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Development of an Extractive Spectrophotometric Method for the Determination of Copper(II) in Leafy Vegetable and Pharmaceutical Samples Using Pyridoxal-4-phenyl-3-thiosemicarbazone (PPT)

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A highly sensitive extractive spectrophotometric method has been developed for the determination of copper(II) using pyridoxal-4-phenyl-3-thilosemicarbazone (PPT) as an analytical reagent. The PPT forms reddish brown species of copper(II) at a pH range of 3.0−5.5, and the complex was extracted into *n*-butanol. The Cu(II)−PPT complex shows maximum absorbance at 440 nm, with molar absorptivity and Sandell's sensitivity being 2.16×10^4 L mol^{-1} cm^{-1} and 2.94×10^{-3} μg cm^{-2}, respectively. The system obeys Beer's law in the range of 0.2−5.0 mg/L. The regression coefficient of the Beer's law straight line is 0.338, and the correlation coefficient is 0.96. The detection limit of the method is 0.0065 μg mL^{-1}. Most of the common metal ions generally found associated with copper do not interfere. The repeatability of the method was checked by finding the relative standard deviation. The developed method has been successfully employed for the determination of copper(II) in leafy vegetable and pharmaceutical samples. The method is evaluated by analyzing samples from the Bureau of Analyzed Samples (BCS 233, 266, 216/1, 207, and 179) and by intercomparison of experimental values using AAS.

KEYWORDS: Copper(II); leafy vegetables; pharmaceuticals; pyridoxal-4-phenyl-3-thiosemicarbazone (PPT); extractive spectophotometric method

INTRODUCTION

Copper is one of several metal ions that play an important role in the biological system. It plays a key role during cell respiration, in the blood of invertebrate animals, and in the formation of hemocyanin, an important respiratory protein, found in the lymph of most animals belonging to Phyla Mollusca and Arthropoda. From the standpoint of human health, its role in three physiological functions is of prime importance. Copper is involved in hemopoiesis and in maintenance of vascular and skeletal integrity in additon to the structure and function of the central nervous system.

Copper occurs naturally in most vegetables, meats, and grains. The study of copper in food items is of great concern, because it plays a definitive role in the intrinsic mechanisms regulating vital biological processes (*1*). Overexposure to copper causes metallic taste, ptyalism, nausea, vomiting, epigastric burning, and diarrhea. Heavy doses of copper cause a series of systematic toxic effects such as hemolysis, hepatic neurosis, gastrointestinal bleeding, oliguria azotemia, hemoglobinuria, hematuria, proteinuria, hypertension, tachycardia, convulsions, and coma. When a congenital deficiency in the homeostatic mechanism for copper exists, the metal accumulates in the liver, discrete areas of the brain, the cornea of the eye, and other tissues, causing Wilson's disease. A wide variety of clinical disorders have been associated with a dietary deficiency of copper, which respond to copper therapy. They include anemia, depressed growth, bone disorders, depigmentation of hair or wool, abnormal wool growth, neonatal ataxia, impaired reproductive performance, heart failure, and gastrointestinal disturbances (*2*). In view of this, the separation and determination of copper from associated elements is indispensable.

For the determination of copper at micro levels there are several frequently adopted methods using analytical techniques such as AAS, ICP-OES, X-ray fluorescence spectroscopy, spectrophotometry, spectrofluorometry, and other such techniques. Among these, the spectrophotometric methods are preferred as they are cheaper and easier to handle and have comparable sensitivity.

A number of spectrophotometric reagents have been used for the determination of copper(II), but a very few number are used

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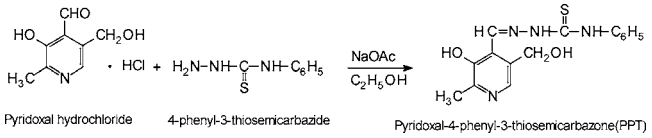
Scheme 1



for the separation and determination of it. Thio and phenyl thiosemicarbazones are important sulfur- and nitrogen-containing organic reagents, where copper coordinates with these reagents to form stable complexes. As it is more stable in its divalent state, it is extracted into organic solvents such as chloroform, *n*-butanol, and others as a divalent complex. The metal chelates of these sulfur- and nitrogen-containing organic reagents find a wide range of applications in medicine (*3*) and agriculture. The reviewed (*4*, *5*) literature revealed that only a few thio and phenyl thiosemicarbazones were employed for extractive determination of copper(II) (*6*−*15*). Hence, the authors introduced a new reagenr, pyridoxal-4-phenyl-3-thiosemicarbazone (PPT), for the selective and spectrophotometric determination of Cu(II) in leafy vegetable, pharmaceutical, and Bureau of Analyzed Samples samples. Our previous studies of transition metal ions such as zinc, cobalt, and palladium extracted from biological and environmental samples using PPT were established (*15*−*19*).

MATERIALS AND METHODS

Apparatus and Reagents. A Shimadzu 240 UV−Vis spectrophotometer with a 1.0 cm quartz cell was used for absorbance studies. An Elico LI-120 digital pH-meter was used for pH adjustment. A Perkin-Elmer 2380 atomic absorption spectrometer was used for the comparison of results. All reagents used were of analytical reagent grade unless otherwise stated.

Synthesis of Pyridoxal-4-phenyl-3-thiosemicarbazone. The chelating agent used for complexation in the present study is PPT. PPT can be prepared as per the procedure reported (*20*). One gram of pyridoxal hydrochloride was dissolved in 15 mL of demineralized double-distilled water and mixed in a flask with 50 mL of ethanol containing 0.8 g of 4-phenyl-3-thiosemicarbazide. The resulting solution was neutralized with sodium acetate and refluxed under heat for 30 min. It was allowed to stand at room temperature until yellow crystals were formed. These were separated and recrystallized from ethanol (**Scheme 1**).

Stock Solution of Copper Sulfate. The stock solution was prepared by dissolving 3.93 g of copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) in double-distilled water containing a few drops of concentrated sulfuric acid. The solution was made up to 1 L and standardized by iodometry (*21*). This stock solution was diluted further, whenever necessary, with double-distilled water.

General Procedure. To an aliquot of solution containing 10.0−150 μg of copper(II) were added buffer of pH 4.5 and 0.5% reagent solutions (0.4 mL); the mixture was shaken with *n*-butanol (2 × 5 mL) for 1 min and allowed to stand for a few minutes. The organic phases were then separated, combined, and made up to 25 mL with *n*-butanol, and its absorbance was measured at 440 nm against the reagent blank.

RESULTS AND DISCUSSION

Copper(II) forms a reddish brown 1:1 (M:L) complex with PPT, which was extracted well into *n*-butanol from sodium acetate−acetic acid buffer of pH 4.5. The colored complex in *n*-butanol showed a maximum absorbance at 440 nm, when the spectrum of the complex was recorded, against the reagent blank. The color of the complex was stable for a minimum of 48 h. The optimum conditions for the effective extraction of copper(II) were established by studying the effects of factors such as pH, reagent concentration, choice of solvent, salting-out agent, and interference of various diverse ions, to develop a sensitive and rapid extractive spectrophotometric method for the determination of copper(II). The proposed method when compared with other spectrophotometric methods (**Table 1**) was found to be more sensitive and selective. It also offers advantages such as reliability and reproducibility in addition to its simplicity, instant color development, and lower levels of interference.

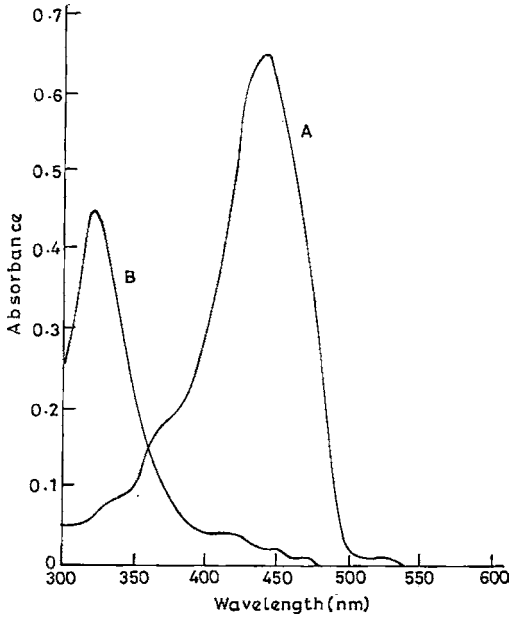


Figure 1. Absorption spectra of (**A**) Cu(II)–PPT complex versus PPT blank and (**B**) PPT versus *n*-butanol blank. Cu(II), 1.0 mL of 0.98×10^{-3} mol/L; pH, 3.0 mL of 4.5; PPT, 2.0 mL of 1.97×10^{-3} mol/L.

Absorption Spectra. The absorption spectra of the Cu(II)−PPT complex are shown in **Figure 1**. From the spectra, it is clear that the Cu(II)−PPT complex and the reagent have maximum absorbances at 440 and 320 nm, respectively. The reagent has a minimum absorbance where the complex has maximum absorbance and does not interfere in the determination of Cu(II). Hence, further absorbance measurements of the complex were made at 440 nm. Hence, all of the spectral measurements were carried out at this wavelength.

Effect of pH. A preliminary study showed that the formation of the Cu(II)−PPT complex was affected by the hydrogen ion concentration. Extraction of copper(II) with PPT was carried out over a wide range of pH (1.0−10.0). It reveals that the extraction of copper(II) with PPT into *n*-butanol was maximum between pH 3.0 and 5.5 (**Figure 2**). Hence, all of the extractions were carried out at pH 4.5, considering it as the optimum pH.

Effect of Solvents. Solvents such as *n*-amyl alcohol, isoamyl alcohol, benzene, *n*-butanol, butyl acetate, carbon tetrachloride, chloroform, chlorobenzene, cyclohexane, cyclohexanol, and methyl isobutyl ketone were prepared to extract 2.5 ppm of copper(II) with PPT. As per the results reported in **Table 2**, *n*-butanol was selected as the suitable solvent for effective extraction of the Cu(II)−PPT complex. Hence, *n*-butanol was chosen for all further studies.

Effect of Reagent Concentration. The effect of reagent concentration on the formation of the Cu(II)−PPT complex has been studied by keeping the amount of metal ion solution (62.5 μg) and 3.0 mL of pH 4.5 buffer constant and 1.0 mol/L of PPT solution containing different concentrations ranging from 0.983×10^{-3} to 9.83×10^{-3} mol/L in order to get the maximum color formation. The total volume of the organic phases was collected into 25 mL standard flasks. The organic phases were

Table 1. Comparison of the Present Method with Other Spectrophtometric Methods for the Determination of Copper

reagent	λ_{max} (nm)	optimum pH range	Beer's law validity range (ppm)	molar absorptivity (L mol^{-1} cm^{-1})	M:L[a]	remarks	ref
phenanthraquinone monothiosemicarbonzone	540	5.5	2.5–3.5	11600	1:2	Zn(II), Ca(II), Mn(II), Al(II), Fe(III), Bi(III), and Co(II) interfere, less sensitive	*6*
8-methoxy-2-chloroquinoline-3-carbaldehyde thiosemicarbazone	410	5.0	3.0	0.0026768	1:1	many metal ions interfere, very poor sensitivity	*7*
p-anisaldehydethiosemicarbazone	398	0.1–0.	0.1–10.0	6100	1:2	Fe(II), Co(II), Zn(II), V(V), Bi(III), Cd(II), and thiocyanate interfere, less sensitive	*8*
quinoline-2-aldehyde thiosemicarbazone	430	7.5	0.5–25.0	13000	1:1	Zn(II), Pd(II), Fe(II), Pb(II), and Ni(II) interfere, less sensitive	*9*
salicyladehyde thiosemicarbazone	375	6.5–7.5	0.5–6.0	9200	1:1	Fe(II), Co(II), Zn(II), V(V), Bi(III), Cd(II), and thiocyanate interfere, less sensitive	*10*
5,5[1]-dimethyl-1,2,3-cyclohexanetrione-1,2-dioxime-3	383		0–11.2	4600	1:3	less sensitive	*11*
2,4-dihydroxy-5-bromoacetophenone thiosemicarbazone	420	6.0	12.7	1450	1:1	very poor sensitivity	*12*
benyaldehyde-4-(2-hydroxy-5-sulfor-phenyl)-3-thiosemicarbazone	325	4.5	7.62	744	1:2	very poor sensitivity	*13*
4-choroisontirosoacetophenone thiosemicarbazone	400	7.5–8.5	0.2–20	2518	1:2	Ag(I), Ni(II), Co(II), Pb(II), Cd(II), cyanide, tartarate, and EDTA interfere,less sensitive	*14*
benzildithiosemicarbasone	380	4.0	0.5–4.0	16347	1:1	Ag(I), Co(II),Ni(II), Pd(II), Pb(II), and Zn(II) interfere, less sensitive	*15*
pyridoxal-4-phenyl-3-thiosemicarbazone	440	3.0–5.5	0.2–5.0	21600	1:1	highly sensitive and selective, the interfering metal ions can be masked by phosphate	PM[b]

[a] M:L, metal/ligand. [b] PM, present method.

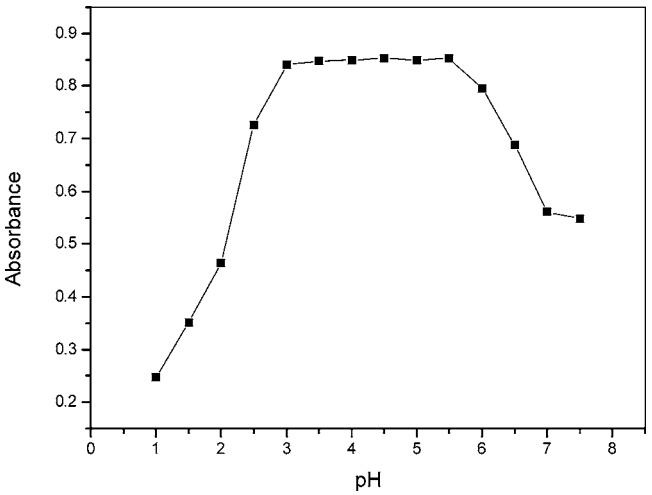


Figure 2. Effect of pH on Cu(II)–PPT complex. Cu(II), 1.0 mL of 0.98×10^{-3} mol/L; PPT, 2.0 mL of 3.5×10^{-3} mol/L; λ_{max}, 440 nm.

made up to 25 mL with *n*-butanol, and the absorbances of these phases were measured at 440 nm, against their corresponding reagent blanks. The results clearly indicate that a 7-fold molar (6.88×10^{-3} mol/L PPT) excess of the reagent to that of metal ion is necessary for maximum color development of the Cu(II)−PPT complex. Hence, a 7-fold molar excess of the reagent was maintained for maximum extraction of copper(II).

Effect of Salting-out Agent. Various salting-out agents, such as magnesium sulfate, lithium nitrate, lithium sulfate, lithium chloride, ammonium sulfate, sodium chloride, and sodium sulfate, were tested to enhance the metal−complex extraction into the organic phase, in a single step. It is noted that these salting-out agents have no significant effect on the absorbance of the colored solution. The presence of copper(II) in the aqueous phase after extraction was tested by using dithizone, spectrophotometrically, which discloses that there is no copper(II) in it.

Table 2. Effect of Solvents on the Extraction of Cu(II)–PPT Complex[a]

solvent	absorbance
n-butanol	0.850
chlorobenzene	0.475
carbon tetrachloride	0.450
butyl acetate	0.424
benzene	0.372
isoamyl alcohol	0.350
chloroform	0.326
methyl isobutyl ketone	0.275
n-amyl alcohol	0.205
cyclohexane	0.154
cyclohexanol	0.142

[a] Cu(II), 1.0 mL of 0.98×10^{-3} M; PPT, 2.0 mL of 3.44×10^{-3} M; pH 4.5; λ_{max} 440 nm.

Validity of Beer's Law, Molar Absorptivity, Sandell's Sensitivity, and Correlation Coefficient. The present studies indicate that Beer's law was obeyed over the concentration range of 0.4−5.0 mg/L of copper(II) with molar absorptivity and Sandell's sensitivity being 2.16×10^4 L mol^{-1} cm^{-1} and 2.94×10^{-3} μg cm^{-2} respectively. The regression coefficient of the Beer's law straight line is 0.338, and the correlation coefficient is 0.96. Good linearity with a correlation coefficient value of 0.96 was obtained for the Cu(II)−PPT complex. The calibration curve was prepared to determine the actual analyte content in real samples. The linear least-squares method, which states that the best straight line through a series of incremental points is that line for which the sum of the squares of the deviations of the points from the line is minimum, was used to prepare the calibration curve.

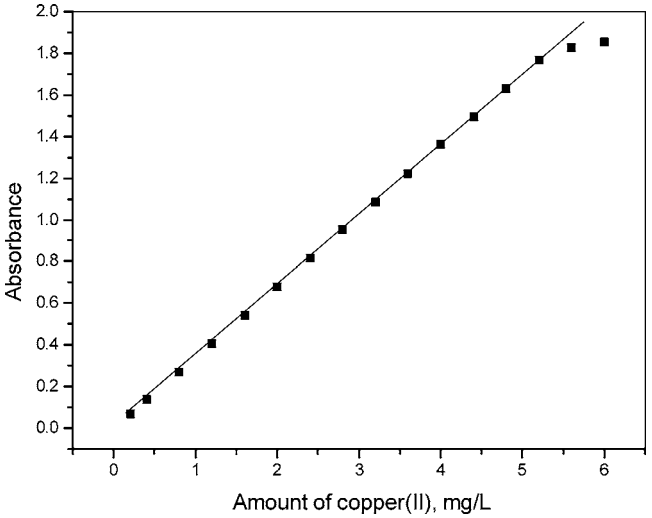


Figure 3. Applicability of Beer's law on Cu(II)–PPT complex. PPT, 2.0 mL of 8.3×10^{-3} mol/L; pH, 4.5; λ_{max}, 440 nm.

Ringbom Plot for Cu(II)−PPT Complex. Ringbom's plot is the standard adapted to know the optimum range of concentration for a system, which obeys Beer's law. The plot is drawn between log C of copper(II) and $(1 - T)$, where T is the transmittance, which is shown in **Figure 3**. The plot has a sigmoid shape with a linear segment, at intermediate concentration values of 0.8−4.4 mg/L of copper(II). The slope of the Ringbom plot is 1.20. On the basis of this value, the ratio between the relative error in concentration and the photometric error is 1.9166. For a photometric error of 1% $\nabla P = 0.01$. Hence, the relative error in concentration is 0.01916.

Precision, Accuracy, and Detection Limit of the Method. Five aliquots of different concentrations with seven samples for each concentration were taken, and copper(II) was determined employing the general procedure in order to assess the accuracy and precision of the method. The standard deviation of the method is found to be not more than 0.006, and the relative standard deviation is less than 1.84%. The values indicate that this method has a greater accuracy and enhanced precision. The detection limit, C_{min}, was determined as the amount of copper(II) corresponding to 3 times the deviation of blank values; a value of 6.5×10^{-2} μg mL^{-1} was obtained.

Determination of the Composition of the Cu(II)−PPT Complex. The composition of the Cu(II)−PPT complex was studied by the method of Job's continuous variation, mole ratio, and Asmus' and slope ratio methods.

Job's Method of Continuous Variation. Equimolar (2.95×10^{-3} mol/L) solutions of copper(II) and PPT were prepared. The metal and reagent solutions were mixed in different proportions, keeping the total volume of metal and ligand constant at 2.0 mL. In each case, 3.0 mL of sodium acetate−acetic acid buffer (pH 4.5) was added to the mixture, and the volume of the aqueous phase was brought to 10.0 mL with double-distilled water. Each of the above aqueous phases was shaken thoroughly with 10.0 mL of *n*-butanol; the organic phase was collected into a 25 mL standard flask and made up to the mark with *n*-butanol. The absorbances of all the organic phases were recorded at 440 nm, against their corresponding reagent blanks. The corresponding graph drawn between absorbance and mole fraction of the metal is shown in **Figure 4**.

Molar Ratio Method. Different aliquots of mixtures containing 1.0 mL of (0.98×10^{-3} mol/L) copper(II) and 3.0 mL of sodium acetate−acetic acid buffer (pH 4.5) were prepared. To each of these solutions was added the required volume of reagent

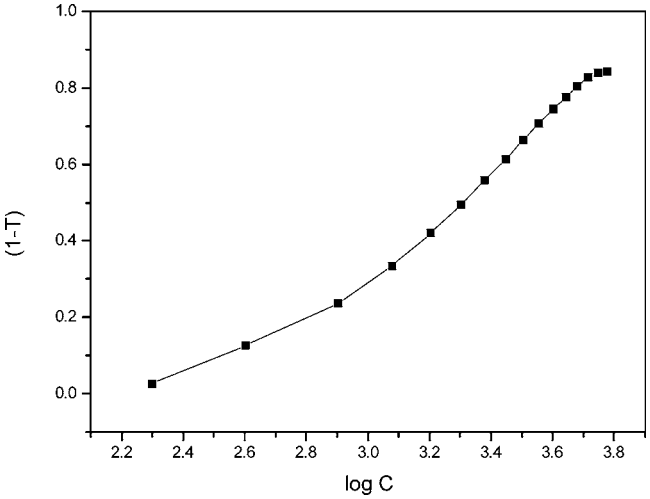


Figure 4. Ringbom plot of Cu(II)–PPT complex. Cu(II), 400–600 μg/L; PPT, 2.0 mL of 8.3×10^{-3} mol/L; pH, 4.5; λ_{max}, 440 nm.

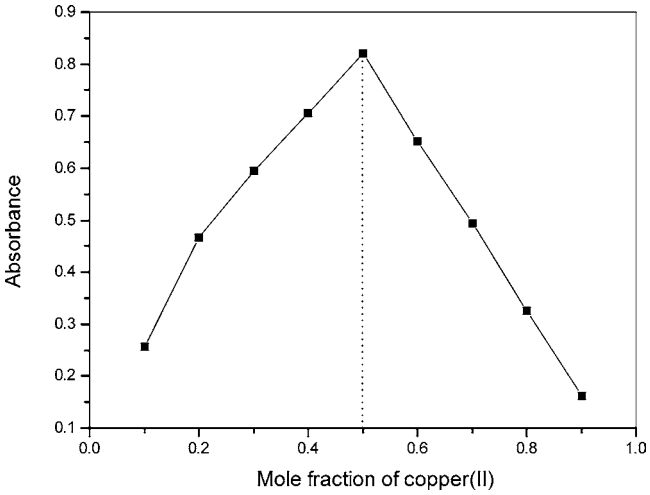


Figure 5. Job's method of continuous variation of Cu(II)–PPT complex. Cu(II) or PPT, 2.95×10^{-3} mol/L; pH, 4.5; λ_{max}, 440 nm.

solution ($0.25-2.95 \times 10^{-3}$ mol/L), and final volumes were made up to 10 mL of *n*-butanol; the organic phase was collected in a 25 mL standard flask and made up to the mark with *n*-butanol. The absorbances of the organic phases obtained were recorded at 440 nm, against their corresponding reagent blanks. A plot drawn between the absorbance and number of moles of the ligand per mole of the metal ion is shown in **Figure 5**.

Slope Ratio Method. Two series of mixtures were prepared using 0.98×10^{-3} mol/L solutions of the reagent and copper(II).

(i) Excess of Metal Ion. In one series, the amount of copper(II) is kept in excess (62.5 μg), and to the samples were added various volumes (0.2−1.0 mL) of 0.98×10^{-3} mol/L PPT solution. To each of these solutions was added 3.0 mL of pH 4.5 buffer, and finally the volumes were made up to 10.0 mL with double-distilled water. These aqueous phases were shaken separately with 10.0 mL portions of *n*-butanol; the extracts were taken into 25 mL standard flasks, and finally the volumes were made up to the mark with *n*-butanol. The absorbances of the colored solutions were recorded at 440 nm, against their corresponding reagent blanks. A plot is drawn between the volume of the reagent and its absorbance, as shown in **Figure 6**.

(ii) Excess of the Reagent. In another case, the volume of 0.98×10^{-3} mol/L PPT solution was kept in excess (1.0 mL),

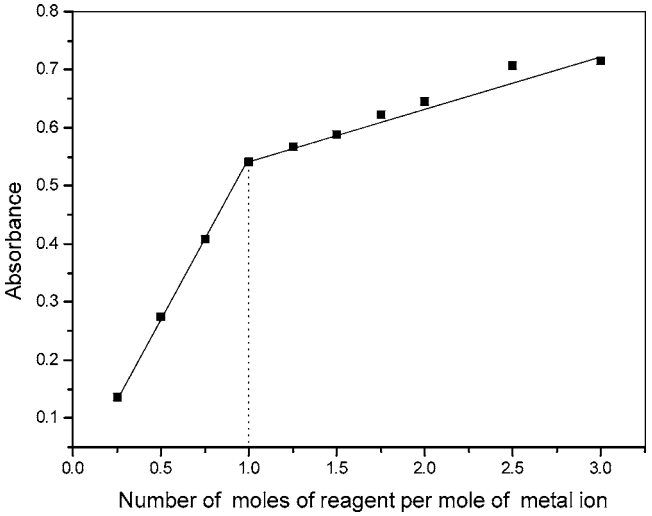


Figure 6. Molar ratio method of CU(II)–PPT complex. Cu(II), 1.0 mL of 0.98×10^{-3} mol/L; PPT, 1.0 mL of 2.5×10^{-3} mol/L; pH, 4.5; λ_{max}, 440 nm.

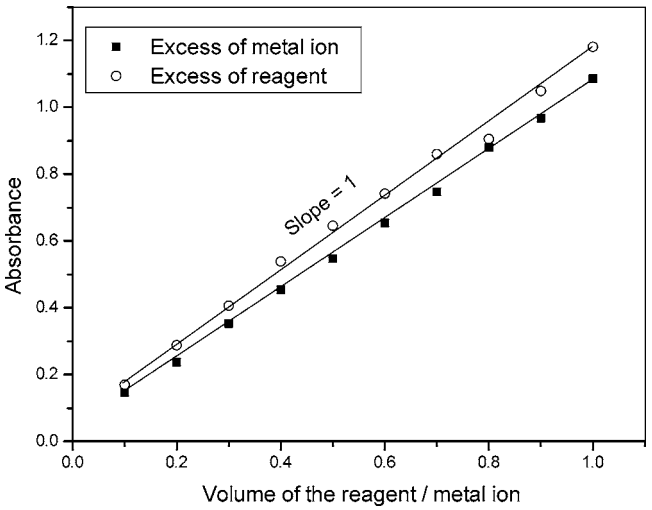


Figure 7. Slope ratio method of Cu(II)–PPT complex. Excess of reagent: Cu(II), 0.1–1.0 mL of 1.97×10^{-3} mol/L; PPT, 1.0 mL of 1.97×10^{-3} mol/L; pH, 4.5; λ_{max}, 440 nm. Excess of metal ion: Cu(II), 1.0 mL of 1.97×10^{-3} mol/L; PPT, 0.1–1.0 mL of 1.97×10^{-3} mol/L; pH, 4.5; λ_{max}, 440 nm.

and to these samples were added various volumes (0.1−1.0 mL) of 0.98×10^{-3} mol/L copper(II) solution; the rest of the procedure adapted is the same as that described under Excess of Metal Ion. The absorbance values of the organic phases recorded at 440 nm, against their corresponding reagent blanks, and the plot drawn between the absorbance and volume of the metal ion are shown in **Figure 7**.

Asmus' Method. For Asmus' method, the data obtained from the molar ratio method was used. Value of $1/m$, where m is extinction modulus, were calculated by dividing the optical density with the cell width along with $1/V$, $1/V^2$, and $1/V^3$. The plots between $1/m$ and $1/V$, $1/V^2$, and $1/V^3$ are indicated in **Figure 8**. Among the plots between $1/m$ and $1/V$, $1/V^2$, and $1/V^3$, only the plot between $1/m$ and $1/V$ was linear plot, indicating the composition of the complex was 1:1 (metal/ligand).

Calculation of the Instability Constant of the Cu(II)−PPT Complex. The instability constant value of the Cu(II)−PPT complex was calculated by using Edmonds and Birnbaum's method and Asmus' method. Absorbance values of the extracts were obtained at 440 nm, after shaking of the solutions

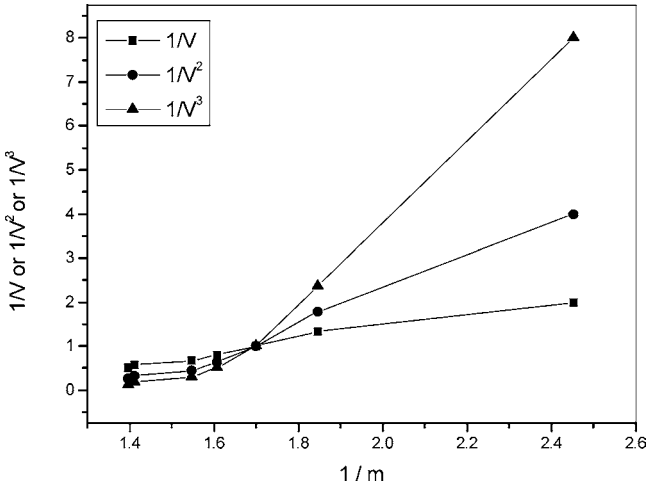


Figure 8. Asmus' method of Cu(II)–PPT complex. Cu(II), 1.0 mL of 0.98×10^{-3} mol/L; PPT, 1.0 mL of 0.5–2.0 mL of 1.48×10^{-3} mol/L; pH, 4.5; λ_{max}, 440 nm.

containing fixed volumes of copper(II) (0.98×10^{-3} mol/L) buffer (pH 4.5) and different known volumes of (1.48×10^{-3} mol/L) PPT with *n*-butanol. The instability constant of the Cu(II)−PPT complex was calculated and found to be 1.26×10^{-3} at room temperature by Edmonds and Birnbaum's method. In Asmus' method the instability constant of the Cu(II)−PPT complex was calculated and found to be 1.23×10^{-3} at room temperature, which is in good agreement with the value obtained by Edmonds and Birnbaum's method.

Effect of Concomitants. The potential interference of diverse ions in the determination of copper(II) was studied by using 62.5 μg of copper(II) and various amounts of each diverse ion in question. A given species was considered to be interfering if it resulted in a ±2% variation of the absorbance, for the established level of copper(II) alone. Cations such as Ca(II), Mg(II), Pb(II), Mn(II), and Bi(III) do not interfere even when present up to 5000 μg. Interference due to Al(III), Cr(III), Ag(I), and Sb(II) can be tolerated up to 2500 μg, whereas Mo(VI) and W(V) can be tolerated up to 2000 μg only. Extraction of copper(II) is not possible in the presence of Co(II), Ni(II), Fe(II), Fe(III), Zn(II), Pd(II), and Cd(II), due to their interference, even when present in trace amounts. Anions such as fluoride, bromide, iodide, chloride, nitrate, sulfate, thiosulfate, citrate, acetate, and tartate do not affect the extraction of copper(II), even when present up to 5000 μg. In the presence of thiocyanate, oxalate, and EDTA, extraction of copper(II) is not possible. One milliliter of 0.2% fluoride was used as a masking agent for Fe(II) and Fe(III). Interference due to Co(II), Ni(II), Zn(II), Pd(II), and Cd(II) can be suppressed by adding 1.0 mL of 0.2% citrate solution. Increasing the amounts of their corresponding masking agents proportionately can mask higher amounts of interfering ions.

APPLICATIONS OF THE DEVELOPED METHOD

The developed extractive spectrophotometric method for copper(II) was applied for the determination in leafy vegetable, pharmaceutical, and of Analyzed Samples samples.

Determination of Cu(II) in Leafy Vegetable Samples. The established optimized conditions of extractive spectrophotometric method were applied to leafy vegetables, for the determination of copper(II) content. The leafy vegetables analyzed were brought from the local market during the month of January. The samples were cleaned and dried in open air,

Table 3. Determination of Copper(II) in Leafy Vegetables

leafy vegetable	amount added (μg/g)	amount of Cu(II) found[a] (μg/g) AAS method	present method	SD	RSD %
cucumber (*Cucumis sitivas*)	10.00	10.52	10.51	0.03	0.28
green peas (*Pisum sativum*)	10.00	11.15	11.14	0.05	0.43
fresh bean (*Dolichos lablab*)	10.00	10.88	10.87	0.04	0.37
white radish (*Raphanus sativus*)	10.00	14.21	14.20	0.06	0.42

[a] Average of four replicants ($n = 4$).

protecting them from mineral contamination. The dried sample was pulverized in a mortar for the purpose of analysis, to a convenient size. Ten grams of each powdered sample was taken into a silica crucible, heated to oxidize the organic matter, and ashed at 550 °C in a muffle furnace for 4−5 h. The ash was dissolved by heating with 10 mL of 2 N hydrochloric acid and filtered through an acid-washed filter paper (Whatman no. 41), and then the residue was washed with hot water. The filtrate and washings were collected in a 25 mL volumetric flask and finally made up to the mark with double-distilled water. An appropriate aliquot of the diluted solution was taken, and its pH 4.5 copper(II) from the leafy vegetable was extracted at 440 nm. The process was repeated four times for each sample, and the results obtained were confirmed by direct atomic absorption spectrometer. The results obtained are presented in **Table 3**.

Determination of Cu(II) in Pharmaceutical Samples. Pharmaceutical samples such as Supradyn, Thesagram-M, Vimgram, and Fersolate were analyzed for copper(II). All of the pharmaceutical samples were brought into solution by adapting the following procedure. The samples were treated separately with concentrated nitric acid on a hot plate, at a low temperature, to avoid violent spurting. The residue of each sample was cooled, and again nitric acid was added. The temperature of the hot plate was kept low to avoid violent spurting. The residue of each sample was cooled, and again nitric acid was added. The temperature of the hot plate was increased to 300 °C. The residue obtained was dissolved in nitric acid (1:1) and then slowly heated for 2 h to procure a dry mass. Finally, the residue was dissolved in a minimum amount of double-distilled water. The same solution was quantitatively transferred into a 500 mL volumetric flask and then made up to the mark with double-distilled water. An appropriate aliquot was analyzed for copper(II) by the recommended procedure using PPT. The results obtained were confirmed by direct atomic absorption spectrometer and are presented in **Table 4**.

Determination of Cu(II) in Bureau of Analyzed Samples Samples. The proposed method was applied for the determination of copper(II) in Bureau of Analyzed Samples samples such as alloy steels (BCS 233 and 266), aluminum base alloy (BCS 216/1), and copper base alloys (BCS 207 and 179). About 0.1 g of each oven-dried (110° C) alloy sample was dissolved in 15 mL of aqua-regia. The solution was heated to near dryness, and the nitrate was expelled from the residue, using 5.0 mL of concentrated hydrochloric acid. Each residue was then extracted into double-distilled water and made up to 500 mL. An appropriate aliquot was analyzed for copper(II) by the recommended procedure using PPT. The results obtained were confirmed by direct atomic absorption spectrometer and are presented in **Table 5**.

Table 4. Determination of Copper(II) in Pharmaceutical Samples

sample	composition	amount of Cu(II) found[a] (mg/tablet) AAS method	present method	SD	RSD %
Supradyn (Roche Chemicals, India)	copper sulfate IP[b] 3.39 mg (equivalent to elemental copper, 0.86 mg); zinc sulfate[c] IP 2.20 mg; sodium borate IP 0.88 mg	0.86	0.85	0.011	1.29
Theragran-M (Sarabhai Chemicals, India)	potassium iodide IP 0.2 mg; dried iron(II) sulfate[c] IP 41 mg; copper sulfate IP 8 mg (equivalent to elemental copper, 0.86 mg)	2.00	1.99	0.013	0.65
Vimgram (Sarabhai Chemicals, India)	calcium carbonate USP 250 mg; iron(II) sulfate[c] IP 34 mg; potassium sulfate IP 10 mg; copper sulfate IP 4.0 mg (equivalent to elemental copper, 0.86 mg); manganese sulfate IP 6.6 mg; magnesium oxide IP 10.0 mg	1.00	0.99	0.012	1.21
Fersolate (Glaxo, India)	iron(II) sulfate[c] IP 195 mg; copper sulfate IP 2.6 mg (equivalent to elemental copper, 0.86 mg); manganese sulfate IP 2.6 mg	0.66	0.65	0.011	1.69

[a] Average of four replicants ($n = 4$). [b] IP, Indian Pharmacopoeia; USP, United States Pharmacopoeia. [c] Masked with phosphate.

Table 5. Determination of Copper(II) in Bureau of Analyzed Samples Samples

alloy	composition %	amount of Cu(II) found[a] (%) AAS method	present method	SD	RSD %
alloy steel (BCS 233)	Cu, 5.09; Co,[b] 23.4; Ni,[b] 11.22; Sn, 7.95; Mn, 0.235	5.00	4.98	0.045	0.90
alloy steel (BCS 266)	Cu, 3.33; Co,[b] 23.4; Ni,[b] 13.3; Al, 7.95	3.30	3.28	0.036	1.09
aluminum base alloy (BCS 216/1)	Cu, 4.42; Mn, 0.73; Fe,[b] 0.40; Zn,[b] 0.11; Ti, 0.10	4.40	4.37	0.044	1.00
copper base alloy (BCS 207)	Cu, 86.84; Sn, 9.8; Zn,[b] 2.53; Pb, 0.41	86.56	86.42	0.824	0.95
copper base alloy (BCS 179)	Cu, 58.8; Zn,[b] 33.9; Sn, 1.75; Al, 1.62; Mn, 1.03; Ni,[b] 1.01; Fe,[b] 0.91	58.62	58.38	0.54	0.92

[a] Average of four replicants ($n = 4$). [b] Masked with phosphate.

CONCLUSIONS

In the present investigation, the researchers have introduced a new reagent, pyridoxal-4-phenyl-3-thiosemicarbazone (PPT), for the extractive spectrophotometric determination of copper(II). This method offers several interesting features such as simplicity, rapidity, and low cost besides sensitivity. The molar absorptivity value of the complex (2.16×10^4 L mol^{-1} cm^{-1}) reveals that the reagent is more sensitive for copper(II) as compared with the earlier reagents. A number of associated elements do not interfere in the determination. The selectivity of the reagent is also improved by the use of suitable masking agents to suppress the interference of some metal ions such as Co(II), Ni(II), Fe(II), Fe(III), Zn(II), Cd(II), and Pd(II). Finally, PPT is strongly recommended for the extractive spectrophotometric determination of copper(II) at minor and trace levels besides its use for the analysis of leafy vegetable and pharmaceutical samples, as described.

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