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DETERMINATION OF TRACE AMOUNT OF CU (II) IN SOIL AND VEGETABLE (TOMATO, CABBAGE AND SPINACH) SAMPLES COLLECTED FROM KATHMANDU USING HETEROCYCLIC THIOSEMICARBAZONE

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ABSTRACT

Copper is an essential trace element that is vital to the health of all living things and is essential to the proper functioning of organs and metabolic processes. While iron and zinc have long been known as metal ions that are important for life, it is clear that Cu is also a critical metal in biology. Given that Cu is also a potentially dangerous toxin exploited by immune cells and that Cu dysregulation causes human disease, the homeostasis of this metal ion must be under exquisite regulatory control. Many questions remain to be deciphered with respect to Cu's role in biology, including an articulation of the entire constellation of Cu-dependent processes, how hosts and microbes interact with respect to Cu, how cells and organs distribute and communicate their Cu status, and precisely how Cu dysregulation contributes to human disease. With the evolution of single-cell eukaryotes came the new challenge of deliverying Cu to an expanding array of metalloproteins located within organelles such as mitochondria, chloroplasts, and the secretory compartments. Furthermore, with the subsequent appearance of multicellular organisms came the requirement to regulate Cu allocations to specific tissues according to varying metabolic needs. Thus, Cu homeostasis became a more complex and tightly controlled process at both an intracellular and intercellular level. Thus Trace amount of Copper has determined by spectrophotometric technique using 1-(2-pyridylazo)-2-naphthal, as a new spectrophotometric reagent. 1-(2pyridylazo)-2-naphthal reacts in highly acidic solution at pH 2.45 to 2.55 with Copper to give a pink chelate which has an absorption maximum at 550 nm. The method has high precision and accuracy.

KEYWORDS: Spectrophotometry, 2-Acetylpyridine thiosemicarbazone (2-APT), 3-Acetylpyridine thiosemicarbazone (3-APT), Soil, Vegetables, Kathmandu, Nepal.

1. INTRODUCTION

Life on Earth has evolved within a complex mixture of organic and inorganic compounds. While organic molecules such as amino acids, carbohydrates and nucleotides form the backbone of proteins and genetic material, these fundamental components of macromolecules are enzymatically synthesized and ultimately degraded.[1] Inorganic elements, such as copper (Cu), iron and zinc, once solubilized from the Earth's crust, are neither created nor destroyed and therefore their homeostatic regulation is under strict control. After the rise of photosynthetic organisms such as the cyanobacteria, oxygen accumulated in the atmosphere and oxygenated the oceans.[2] This led to a decrease in the solubility of iron and an expansion of the biological role of Cu, suggesting there was a shift from the exclusive use of iron in biology to embrace similar, though not identical, roles for Cu. One property of Cu that drives its diverse roles in structure and catalysis is its existence in either a reduced (Cu+) state or an oxidized (Cu2+) state. Copper can be considered either essential or hazardous to life and plays a substantial role in the environment.[3] Excessive intake of copper can cause accumulation especially in liver cells and cause hemolytic crisis and neurological disturbances.[4]

Copper (Cu) is found in a variety of cells and tissues with the highest concentrations in the liver and brain.[5] Cu is largely present in biological systems as cupric form (Cu++), although several distinct types of the bound cation can be found in Cu containing enzymes, often in combination within a single protein.[6] Several analytical techniques have been used for determination of copper, including atomic absorption spectrometry, atomic emission spectrometry, electroanalytical techniques, spectrophotometry, inductive coupled plasma-emission spectrometry, inductive coupled plasma-mass spectrometry, flow injection diode array spectrophotometry and X-ray fluorescence spectrometry.[7] However, spectrophotometry methods are often preferred, as they involve inexpensive instrument, less labor-intensive, and provide comparable sensitivity when appropriate chromogenic reagents are available.[8]

Thiosemicarbazone derivatives are of considerable interest due to their versatility as ligands bearing suitable donor atoms for coordination to metals with strong coordinating ability toward different metal ions.[9] They show a wide range of chemical properties depending on the parent aldehyde or ketone; in particular, if these are heterocyclic aromatic systems, their nature seems to enhance their activity.[10] Thiosemicarbazones are basically Schiff bases obtained by the condensation of a thiosemicarbazide, prepared from an aryl, aralkyl, or alkyl isothiocyanate and hydrazine, with an aldehyde or ketone under ambient conditions.[11] Therefore the aim of this work was to develop a highly sensitive, efficient and direct spectrophotometric method for Cu(II) determination using chromogenic reagents containing a Schiff base. The conditions for the direct spectrophotometric determination of Cu(II) with 2-APT and 3-APT were described. Various factors influencing the sensitivity of the proposed method such as the pH and ranges of applicability of Beer's law on the determination of Cu(II) were also studied. The method was applied to soil and vegetable samples (tomato, cabbage and spinach) as well and compared to standard method (F-AAS).

2. MATERIALS AND METHODS

2.1 Sample collection

Kathmandu is the largest metropolis capital city of Nepal with a population of 1.5 million in the city proper, and 3 million in its urban agglomeration across the Kathmandu Valley, which includes the towns of Lalitpur, Kirtipur, Madhyapur Thimi, Bhaktapur making the total population to roughly 5 million people and the municipalities across Kathmandu valley. Kathmandu is also the largest metropolis in the Himalayan hill region. Nepali is the most spoken language in the city, while English is understood by the city's educated residents. Thus the soil samples were collected from three sampling spots (Lalitpur, Kirtipur and Bhaktapur) districts in the Valley while the vegetable samples were collected from the local Kalimati market. They were kept in plastic bags, kept in ice-box, and once in the laboratory, oven-dried, sieved to powdered and stored in double-cup polyethylene bottles prior to analysis.

2.2 Chemicals

The chemicals used were 99% N, N-dimethylformamide (DMF), 99.9% dimethyl sulphoxide (DMSO), 98% 2-acetylpyridine, 98% 3-acetylpyridine, 99% thiosemicarbazone, 37% hydrochloric acid, 65% nitric acid, 30% hydrogen peroxide, 98% sulphuric acid, ethanol, ammonium chloride, ammonium hydroxide, 99% acetic acid, sodium acetate, 98% copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$). All the chemicals were analytical grade reagents. All glassware were cleaned with 5% HNO_3.

2.3 Preparation of Cu(II) and buffer solutions

A stock solution of Cu (II) (1 mg/mL) was prepared by dissolving 3.93 g of CuSO4·5H2O in distilled water containing a few drops of conc. H2SO4. The solution was made up to 1 L and standardized by iodometry.[12] This stock solution was further diluted, whenever necessary, with distilled water. The buffer solutions were prepared by mixing 1 M HCl and 1 M sodium acetate (pH 1-3), 0.2 M acetic acid and 0.2 M sodium acetate (pH 3.2–6.0), 1M sodium acetate and 0.2 M acetic acid (pH 7.0), and 2 M ammonium hydroxide and 2 M ammonium chloride (pH 8.0-12.0). Suitable portions of these solutions were mixed to get the desired pH.

2.4 Synthesis of Schiff base ligands and their respective metal complexes

The Schiff base, 2-APT was prepared using 3 mmol of 2-acetylpyridine in ethanol (10 mL) and mixed with equimolar amount of thiosemicarbazone in hot water (20 mL). The mixture was refluxed for 5 h and cooled to room temperature. The crystals obtained were subjected to filtration, washed with cold ethanol and dried.[13] The same procedure was used for preparation of 3-APT using 3-acetylpyridine. A 1×10^{-2} M stock solution of each reagent was prepared by dissolving 0.049 g of the specific reagent with DMF and suitably diluted to get the required concentrations wherever necessary. For the synthesis of metal complexes, a hot ethanolic solution (25 mL) of free ligand (2 mmol) and a hot ethanolic solution (25 mL) of the metal salt (1 mmol) were refluxed for 4 h at 50°C. After cooling to room temperature, the yellow solid was collected by filtration and washed sequentially with water and ethanol to give the desired product.[14]

2.5 Absorption spectra of reagent solutions and metal complexes

An aliquot of reagent (usually 1 mL of 1×10^{-2} M) solution was taken in a 25-mL volumetric flask containing 10 mL of buffer solution and made to the mark with distilled water. The absorbance of the reagent solution was measured against water blank. For measuring the absorption spectrum of complex, in a 25-mL volumetric flask, the metal complex in solution was prepared by taking 10 mL of buffer and suitable concentration of metal ion and reagent (tenfold molar excess to metal ion) solutions. The contents were diluted to the mark with distilled water and the absorbance was measured against the reagent blank.

2.6 Determination of the stoichiometry of the complex

The composition of the Cu(II)–2-APT and Cu(II)–3-APT complexes were determined by Job's continuous variation[15] and mole ratio methods.[16] For Job's method of continuous variation 10 mL of buffer solution, and equimolar solutions of Cu(II) and the reagent were prepared in a series of 25-mL volumetric flasks. The metal and reagent solutions were mixed in different proportions and diluted to the mark with distilled water. The absorbances were recorded at λmax, against the corresponding reagent blank. A plot between mole fraction of the metal ion and the absorbance was made. The stability constants of the complexes were also calculated. For mole ratio method 10 mL of buffer solution, constant amount of metal ion and known and varying aliquots of the reagent solutions were added in a series of 25-mL volumetric flasks. The contents of each flask were made to the mark with distilled water. The absorbances were recorded at λmax, against the corresponding reagent blank. The composition of the complex was ascertained from the plot between the absorbance and the volume of the reagent.

2.7 Instrumentation

The UV-Vis absorption spectra were recorded using Shimadzu 2450 double beam spectrophotometer at 800–200 nm range. An ELICO digital pH meter (Model LI-120) with combined glass electrode was used for measurement of pH. In order to characterize the synthesized reagent, the IR spectrum was recorded as KBr discs using Perkin-Elmer (spectrum 100) IR spectrophotometer in the 4000–200 cm^{-1} range. The 1 HNMR spectra were taken at 400 MHz on a JEOL GSX-400 high resolution spectrometer at room temperature using tetramethylsilane as the internal standard. The mass spectra were recorded on Micro Mass VG-7070 H Mass spectrometer. The copper concentration was determined using Varian AA 240FS fast sequential atomic absorption spectrometer.

2.8 Statistical analysis

All mathematical and statistical computations were made using Excel 2007 (Microsoft Office) and Origin Pro 8.5.0 SR1 (Origin Lab Corporation, USA). Data were reported as mean ± SD. Student t test was used for comparison of the developed method with standard method.

3. RESULTS AND DISCUSSION

3.1 Characterization of 2-ATP and 3-ATP

The 2-ATP and 3-ATP were characterized by IR, 1 HNMR and MS. The IR spectrum of 2-APT exhibits absorption bands corresponding to υ (N–H, asym), υ (N–H, sym), υ (C–H, pyridine), υ (C=N, Schiff's base), υ (C–C, pyridine), δ (C–H, aromatic ring), υ (N–H, primary amide), υ (C=S) and δ (C–H, aromatic ring) at 3373 (m), 3261 (m, br), 3183 (s), 1608 (s), 1501 (s), 1466 (s), 1244 (w), 1086 (m) and 783 (m) cm−1 , respectively. Similarly, the 3-APT shows bands corresponding to υ (N–H, asym), υ (N–H, sym), υ (C–H, pyridine), υ (C=N, Schiff's base), υ (C–C, pyridine), υ (C–H, aromatic ring), υ (N–H, primary amide), υ (C=S) and δ (C–H, aromatic ring) at 3434 (m), 3387 (m, br), 3270 (s), 1611 (s), 1504 (s), 1406 (s), 1104 (w), 1088 (m) and 704 (m) cm^{-1}, respectively. These results were comparable with the one reported by Cobeljic et al.[11], Manikandan et al.[13] and Singh and Singh.[9]

The 1 H NMR spectra of free ligand, 2-APT showed signal at δ 6.8–7.9 ppm, characteristics of the protons of aromatic moieties (pyridine ring protons) of the ligand were observed as multiplets. The peak appeared at 2.4 ppm has been assigned to methyl group.[13] Similarly, the 1 H NMR spectra of free ligand, 3-APT showed a signal at δ 7.1–8.6 ppm (multiplets), characteristics of the protons of aromatic moieties (pyridine ring protons) of the ligand, while the methyl group peak appeared at 1.1 ppm. The mass spectrum of 2-APT and 3-ATP shows signal at 195 (M + 1) corresponding to its molecular ion peak. The molecular formula of the reagent is C8H10N4S (M. Wt 194).

The light yellow Cu(II)–2-APT and Cu(II)–3-APT complexes has a maximum absorbance at 370 and 350 nm respectively. These complexes are stable for 46 h. It is expected that the efficiency of the extraction process should mainly depend on the metalextractant complex formation and the concentration of the extractable species (closely related to pH of the system). Therefore, the effect of the equilibrium pH on the extraction of Cu (II) was examined in the range from 1.0 to 10.0 for both methods. The results indicated that the complexes required alkaline (8.0–10.0) condition and hence pH of 9.0 was selected as the optimal condition for further experiments. The effects of reagent concentration on the absorbance of the complex were studied at λmax. The obtained results indicate that fivefold molar excess of reagent is required for full color development. Therefore further studies were carried out using at least fivefold molar excess of reagent to Cu (II).

3.2 Beer's law and sensitivity of Cu(II)–2-APT and Cu(II)–3-APT complexes

A calibration graph for the determination of copper was prepared under the optimum experimental conditions. The Cu(II)–2-APT system obeys Beer's law in the concentration range of 0.16–1.3 µg/mL with the equation A370 = 0.66194C + 0.09596. The molar absorptivity and Sandell's sensitivity of the method are 2.1 × 104 L/mol cm and 0.009 µg/cm^2 of Cu(II), respectively. The replicate (n = 10) analyses of a solution containing 1.0 µg/mL of Cu(II) gave 1.08 ± 0.0123 (%RSD = 1.14%). The Cu(II)–3-APT system obeys Beer's law in the concentration range of 0.44–1.05 µg/mL with the equation A350 = 0.51782C + 0.10188. The molar absorptivity and Sandell's sensitivity of the method are 6.7 × 10^3 L/ mol cm and 0.029 µg/cm2 of Cu(II), respectively. The replicate (n = 10) analyses of a solution was 1.028 ± 0.01345 (%RSD = 1.31%). The correlation coefficient values of the standard curves for Cu(II)–2-APT and Cu(II)–3-APT complexes were found to be 0.999 and 0.998, respectively, showing excellent linearity of the developed methods. The detection limit of the Cu(II)-2-APT and 3-APT methods are 0.053 and 0.147 µg/mL respectively. Comparing the two reagents, 2-APT is more sensitive than 3-APT for Cu (II) determination.

3.3 Analytical applications

In order to confirm the applicability of the proposed method, it has been applied to the determination of Cu(II) in vegetable and soil samples. The results for this study are presented in Tables 1 and 2. A flame atomic absorption spectrometry (F-AAS) method was used as a standard reference method and the results are also shown in Tables 1 and 2. The performance of the proposed method was compared with F-AAS method using student t test.

Table 1: Determination of Cu(II) (mean±SD, n=3) in vegetable samples.

Vegetable	Amount of Cu (II) found (µg/g) by		
	F-AAS method	2-ATP method	3-ATP method
Tomato	15.4±0.35	15.7±0.51	16.4±0.15
Cabbage	18.1±0.26	18.5±0.23	18.3±0.41
Spinach	15±0.39	16.1±0.39	16.4±0.17

Using paired sample t test at the 0.05 level, the developed methods are not significantly different with the standard method of F-AAS. Therefore, the results of the developed methods are in good agreement with the standard method. Moreover, comparing the results obtained from the two spectrophotometric reagents, the differences of the developed methods are not significantly different.

Table 2: Determination of Cu(II) (mean±SD, n=3) in soil samples.

Vegetable	Amount of Cu (II) found (µg/g) by		
	F-AAS method	2-ATP method	3-ATP method
Lalitpur	0.47±0.23	0.49.7±0.11	0.48.4±0.35
Kirtipur	0.81±0.72	88.5±0.21	87.3±0.29
Bhaktapur	0.17±0.18	16.1±0.39	16.4±0.17

The present method was also compared with other existing spectrophotometric methods in the literature Nalawade et al.[18] and Kamble et al.[19] The developed methods are comparable with the reported methods with respect to the aforementioned analytical performances. Therefore, the proposed method could be used as an alternative method for copper determination in various media.

4. SUMMARY AND CONCLUSION

Copper (Cu) is a vital mineral essential for many biological processes. The vast majority of all Cu in healthy humans is associated with enzyme prosthetic groups or bound to proteins. Cu homeostasis is tightly regulated through a complex system of Cu transporters and chaperone proteins. Excess or toxicity of Cu, which is associated with the pathogenesis of hepatic disorder, neurodegenerative changes and other disease conditions, can occur when Cu homeostasis is disrupted. Cu homeostasis is generally well-maintained with effective regulatory mechanisms, and Cu toxicity resulting from disturbed homeostasis is an important contributor to numerous different symptoms and disease conditions. These disease states are most often linked to the role of Cu as a redox-active transition metal that may initiate oxidative damage. The capacity to initiate oxidative damage is most commonly attributed to Cu-induced cellular toxicity. So, in this experiment 2-APT and 3-APT have been synthesized and characterized. The proposed ligands have been successfully applied as complexing agent to determine Cu(II) using spectrophotometry. The developed methods are practical and valuable for determination of copper. The results showed good agreement with the results obtained by F-AAS methods for soil and vegetable samples.

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