



Second Derivative Spectrophotometric Determination of Copper(II) using 2-Acetylpyridine Semicarbazone in Biological, Leafy vegetable and Synthetic Alloy Samples

S. Vidyasagar Babu, K. Hussain Reddy*

Department of Chemistry, Sri Krishnadevaraya University, Anantapur-515 003. A.P. India.

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ABSTRACT

2-Acetylpyridine semicarbazone (APS) has been used for the spectrophotometric determination of copper(II) in aqueous medium. APS react with copper(II) in slightly acidic (pH,6.0) medium. The colour reactions between APS and copper(II) is instantaneous and the absorbance of complex remains constant for over 24h. The maximum absorbance (λ_{max}), composition, molar absorptivity and sandells sensitivity of Cu-APS complex respectively are 355 nm, 1:2, $9.8 \times 10^3 \text{ L. mol}^{-1} \cdot \text{cm}^{-1}$ and $0.064 \mu\text{gcm}^{-2}$ of Cu(II) respectively. The systems obey Beer's law for 0.254-5.183 $\mu\text{g/ml}$ of Cu(II). Spectrophotometric data of colour reaction between copper(II) and 2-acetylpyridinethiosemicarbazone(APT) are included for comparison. Large number of cations, anions and complexing agents (e.g. triethanolamine, thiourea) do not interfere in the determination of APS method. The method is successfully applied for the determination of copper in biological, leafy vegetable and synthetic alloy samples.

Key Words: Semicarbazone, spectrophotometry, copper, biological, leafy and synthetic alloy samples.

1. INTRODUCTION

Copper is an essential metal for plants, microorganisms, animals and human beings to perform specific biological functions. Copper is often added to fertilizer to serve as a supplement to plants. The failure to supply adequate amounts of copper leads to a variety of biochemical and physiological disorders in plants[1,2]. In humans, it not only facilitates the conversion of iron to hemoglobin but also stimulates the growth of red blood cells. Copper is an integral part of certain digestive enzymes. copper deficiency[3,4] results in bodily weakness, digestive disturbances and impaired respiration. The effects of deficiencies vary depending on the nature of the living being, the factors affecting metal concentrations and the level of deficiency[5]. As a toxicant at elevated levels of biologically available form, it produces a physiological response. However, excess copper because of its potential incorporation in component organisms of food webs is of concern[6,7].

The review of literature [8-10] suggested that 2-acetylpyridine semicarbazone (APS) is not used for the spectrophotometric determination copper(II). Recently we have reported [11,12] spectrophotometric determination of mercury(II), cobalt(II) using APT and APS. In continuation of our ongoing research on spectrophotometric determination of transition metal ions using thiosemicarbazones and semicarbazones, here in

we report second derivative spectrophotometric determination of copper(II) using 2-acetylpyridine semicarbazone(APS) in biological, leafy vegetable and synthetic alloy samples. Spectrophotometric data of colour reaction between copper(II) and 2-acetylpyridine thiosemicarbazone(APT) are also included for the sake of comparison.

2. EXPERIMENTAL

The reagents APT and PAS were synthesized as described earlier[11,12] pKa values of reagents were determined spectrophotometrically by using Phillips and Merrit method[13]. The values of deprotonation of APT and APS were 5.46 and 6.61 (pK₁); 8.56 and 8.30 (pK₂) as shown in scheme 1.

The reagents (APT or APS) solution (0.01 M) was prepared by dissolving 50 mg of the compound in dimethylformamide (DMF) in 25-ml standard flask. The reagent solution is stable for at least 12 h.

Hydrochloric acid (1 M)-sodium acetate (1 M) (pH 0.5-3.5); 0.2 M NaOAc-0.2 M AcOH (pH 4-6) and 2 M NH₄Cl-2 M NH₄OH (pH 7-10) solutions were used.

Copper(II) Solution

Cu(CH₃COO)₂·2H₂O (0.1997g) was dissolved in doubly distilled water containing a few drops of conc. CH₃COOH in a 100 ml standard flask to get 1×10^{-2} M solution, which was standardized

*Corresponding Author:

Email: khussainreddy@yahoo.co.in

titrimetrically[14] the working solutions were prepared daily by diluting the stock solution to an appropriate volume. All other chemicals used were of AR grade.

2.1. Recommended Procedures

(a) Determination of copper(II) (Zero order)

An aliquot of the solutions containing copper (II) in Beer's law validity range Table 2 and buffer solution in optimum pH range and 1 ml of 0.01 M reagent (APT or APS) were combined in a 25-ml volumetric flask and the resulting solution was diluted to the mark with distilled water and absorbencies were measured at 376 and 355 λ_{\max} against corresponding reagent blank. The measured absorbance is used to compute the amount of copper present in the samples using predetermined calibration plot.

(b) Determination of copper(II) using second derivative spectrophotometry

A sensitive second order derivative spectrophotometric method was developed for the determination of copper(II). For the Cu-APT and Cu-APS second derivative spectra, the derivative amplitudes at 426 nm, 440nm (peak) and at 438 nm, 415nm (valley) were proportional to the concentration of Cu(II). The derivative amplitudes were measured for different concentration of Cu(II) and plotted against the amount of Cu(II). The plots were linear and obeyed Beer's law in the range 0.1762-0.0035, 0.1009-0.0015 $\mu\text{g ml}^{-1}$ at 426nm, 440nm and 0.04-0.0001, 0.01-0.0001 $\mu\text{g ml}^{-1}$ at 438 nm, 415 nm. In the second order derivative method

Apparatus

Schimadzu 160A UV-visible spectrophotometer equipped with 1.0 cm quartz cell and an ELICO model LI-610 pH meter were used in the present study.

3. RESULTS AND DISCUSSION

The APS and APT reagent solutions (0.01M) are stable for 12 h. The bathochromic shift of absorption band from 290 to 300 nm indicates that in solution on increasing the pH, the acid is neutralized and the $>\text{C}=\text{X}$ group (where X= O or S) is enolized and dissociated[15]. The colour reactions of some important metal ions with APT and APS are summarized in Table 1. In basic medium (above pH 8.56) the ligands presumably exist in enolic form and coordinates the divalent metal ion as mono anion to give neutral complexes.

3.1 Determination of Copper(II)

Copper (II) react with APT and APS in slightly acidic to give colored complexes in Table 1. The colour reactions are instantaneous at room temperature. The change in the order of addition of

metal ion, reagent (APT or APS), buffer has no adverse effect on the absorbance of complexes.

Absorption spectra of APS blank and Cu-APS complex are given in Figure 1. Various physico-chemical and analytical characteristics of the complexes are summarized in Table 2. Second derivative spectra of Cu-APT and Cu-APS systems are given in Figure 2 and 3 respectively. The derivative amplitudes are proportional to concentration of copper(II). The stoichiometry of the complexes was determined by well known Job's continuous variation, molar ratio methods. Sodium acetate (0.2 M)-acetic acid(0.2 M)(pH 5.5, 6.0) (APT and APS) buffer solution and equimolar (4×10^{-4} and 2×10^{-4} M) solution of Cu(II) and APT or APS were used in these methods. The data obtained in Job's method were used in the calculation of stability constants of the complexes.

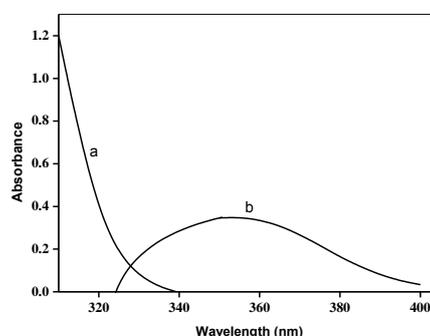


Figure 1. Absorbance spectrum of (a) APS Vs Water blank, (b) Cu (II) APS complex Vs APS solution. ($[\text{Cu (II)}] = 4 \times 10^{-5}\text{M}$, $[\text{APS}] = 4 \times 10^{-4}\text{M}$, pH = 6.0)

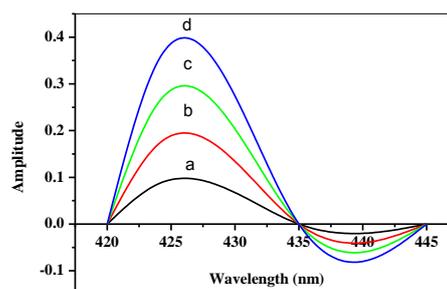


Figure 2. Second derivative spectra of Cu(II) - APT Vs reagent blank Cu(II), $\mu\text{g/ml}$ (a) 0.5083; (b) 1.0166; (c) 1.5249; (d) 2.0332. $[\text{APT}] = 6 \times 10^{-4}$, pH = 5.5

3.2 Interference

The effects of various cations and anions which are generally associated with the copper(II) are studied by measuring the absorbance of the copper complex in the presence of foreign ions. The colour

Table 1 . Chromogenic characteristics of APS.

Metal ion	λ_{\max}	$\epsilon \times 10^4$ *	pH range	Colour of the complex
Cu(II)	376	0.26	5.0-7.0	Pale greenish yellow
Ni(II)	375	1.6	5.0-7.0	Yellow greenish
Co(II)	355	1.25	5.0-8.0	Orange yellow
Zn(II)	360	4.06	5.0-7.0	Pale greenish yellow
Fe(II)	370	0.17	4.0-7.0	Deep yellow with red tinge
Fe(III)	375	0.14	4.0-7.0	Deep yellow with red tinge
V(V)	360	0.37	3.0-7.0	Yellow coloured

* L. mol⁻¹ cm⁻¹

reaction is performed as described in the standard procedure. An error of $\pm 2\%$ in the absorbance or amplitude reading in the case of derivative methods considered tolerable. The results are given in Table 3. The data obtained in second derivative method are also incorporated. The data suggest that several associated anions and cations do not interfere when they are present in large excess. The tolerance limit values for many anions and cations are high in derivative methods. The interference of associated metal ions such as Fe(III) and Al(III) is decreased with triethanolamine. Larger amounts of nickel(II) do not interfere in the presence of thiourea.

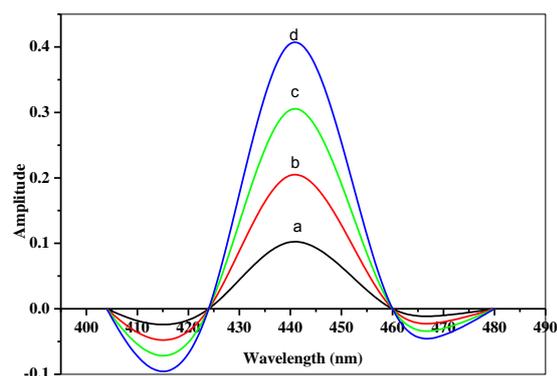


Figure 3. Second derivative spectra of Cu(II)-APS Vs reagent blank Cu(II), $\mu\text{g/ml}$; (a) 1.0166; (b) 2.0332; (c) 3.0499; (d) 4.0665. ([APS] = 6×10^{-4} pH = 6.0)

3.3 Applications

The present method was successfully applied to the determination of copper when present alone. The method is extended to the determination of copper in a leafy vegetable samples, biological samples and synthetic alloy samples.

3.4 Biological samples

A 2-5 g of dried fish and sheep liver samples were taken in a 250-ml beaker. A 6 ml of concentrated nitric acid was added and gently heated for half-an-hour. After the disappearance of the forth, 6 ml of 1:1 nitric acid and perchloric acid were added[16]. The contents were digested for one hour and repeatedly treated with 6 ml portions of nitric acid and perchloric acid mixture until the solution

becomes colorless. The acid solution was evaporated to dryness and the resulting white residue was dissolved in minimum volume of 1M nitric acid and made upto the volume in a 50-ml volumetric flask. Aliquots of this solution were taken for analysis by following recommended procedure. The results are given in Table 4.

3.5 Leafy vegetable sample

Dry ashing method was used in the analysis of organic samples. A 10 g of dried leafy vegetable sample was taken in a silica dish. The sample was heated over a low burner until the material chars. The charned mass was moistened with 1:1 HNO₃. Occasionally a 20 percent solution of magnesium nitrate was used for this purpose, particularly if the ash content is very low. Again evaporated to dryness, and transferred to a muffle furnace. The temperature to about 500 °C is reached in the course of about 3 hours and continued to heat at that temperature until the ash is white. The dish was cooled and the ash was dissolved in few drops of dilute HCl and filtered the solution was diluted to 50 ml in a standard flask. Aliquots of this solution were taken for analysis of copper by following recommended procedure [17,18]. The results are given in Table 5.

3.6 Synthetic alloy samples

The samples solutions whose composition corresponding to NTPC and aluminum alloys was prepared by using 1×10^{-3} M solutions of each metal ion present in the alloy. The results are given in Table 6.

Known aliquot of the above alloy sample solution was taken in a 10 ml flask containing 5 ml of buffer solution(pH 5.5), 70 $\mu\text{g/ml}$ of fluoride to mask Fe(II) and 1 ml of the APS solution (4×10^{-4} M).The contents were made up to the mark with distilled water. The contents, if necessary were filtered and the absorbance of the resulting solution was measured at 376 nm against the reagent blank. The amount of copper(II) present in the sample was determination from the predetermined calibration plot.

Table 2. Physico-chemical and analytical characteristics of Cu(II) complexes with APT and APS.

S.N	Characteristics	Results	
		Cu-APT	Cu-APS
1	λ_{\max} (nm)	376	355
2	pH range (optimum)	5.0-7.0	5.0-7.0
3	Mole of reagent required per mole of metal ion for full colour development	10 fold	10 fold
4	Time stability of the complex (in minutes)	120	75
5	Beer's law validity range ($\mu\text{g/ml}$)	0.25-2.54	0.51-5.18
6	Molar absorptivity ($\text{lit mol}^{-1}\text{cm}^{-1}$)	2.6×10^3	9.8×10^3
7	Specific absorptivity ($\text{ml g}^{-1}\text{cm}^{-1}$)	0.042	0.154
8	Sandell's sensitivity (μgcm^{-2} of Cu(II))	0.023	0.064
9	Composition of the complex as obtained in Job's and molar ratio methods (M : L).	1 : 1	1:2
10	Stability constant of the complex	1.03×10^7	1.05×10^{13}
11	Standard deviation	0.014	0.040
12	Relative standard deviation (RSD),(co-efficient of variation)	0.61%	2.54%

Table 3. Tolerance limit of foreign ions in the determination of 1.42 ($\mu\text{g/ml}$) of copper Masked with 800 $\mu\text{g/ml}$ of thiourea.

Ions	Tolerance limit (ppm)				
	APS Method	APS 2 nd . D	Ions	APS Method	APS 2 nd . D
Triethanolamine	1250	1350	Fe(III) ^b	6.0	8.0
Citrate	210	250	Ni(II) ^a	3.4	3.8
Ascarbate	220	250	Hg(II)	4.8	5.2
Acetate	120	150	Co(II)	1.4	2.0
Nitrate	250	280	Ba(II)	3.1	3.4
Sulphate	390	420	Al(III) ^b	26	28
Oxalate	530	550	Cd(II)	27	30
Tartrate	600	620	Mg(II)	5.0	11
Iodide	410	430	Cr(VI)	12	22
Carbonate	360	380	Mo(VI)	21	43
Bromide	320	340	Pd(II)	11	14
Thiourea	850	870	Ag(I)	7.6	7.8
Fluoride	80	100	Zn(II)	4.7	12

^bMasked with 1000 $\mu\text{g/ml}$ of triethanolamine.

4. CONCLUSIONS

The present methods for the determination of copper (II) are compared with other recently reported spectrophotometric methods [19-28]. The present APS method seems to rank among highly sensitive methods for the determination of copper(II). The results obtained in the analysis of different samples using present method are comparable to the data obtained Table 7. The present ligands containing heterocyclic ring are found to be potential and cost effective for the determination of copper(II) without the need for extraction using the toxic solvents. Further, the reagents are easy to synthesize using commercially available precursors. Moreover, the present method are simple, rapid and very sensitive for non-extractive spectrophotometric determination of copper(II) in aqueous medium.

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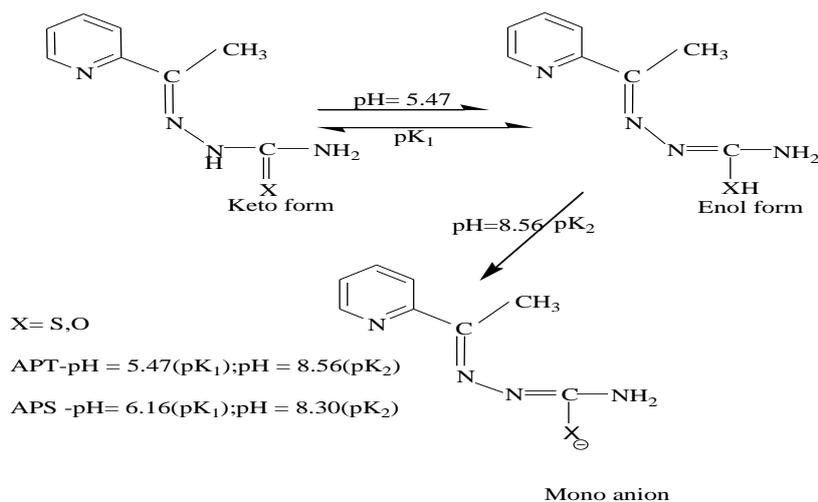
Table 4. Analysis of Fish and Sheep liver samples

Sample	Copper ^a / $\mu\text{g/g}$		Recovery(%)
	Added	Found	
Sheep liver	0	0.172	-
	100	101.02	100.80
	500	500.92	100.15
Fish liver	0	1.10	-
	100	101.21	100.10
	500	502.10	101.20

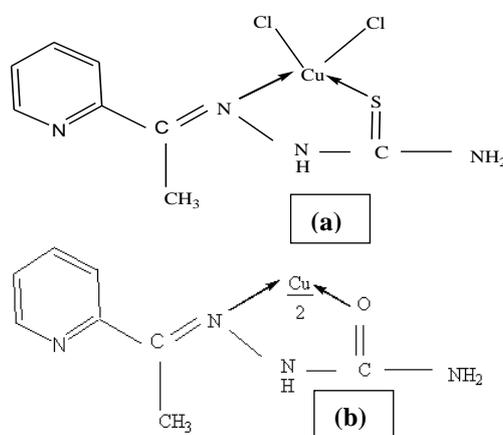
^aAverage of five determinations

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Scheme 1. Different species of APT and APS in solution at different pH values



Scheme 2. Structure of (a) $[Cu (APT)Cl_2]$ (b) $[Cu (APS)_2]^{2+}$ complexes

Table 5. Determination of copper in leafy vegetable samples.

Name of the sample	Amount of copper ^a found ($\mu\text{g/g}$ in dried leaves)	
	APS Method	AAS Method
Thotakura (<i>Amaranthus gangeticus</i>)	0.268	0.274
Chukkaku (<i>IRumex vesicarius</i>)	0.250	0.259
Tutikura (<i>Ipomoea reptans</i>)	0.265	0.269
Cauliflower green (<i>brassicaderaceavar botmtis</i>)	0.296	0.292
Khesari (<i>Latyrus sativus</i>)	0.175	0.193
Vepaku(Medicinal leaves) (<i>Azadirachata indica</i>)	0.266	0.271
Gaddi chamanti (<i>Tridax procumbens L</i>)	0.121	0.120

^aAverage of five determinations.

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Table 6. Deterimination of copper (II) in synthetic alloy.

Sample	Sample Cetr.Comp.(%)	Amountofcopper (II) µg /g		Recovery (%)
		Taken	Found ^a	
NTPC alloy	Ni 10; Cr 15; Fe 6.5; Cu 4.5; Mn 2.0	0.457	0.438	95.00
		0.915	0.864	94.00
		1.829	1.712	93.00
Aluminum alloy	Ni 1.93; Cu 4.10; Fe 0.4; Mn 0.2; Mg 1.6; and rest Al	1.003	0.998	99.00
		2.006	1.990	99.00
		3.009	3.005	99.00

^aAverage of 3 determinations

Table 7. Comparison of spectrophotometric methods for the determination of copper(II).

Reagent	Max nm	Beer's law range	Molar absorptivity L mo l ⁻¹ cm ⁻¹	M:L	Remark	Ref.
3-hydroxybenzaldehyde thiosemicarbazone	351	0.51-4.1 µg/mL	8.75×10 ³	1:2	Poor sensitivity	19
2, 4-Dihydroxy benzo phenone benzoic acid hydrazone	410	0.31-2.20 µg/mL	1.5×10 ⁴	1:1	Poor sensitivity	20
Benzil- α -monoxime isonicotinoyl hydrazone	346	0.50-5.08 mg/mL	1.2×10 ⁴	1:1	Poor sensitivity	21
2, 5-Dihydroxy aceto phenone benzoic acid hydrazone	400	0.30-6.00 µg/mL	1.1×10 ⁴	1:1	Poor sensitivity	22
3-Methoxy, 4-hydroxy Benzaldehyde 4-bromophenyl hydrazone	462	2.0-4.0 µg/mL	2.05×10 ⁴	1:1	Extraction step Poor sensitivity	23
2-Pyridine carboxaldehyde Isonicotinyl Hydrazone	352	0.01-1.5 mg/mL	5.2×10 ⁴	1:2	Poor sensitivity High reagent Consuming	24
a-(2-Benzimidazolyl) - α', α''-(N-5-nitro-2-pyridyl hydrazone)	410	0-2.5 µg/mL	3.81×10 ⁴	1:2	Extraction step, Complexis unstable high reagent consuming	25
Benzothiazole-2-aldehyde-2-quinolylylhydrazone	523	0.35-2.95 µg/mL	7.5×10 ⁴	1:2	Extraction step, Poor sensitivity, high reagent consuming	26
5-(2-benzothiazolylazo)-8-hydroxyquinoline	667	0.2-3.7 µg/mL	1.80×10 ⁹	1:1	Complicated procedure, poor sensitivity	27
Diacetylmonoxime-4-phenyl -3-thiosemicarbazone	385	0.10-0.51 µg/mL	2.92×10 ⁴	1:1	Poor sensitivity	28
2-Acetylpyridine semicarbazone	355	0.5-5.0 µg/mL	9.8×10 ³	1:1	Poor Sensitivity	PM

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