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Spectrophotometric Determination of Copper(II) with 4-(2-Pyridylazo)-resorcinol Disodium in Pharmaceuticals

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Spectrophotometric determination of copper(II) with 4-(2-pyridylazo)-resorcinol disodium salt dihydrate (PAR) in aqueous phosphate buffer at pH = 10 was studied. It was suggested in this study that two complexes are formed. The first is Cu(PAR) with absorption maxima at 510 nm, $\varepsilon = 4.0 \times 10^4$ mol^{-1} cm^{-1} L and the second is $Cu(PAR)_2$ with absorption maxima in the range 495-500 nm, $\varepsilon = 7.62 \times 10^4$ and 7.12×10^4 mol^{-1} cm^{-1} L in absence and presence EDTA respectively. The formation constant pK for the $Cu(PAR)_2$ complex is in order of 10^{12}. Beer's law is obeyed for Cu-PAR complex, ratio of 1:2, on the range of 1×10^{-6}-2.5×10^{-5} M and 0.1×10^{-6}-2.5×10^{-5} M with relative standard deviation 1.7 and 2.2 % in absence and presence of EDTA respectively. Cr^{3+}, As^{3+}, Ni^{2+}, Co^{2+}, Fe^{3+} and Pb^{2+} do not cause any considerable interference. Whereas, Hg^{2+}, Zn^{2+} and Cd^{2+} do not interfere in presence of EDTA. This method was also applied successfully for determination of copper(II) in some pharmaceuticals in the presence of EDTA. The relative standard deviation did not exceed ± 1.5 %.

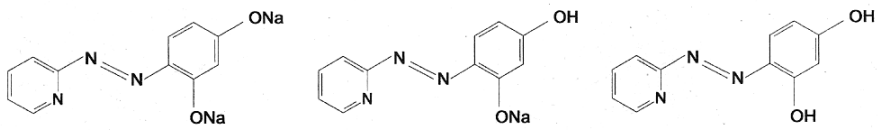
Key Words: 4-(2-Pyridylazo)-resorcinol (PAR), Copper, Spectrometry, Pharmaceuticals.

INTRODUCTION

Copper(II) is a necessary element for some biological operation in human body, animals and plants[1,2]. Various techniques such as polarography, voltammetry, UV-visible and AAS with high sensitivity for the determination of copper(II) are reported[3-6]. The formation of complex between copper(II) and 4-(2-pyridylazo)-resorcinol monosodium (PAR) as monohydrate salt with ratio of 1:1 at pH = 6.5 (λ_{max} = 510 nm) or 1:2 at pH = 9.2 (λ_{max} = 495 nm) was studied using spectrophotometric method[7,8]. 4-(2-Pyridylazo)-resorcinol monosodium was used directly for the determination of copper(II)[9-12] and formation of complex Cu:(PAR)[9-18]. Fig. 1 shows three structure of 4-(2-pyridylazo)-resorcinol and its sodium derivatives.

EXPERIMENTAL

Spectrophotometric measurements was made in a Biotech E.M. UV-Visible spectrophotometer with 1.00 cm quartz cells. The pH measurement was performed with EUTECH COPERSCAN-500. A ultrasonic processor model POWERSONIC 405 was used to sonicate the sample solutions.



4-(2-pyridylazo)-resorcinol disodium | 4-(2-pyridylazo)-resorcinol monosodium | 4-(2-pyridylazo)-resorcinol

Fig. 1. Structures formula of PAR

All solutions are prepared with deionized water from analytical-reagent grade chemicals as the following: 0.01 M PAR (BDH), 0.01 M ethylenediaminetetraacetic acid disodium salt (EDTA), 0.001 M $Cu(NO_3)_2.3H_2O$, HCl and HNO_3 (Merck). The medium pH is controlled by using phosphate buffers pH = 10 prepared from 0.25 M Na_3PO_4 and 0.2 M Na_2HPO_4 (Merck).

Procedures: The test solution is prepared by adding 0.8 mL of 0.01 M EDTA to 0.1 mL sample solution (content Cu^{2+}), 2 mL phosphate buffer, 0.8 mL of 0.01 M PAR and made up to 10 mL by deionized water.

A blank prepared in the same way but without copper(II). The absorbance of the solution is measured at 495-500 nm and the amount of copper in the samples is determined using standard additions.

RESULTS AND DISCUSSION

Effect of pH and time: The effect of pH values in the range 6.0-12.5 on the absorbance (A) of copper(II)-PAR complex was examined. It was found that, the optimum value of pH is 10 for highest absorbance (Fig. 2).

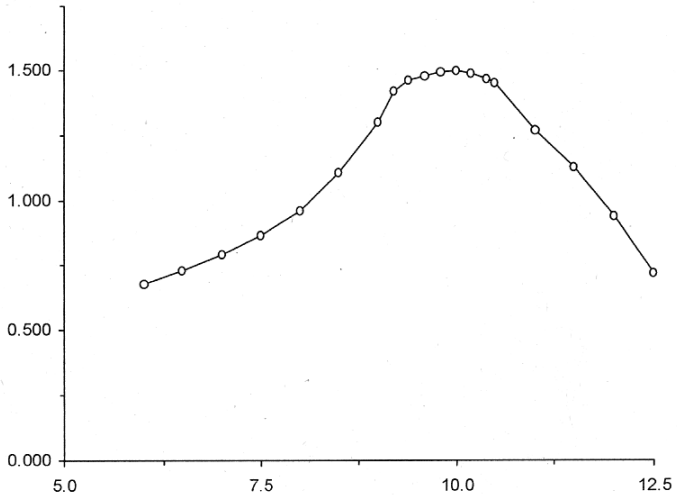


Fig. 2. Effect of pH on the determination of Cu^{2+} (2×10^{-5}) M, reagent concentration (2×10^{-4}) M, λ_{max} = (495-500) nm, l = 1 cm

The effect of time on stability of the copper(II)-PAR complex solution was studied. It was found that, the complex was stable for 1 week.

Spectrophotometric results: UV-Vis spectra by using distilled water as blank were studied. The Cu^{2+} solutions do not absorb in range 330-600 nm, while PAR solutions has good absorption at 414 nm with molar absorptive coefficient $\varepsilon = 4.45 \times 10^4$ mol^{-1} cm^{-1} L. When the concentration of Cu^{2+} increases to $C_{Cu^{2+}} \leq ½ C_{PAR}$, the PAR peak decreases and new peak for Cu-PAR complex at λ_{max} = 495-500 nm sharply increases. After that, when $C_{PAR} \geq C_{Cu^{2+}} > ½ C_{PAR}$, PAR peak disappears and the peak for Cu-PAR complex slowly increases. Finally, when $C_{Cu^{2+}} > C_{PAR}$, the peak for Cu-PAR complex appears at λ_{max} = 510 nm and stays constant (Fig. 3).

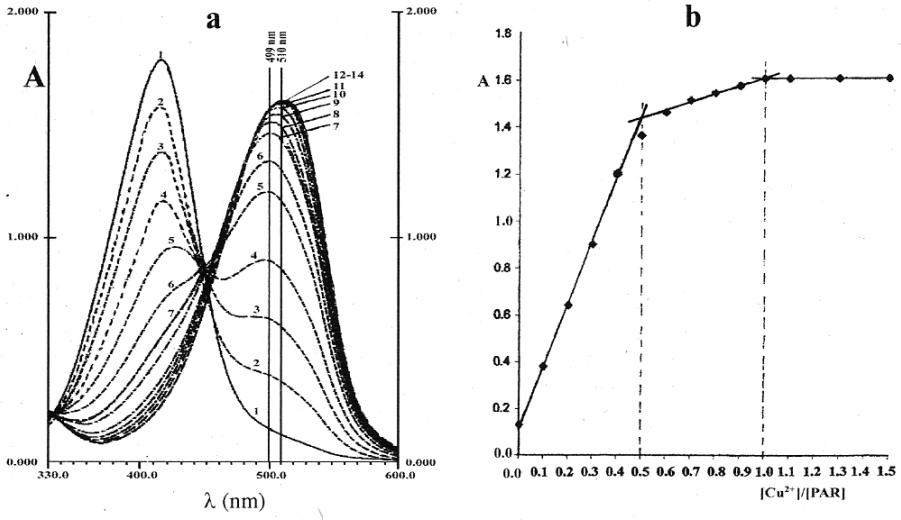


Fig. 3. (a) Uv-vis spectra for Cu^{2+} ($C_{Cu^{2+}}$: 1-0, 2-0.4 × 10^{-5}, 3-0.8 × 10^{-5}, 4-1.2 × 10^{-5}, 5-1.6 × 10^{-5}, 6-2.0 × 10^{-5}, 7-2.4 × 10^{-5}, 8-2.8 × 10^{-5}, 9-3.2 × 10^{-5},10-3.6 × 10^{-5},11-4.0 × 10^{-5}, 12-4.4 × 10^{-5}, 13-5.2 × 10^{-5}, 14-6.0 × 10^{-5} M), PAR 4 × 10^{-5} M. (b) Molar ratio method to calculate coupling ratio for complex PAR-Cu, PAR concentration is constant 4 × 10^{-5} M (in phosphate buffer pH = 10 by using distilled water as blank, λ_{max} = 495-500 nm, l = 1 cm)

Composition of copper(II)-PAR complex

Molar ratio method: The stoichiometry of copper(II)-PAR complex by molar ratio method, $A_{max} = f\left(\frac{[Cu^{2+}]}{[PAR]}\right)$, confirms that the ratios of complex Cu:PAR are equal to 1:2 and 1:1. Where the concentrations of Cu^{2+} change from 0 to 6.0 × 10^{-5} M and the PAR concentration is constant 4 × 10^{-5} M (Fig. 3).

Continuous variation method: The ratios of copper(II)-PAR complex by continuous variation method, $A_{max} = f\left(\frac{[Cu^{2+}]}{[Cu^{2+}]+[PAR]}\right)$, in which $C_{Cu^{2+}} + C_{PAR} = 3 \times 10^{-5}$ M (constant) were studied. The stoichiometry of complex are equal to 1:2 and 1:1 (Fig. 4).

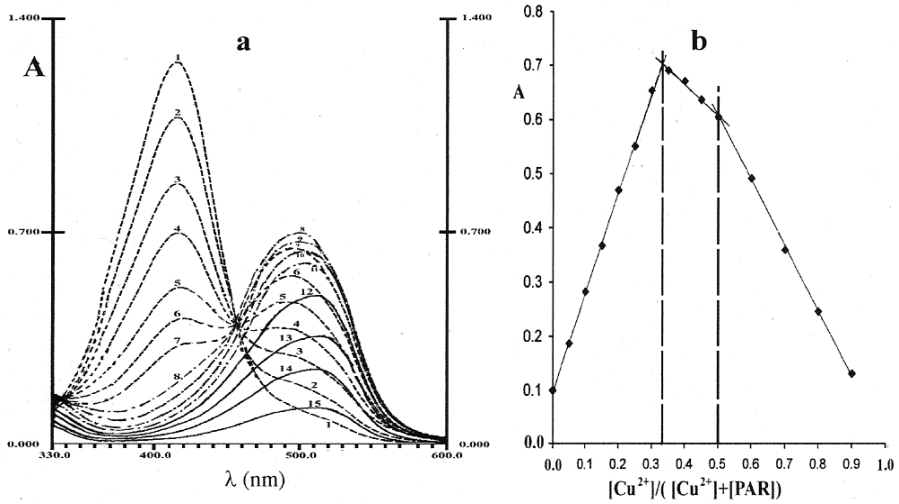


Fig. 4. (a) Absorption spectra (UV-vis) for Cu^{2+} solutions when $C_{Cu^{2+}}$ + CPAR = 3×10^{-5} M, ($C_{Cu^{2+}}$: 1-0, 2-0.15×10^{-5}, 3-0.30×10^{-5}, 4- 0.45×10^{-5}, 5-0.60×10^{-5}, 6- 0.75×10^{-5}, 7-0.90×10^{-5}, 8-1.05×10^{-5}, 9-1.20×10^{-5}, 10-1.35×10^{-5}, 11-1.50×10^{-5}, 12-1.80×10^{-5}, 13-2.10×10^{-5}, 14-2.40×10^{-5}, 15-2.70×10^{-5} M), (b) Continuous variation method to calculate coupling ratio for Cu-PAR complex (by using distilled water as blank, in phosphate buffer pH = 10, λ_{max} = 495-500 nm, l = 1 cm)

Calculation of the complex formation constant: The complex formation constant (K) is calculated by the following formula: $K = [Cu(PAR)_2]/[Cu^{2+}].[PAR^{-2}]^2$ (Table-1).

TABLE-1
FORMATION CONSTANT LOGARITHM DETERMINATION
FOR THE COPPER COMPLEX

Method	Coupling ratio	log K
Molar ratio	1:2	12.31
Continuous variation	1:2	11.83

Effect of interfering ions: The effect of some interfering ions such as Cr^{3+}, As^{3+}, Ni^{2+}, Co^{2+}, Pb^{2+}, Fe^{3+}, Hg^{2+}, Zn^{2+}, Cd^{2+} on $Cu(PAR)_2$ complex was studied. Cr^{3+}, As^{3+}, Ni^{2+}, Co^{2+}, Fe^{3+} and Pb^{2+} do not cause any considerable interference. Whereas, Hg^{2+}, Zn^{2+} and Cd^{2+} interfere, but they can be masked by adding of EDTA (Fig. 5).

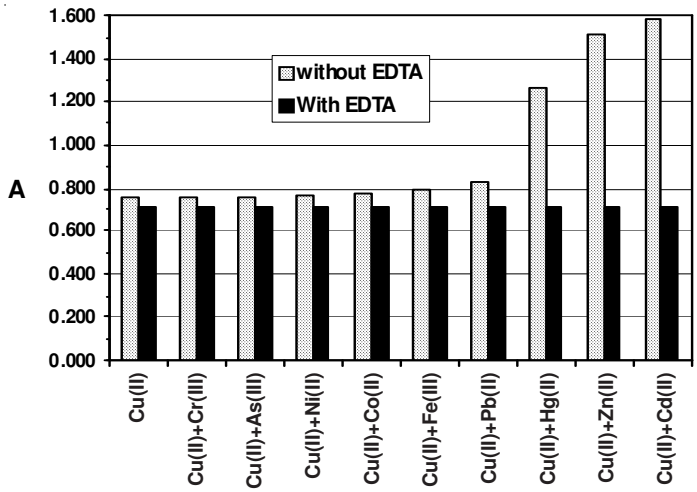


Fig. 5. Absorption in phosphate buffer (pH = 10) for $Cu(PAR)_2$ 2×10^{-5} M in presence 2×10^{-5} M of another ions (PAR and EDTA 2×10^{-4} M, l = 0.5 cm)

Calibration curve: The calibration curves for $Cu(PAR)_2$ showed excellent linearity over concentration ranges of 1-25 and 0.1-25 μM in the absence and presence of EDTA. The spectra characteristics of the $Cu(PAR)_2$ solutions as ε, λ_{max}, Beer's law limits, the equation (Y = mX + b; Y- absorbance, X- concentration μM, b- intercept and m- slope) and the correlation coefficient (R) are summarized in Table-2.

TABLE-2
QUANTITY FACTORS FOR COMPLEX Cu $(PAR)_2$

Factors	Method	
	Spectrophotometer in absence of EDTA	Spectrophotometer in presence of EDTA
pH	10	10
Linearity	1-25 μM	0.1-25 μM
Detection limit	0.1 μM	0.08 μM
ε, mol^{-1} cm^{-1} L	7.62×10^4	7.12×10^4
Sandell's sensitivity, μg cm^{-2} (A = 0.001)	0.83×10^{-3}	0.89×10^{-3}
λ_{max}, (nm)	500	495-500
m	0.0762	0.0712
b	-0.0244	-0.0008
R^2	0.9999	0.9998
RSD %	1.7	2.2

The precision and accuracy of two methods (in the absence and the presence of EDTA) were tested by determining the Cu(II) concentration, ranged within the Beer's law limit, for five replicates time for each concentration (n = 5) are given in Tables 3 and 4.

TABLE-3
DETERMINATION OF COPPER(II) BY SPECTROPHOTOMETER METHOD IN PRESENCE CONSTANT CONCENTRATION OF REAGENT 2×10^{-4} M IN ABSENCE OF EDTA

C_{Cu} (μM) taken	C_{Cu} (μM) found ± SD	RSD (%)	Analytical standard error $\frac{SD}{\sqrt{n}},10^{-6}$	Confidence limit $\left(\overline{X} \pm \frac{SD}{\sqrt{n}} \times t\right),10^{-6}$	Recovery (%)
1.00	1.00 ± 0.017	1.7	0.0076	1.00 ± 0.0211	100.0
3.00	3.00 ± 0.048	1.6	0.0215	3.00 ± 0.0595	100.0
5.00	5.00 ± 0.075	1.5	0.0335	5.00 ± 0.0930	100.0
7.00	7.00 ± 0.105	1.5	0.0470	7.00 ± 0.1300	100.0
10.00	10.00 ± 0.140	1.4	0.0626	10.00 ± 0.1730	100.0
15.00	15.01 ± 0.190	1.3	0.0850	15.01 ± 0.2350	100.1
20.00	20.02 ± 0.260	1.3	0.1160	20.02 ± 0.3220	100.1
25.00	25.00 ± 0.300	1.2	0.1340	25.00 ± 0.3720	100.0

n = 5, t = 2.776 [Ref. 17].

TABLE-4
DETERMINATION OF COPPER(II) BY SPECTROPHOTOMETER METHOD IN PRESENCE CONSTANT CONCENTRATION OF REAGENT 8×10^{-4} M AND 8×10^{-4} M OF EDTA

C_{Cu} (μM) taken	C_{Cu} (μM) found ± SD	RSD (%)	Analytical standard error $\frac{SD}{\sqrt{n}},10^{-6}$	Confidence limit $\left(\overline{X} \pm \frac{SD}{\sqrt{n}} \times t\right),10^{-6}$	Recovery (%)
0.100	0.100 ± 0.0022	2.2	0.00098	0.100 ± 0.0027	100.0
0.500	0.501 ± 0.0100	2.0	0.00450	0.500 ± 0.0124	100.2
0.800	0.800 ± 0.0160	2.0	0.00715	0. 800 ± 0.0198	100.0
1.000	1.006 ± 0.0170	1.7	0.00760	1.006 ± 0.0211	100.6
2.00	2.00 ± 0.0300	1.5	0.01340	2.00 ± 0.0372	100.0
4.00	4.00 ± 0.0560	1.4	0.02500	4.00 ± 0.0694	100.0
8.00	8.01 ± 0.1040	1.3	0.04650	8.01 ± 0.1290	100.1
10.00	10.00 ± 0.1300	1.3	0.05810	10.00 ± 0.1610	100.0
20.00	20.02 ± 0.2400	1.2	0.10700	20.02 ± 0.2970	100.1
25.00	24.98 ± 0.2700	1.1	0.12100	24.98 ± 0.3340	99.9

n = 5, t = 2.776 [Ref. 17].

Application

Determination of Cu in some pharmaceuticals as vitamins: Each analyzed vitamin contains multivitamins and minerals as following: **Adavit silver:** 2 mg Cu, 120 mg Ca, 100 mg Mg, 105 mg Se, 94 μg Mo, 37.5 mg K and 22.5 mg Zn, **Centaraz:** 2 mg Cu, 18 mg Fe, 162 mg Ca, 125 mg P, 10 μg Si, 10 μg V, 100 mg Mg, 15 mg Zn, 2.5 mg Mn, 40 mg K, 25 μg Cr, 25 μg Mo, 25 μg Se, 5 μg Ni and 10 μg Sn, **Supradyn:** 900 μg Cu, 120 mg Ca, 25 μg Cr, 8 mg Fe, 45 mg Mg, 1.8 mg Mn, 45 μg Mo, 126.3 mg P, 20.4 mg K, 55 μg Se and 8 mg Zn, **Ophtavite:** 2 mg Cu, 40 mg Zn and 40 μg Se and **Supravit forte:** 1 mg Cu, 2 mg Mn, 4.9 mg Zn, 100 mg Fe(II) and 20 mg Mg.

Suitably weighed the medicine tablets (or capsules) were crushed and then burned in platinum bowl until ash become grey white. Ash was treated with 10 mL HCl and boiled it gently for 10 min. Transferred the solution into a beaker, added a few drops of HNO_3 and heated to boil again until dry and finally dissolved in distilled water. Final volume was increased to 25 mL in a standard flask. An aliquot of the resulting solution was determined by the general procedure and results were compared with atomic absorption spectrometry (AAS). The results obtained by standard addition were shown in Table-5.

TABLE-5
DETERMINATION OF COPPER IN SOME PHARMACEUTICALS (AS VITAMINS)

Pharmaceuticals samples	Copper present (mg)	Copper found (mg)	RSD (%)
Adavit silver	2.00	2.08	1.3
Centaraz	2.00	2.02	1.3
Supradyn	0.90	0.89	1.4
Ophtavite	2.00	2.04	1.3
Supravit forte	1.00	0.98	1.5

It can be observed that the difference between the results by AAS and the found values by this method are less than 4 % and the relative standard deviation is did not exceed ± 1.5 %. Therefore, this method can be successfully applied for the determination of copper in pharmaceuticals.

Conclusion

A highly sensitive spectrophotometric method for copper(II) determination is developed using 4-(2-pyridylazo)-resorcinol disodium (PAR) as dihydrate salt. This method was applied successfully for determination 1×10^{-7} M of copper(II) with relative standard deviation not exceeding 2.2 % with no ionic interference (Cr^{3+}, As^{3+}, Ni^{2+}, Co^{2+}, Fe^{3+}, Pb^{2+}, Hg^{2+}, Zn^{2+} and Cd^{2+}) in the presence of EDTA and phosphate buffer (pH = 10), the procedure was applied successfully for determination of copper in the presence of EDTA in some pharmaceuticals (as vitamins). The relative standard deviation did not exceed ± 1.5 %.

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