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Research Article

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A simple spectrophotometric method for the determination of copper in environmental samples using flower extract of *Caesalpinia pulcherrima*

Shivaji H. Burungale

Laboratory of Analytical Chemistry, PG Department of Chemistry, Yashwantrao Chavan College of Science, Karad, Maharashtra, India

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ABSTRACT

A very simple, ultra-sensitive and selective new spectrophotometric method has been developed for the rapid determination of copper (II) at ultra-trace level using extract of flower in presence of aqueous solutions. The extract of flower has been proposed as a new analytical reagent for the direct non extractive spectrophotometric determination of copper II. The extract of flower reacts with copper in an aqueous media to give a highly absorbent pinkish chelate complex. The maximum absorption was obtained at 510 nm and remains stable for 72h. The average molar absorptivity and Sandell's sensitivity were found to be $4.71\times10^{5} mL^{-1}$ cm $^{-1}$ and5µg $cm^{-2}$of copper II respectively. Linear calibration graphs were obtained for 0.01-200 mg L^{-1} of CuII. A very large excess of cations, anions and complexing agent do not interfere in the determination. The method is highly selective for copper and was successfully used for the determination of copper in several standard reference materials as well as in some environmental samples. The results of the proposed method for biological and food samples were comparable with AAS and to be in good agreements. The method has high precision and accuracy.

Keywords: spectrophotometry, Extracts of Flower, copper, real environmental samples.

INTRODUCTION

Copper is an essential trace nutrient to all plants and animals, copper is an industrially important metal. It is used in coin, wire making, alloys, fashioning, medicine products, transportation industry and thermal conductors [1]. The amount of copper that contaminates various biological and environmental substances is of concern since copper traces promote rancidity and off flavours in foods beverages. The levels of copper in biological samples may indicate malefaction or contamination, in addition, the accumulation of copper in the human liver is a characteristic of Wilson's disease, jaundice which produces neurologic and psychiatric defects, and hence there is a great need to develop simple, sensitive, selective,and inexpensive method for the determination of copper in environmental, biological, soil and industrial samples for continuous monitoring to establish the levels of copper in environmental, biological matrices.

A simple spectrophotometric method for the determination of copper in industrial, environmental, biological and soil samples using 2,5-dimercapto-1,3,4-thiadiazole has been reported by Ahmed et al.[2]. Spectrophotometric determination of zinc and copper using a liquid wave guide capillary cell by Pascoa and his coworkers [3] and it was successfully applied to natural water samples.To improve the performance analytically a rapid synergistic cloud point extraction of trace amounts of copper in various samples using spectrophotometry[4]. Copper dithiocarbomate is used as a reagent for simultaneous determination of copper and zinc in environmental samples was developed by Uddain et al[5]. From literature survey [6-14] it reveals that those methods are lengthy, time consuming, pH dependant and in most of interference was high and applied on limited samples .it is needless to emphasize further that the direct spectrophotometric method is very more useful. Turkoglu et al [15] reported a simple spectrophotometric determination of copper in natural water and pharmaceutical samples with chloro (phenyl)

glyoxime. Copper is quantitatively retained with 1, 5-diphenylcarbazone (DPC) on microcrystalline naphthalene in the pHrange 6.5 - 8.0 from a large volume of aqueous solutions of various samples as reported by Shishehborea et al [16]. The liquid-liquid extraction of Cu^{2+} ions withorganic solutions containing differentchelating agents was reported by Marczenko and Eaton [17].Spectrophotometric determination of copper in environmental water samples by solvent extraction of an ion association complex of the dichlorocuprate (I) ion with ethyl violet has been developed by Yamamoto and Kumamaru [18]. Shishehborea et al [19] developed a Spectrophotometric method for the determination of trace copper after preconcentration with 1,5-Diphenylcarbazone on microcrystallinenaphthalene. The very simple, highly sensitive and selective Spectrophotometric procedure was developed by Ghazy et al [20] for the determination of copper (II) in natural water, vitamins and certified steel scrap samples. A facile, sensitive and selective extractive Spectrophotometric method was developed by Rekha et al [21] for the determination of copper (II)in various water and alloy samples using a newly synthesizedreagent,3-methoxy-4-hydroxybenzaldehyde4-bromophenylhydrazone.

In the present work, a sensitive and simple method for the determination of trace amounts of a copper (II) – Caesalpinia pulcherrima extracts of flowers by spectrophotometry. The influences of some analytical parameters including metal ion concentration, volume of reagent, etc. on the colour formation were investigated. All seeds of Caesalpinia are poisonous. However the seeds of some species are edible before the seed reach maturity (e.g. immature seeds of C. pulcherrima) or with treatment (C. bonduc toxicity is reduced after roasting). C. pulcherrima is the most widely cultivated species in the genus Caesalpinia. It is a striking ornamental plant, widely grown in domestic and public gardens and has a beautiful in florescence in yellow, red and orange. Its small size and the fact that it tolerates pruning well allow it to be planted in groups to form a hedgerow; it can be also used to attract humming birds.

Leaves: Even-bipinnate, alternate, to 24 inches long, with 4-9 pairs of even pinnae, 5-12 pairs of oblong to obviate leaflets

Flowers: Caesalpiniaceous, yellow, red or pink, with 10 long thread-like stamens, on terminal racemes to 22 inches long Fruits: Pods, flatten, to 5 inches long with 5-8 shiny brown, flat seeds

EXPERIMENTAL SECTION

Apparatus

Systronic (106) visible spectrophotometer Shimadzu (model) atomic absorption spectrophotometer equipped with a microcomputer controlled air –acetylene flame at 324.8 nm was used for comparing the results.

Reagent and Solutions

All of the chemicals used were of analytical reagent grade or the highest purity available. Doubly distilled deionized water, which is non-absorbent under ultraviolet radiation, was used throughout. Glass vessels were cleaned by soaking in acidified solution of $KMnO_4$ or $K_2Cr_2O_7$followed by washing with concentrated HNO_3 and rinsed several times with deionized water. Stock solutions and environmental water samples (1000- mL each) were kept in polypropylene bottles

Copper (II) standard solution 1.57×10-2 mol L^{-1}

A 100-mL amount of stock solution (1 mgmL-1) of Cu(II)was prepared by dissolving 392.9mg of copper sulfate pentahydrate ($CuSO_4$. 5 H_2O)in doubly distilled deionized water. Aliquots of thissolution were standardized by iodometric titration.Working standard solutions were prepared by suitable dilutions of the stock solution.

Plant extracts: Crushed Caesalpinia pulcherrima of flowers were extracted by the following methods.

Extraction at room temperature or cold extraction: 50 g Caesalpinia pulcherrima of flowers were dipped in 100 ml petroleum ether and hexane respectively for 2 hr at roomtemperature in a stoppered conical flask and shaken periodicallyby electrical stirrer. The extracts were filtered and filtrate was evaporated under reduced pressure on water bath to obtain crude.

Hot extraction or reflux extraction: 50 g Caesalpinia pulcherrima of flowers were refluxed in 100 ml petroleum etherand hexane respectively for 2 hr, in a round bottom flask. The extracts were filtered and filtrate was evaporated under reducedpressure on water bath to obtain crude.

Soxhlet extraction: In this method, 50 g Caesalpinia pulcherrima of flowers were extracted in 100 ml petroleum ether by soxhlet extraction technique for 2 hr. The extracts were filtered and filtrate was evaporated under reduced pressure to obtain crude.

General procedure

A volume of 0.01-1.0mL of neutral aqueous solution containing 10-180 µg of copper (II) in a 10-mL volumetric flask was mixed with a Caesalpinia pulcherrima of flowers of extracts used as reagent. The solution was mixed well. After few seconds, the mixture was diluted up tothe mark with deionized water. After 1 min the absorbance was measured at 510 nm against a corresponding reagent blank solution. The copper content in an unknown sample was determined using a prepared calibration graph.

RESULTS AND DISCUSSION

Absorption spectra

The absorption spectra of a copper (II) - Caesalpinia pulcherrima of flowers of extracts solution was recorded using the spectrophotometer. The absorption spectra of the copper (II)-Caesalpinia pulcherrima of flowers of extracts are a asymmetric curve with maximum absorbance at 510nm and an average molar absorptivity of 1.4 x 105 L mol-1 cm-1 (Fig.1). The reagent blank exhibited negligible absorbance despite having wavelength at 404 nm. The reaction mechanism of the present method is as reported earlier.

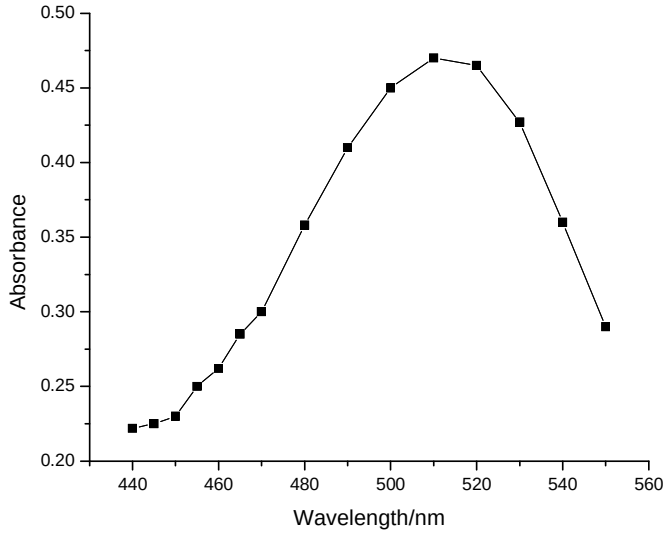

Fig.1 Absorption spectra of Cu-Flower extract in aqueous solution

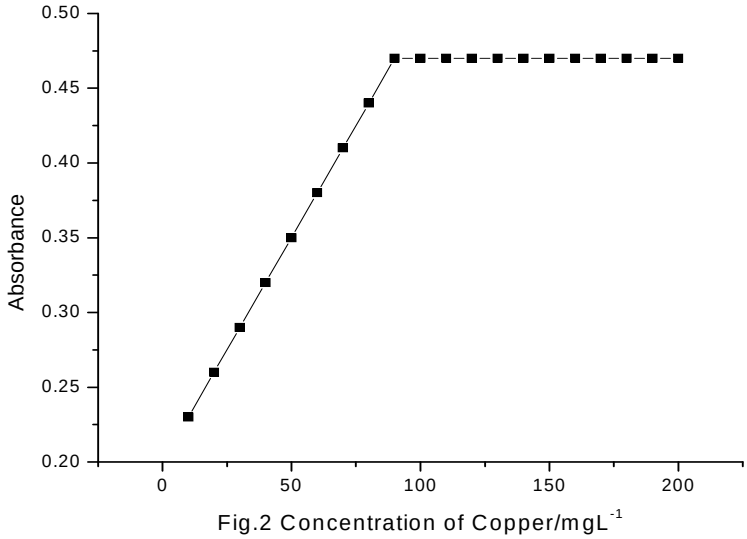

Fig.2 Concentration of Copper/mgL^{-1}

Calibration graph (Beer's law and sensitivity)

The well known equation for a Spectrophotometric analysis in a very dilute solution was derived from Beer's law. The effect of the metal concentration was studied over 0.01-200 mg L^{-1} for convenience of the measurement. The absorbance was linear for 0.01-90 mg L^{-1} at 510nm. Of the four calibration graphs one showing the limit of the linearity is given in (Fig. 2). The molar absorption co-efficient and the Sandell.s sensitivity were found to be 1.4 x 105 L mol^{-1} cm^{-1} and 5 ng cm^{-2} of copper(II), respectively. The selected analytical parameters obtained with the optimization experiments are summarized in (Table.2)

Table 2.Selected analytical parameters obtained by optimization experiments

Parameters Studied	Parameters Studied	Parameters Studied
Wavelength / λmax (nm)	400-560nm	510nm
Solvent	water	Water
Time / h 1-24h	1-24h	1 min.-24 h (preferably 2 min.)
Temperature /ºC	25±5ºC	25±5ºC
Reagent	1-10 mL	5mL
Molar absorption Coefficient/ L $mol^{-1}cm^{-1}$	$0.7\times10^5 4.7\times10^5$	4.71×10^5
Linear range/mg L^{-1}	0.001-100	0.01-18
Detection limit /mg L^{-1}	10-200	100
Sandell's Sensitivity /µgcm^{-2}	0.1 - 100	5
Relative Standard Deviation	0 -2	0 - 2
Regression Co-efficient	0.998-0.9999	0.999

Effect of reagent concentration

Different concentration of Caesalpinia pulcherrima of flowers of extracts were added to a fixed metal ion concentration and the absorbance was measured according to the general procedure. It was observed that a 1mg L^{-1} of copper metal, the reagent concentration Caesalpinia pulcherrima produced a constant absorbance of Cu - Caesalpinia pulcherrima (Fig.3). For different copper concentration (0.5 and 1mg L^{-1}) an identical effect of varying the reagent concentration was noticed. A greater excess were not studied. For all subsequent measurements, 5 mL of reagent was added.

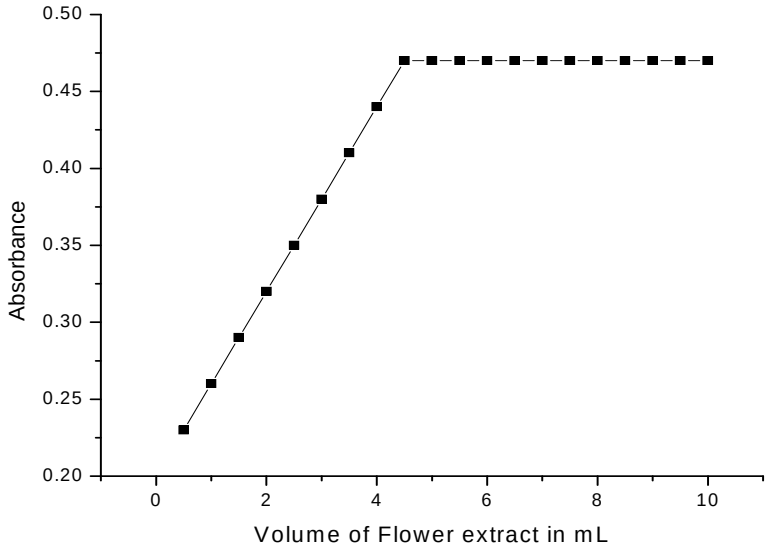

Fig.3 Effect of reagent as a flower extract on the absorbance

Effect of time

The reaction is very fast. A constant maximum absorbance was obtained just after dilution within few seconds to volume and remained strictly constant for over 24 h; a longer period of time was not studied.

Effect of foreign ions

The effect of over 50 ions and complexing agents on the determination of only 1 mg L^{-1} of Copper (II) was studied. The criterion for interference was an absorbance value varying by more than 5% from the expected value for copper alone. As can be seen, a large number of ions have no significant effect on the determination of copper. The tolerance limit of NO_3^-, ClO_4^- SO_4^{2-} and PO_4^{3-} are especially high which is advantageous with respect to the digestion of samples. The quantities of these diverse ions mentioned were the actual amounts added and not the

tolerance limits. However, for those ions whose tolerance limits have been studied, their tolerance ratios are mentioned in (Table 3).

Table 3.Tolerance limits of foreign ions, tolerance ratio [Species(x)]/Cu (w/w)

Species x	Tolerance mg	Species x	Tolerance mg	Species x	Tolerance mg
Acetate	50	Strontium	200	Lead	85
Bromide	45	Beryllium	125	Thallium	110
Chloride	125	Aluminum	160	Tin	135
Carbonate	40	Bismuth	140	Antimony	165
Nitrate	35	Iron	100	Vanadium	100
Nitrite	20	Cerium	25	Manganese	100
Thiocyanate	100	Chromium	40	Cobalt	100
Sulphate	200	Calcium	150	Nickel	150
Sodium	300	Magnesium	200	Tungsten	100
Potassium	250	Barium	175	Zinc	200

Applications

The present method was successfully applied to the determination of copper (II) in series of synthetic mixtures of various compositions (Table 4) and also in number of real samples, e.g. several standards alloys and steels (Table 5). The method was also extended to the determination of copper in a number of environmental water samples, biological and soil samples. In view of the unknown composition of environmental water samples, the same equivalent portions of each sample was analyzed for copper content; recoveries in both .spiked. (Added to the samples before the mineralization and dissolution) and the .unspiked. Conditions are in good agreement (Table 6). The results of biological and food analyses by spectrophotometric method were found to be in excellent agreement with those obtained by AAS (Table 7). The results of soil samples analysis by the spectrophotometric method are shown in (Table 8). The speciation of Cu(I) and Cu(II) in mixtures are shown in(Table 9)

Determination of copper in synthetic mixture

Several synthetic mixtures of varying compositions containing copper (II) and diverse ions of known concentrations were determined by the present method using tartrate as a masking agent and the results were found to be highly reproducible. The results are shown in (Table 4).Accurate recoveries were achieved in all solutions.

Table 4 :Determination of copper in synthetic mixture

Sr. No.	Synthetic Mixtures	Recovery of Copper(%)a
1	Cu (II) + Zn (II)	99.67
2	Cu (II) + Fe(II)	99.45
3	Cu (II) + Cd (II)	99.60
4	Cu (II) + Fe (III) + Al (III)	98.97
5	Cu (II) + Zn (II) + Cd (II)	98.85

a. Average of the five replicate determinations

Table 5.Deatermination of copper in certified reference materials

Sample	Certified Reference	Material (Composition, %)	Copper (%)a		RSD (%)
			Certified value	Found (n=5)	
	BAS-CRM-10g (high tensile):	Sn, 0.21.Zn, 30. Al, 3.34.Pb, 0.023. Ni, 0.06. Fe, 1.56. Mn, 1.36. Cu, 60.8.	60.8.	60.3.	1.3
	BAS-CRM-5g	Cu, 67.4. Sn, 1.09. Pb,2.23. Zn, 28.6, Ni, 0.33. P, 0.01.	Cu, 67.4	Cu. 64.	1.2
	Brass, Class-1	Pb, 0.00. Fe, 0.01. Cu, 70.61.	Cu, 70.61.	Cu, 70.56.	1.4

a.Average of the five replicate determinations
b.The measure of precision is the relative standard deviation (RSD).

Determination of copper in alloys, steels and brass (Certified reference materials)

A 0.1g amount of an alloy or steel or brass sample containing 0.18 - 70.61% of copper was accurately weighed and placed in a 50mLErlenmeyer flask. To it, 10mL of concentrated HNO_3 and 1-mL of concentrated H_2SO_4 were carefully added and then covered with a watch glass until the brisk reaction subsides. The solution was heated and simmered gently after the addition of another 5mL of concentrated HNO_3 until all carbides were decomposed. The solution was carefully evaporated to dense white fumes to drive off the oxides of nitrogen and then cooled to room temperature (25±5)0C. After suitable dilution with deionized water, the contents of the Erlenmeyer flask were warmed to dissolve the soluble salts.The solution was then cooled and neutralized with a dilute NH_4OH solution in the presence of 1-2mLof 0.01% (w/v) tartrate solution. The resulting solution filtered, if necessary, through Whatman no. 40 filter paper into a 25mL calibrated flask.The residue (silica and tungstic acid) was washed with a

small volume (5mL) of hot (1:99) sulfuric acid, followed by water, the filtration and washing were collected in the same calibrated flask and the volume was made up to the mark with deionized water. A suitable aliquot (1-2mL) of the above solution was taken into a 10mL calibrated flask and the copper content was determined as described procedure using Caesalpinia pulcherrima as agent. Based on five replicate analyses, the average concentration of copper determined by spectrophotometric method and was in good agreement with the certified values. The results are given in (Table5).

Determination of copper in environmental water samples

Each filtered (with Whatman No.40) environmental water sample (1000mL) wasevaporated nearly to dryness with a mixture of 2mL of concentrated H_2SO_4 and 5mL ofconcentrated HNO_3 to sulfur trioxide fumes in a fume cupboard following a method recommended by Greenberg et al]. After cooling additions of5mL of concentrated HNO_3 was repeated and heating to a dense fume continued or until the solution became colorless. The solution was then cooled and neutralized with dilute NH_4OH in thepresence of 1-2mL of a 0.01% (w/v) tartarate solution. The resulting solution was then filtered and quantitatively transferred into a 25mLcalibrated flask and made up to the mark with deionized water. An aliquot (1-2mL) of this preconcentrated water sample was pipetted into a 10mL calibrated flask and the copper content was determined as described under the general procedure using Caesalpinia pulcherrima agent. The results are given in (Table 6).

Table 6.Determination of copper in some environmental water samples

Sample	Cu/µg L^{-1}		Recovery ± s (%)
	Added	Found	
Tap water	0 100 500	40 140 542	100±0.0 102±0.2
Well water 0	0 100 500	35 136 535	100.7±0.6 100.6±0.5
River water	0 100 500	60.0 161.0 566.0	100.6±0.4 100.±0.6
Lake water	0 100 500	43.0 144.0 545.0	100±0.1 100.9±1.2
Drain water	0 100 500	130.0 238.0 640.0	101.3±1.0 100.7±0.8

Table 6.Determination of copper in pharmaceutical preparations

Pharmaceuticals	Form	Certified value mg / tablet	Found mg /tablet	Recovery %
SUPRADYNE	$CuSO_4$. 5H2O	3.39	3.41 3.57 3.78 3.10	100.6 105.3 111 91.3
MULTIRICH	Copper	50	53 48.86 49.47 52.07	106 97.72 98.94 104.14
MULTIVITE	$CuSO_4$. 5H2O	0.1	0.108 0.092 0.101 0.105	108 92 101 105
GBION	Copper	2.0	1.36 0.8623 0.8922 0.954	68.0 43.1 44.6 47.7
NEXBLEND	CuO	0.5	0.40 0.3047 0.3542 0.3148	80 60.94 70.84 62.96

Determination of copper in pharmaceutical samples

The sample was then ashes in a Muffle furnace at 500^{0C} for a 2h in the presence of 10mL concentrated nitric acid. Then at the following content of each beaker were completely evaporated and cooled at room temperature, 5mL of distilled water to each beaker and warmed slightly. The content of each beaker was filtered and neutralized with dilute ammonia in the presence of 1-2mL of 0.01% (w/v) tartrate solution, transferred quantitatively into a 10mL

calibrated flask and made up to the mark with deionized water.A suitable aliquot (1-2mL) of the final solution was pipetted into a 10-mL calibrated flask and the copper content was determined as described under the general procedure using a Caesalpinia pulcherrima solution as agent. The results of the pharmaceutical analyses by the Spectrophotometric method were found to be in excellent agreement with those obtained by AAS.

Determination of copper in soil samples

An air-dried homogenized soil sample (100g) was accurately weighed and placed in a100mL micro-Kjeldahl flask. The sample was digested in the presence of an oxidizing agent. The content of flask was filtrated through Whatman No. 40 filter paper into a 25mLcalibrated flask, and neutralized with dilute ammonia in the presence of 1-2mL of a 0.01 %(w/v) tartrate solution. It was then diluted up to the mark with deionized water. A suitable aliquots (1-2mL) were transferred into a 10mL calibrated flask and the copper content was determined as described under general procedure using a Caesalpinia pulcherrima solution as agent. The results are given in (Table 8).

Table 8.Determination of copper in some surface soil samples

Serial No.	Copper µg/100 g	Sample source
S1	20.5±1	Agriculture soil
S2	50.8±1.8	River soil
S3	100.0±1.6	Traffic soil
S4	75.9±2.0	Roadside soil

CONCLUSION

It is a new approach and alternative of standard method for copper. In the present work, a simple sensitive, selective and inexpensive method with Cu (II) - Caesalpinia pulcherrima was developed for the determination of copper in environmental, industrial, pharmaceutical and soil samples. The method also offers a very efficient procedure for speciation analysis, factors such as the low cost of the instrument, easy handling, portable, lack of any requirement for consumables, and almost no maintenance, have caused spectrophotometry to remain a popular technique, particularly in laboratories of developing countries with limited budgets. The sensitivity in terms of the molar absorptivity ($\epsilon = 4.71\times10^5$L mol^{-1} cm^{-1}) and precision in terms of the relative standard deviation of the present method are very reliable for the determination of copper in real samples.

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