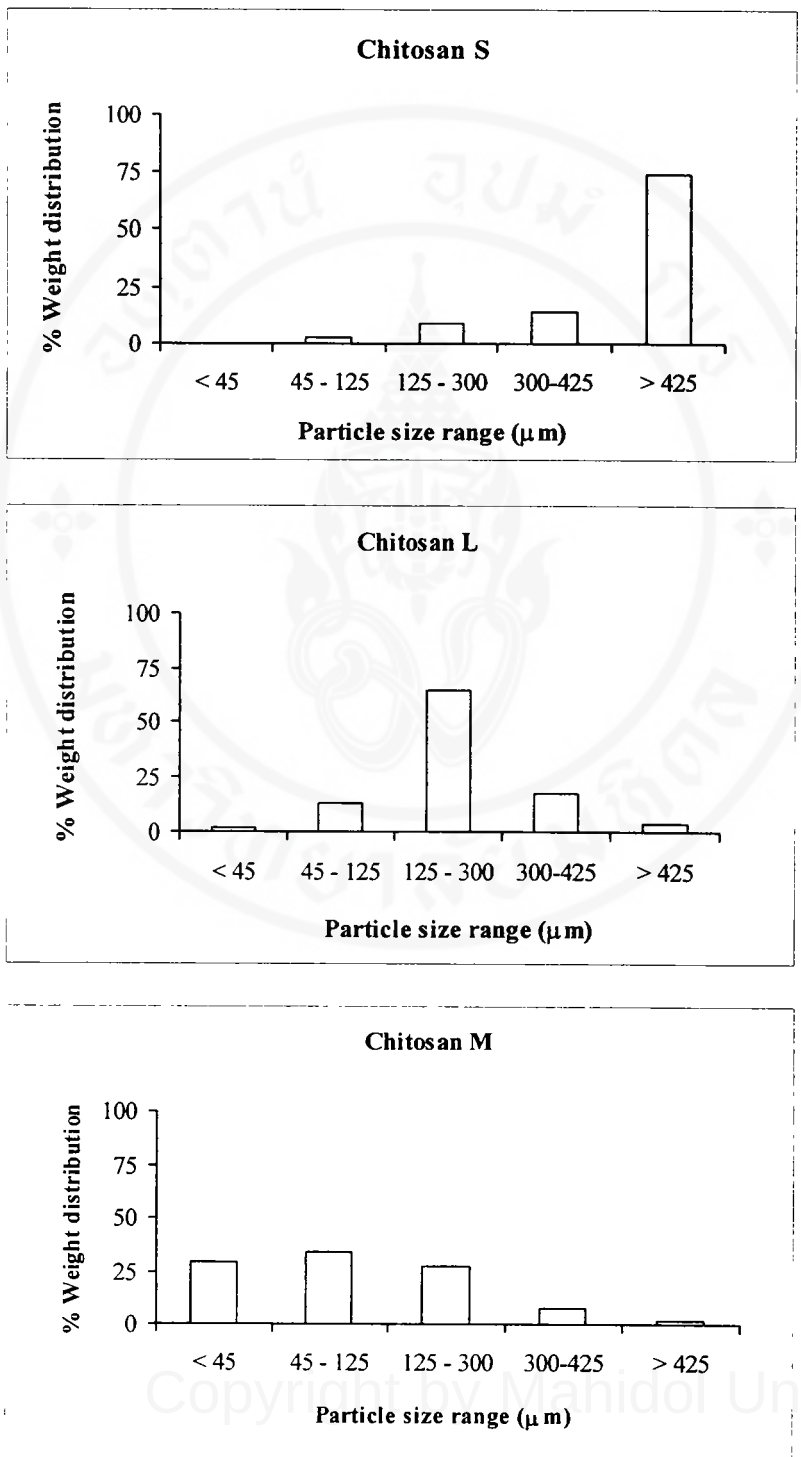


Figure 3.1 Particle size distribution of three chitosan samples.

3.1.2 Determination of molecular weight

Molecular weight of commercial chitosans usually lies between 100,000 and 1,200,000. Some methods such as light scattering [20] and viscosity [21] can be used to determine the molecular weight of chitosan. In this work, gel permeation chromatography (GPC) was used to determine molecular weight of chitosans. The work was carried out with compliment by the National Metal and Materials Technology Center (MTEC). The details of the operating condition are described in Section 2.3.3.

Gel permeation chromatography (GPC) or size exclusion chromatography (SEC) has been developed into one of the most useful methods for routine determination of average molecular weights and molecular weight distribution of polymers. GPC is a form of liquid chromatography in which the molecules are separated according to their molecular size. The procedure involves injecting a dilute solution of a polydisperse polymer into a continuous flow of solvent passing through a column containing tightly packed microporous gel particles. Separation of the molecules occurs by preferential penetration of different size of molecules into the pores; small molecules are able to penetrate more easily through the pores compared to the larger molecules so that their rate of passage through the column is correspondingly slower. The continuous flow of solvent leads to separation of the molecules according to size with the larger molecules being eluted first followed by the smaller molecules, which have penetrated more deeply into the pores, and thus requires longer elution times. It follows that the time or, more usually volume of elution (V_e), is inversely proportional to the molecular size. If the pore size is too

small to permit penetration by any of the molecules, or if the pore size is so large that all of the molecules can penetrate by with the same relative ease, there would be little or no separation of the molecules [46].

Although GPC separates molecules according to their molecular size, with the result being presented as a size distribution curve, the technique does not give absolute values of molecular weight and there is a need to calibrate with polymer standards of known molecular weight. This is one of the major limitations of the technique since only a limited number of polymer standards are available. Polystyrene standards are most commonly used but Pullulan standards (polysaccharide) were used for the calibration in this work. Pullulan standards were dissolved in 0.50 M acetic acid and 0.50 M sodium acetate (1:1 v/v). These standards provided molecular weight range of $5.80 \times 10^3 - 1.66 \times 10^6$ Dalton. The calibration curve was the plot between the logarithm of molecular weight of Pullulan (Log Mp) against the retention time (R_T). Figure 3.2 shows the calibration plot obtained.

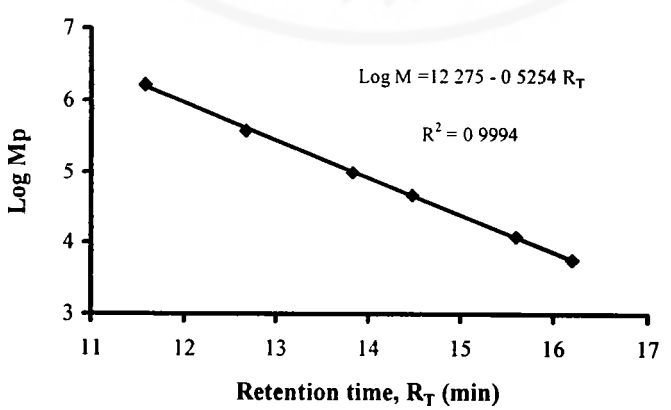


Figure 3.2 The calibration curve of Pullulan standards obtained from the gel permeation chromatography. The standard ranged from 5.80×10^3 to 16.6×10^5 Dalton.

The samples that are chitosan S, chitosan L and chitosan M were dissolved in 0.50 M acetic acid and 0.50 M sodium acetate (1:1 v/v). The liquid samples were duplicately injected into the GPC system (Section 2.3.3). Chromatograms of a Pullulan standard and chitosan samples are shown in Appendix I. The average retention time of a samples was used to calculate the average molecular weight specific to GPC (GPC molecular weight), $\overline{Mp}$, from the calibration equation. The results of the three samples are shown in Table 3.1.

Table 3.1 Average GPC molecular weights ($\overline{Mp}$) of three chitosan samples determined by gel permeation chromatography.

Sample	Retention time (minutes)		Average molecular weight, $\overline{Mp}$ ($\times 10^5$) (n =2)
	No. 1	No. 2	
Chitosan S	12.97	12.82	3.19 ± 0.41
Chitosan L	13.02	12.97	2.82 ± 0.12
Chitosan M	13.34	13.30	1.89 ± 0.05

The results in Table 3.1 indicate that the order of average molecular weight is chitosan S > chitosan L > chitosan M.

3.1.3 Degree of deacetylation

Degree of deacetylation is a measure of percentage of deacetylation. At that site where deacetylation has taken place, there is free amino group left in the polysaccharide repeat unit. Different degree of deacetylation of chitin gives chitosan with different chemical and physical properties. The amount of free amino group may govern the adsorption capacity of a sample for metal ions. In this work, it was therefore necessary to determine the degree of deacetylation of chitosan.

As described in the Section 1.2.3.1, several methods such as infrared spectrophotometry (IR) [7-9], first derivative ultraviolet spectrophotometry (1DUVS) [12], ^{1}H NMR) [16], solid-state ^{13}C NMR [17], and ninhydrin test [18] have been used to determine the degree of deacetylation of chitosan. Each of these methods has its own advantages and disadvantages. Up to date there is still no standard method for the determination of degree of deacetylation of chitosan yet. In this work, the three chitosan samples that were washed for elimination fillers (described in Appendix II), were analyzed by the four techniques. This work is a co-operative work carried out together with Chantore W. [47]. The operational procedures are described in Section 2.3.4. The ninhydrin test and the first derivative ultraviolet spectrophotometry were used to calculate the degree of deacetylation on a per mole basis. The degrees are presented as percentage of deacetylation (%deacetylation). Percentage of deacetylation obtained by the other two techniques, IR spectrophotometry and solid-state ^{13}C NMR, were calculated using sizes of signal of the indicating signal and the signal of an internal standard. Results of the degree of deacetylation obtained using the four techniques are summarized in Table 3.2. The results show that percentages of deacetylation obtained by ninhydrin test were much lower than the results obtained

from the first derivative ultraviolet spectrophotometry, although calculations were similarly made based on per mole basis. This finding agree with the work reported by Khor E. et al. [48]. Results of the IR spectrophotometry correspond to the results of the solid-state ^{13}C NMR. Description of results of each method are as followings.

3.1.3.1 By ninhydrin test [18]

Ninhydrin test is the method, which estimates the amount of $-NH_2$ group on the glycoside repeat unit of chitosan. The reaction of ninhydrin with a primary amino group will form a colored reaction product, diketohydrindylidene-diketohydrindamine, also called Ruhemann's purple [49]. This reaction has been extensively used for amino acid analysis. The procedure of this method is described in Section 2.3.4.1. The calibration curve plotted between concentration of *D*-glucosamine and the absorbance of the purple product is shown in Figure 3.3

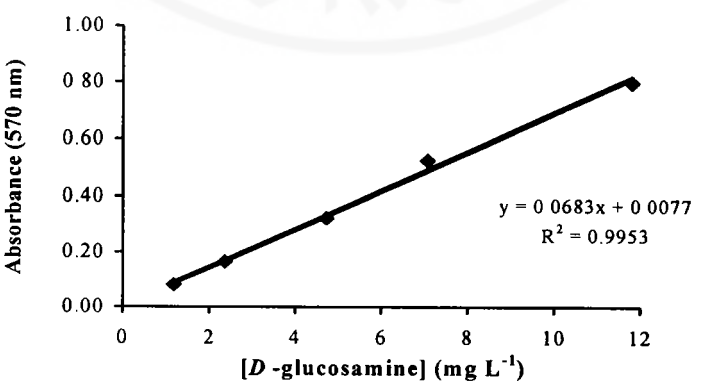


Figure 3.3 A linear calibration curve obtained by plotting the absorbance at 570 nm against the corresponding *D*-glucosamine concentrations.

The percentage of degree of deacetylation was calculated based on a per mole basis as shown in equation 3.1 (derivation of this formula is shown in Appendix III). The results are shown in Table 3.2.

$$\% \text{ Deacetylation} = \left[\frac{\Phi}{\Phi + [(w - 179\Phi)/221]}\right] x100 \qquad (3.1)$$

where Φ is the weight of GlcN determined/179

w is the weight of chitosan sample used

179 and 221 are the molecular weight of GlcN and GlcNAc, respectively.

3.1.3.2 By first derivative ultraviolet spectrophotometry (1DUVS) [12]

The degree of acetylation of chitosan can be determined in solution of chitosan in acetic acid by first derivative ultraviolet spectrophotometry at zero crossing point (ZCP). At this wavelength, the *N*-acetyl-*D*-glucosamine (GlcNAc) signal readings are linearly dependent on concentration and are not influenced by the presence of acetic acid.

The zero order absorption spectra of GlcNAc show maxima at 200 nm. The presence of acetic acid greatly disturbs the determination of GlcNAc, its contribution being particularly high around 195 nm. By switching the mode of spectrophotometer from zero order adsorption scan to first order mode, determination of the signal of GlcNAc could be directly measured at the ZCP where the acetic acid

solvent does not interfere. The first derivative spectra for GlcNAc and acetic acid are shown in Figure 3.4

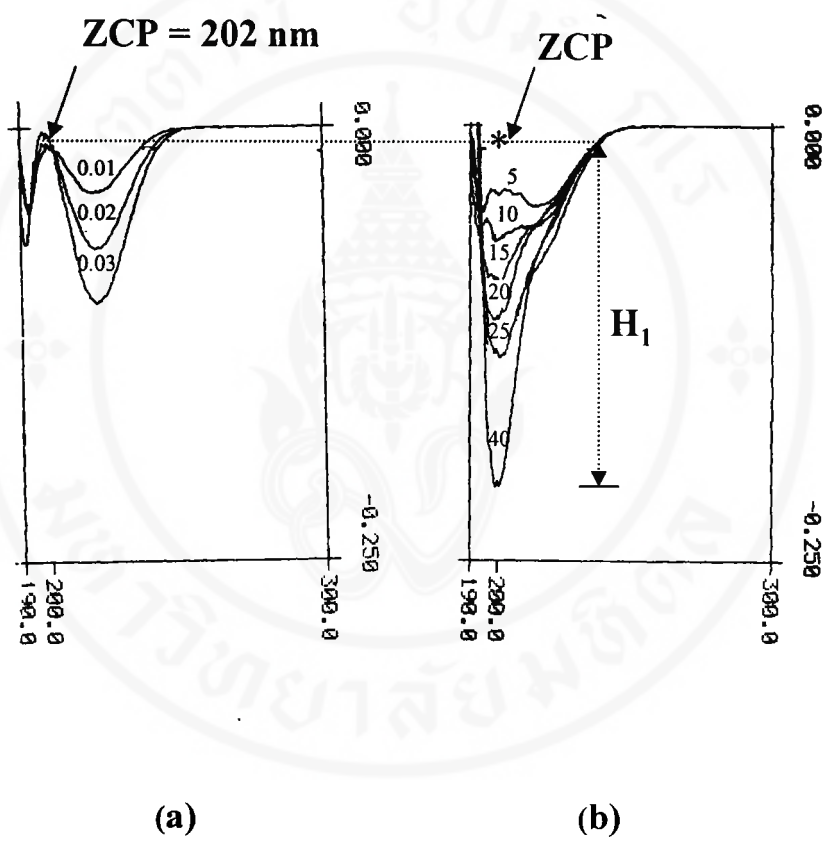


Figure 3.4 First derivative spectra of:

a) 0.01, 0.02, and 0.03 M acetic acid solutions.

b) GlcNAc at various concentration (5, 10, 15, 20, 25 and 40 mg L^{-1}) in 0.01 M acetic acid. An example of H_1 taken for the concentration of 40 mg L^{-1} is depicted.

From the result in Figure 3.4a, when the first derivative spectra of acetic acid solutions (0.01 to 0.03 M) were recorded against water, all the acetic acid spectra shared a common point at 202 nm. This wavelength was used by Muzzarelli R.A.A. et al. [12] as the zero crossing point (ZCP). The zero crossing point is closed to the GlcNAc maximum on the wavelength axis, this makes the GlcNAc determination independent of the acetic acid concentration in the concentration interval usually encountered in the dilute chitosan solutions.

To obtained a calibration curve for GlcNAc, the first derivative spectra of GlcNAc solutions were taken for six concentrations in the range 5.0 to 40.0 mgL^{-1} of GlcNAc in 0.01 M acetic acid as shown in Figure 3.4b. The spectra of 0.01, 0.02, and 0.03 M of acetic acid solutions were superimposed and the vertical distance from ZCP to each GlcNAc solution spectrum, H_1, was measured (mm). A linear calibration curve was obtained by plotting the H_1 values against the corresponding GlcNAc concentrations as shown in Figure 3.5.

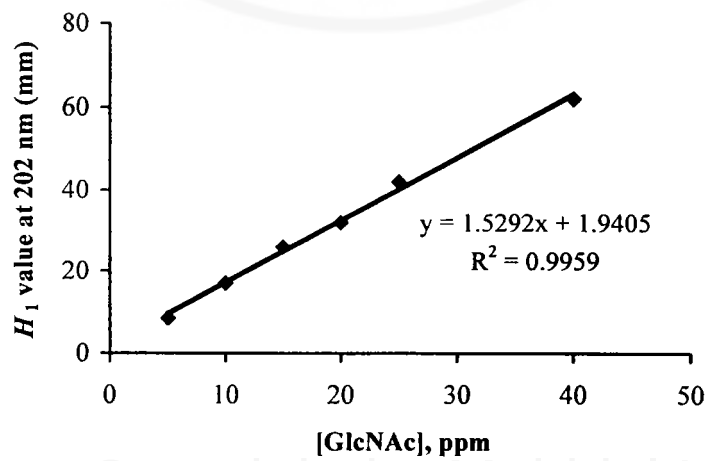


Figure 3.5 A linear calibration curve by plotting the H_1 values at zero crossing point (202 nm) against the corresponding GlcNAc concentrations.

As noted by Muzzarelli R.A.A. et al. [12], the presence of *D*-glucosamine (GlcN) may give rise to a large H value for GlcNAc than expected. Therefore, a reference curve for correcting this discrepancy was necessary. A fixed concentration of 10.0 mgL^{-1} GlcNAc in 0.01M acetic acid solution was prepared and varying amounts of GlcN was dissolved in GlcNAc solution to give a series of different percentages of GlcNAc solutions (w/w). The H values of the pure GlcNAc solution, H_1, and the H values of the solutions of different percentages of GlcNAc, H_2, were measured. The reference curve was obtained by plotting H_1/H_2 against the corresponding GlcNAc percentage. The results are shown in Figure 3.6.

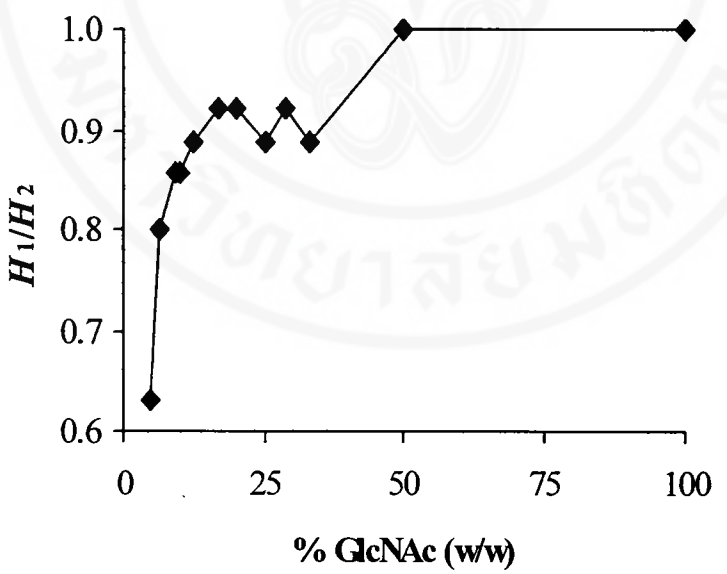


Figure 3.6 Correction curve for GlcNAc by plotting H_1/H_2 against the corresponding GlcNAc percentage.

Three chitosan samples that were washed as described in Appendix III, were accurately weighed approximately 0.01 g. 10.0 ml of 0.10 M acetic acid was added to chitosan samples. The mixture was heated until chitosan sample dissolved, the chitosan solution was cooled down and made up to 100.0 mL with deionized distilled water. Triplicate measurements were performed for each sample. The *H* values of the chitosan samples, H_2, were measured. The estimation of GlcNAc percentage (w/w) in chitosan samples was obtained from calibration curve in Figure 3.5.

From the approximate percentage of GlcNAc (w/w), the *H* values obtained from chitosan samples, H_2, were corrected by using correction curve from Figure 3.6. The *H* values (no effect of GlcN), H_1, were obtained by equation as following:

$$H_1/H_2 = \text{correction factor} \qquad (3.2)$$

Hence,

$$H_1 = \text{correction factor} \times H_2 \qquad (3.3)$$

where H_1 is the *H* values of the pure GlcNAc solution

H_2 is the *H* values of the solutions of different percentages of GlcNAc

The H_1 values obtained from equation 3.3 were used for determination of GlcNAc concentration in chitosan samples using calibration curve in Figure 3.5. The degree of deacetylation was calculated using equation 3.4 (derivation of this equation is shown in Appendix III). The results are summarized in Table 3.2.

$$\% \text{ Deacetylation} = 100 - \left(\frac{A}{A + [(w - 221A)/179]} x100 \right) \quad (3.4)$$

where A is the weight of GlcNAc determined/221

w is the weight of chitosan sample used

179 and 221 are the molecular weight of GlcN and GlcNAc, respectively.

3.1.3.3 By FT-IR spectroscopic technique

IR spectra of chitosans were recorded from KBr disc technique as described in Section 2.3.4.3. The IR spectra of three chitosan samples obtained by this technique are shown in Figure 3.7. The extent of *N*-acetylation of chitosan was determined from the absorbance of the amide I band at 1655 cm^{-1}. The 3450 cm^{-1} absorbance characteristic of hydroxyl group of sample was used as internal standard to correct for differences in chitosan concentration when the KBr disc techniques was used. The degree of deacetylation of the sample was calculated from equation 3.5. The degree of deacetylation of chitosan has shown in Table 3.2.

$$\% \text{ Deacetylation} = [100-(A_{1655}/A_{3450}) \text{ x } (100/1.33)] \quad (3.5)$$

where A_{1655} is the area of the amide I band having the maximum absorption at 1655 cm^{-1}

A_{3450} is the area of the hydroxyl group having the maximum absorption at 3450 cm^{-1}.

The absorption band at 3450 cm^{-1} was determined from 1902 to 3837 cm^{-1} as an internal standard. The absorbance of the amide I band at 1655 cm^{-1} was calculated from 1277 to 1902 cm^{-1}. The ratio was 1.33, using the baselines indicated in Figure 2.1 of fully *N*-acetylated chitosan (A_{1655}) and maximum absorption values of hydroxyl band (A_{3450}) [7].

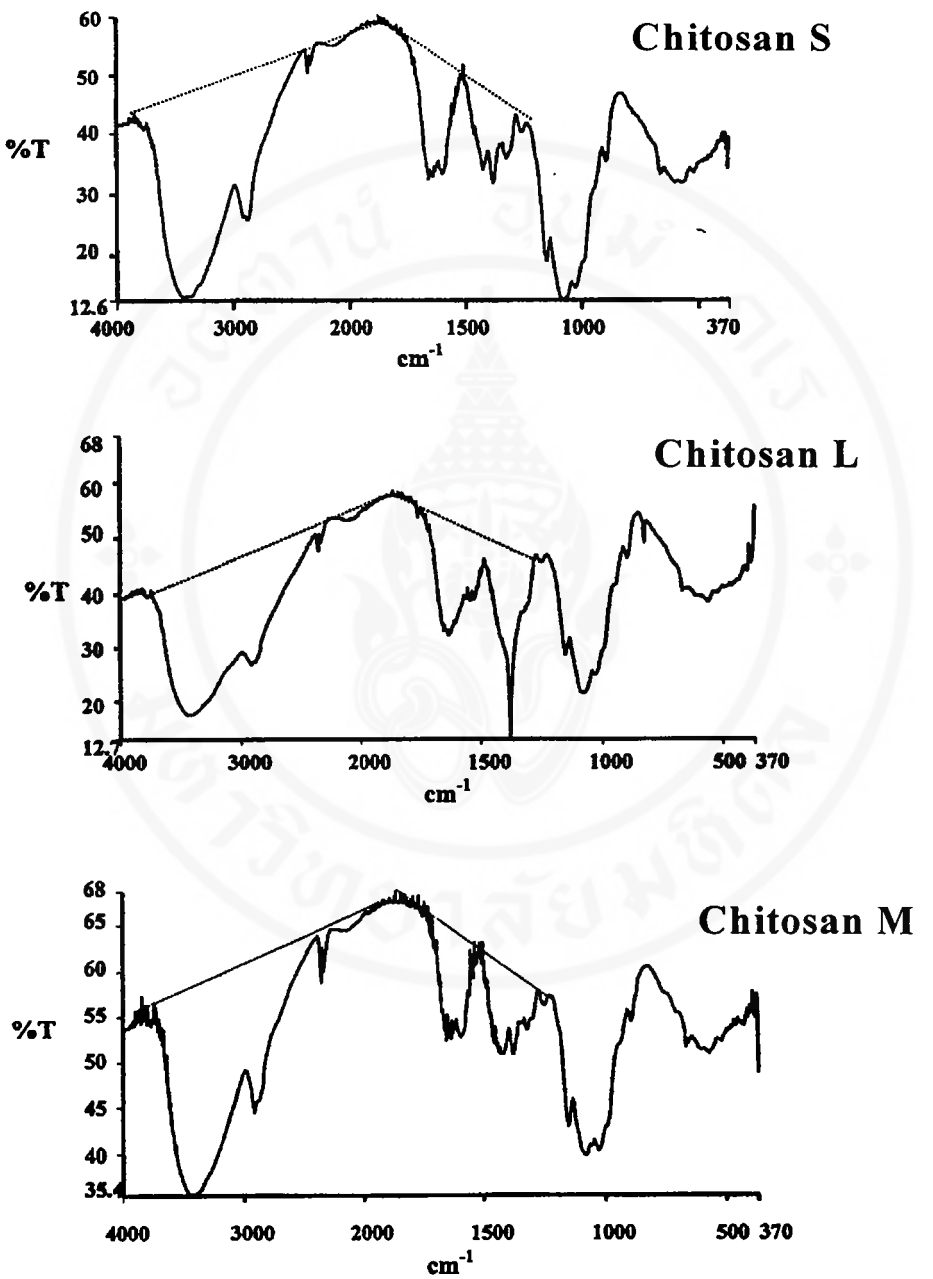


Figure 3.7 IR spectrum of three chitosan samples.

3.1.3.4 By solid-state ^{13}C NMR technique

The degree of deacetylation of chitosan was calculated from solid-state ^{13}C NMR [17] data by comparing the area of the CH_3 resonance to the resonance of the glucose carbons as described in Section 2.3.4.4. Solid-state ^{13}C NMR spectra of chitosan samples are shown in Figure 3.8.

The percentage of deacetylation of chitosan was calculated using equation 3.6 and the results are summarized in table 3.2.

$$\% \text{ Deacetylation} = \{100 - [(I_{CH3})/(I_{C1-C6}/6)]x100\} \qquad (3.6)$$

where I_{CH3} is the area of the CH_3 resonance of acetyl group

I_{C1-C6} is the sum of area of the glucose carbons

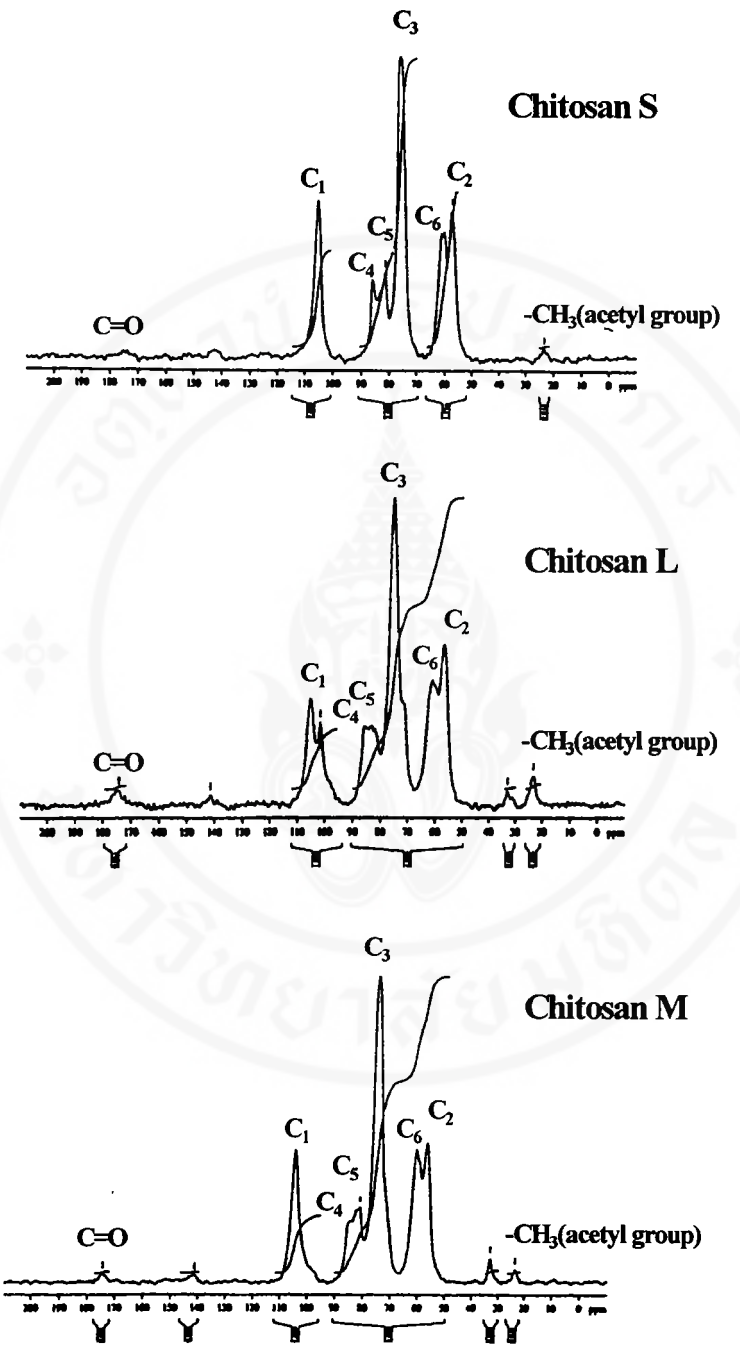


Figure 3.8 Solid-state ^{13}C NMR spectrum of three chitosan samples.

3.1.4.5 Comparison of degree of deacetylation determined by four techniques.

Results of the degree of deacetylation obtained using the four techniques are summarized in Table 3.2.

Table 3.2 Degree of deacetylation of chitosan samples determined by ninhydrin test, first derivative ultraviolet spectrophotometry, IR spectrophotometry, and solid-state ^{13}C NMR.

Sample	% Deacetylation of chitosan samples determined by			
	Ninhydrin test (n = 2)	1DUVS (n = 3)	FT-IR (n = 2)	^{13}C NMR (n = 2)
Chitosan S	27.9 ± 4.4	93.4 ± 0.1	91.8 ± 0.1	98.2 ± 0.1
Chitosan L	23.7 ± 2.8	91.1 ± 0.5	85.1 ± 0.9	91.7 ± 0.7
Chitosan M	10.4 ± 2.9	93.7 ± 0.2	92.1 ± 0.2	97.2 ± 0.3

The degree of deacetylation determined by ninhydrin test was calculated on the per mole basis. However, the values of degree of deacetylation obtained were the lowest among the other techniques although when these results were compared with the first derivative ultraviolet spectrophotometry, which calculated based on the per mole basis. These two techniques are applicable to determination of degree of deacetylation of chitosan solution. It is found that the degrees from first derivative ultraviolet spectrophotometry are much greater than the ninhydrin test. This may be attributed to the fading of color intensity of the sample

solution with time, after the boiling process. This decolorization was observed visually about 30 minutes after the boiling process and therefore, contributed to errors in the absorbance values. This finding agree with the work of Khor E. et al. [48] and was not recommended to used. The results from this method were not taken into consideration for further discussion.

First derivative ultraviolet spectrophotometry is simple and convenient among all four techniques. It requires very small amounts of sample and relies on simple reagents and instrumentation. This technique is very sensitive for *N*-acetyl-*D*-glucosamine detection. Degree of deacetylation determined by this technique were calculated based on the per mole basis. However, the value of degree of deacetylation obtained by this method is only approximate. This technique is not suitable for determination of degree of deacetylation in chitosan samples that have too low *N*-acetyl content since the measurement of signal is relied on the *N*-acetyl content. If the amount of GlcNAc in sample is too low, the signal height is greatly disturbed by GlcN. Although the effect of GlcN can be resolved by using correction curve, low value of correction factor also incurs larger experimental errors.

IR spectrophotometry is the method that is also commonly used for determination of degree of deacetylation of chitosan. This technique is simple. The difficulty of using IR technique is in the step of mixing between chitosan sample and KBr. Chitosan flake is stiff and difficult to grind with KBr to make a homogeneous form of disk. This technique is a solid state method and may not appealing because variations can be found in the results obtained using different baselines. Different

baselines have been suggested for samples with different ranges of degree of deacetylation but the choice of the baseline is debatable, especially when its range for individual samples are unknown [50]. The possibility of other error in the using IR spectrophotometry is that the 3450 cm^{-1} absorption band might be sensitive to adsorbed water.

Solid-state ^{13}C NMR is a highly sensitive technique and has the advantage of being applicable to insoluble products. The limitations of solid-state ^{13}C NMR technique for quantitative purposes becomes important at low acetyl contents. Therefore, solid-state ^{13}C NMR is recommended for determinations at high acetyl content in chitosan.

The degree of deacetylation determined by IR spectrophotometry and solid-state ^{13}C NMR were calculated based on the ratio of indicator signal against internal standard signal. These two techniques are applicable to solid sample. The results show that the degree of deacetylation determined by both techniques correlate well with each other.

Although the first derivative ultraviolet spectrophotometry was used to determine degree of deacetylation of chitosan in liquid solution whereas the IR spectrophotometry and solid-state ^{13}C NMR techniques were used in solid sample, all of these results were close (Table 3.2). The results have shown that the order of degree of deacetylation of three chitosan samples is: chitosan S ≈ chitosan M > chitosan L.

3.2 Kinetic studies

The propose of kinetic experiments is to determine the time required for each adsorption system to reach equilibrium. Kinetic of adsorptions of Cu(II) and Zn(II) on three chitosans were studied as described in Section 2.4. The results of kinetics of adsorption of copper(II) and zinc(II) ions on the three chitosans are shown in Figure 3.9. The plots between adsorptivity and time in Figure 3.9 were base on the data summarized in Table 5A and Table 6A, Appendix V.

The Cu(II) results, depicted in Figure 3.9a, have shown that the adsorption equilibrium was reached within two hours for chitosan S and chitosan M. Contact time of approximately three hours was required for the adsorption of on chitosan L to reach its equilibrium. The results for kinetics of Zn(II) adsorptions are illustrated in Figure 3.9b. The results indicated that the equilibriums were reached within approximately two hours for every sample.

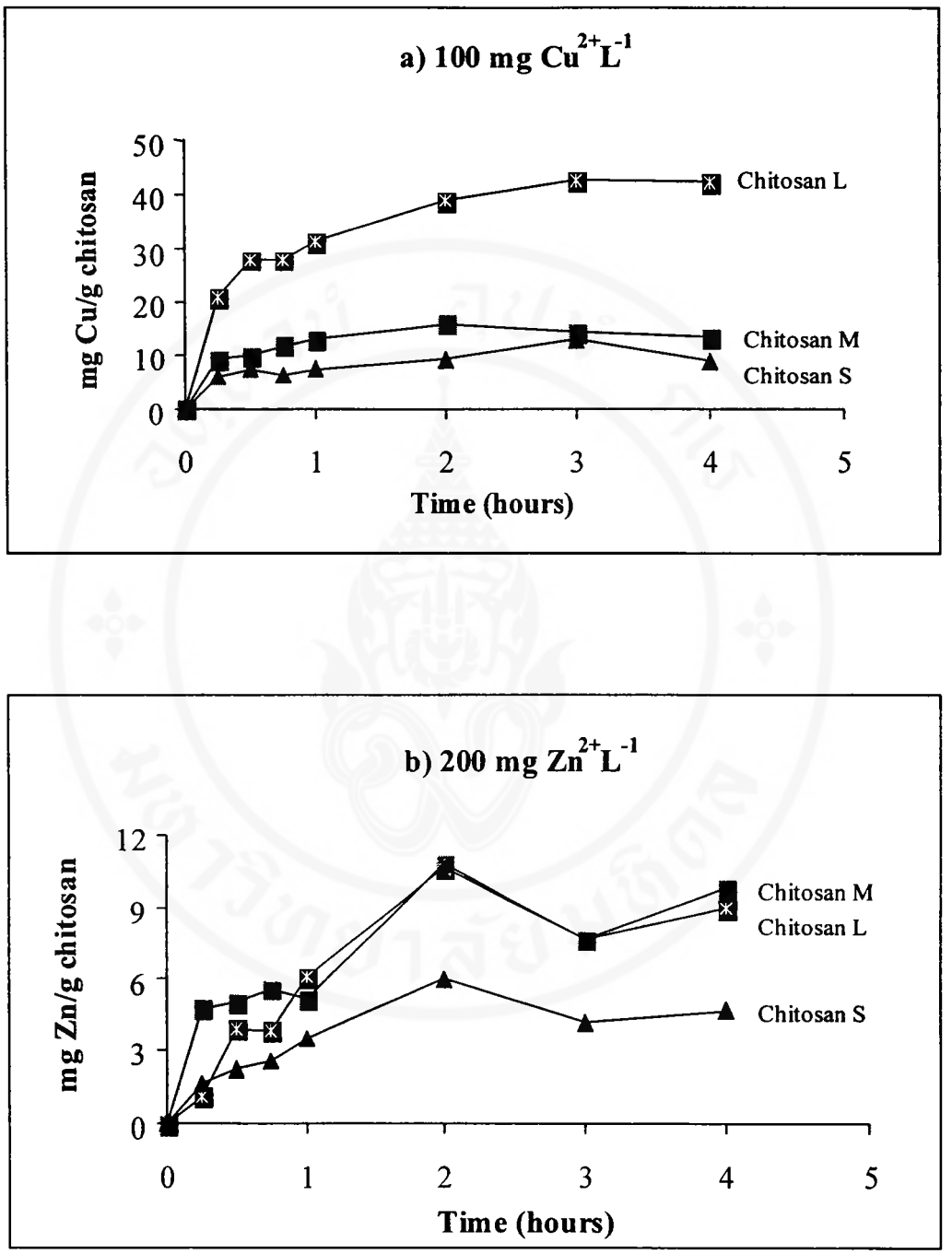


Figure 3.9 Adsorption kinetics of Cu(II) and Zn(II) on three chitosans studied at the following condition : in 0.01 M HCl, 37 °C.

3.3 Adsorption studies of Cu(II) ions and Zn(II) ions on chitosans

3.3.1 Adsorption capacities

Adsorption capacity of Cu(II) in 0.01 M HCl solution were determined on chitosan S, chitosan L, and chitosan M by constructing the isotherms. For this experiment, chitosan S which lied in the main size range (>300 μm) was used. After the opening of capsules chitosan L and chitosan M was homogeneously mixed before use. A portion of chitosan was accurately weighed at approximately 0.1 g and was allowed in contact with 25.0 mL of Cu(II) solutions at 37 °C for two hours. The initial concentration of Cu(II) in 0.01 M HCl were from 0.5 to 1,200 mg L^{-1}. The experiments were carried out in duplicate. The initial concentration (Ci) and the residual concentration (Ce) of Cu(II) ion were determined using FAAS by standard addition method. These concentration values of Ci and Ce were then used to calculate the amount of Cu(II) adsorbed on chitosan in milligram per one gram sample (Qe).

Figure 3.10 are the adsorption isotherms, which represent the relationship between the amount of copper(II) ion adsorbed (Qe) on chitosan and the residual concentration of copper(II) ion (Ce). Each isotherm was plotted using all the data points obtained from duplicate experiments (Table 7A-9A, Appendix VI). The adsorption capacities of Cu (II) on chitosans in 0.01 M HCl were obtained using the non-linear fitting program called ENZFITTER. The fitting was based on the Langmuir equation described in Section 1.4. The capacities reported in Table 3.3 were obtained from weight correction using the data of percentage of weight loss in Table 4A, Appendix IV.

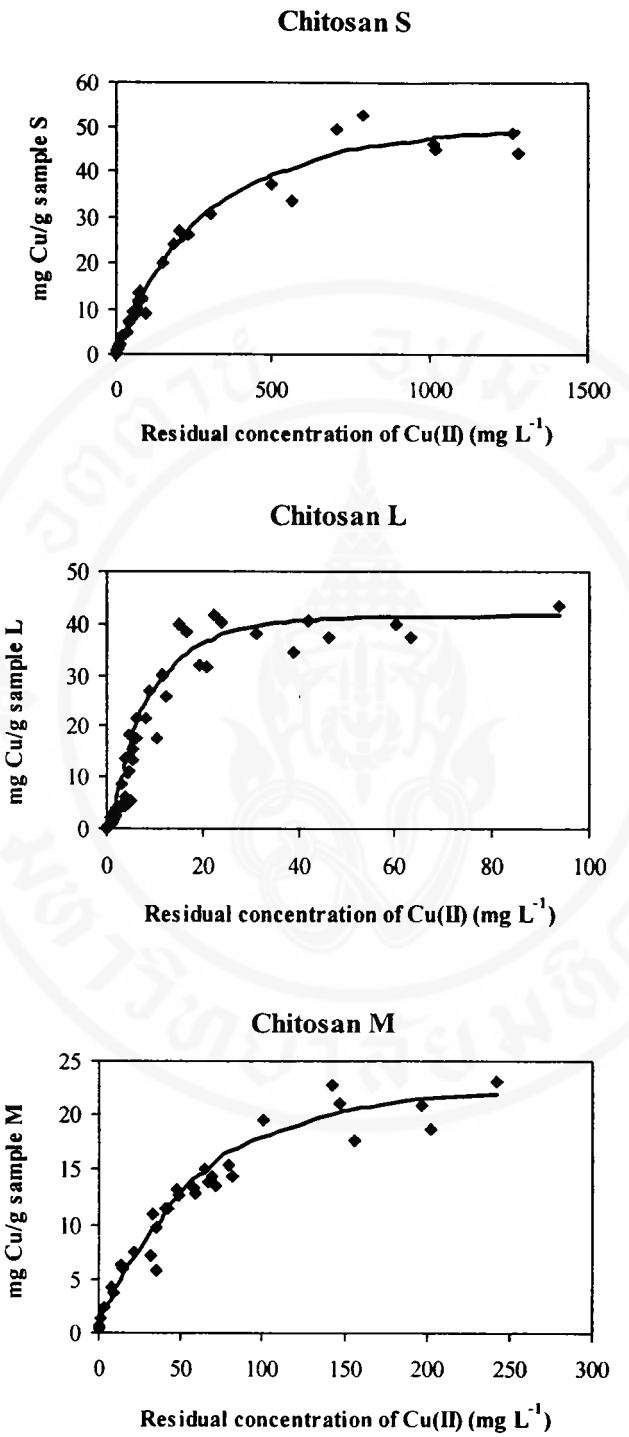


Figure 3.10 Adsorption isotherm of Cu(II) on chitosan S, chitosan L, and chitosan M at the following condition: in 0.01 M HCl, 37 °C, two hours contact time. Weights of chitosan L and chitosan M were of the products and the fillers.

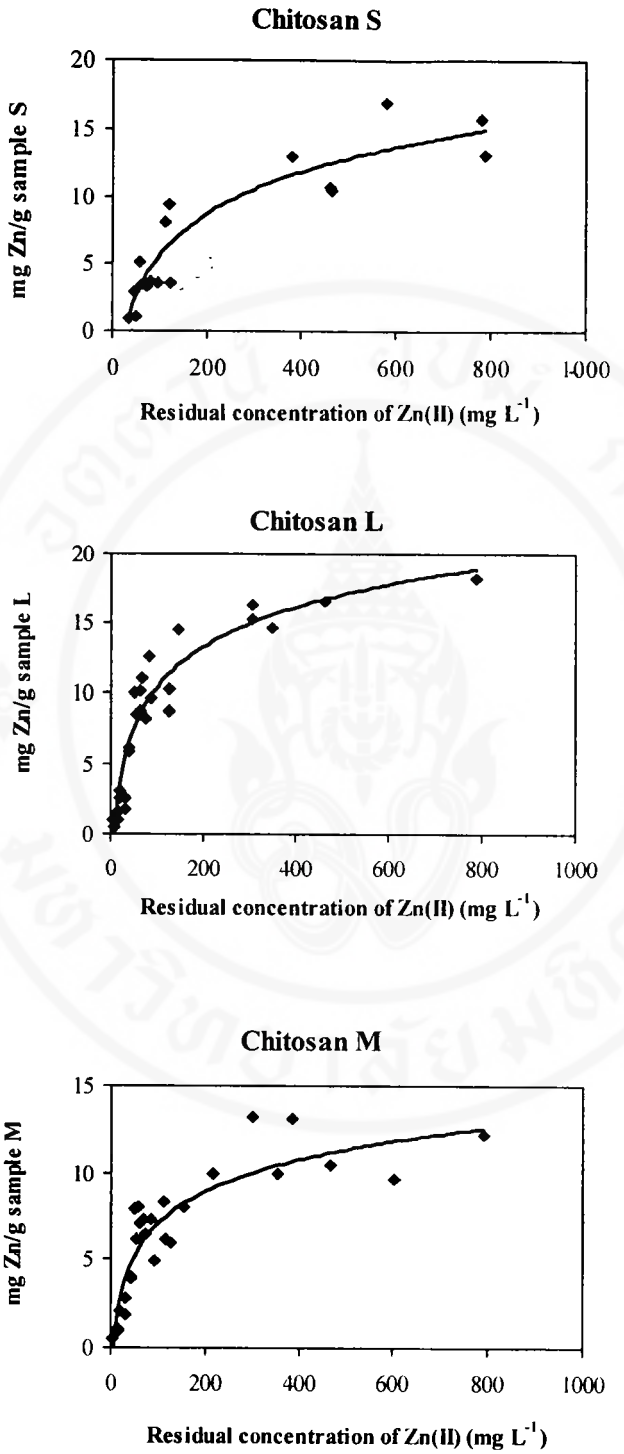


Figure 3.11 Adsorption isotherm of Zn(II) on chitosan S, chitosan L, and chitosan M at the following condition: in 0.01 M HCl, 37 °C, two hours contact time. Weights of chitosan L and chitosan M were of the products and the fillers.

Similar experiment and calculations were carried out to determine the adsorption capacities of Zn(II) on chitosans. Initial concentration of Zn(II) in 0.01 M HCl solutions were from 5 to 800 mg L^{-1}. The results for zinc(II) adsorption are also presented in Table 3.3. The isotherms for all three chitosans are depicted in Figure 3.11. Details of the experiment data are presented in Appendix VII.

Table 3.3 Maximum adsorption capacities of Cu(II) and Zn(II) ions on chitosan S, chitosan L, and chitosan M. The experiments were carried out in the solution of 0.01 M HCl at 37 °C, two hours contact time.

Sample	Maximum adsorption capacity [a] ± error [b] (mg metal/g chitosan[c])	
	Cu(II)	Zn(II)
Chitosan S	59.8 ± 3.0	20.3 ± 2.8
Chitosan L	52.2 ± 3.2	19.8 ± 1.6
Chitosan M	24.9 ± 1.4	13.6 ± 1.1

[a] The capacities were calculated based on weight loss during the two hours of contact time

[b] The errors were given by the fitting of ENZFITTER program.

[c] Weights of chitosan L and chitosan M were of the products with fillers.

The capacities of adsorption for Cu(II) and (II) ions in Table 3.3 are also presented as bar chart in Figure 3.12.

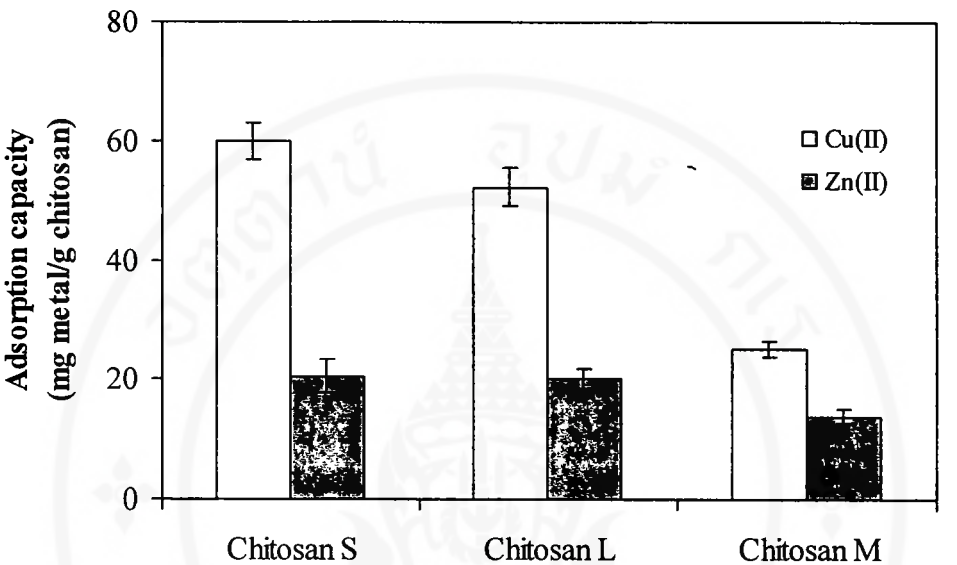


Figure 3.12 Adsorption capacities determined on chitosan S, chitosan L, and chitosan M for adsorptions of Cu(II) and Zn(II) ions. The capacities were calculated based on weights of chitosans with fillers.

The results in Table 3.3 and Figure 3.12 indicated that the adsorption capacities of Cu(II) on three chitosans are greater than the adsorption capacities of Zn(II). These results agree with the work of Ni C. et al. [51]. In their work, adsorption of Cu(II) and Zn(II) on chitosan were carried out in aqueous solution at pH 5.6. They found that adsorption capacity of Cu(II) (1.46 mmol Cu/g chitosan) was greater than that of Zn(II) (1.30 mmol Zn/g chitosan). This result has shown that Cu(II) ion is preferentially adsorbed over Zn(II) ion. As described in Section 1.2.3.5, the amine group ($-NH_2$) on chitosan can serve as coordination site for metal adsorption including

Cu(II) and Zn(II). Generally, binding constant between Cu(II) and RNH_2 is greater than between Zn(II) and RNH_2. The binding constant between Cu(II)-NH_2 on chitosan should be also greater than Zn(II)-NH_2 on chitosan. This resulted that interaction of Cu(II) and –NH_2 on chitosan was stronger than Zn(II).

The order of capacity for Cu(II) adsorbed on chitosan is: chitosan S > chitosan L > chitosan M. Nevertheless, according to *t*-test, at 95% confidence (P=0.05), the difference in adsorption capacities for Cu(II) ion between chitosan S and chitosan L are not significant. The order of capacity for Zn(II) adsorbed on chitosan is: chitosan S ≈ chitosan L > chitosan M.

It can be observed from all of the isotherms that some data points exhibited deviations from the Langmuir fitting. This could possibly be due to the difference in conditions during each course of the experiment. Also some of chitosans may have been solubilizrd during the two hours.

3.3.2 Possible mechanism of adsorption

It is known that the uptake of transition metals is mainly effected via coordination with the amine group (-NH_2) on chitosan [27,52-53]. Two -OH groups and one -NH_2 group are grabbed by the metal ion, Cu(II) or Zn(II), and the fourth site is probably occupied by a water molecule or the -OH group on the third carbon atom. The reaction below can be written to describe the adsorption at the amine site of chitosan.

$$M^{2+} + RNH_2 \longrightarrow M(RNH_2)^{2+} \quad (3.7)$$

where M^{2+} are Cu^{2+} or Zn^{2+}

However, or in an acidic solution like 0.01 M HCl, the two following reactions are also possible,

$$H^+ + RNH_2 \longrightarrow RNH_3^+ \quad (3.8).$$

$$M^{2+} + RNH_3^+ \longrightarrow M(RNH_2)^{2+} + H^+ \quad (3.9)$$

Reaction 3.8 can take place via protonation of H^+ on the amine groups of chitosan.

To support the reaction written as equation 3.8, the data of changes in solution pH could be used. Figure 3.13 and Figure 3.14 were the plot between the solution pH measured in the metal solutions with and without two-hours of contact with chitosans.

Figure 3.13 and Figure 3.14 were the data obtained during the construction of isotherms for adsorptions of Cu(II) and (Zn), respectively. These Figures illustrate that the pH of all solutions without chitosan (full line) rose to approximately three units (from 2 to 5) in the systems where chitosans were present. This means that after allowing the solutions in contact with chitosans, protonation took place, resulted the rise in pH. This phenomena was found for all solutions of 0.01 M HCl and was independent upon the presence of metal ions. From the structure of chitosan shown in Figure 1.1, Section 1.2.1, protonation could occur at the $-NH_2$ group as written in equation 3.8. Muzzarelli R.A.A. [5] has determined the protonation constant (log *Kp*) and found to be equal to 6.3. The author concluded that about 20% of amino group of chitosan is protonated even at pH 6.9. Thus, the reactions written in equation 3.7 or equation 3.8 and equation 3.9 do compete during the adsorption studies.

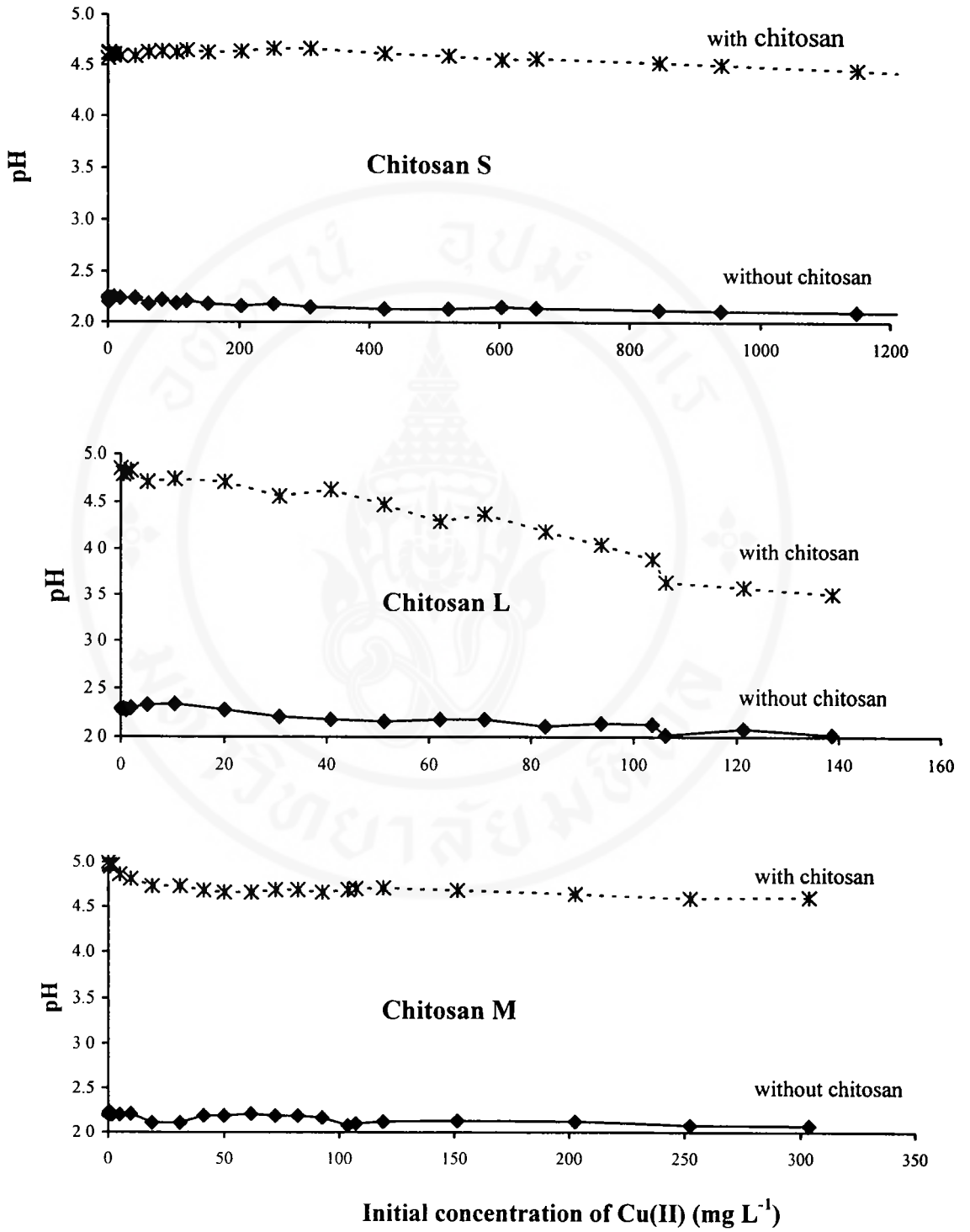


Figure 3.13 Plot between the final pH of solution mixture after two hours of contact of three chitosan samples and initial concentration of Cu(II) in 0.01 M HCl. These results were obtained during the isotherm experiment.

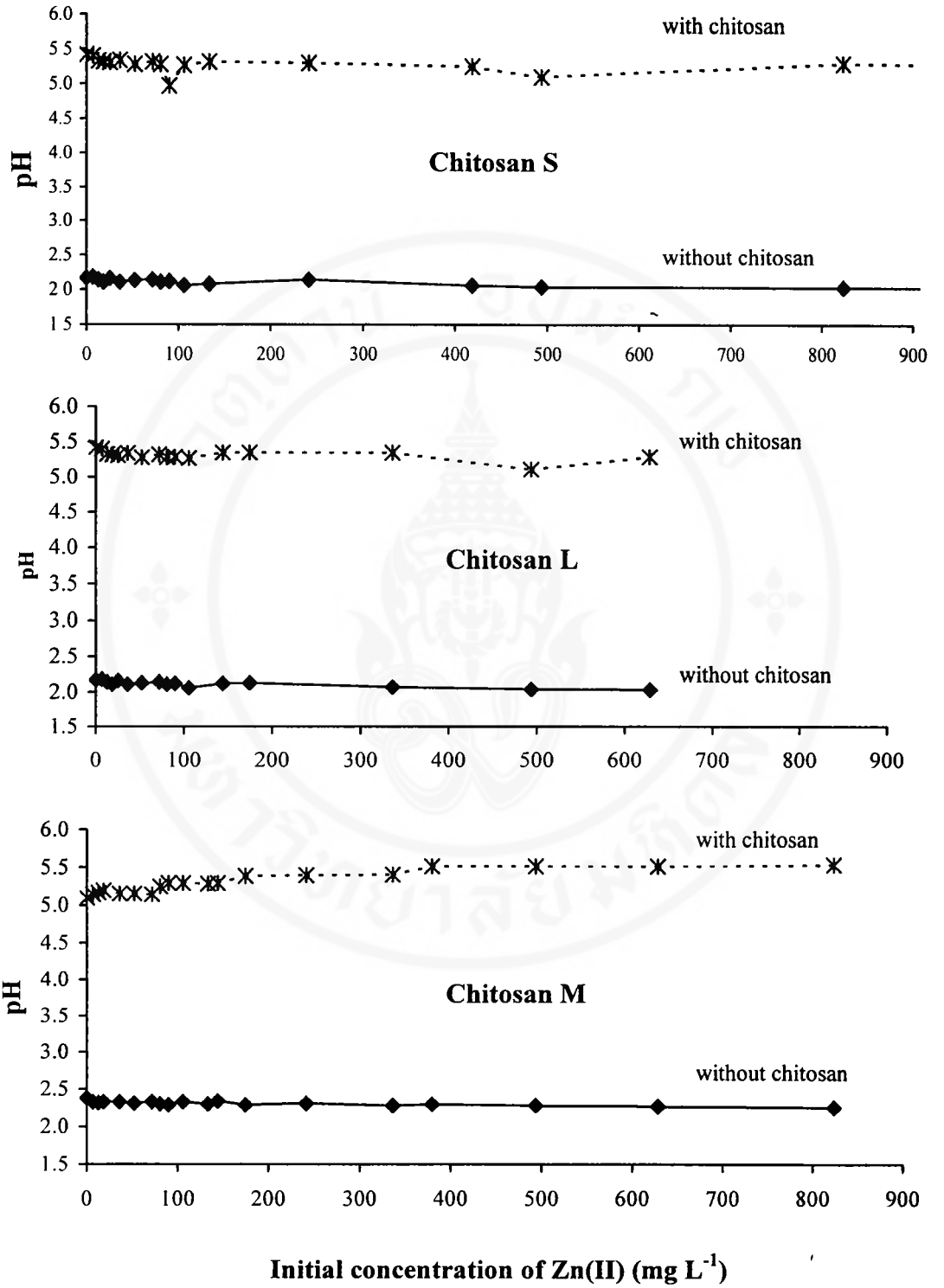


Figure 3.14 Plot between the final pH of solution mixture after two hours of contact of three chitosan samples and initial concentration of Zn(II) in 0.01 M HCl. These results were obtained during the isotherm experiment.

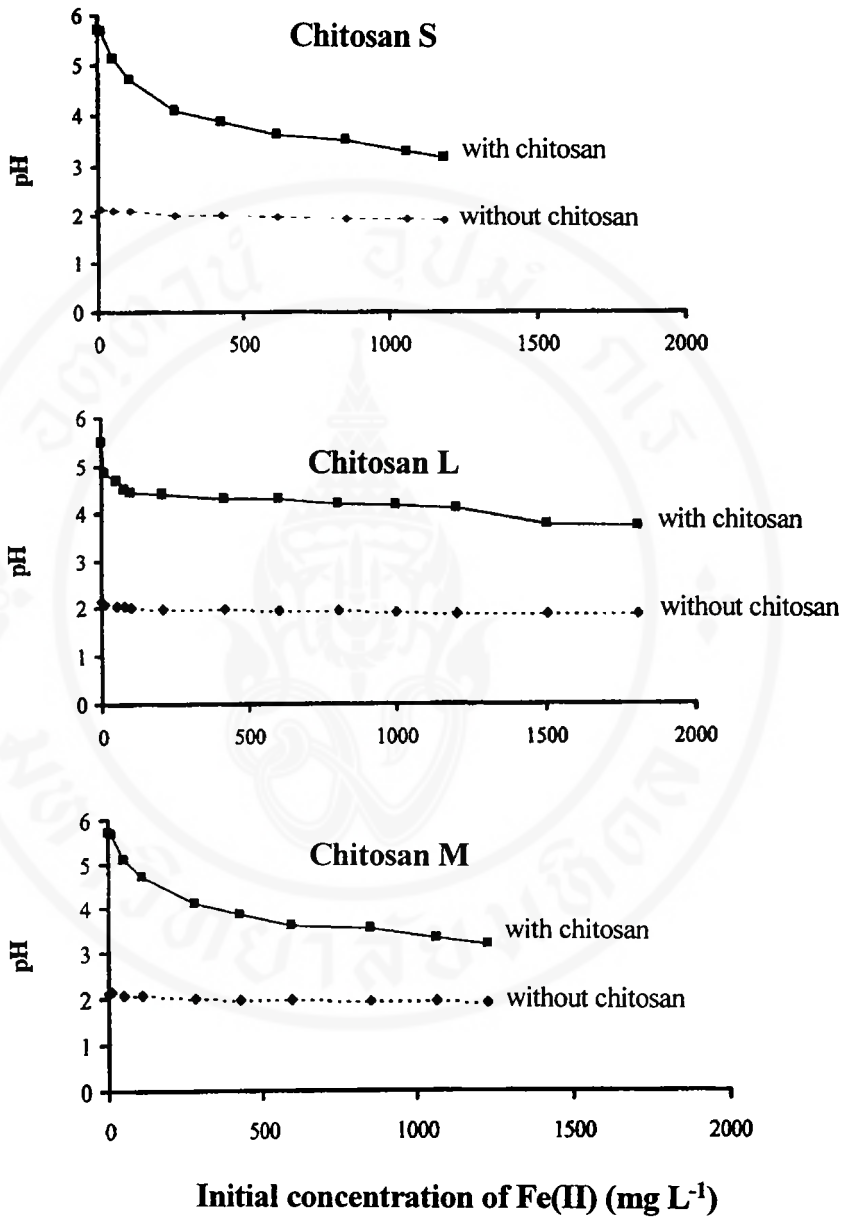


Figure 3.15 Plot between the final pH of solution mixture after two hours of contact of three chitosan samples and initial concentration of Fe(II) in 0.01 M HCl. These results were obtained during the isotherm experiment that was carried out by Chantore W.[47].

For the system of Cu(II) ions and chitosan (dotted line), in Figure 3.13, the pH fell with increasing in Cu(II) concentration. This was due to the increase in adsorption of Cu(II) ions on chitosans. The adsorption of Cu(II) ions took place via the reactions written in equation 3.7 or equation 3.8 and 3.9, thus resulted in the drop of pH as Cu(II) ion was more concentrated. This means that H^+ is released after the adsorption of Cu^{2+}. Although the drop of pH was not dominant for chitosan S, it can be seen that the pH did fall for all of the isotherms of Cu(II) system. This phenomena was also discovered in the systems where the same chitosans were allowed in contact with Fe(II) ions in 0.01 M HCl [47]. Figure 3.15, were the results obtained by Chantore W. who has measured capacities of adsorption for Fe(II) ions. The results show that there were the drop of pH as Fe(II) concentration was increased. This indicated that adsorption of a metal on the $-NH_2$ site of chitosan could possibly occur via equation 3.9.

The drop of solution pH was scarcely observed for the system of Zn(II), Figure 3.14. One possible explanation for this is that because Zn(II) ions only slightly adsorbed on the three samples or in other words all of the samples contributed rather low capacities for Zn(II) ions. Thus, there is less number of moles of Zn(II) ions to replace H^+ on the $-NH_2$ sites.

It was found by Chantore W. [47], that the capacities of Fe(II) ions for the same set of chitosans were much greater than for Cu(II) ions and for Zn(II) ions. The capacities for these three metals are summarized in Table 3.4 as in mmol M^{2+}/g.

Table 3.4 Comparison of adsorption capacities of metal ions on three chitosan samples.

Sample	Capacities of adsorption in 0.01 M HCl (mmol M^{2+}/g chitosan)[a]		
	Fe(II)[b]	Cu(II)	Zn(II)
Chitosan S	0.65 ± 0.07	0.94 ± 0.05	0.31 ± 0.04
Chitosan L	4.43 ± 1.21	1.21 ± 0.09	0.44 ± 0.03
Chitosan M	3.41 ± 0.31	0.80 ± 0.06	0.43 ± 0.04

[a] The capacities were calculated based on weights of chitosans without fillers.

[b] The results were reported by Chantore W. [47].

3.4 Adsorption of copper(II) and zinc(II) ions on chitosans in the binary system

Effect of competing cation on adsorption of metal on chitosan was studied in a binary system of Cu(II) and Zn(II) prepared in 0.01 M HCl. Adsorption of a metal on chitosan was studied when the other metal ion, or so-called competing ion, was added to the system at different concentration. The experiments were carried out according to the design described in Section 2.6 and Appendix VIII. In the design the possible meal intakes of a metal (in milligram) and of chitosan (in gram) were considered and fixed constant. Adsorptivity of this metal was studied at various loading of the competing ions. The experimental data are shown in Appendix VIII. Following graphs in Figure 3.16 and Figure 3.17 were based upon those data and are used for the following discussion in this section.

3.4.1 Effect of Zn(II) on the adsorptivity of Cu(II) at the meal intake (1.5 mg Cu/g chitosan).

The results in Figure 3.16a shows that the amount of Cu(II) ion bound onto the surface of all chitosans did not decreased significantly as number of moles of Zn(II) ions was greater in the system. Hence, it can be concluded that although the adsorption of Zn(II) foreign ion on three chitosan samples seemed to increase to the ratio of Cu : Zn at 1 : 19, adsorption of Zn(II) had very little or no affect on Cu(II) adsorptivity.

3.4.2. Effect of Cu(II) on the adsorptivity of Zn(II) at the meal intake (8.4 mg Zn/g chitosan).

The results of chitosan S and chitosan L in Figure 3.16b indicated that over the mole ratio of the metal of interest : the competing metal studied (1 : 0 to 1 :9), the adsorptivities of Zn(II) on these samples decreased as more Cu(II) ions were added to the system. Thus, this support that the two cations adsorb on the same site.n Cu(II) ions can replace on the sites formerly occupied by Zn(II)n ions. However, this effect was not obvious on chitosan M since the sample contain rather high percentage of fillers (approximately 50%). This caused large experimental errors as seen in Figure 3.16b. Since the adsorptivity of Zn(II) on all chitosans were very low for chitosan M (at zero concentration of Cu(II), thus the effect of addition Cu(II) ions was therefor ambiguous.

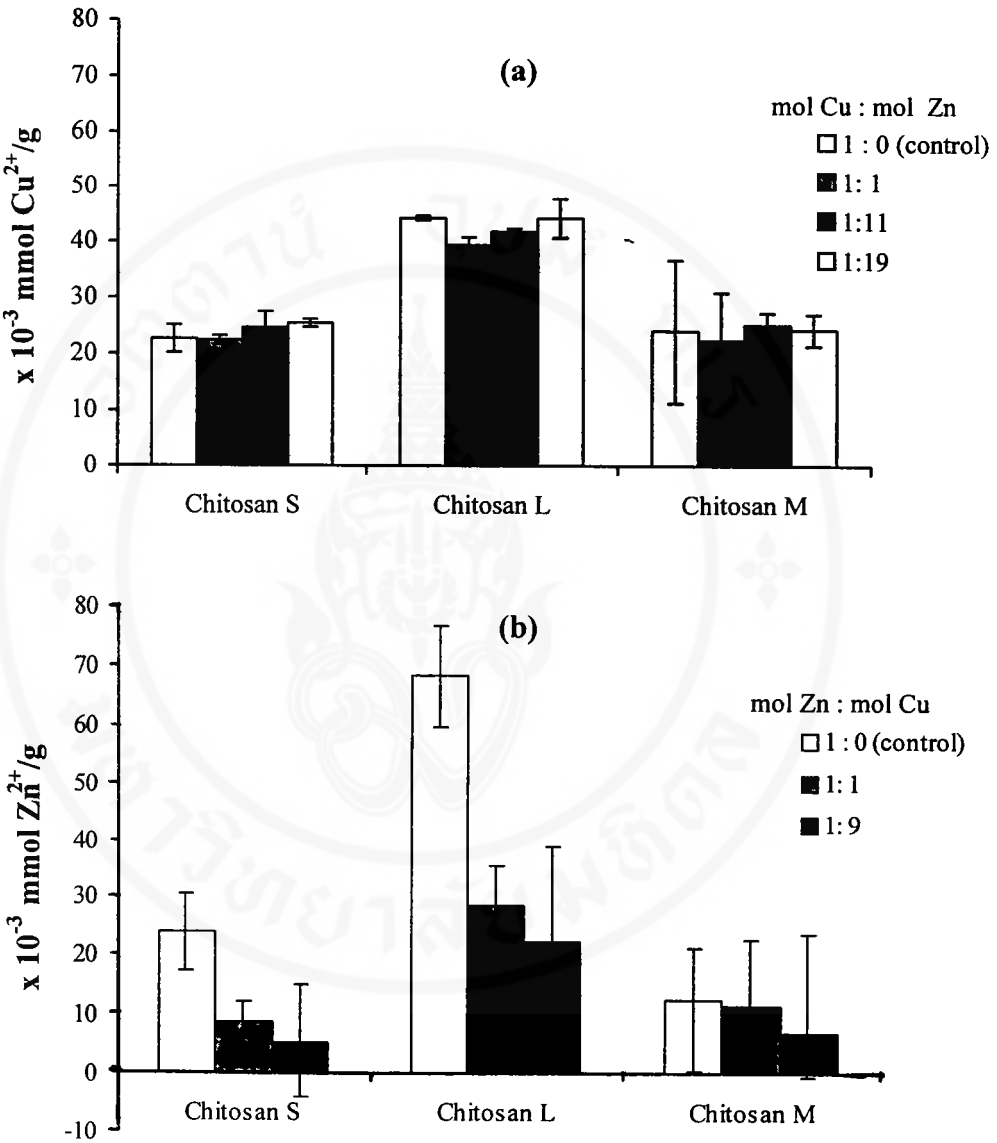


Figure 3.16 Effect of addition of competing cation on the adsorptivity of a metal of interest on chitosans studied at meal intakes:

(a) Effect of Zn(II) on the adsorptivity of Cu(II) on chitosans at the meal intake (1.5 mg Cu/g chitosan) and

(b) Effect of Cu(II) on the adsorptivity of Zn(II) on chitosans at the meal intake (8.4 mg Zn/g chitosan).

3.4.3 Preferential of adsorption of Cu(II) over Zn(II) on chitosan.

Figure 3.17 is the bar charts plotted from the same data set obtained, however the results of adsorptivity of a metal were taken from the set having the mole ratio at 1 : 1. At these 1 : 1 mole ratios, there were two sets of data studied at different concentration of Cu(II) and Zn(II) at 0.09 mmol M^{2+} L^{-1} and at 0.50 mmol M^{2+} L^{-1}. This resulted that number of mole of a metal at 0.02 and 0.13 mmol/g chitosan, respectively.

The bar charts in Figure 3.17 indicated that Cu(II) ion is more preferentially sorbed onto $-NH_2$ sites of chitosans than Zn(II) ions although the sizes of these two ion were slightly different. The results agree well with the adsorption capacities shown in Table 3.3 and in Figure 3.12 where the capacities for Cu(II) ion were mostly greater that those for Zn(II).

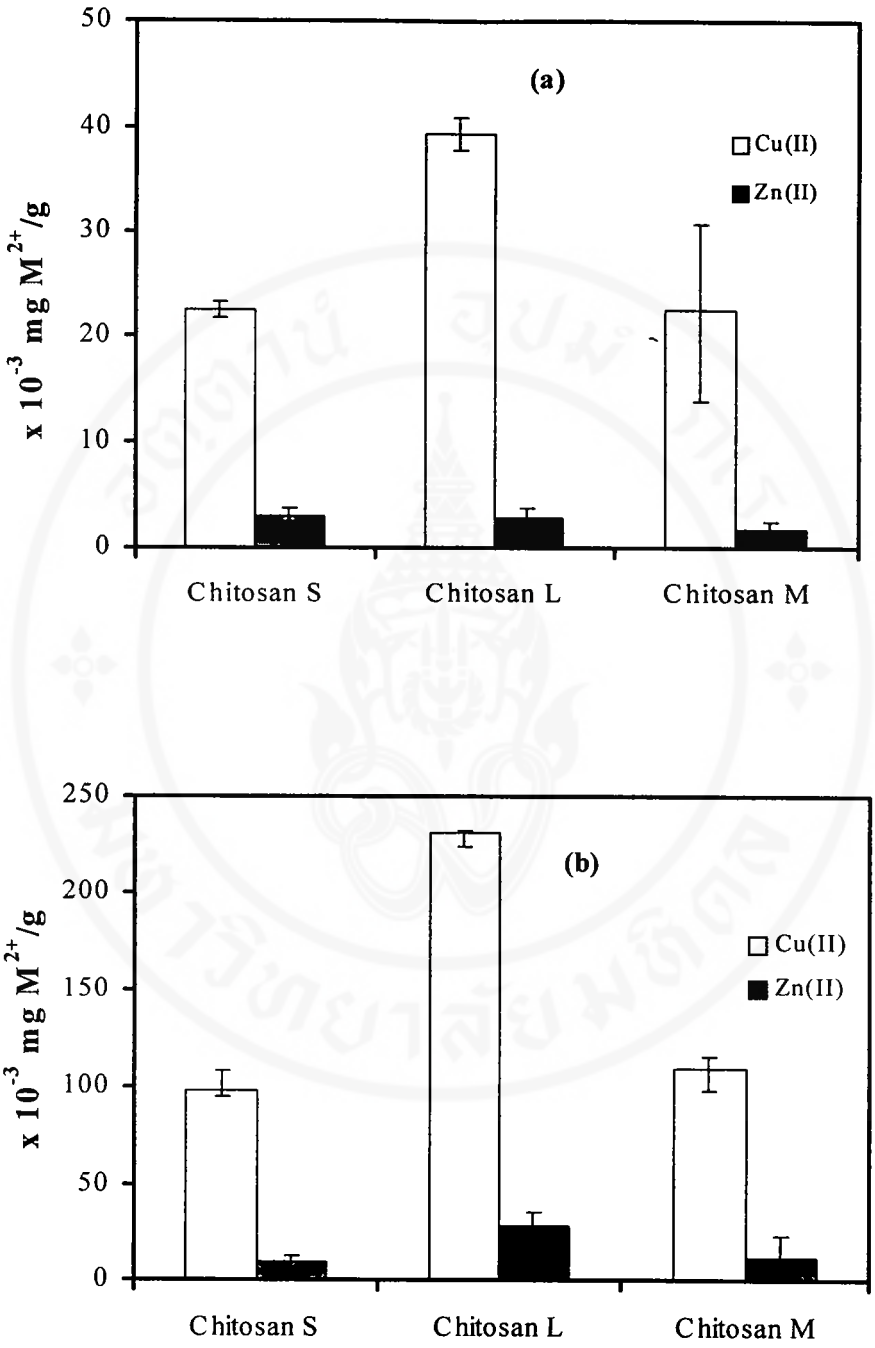


Figure 3.17 Preferential of adsorption of Cu(II) over Zn(II) on chitosan.

a) Concentration of Cu(II) and Zn(II) at 6 mg L^{-1}.

b) Concentration of Cu(II) and Zn(II) at 32 mg L^{-1}.

CHAPTER IV

CONCLUSION

In this work, adsorption of Cu(II) and Zn(II) were studied on three chitosans namely, chitosan S, chitosan L and chitosan M in 0.01 M HCl. This type of acid solution at pH 2 was used as the media to imitate the pH of human stomach. The three samples differ from one another in terms of purity. Chitosan S is a pure sample and contains no filler. The sample was supplied by a local producer in Thailand. The other two samples, which are chitosan L and chitosan M, are commercially available in supermarkets. Chitosan L contains ascorbic acid of 5% (w/w) with unknown fillers of 27.5 % (w/w), whereas chitosan M contains carbohydrate of 32% (w/w) and fiber of 18.4 %(w/w). These two samples were used as dietary supplements.

Kinetics of adsorption of Cu(II) and Zn (II) were studied on three chitosans. It was found that adsorption equilibrium of Cu(II) was reached within two hours for chitosan S and chitosan M. The contact time of approximately three hours was required for chitosan L to reach the equilibrium. The results for kinetics of Zn(II) adsorptions indicated that the equilibriums were reached within approximately two hours for every samples.

Adsorption capacities of Cu(II) and Zn(II) ions on three chitosans were measured in 0.01 M HCl after two hours of contact with solutions. This duration time was chosen to be similar to the time food spends inside the stomach. The adsorption

capacities of Cu(II) and Zn(II) on chitosans were obtained using ENZFITTER program based on Langmuir plot. The order of capacities of Cu(II) ion were: chitosan S (59.8 ± 3.0 mg/g) > chitosan L (52.2 ± 3.2 mg/g) > chitosan M (24.9 ± 1.4 mg/g). The order of capacities of Zn(II) ion were: chitosan S (20.3 ± 2.8 mg/g) ≈ chitosan L (19.8 ± 3.2 mg/g) > chitosan M (13.6 ± 1.1 mg/g). For all samples, the adsorption capacities for Cu(II) ions were greater than for Zn(II) ions.

Chitchumroonchokchai C. [3] had studied daily intake of copper and zinc in Thai nutrition. The value of daily intake was used for estimation of milligram metal intake per meal. The maximum intakes of copper and zinc, when calculated per meal, are approximately 1.34 mg Cu/meal and 6.63 mg Zn/meal, respectively. From the capacity data obtained in this work, chitosan L and chitosan M which are used as meal supplement, may inhibit gastric absorption of Cu(II) and Zn(II) (one gram is the recommended meal dose). However, the experiments were not carried out in the exact condition of the stomach condition. An in vivo study should be carried out to confirm this.

Mechanism of adsorption of the two metals was proposed as described through the following reactions.

- Direct adsorption of metal ion on the $-NH_2$ group.

$$M^{2+} + RNH_2 \longrightarrow M(RNH_2)^{2+} \quad (5.1)$$

where M^{2+} are Cu^{2+} or Zn^{2+}

- Adsorption via protonation followed by de-protonation.

$$H^+ + RNH_2 \longrightarrow RNH_3^+ \qquad (5.2)$$

$$M^{2+} + RNH_3^+ \longrightarrow M(RNH_2)^{2+} + H^+ \qquad (5.3)$$

The above mechanism was written based on the changes in pH of solution as the metal concentrations were increased. The results of Cu(II) system was similar to the system of Fe(II) [47]. The adsorption of Cu(II) ions took place via the reactions written in equation 5.1 or equation 5.2 and 5.3, thus resulted in the drop of pH as Cu(II) ion was more concentrated. This means that H^+ is released after the adsorption of Cu^{2+}. Although the drop of pH was not dominant for chitosan S, it can be seen that the pH did fall for all of the isotherms of Cu(II) system.

The drop of solution pH was scarcely observed for the system of Zn(II). One possible explanation for this is that because Zn(II) ions only slightly adsorbed on the three samples or in other words all of the samples contributed rather low capacities for Zn(II) ions. Thus, there is less number of moles of Zn(II) ions to replace H^+ on the $-NH_2$ sites.

Effect of competing cation on metal adsorption in 0.01 M HCl were studied in the binary system of Cu(II) and Zn(II). Fixed amount of a metal of interest and chitosan were designed to be equal to the meal intake, 1.34 mg Cu/g chitosan and 6.63 mg Zn/g chitosan. The values of intake load were fixed whereas the concentrations of the competing species were varied to give different loading of the competing cation in one gram of chitosan. The following conclusions can be drawn.

- Since Cu(II) is preferentially sorbed on chitosans, increasing in Zn(II) concentration (as competing cation) had very little or no affect on Cu(II) adsorptivity, although the mole ratio of Cu : Zn was at 1 : 19.

- The adsorptivities of Zn(II) on three chitosan samples decreased as more Cu(II) ions (as competing cation) were added to the system. However, this effect was not obvious for chitosan M since the sample contains rather high percentage of fillers (approximately 50%). This caused large experimental errors. Also the adsorptivity of Zn(II) on all chitosans were very low for chitosan M (at zero concentration of Cu(II), thus the effect of addition the foreign Cu(II) ions in Zn(II) system was therefore ambiguous.

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APPENDIX I

Gel permeation chromatograms

Average molecular weights of three chitosan samples were determined by gel permeation chromatography (GPC), model PL-GPC 110, using an Ultralinear hydrogel column with a reflective index (RI) detector. An aqueous solution combining 0.50 M acetic acid and 0.50 M sodium acetate (1:1 v/v) was used as the eluent delivering system. The procedure and condition were describe in Section 2.3.3.

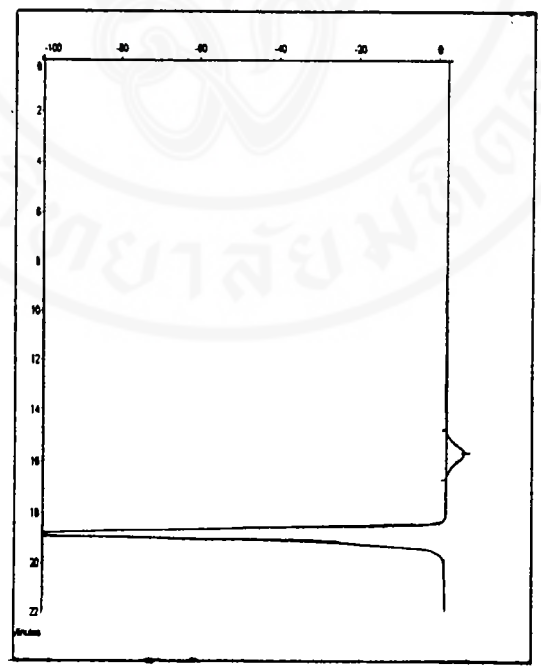

Figure 1A Gel permeation chromatogram of Pullulan standard at molecular weight 1.22×10^4 Dalton.

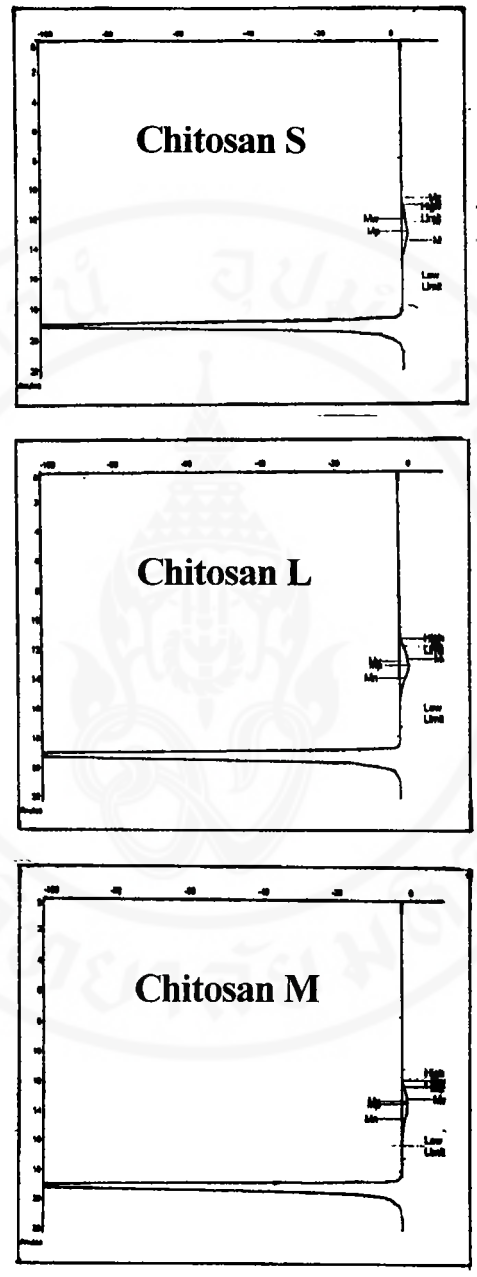

Figure 2A Gel permeation chromatogram of three chitosan samples.

APPENDIX II

Washing of chitosans

Three chitosan samples (S, L, and M) were washed to separate chitosan from other chemical added as fillers during processing. These washed samples were used for determination of degree of deacetylation in Section 2.3.4.

Chitosan L is a mixture and contains chitosan (as deacetylated chtiosan) at 67.5% (w/w). The package of this sample is labeled that the sample also contains 15 mg ascorbic acid per 250 mg capsules (5.0% w/w). The sample contains unknown filler at 27.5% (w/w).

In this experiment, approximately 4 grams of chitosan L was washed with 30 mL of deionized distilled water several times until the sample becomes ascorbic acid free. To confirm this, the rinsing solution was tested by adding 1.0 mL of 0.001 M $KMnO_4$ to 5.0 mL of this solution. The test employs the redox reaction:

$$2MnO_4^- + C_6H_8O_6 + 14H^+ \longrightarrow C_6H_6O_6 + 2Mn^{2+} + 8H_2O \quad (1A)$$

Ascorbic acid — Dehydroascorbic acid

The sample was washed until the color of permanganate ion (MnO_4^-) persisted in the rinsing solution, or until the concentration of MnO_4^- is constant. A spectrophotometer was used to measure the absorbance of permanganate ion (purple in color) at 525 nm. The results of this test are shown in Table 1A and Figure 3A. It was observed that the test solution of chitosan L increased as the number of washing

step increased. The absorbance finally became constant which confirmed that elution of ascorbic acid from chitosan L was complete.

It was found that the absorbance of MnO_4^- never changed throughout the step of washing. This means that there were no other reducing agents (such as ascorbic acid) being washed away from chitosan S nor chitosan M. Therefore, it can concluded that both chitosan S and chitosan M do not contain ascorbic acid like chitosan L.

Table 1A Absorbance value of rinsing solution from chitosan S, chitosan L, and chitosan M that were tested with 0.001 M $KMnO_4$

Washing step	Absorbance at 525 nm		
	Chitosan S	Chitosan L	Chitosan M
1	0.650	0.000	0.645
2	0.655	0.000	0.649
3	0.653	0.016	0.640
4	0.656	0.140	0.651
5	0.648	0.429	0.647
6		0.539	
7		0.602	
8		0.593	
9		0.576	
10		0.644	
11		0.608	
12		0.617	
13		0.654	
14		0.654	
15		0.652	
15		0.652	
Control	0.652	0.652	0.652

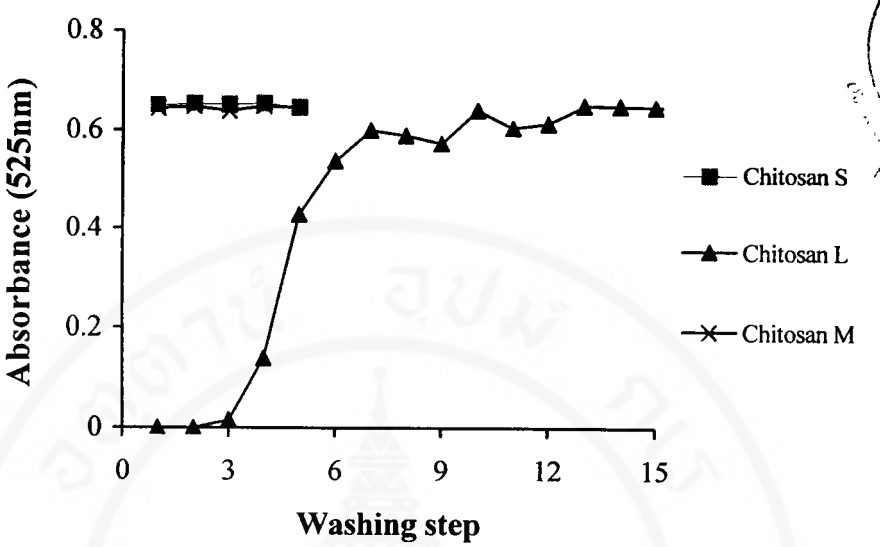

Figure 3A The plotting between washing step and absorbance of rinsing solution from chitosan S, chitosan L, and chitosan M when using 0.001 $KMnO_4$ for testing.

Chitosan M is a mixture and contains chitosan (as deacetylated chitosan) at 49.02% (w/w). The label on the package of this sample indicated that the sample also contains carbohydrate at 32.6%(w/w). In this experiment, chitosan M was washed with warm deionized distilled water (60 °C) several times (5 grams sample per 30 mL water) until the sample become starch free. To confirm this, the rinsing solution was tested by adding 100 μL of 0.01 M I_2 and 500 μL of 0.008 M I^- to 5.0 mL of this solution. The test employs the starch-I_3^- reaction.

Chitosan M was washed until the color of tri-iodide (I_3^-), yellow in color, persisted in the rinsing solution or until there was no blue color of starch-I_3^- present. A spectrophotometer was used to measure the absorbance of starch-I_3^- complex (brilliant

blue in color) at 582 nm and the absorbance of I_3^- (yellow in color) at 289 and 352 nm, which is one of the maximum absorption band of tri-iodide. The results of this test are shown in Table 2A and Figure 4A.

Table 2A Absorbance value of rinsing solution from chitosan S, chitosan L, and chitosan M that were tested by using starch-I_3^- reaction.

Washing step	Absorbance at 582 nm		
	Chitosan S	Chitosan L	Chitosan M
1	0.000	0.000	0.170
2	0.000	0.000	0.210
3	0.000	0.000	0.127
4	0.000	0.000	0.0081
5	0.000	0.000	0.049
6			0.043
7			0.050
8			0.060

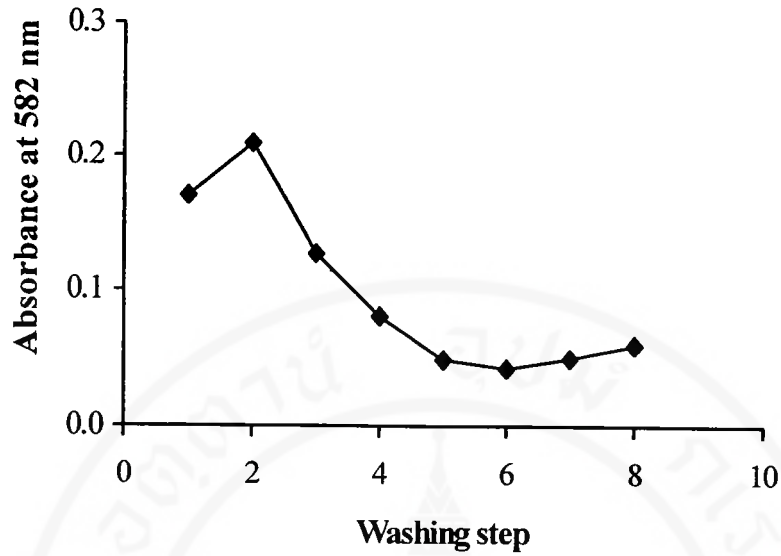

Figure 4A The plotting between washing step and absorbance of rinsing solution from chitosan M when using starch-I_3^- reaction.

It was observed that the absorbance of starch-I_3^- complex measured at 582 nm on the rinsing solution of chitosan M decreased as the number of washing step increased. When, the absorbance of this almost became constant and near to the absorbance of reagent blank, the absorbance of I_3^- measure at 352 were used. The absorbance of I_3^- should not change if there is no starch present in the rinsing solution. From these results, can be concluded that the separation of starch from chitosan M was nearly complete.

APPENDIX III

Derivation of the equation for calculation of degree of deacetylation by ninhydrin test and first derivative ultraviolet spectrophotometry

The degree of deacetylation is the mole fraction of the glucosamine residues (a unit of chitosan) in the polymer chain. The degree of deacetylation that determined by ninhydrin test and first derivative ultraviolet spectrophotometry were calculated based on per mole basis. In this Appendix, the equations used for calculation the degree of deacetylation by ninhydrin test and first derivative ultraviolet spectrophotometry are derived.

a) Ninhydrin test

Ninhydrin test is the method, which estimates the amount of $-NH_2$ group on the glycoside repeat unit of chitosan.

The partially *N*-acetylated chitosan is consisted *N*-acetyl-*D*-glucosamine (GlcNAc) and *D*-glucosamine (GlcN) on polymer chain. Therefore,

$$w = x + y \qquad (2A)$$

where w is the total weight of chitosan (g)

x is the weight of GlcN (g)

y is the weight of GlcNAc (g)

From equation 2A can be rewritten as following:

$$w = x + (w-x) \qquad (3A)$$

In order to determine percentage of deacetylation in mole, it is convenient to define the total mole of chitosan (N). In terms of mole of chitosan, equation 4A can be written:

$$N = (x/179) + [(w-x)/221] \quad (4A)$$

where 179 and 221 are the molecular weight of GlcN and GlcNAc, respectively.

Since degree of deacetylation is defined as the ratio of mole of GlcN per mole total of chitosan. Hence, the percentage of deacetylation can be determined by

$$\% \text{ Deacetylation} = \frac{\text{mole of GlcN x 100}}{\text{total mole of chitosan}} \quad (5A)$$

$$= \left[\frac{(x/179)}{(x/179)+[(w-x)/221]}\right] x100 \quad (6A)$$

$$= \left[\frac{\Phi}{\Phi+[(w-179\Phi)/221]}\right] x100 \quad (7A)$$

where Φ is the weight of GlcN determined/179

b) First derivative ultraviolet spectrophotometry

The degree of deacetylation of chitosan obtained by using first derivative ultraviolet spectrophotometry, this method determined *N*-acetyl-*D*-glucosamine (GlcNAc) residues in the partially acetylated chitosan.

A general equation, which represents the total weight of chitosan (w), could be written as:

$$w = y + (w-y) \quad (8A)$$

where y is the weight of GlcNAc (g) and the weight of *D*-glucosamine (GlcN), (w-y), was obtained.

In order to determine percentage of deacetylation in mole, it is convenient to define the total mole of chitosan (N). In terms of mole of chitosan, equation 9A can be written:

$$N = (y/221) + [(w-y)/179] \quad (9A)$$

where 179 and 221 are the molecular weight of GlcN and GlcNAc, respectively.

Since degree of acetylation is defined as the ratio of mole of GlcNAc per total mole of chitosan. Hence, the percentage of acetylation can be determined by

$$\%\ \text{Acetylation} = \frac{\text{mole of GlcNAc}}{\text{total mole of chitosan}} \times 100 \quad (10A)$$

$$= \left[\frac{(y/221)}{(y/221)+[(w-y)/179]}\right] x100 \quad (11A)$$

$$= \left[\frac{A}{A+[(w-221A)/179]}\right] x100 \quad (12A)$$

where A is the weight of GlcNAc determined/221

Thus, $\%\ \text{Deacetylation} = 100 - (\%\,Acetylation)$ (13A)

$$= 100 - \left(\frac{A}{A+[(w-221A)/179]} x100\right) \quad (14A)$$

APPENDIX IV

Solubility studies of chitosans

Chitosan is insoluble in some solvents such as water, alkali, and inorganic acids, but soluble in most solutions of organic acid when the pH of the solution is less than 6. Acetic acid and formic acid are two of the most widely used acids for dissolving chitosan. Some dilute inorganic acids such as nitric acid, perchloric acid, phosphoric acid, and hydrochloric acid can also be used to prepare chitosan solution, but after prolonged stirring and warming. In this study, 0.01 M hydrochloric acid was selected as the medium for determination of chitosan capacity on adsorption of Cu(II) and Zn(II). Thus, it was necessary to determine solubilities of chitosan sample.

Solubility of three chitosan samples was studied both in deionized distilled water and in 0.01 M HCl solution, as described as followings.

The solubility studies of chitosans were carried out in 125 mL stoppered conical flasks, each containing 0.4 g of accurately weighed chitosan and 100 mL of 0.01 M hydrochloric solution. This ratio of chitosan to the solution was similar to the actual ratio used in the isotherm studies. The mixtures were incubated at 37 °C using shaking incubator for two hours. The mixtures were then centrifuged to separate chitosan solid from the supernatant. Chitosan solids were washed with deionized distilled water approximately 5 times for removal of excess chloride ion. The rinsing solution was tested for chloride ion using a few drops of 6 M HNO_3 and 0.1 M

$AgNO_3$. The chitosan solids were dried at 60 °C in an oven until the constant weight was achieved.

Solubilities of chitosans were calculated as in gram of solubilized chitosan per 100 ml of solvent. The results are shown in Table 3A

Table 3A Solubility of chitosans in deionized water and 0.01 M HCl.

Sample	Solubility ± variation (n = 3) (g chitosan/100 ml solvent)	
	In deionized water	in 0.01 M HCl
Chitosan S	0.034 ± 0.006	0.151 ± 0.003
Chitosan L	0.075 ± 0.001	0.232 ± 0.005
Chitosan M	0.059 ± 0.004	0.171 ± 0.005

The results indicated that each sample could slightly solubilize in 0.01 M HCl solution after incubation at 37 °C for two hours. Chitosan L was solubilized greater than other chitosans both in deionized water and 0.01 M HCl solution.

From the solubility values in Table 3A, percentage of weight loss of chitosans were also obtained during the experiment. The weight loss contents are shown in Table 4A. These results were used in the calculation of adsorption capacities of chitosans for Cu(II) and Zn(II) in Section 3.3.

Table 4A Weight loss content of chitosans (%w/w) in deionized water and 0.01 M HCl

Sample	Weight loss ± variation (n = 3) (%w/w)	
	In deionized water	In 0.01 M HCl
Chitosan S	8.00 ± 1.33	35.03 ± 1.24
Chitosan L	17.66 ± 0.32	54.15 ± 1.18
Chitosan M	13.95 ± 0.90	38.94 ± 1.55

APPENDIX V

Experimental data obtained form kinetic studies of adsorption of Cu(II) and Zn(II) on chitosans

Table 5A Adsorption kinetics of Cu(II) on three chitosans. The experiments were carried out using initial Cu(II) concentration of 100 mg L^{-1} in 0.01 M HCl, 37 °C.

Contact time (hr)	mg Cu/g chitosan		
	Chitosan S	Chitosan L	Chitosan M
0.25	6.02	21.04	9.25
0.50	7.47	27.91	9.96
0.75	6.36	28.08	12.08
1	7.51	31.49	12.89
2	9.25	38.74	16.12
3	12.92	42.56	14.43
4	9.02	42.12	13.26

Table 6A Adsorption kinetics of Zn(II) on three chitosans. The experiments were carried out using initial Zn(II) concentration of 200 mg L^{-1} in 0.01 M HCl, 37 °C.

Contact time (hr)	mg Zn/g chitosan		
	Chitosan S	Chitosan L	Chitosan M
0.25	1.60	1.09	4.67
0.50	2.21	3.84	4.94
0.75	2.55	3.80	5.56
1	3.43	6.08	5.11
2	5.96	10.69	10.83
3	4.10	7.61	7.62
4	4.58	8.95	9.80

APPENDIX VI

Experimental data obtained form isotherm studies of Cu(II) and pH changes

Table 7A Adsorption capacities for Cu(II) on *chitosan S.* The capacities were measured under constant temperature at 37 °C where the media is 0.01 M HCl.

Set I

Initial concentration of Cu(II) (mg L^{-1})	Residual concentration of Cu(II) (mg L^{-1})	Adsorption capacity (mg Cu/g chitosan)	Initial pH	Final pH
0	0	0	2.24	4.53
0.51	0.15	0.13	2.24	4.47
1.07	0.29	0.28	2.20	4.61
1.88	1.14	0.26	2.23	4.73
4.92	2.67	0.79	2.24	4.55
9.55	5.60	1.40	2.25	4.60
18.69	11.88	2.43	2.24	4.61
30.75	19.67	3.93	2.23	4.58
40.95	27.83	4.62	2.24	4.59
49.87	36.27	4.79	2.20	4.59
61.50	41.41	7.17	2.18	4.59
71.99	50.36	7.67	2.21	4.60
81.78	57.01	8.70	2.22	4.63
92.39	63.82	10.13	2.21	4.63
103.69	70.28	11.77	2.19	4.61
119.04	84.32	12.18	2.21	4.54
151.21	111.90	13.92	2.18	4.60
202.49	145.31	20.19	2.16	4.58

Table 7A (continued)

Set I (continued)

Initial concentration of Cu(II) (mg L^{-1})	Residual concentration of Cu(II) (mg L^{-1})	Adsorption capacity (mg Cu/g chitosan)	Initial pH	Final pH
252.24	183.54	24.12	2.18	4.60
303.58	228.98	26.14	2.15	4.58
422.85	290.99	46.94	2.13	4.39
521.52	359.36	56.93	2.13	4.55
603.61	467.41	48.13	2.15	4.57
845.01	702.79	49.35	2.14	4.59
939.50	832.58	37.27	2.11	4.47
1148.03	1020.12	44.78	2.10	4.46
1403.94	1264.38	48.69	2.09	4.39

Set II

Initial concentration of Cu(II) (mg L^{-1})	Residual concentration of Cu(II) (mg L^{-1})	Adsorption capacity (mg Cu/g chitosan)	Initial pH	Final pH
0.00	0.00	0.00	2.25	4.57
0.51	0.08	0.16	2.24	4.57
1.07	0.34	0.27	2.20	4.62
1.88	0.70	0.43	2.23	4.63
4.92	2.71	0.80	2.24	4.61
9.55	5.54	1.44	2.25	4.61
18.69	13.23	1.97	2.24	4.59
40.95	28.79	4.43	2.24	4.59
61.50	41.89	7.09	2.18	4.63
81.78	55.56	9.55	2.22	4.64
103.69	72.63	11.31	2.19	4.63
119.04	94.18	9.01	2.21	4.65
151.21	114.74	13.35	2.18	4.63
202.49	147.64	19.95	2.16	4.64
252.24	184.86	24.25	2.18	4.67
308.73	232.59	27.05	2.15	4.67
422.85	338.75	30.62	2.13	4.62

Table 7A (continued)

Set II (continued)

Initial concentration of Cu(II) (mg L^{-1})	Residual concentration of Cu(II) (mg L^{-1})	Adsorption capacity (mg Cu/g chitosan)	Initial pH	Final pH
603.61	499.24	37.29	2.15	4.56
656.69	562.29	33.67	2.14	4.57
939.50	790.91	52.65	2.11	4.51
1148.03	1016.78	46.21	2.10	4.46
1403.94	1279.46	44.06	2.09	4.39

Table 8A Adsorption capacities for Cu(II) on *chitosan L.* The capacities were measured under constant temperature at 37 °C where the media is 0.01 M HCl.

Set I

Weight of chitosan L = 0.5 g

Initial concentration of Cu (II) (mg L^{-1})	Residual concentration of Cu(II) (mg L^{-1})	Adsorption capacity (mg Cu/g chitosan)	Initial pH	Final pH
0.00	0.00	0.00	2.23	5.69
1.96	0.05	0.12	2.23	5.83
5.13	0.22	0.30	2.16	5.88
10.40	0.39	0.62	2.16	5.94
20.08	0.76	1.20	2.18	6.00
30.70	1.62	1.80	2.18	6.01
40.80	1.96	2.40	2.17	5.96
51.23	2.09	3.04	2.10	5.95
62.14	2.25	3.70	2.11	5.90
70.89	3.31	4.17	2.10	5.90
82.81	3.43	4.91	2.12	5.88
93.71	5.00	5.48	2.13	5.82
103.57	4.60	6.12	2.20	5.85

Table 8A (continued).

Set I (continued)

Weight of chitosan L = 0.1 g

Initial concentration of Cu(II) (mg L^{-1})	Residual concentration of Cu(II) (mg L^{-1})	Adsorption capacity (mg Cu/g chitosan)	Initial pH	Final pH
0.00	0.00	0.00	2.20	4.77
25.39	3.18	11.21	2.21	4.45
30.70	3.91	13.50	2.21	4.30
36.10	5.56	15.64	2.12	3.99
40.80	4.75	18.18	2.18	4.37
45.32	10.60	17.59	2.21	4.15
51.23	8.00	21.66	2.16	4.20
62.14	9.00	27.03	2.18	4.14
70.89	11.67	29.79	2.18	3.86
82.81	19.18	32.07	2.11	4.01
93.71	16.47	38.64	2.14	3.77
103.57	23.92	40.33	2.13	3.71
121.30	46.34	37.46	2.08	3.20
106.15	31.34	38.27	2.02	4.14
138.63	63.46	37.40	2.02	4.29
189.90	93.66	43.51	2.02	4.05

Table 8A (continued).

Set II

Initial concentration of Cu(II) (mg L^{-1})	Residual concentration of Cu(II) (mg L^{-1})	Adsorption capacity (mg Cu/g chitosan)	Initial pH	Final pH
0	0	0	2.29	4.85
0.51	0.15	0.19	2.29	4.79
1.07	0.27	0.41	2.28	4.81
1.96	0.28	0.87	2.30	4.83
5.13	0.63	2.33	2.33	4.71
10.40	2.44	4.11	2.34	4.74
20.08	2.97	8.81	2.28	4.71
30.70	5.43	13.14	2.21	4.56
40.80	6.72	17.60	2.18	4.63
51.23	8.69	21.74	2.16	4.47
62.14	12.19	25.86	2.18	4.29
70.89	11.45	30.32	2.18	4.37
82.81	20.88	31.65	2.11	4.18
93.71	16.16	39.97	2.14	4.04
103.57	22.28	41.66	2.13	3.88
106.15	38.82	34.67	2.02	3.63
121.30	41.91	40.68	2.08	3.57
138.63	60.24	39.80	2.02	3.50

Table 9A Adsorption capacities for Cu(II) on *chitosan M.* The capacities were measured under constant temperature at 37 °C where the media is 0.01 M HCl.

Set I

Initial concentration of Cu(II) (mg L^{-1})	Residual concentration of Cu(II) (mg L^{-1})	Adsorption capacity (mg Cu/g chitosan)	Initial pH	Final pH
0	0	0	2.20	4.68
0.50	Nd	-	2.29	4.60
1.20	0.09	0.43	2.28	4.61
1.88	0.29	0.62	2.30	4.60
4.92	1.26	1.40	2.33	4.60
9.55	3.42	2.38	2.34	4.61
18.69	7.46	4.32	2.28	4.56
30.75	14.12	6.42	2.29	4.53
40.95	21.49	7.45	2.24	4.51
49.87	34.64	5.78	2.21	4.49
61.50	35.61	9.82	2.23	4.51
71.99	41.71	11.56	2.11	4.51
81.78	48.84	12.70	2.17	4.49
92.39	57.27	13.38	2.16	4.37
103.69	64.16	15.06	2.16	4.31
107.16	69.28	14.36	2.25	4.51
119.04	78.86	15.41	2.27	4.52
151.21	114.05	14.19	2.07	4.40
151.21	117.56	12.85	2.14	4.48
202.49	146.98	20.99	2.18	4.46
202.49	156.70	17.65	2.18	4.55
252.24	197.16	20.92	2.07	4.57
303.58	241.93	23.07	2.08	4.56

Table 9A (continued).

Set II

Initial concentration of Cu(II) (mg L^{-1})	Residual concentration of Cu(II) (mg L^{-1})	Adsorption capacity (mg Cu/g chitosan)	Initial pH	Final pH
0	0	0	2.20	5.00
0.51	Nd	-	2.23	4.97
1.07	0.08	0.38	2.20	4.95
1.88	0.24	0.63	2.20	4.97
4.92	1.16	1.45	2.20	4.86
9.55	3.33	2.37	2.21	4.81
18.69	9.05	3.70	2.11	4.73
30.75	15.00	6.03	2.11	4.73
40.95	21.00	7.61	2.19	4.68
49.87	31.15	7.19	2.19	4.66
61.50	33.13	10.91	2.21	4.66
71.99	42.07	11.47	2.19	4.69
81.78	47.32	13.18	2.19	4.69
92.39	58.89	12.76	2.17	4.66
103.69	66.89	13.94	2.08	4.69
107.16	71.86	13.52	2.10	4.70
119.04	81.49	14.34	2.12	4.71
151.21	100.48	19.45	2.13	4.68
202.49	142.87	22.86	2.12	4.64
252.24	202.35	18.67	2.08	4.59
303.58	257.44	17.25	2.07	4.60

APPENDIX VII

Experimental data obtained form isotherm studies of Zn(II) and pH changes

Table 10A Adsorption capacities for Zn(II) on *chitosan S.* The capacities were measured under constant temperature at 37 °C where the media is 0.01 M HCl.

Set I

Initial concentration of Zn(II) (mg L^{-1})	Residual concentration of Zn(II) (mg L^{-1})	Adsorption capacity (mg Zn/g chitosan)	Initial pH	Final pH
0	0	0	2.17	5.42
6.88	6.07	0.30	2.18	5.40
12.96	12.41	0.20	2.14	5.33
18.51	17.48	0.38	2.11	5.32
25.32	23.98	0.47	2.16	5.31
36.18	33.67	0.90	2.11	5.34
52.45	49.42	1.08	2.13	5.28
71.98	62.36	3.49	2.14	5.32
80.75	72.49	2.99	2.11	5.28
90.00	85.74	1.51	2.12	4.97
105.83	98.71	2.56	2.06	5.27
133.45	123.21	3.60	2.08	5.32
241.34	227.67	4.94	2.14	5.30
418.70	381.75	13.05	2.06	5.26
493.91	464.09	10.57	2.04	5.11
823.79	787.28	13.17	2.03	5.31

Table 10 A (continued).

Set II

Initial concentration of Zn(II) (mg L^{-1})	Residual concentration of Zn(II) (mg L^{-1})	Adsorption capacity (mg Zn/g chitosan)	Initial pH	Final pH
0	0	0	2.38	5.10
6.88	6.28	0.21	2.33	5.15
12.96	12.09	0.31	2.32	5.17
18.51	16.61	0.69	2.33	5.20
25.32	23.46	0.66	2.33	5.16
36.18	33.40	1.00	2.31	5.16
52.45	44.36	2.88	2.33	5.15
71.98	57.70	5.16	2.30	5.25
80.75	71.41	3.28	2.29	5.30
90.00	79.58	3.66	2.33	5.30
105.83	95.94	3.57	2.30	5.29
133.45	110.74	8.16	2.34	5.30
144.31	117.87	9.42	2.29	5.39
241.34	194.91	16.60	2.28	5.40
418.70	373.32	16.37	2.28	5.52
493.91	463.29	10.85	2.28	5.52
628.58	580.89	16.95	2.27	5.52
823.79	780.04	15.81	2.26	5.54

Table 11A Adsorption capacities for Zn(II) on *chitosan L.* The capacities were measured under constant temperature at 37 °C where the media is 0.01 M HCl.

Set I

Initial concentration of Zn(II) (mg L^{-1})	Residual concentration of Zn(II) (mg L^{-1})	Adsorption capacity (mg Zn/g chitosan)	Initial pH	Final pH
0	0	0	2.17	5.42
6.88	5.04	0.96	2.18	5.40
12.96	11.04	0.99	2.14	5.33
18.51	16.49	1.05	2.11	5.32
25.32	20.44	2.52	2.16	5.31
36.18	32.65	1.80	2.11	5.34
52.45	41.00	5.88	2.13	5.28
71.98	55.54	8.45	2.14	5.32
80.75	63.64	8.77	2.11	5.28
90.00	74.15	8.16	2.12	4.97
105.83	81.45	12.58	2.06	5.27
144.31	124.22	10.27	2.12	5.35
174.86	146.71	14.45	2.13	5.35
336.83	307.27	15.25	2.07	5.35
493.91	461.34	16.55	2.04	5.11
628.58	581.23	24.20	2.03	5.29

Table 11A (continued).

Set II

Initial concentration of Zn(II) (mg L^{-1})	Residual concentration of Zn(II) (mg L^{-1})	Adsorption capacity (mg Zn/g chitosan)	Initial pH	Final pH
0	0	0	2.20	4.95
6.88	5.94	0.49	2.19	4.97
12.96	10.13	1.46	2.19	5.06
18.51	15.27	1.68	2.18	5.11
25.32	19.29	3.12	2.17	5.14
36.18	31.21	2.54	2.17	5.15
52.45	40.51	6.13	2.14	5.18
71.98	52.51	10.01	2.16	5.19
80.75	60.90	10.18	2.14	5.25
90.00	68.49	11.07	2.15	5.26
105.83	87.28	9.57	2.12	5.27
133.45	103.81	15.15	2.11	5.28
144.31	127.16	8.77	2.09	5.29
174.86	159.89	7.68	2.11	5.30
336.83	305.20	16.31	2.07	5.29
379.85	350.89	14.63	2.05	5.29
628.58	581.22	24.20	2.04	5.31
823.79	788.06	18.24	2.05	5.32

Table 12A Adsorption capacities for Zn(II) on *chitosan M.* The capacities were measured under constant temperature at 37 °C where the media is 0.01 M HCl.

Set I

Initial concentration of Zn(II) (mg L^{-1})	Residual concentration of Zn(II) (mg L^{-1})	Adsorption capacity (mg Zn/g chitosan)	Initial pH	Final pH
0	0	0	2.40	5.02
6.88	5.63	0.48	2.33	5.13
12.96	10.24	1.05	2.32	5.16
18.51	16.19	0.89	2.33	5.17
25.32	19.76	2.09	2.33	5.21
36.18	28.97	2.76	2.31	5.24
52.45	42.31	3.90	2.33	5.20
71.98	55.88	6.19	2.30	5.18
80.75	59.68	7.99	2.29	5.23
90.00	73.12	6.50	2.33	5.27
105.83	86.81	7.29	2.30	5.25
133.45	117.49	6.17	2.34	5.34
144.31	128.78	5.95	2.29	5.36
174.86	153.68	8.06	2.31	5.36
241.34	215.38	9.97	2.28	5.42
336.83	302.32	13.25	2.30	5.41
418.70	384.69	13.16	2.28	5.47
628.58	584.19	17.16	2.27	5.54
823.79	792.14	12.24	2.26	5.56

Table 12A (continued).

Set II

Initial concentration of Zn(II) (mg L^{-1})	Residual concentration of Zn(II) (mg L^{-1})	Adsorption capacity (mg Zn/g chitosan)	Initial pH	Final pH
0	0	0	2.38	5.10
6.88	5.59	0.50	2.33	5.15
12.96	10.19	1.07	2.32	5.17
18.51	15.94	0.99	2.33	5.20
36.18	31.38	1.84	2.33	5.16
52.45	41.90	4.02	2.31	5.16
71.98	51.26	7.92	2.33	5.15
80.75	62.20	7.07	2.30	5.25
90.00	70.97	7.25	2.29	5.30
105.83	93.06	4.89	2.33	5.30
133.45	111.73	8.27	2.30	5.29
144.31	128.75	5.95	2.34	5.30
241.34	222.77	6.96	2.31	5.40
336.83	299.88	14.03	2.28	5.41
379.85	353.97	9.96	2.30	5.52
493.91	465.90	10.47	2.28	5.52
628.58	603.16	9.67	2.27	5.52

APPENDIX VIII

Effect of competing cation on metal adsorption in the binary system

Effect of competing cation on metal adsorption in 0.01 M HCl solution was studied in a binary system of Cu(II) and Zn(II). Adsorption of a metal on chitosans was measured when the other metal was added to the system. Adsorption of a metal on chitosans was measured when the other metal was added to the system as described in Section 2.6. Table 13A is the summary of the design when 50 milliliters of the metal mixture was added into 0.2 gram of chitosan.

Table 13A Experimental conditions for adsorption of Cu(II) and Zn(II) ions on chitosans in the binary system.

I) Variation of Zn(II) loading, as the competing ion, when Cu(II) concentration was fixed at approximately 1.5 mg/g chitosan

mg intake Cu^{2+}/g chitosan [a]	$[Cu^{2+}]$ in mg/L[a] for 0.2 gram chitosan	mg Zn^{2-} loaded/g [b]	$[Zn^{2+}]$ in mg/L[b] for 0.2 gram chitosan	Mole ratio of Cu to Zn
1.58	6.30	0.00	0.00	1 : 0
1.44	5.78	1.63	6.52	1 : 1
1.45	5.84	16.2	64.7	1 : 11
1.46	5.84	27.0	108	1 : 19

[a] The values were calculated after FAAS measurement. The values were aimed for 1.3 mg/g chitosan.

[b] The values were calculated after FAAS measurement. The values were aimed for 0, 1.34, 12.5, and 20 mg/g chitosan to give the mole ratio of Cu to Zn at 1: 0, 1: 1, 1: 10 and 1: 20, respectively.

II) Variation of Cu(II) loading, as the competing ion, when Zn(II) concentration was fixed at approximately 8 mg/g chitosan.

mg intake Zn^{2+}/g chitosan [a]	$[Zn^{2+}]$ in mg/L[a] for 0.2 gram chitosan	mg Cu^{2+} loaded/g [b]	$[Cu^{2+}]$ in mg/L[b] for 0.2 gram chitosan	Mole ratio of Zn to Cu
8.36	33.4	0.00	0.00	1 : 0
8.16	32.6	7.92	31.7	1 : 1
8.07	32.3	68.8	275	1 : 9

[a] The values were calculated after FAAS measurement. The values were aimed for 6.63 mg/g chitosan.

[b] The values were calculated after FAAS measurement. The values were aimed for 0, 6.63, and 60 mg/g chitosan to give the mole ratio of Cu to Zn at 1: 0, 1: 1 and 1: 10, respectively.

In each table, the last value of the competing cation loading was greater than the maximum adsorption capacity of all chitosan (the capacities were found to be from 25 to 60 mg Cu/g and from 14 to 20 mg Zn/g (Table 3.3, Section 3.3.1).

According to Table 13A, all solutions were allowed in contact with 0.2 gram of a chitosan sample for two hours. Residual concentrations of Cu(II) and Zn(II) in the supernatant were determined by FAAS using standard addition method. The experiments were carried out in triplicate for each chitosan. Data obtained from these experiments are shown in Table 14A-19A. The results were discussed in Section 3.4.

Table 14A Effect of Zn(II) as the competing cation on the adsorption of Cu(II) on chitosan S.

Ci of Cu(II) ($mg\ L^{-1}$)	Added Zn(II) ($mg\ L^{-1}$)	Qe ($mg\ M^{2+}$/ g chitosan S) (n =3)		Qe ($x10^{-3}$mmol M^{2+}/g chitosan S) (n = 3)	
		Cu	Zn	Cu	Zn
6.30	0	1.44 ± 0.15	0 ± 0.00	22.62 ± 2.43	0 ± 0.00
5.78	6.52	1.42 ± 0.05	0.20 ± 0.04	22.37 ± 0.78	3.10 ± 0.68
5.84	64.72	1.56 ± 0.18	3.83 ± 0.37	24.62 ± 2.79	58.54 ± 5.71
5.84	107.84	1.61 ± 0.05	5.66 ± 0.80	25.39 ± 0.74	86.60 ± 12.27

Table 15A Effect of Zn(II) as the competing cation on the adsorption of Cu(II) on chitosan L.

Ci of Cu(II) ($mg\ L^{-1}$)	Added Zn(II) ($mg\ L^{-1}$)	Qe ($mg\ M^{2+}$/ g Chitosan L) (n =3)		Qe ($x10^{-3}$mmol M^{2+}/g Chitosan L) (n = 3)	
		Cu	Zn	Cu	Zn
6.30	0	2.81 ± 0.03	0 ± 0.00	44.27 ± 0.43	0 ± 0.00
5.78	6.52	2.50 ± 0.09	0.19 ± 0.06	39.36 ± 1.47	2.92 ± 0.95
5.84	64.72	2.66 ± 0.04	1.54 ± 0.80	41.82 ± 0.61	23.58 ± 12.16
5.84	107.84	2.81 ± 0.22	5.73 ± 0.66	44.24 ± 3.54	87.62 ± 10.04

Table 16A Effect of Zn(II) as the competing cation on the adsorption of Cu(II) on chitosan M.

Ci of Cu(II) ($mg\ L^{-1}$)	Added Zn(II) ($mg\ L^{-1}$)	Qe ($mg\ M^{2+}$/ g (chitosan M) (n =3)		Qe ($x10^{-3}$mmol M^{2+}/g chitosan M) (n = 3)	
		Cu	Zn	Cu	Zn
6.30	0	1.53 ± 0.81	0 ± 0.00	24.03 ± 12.71	0 ± 0.00
5.78	6.52	1.42 ± 0.09	0.11 ± 0.06	22.38 ± 8.47	1.69 ± 0.85
5.84	64.72	1.59 ± 0.13	1.79 ± 0.61	25.08 ± 2.11	27.44 ± 9.33
5.84	107.84	1.54 ± 0.18	2.32 ± 0.85	24.19 ± 2.79	35.52 ± 12.96

Table 17A Effect of Cu(II) as the competing cation on the adsorption of Zn(II) on chitosan S.

Ci of Zn(II) ($mg\ L^{-1}$)	Added Cu(II) ($mg\ L^{-1}$)	Qe ($mg\ M^{2+}$/ g chitosan S) (n = 3)		Qe ($x10^{-3}$mmol M^{2+}/g Chitosan S) (n = 3)	
		Zn	Cu	Zn	Cu
33.43	0	1.56 ± 0.43	0 ± 0.00	23.92 ± 6.63	0 ± 0.00
32.64	31.69	0.58 ± 0.23	6.23 ± 0.69	8.81 ± 3.44	97.98 ± 0.94
32.56	125.08	1.38 ± 0.08	21.74 ± 1.56	21.18 ± 1.21	342.16 ± 24.58
32.28	275.22	0.35 ± 0.63	37.98 ± 1.50	5.36 ± 9.57	597.72 ± 23.68

Table 18A Effect of Cu(II) as the competing cation on the adsorption of Zn(II) on chitosan L.

Ci of Zn(II) ($mg\ L^{-1}$)	Added Cu(II) ($mg\ L^{-1}$)	Qe ($mg\ M^{2+}$/ g chitosan L) (n = 3)		Qe ($x10^{-3}$mmol M^{2+}/g chitosan L) (n = 3)	
		Zn	Cu	Zn	Cu
33.43	0	4.46 ± 0.55	0 ± 0.00	68.14 ± 8.41	0 ± 0.00
32.64	31.69	1.85 ± 0.45	14.67 ± 0.12	28.30 ± 6.93	230.93 ± 1.82
32.56	125.08	1.90 ± 0.97	54.00 ± 0.94	29.13 ± 14.81	849.75 ± 14.79
32.28	275.22	1.45 ± 1.08	84.04 ± 1.62	22.21 ± 16.59	1322.54 ± 25.51

Table 19A Effect of Cu(II) as the competing cation on the adsorption of Zn(II) on chitosan M.

Ci of Zn(II) ($mg\ L^{-1}$)	Added Cu(II) ($mg\ L^{-1}$)	Qe ($mg\ M^{2+}$/ g chitosan M) (n = 3)		Qe ($x10^{-3}$mmol M^{2+}/g chitosan M) (n = 3)	
		Zn	Cu	Zn	Cu
33.43	0	0.83 ± 0.81	0 ± 0.00	12.67 ± 12.45	0 ± 0.00
32.64	31.69	0.74± 0.73	6.97 ± 0.41	11.29 ± 11.11	109.64 ± 6.46
32.56	125.08	1.40 ± 0.25	21.14 ± 2.78	21.43 ± 3.82	332.66 ± 43.79
32.28	275.22	0.46 ± 0.52	28.10 ± 3.51	7.08 ± 7.90	442.24 ± 55.21

APPENDIX IX

Adsorption capacities of some metal ions on chitosan

a) Adsorption of copper(II) on chitosan

The adsorption of copper(II) on chitosan was reported by Wan Ngah W.S. et al.[54]. The capacity of the copper ions on chitosan was determined in aqueous solution of $CuSO_4$ at pH 6.2. Different volumes of the stock solution of Cu(II) ion (100 mg L^{-1}) were passed through a 0.2 g chitosan column (i.d. 12 mm, glass tube) with flow rate at 1.0 mL min^{-1}. The effluents were collected in a 100 mL volumetric flask. Column was washed with distilled water. The amount of copper remaining in the combined effluent was determined using the atomic absorption spectrometer. The adsorption isotherm is shown in Figure 5A. The capacity of Cu(II) ion on chitosan was compared with commercially resin, Dowex A-1 and Zerolit 225. The capacities of Cu(II) ion on chitosan, Dowex A-1 and Zerolit 225 were 4700, 2.3 and 440 μg/g, respectively. The results clearly demonstrated that chitosan had a greater specific adsorption capacity than the commercial chelating resin used.

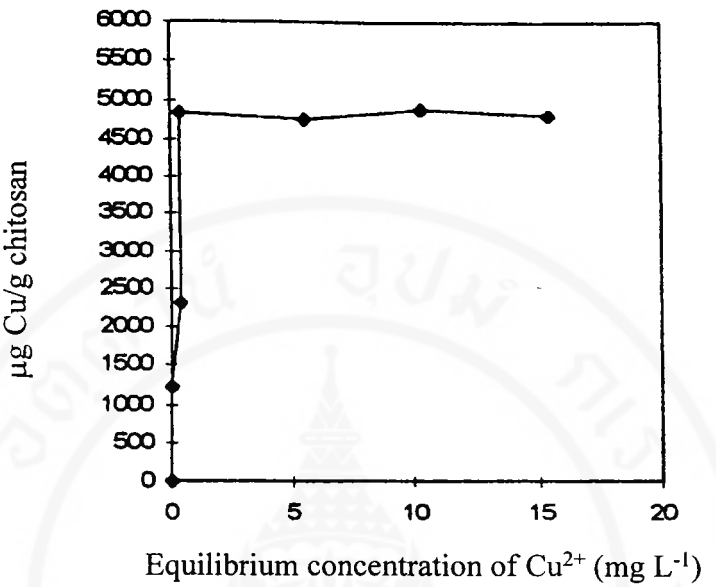

Figure 5A Adsorption isotherm of Cu(II) ion on chitosan [54].

b) Adsorption of mercuric ion on chitosan

The adsorption of mercuric ion on chitosan was investigated by Peniche-Covas C. et al. [25]. The adsorption isotherm experiments were performed by suspending 0.2 g of chitosan (%deacetylation = 90 ± 1) in 20 mL of various concentrations of mercuric chloride solutions (10 to 24 x 10^3 mg L^{-1}). The mixtures were stirred at 25 °C for three hours. In all case, the working pH was that of the solution. The solid was separated by filtration and the amount of mercury remaining in solution was determined by Flame Atomic Absorption Spectrometer (FAAS) at ,253 nm. Low concentrations of Hg^{2+} were determined by the cold-vapor techniques. Adsorption isotherm of Hg^{2+} on chitosan is shown in Figure 6A.

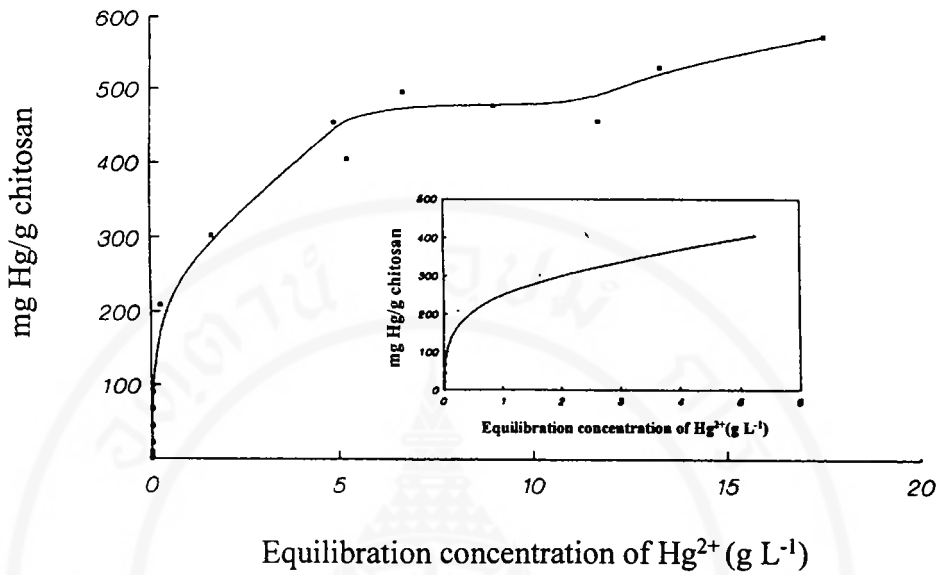

Figure 6A Adsorption isotherm of Hg^{2+} on chitosan. The first part of the curve is shown enhanced inside the figure [25].

In Figure 6A, The adsorption capacity increases as the concentration of mercuric chloride increases until an equilibrium concentration of approximately 5 x 10^3 $mgHg^{2+}$ L^{-1} is reached. For equilibrium concentrations above 12 x 10^3 $mgHg^{2+}$ L^{-1}, there is a further increase in the amounted of mercury retained. The first part of this curve, which is shown in enhanced inside the figure, exhibits the form of Langmuir isotherm. The adsorption in this stage is due mainly to complexation of mercuric ions with the amino groups of chitosan. The adsorption capacity that calculated from Langmuir model is 430 mg Hg^{2+} per gram of chitosan.

c) Adsorption of hexavalent chromium on chitosan

The adsorption of Cr(VI) on chitosan was investigated by Udaybhaskar et al. [56]. Adsorption isotherm experiment was carried out in aqueous solution of $K_2Cr_2O_7$ at room temperature (25 ± 2 °C) for 12 hours in rotary shaker using 300 mL glass bottles. Isotherm studies were conducted with a constant weight of chitosan and varying initial concentration of Cr(VI). Adsorption isotherm of Cr(VI) were studied in two different particle sizes are presented in Figure 7A. A slight increase in capacity for the smaller particles can be due to the increase in surface area. The adsorption capacity of Cr(VI) on chitosan (particle size 370 μm) that calculated based on Langmuir equation was 34.96 mg Cr(VI)/g chitosan. It was found that presence of electrolytes and chloride significantly affected the Cr(VI) adsorption, indicating the electrostatic attraction as the main adsorption mechanism.

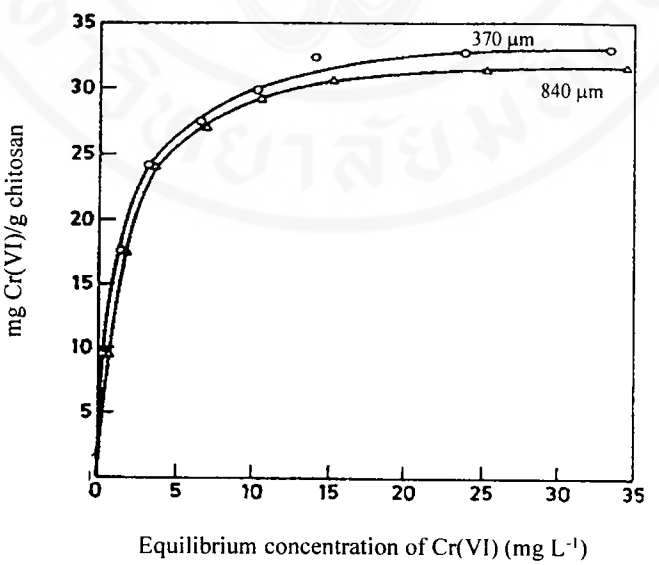

Figure 7A Adsorption isotherm of Cr(VI) on two different particle sizes of chitosan, 370 and 840 μm [56].

BIOGRAPHY

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