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A Simple Spectrophotometric Method for the Determination of Copper in Some Real, Environmental, Biological, Food and Soil Samples Using Salicylaldehyde Benzoyl Hydrazone

M. Jamaluddin Ahmed* and Tasnima Zannat

Laboratory of Analytical Chemistry, Department of Chemistry, University of Chittagong, Chittagong - 4331, Bangladesh

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Abstract

A very simple, ultra-sensitive, highly selective and non-extractive spectrophotometric method for the determination of trace amounts copper(II) has been developed. Salicylaldehy debenzoyl hydrazone (SAL-BH) has been proposed as a new analytical reagent for the direct non-extractive spectrophotometric determination of copper(II). SAL-BH reacts with copper in a slightly acidic (0.0001-0.005 M H_2SO_4) in 40% 1,4-dioxane media with copper(II) to give a highly absorbent greenish yellow chelate with a molar ratio 1:1(Cu^{II}: SAL-BH) The reaction is instantaneous and the maximum absorption was obtained at 404 nm and remains stable for 72 h. The average molar absorptivity and Sandell's sensitivity were found to be 1.4×10^5 L mol^{-1} cm^{-1} and 5.0 ng cm^{-2} of copper(II), respectively. Linear calibration graphs were obtained for 0.01 – 18 mg L^{-1} of Cu^{II}. The detection limit and quantification limit of the reaction system were found to be 1 ng mL^{-1} and 10 µg L^{-1}, respectively. A large excess of over 50 cations, anions and complexing agents (e.g., tartrate, oxalate, citrate, phosphate, thiocyanate etc.) 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 (steels and alloys) as well as in some environmental waters (portable and polluted), biological (human blood and urine), food and soil samples and solutions containing both copper(I) and copper(II) as well as some complex synthetic mixtures. The results of the proposed method for biological and food samples were comparable with AAS and were found to be in good agreement. The method has high precision and accuracy (s = ± 0.01 for 0.5 mg L^{-1}).

Keywords: Spectrophotometry; Salicylaldehydebenzoylhydrazone; Copper; Real; Environmental; Biological; Food samples; Soil samples.

Introduction

Copper is an essential trace nutrient to all plants and animals [1]. Copper is an industrially important metal, it is used in coin making, wire making, medicine, alloys, fashioning metal products, transportation industry and thermal conductance [2]. On the other hand, toxic role of the metal ion is well recognized [3]. Increasing accumulation of copper(II) in the environment through numerous industrial sources, poses danger to public health. The amount of copper that contaminates various biological and environmental substances is of concern since copper traces promote rancidity and off-flavors in foods and 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. Hence, there is a great need to develop, simple, sensitive, selective and inexpensive method for the determination of copper in environmental, biological, soil, and

*Corresponding Author Email: pmjahmed55@gmail.com

industrial samples for continuous monitoring to establish the levels of copper in environmental and biological matrices.

Spectrophotometry is essentially a trace-analysis technique and is one of the most powerful tools in chemical analysis. Salicylaldehyde benzoyl hydrazone (Sal-BH) has been reported as a metallo complexing Schiff base reagent [4], but it has not previously been used for the spectrophotometric determination of copper. This paper reports on its use in a very sensitive, highly specific spectrophotometric method for the trace determination of copper. This method is far more selective, non-extractive, simple and rapid than all of the existing spectrophotometric methods [5-25]. The method is based on the reaction of non-absorbent Sal-BH in a slightly acidic solution (0.0001-0.005 M) with copper(II) to produce a highly absorbent greenish yellow product followed by a direct measurement of the absorbance in an aqueous solution with suitable masking, the reaction can be made highly selective and the reagent blank solution do not show any absorbance.

From above mentioned literature survey (Table 1) it reveals that those methods are lengthy, time-consuming, pH dependent and in most of above mentioned method, interference was high and applied on limited samples. It is needless to emphasize further that the direct spectrophot-ometric method in non-extractive way is more useful if it offers high sensitivity and selectivity. Search should be directed a new in order to develop simpler spectrophotometric method for non-extractive estimation of copper in very selective and sensitive ways.

Table 1. **Summary of review on the existing spectrophotometric methods for the determination of copper.**

Reagent	λ_{max} (nm)	€ ($Lmol^{-1}cm^{-1}$)	Beer's law (mg L^{-1})	Interference	Remarks	Ref.
Cysteine(RSH) by hexacyanoferrate	420	-	0-6.35	-	i) Limited application ii) Interference was not studied. iii) Less sensitive.	6
Thiomichlerketone (TMK)	500	5.7×10^4	0-15.0	-	i) pH- dependent ii) Limited Application iii) Less sensitive.	7
2-aminocyclopentenel - dithocaeboxylate	426	1.6×10^4	0.04-4.0	Many	i) pH- dependent ii) Less selective due to much interference iii) Lengthy and time consuming iv) limiter applications	8
3- methoxy-4- hydroxy bengaldehyed 4-bromophenyl hydrazone	462	2.052×10^4	0.2.-4.0	-	i) Solvent extractive ii) Lengthy and time consuming. iii) pH-dependent iv) Application was limited.v) Interference was not studied	9
Leucocrystal violet	590	1.47×10^6	0.004-0.04	-	i) pH- dependent , less selective and limited application	10
Acetophenone-p-chlorophenylthiosemicarbazone.	600	5.5×10^3	0.25-6.35	Many	i) Less selective ii) Limited applications	11
Alizarin Red s	510	3.5×10^4	0.011-0.320	-	i) pH- dependent ii) Limited application iii) Interference was not studied.	12
1,5-diphenylcarbazon (DPC)	542	2.5×10^3	0.04-5.0	Al(III), Fe(II) Ni(II), Hg(II)	i) Less selective ii) pH dependent,and preconcentration method. iii) Less sensitive.	13
Pyridylazo-4-phenyl-3-thiosemicarazone	440	2.16×10^4	0.2-5.0	-	i) pH- dependent ii) Interference was not studied.	14
1,3-diaminepropen-3-propyl-anchored sillca gel	460	6.5×10^3	-	Many	i) Preconcentration method ii) Time consuming iii) Less selective due to much interference.	15
Bromosulphonazo	616.8	3.3×10^5	0-1.024	Many	i) Solvent extractive. ii) Lengthy & time consuming iii) Less selective due to much interference.	16
2-(5-bromo-2-pyridylazo-5- diethylaminophenol	470	2.5×10^4	0.15-0.9	-	i) pH- dependent ii) Interference was not studied. iii) Detection limited was not mentioned iv) Less sensitive	17

DBH-PF	570	1.2×10^5	0.0-0.24	Many	i)	pH- dependent ii) Applied to the limited samples iii) Detection limited was not mentioned.iv) Less selective due to much interference	18
Butyllhidamine B Cation	570	1.66×10^6	0.0-0.02	Many	i) iii)	Solvent extractive. ii) Less selective due to much interference. Lengthy & time consuming.	19
Diantipyryl-(2-bromophenol) methan	-	-	0.02-0.2	Many	i)	Less selective due to much interference.ii) Molar absorptivity was not mentioned.iii) pH-dependent	20
Hydroxy-3-carboxy-5-sulfobenzenediazoaqminoazbenzene	530	1.31×10^4	0-0.24	Many	i)	pH- dependent, less selective and Limited applications.	21
O-bromophenylfluorene	570	3.64×10^4	0.08-1.6	Many	i)	Less selective ,ii)Less sensitive	22
5-(2-benzothiazolylazo)-8-hydroxyquinoline	667	3.7×10^3	0.2-3.7	many	i) ii)	Less selective Less sensitive.	23
2-ketobutyric acid thiosemicarbazone	387.2	1.85×10^4.	0.30-2.40	Many	i)	Less selective ii) Lengthy and pH-dependent.	24
Salicylalde hidebenzoyl hydrazone (present method)	404	1.4×10^5	0.01-18.0	Using suitable masking agent, the reaction can be made highly selective	i) ii) v)	Non-extractive, highly selective and sensitive Aqueous reaction medium simple and rapid	Present method

Experimental Section

Apparatus

Shimadzu (Kyoto, Japan) (Model-1800) double beam UV/VIS the recording spectrophotometer and a Jenway (England, U.K) (Model-30100) pH meter with a combination of electrodes were used for the measurements of absorbance and pH, respectively. A Shimadzu (Model-AA7000) atomic absorption spectrophotometer equipped with a microcomputer controlled air-acetylene flame at 324.8 nm was used for comparing the results. (Experimental conditions were: Slit width, 2 nm; lamp current, 3 mA; wavelength, 324.8 nm; flow rate of carrier gases are- air, 6.5 L min^{-1}; acetylene, 2 L min^{-1}; sample volume, 10 µL). A Shimadzu (Model-Prestige 21) FTIR spectrophotometer, range 7500 – 350 cm^{-1} were used to record the FTIR spectrum.

Synthesis and characterization of the reagent

The reagent was synthesized in the laboratory according to the method of Sacconi [26] and Salam [27]. The reagent salicylalde hydebenzoyl hydrazone (Sal-BH) was synthesized by two steps. First benzoyl hydrazone (BH) was prepared by refluxing ethylbenzoate (700 mmol) was added to hydrazine hydrate (700 mmol) in a round bottomed flask equipped with a reflux condenser. It was heated under reflux at 140°C for about 24 hours with continuous stirring using a magnetic stirrer. Then it was kept to stand overnight when white product separated out. The product so obtained was then filtered off, washed with ethanol and was dried first in air and then in a desiccator over silica gel. The collected crystalline product was then re-crystallized twice from the ethanol. The off-white crystalline product of benzoylhydrazone was thus washed, dried in air and finally in a desiccator under vacuum over silica gel whose melting point 114.5^0C (Lit. 112.5^0C).

Finally, salicylaldehyde benzoyl hydrazone (Sal-BH) was prepared by dissolving benzoyl hydrazone (30 mmol) in 50 mL of ethanol, and salicylaldehyde (30 mmol) was added dropwise in this solution with continuous stirring. The solution was refluxed for about one hour. Then it was cooled, allowed to stand for crystallization when a white crystalline product is separated out. A white crystalline product was obtained which then recrystallized and filtered off, washed with ethanol and dried in desiccators over silica gel and calcium chloride. Yield of product was 80%.

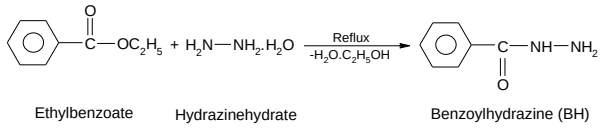
The reagent was characterized by taking melting point, elemental analysis, FTIR spectrum

and thermogravimetric analysis. The melting point of the reagent was 181^{0}C (Lit. 182^{0}C).

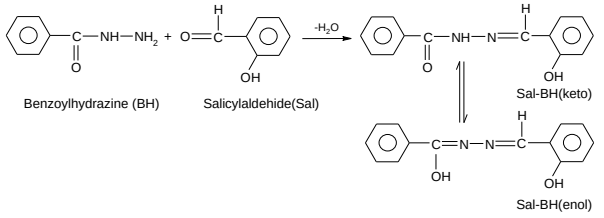
The results of elemental analysis (C = 49.59, N = 8.05, H = 3.34) % of the reagent was in good coincidence with the calculated values (C=49.77, N=8.45, H=4.14) %. The presence of FTIR peak at 1604.77 cm^{-1} was due to the characteristic C=N double bond (νC=N, 1590 – 1660 cm^{-1}) [28] of the Sal-BH. Both FTIR spectrum and elemental analysis data indicated the formation of the reagent. The steadiness of the thermogravimetric curve obtained for about 1g of the reagent at 80-90^{0}C indicated that the reagent didn't contain any moisture.

Synthesis of the Reagent

Step-1



Step-2



Reaction scheme of salicylaldehydebenzoylhydrazone(Sal-BH)

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 containing 1mL of concentrated HNO_3. More rigorous contamination control was applied when the copper levels in the specimens were low.

Sal-BH solution 3.95x10^{-3} mol L^{-1}

This solution was prepared by dissolving the requisite amount of salicylalde hydebenzoyl hydrazone in a known volume of 1,4 dioxane. More dilute solution of the reagent was prepared as required.

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

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

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

A 100-mL amount of stock solution (1 mg mL^{-1}) of Cu(I) was prepared by dissolving 155.7 mg of cuprous chloride (CuCl) in doubly distilled deionized water. Aliquots of this solution were standardized by iodometric titration. Working standard solution was prepared by suitable dilutions of stock solution.

Sodium azide solution

A 100mL sodium azide solution (2.5 % w/v) (Fluka purity > 99%) was freshly prepared by dissolving 2.5 gm in 100-mL of deionized water

EDTA solution

A 100mL stock solution of EDTA (0.01%) was prepared by dissolving 10 mg of A.C.S. grade (≥90%) ethylenediaminetetraacetic acid, dissodium salt dehydrate in (100mL) deionized water.

Tartrate solution

A 100mL stock solution of tartrate (0.01%) was prepared by dissolving 10 mg of A.C.S. grade (99%) potassium sodium tartrate tetrahydrate in (100mL) deionized water.

Dilute ammonium hydroxide solution

A 100mL solution of dilute ammonium hydroxide was prepared by diluting 10mL concentration. NH_4OH (28-30% A.C.S. grade) to 100mL with deionized water. The solution was stored in a polypropylene bottle.

Other solutions

Solutions of a large number of inorganic ions and complexing agents were prepared from their Analytical grade or equivalent grade water soluble salts (or the oxides and carbonates in hydrochloric acid); those of niobium, tantalum, titanium, zirconium and hafnium were specially prepared from their corresponding oxides (Specupure, Johnson Matthey) according to the recommended procedures of Mukharji [29]. In the case of insoluble substances, special dissolution methods were adopted [30].

General procedure

A volume of 0.01-1.0mL of neutral aqueous solution containing 0.1-180 µg of copper (II) in a 10-mL volumetric flask was mixed with a 1:400 to 1:1200 fold molar excess of Sal-BH reagent solution (preferably 1mL of 3.95×10^{-3} M) followed by the addition of 1 – 3.5mL (preferably 1mL) of 0.001 M sulfuric acid. The solution was mixed well. After few seconds 4-mL of 1,4-dioaxne was added. The mixture was diluted up to the mark with deionized water. After 1 min the absorbance was measured at 404 nm against a corresponding reagent blank. The copper content in an unknown sample was determined using a concurrently prepared calibration graph.

Sample collection and preservation

Water: Water samples were collected in polythene bottles from shallow tube-wells, tap-wells, river, sea and drain of different places of Bangladesh. After collection, HNO_3 (1mL L^{-1}) was added as preservative.

Blood and Urine: Blood and urine samples were collected in polypropylene bottles from effected persons of Chittagong Medical College Hospital, Bangladesh. Immediately after collection they were stored in a salt-ice mixture and latter, at the laboratory, were kept at-20^0C.

Soil: Soil (surface) samples were collected from different locations in Bangladesh. Samples were dried in air and homogenized with a mortar.

Food: Food samples were collected from local market of Chittagong in Bangladesh.

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 50mL Erlenmeyer 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 $(25\pm5)^0$C. 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 1-2mL of 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 under general procedure using citrate or fluoride as masking agent. Based on five replicate analyses, the average copper concentration determined by spectrophotometric method was in good agreement with the certified values. The results are given in (Table 5).

Determination of copper in environmental water samples

Each filtered (with Whatman No. 40) environmental water sample (1000mL) was evaporated nearly to dryness with a mixture of 2mL of concentrated H_2SO_4 and 5mL of concentrated HNO_3 to sulfur trioxide fumes in a fume cupboard following a method recommended by Greenberg et al [31]. After cooling additions of 5mL 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 the presence of 1-2mL of a 0.01% (w/v) tartrate solution. The resulting solution was then filtered and quantitatively transferred into a 25mL calibrated 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 citrate or fluoride as a masking agent. The results of analyses of environmental water samples from various sources for copper are given in (Table 6).

Most spectrophotometric methods for the determination of copper in natural and sea water require the preconcentration of copper [32]. The concentration of copper in natural and sea water is a few $\mu g\ L^{-1}$ in developed countries [31]. The mean concentration of copper in natural found in U.S. drinking water is greater than 20 $\mu g\ L^{-1}$[31].

Determination of copper in biological samples

Regarding human blood (2-5mL), urine (20-50mL) and food sample [Apple (50 gm), and egg (1 piece)] were transferred into a 25mL beaker. The sample was then ashes in a Muffle furnace at 500^0C for a 4 h in the presence of 10mL concentrated nitric acid following a method recommended by Stahr [33]. Then at the following content of each beaker were cooled at room temperature, 1.5mL of concentrated hydrochloric acid 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 fluoride or thiocyanate solution as masking agent. The results of the biological analyses by the spectrophotometric method were found to be in excellent agreement with those obtained by AAS. The results are given in (Table 7).

A deficiency of copper causes diseases such as anemia while excess of it causes “Jaundice and Wilson’s disease”. An excess of copper can contribute to many symptoms: depression, spaciness, paranoia, alternating moods, anxiety, panic, fearfulness, schizophrenia, phobias, etc [34]. The abnormally high value for the Wilson’s disease patient is probably due to the involvement of high copper concentration with As and Zn. Occurrence of such high copper contents are also reported in Wilson’s disease patient from some developed countries [35].

Determination of copper in soil samples

An air-dried homogenized soil sample (100g) was accurately weighed and placed in a 100mL micro-Kjeldahl flask. The sample was digested in the presence of an oxidizing agent following a method recommended by Jackson [36]. The content of flask was filtrated through Whatman No. 40 filter paper into a 25mL calibrated 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 fluoride or thiocyanide solution as a masking agent and quantified from a calibration graph prepared concurrently. The results are given in (Table 8).

Determination of copper (I) and copper (II) speciation in mixture

Suitable aliquots (1-2mL) of copper(I+II) mixtures (preferably 1:1, 1:5 and 1:10) were taken in 25mL conical flasks. A few drops of 1 mol L^{-1} sulfuric acid and 1-3mL of 1% (w/v) potassium permanganate solution were added to oxidize the monovalent copper, 5mL of water was added to the mixtures and heated on a steam bath for 10-15 min. with occasional gentle shaking and the cooled to room temperature. Then, 3-4 drops of freshly prepared sodium azide solution (2.5% w/v) were added and gently heated with a further addition of 2-3mL of water if necessary for 5 min. to drive off the azide, cooled to room temperature. The reaction mixture was neutralized with dilute ammonia and transferred quantitatively into a 10mL volumetric flask; 1mL of 3.95×10^{-3} M Sal-BH reagent solution was then added, followed by the addition of 1mL of 0.001 mol L^{-1} sulfuric acid and 4ml 1,4 dioxane was made up to the mark with deionized water. The absorbance was measured after 1 min. at 404 nm against the reagent blank. The total copper content was calculated with the help of a calibration graph.

An equal aliquot of the above mentioned copper (I+II) mixture was taken into a 25mL beaker. A 1mL volume of 0.05 % (w/v) thiocyanide (SCN^-) was added to mask copper(I) and was neutralized with dilute NH_4OH. The content of the beaker was transferred into a 10mL volumetric flask. Then, 1mL of a 0.001M sulfuric acid solution and 4 mL 1,4 dioxane was added followed by the addition of 1mL of 3.95×10^{-3} M Sal-BH and made up to the volume with deionized water. After 1 min. the absorbance was measured against a reagent blank as before. The copper concentration was calculated in mg L^{-1} or µg L^{-1} with the aid of a calibration graph. This gave a measure of the copper(II) originally present in the mixture. This value was subtracted from that of the total copper to obtain the copper(I) present in the mixture. The results were found to be highly reproducible. The occurrences of such reproducible results are also reported for different oxidation states of copper [37]. The results of a set of determination are given in (Table 9).

Result and Discussion

Factors Affecting the Absorbance

Absorption spectra

The absorption spectra of a copper (II)-Sal-BH system in aqueous medium in presence of 1mL 0.001M sulfuric acid solution, was recorded using the spectrophotometer. The absorption spectra of the copper (II)-Sal-BH is a assymmetric curve with maximum absorbance at 404nm and an average molar absorptivity of 1.4 x 10^5 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.

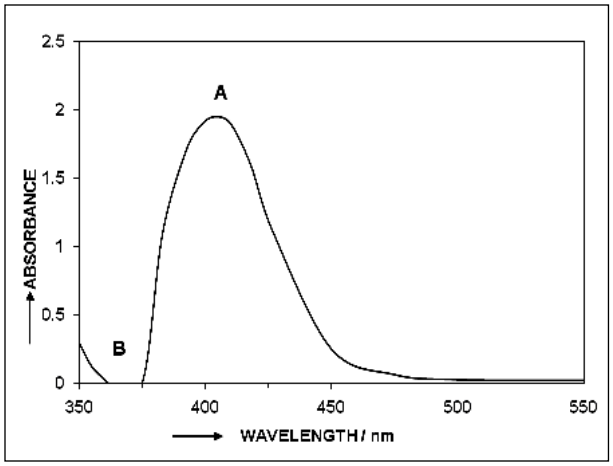


Figure 1. **A and B absorbation spectra of Cu^{II}- Sal-BH system (λ_{max} =404nm) and the reagent blank, respectively in aqueous solutions.**

Effect of solvent

Because Sal-BH is partially soluble in water, an organic solvent was used for the system, of the various solvents (acetone, benzene, carbon tetrachloride, chloroform, 1-butanol, isobutyl methyl ketone, N,N-dimethylformamide, methanol, ethanol and 1, 4-dioxane) studied, 1,4-dioxane was found to be the best solvent for the system. Different volumes (0-7mL) of was added to fixed metal ion concentration and the absorbance were measured according to the general procedure. It was observed that at 1mg L^{-1} Cu(II)-chelate metal, 4-7mL(40-70%) 1, 4-dioxane produced a constant absorbance of the Cu-chelate (Fig. 2). For all subsequent measurements, 4mL(40%) of 1, 4-dioxane was added.

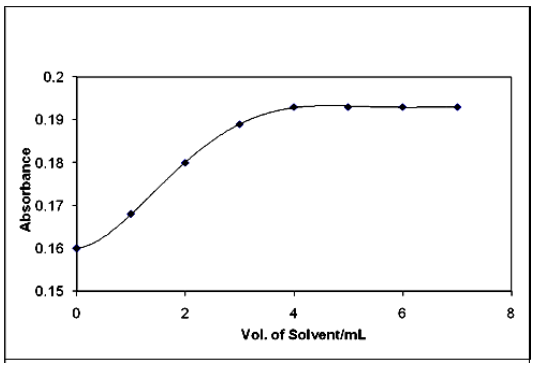


Figure 2. **Effect of solvent on the absorbance of Cu^{II}-Sal-BH system.**

Effect of acidity

Of the various acids (nitric, sulfuric, hydrochloric and phosphoric) studied, sulfuric acid was found to be the best acid for the system. The variation of the absorbance was noted after the addition of 0.05-5.0mL of 0.001M sulfuric acid to every 10mL of test solution. The maximum and constant absorbance was obtained in the presence of 1-3.5mL of 0.001M sulfuric acid at room temperature $(25\pm5)^0$C. Outside this range of acidity, the absorbance is decreased (Fig. 3). For all subsequent measurements 1mL of 0.001M sulfuric acid was added.

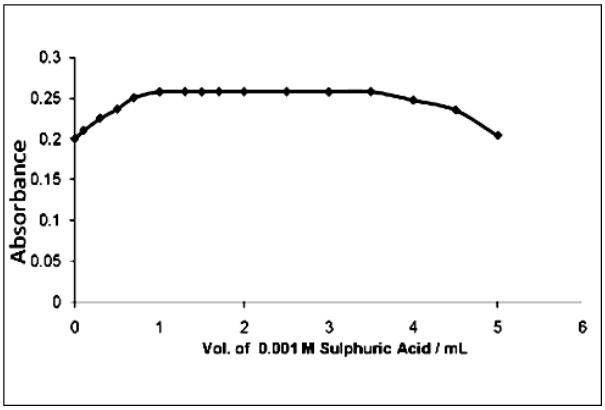


Figure 3. **Effect of the acidity on the absorbance of Cu^{II}-Sal-BH system.**

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 reagent concentration

Different molar excesses of Sal-BH 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 molar ratio of 1:400 to 1:1200 produced a constant absorbance of Cu - chelate (Fig. 4). 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, 1mL of 3.95×10^{-3} M Sal-BH reagent was added.

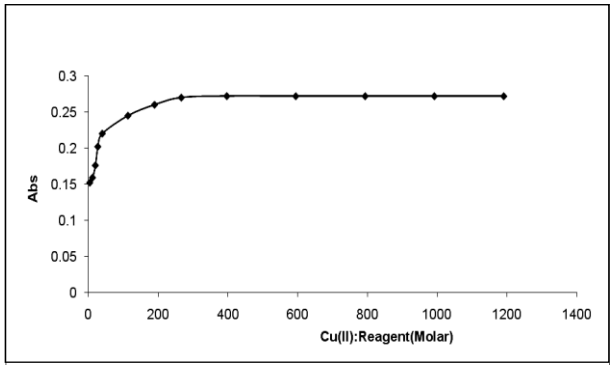


Figure 4. **Effect of reagent on the absorbance of Cu^{II}-Sal-BH system.**

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 0.01-100 mg L^{-1} distributed in four different sets (0.01 -0.1, 0.1-1.0, 1.0-10, 10-100 mg L^{-1}) for convenience of the measurement. The absorbance was linear for 0.01-18.0 mg L^{-1} at 404nm. Of the four calibration graphs one showing the limit of the linearity is given in (Fig. 5). The next three are straight-line graphs passing through the origin. The molar absorption co-efficient and the Sandell's sensitivity [38] were found to be 1.4 x 10^5 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).

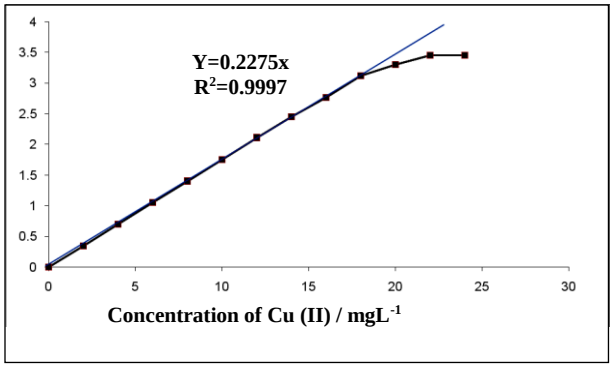


Figure 5. **Calibration graph, 1-18 mgL^{-1} of copper(II).**

Table 2. **Selected analytical parameters obtained by optimization experiments.**

Parameters	Studied range	Selected value
Wavelength / $\lambda_{max\ (nm)}$	200-800	404
Solvent / (%)	0-70	40-70 (preferably 40)
Acidity H_2SO_4 / M	0.0-0.006	0.001-0.004 (preferably 0.001)
pH	2.58-1.21	2.58-1.21(preferably 2.27)
Time / h	1-24h	1 min.-24 h (preferably 2 min.)
Temperature / ^oC	25±5 ^oC	25±5 ^oC
Reagent(fold molar excess, M:R)	1:10-1:1200	1:400-1:1200 (preferably 1:400)
Molar absorption Coefficient / L mol^{-1} cm^{-1}	0.7×10^5 – 1.7×10^5	1.4×10^5
Linear range/mg L^{-1}	0.001-100	0.01-18
Detection limit /µg L^{-1}	0.01-100	1
Sandell's Sensitivity /ng cm^{-2}	0.1 - 100	5
Relative Standard Deviation	0 -2	0 - 2
Regression Co-efficient	0.998-0.9999	0.999

Precision and accuracy

The precision of the present method was evaluated by determining different concentration of copper (each analyzed at least five times). The relative standard deviation (n=5) was 0-2% for 0.1-180 µg of copper in 10-mL indicating that this method is highly precise and reproducible. The detection limit (3s/S of the blank) and Sandell's sensitivity (concentration for 0.001 absorbance unit) for copper were found to be 1.0 µg L^{-1} and 5 ng cm^{-2}, respectively. The analytical results must be evaluated with regard to the validity of analytical method. Poor analytical quality may lead to false conclusions. Keeping this in our mind the validity of our method was tested by analyzing several Standard Reference Materials (Table 5), Recovery Studies (Table 6) and also comparing the results with conventional analysis (AAS) (Table 7). With suitable masking, the reaction can be made highly selective.

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 [39] 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. Only Fe(III) interferes due to complex formation with Sal-BH. In order to eliminate the interference of Fe(III) thiocyanate, can be used as a masking agent. Moreover, 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. During interference studies, if a precipitate was formed, it was removed by centrifugation. 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).

Composition of the absorbent complex

Job's method [40] of continuous variation and the molar-ratio [41] method were applied to ascertain the stoichiometric composition of the complex. A Cu: Sal-BH (1:1) complex was indicated by both methods.

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

Species x	Tolerance ratio / x/Cu (w/w)	Species x	Tolerance ratio / x/Cu (w/w)	Species x	Tolerance ratio / x/Cu (w/w)
Acetate	100	Citrate	20	Oxalate	1000
Arsenic(III + V)	100	Chromium(III +VI)	100	Lead(II)	100
Ascorbic acid	200	Citric acid	1000	Perchlorate	1000
Aluminum	100	Cyanide	100	Phosphate	1000
Azide	1000	Carbonate	1000	Strontium	100
Antimony(III)	100	EDTA	50	Silver	100
Ammonium	100	Fluoride	1000	Selenium(IV+VI)	100
Bromide	1000	Iodide	500	Sulfate	1000
Barium	10	Iron(II)	50	Sodium	1000
Bismuth(III)	50	Iron(III)	10[b]	Tartrate	1000
Beryllium	100	Mercury(II)	100	Thiocyanate	1000
Chloride	1000	Magnesium	100	Tin(II)	100
Cerium(III)	100	Manganese(II)	100	Thallium (I)	100
Cesium	100	Molybdenum(V+VI)	100	Tellurium(IV)	100
Calcium	1000	Nitrite	1000	Vanadium(V)	100
Cobalt(II+III)	100	Nickel	100	W(VI)	50
Cadmium	100	Nitrate	200	Zinc	100

[a]Tolerance limit defined as ratio that causes less than 5 percent interference.
[b]with 100 mg L^{-1} NH_4CNS

Table 4. **Determination of copper(II) in synthetic mixtures.**

Sample	Composition of mixture/mg L^{-1}	Copper (II)/mg L^{-1}		Recovery ± s[b](%)
		Added	Found[a]	
A	Cu(II)	0.5	0.49	98±0.2
		1.0	1.00	100±0.0
B	As in A+ Mn^{2+}(25)+Al (25)	0.5	0.48	97±0.3
		1.0	0.98	98±0.2
C	As in B+ Mo^{VI} (25) +Hg^{2+} (25)	0.5	0.51	101±0.2
		1.0	1.02	102±0.2
D	As in C+Ca (25)+ Mg (25)	0.5	0.52	104±0.4
		1.0	1.04	104±0.4
E	As in D+ K (25)+Se^{VI} (25)	0.5	0.52	104±0.4
		1.0	1.03	103±0.3

[a]Average of five analysis of each sample
[b]The measure of precision is the standard deviation (s).

Table 5. **Deatermination of copper in certified reference materials.**

Sample	Certified Reference Material [42] (Composition, %)	Copper (%)		RSD[b](%)
		Certifid value	Found[a] (n=5)	
1	Bureau of Analysed Samples Ltd. No., 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	60.4	1.4
2	Bureau of Analysed Samples Ltd. No., BAS-CRM-5g: Cu, 67.4. Sn, 1.09. Pb, 2.23. Zn, 28.6, Ni, 0.33. P, 0.01.	67.4	67.01	1.2
3	Brass, Class-1: Pb, 0.00. Fe, 0.01. Cu, 70.61.	70.61	70.5	1.4
4	YSBC20a-95[c]: Mn, 0.81, Cr, 16.30, Mo, 0.52, V, 0.24, Co, 1.45, Cu,1.35	1.35	1.3	1.3
5	GSBH-40101-96[c]: C,1.5, Mn, 0.15 Cr, 11.63, Ni, 0.1, Mo, 0.99, V, 0.41, Co, 0.02, Cu,0.18	0.18	0.17	2.0

[a]Average of the five replicate determinations
[b]The measure of precision is the relative standard deviation (RSD).
[c]These CRMs were from Beijing NCS Analytical Instruments Co. Ltd., China

Table 6. **Determination of copper in some environmental water samples.**

Sample		Cu/µg L^{-1}		Recovery ± s (%)	s_r[b] (%)
		Added	Found[a]		
Tap water		0	40.0		
		100	140.0	100±0.0	0.00
		500	542.0	102±0.2	0.21
Well water		0	35.0		
		100	136.0	100.7±0.6	0.25
		500	535.0	100.6±0.5	0.21
Rain Water		0	10		
		100	110.0	100±0.0	0.21
		500	512.0	100.5±0.8	0.26
River water	Karnafuly	0	60.0		
		100	161.0	100.6±0.4	0.24
		500	566.0	100.±0.6	0.29
	Halda	0	61.0		
		100	160.0	99.4±0.3	0.15
		500	565.0	100.7±0.5	0.18
Sea water	Bay of Bengal (upper)	0	42.0		
		100	145.0	101.4±0.8	0.26
		500	547.0	100.9±1.0	0.35
	Bay of Bengal (lower)	0	43.0		
		100	144.0	100±0.1	0.20
		500	545.0	100.9±1.2	0.45
Lake water	Kaptai	0	75.0		
		100	180.0	97±0.5	0.29
		500	585.0	100.8±0.6	0.37
Drain water	Cable Factory[c]	0	130.0		
		100	238.0	101.3±1.0	0.29
		500	640.0	100.7±0.8	0.25

[a]Average of five replicate determinations.
[b]The measure precision is the relative standard deviation (s_r)
[c]Estern Cable Factory, Chittagong.

Table 7. **Determination of copper in some human fluids and food samples.**

Serial No.	Sample	Copper / µg L^{-1}		Sample source[a]
		AAS	Proposed method[b]	
1.	Blood	525.0±1.2	530±1.5	Wilson's diseases patient (Male)
2.	Blood	165.0±1.0	160.0±1.2	Hypertension (Male)
3.	Blood	260.0±1.3	250.5±1.3	Lung cancer (Female)
4	Blood	132.8±1.6	135.0±1.5	Normal adult (Male)
5	Apple	11.2[c]±1.2	10.3[c]±1.9	Apple(China)
6	Egg	0.64[c]±0.5	0.58[c]±0.6	Egg(Boiler)

[a] Human fluids were from Chittagong Medical College Hospital and food samples were from local market.
[b]Average of five replicate determinations ± s
[c]Values in mg kg^{-1}

Table 8. **Determination of copper in some surface soil samples.**

Serial No.	Copper / µg g^{-1} (n = 5)[a]	Sample source
S_1[c]	20.5±1.5[b]	Agriculture soil (Chittagong University Campus)
S_2	50.8±1.8	Esturine soil (Karnafuli River)
S_3	22±1.4	Marine soil (Chittagong Sea Beach)
S_4	100.0±1.6	Traffic soil (Kadamtali Bus Station)
S_5	75.9±2.0	Roadside soil (Chittagong – Rangamati road)

[a]Average of five analysis of each sample
[b]The measure of precision is the standard deviation (±s).
[c]Composition of soil samples: C, N, P, K, Na, Ca, Mg, Fe, Pb, Cu, Zn, Mn, Mo, Co,NO_3, NO_2, SO_4, etc.

Table 9. **Determination of copper (I) and copper (II) speciation in mixtures.**

Serial No	Cu(II): Cu(I)	Cu, taken/mg L^{-1}		Cu, found/mg L^{-1}		Error/mg L^{-1}	
		Cu(II)	Cu(I)	Cu(II)	Cu(I)	Cu(II)	Cu(I)
1	1:1	1.00	1.00	1.00	0.99	0.00	0.01
2	1:1	1.00	1.00	0.99	1.00	0.01	0.00
3	1:1	1.00	1.00	1.00	0.98	0.00	0.02
		Mean error: Cu(II) = ±0.0068; Cu(I) = ± 0.014 Standard deviation: Cu(II) = ± 0.006; Cu(I) = ±0.011					
1	1:5	1.00	5.00	0.99	4.98	0.01	0.02
2	1:5	1.00	5.00	0.99	5.99	0.01	0.01
3	1:5	1.00	5.00	0.98	5.97	0.01	0.03
		Mean error: Cu(II) = ±0.013; Cu(I) = ± 0.016 Standard deviation:Cu(II)= ±0.0058;Cu(I) = ±0.0058					
1	1:10	1.00	10.00	0.98	9.98	0.02	0.02
2	1:10	1.00	10.00	0.99	9.99	0.01	0.01
3	1:10	1.00	10.00	0.98	9.98	0.02	0.02
		Mean error: Cu(II) = ±0.016; Cu(I) = ± 0.016 Standard deviation:Cu(II) = ±0.0058; Cu(I) = ±0.006					

Conclusions

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) - Sal-BH complex was developed for the determination of copper in environmental, industrial, biological, food and soil samples for continuous monitoring to establish trace level of copper in difficult sample matrices. The method also offers a very efficient procedure for speciation analysis. Although many sophisticated techniques, such as pulse polarography, HPLC, NAA, AAS, and ICP-MS, are available for the determination of copper at trace levels in numerous complex materials,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 ($\varepsilon = 1.4 \times 10^5$ 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 down to (ng g^{-1}) levels in an aqueous medium at room temperature(25 ± 5^0 C).

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