



SIMPLE, SENSITIVE, AND COST-EFFECTIVE TRACE LEVEL DETERMINATION OF COPPER IN ALCOHOLIC BEVERAGES AND BIOLOGICAL SAMPLES WITH 4-(2- PYRIDYLAZO)RESORCINOL IN PRESENCE OF NEUTRAL MICELLAR MEDIUM USING DERIVATIVE SPECTROPHOTOMETRY

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ABSTRACT: A reagent, 4-(2-pyridylazo) resorcinol has been reported to determine trace amounts of Cu(II) in the presence of a non-ionic surfactant, Triton X-100 using derivative spectrophotometry. Water-insoluble Cu(II) complex with 4-(2-pyridylazo) resorcinol has been solubilized in an aqueous phase in presence of a neutral surfactant, Triton X-100 and studied in normal and derivative mode. Important analytical figures, such as molar absorption coefficient (ϵ), specific absorptivity (a), and Sandell's sensitivity (S) of the Cu(II)-PAR complex at 510 nm (λ_{\max}) in neutral micellar media are $4.88 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$, $0.77 \text{ mL g}^{-1} \text{ cm}^{-1}$ and 1.30 ng cm^{-2} , respectively. The IUAPC detection limit and Beer's law range of Cu(II) determination are 1.40 ng mL^{-1} and $0.13 - 2.54 \text{ } \mu\text{g mL}^{-1}$, respectively. The conditional stability constant of the 1:2 complex formed at optimum experimental conditions is $5.67 \times 10^8 \text{ L}^2 \text{ mol}^{-2}$. Interference due to the presence of cations, anions, and complexing agents has been studied in normal as well as derivative modes. The proposed cost-effective, sensitive, and simple derivative spectrophotometric procedure is successfully applied to determine Cu(II) contents in commercially available alcoholic beverages and a biological

specimen. The analysis results are in good agreement with those obtained with atomic absorption spectrometry.

KEYWORDS: 4-(2-pyridylazo) resorcinol; Copper; Triton X-100; Non-ionic micellar media; Trough depth; Peak height.

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1. INTRODUCTION

Although copper is an essential micronutrient for humans, it is highly toxic to both plant and animal life at high concentrations, which can damage the central nervous system, leading to Wilson and Parkinson's disease.¹ Its deficiency also causes ischemic heart disease, anaemia, and bone disorders.² The World Health Organization (WHO) has notified the permissible limit of Cu(II) in drinking water at 2.0 ppm.³ As copper is necessary and toxic for biological systems, it's the concentration range between essential and toxic levels is narrow. Therefore, sensitive, simple, and cost-effective methods for trace level determination of Cu(II) in various matrices are highly in demand in developing countries.

Methods using costly and expensive instruments such as F-AAS,⁴⁻⁸ ICP-OES,^{9,10} ICP-MS,^{11,12} are reported in the literature for the determination of Cu(II) in diverse matrices. The determination of Cu(II) using these analytical instruments is not only carried out in well cleaned expensive sophisticated infrastructure but also requires skilled manpower. The liquid-liquid microextraction/or extraction,⁴⁻⁶ solid-phase extraction/or liquid-solid extraction,^{7,8,10,11} and cloud-point extraction⁹ are generally used in these expensive methods to amplify the selectivity and sensitivity of the procedure. Moreover, mostly organic solvents used for preconcentration and extractions/or separations are toxic and notified by Environment Protection Agency (EPA) are carcinogenic. Therefore, interest in developing newer simple and rapid green methods has grown as these costly analytical instruments are not much helpful in rapid and onsite detection and determination of Cu(II) at trace levels in a wide range of environmental matrices.

The cost effective spectrophotometric methods are sensitive, simple, and rapid have also been reported in the literature for determination of Cu(II) using reagents include 2,4-bis(4-phenylazophenylaminodiazo)phenol,¹³ isonitrosopropiophenone thiosemicarbazone,¹⁴ 1,3,3-trimethyl-2-[5-

(1,3,3-trimethyl-1,3-dihydroindol-2-ylidene)-penta-1,3-dienyl]-3H-indolium,¹⁵ lead diethyldithiocarbamate,¹⁶ tetrabutylammonium/tetrathiocyanatocuprate(ion-pair),¹⁷ pyridoxal-4-phenyl-3-thiosemicarbazone,¹⁸ 4-[N,N-(Dimethyl)amino]benzaldehyde thiosemicarbazone,¹⁹ neocuproine (2,9-dimethyl-1, 10-phenanthroline) / N-phenyl benzimidoylthiourea,²⁰ N'',N'''-bis[(E)-(4-fluorophenyl)methylidene]thiocarbonohydrazide,²¹ 4-(4'-nitrobenzylideneimino)-3-methyl-5-mercapto-1,2,4-triazole,²² 1-(2-Pyridylazo)-2-naphthol,²³ 4-(4'-chlorobenzylideneimino)-3-methyl-5-mercapto-1,2,4-triazole,²⁴ 4-((furan-2-ylmethylene)amino)-5-methyl-4 H-1,2,4-triazole-3-thiol,²⁵ 2-chlorobenzaldehyde thiocarbohydrazide,²⁶ (4-hydroxy-2-oxo-2H-chromen-3-yl)methyl pyrrolidine-1-carbodithioate,²⁷ and 6-hexyl-4-(2-thiazolylazo)resorcinol²⁸. Dithizone, dithiocarbamates, cuproine and related compounds are also reported for separation/ preconcentration of Cu(II) by extraction and then spectrophotometric determination.²⁹ Number of photometric methods based on azo reagents, such as PAN,^{30,31} PAR,^{32,33} and 5-Br-PADAP³⁴ have also been reported as sensitive methods for the determination of Cu(II). The methods reported for the spectrophotometric determination of Cu(II) are based on the formation of a Cu(II) complex with a reagent which has to be extracted using liquid-liquid extraction,^{14,17-20,22,24,26} dispersive liquid-liquid microextraction,^{15,27} solid-phase extraction,^{13,16,25,32} cloud-point extraction,²⁸ and synergic extraction,²¹ into non-aqueous solvents for absorbance measurement. Sometimes preconcentration of Cu(II) is also carried out by solid-phase extraction before its determination by spectrophotometric methods and other spectrometric methods. Several spectrophotometric methods for determination of Cu(II) uses chloroform^{16,17,19,22,24} an organic solvent, listed as a carcinogenic by EPA³⁵. Its vapours can cause fatigue, headaches, dizziness and have adverse effect on central nervous system.³⁵ The sensitivity and determination range of developed derivative spectrophotometric method has been compared with the methods reported in the literature is summarised in Table 1.¹³⁻³⁴ Therefore, newer simple, sensitive, and rapid methods for determination of Cu(II) in wide range of different samples is of great importance.

4-(2-Pyridylazo) resorcinol (PAR), water-soluble, non-selective azo dye generally used as a photometric reagent for several metal ions, is also reported in the literature for determination of Cu(II),^{32,33,36} forms a water-insoluble complex which requires tedious and time-consuming extraction steps before the photometric study to increase the sensitivity and selectivity of the developed procedure.

Table 1. Comparison of reported spectrophotometric methods for determination of Cu(II)

Reagent	Solvent	λ_{\max} (nm)	$\epsilon \times 10^4$, (L mol ⁻¹ cm ⁻¹)	Determination Range/Detection limit	Samples	Remark ^{Ref}
2,4-bis(4-phenylazophenylamino)phenol	Ethanol	540	18.6	0-0.2 μg 100mL ⁻¹ /2.0 ng mL ⁻¹	Biological (hair, blood, urine) samples	Solid-phase extraction ¹³
isonitrosopropiophenone thiosemicarbazone	isoamyl alcohol	390	0.5826	0.5-6.0	Synthetic mixtures, pharmaceuticals, aluminium alloys samples	Liquid-liquid extraction ¹⁴
1,3,3-trimethyl-2-[5-(1,3,3-trimethyl-1,3-dihydroindol-2-ylidene)-penta-1,3-dienyl]-3H-indolium	amyl acetate, tetrachloromethane, and methanol	640		0.020-0.090 mg L ⁻¹ / 0.005 mg L ⁻¹	Water samples	Dispersive liquid-liquid microextraction ¹⁵
lead diethyldithiocarbamate	Chloroform	435	1.0689	0.2-5 mg L ⁻¹	Alloys and water samples	Solid-phase extraction ¹⁶
Tetrabutylammonium + tetrathiocyanatocuprate (ion-pair)	Chloroform	485	-	0.5-25 mg L ⁻¹	Environmental, alloys, pharmaceutical samples	Liquid-liquid extraction ¹⁷
pyridoxal-4-phenyl-3-thiosemicarbazone	n-butanol	440	2.16	0.2-5.0 mg L ⁻¹ / 0.0065 μg mL ⁻¹	Water, alloys, and natural samples	Liquid-liquid extraction ¹⁸
4-[N,N-(Dimethyl)amino]benzaldehyde thiosemicarbazone	Chloroform	420	1.72	1.2-3.8 μg mL ⁻¹	Alloys, complexes, water,	Liquid-liquid extraction ¹⁹

					pharmaceutical samples	
Neocuproine (2,9-dimethyl-1, 10-phenanthroline)	N-phenyl benzimidoylthiourea/Chloroform	460	14.5	0.01-0.4 mg L ⁻¹	Water and soil samples	Liquid-liquid extraction ²⁰
N'',N'''-bis[(E)-(4-fluorophenyl)methylidene]thiocarbonyldrazide	Pyridine/amyl acetate	375	4.25	2.0-14 µg mL ⁻¹	Alloys, fertilizers, pesticides, pharmaceuticals, food materials and synthetic mixtures	Synergistic extraction ²¹
4-(4'-nitrobenzylidene imino)-3-methyl-5-mercapto-1, 2, 4-triazole	Chloroform	470	0.282	4.75-16.13 µg mL ⁻¹	synthetic mixtures, pharmaceutical and alloys samples	Liquid-liquid extraction ²²
5-(4-nitrophenylazo) salicylic acid	2,2'-Dipyridyl in ethanol medium	525	2.60	2.2 - 6.3 mg L ⁻¹	Pharmaceutical, biological, and water samples	ternary complex ²³
4-(4'-chlorobenzylideneimino)-3-methyl-5-mercapto-1, 2, 4-triazole	Chloroform	414	0.33813	5-17.5 µg mL ⁻¹	Food samples, leafy vegetables, fertilizers and environmental samples	Liquid-liquid extraction ²⁴
4-((furan-2-ylmethylene) amino)-5-methyl-4 H-1,2,4-triazole-3-thiol	Amberlite IR-120 / reversed-phase	599	649	5.0-190 ng mL ⁻¹ / 1.64 ng mL ⁻¹	Water, vegetables, food, and biological samples	solid-phase extraction ²⁵
2-Chlorobenzaldehyde thiocarbohydrazone	1-butanol	470	0.3429	Up to 17.5 µg mL ⁻¹	alloy, pharmaceutical, fertilise,	Liquid-liquid extraction ²⁶

					leafy vegetables and water samples	
(4-hydroxy-2-oxo-2H-chromen-3-yl)methyl pyrrolidine-1-carbodithioate	Tetrachloroethylene(extraction solvent) / acetone(dispersive solvent)	445	1.3	1.0 – 200 $\mu\text{g mL}^{-1}$ / 0.3 $\mu\text{g L}^{-1}$	Water, fruit juice samples	Dispersive liquid-liquid microextraction ²⁷
6-hexyl-4-(2-thiazolylazo)resorcinol	Triton X-114	535	25.4	4.5–254 ng mL^{-1} / 1.34 ng mL^{-1}	Water and saline solution samples	Cloud-point extraction ²⁸
1-(2-pyridylazo)-2-naphthol	Triton X-100	555	5.21	0.8-4.00 $\mu\text{g mL}^{-1}$ / 4.0 ng mL^{-1}	Beverages, biological, alloys, and certified samples	Aqueous phase ³⁰
4-(2-pyridylazo)resorcinol	styrene-divinylbenzene anion-exchange resin sol-gel optical sensor	500	0.3–4.5 $\mu\text{g L}^{-1}$	5.0-80 $\mu\text{g L}^{-1}$ / 3.0 $\mu\text{g L}^{-1}$	Mushrooms, tea, drugs, and waters samples	Solid-phase extraction ³²
2-(5-bromo-2-pyridylazo)-5-diethylaminophenol	Water-acetone	556	6.87	0-20 $\mu\text{g per 25 mL}$	Different acidic soil samples	³⁴
4-(2-pyridylazo)resorcinol	Triton X-100	510	4.88	0.13-2.54 $\mu\text{g mL}^{-1}$ / 1.40 ng mL^{-1}	Beverages and biological samples	Aqueous phase [Present Study]

The present study is based on utilizing the solubilization capability of neutral surfactant, Triton X 100,³⁷ to solubilize the reagent 4-(2-pyridylazo) resorcinol (PAR) and its Cu(II)-PAR complex in the aqueous phase. The sensitivity and selectivity of the developed method are considerably enhanced by using non-ionic micellar media formed by Triton X-100.^{30,37} High order derivative spectrophotometry is also used to

improve the sensitivity and selectivity of the present method besides overcoming interference due to overlapping spectral profiles of interfering ions, matrix background effect, sample turbidity, and improvement in minute details of the spectra.³⁸ The above-mentioned techniques have been utilized to make the present procedure a sensitive, simple rapid, and green method as it avoided extraction or pre-concentration steps using toxic organic solvents.^{39,40} In the present work, the precision, accuracy, and applicability have been verified by determining Cu(II) contents in commercially available beverages and biological samples and comparing the results with atomic absorption spectrometry (AAS). The present developed green method is cost-effective, simple, sensitive in comparison with other spectrophotometric and spectrometric methods reported in the literature.

2. EXPERIMENTAL

2.1. Instruments

Absorption spectra in the normal and derivative modes were recorded on a thermal paper using a Shimadzu (Model UV-260, Japan) recording spectrophotometer. A pair of 10 mm matched quartz cells was used to record absorption spectra. A bandwidth of 2 nm was selected for recording spectra. $\Delta\lambda$ 2 nm and 4 nm were selected for recording spectra in first- and second-order derivative mode, respectively. The pH of all test solutions was adjusted using an ECIL digital pH meter (Model No. 5651, India) using a combined glass calomel electrode. Cu(II) contents in sample solutions were determined by using Shimadzu Atomic Absorption Spectrometer (Model AA-640-13, Japan).

2.2. Reagents

All chemicals used were of analytical grade purity. A Stock solution of Cu(II) (4.00×10^{-4} M) was prepared from $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. Equimolar solutions (100 mL) of 4-(2-pyridylazo) resorcinol (PAR) were prepared in the minimum amount of H_2SO_4 . Neutral surfactant, Triton X-100 (4.0 % m/v) was then added and the solution diluted with double distilled water.

2.3. Working Solutions

The optimum pH of complexation was determined by preparing two sets of solutions containing Triton X-100 (4.0% m/v) in the pH range 1.0 to 12.0, one set containing only ligand, PAR, and the other containing PAR and Cu(II).

The effect of increasing PAR concentration at the optimum pH was examined at 510 nm, λ_{\max} of complex and by preparing a set of solutions containing increasing amounts from 2.00×10^{-6} to 2.00×10^{-4} M of the reagents and fixed amount (8.00×10^{-6} M) of Cu(II) ions in presence of Triton X-100 4.0% (m/v).

The linear range of determination of Cu(II) has been determined at 510 nm, λ_{\max} of Cu(II)-PAR complex; (i) by varying metal ion concentration (4.00×10^{-6} to 4.00×10^{-5} M) at a fixed ligand concentration (2.00×10^{-4} M) and (ii) keeping a fixed metal to ligand ratio.

The effect of the presence of foreign ions (cations, anions, or complexing ligands) on the absorbance of the Cu-PAR complex was studied by adding the foreign ion to the metal solution before forming the complex and then recording the spectra in normal mode and derivative mode. A change of $\geq 4.0\%$ in the absorbance was taken as the criteria for causing interference.

3. RESULTS AND DISCUSSION

3.1. Absorption Spectra and their dependence on pH

The ligand, PAR in presence of neutral micellar media gives a peak at 390 nm which shifts bathochromically to 400 nm, the intensity of which gradually decreases with an increase in pH up to 4.5. Further, an increase in pH (up to 8.0) shifts maximum at 400 nm to 405 nm. Its Cu(II) complex also has pH-dependent peaks and spectra similar to ligand blank except for higher absorbance near 510 nm. However, on increasing the pH, a marked increase in absorbance is observed at 510 nm up to pH 11.5 and decreasing sharply thereafter. This decrease coupled with an increase at 405 nm, λ_{\max} of the ligand, and the presence of an isosbestic point at 440 nm indicate dissociation of the complex with an increase in pH.

The relative increase in absorbance of the solutions containing metal ions over ligand alone was plotted against pH. The plot showed maxima at pH 7.0 and therefore, all subsequent studies have been carried out at 7.0 pH.

A neutral surfactant, Triton X-100 was selected as a solubilizing agent during the present study as there is negligible /or no electrostatic interaction present between the neutral surfactant and metal ion,³⁷ and complexation reaction between Cu(II) ion and PAR is not affected.

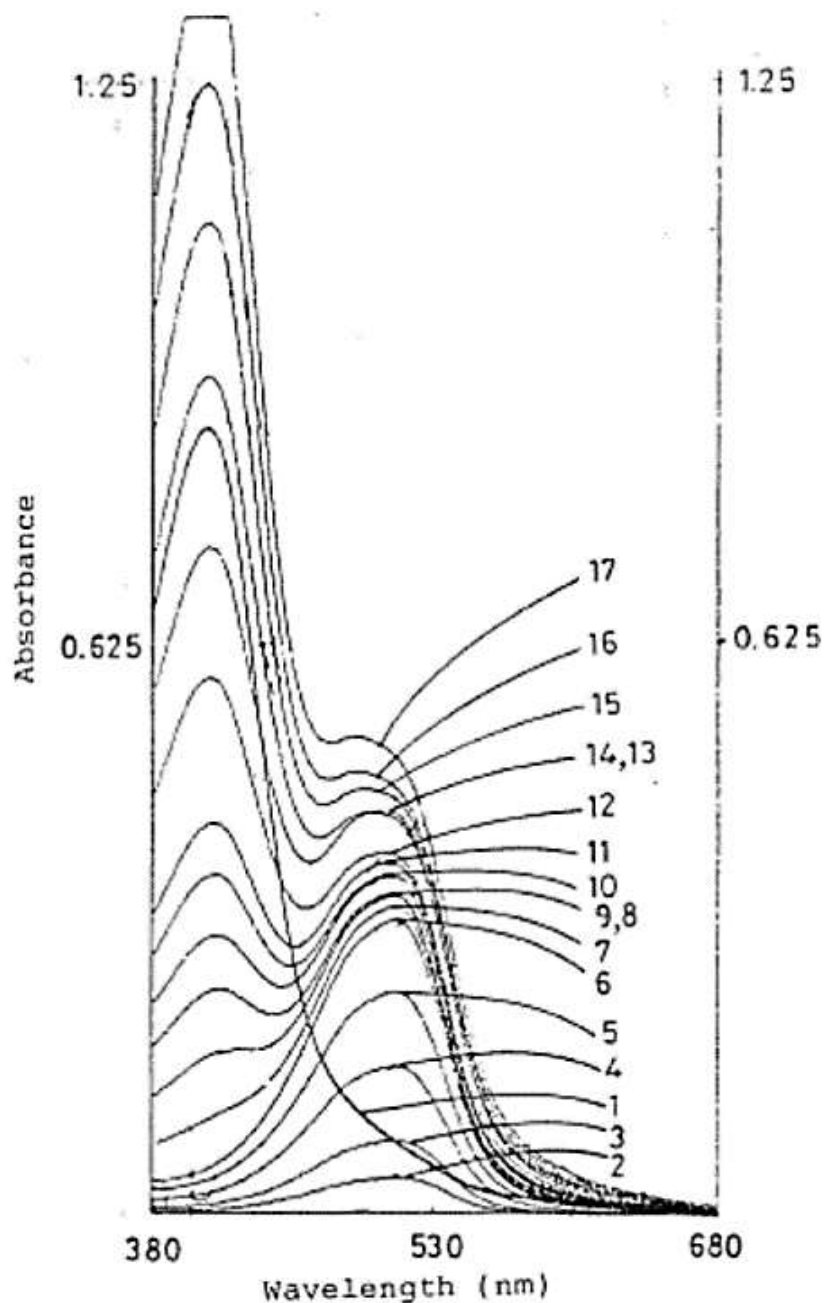


Fig. 1. Absorption spectra of the solutions containing an increasing amount of PAR (4.00×10^{-6} - 4.00×10^{-5} M) and fixed amount of Cu(II) ion (8.00×10^{-6} M) in presence of Triton X-100 (4% m/v).

3.2. Varying PAR concentration

The absorbance of the system has been found to increase with an increase in the concentration of the PAR, as absorption spectra were recorded against water blank and the ligand also absorbs significantly at λ_{\max} , 510 nm (Fig. 1). So, on increasing the concentration of PAR, the contribution of absorbance of free ligand has been corrected for absorbance at λ_{\max} . The graph of corrected absorbance at 510 nm was plotted against

the mole ratio of ligand, which remains practically flat line from 4-fold to 25-fold excess of the PAR. During subsequent studies, the PAR concentration has, therefore, been kept between 4.00 to 25.0 times the Cu(II) concentration wherever possible.

3.3. Varying Cu(II) ion concentration

The complex, Cu(II)-PAR in the presence of Triton X-100 shows a well-defined isosbestic point at 445 nm (Fig. 2) which indicates the existence of a single complexation equilibrium. The ligand peak at 410 nm

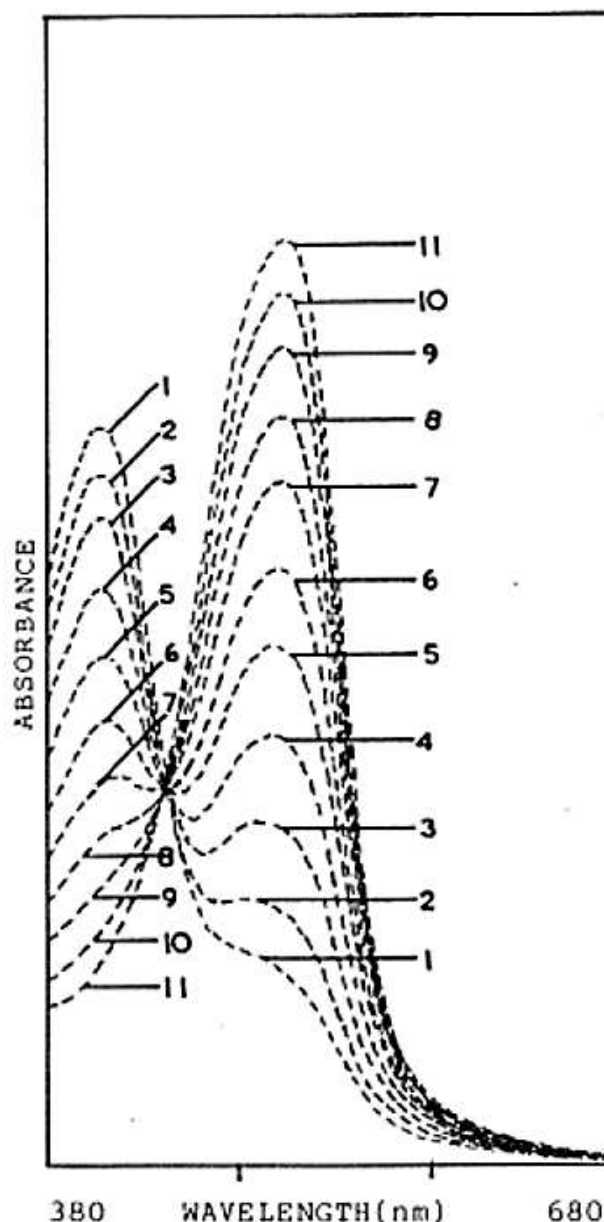


Fig. 2. Absorption spectra of the solutions containing a fixed amount of PAR (2.00×10^{-4} M) and increasing concentration of Cu(II) ion (4.00×10^{-6} - 4.00×10^{-5} M) in presence of Triton X-100 (4% m/v).

disappears with increasing metal concentration and reduces to a shoulder to the peak of the complex (Fig. 2). The absorbance of the complex at its λ_{\max} , 510 nm was plotted against the concentration of the Cu(II) ions, showing a good linear relationship (Table 2). The linear relationship between absorbance at 510 nm and concentration of Cu(II) ($\mu\text{g mL}^{-1}$) with a residue square of 0.9926 is observed. The negative intercept in the linear equation indicates that the ligand also has absorption at λ_{\max} of the complex (Table 2).

Table 2. The linear regression equation and detection limit for determination of Cu(II) in normal and derivative mode

Mode of Absorption spectra	Linear equation	Regression Coefficient	Detection limit (ng mL^{-1})
Normal Mode	$C = 1.942 \times 10^0 [A]_{510\text{nm}} - 6.755 \times 10^{-1}$	0.9926*	3.14
First-order derivative Mode	$C = 4.357 \times 10^{-1} [\text{PH}]_{470\text{nm}} + 4.005 \times 10^{-2}$	0.9944	1.40
	$C = 3.056 \times 10^{-1} [\text{TD}]_{435\text{nm}} - 1.121 \times 10^{-2}$	0.9926	
	$C = 3.073 \times 10^{-1} [\text{TD}]_{535\text{nm}} + 5.634 \times 10^{-2}$	0.9967*	
Second-order derivative mode	$C = 4.593 \times 10^{-1} [\text{PH}]_{550\text{nm}} + 1.071 \times 10^{-1}$	0.9929	1.40
	$C = 2.997 \times 10^{-1} [\text{TD}]_{515\text{nm}} + 5.490 \times 10^{-2}$	0.9967*	

* Recommended for the quantitative determination of Cu(II) in a neutral micellar medium.

[C] – Concentration of Cu(II) in $\mu\text{g mL}^{-1}$; [PH] – Peak Height; [TD] – Trough Depth.

The first-order derivative spectra of the above set of solutions show a peak at 470 nm, and two troughs at 435 nm and 535 nm (Fig. 3. A). As the concentration of the complex increases peak height at 470 nm and trough depth at 435 nm increases with a slight bathochromic shift. The λ_{\max} of Cu(II)-PAR in the normal

spectra can be precisely predicted from the position of cross-over point in the first-order derivative spectra. The cross-over points at 510 nm and 410 nm correspond to the λ_{\max} of the complex and the ligand, respectively. Linear regression of peak height at 470 nm [PH] and trough depths at 435 nm [TD₁] and 535

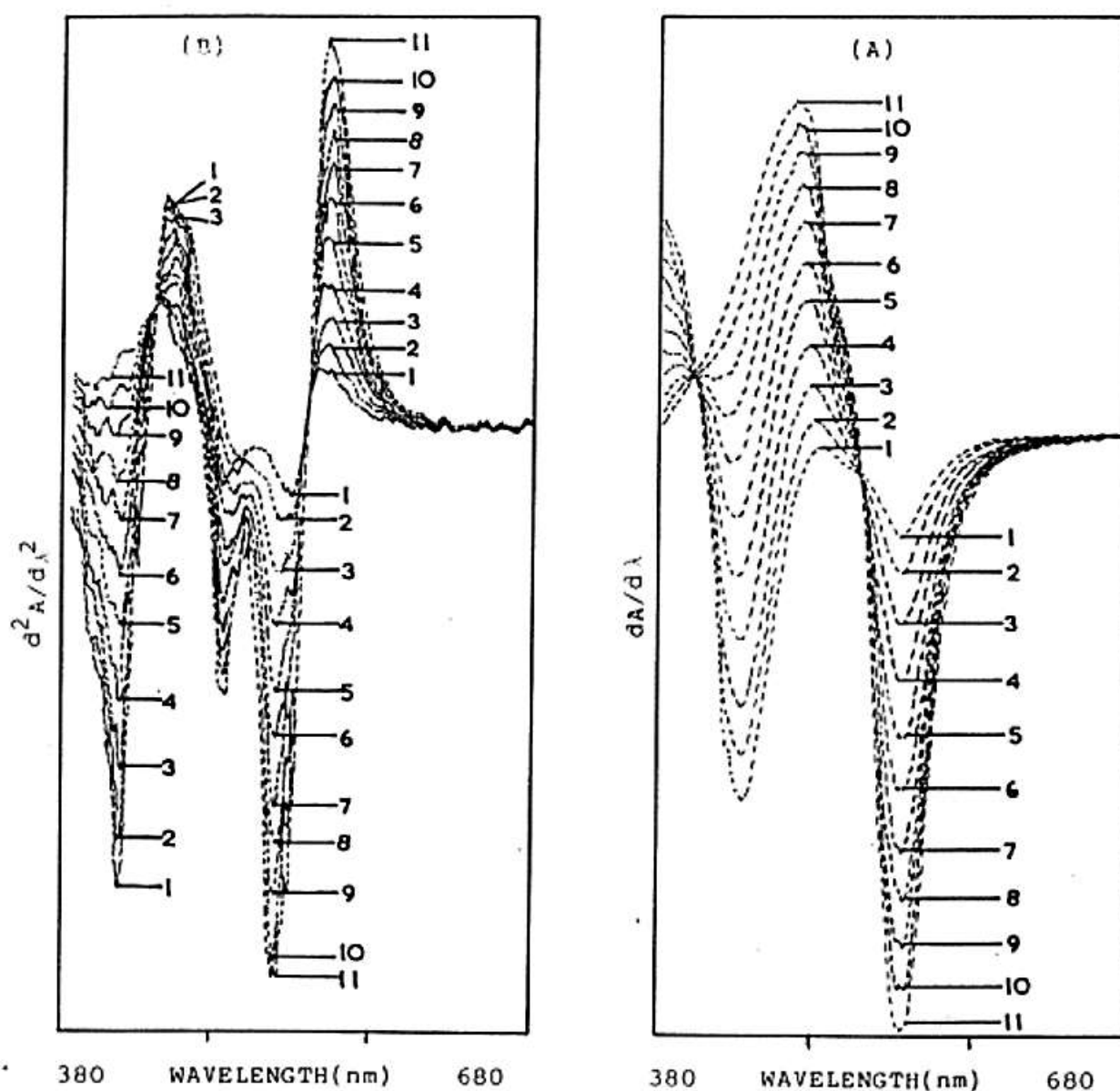


Fig. 3. (A) First-order derivative spectra and (B) Second-order derivative spectra of the solutions containing a fixed amount of PAR (2.00×10^{-4} M) and increasing concentration of Cu(II) ion (4.00×10^{-6} - 4.00×10^{-5} M) in presence of Triton X-100 (4% m/v).

nm [TD₂] on metal ion concentration gives a good linear fit, shown in Table 2. The second-order derivative spectra (Fig. 3. B) show a clear peak at 550 nm and a trough at 515 nm. The height of the peak at 550 nm and the depth of the trough at 515 nm are proportional to the metal ion concentration ($\mu\text{g mL}^{-1}$) with a good

fit (Table 2). The measuring absorbance at 510 nm in normal mode as well as measuring the trough depths at 535 nm and 515 nm in first- and second-order derivative mode, respectively is recommended for trace determination of Cu(II). The developed procedure is more sensitive in derivative modes as evidenced by the lower detection limit (Table 2). The method shows a good precision (R.S.D., n=6) between 1.15% and 2.74% for the determination of $0.65 \mu\text{g mL}^{-1}$ Cu(II).

Table 3. Important analytical figures of Cu(II)–PAR complex in the presence of a neutral micellar medium of Triton X-100

Analytical Figures	Cu(II)-PAR-Triton X-100 complex at 7.0 pH
λ_{max}	510 nm
Molar absorption coefficient, ϵ	$4.88 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$
Sandell's Sensitivity, S	1.30 ng cm^{-2}
Specific absorptivity (a)	$0.77 \text{ mL mol}^{-1} \text{ cm}^{-1}$
Analytical Sensitivity, $S_A = S_s/m$	1.05 ng mL^{-1}
Beer's Law	$0.13 - 2.50 \mu\text{g mL}^{-1}$
Detection Limit, $C_{L(k=3)}$	1.40 ng mL^{-1}
Limit of Quantification, $C_{Q(k=10)}$	10.5 ng mL^{-1}
Linear Dynamic range	$0.01 - 2.50 \mu\text{g mL}^{-1}$
RSD (%) (n=6; $0.65 \mu\text{g mL}^{-1}$)	2.74

The important analytical figures, IUPAC detection limit, molar absorption coefficient (ϵ), Sandell's sensitivity (S), and specific absorptivity (a) of Cu(II)-PAR-Triton X-100 at 510 nm under optimum working conditions are 1.40 ng mL^{-1} , $4.88 \times 10^{-4} \text{ L mol}^{-1} \text{ cm}^{-1}$, 1.30 ng cm^{-2} , and $0.77 \text{ mL g}^{-1} \text{ cm}^{-1}$, respectively (Table 3). The system obeys Beer's law from $0.13 - 2.54 \text{ } \mu\text{g mL}^{-1}$ of Cu(II) ion concentration.

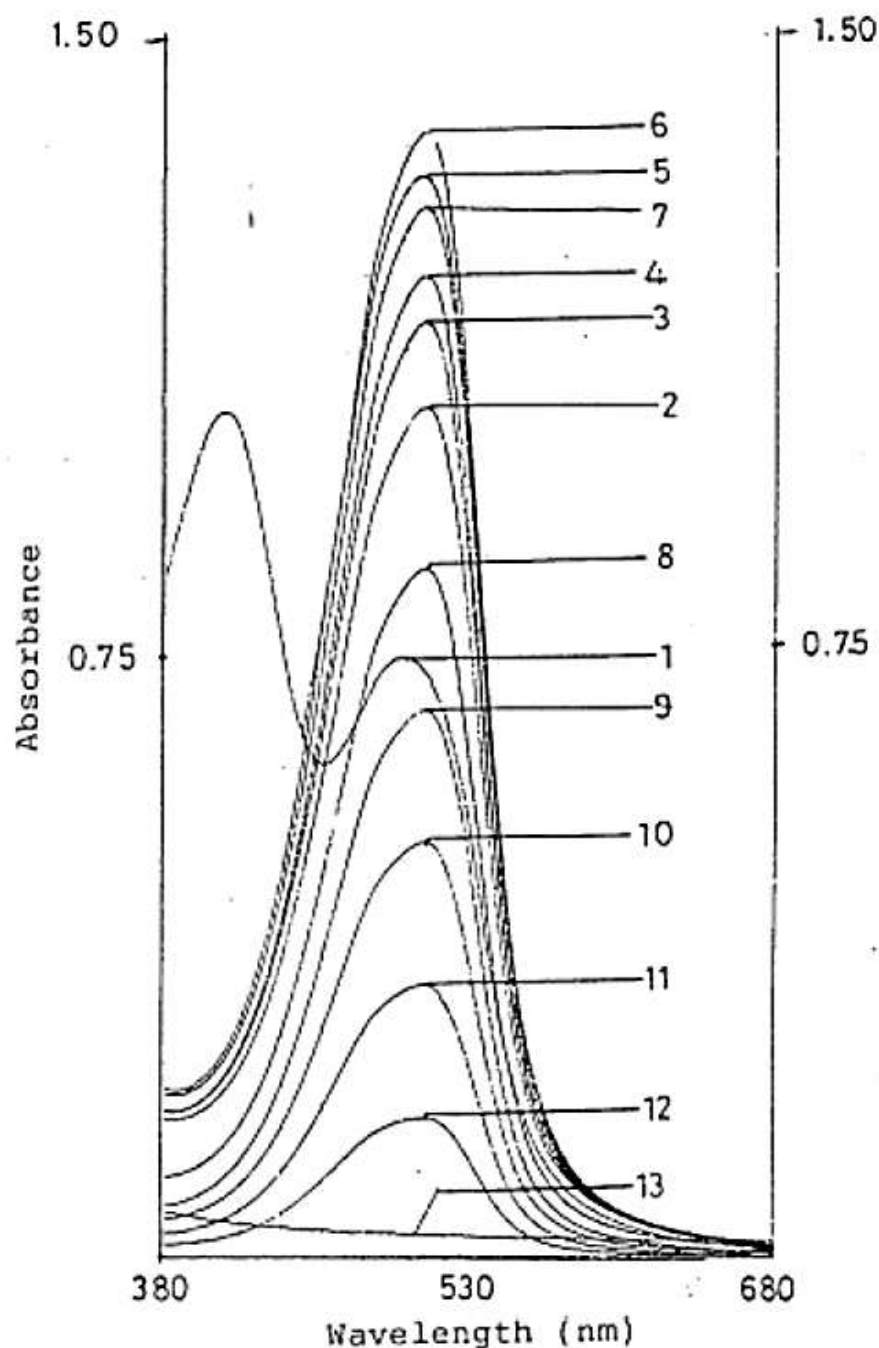
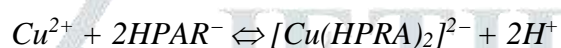


Fig. 4. Absorption spectra of the solutions with increasing mole fraction of metal ion amount of PAR ($4.00 \times 10^{-6} - 4.00 \times 10^{-5} \text{ M}$) and fixed amount of Cu(II) ion ($4.00 \times 10^{-6} - 4.00 \times 10^{-5} \text{ M}$) in presence of Triton X-100 (4% m/v).

3.4 Composition and stability constant

The composition of the complex formed in the neutral micellar medium under the optimum experimental conditions was determined using Job's method of continuous variations graph. The absorbance at λ_{\max} was plotted against the mole fraction of Cu(II) ions shows a maximum at 0.33 (Fig. 4). The mole fraction of 0.33 corresponds to the composition of 1:2 (M:L) metal to the ligand. The complex of Cu(II) with PAR may represent as $[\text{Cu}(\text{PAR})_2]$ in a micellar medium of neutral surfactant.

The stability constant of the Cu(II) complex formed with PAR under experimental conditions was calculated as conditional stability constant⁴¹ and using the following equilibrium.



$$K' = \frac{[\text{Cu}(\text{PAR})_2]}{[\text{Cu}][\text{PAR}]_T^2}$$

Where $[\text{PAR}]_T^2$ total concentration of all forms of PAR present in the solution.

The conditional stability constant of the Cu(II)-PAR complex in presence of neutral micellar medium has been calculated using concentration terms instead of activity terms since the concentration of metal ion and PAR in presence of neutral micellar medium is very low.⁴¹ Conditional stability constant, K' is calculated by taking an average of six values and found to be $5.673 \times 10^8 \text{ L}^2\text{mol}^{-2}$.

3.5. Effect of foreign ions

The effect of foreign ions/ligands on the absorbance of the Cu(II)-PAR complex has been investigated to evaluate the practical utility of the developed method for trace level determination of Cu(II). Interference is tested from 10-fold to 25-fold excess of the added ion/ligand under optimum experimental conditions in normal as well as the derivative modes.

The cations investigated are Al^{3+} , Be^{2+} , Ca^{2+} , Cd^{2+} , Cr^{3+} , Co^{2+} , Fe^{2+} , Fe^{3+} , Hg^{2+} , Mg^{2+} , Mn^{2+} , Ni^{2+} , Pb^{2+} , Tl^+ , VO^{2+} , and Zn^{2+} . Among these Cd^{2+} , Cr^{3+} , Co^{2+} , Fe^{2+} , Fe^{3+} , Hg^{2+} , Mn^{2+} , Ni^{2+} , Pb^{2+} , VO^{2+} , and Zn^{2+} interfered in the determination of Cu(II) even if present at 25-fold excess. While Al^{3+} , Be^{2+} , Ca^{2+} , Mg^{2+} , and Tl^+ , and shows only significant interference at 125-fold excess that of Cu(II) ions. The anions/complexing ligands

studied include acetate, ascorbic acid, borate, bromide, chloride, fluoride, citrate, EDTA, iodide, oxalate, tartrate, and thiocyanate. Among these EDTA masked the complex formation almost completely between Cu(II) and PAR but chloride, thiocyanate, and boric acid interfere seriously at 25-fold excess, and fluoride masked the reaction between Fe and PAR. No significant interference observed by bromide, citrate, iodide, and tartaric acid.

3.6. Determination of Cu(II) in Beverages and Biological Samples

Photometric data reported above suggest that PAR is sensitive for trace determination of Cu(II) in presence of neutral surfactant. The developed method has been successfully used to determine the Cu(II) content in beverages and biological samples.

A 10 mL beverage sample was digested by refluxing for six hours in a solution containing a mixture of 10 mL nitric acid and 3 mL H₂O₂. The resultant digested mixture was evaporated to dryness.

A sample of goat liver (freeze-dried) was heated in a furnace at 500-600 °C for 12 hours. The residue obtained was liquified in HNO₃ (concentrated) and slowly heated on a heating plate to dryness.

The contents of both samples were filtered and made up to a suitable volume with double distilled water. Test solutions were prepared at optimum working conditions by procuring an appropriate aliquot of the sample solution and the PAR in presence of Triton X-100 (4.0% m/v). Absorption spectra of the sample solutions were then recorded against water blank in normal and derivative modes. The respective linear regression equations obtained with standard Cu(II) solutions were used to calculate the Cu(II) contents in beverages and biological samples. The values of absorption at 510 nm ($[A]_{510\text{nm}}$) or trough depth at 535 nm ($[TD]_{535\text{nm}}$, first-order derivative mode) or at 515 nm ($[TD]_{515\text{nm}}$, second-order derivative mode) were used to determine Cu(II) contents in the sample solutions. The analytical results obtained are in good agreement with those obtained using an atomic absorption spectrometer (AAS) shown in Table 4.

4. CONCLUSION

The ligand PAR in presence of Triton X-100 is advantageous for trace determination of Cu(II) in beverage and biological matrices without any prior separation or preconcentration. The presence of a single equilibrium in the solution is indicated by the isosbestic point at 440 nm of the Cu(II)-PAR complex in the

Table 4. Spectrophotometric and atomic absorption spectrometric (AAS) determination of Cu(II) in alcoholic beverages and biological matrices with the PAR in presence of Triton X-100

Sample	Cu(II) contents (ppm)		Error (%)*
	Developed method	AAS	
Whisky (5 ppp) Ajudhia Distillery, Moradabad, India	4.63	4.64	+0.215
	4.66		-0.431
	4.63		+0.862
	4.68		+0.431
Rum (10 ppm) Ajudhia Distillery, Moradabad, India	9.09	9.11	+0.219
	9.15		-0.439
	9.14		-0.329
	9.10		+0.109
Gin (10 ppm) Ajudhia Distillery, Moradabad, India	9.25	9.30	+0.537
	9.34		-0.430
	9.32		-0.215
	9.27		+0.322
Brandies (5ppm), Ajudhia Distillery Moradabad, India.	4.52	4.54	+0.440
	4.54		±0.000
	4.50		+0.881
	4.56		+0.440
Goat Liver	3.74	3.72	-0.537
	3.70		+0.537
	3.75		+0.806
	3.71		+0.268

*- Average of 3 determinations; CV ≤ 1%.

neutral micellar medium. The measurement of absorbance at 510 nm, trough depths at 535 nm, and 515 nm, in first- and second-order derivative spectra, respectively are recommended for trace determination as evidenced by respective linear regression equations and lower detection limit of 1.40 ng mL^{-1} (Table 2). First-order derivative spectra of the Cu(II)-PAR system show the exact position of λ_{max} by a sharp cross-over point at 510 nm. The presence of Triton X-100 (a neutral surfactant), not only increases the sensitivity of the method but also makes the present developed procedure, a green spectrophotometric method as it is free from lengthy and tidy pre-concentration or extraction steps using toxic organic solvents. The molar absorptivity ($4.88 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$) of the 1:2 Cu(II)-PAR complex in presence of neutral micellar media in terms of sensitivity is higher than the reported Cu(II)-PAR ($2.7 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$) complex.⁴² The detection limit of the developed green and inexpensive method is 1.40 ng mL^{-1} which is higher than most of the reported spectrophotometric methods (0.005 mg L^{-1} , $0.0065 \text{ } \mu\text{g mL}^{-1}$, 1.64 ng mL^{-1} , $0.3 \text{ } \mu\text{g L}^{-1}$, 1.34 ng mL^{-1} , and 4.0 ng mL^{-1})^{15,18,25,27,28,30} and comparable to other reported expensive spectrometric methods F-AAS ($0.60 \text{ } \mu\text{g L}^{-1}$, $0.7 \text{ } \mu\text{g L}^{-1}$, $0.36 \text{ } \mu\text{g L}^{-1}$, and 2.9 ng mL^{-1}),^{4,5,6,7} ICP-OES ($0.149 \text{ } \mu\text{g g}^{-1}$),⁹ ICP-MS (0.6 ng L^{-1} and 3 ng g^{-1}),^{11,12} and ET-AAS (26 ng g^{-1})¹². The present cost-effective and green spectrophotometric method has been excellently suitable for the simple and rapid trace level estimation of Cu(II) in biological and alcoholic beverages samples.

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