# Biosorption using chitosan thiourea polymer as an extraction and preconcentration technique for copper prior to its determination in environmental and food samples by flame atomic absorption spectrometry: Synthesis, characterization and analytical applications

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## ARTICLE INFO

## ABSTRACT

The present work describes the synthesis and use of the chitosan thiourea polymer (CT polymer) as a novel biosorbent for copper preconcentration prior to its determination by flame atomic absorption spectrometry (FAAS). CT polymer submitted in the present study was synthesized by the direct reaction between chitosan isolated from penaeus monodon chitin and ammonium thiocyanate, then the chemical structure and morphology of polymer were investigated by spectroscopic measurements, thermal analysis, X—ray diffraction (XRD) and scanning electron microscope (SEM). The results of SEM and XRD show that the prepared CT polymer has rough and hard surface with pores and nanofibers structure in the α—form. The height equivalent to the theoretical plates (HETP), the plates number (N), LOD and LOQ of CT polymer packed column were calculated to be $0.10 \pm 0.04$ mm, $100 \pm 2.02$, $0.3\,\mu g\,L^{-1}$ and $0.99\,\mu g\,L^{-1}$, respectively. The developed column was employed in combination with FAAS for the analysis of copper in a certified reference material (NRCC—SLRS-4 Riverine water), potato, tea, and rice. Furthermore, the structure of the copper complex with CT polymer was proven with the aid of different spectroscopic and magnetic measurements.

## 1. Introduction

Copper is one of the basic and necessary elements which has wide applications in our life. The excellent electric conductivity of this element makes it widely used material in many electrical industries. As a result of these applications, copper ions release to environment and subsequently accumulate in living organisms causing problems for human, animal, and plant as reported by world health organization [1]. In general, the trace elements determination is one of the most important targets in analytical chemistry because the direct use of conventional analysis techniques e.g. atomic absorption/emission spectrometry and spectrophotometry is inappropriate for monitoring extremely low concentration levels in the real samples [2]. However, the determination of such elements at sub-parts per million (sub-ppb) levels may be performed by the different separation and preconcentration techniques. Among these techniques, solid phase extraction (SPE) has found a wide range of applications due to low cost, easy

to use, high extraction efficiency, and the ability of combination with many detection techniques [3].

Biosorption is an extraction technique in which the materials of biological origin are employed as stationary phases for the extraction of analytes by different mechanisms like chelation, and ion exchange [4]. Although, biosorption has several benefits such as low cost, and simple operation, the selectivity of this technique is low since most biological materials have affinity to react with a relatively large number of metal ions [5]. However, the chemical modification of raw biological materials may make them more selective for certain ions or species.

Chitosan composed of glucosamine units [β-(1 → 4)-2-amino-2-deoxy-ᴅ-glucopyranose] and acetyl glucosamine units [β-(1 → 4)-2-acetamido-2-deoxy-ᴅ-glucopyranose] may be derived from the chitin isolated from exoskeletons of some crustaceans like prawns, crabs, and shrimps by deacetylation with strong alkali [6]. However, the raw chitosan suffers from some disadvantages. As an example, the low selectivity of metal ions, therefore, it requires chemical modifications of the type of functionalization with suitable functional groups and graft copolymerization to improve its efficiency [7]. Thus, various chitosan derivatives have been pre-

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pared and used for the selective extraction of some heavy metal ions [8]. However, a recent literature on the applications of chitosan derivatives to analytical chemistry has revealed that no study on the use of the chitosan thiourea polymer ligand abbreviated as CT polymer for copper(II) determination. Therefore, the main goals of the present work will be focused on: (i) isolation of chitin from tiger shrimp (Penaeus monodon) and conversion of it to chitosan by deacetylation; (ii) the synthesis and characterization of CT polymer yielded by reaction of chitosan with $NH_4SCN$; (iii) synthesis of Cu(II) complex with CT polymer and studying its chemical structure using known characterization techniques; and finally (iv) developing a simple, low cost and precise method based upon a column packed with CT polymer for separation, and determination of copper at ultra trace concentration levels in water and food samples.

## 2. Experimental

### 2.1. Instrumentations

A PerkinElmer (AAnalyst 800, Norwalk, CT, USA) flame atomic absorption spectrometer containing a deuterium background correction system was operated according to the guidelines of the manufacturer for copper determination. A PerkinElmer ICP-MS Sciex model Elan DRC II (California, CT, USA) was employed as a reference procedure for the determination of copper in food samples and measurement of other elements concentrations when studying metal uptake by CT polymer under non-competitive and competitive conditions. The FT – IR spectra in the range of 4000–400 $cm^{-1}$ of the investigated compounds were recorded as KBr discs by FT – IR Shimadzu spectrophotometer model 4000. Carbon, hydrogen, nitrogen and sulfur contents were measured by elemental analyzer model PerkinElmer 2400C series (California, CT, USA). The electronic spectra of the compounds were recorded by Angstrom spectrophotometer model UV1900 (Angstrom Advanced Inc., Boston, USA) with one centimeter quartz cell. The X – ray analysis and the morphology of CT polymer were done in center of Nanotechnology, King Abdulaziz University, KSA and using an ultima-IV (Rigaku, japan) multipurpose X – ray diffraction system and field emission – scanning electron microscope (FESEM) (JSM-7500F; JEOL Japan), respectively. Cu K-$\alpha$ source ($\lambda$ = 0.154060 nm) was used with X – ray diffraction system to scan in 2θ range of 5° to 90°, while, the electron microscope studied the morphology of synthesized polymer using parallel beam geometry and a multi-purpose thin film attachment. Electron spin resonance (ESR) spectrum of Cu – CT complex was recorded by Bruker spectrometer model EMXplus (Billerica, Massachusetts, USA) with 100 kHz modulation frequency. Magnetic susceptibility of the complex was measured by the Gouy method at room temperature using Alfa Aesar magnetic susceptibility balance model MKII (Ward Hill, Massachusetts, USA). The effective magnetic moment ($\mu_{eff}$) was calculated by the mathematical expression of $\mu_{eff} = 2.828 (\chi_M.T)^{1/2}$ B.M., where $\chi_M$ is the molar susceptibility corrected using Pascal's constants for the diamagnetism of all atoms in the compound and T is the absolute temperature [9].

### 2.2. Materials and reagents

Unless otherwise stated, all chemicals and solvents employed in this work were of analytical reagent-grade quality and were used without further purification. They were all purchased from Merck (Darmstadt, Germany). Stock aqueous standard solution (1000 $\mu g\,mL^{-1}$) of $Cu^{2+}$ ions was prepared by dissolving an appropriate amount of $Cu(NO_3)_2 \cdot 3H_2O$ in deionized water, and more diluted standard solutions were prepared by suitable dilution of the stock solution. Stock solutions (1000 $\mu g\,mL^{-1}$) of metal ions

employed to test the selectivity were prepared from their nitrate or chloride salts in deionized water. Penaeus monodon was collected from El-Kaakia fish market in Makkah, Saudi Arabia. The shell structure was separated from the body and washed several times with distilled water. Sample was then oven-dried at 60 °C for 36 h. The dried sample was crushed into a fine powder by a porcelain mortar.

### 2.3. Isolation of chitin

For demineralization, powdered raw material (25 g) was treated by HCl (250 mL, 2 mol $L^{-1}$) at ambient temperature for 2 h. After washing sample with distilled water, and drying at 60 °C for 24 h in an oven, deproteinization was performed by refluxing with NaOH (2 mol $L^{-1}$) for 19 h, and the sample was then washed with deionized water until a neutral pH was achieved. The chitin isolated was highly pink, So, pigment traces responsible for this color were removed using an oxidizing treatment by the mixture of $KMnO_4$, oxalic acid, and $H_2SO_4$. Finally, the purification of the obtained chitin was carried out by washing with hot ethanol and boiling in acetone to remove any impurities. The purified chitin was dried in a vacuum oven at 60 °C to constant weight. Chitin content was determined by weight difference between the raw material and the chitin obtained after acidic and alkaline treatments.

### 2.4. Conversion of chitin into chitosan by N-deacetylation

The chitin extracted from penaeus monodon was treated by 50% NaOH (15 mL/g) at 100 °C for 18 h. After filtration, the residue was washed to neutrality with hot distilled water for several times. The obtained chitosan was dried in a vacuum oven at 50 °C for 24 h, and purified by dissolving in 4% acetic acid and re-precipitation using 20% NaOH solution. Sample was then washed with deionized water until neutralization and freeze-dried.

### 2.5. The synthesis of CT polymer

A solution of ammonium thiocyanate (1.522 g) in 50 mL ethanol was added drop wise to a suspension of chitosan (1.612 g) in 50 mL ethanol. The reaction mixture was heated to reflux for 18 h. After cooling down to room temperature, the precipitate was collected by filtration and repeatedly washed with ethanol. The filtrate was checked for excess of ammonium thiocyanate by iron(III) ions. A white product was obtained by filtration and then left in drying oven at 90 °C until complete dryness and then kept in a desiccator until used. The yield was 1.725 g (78.31%) and its melting point was above 300 °C. Scheme 1 represents the formation of CT polymer. Elemental analysis; calculated (found)%, C; 38.18 (37.84), H; 5.49 (5.82), N; 12.72 (12.97) and S; 14.56 (14.33).

### 2.6. Determination of the deacetylation degree of CT polymer

The degree of deacetylation (DDA) of chitosan isolated from penaeus monodon chitin was determined using FT – IR spectra and with the aid of the following equation to be 93% [10]:

$$DDA\% = \left[ 100 - \left( \frac{A_{1658}}{A_{3450}} \right) \times 115 \right] \tag{1}$$

where, $A_{1658}$ and $A_{3450}$ are the absorbance values at the wave numbers corresponding to the amide and the primary amino groups of chitosan, respectively, and the factor (115) represents the value of the ratio of $A_{1658}/A_{3450}$ for the fully N-acetylated chitosan.