International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol. 3, No.2, pp 661-670, April-June 2011

Resorcinol Method for Colorimetric Microdetermination of Copper in Pure Forms

Shabir A. Mir[1]

**Division of Veterinary Pharmacology & Toxicology,
Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir,
F.V.Sc. & A.H., Shuhama, Srinagar-190001, J & K, India**

***[1]Corres. author : mirsamir.19@rediffmail.com
Telephone/Telex No: 91-0194-2262216***

Abstract: A simple colorimetric method is proposed for the micro-determination of copper in pure forms. The assay is based on the formation of a color complex between copper and resorcinol in presence of ammonia with peak absorption at 450 nm. The method is linear over 0.05 through 1.0 µg Cu ($R^2 \geq 0.99$). The regression coefficients vary from 0.275 to 0.920 depending upon the choice of experimental conditions. The assay can be conducted either at room temperature or rapidly by incubating the samples in boiling water bath for 3 minutes. The optimum conditions for the assay and the potential interfering have been identified. The method has been tested for determining copper (I & II) in known aqueous solutions, in freshly synthesized copper (II) nitrate and copper (II) chloride, and for determining traces of copper in reagent grade sodium chloride. The results obtained are comparable to those provided by the dithiocarbamate method with respect to per cent recovery and precision ($P > 0.1$) with obvious advantages: (i) having at least ten times better detection limit and sensitivity, and (ii) being unaffected by the presence of Cd or Co when used up to 20 µg each per sample.

Key words: Copper, Resorcinol, Micro-determination.

Introduction

Copper is essential for plant, animal and human health. It has a wider importance from research, therapeutic, industrial and toxicological considerations. Consequently, a variety of methods are available for its determination. Iodometric[1] and acidimetric methods[2] are employed for per cent purity assays. Micro-determinations are commonly accomplished with different versions of atomic absorption spectrometry (AAS) [3-10] or with colorimetry employing a variety of test reagents[1, 11-27]. AAS is highly sensitive and reliable but requires expensive instrumentation and technical expertise so remains beyond the reach of common laboratories. The detection limits for different colorimetric versions are generally in the range of 0.1 through 1 ppm[13, 19, 21, 25] that suffice their use for routine analytical works. The method using sodium diethyl dithiocarbamate reagent is more popular[1, 28], constituting an official method[29, 30]and serves as a reference standard for evaluation of new methods[21]. An alternative colorimetric method using resorcinol has been rationalized on two observations. Firstly, the derivatives of resorcinol principally 4-(2-pyridylazo) resorcinol, 4-(2-thiazolylazo) resorcinol, 5-Methyl-4-(2-thiazolylazo) resorcinol and 4, 6-diacetyl resorcinol have been employed to facilitate concentration and determination of many metals including Cu[31-36]. Secondly, during an investigation on the reactivity of copper (II) ions with different plant phenolics, the copper ions showed selective interaction with resorcinol, in preference to other phenolics including its structural isomers viz., catechol and quinol, forming a color complex with maximum absorbance at 450 nm. Consequently, the experiments were designed to

investigate and optimize the reaction for quantitative determination of copper in test samples.

Materials and Methods

The experiments were conducted at ambient temperatures ranging from 10 through 34 ^{0}C with overall mean of 21.3 ± 0.7 0C (n= 106).

Reagents

The reagents used were of standard purity. Double distilled water, boiled and cooled to room temperature, has been used for preparation of the reagents. Deionized water was used for washing of the glassware.

The stock solutions included Cu (II) 500 µg mL^{-1} from copper sulfate pentahydrate (labeled purity 99.5 %), Cu (I) 355.3 µg mL^{-1} from copper (I) oxide in 2.5 M ammonia, ammonia 5 M (standardized against standard sulfuric acid by back titration), resorcinol 5% (w/v) in 10 % ethanol, and carbamate reagent containing 0.4 % (w/v) disodium diethyl dithiocarbamate in water. The required working solutions were made by dilution in water at the time of use. Working solutions for resorcinol were always prepared in 10% ethanol.

Resorcinol vis-à-vis carbamate methods for copper analysis

The optimized resorcinol procedure involves addition of 0.6 mL of 0.2 M ammonia to a 2.6 mL aqueous sample containing 0 through 1.0 µg copper. The samples are added 0.2 mL of 0.1 % resorcinol reagent, well mixed and allowed standing at room temperature. The color is monitored at 450 nm (UV-Visible Spectrophotometer SL-150, Elico (India) Ltd, Hyderabad) following one hour standing at room temperature following the addition of resorcinol. Rapid version of the technique includes incubating the reaction mixture in boiling water bath for exactly 3 minute period, and reading the samples within 5 minutes following their cooling to room temperature.

The resorcinol method was optimized with respect to monitoring change in absorbance over 0.5 through 2 hour observation period, and for optimizing the requirements for ammonia and resorcinol while using standard mass of copper 0.5 µg. The samples containing varying ammonia were analyzed for pH following monitoring of absorbance by a pocket pH meter (pH Scan 3, Eutech Instruments, Malaysia) with sensitivity 0.01 pH unit. Based on the results, the two assay versions were compared while using ammonia 2 mg with resorcinol 0.2 mg or 1 mg. The routine procedure employing resorcinol 0.2 mg was also conducted with 5 mg ammonia to elucidate effect of increased ammonia on the regression parameter of the assay.

The carbamate method[1] has been scaled down to match the volume of 3.4 mL as employed for the resorcinol method. The optimized protocol involves addition of 0.4 mL 5 M ammonia to a neutralized aqueous solution of 2.5 mL containing copper 0 through 20 µg. The samples are added each 0.5 mL carbamate reagent, mixed up, allowed standing at room temperature for 15 minutes and monitored for absorbance at 450 nm.

Interaction and interference studies

Interaction studies between cupricamine and different other phenolics including catechol, quinol, phenol, phlorglucinol, methyl catechol, pyrogallol, gallic and tannic acids have been undertaken to demonstrate the selectivity of interaction between cupricamine and resorcinol.

Optimized method has been evaluated for possible interferences with different test chemicals including acids (hydrochloric acid, acetic acid, sulfuric acid), acids with copper reducing/complexing potential (citric acid, ascorbic acid, thioglycollic acid, oxalic acid), copper complexing / sequestering agents (disodium EDTA, potassium sodium tartrate), anionic chelators (acetate, phosphate), reducing agents (sodium metabisulphite, hydroxylamine HCl), oxidizing agent (hydrogen peroxide) and ethanol. The comparative performances of the resorcinol and carbamate methods have been evaluated in presence of other metallic ions including Cd, Zn, Co, Ni and Mo.

Solubility and adsorption properties of copper-resorcinol complex

The solubility of color complex in organic solvents has been checked by shaking the samples vigorously with equal volumes of test organic solvents including diethyl ether, petroleum ether, benzene, chloroform, isoamyl alcohol, ethanol, methanol, carbon tetrachloride and n-heptane, and monitoring the color intensities of the respective solvent phases.

Adsorption of the color complex to various adsorbents such as activated charcoal, bentonite, kaolin, barium sulfate, silica gel and neutral alumina oxide was studied by shaking the color complex in aqueous solution with each test adsorbent added as 10 mg $^{-1}$ mL solution, and observing the color intensity of the supernatant. Further preliminary adsorption studies were carried out while using activated charcoal.

Verification of resorcinol method

The technique was employed to determine copper content in aliquots of aqueous solutions of copper acetate, and those containing freshly synthesized chloride and nitrate salts of copper from copper sulfate. For latter studies, copper sulfate solution as 10 mL containing total Cu as 500 µg was added 10 mL

of 0.1N NaOH to precipitate copper hydroxide, and the volume was made 100 mL with water. The precipitate was allowed to grow for 15 minute, removed by filtration over Whatman No. 1 filter paper, and freed off the residual alkali by repeated washing with about 100 mL water till effluent failed to react to phenolphthalein indicator. The precipitate was carefully reconstituted in 25 mL of 0.005N HCl or 0.005N HNO_3 . The reconstituted salts were appropriately diluted with water and assayed approximately for copper content by neutralization titrimetry[2]. Appropriately diluted samples were assayed for copper content by the outlined method against the standard copper sulfate solution.

The method was employed to determine copper in tap water. The samples were collected in acid-washed and water-rinsed plastic containers. Each 10 mL aliquot was added copper to provide 0 or 0.3 ppm concentration, acidified with 1 mL of ca. 1M HCl. The acid extracts were obtained by filtration over Whatman filter paper No.1, rinsed with ca. 0.1 M HCl. Each 8 or 10 mL of acid extract was neutralized with standard NaOH (checked by titration of an aliquot of the extract against standard alkali hydroxide). The neutralized sample extracts were added standard ammonia to provide ca. 0.3 M concentration when made to volume of 18 mL with water. The samples were mixed, allowed to stand for five minutes, centrifuged at 5000 rpm for about ten minutes to remove any insoluble material, and to get clear supernatant. Filtration was avoided, as cupricamine tends to interact with cellulose. The extracts obtained were assayed by resorcinol method using 2 or 2.5 mL aliquots.

The resorcinol method has been compared with dithiocarbamate method in three assay settings. Assay -1 included addition of standard copper (II) directly to distilled water and proceeding with recovery estimations by the two methods. Assay-2 employed appropriate aliquots of solutions of copper (I) in ammonia. The samples were assayed in terms of standard copper (II) to elucidate the suitability of methods in determining either forms of copper from aqueous solutions. Assay-3 involved determination of traces of copper (II) present in reagent grade sodium chloride. For this assay, sodium chloride was prepared as 20 % (w/v) solution containing 0.24 M ammonia. For resorcinol method, one mL aliquot was used for determination of copper. For dithiocarbamate assay, 5 mL aliquots were added 0 and 3 µg copper, and processed simultaneously with standard calibration assay including one with copper 3 µg. This procedure enabled determination of copper that otherwise remained undetectable by dithiocarbamate method.

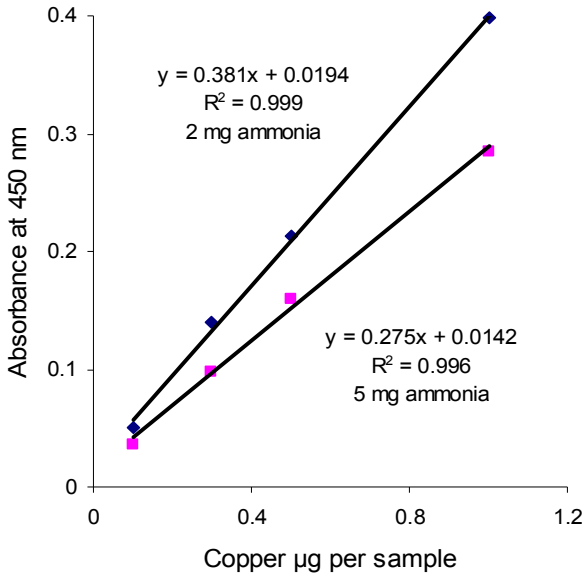
Table I. Copper-resorcinol linearity in presence of ammonia

Copper mass, µg	**Absorbance values**
0.1	0.051 ± 0.002
0.3	0.140 ± 0.005
0.5	0.213 ± 0.006
1.0	0.398 ± 0.009
2.0	0.593 ± 0.006
3.0	0.718 ± 0.006
5.0	0.848 ± 0.005
10.0	0.972 ± 0.005

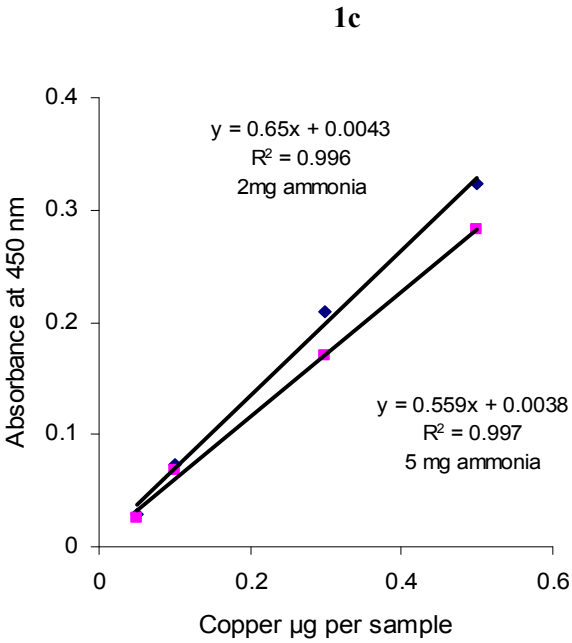
Statistical analysis

Concentration range, µg Cu	**r ± S.E.**	**b ± S.E**
0.1 – 0.5	0.998 ± 0.002	0.405 ± 0.013
0.1 – 1.0	0.999 ± 0.001	0.381 ± 0.007
1.0 – 3.0	0.992 ± 0.009	0.160 ± 0.012
3.0 – 10.0	0.967 ± 0.037	0.034 ± 0.005

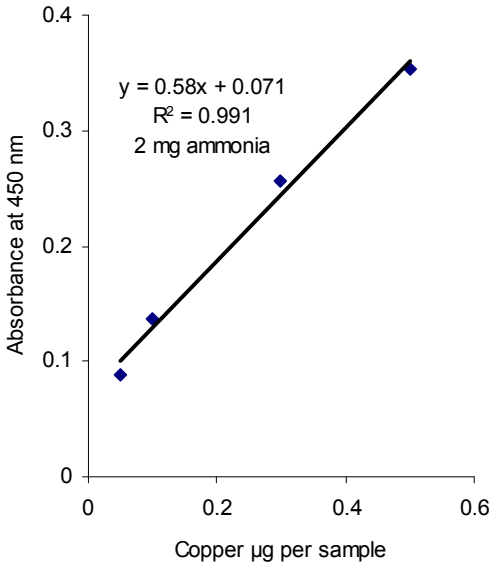
The values are mean ± se of six observations each with assay at room temperature using 2 mg ammonia and 0.2 mg resorcinol



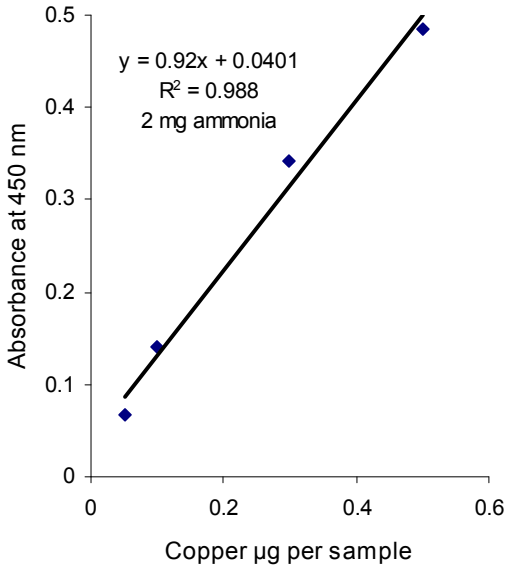
1a



1c



1b



1d

Fig.1 Resorcinol assays over 0.05 - 0.5 µg Cu (**1b, 1c & 1d)** or 0.1 - 1.0 µg Cu (**1a)** conducted at room temperature (**1a** & **1b**) and by 3-minute incubation in boiling water bath (**1c** & **1d**) using resorcinol 0.2 mg (**1a**, **1c**) and 1 mg (**1b**, **1d**).

Results and Discussion

Optimization studies

Resorcinol assay is quite sensitive in detecting copper as low as 0.05 µg in a 2.6 mL sample volume. The assay conducted at room temperature exhibits perfect linearity over 0.1 through 1 µg copper while using 0.2 mg resorcinol and 2 or 5 mg ammonia (Table 1, Fig. 1a) with improvement in detection down to 0.05 µg with 1 mg resorcinol (Fig 1b). The assay shows drop in regression coefficient with varying reactant concentrations from 0.446 (1 mg resorcinol, 2 mg ammonia) to 0.382 (0.2 mg resorcinol, 2 mg ammonia) to 0.275 (0.2 mg resorcinol and 5 mg ammonia) (Fig 1a, 1b). With 3-minute heat incubation, the assay obeys perfect linearity over 0.05 through 0.5 µg copper. The absorbance values due to 1 µg copper undergo departure from the linearity under the test conditions. The regression coefficient increases as a function of concentrations of ammonia and resorcinol, from 0.559 (0.2 mg resorcinol, 5 mg ammonia) to 0.649 (0.2 mg resorcinol, 2 mg ammonia) to 0.920 (1mg resorcinol, 2 mg ammonia). The sensitivity of the assay undergoes a decline at higher mass range for copper. Consequently, at 0.2 mg resorcinol and 2 mg ammonia at room temperature, the coefficient drops from 0.41 (0.1 - 0.5 µg copper, r = 0.998) to 0.38 (0.1 – 1.0 µg copper, 0.999) to 0.16 (1-3 µg copper, r =0.992) and to 0.034 (3-10 µg copper, r = 0.97) (Table 1). The results reveal that the assay can be conveniently conducted at room temperature or at elevated temperature employing 2 mg ammonia with either 0.2 or 1 mg resorcinol. The color development is unaffected by exposure to light and air – the mean absorbance values of the tubes exposed to air and light were comparable to those kept stoppered, sealed under liquid paraffin layer and held in dark in the sealed rotor of the centrifuge over 2 hour observation period ($P>0.1$, n = 4 each). Time optimization study with room temperature assay reveals stable absorbance values between one and two hours following addition of resorcinol. With copper 0.5 µg, resorcinol 0.2 mg and ammonia 2 mg, the mean absorbance value at one hour, 0.212 ± 0.005, does not differ from those observed at 1.5 hour, 0.207±0.003, and two hours, 0.218 ± 0.002 ($P>0.1$, n=4 each). The values over 1 to 2 hour period are significantly more than the mean, 0.124 ± 0.003, observed at 30 min ($P<0.01$, n = 4 each).

The mean absorbance, with constant mass of Cu 0.5 µg and resorcinol 0.2 mg, increases with increase in ammonia from 0.1 through 1 mg (r =0.86) and then remains fairly stable up to 5 mg ammonia with peak absorbance at 2 mg ammonia than at 1 or 5 mg ammonia (Table 2). The values beyond 1 and 10 mg ammonia are significantly lower than those at 1 to 5 mg ammonia ($P<0.01$, n = 4 each). The pH over the range of test concentrations of ammonia ranges from 9.90 ± 0.01 through 12.60±0.01 with optimal pH at 2 and 5 mg of ammonia, respectively, as 11.50± 0.01 and 11.96 ±0.01 (n = 4 each) . At constant mass of copper 0.5 µg and ammonia 2.0 mg, the absorbance increases with increase in resorcinol from 0.1 through 5.0 mg (r = 0.68), and the mean absorbance value at 10 mg is significantly lower than the value at 5 mg ($P<0.01$, n=4 each) (Table 3) . Increase in resorcinol beyond 1 mg increases background color in the blanks in a concentration related manner. In view of these observations, resorcinol concentration of 0.2 mg, maximally 1 mg, is optimal for routine use. Stoichiometrically each copper atom requires six molecules of ammonia to form a soluble cupricamine bivalent cation[39]. Apparently one molecule of cupricamine would require one molecule of resorcinol. Increase in absorbances with increased concentrations of resorcinol, or with prolonged incubation as reflected by alterations in regression coefficients may suggest formation of different arrangements of the complex under varying experimental conditions (Fig 1).

Table II. Effect of varying ammonia mass on absorbance values with standard protocol [a]

Ammonia mass, mg	Absorbance values	pH
0.1	0.070 ± 0.004	9.87 ± 0.04
0.3	0.160 ± 0.008	10.50 ± 0.03
1.0	0.200 ± 0.006	11.12 ± 0.01
2.0	0.218 ± 0.006	11.50 ± 0.01
5.0	0.200 ± 0.004	11.96 ± 0.01
10.0	0.183 ± 0.005	12.29 ± 0.01
20.0	0.150 ± 0.004	12.59 ± 0.01

[a] Copper 0.5 µg, resorcinol 0.2 mg. The values are mean ± se of four observations each.

Table III. Effect of varying resorcinol on absorbance values with standard protocol [a]

Resorcinol, mg	Absorbance values
0.1	0.120 ± 0.005
0.2	0.220 ± 0.003
0.5	0.322 ± 0.009
1.0	0.380 ±0.004
2.0	0.402 ±0.006
5.0	0.438 ± 0.006
10.0	0.386 ±0.005

[a] Copper 0.5 µg, ammonia 2 mg. The values are mean ± se of four observations each.

Selective interaction of copper with resorcinol

Experimental evidence has demonstrated selective interaction between copper and resorcinol in presence of ammonia for generation of copper resorcinol complex. Firstly, resorcinol with copper in absence of ammonia, or with ammonia in absence of copper fails to produce any coloration upon incubation at room temperature or upon incubation in boiling water bath for 3 minutes. The color appears only when the three components, viz., copper, ammonia and resorcinol, are incubated together. Secondly, copper in presence of ammonia shows remarkable selectivity towards resorcinol (1, 3 –dihydroxybenezene) in preference to its structural isomers such as catechol (1, 2-dihydroxybenzene) and quinol (1, 4-dihydroxybenzene).Other phenolics such as monohydroxybenzenes (phenol, methyl catechol), trihydroxybenzenes (pyrogallol, phlorglucinol) and phenolic acids (gallic acid, tannic acid) upon testing up to 5 mg of each test compound in 10% ethanol with 2 mg ammonia and 1 µg copper in 3.4 mL volume over one hour observation period have failed to exhibit any response. Phenol, catechol and methyl catechol hardly show any interaction with cupricamine or ammonia. Distinct colors produced by hydroquinone (deep red), pyrogallol (deep yellowish), tannic acid (light pale) and gallic acid (bluish green) are attributable to the effect of ammonia as colors develop uniformly in simultaneously run blanks in absence of copper. Phlorglucinol at 1 mg demonstrates a distinct coloration with maximum absorbance at 540 nm with linear increase in color intensity as function of copper concentration over 0.1 through 2.5 µg. However, the absorbance values remain too low, 0.05 to 0.14, over the test copper range to be of any practical utility. The interaction of copper and resorcinol in presence of ammonia provides a distinct color with maximum absorbance at 450 nm. The absorbance values show greater and wider magnitude over test concentration range of copper to be of practical utility. The lack of reactivity of catechol and quinol to cupricamine implies that either the specific orientation of hydroxyl functions on benzene in resorcinol is critical for its bonding with cupricamine, and / or relatively more powerful reducing potential of catechol and quinol compared to that of resorcinol[38] is presumably offsetting their interaction with cupricamine.

Effect of various agents on copper-resorcinol reactivity/stability

Formation of color complex is sensitive to acids, oxidizing and reducing agents, anionic chelators (acetate and phosphate) and copper sequestering/complexing agents. All test agents reduced or completely prevented formation of copper resorcinol complex. High ammonium chloride also impaired color development. Additon of these agents to already developed color complex lead to its quantitative decolorization. High ammonia (ca. 500 mmoles in excess to test requirement) completely prevented decolorization produced by TGA (30 mmoles), oxalic acid (50 mmoles), disodium EDTA (50 mmoles), citric acid (3 mmoles) or ascorbic acid (0.3 mmoles), but failed to prevent decolorizing effect of hydroxylamine HCl (135 mmoles). At equimolar concentrations (300 mmoles), potassium acetate and trisodium phosphate caused significant reduction in color complex formation ($P<0.01$, $n=4$ each) compared to sodium chloride and sodium sulfate. The effect of acetate was further confirmed by noting a quantitative decrease in complex formation in presence of ammonium acetate (0.04 -0.4 mmoles, $r = -0.93$). Presence of HCl during color development produced similar decolorizing effect (12 -120 mmoles, $r = -.98$). Ethanol 0.2 to 0.7 mL failed to alter color development but reduced background color in blanks that develops otherwise with increasing concentration of ammonia and / or resorcinol. Impairment in color complex formation by ammonium chloride provided rationale for suppressing its formation while neutralizing acid extracts. Thus, NaOH was used to neutralize the acid, and then ammonia was added to form cupricamine.

The foregoing observations indicate the necessity of absence of acids (mineral and organic), anionic chelators (notably phosphates and acetate), ammonium salts, and agents with redox potential (oxidizing and reducing agents, and of copper chelating agents (citrate, tartrate, EDTA) in the samples for development of color complex.

Solubility and adsorption studies

The color complex does not extract into organic solvents viz., m-amyl alcohol, benzene, petroleum ether, diethyl ether, n-heptane, carbon tetrachloride and chloroform when color complex formed from 2 µg copper in 4 mL reaction volume was shaken with equal volume of organic solvent. The color complex is retained in aqueous phase. The complex is soluble in methanol and ethanol. The adsorption of the color complex to different adsorbents has been in the order:

Activated charcoal > bentonite =
kaolin>>alumina>silica G >> barium sulfate

Activated charcoal as low as 5 mg mL^{-1} causes complete adsorption of the complex when added 10 to 15 min following addition of resorcinol. The adsorbed complex is separated by filteration on Whatman filter paper no. 1, and is washed with plenty of water and with ammonia solution up to 0.5 M strength without

any loss in adsorbed copper. The copper is eluted with either 0.5 M sulfuric acid or 1 M ammonia in 80 % ethanol. With 5 mL 0.5 M sulfuric acid 5 µg copper adsorbed to the charcoal has been recovered as 4.5 ± 0.1 µg (n= 6). The method also works with dilute copper solutions. For instance, copper complex containing 1 µg copper in 25 or 30 mL water was completely adsorbed to 25 mg activated charcoal. The adsorbed copper on elution either with 5 mL 1 M ammonia in 80% ethanol for direct monitoring of copper resorcinol complex or with 2 mL 0.5 M sulfuric acid for dithiocarbamate method has shown, respectively, 61 and 63 per cent recovery.

Applied aspects

Per cent purity of copper acetate in a solution of known strength was estimated to be 98.6 ± 2.7 (n =12) with respect to standard copper (from copper sulfate) using matching concentration ranges 0.3 through 2.0 µg of each (n=3 each). Estimated mass in synthesized copper nitrate and copper chloride solutions, with calculated mass respectively as 0.5 and 0.3 µg respectively, were found as 0.53 ± 0.01 and 0.32 ± 0.02 µg (n= 6 each) revealing per cent recovery or purity of synthesized materials as 106.3 ± 1.4 and 107.0 ± 5.8 respectively. Ammonia extraction of neutralized acid extracts worked well with tap water. The copper content in tap water remained undetectable indicating concentration if any to be less than the detection limit of the technique. Copper added to tap water providing 0.3 ppm was recovered as 0.30 ± 0.01 ppm indicating per cent recovery of 100 ± 3 (n = 6 each). Neutralization of acid-extracts with NaOH was necessitated to limit formation of ammonium chloride that interferes with the assay, and is otherwise formed while neutralizing the samples directly with ammonia. The applied protocol with simulated experiments using 2 to 5 mg of each metal species completely precipitated Fe (ferrous and ferric 1:1), Mn, Cd, Pb and Hg (II). Metals like Co, Ni, Zn and Cd are known to form ammine complexes with ammonia while Mo remains solubilized in ammonia[37]. These observations necessitated evaluation of their possible interferences. Mo, Cd, Zn, and Co up to test mass of 20 µg each per sample failed to interfere with absorbance values. Ni up to 5 µg did not interfere while 10 and 20 µg caused 40 and 60 % reduction in absorbance with standard concentration of Cu 0.5 µg conducted at room temperature ($P<0.01$, n=5 each).

Table IV. Comparative evaluation of resorcinol and dithiocarbamate methods for copper analysis

Parameter	Analytical methods	
	Resorcinol	Dithiocarbamate
Linear range Cu µg	0.1 – 1.0	1 - 20
b ± s.e.	0.381 ± 0.007[a]	0.046 ± 0.001
RSD, %	4.5	5.3
Assay 1 (n=6 each) Copper (II) added per sample µg Copper (II) recovered µg Per cent recovery	 0.5 0.51± 0.01 101.6 ± 1.5	 5.0 5.1 ± 0.1 102.4 ± 1.4
Assay 2 (n = 5 each) Copper (I) added per sample µg Copper (I) recovered µg Per cent recovery	 1.0 0.93± 0.02 93.4 ± 2.0	 5.0 4.63 ± 0.05 92.6 ± 1.1
Assay 3 (n = 5 each) Copper in NaCl (GR)	 0.50±0.04	 0.51±0.05

[a] Room temperature assay; per cent recovery and estimated copper with either method for all assays comparable ($P>0.1$); $R^2 > 0.99$ for the two methods; RSD, relative standard deviation.

Comparative evaluation with carbamate assay

The carbamate method is one of the most widely used spectrophotometric methods for determination of copper[1, 30-32]. The assay[1] with reactants scaled down to match the reaction volume of the resorcinol method has been linear over 1 through 20 µg copper per sample (r ± s.e. = 0.999 ±0.001; b ± S.E. = 0.046 ±0.001, n = 6 at each concentration of copper). The detection limit for copper with resorcinol method is 10 to 20 times lower, and the sensitivity, as reflected by regression coefficient values, 6 to 20 times more than with dithiocarbamate method. The precision of the resorcinol method as indicated by RSD, 4.5 %, is comparable to that, 5.3 %, obtained with dithiocarbamate assay (Table 4). As evident (Table 4), all test assays provide comparable values while determining copper (I) or copper (II) added to the water, or while determining traces of copper in reagent grade sodium chloride. Estimated per cent copper (I), about 93, in test aliquots with the two methods ($P>0.1$, n= 6 each) is closer to the labeled per cent purity, minimum of 96, of the copper (I) oxide salt. The mean absorbance by resorcinol method due to copper 0.5 µg is not affected by presence of Cd, Mo, Co and Zn up to 20 µg test concentration ($P>0.1$, n = 5 each). The carbamate method tolerated presence of Mo and Zn. Co and Cd cause concentration related increase in opalescence over 5 to 40 µg by direct interaction with the diethyl dithiocarbamate reagent consequently at 20 µg , the metals respectively increase mean absorbance of 5 µg of copper by about 56 and 40 % respectively ($P<0.01$, n = 5 each). Ni up to 5 µg is tolerated by resorcinol method while higher concentrations cause concentration related decrease in absorbance over 10 through 50 µg. The carbamate method tolerates Ni only up to 3 µg mass per sample, and higher concentrations impart serious opalescence such that and 5 µg Ni increased absorbance due to 5 µg copper by 12 to 15 per cent ($P<0.01$, n = 5 each). Ni and Co are known interfering agents for determination of copper by diethyl dithiocarbamate reagent[39]. The foregoing observations imply that the resorcinol method like carbamate method can be used to determine both copper (I) and copper (II) in aqueous solutions with comparable results. Besides, the resorcinol method enables determination of traces of copper in reagent grade sodium chloride.

Conclusions

The study provides an alternative simple and inexpensive micro-method for colorimetric determination of copper based on formation of a color complex between copper and resorcinol in presence of ammonia. The optimized method is suitable for determination of copper as low as 0.05 µg in 2.6 mL sample extracts implying lowest detection limit for Cu as 0.02 ppm. The reaction is sensitive to acids, oxidizing and reducing agents, copper complexing and sequestering agents, and anionic chelators notably acetate and phosphate. Therefore, the method is most suited for copper analysis in purer samples where these interfering agents are missing. The color complex is fairly stable to heat, insoluble in organic solvents, soluble in ethanol and methanol, and efficiently adsorbed by activated charcoal. The various versions of the technique can be safely used with perfect linearity over 0.1 through 0.5 µg of copper. The method in its present form is suitable for determination of copper (I) or copper (II) in ammoniated aqueous solutions. The method is comparable to dithiocarbamate method with at least ten times better detection limit, and shows better tolerance in presence of Cobalt than that of carbamate method. Metallic interferences reveal that the method works well in presence of Cd, Co, Mo and Zn up to test concentration of 20 µg each per sample while Ni is tolerated only up to 5 µg. Adsorption of copper-resorcinol complex to activated charcoal has practical utility. It would enable separation of copper from other undesirable chemicals, or its concentration from dilute solutions. Preliminary studies have provided encouraging results.

References

1. Jeffrey GH, Bassett J, Mendham J, Denney RC. Vogel's Textbook ofQuantitative Chemical Analysis. Longman Group UK Ltd., London; 1989.pp. 177, 393.
2. Mir SA, Shakoor A. A New Assay for Copper (II) sulfate by Acidimetry and Its Comparison with Iodometric Assay. Asian J.Chem. 2001; 13(1):255-258.
3. Parker MM, Humoller FL, Mahler DJ. Determination of Copper and Zinc in Biological Material. Clin. Chem. 1967; 13: 40-48.
4. Tautkus S, Kazlauskas R, Kareiva A. Determination of Copper in Tea Leaves by Flame Atomic Absorption Spectrometry. Chemija 2004; 15(4): 49-52.
5. Yamini Y, Hassan J, Karbasi M-H. Solid-Phase Extraction of Copper with Cupron on Octadecyl

Silica Cartridge and its Determination with Atomic Absorption Spectrometry. Microchim. Acta 2004; 148: 305-309.

6. Metin O, Ozcan O, Gultepe M. A Copper Determination Method Based on the Reaction Between 2-(5-Nitro-2-Pyridylazo)-5-(N-Propyl-NSulfopropylamino) Phenol (Nitro- PAPS) and Copper for Use in Urine Copper Measurement and Application to Automation. Turk. J. Med. Sc. 2007; 37 (2): 83-86.
7. Shabani AMH, Dadfarnia S, Nasirizadeh N, Shishehbore MR. Preconcentration of Copper with Dithizone-Naphthalene for Subsequent Determination by Atomic Absorption Spectrometry. J. Anal. Chem. 2007; 2(1): 46-50.
8. Jadid AP, Eskandari H. Preconcentration of Copper with Solid Phase Extraction and its Determination by Flame Atomic Absorption Spectrometry. E-J. Chem. 2008; 5(4): 878-883.
9. Shokrollahi A, Ghaedi M, Gharaghani S, Fathi MR, Soylak M. Cloud point extraction for the Determination of Copper in Environmental Samples by Flame Atomic Absorption Spectrometry. Quím. Nova 2008; 31(1): 1 9-30.
10. De Silva EGP, Hatje V, Dos Santos WNL, Costa LM, Nogueira ARA, Ferreira SLC. Fast Method for the Determination of Copper, Manganese and Iron in Seafood Samples. J. Food Compos. Ana. 2008; 21(3): 259-263.
11. Kimura K, Murakami Y. New Modified Colorimetric Method of Determination of Copper in Biological Materials with Sodium Diethyldithiocarbamate. Microchim. Acta1951; 36(2): 958-966.
12. Lofberg RT. A Sensitive Visible Spectrophotometric Method for Copper Using Bis-Substituted Thiocarbamoyl Trisulfides. Anal. Lett. 1969; 2(8): 439-448.
13. Baker JM, Teggins JE, Hoffman JW. A Colorimetric Method for the Determination of Trace Copper Concentrations in Water. J. Am. Water Resour. As. 1971; 7(6): 1246-1249.
14. Karapetian JV, Badalians SA. Spectrophotometric Determination of Copper in Milk. J. Dairy Sci. 1981; 64, 130-131.
15. Makino T, Itoh J-I. A Highly Sensitive Colorimetric Determination of Serum Copper Using α, β, γ, δ-Tetrakis (4-N-Trimethylaminophenyl)-porphine. Clin. Chim. Acta 1981; 111(1): 1-8.
16. Mochón MC, Ariza. LG. Girard-P Derivative of 2-Pyridinecarboxaldehyde as Analytical Reagent. Spectrophotometric Determination of Copper. Microchem. J.1981; 26(4): 463-471.
17. Singh I, Poonam M, Kadyan PS. Spectrophotometric Determination of Iron and Copper in Milk, Foodstuffs and Body Tissues with 1-(2-quinolylazo)-2, 4, 5- Trihydroxybenzene. Talanta 1985; 32(5): 387-390.
18. Ojeda CB, Torres AG, Rojas FS, Pavón JMC. Determination of Copper with 1, 5-bis (di-2-pyridylmethylene) thiocarbonohydrazide: Determination of Copper in Alloys. Microchem. J. 1987; 35(2):164-167.
19. Abe A, Yamashita S, Noma A. Sensitive, Direct Colorimetric Assay for Copper in Serum. Clin. Chem.1989; 35:552-554.
20. Kumar B, Singh HB, Katyal M, Sharma.RL. Spectrophotometric and Derivative Spectrophotometric Determination of Copper (II) with Dithizone in Aqueous Phase. Microchimica Act 1991; 105(1-3): 79-87.
21. Rizk M, Zakhari NA, Toubar SS, el-Shabrawy Y. Sensitive Colorimetric Determination of Copper (II) ions in Some Chemicals and Multivitamins. Acta Pharm. Hung. 1992;62(4): 158-166.
22. Li S, Li S, Chen. A. Spectrophotometric Determination of Trace Copper with a Cu- -Diethyldithiocarbamate-β-cyclodextrin Colour System. Talanta 1993; 40(7):1085- 1090.
23. Toral MI, Richter P, Munoz B. Derivative Spectrophotometric Method for Copper Determination. Environ. Monit. Assess. 1995; 38: 1-10.
24. Marczenko Z, Balcerzak M, Kloczko E. Separation, Preconcentration and Spectrophotometry in Inorganic Analysis. Elsevier, Amsterdam, 2000. p.178.
25. Sarma LS, Kumar JR, Reddy KJ, Reddy AV. 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)J. Agric. Food Chem. 2005; 53 (14): 5492 - 5498.
26. Shishehbore MR, Nasirizadeh N, Shabani AMH, Tabatabaee M. Spectrophotometric Determination of Trace Copper after Preconcentration with 1, 5-Diphenylcarbazone on Microcrystalline Naphthalene. Can. J. Anal. Sci. Spect.2005; 50(3): 130-134.

27. Fu D, Yuan D. Spectrophotometric Determination of Trace Copper in Water Samples with Thiomichlersketone. Spectrochim. Acta A 2007; 66(2): 434-437

28. Winton AL, Winton KB. Techniques of Food Analysis. Agrobios (India), Jodhpur; 2001. p. 259.

29. AOAC Official Methods of Analysis, 17th Ed., AOAC Int., Maryland, USA; 2000. p.8.

30. Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission,27th Session. Report of the 25th Session of the Codex Committee on Methods of Analysis and Sampling, Geneva; 2004.

31. Pandey G, Narang KK. Synthesis, Characterization, Spectral Studies, and Antifungal Activity of Mn (II), Fe (II), Co (II), Ni (II), Cu (II), and Zn (II) Complexes with Monosodium 4-(2-pyridylazo) resorcinol. Synt. React. Inorg. Met.-Org. Chem. 2004; 34: 291-311.

32. Ince M, Kaplan O, Yaman M. Solid-phase Extraction and Preconcentration of Copper in Mineral Waters with 4-(2-pyridyl-azo) Resorcinol-loaded Amberlite XAD-7 and Flame Atomic Absorption Spectrometry. Water Environ. Res. 2008; 80: 2104 -2110.

33. Lee CH, Kim JS, Suh MY, Lee W. A Chelating Resin Containing 4-(2-thiazolylazo) Resorcinol as the Functional group Synthesis and Sorption Behaviour for Trace Metal Ions. Anal. Chim. Acta 1997; 339(3): 303-312

34. Chung Y, Chung W. Determination of Co (II) Ion as a 4-(2-Thiazolylazo) resorcinol or 5-Methyl-4-(2-thiazolylazo) resorcinol Chelate by Reversed-Phase Capillary High- Performance Liquid Chromatography. B. Kor. Chem. Soc. 2003; 24:1781-1784.

35. Emara AAA, El-Sayed BA, Ahmed E.-SAE. Syntheses, Spectroscopic Characterization and Thermal Behavior on Novel Binuclear Transition Metal Complexes of Hydrazones Derived from 4, 6-Diacetylresorcinol and Oxalyldihydrazine. Spectrochim. Acta A. 2008; 69: 757-769.

36. Shebl M. Synthesis and Spectroscopic Studies of Binuclear Metal Complexes of a Tetradentate N_2O_2 Schiff base Ligand Derived from 4, 6-Diacetylresorcinol and Benzylamine. Spectrochimica Acta A 2008; 70: 850-859.

37. Soni PL, Katyal M. Textbook of Inorganic Chemistry. Sultan Chand & Sons, New Delhi; 1991. pp.3.155, 3.195, 3.275, 3.321, 3.323.

38. Soni PL, Chawla HM. Textbook of Organic Chemistry. Sultan Chand & Sons, New Delhi; 1994. p.2.637.

39. Bendix GH, Grabenstetter D. A Dithizone Method for the Rapid Determination of Copper. Ind.Eng. Chem. Anal.Ed. 1943; 15910): 649-652.

*****