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High Sensitive and Selective Spectrophotometric Method for the Determination of Copper in Industrial, Environmental, Biological and Soil Samples Using 2-Hydroxynaphthaldehydebenzoylhydrazone

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Abstract

A very simple, ultra-sensitive and highly selective spectrophotometric method is presented for the rapid determination of copper at a trace level. 2-hydroxynaphthaldehyde- benzoylhydrazone (HNABH) has been proposed as new analytical reagent for the direct non-extractive spectrophotometric determination of copper (II). HNABH reacts with copper in a slightly acidic (1×10^{-4}-3.5×10^{-4} mol L^{-1} H_2SO_4) in 50 % N,N-dimethylformamide (DMF) media with copper (II) to produce a highly absorbent green chelate with a molar ratio 1:1. The reaction is instantaneous and the maximum absorption was obtained at 427 nm and remains stable for 24 h. The average molar absorptivity and Sandell's sensitivity were found to be 4.35×10^4 L mol^{-1} cm^{-1} and 5.0 ng cm^{-2} of copper (II), respectively. Linear calibration graphs were obtained for 0.01 – 12.0 mg L^{-1} of Cu (II). The detection limit and quantification limit of the reaction system were found to be 1 µg L^{-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 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) 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 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; 2-hydroxynaphthaldehydebenzoylhydrazone copper; Industrial; environmental; biological; soil samples

1. Introduction

Copper is an essential trace nutrient to all high plants and animals. In animals, including human it is found in primarily in the bloodstream, and in copper-based pigments. However, insufficient amounts; copper can be poisonous and even fatal to organisms. Copper also has a significant presence as a decorative metal art. It can also be used as an anti-germ surface that can add to the anti-bacterial and antimicrobial feature of buildings such as hospitals [1]. Copper has a high electrical and thermal conductivity, among pure metals at room temperature [2]. On the other hand, toxic rule of the metal ion is well recognized [3]. Increasing accumulation of copper (II) in the environment through numerous industrial

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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 neurological and psychiatric defects. Hence; there is a great need to develop, simple, sensitive, selective and inexpensive methods for the determination of copper in environmental, biological, soil, and 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. 2-hydroxynaphthaldehydebenzoylhydrazone has been reported as a spectrophotometric reagent for aluminum [4]; 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 [5-24]. Some of the methods are not sensitive, some are from interference, some have limited application, some method use surfactant, some are solvent extractive and there are some methods where they used buffer solution and chelate stabilizer. Some are very lengthy process, time consuming, pH and temperature dependent. A comparison of few selected process; their spectral characteristic and draw backs are summarized in Table 1. The Shiff-base reagents had widely been applied for the determination of noble metal ions, this type of reagents generally have higher sensitivity and selectivity for the determination of metal ions [25]. In the search for more Shiff-base reagent, in this work, a new reagent 2- hydroxynaphthaldehydebenzoylhydrazone (HNABH) was synthesized according to the method Sacconi [26] and Salam [27] and color reaction of HNABH with Cu in aqueous media was carefully studied. The method is based on the reaction of non-absorbent HNABH in a slightly acidic solution (1×10^{-4}-3.5×10^{-4}) mol L^{-1} with copper(II) to produce a highly absorbent green chelate 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.

2. Experimental

2.1. Apparatus

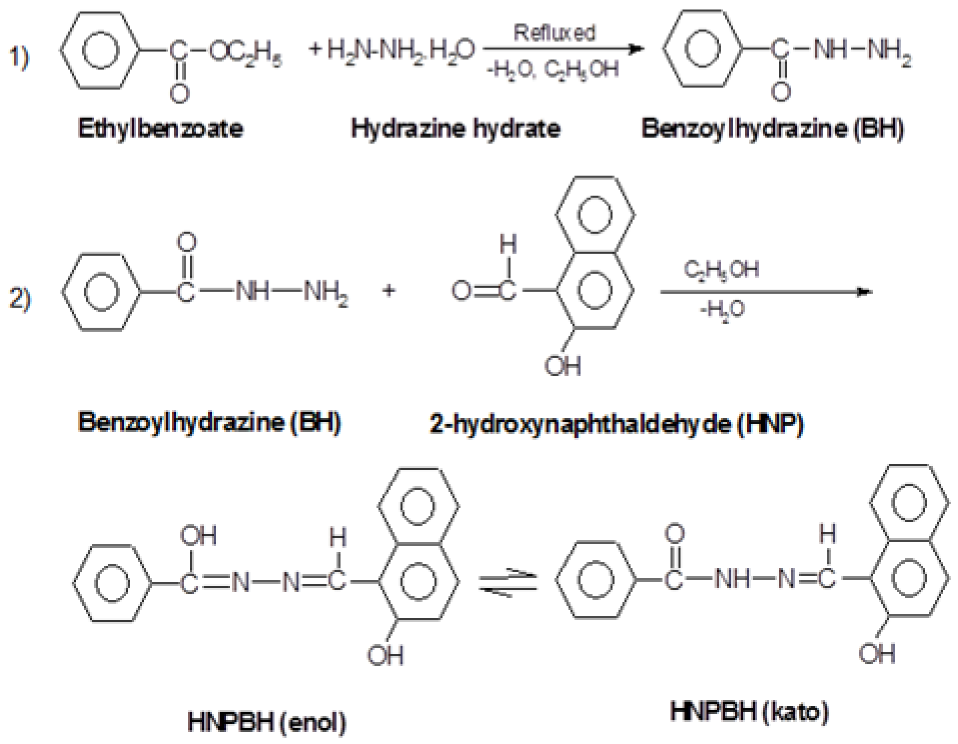
Shimadzu (Kyoto, Japan) (Model-1800) double beam UV/VIS the recording spectrophotometer and a Jenway (England, U.K) (Model-30100) pH meter with a combination of electrodes were used for the measurements of absorbance and pH, respectively. A Shimadzu (Model-AA640) atomic absorption spectrophotometer equipped 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.

Table 1. Summary of the comparison between present and existing spectrophotometric methods for the determination of copper

Reagent	solvent	Medium Aqueous/ Surfactant/ Organic	Acidity/ pH	λ_{max} (nm)	Molar absorption co-efficient (L.mol^{-1} cm^{-1})	Beer's Law (mgL^{-1})	Detection Limit (µgL^{-1})	RSD %	Interference	Remarks	References
2-aminocyclopentene-1-dithocaeboxylate	DMF	Aqueous	pH-4.5	426	1.6×10^4	0.04-4.0	0.8	1.53	Many	i) pH- dependent ii) Less selective due to much interference iii) Lengthy andtime consuming	8
Acetophenone-p-chlorophenylthiosemicarbazone	-	Oleic acid surfactant	pH-4.9	600	5.5×10^3	0.25-6.35	21	0.19	Many	i) Less selective due to much interference. ii) Limited applications iii) Interference was not studied.	11
1-,5-diphenylcarbazone (DPC)	DMF	Aqueous	pH-6.5-8.0	542	2.5×10^3	0.04-5.0	30	1.4	Al(II) Fe(II) Ni(II) Hg(II)	i) Less selective due to much interference. ii) Preconcentration method iv) pH- dependent.	13
4-benzylpiperidinedithiocarbamate	Chloroform	Solvent extractive	-	437	8.19×10^3	0.004-0.1	50	5.0	Many	i) Solid phase extractive method ii) Preconcentrationmethod iii) Less selective due to much interference.	16
Pyridylazo-4-phenyl-3-thiosemicarazone	Ethanol	Aqueous	pH-3.0-5.5	440	2.16×10^4	0.2-5.0	65	6.0	Many	i) pH- dependent ii) Interference was not studied. iii)Limited applications.	17
Bromosulphonazo	-	Solvent extractive	pH 4.5	616.8	3.3×10^5	0-1.024	7.03	4.68	Many	i) Solvent extractive. ii) Lengthy &time consuming.	19
5-(2-benzothiazolylazo)-8-hydroxyquinoline	-	Aqueous	-	667	3.7×10^{-8}	0.2-3.7	67	1.28	Many	i) Less selective due to much interference. ii) Less sensitive.	21
2,5-dimercapto-1,3,4-hiadiazole(DMTD)	-	Aqueous	0.002 0.014 M H_2SO_4	390	5.64×10^4	0.1-20	15	2	Fe(III) Hg(II) at high conc.	i) Less selective due to some interference ii) Less sensitive	23
2-Hydroxynaphthaldehyde-benzoylhydra-zone (HNABH) (present method)	DMF	Aqueous	0.0005 -0.05 M H_2SO_4	427	4.35×10^4	0.01-12.0	1.0	0-2	Using suitable masking agent, the reaction can be made highly selective	i) Non-extractive ii) Highly sensitive. iii) Fairly selective iv) Aqueous reactionmedium v) simple andrapid	present method

2.2. Synthesis and characterization of the reagent

The reagent was synthesized in our laboratory according to the method of Sacconi [26] and Salam [27]. The reagent 2-hydroxynaphthaldehydebenzoylhydrazone (HNABH) was synthesized by two steps. First, benzoylhydrazine (BH) was prepared by refluxing ethylbenzoate (700 mmol) with hydrazine hydrate (700 mmol) at 140 °C for 20 hours in a round bottle flux equipped with a condenser and then recrystallizing twice from ethanol. An off-white crystalline product was obtained whose melting point was 115 °C (Literature value was 113-117 °C [26]). The reagent was characterized by taking melting point, elemental analysis, FTIR spectrum and thermogravimetric analysis. The melting point of the reagent was 204 ºC (Literature value was 206 ºC [26]).



Schema 1: Synthesis of 2-hydroxynaphthaldehydebenzoylhydrazone

2.3. 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 1-mL of concentrated HNO_3. More rigorous contamination control was applied when the copper levels in the specimens were low.

2.3.1. HNABH solution $3.9x10^{-3}$ mol L^{-1}

This solution was prepared by dissolving the requisite amount of 2-hydroxynaphthaldehydebenzoylhydrazone in a known volume of dimethylformamide (DMF). More dilute solution of the reagent was prepared as required.

2.3.2. 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 solution was prepared by suitable dilutions of the stock solution.

2.3.3. 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.

2.3.4. 1,10-Phenanthrolin solution

A 0.1% 1,10-phenanthrolin solution was prepared by dissolving 0.1 gm amount in 100-mL slightly hot deionized water.

2.3.5. Sodium Azide Solution

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

2.3.6. EDTA Solution

A 100-mL stock solution of EDTA (0.01%) was prepared by dissolving 10 mg of American Chemical Society grade (≥90%) ethylenediaminetetraacetic acid, di-sodium salt dehydrate in (100-mL) deionized water.

2.3.7. Tartrate Solution

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

2.3.8. Dilute Ammonium Hydroxide Solution

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

2.3.9. Dimethylglyoxime(DMG) solution

A 100-mL stock solution of (0.1%) DMG was prepared by dissolving 0.1 g of A.C.S. grade DMG in 100-mL rectified spirit.

2.3.10. 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 [28]. In the case of insoluble substances, special dissolution methods were adopted [29].

2.4. General Procedure

A volume of 0.1-1.0-mL of neutral aqueous solution containing 0.1-120 µg of copper (II) in a 10-mL volumetric flask was mixed with a 1:100 to 1:1000 fold molar excess of 2-hydroxy- naphthaldehydebenzoylhydrazone (HNABH) reagent solution (preferably 1-mL of 3.9×10^{-3} mol L^{-1}) followed by the addition of 1 – 3.5-mL (preferably 1-mL) of 0.001 mol L^{-1} sulfuric acid. The solution was mixed well. After few seconds 5-mL of N,N-dimethylformamide (DMF) was added. The mixture was diluted up to the mark with deionized water. After 1 min the absorbance was measured at 427 nm against a corresponding reagent blank. The copper content in an unknown sample was determined using a currently prepared calibration graph.

2.5. Sample Collection and Preservation

Water: Water samples were collected in polyethylene bottles from shallow tube-wells, tap-wells, river, sea and drain of different places of Bangladesh. After collection, 1-mL HNO_3 (1 mL 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 °C.

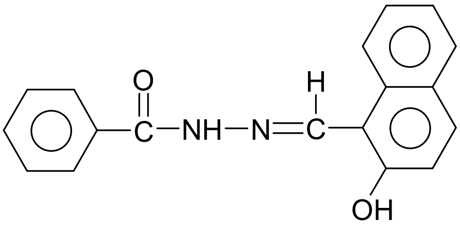
Soil: Soil (surface) samples were collected from different locations in Bangladesh. Samples were dried in air and homogenized with a mortar.

3. Result and Discussion

3.1. Factors Affecting the Absorbance

3.1.1. Absorption spectra

The absorption spectrum of a copper (II)-HNABH system in aqueous medium in presence of 1-mL 0.001 mol L^{-1} sulfuric acid solution, was recorded using the spectrophotometer. The absorption spectra of the copper (II)-HNABH is a asymmetric curve with maximum absorbance at 427 nm and an average molar absorptivity of 4.35×10^4 L mol^{-1} cm^{-1} (Fig. 1). The reagent blank exhibited negligible absorbance despite having wavelength at 427 nm. The reaction mechanism of the metal - Shiff-base reagent is as reported earlier [30].



The molecular structure of HNABH

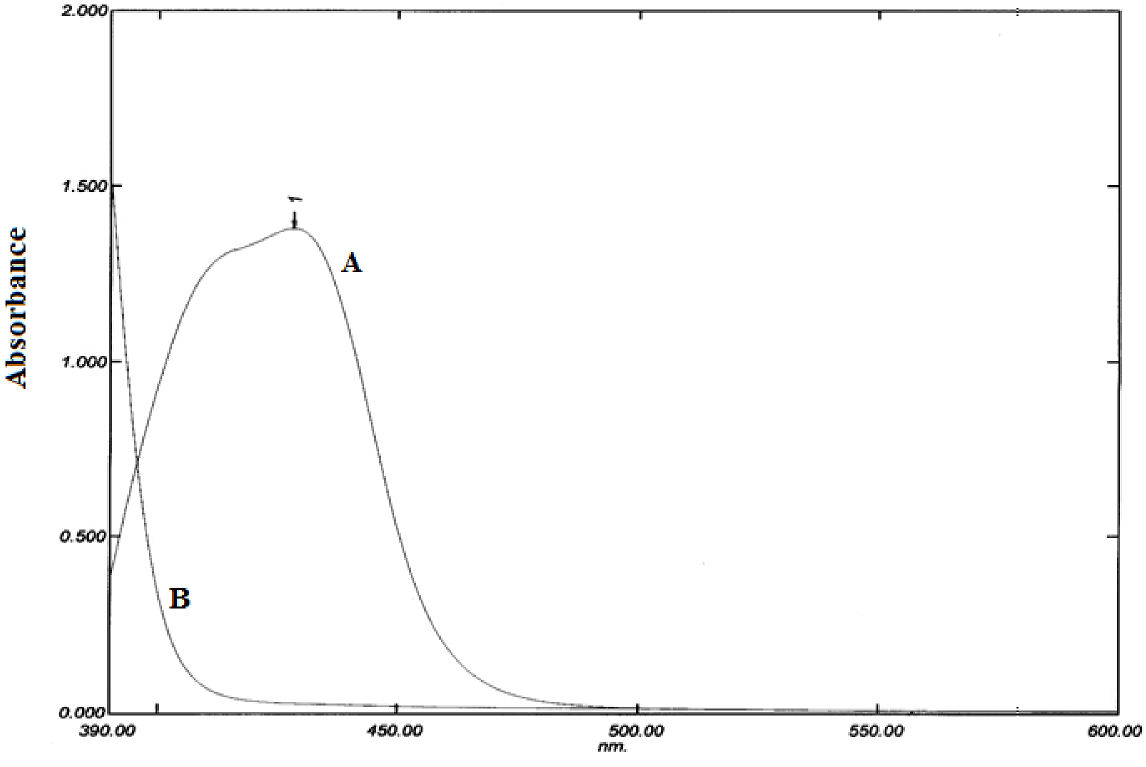


Fig 1. A and B absorbance spectra of Cu (II) - HNABH system and the reagent blank (λ_{max} = 427nm) in aqueous solutions

3.2. Effect of Solvent

Because HNABH was partially soluble in water, an organic solvent was used for the system and the various solvents (acetone, benzene, carbon tetrachloride, chloroform, 1-butanol, isobutyl methyl ketone, dimethylformamide, methanol, ethanol and 1, 4-dioxane) studied, dimethylformamide was found to be the best solvent for the system. Different volumes (0-7-mL) of dimethylformamide were added to fixed metal ion concentration and the absorbance were measured according to the general procedure. It was observed that at 1 mg L^{-1} Cu(II)-chelate metal, 3-7 mL dimethylformamide produced a constant absorbance of the Cu-chelate (Fig. 2). For all subsequent measurements, 5 mL of dimethylformamide was added.

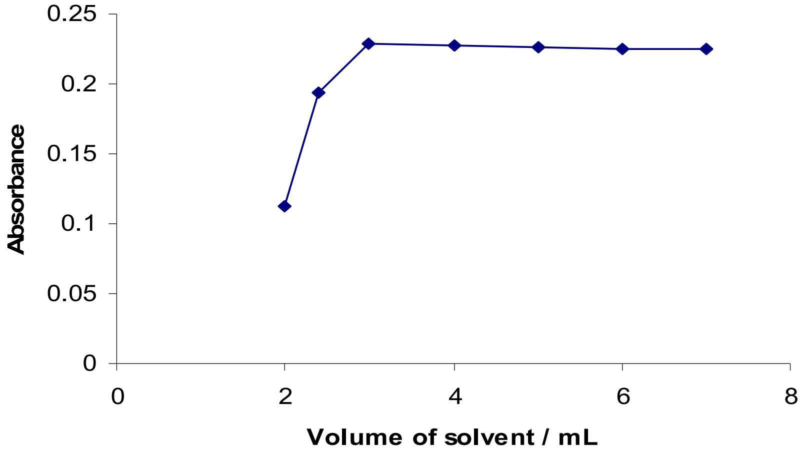


Fig 2. Effect of solvent on the absorbance of Cu (II) - HNABH system

3.3. 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 0.05-5.0 mL of 0.001mol L^{-1} sulfuric acid to every 10 mL of test solution. The maximum and constant absorbance was obtained in the presence of 1-3.5 mL of 0.001 mol L^{-1} sulfuric acid (pH 2.58 – 1.21) at room temperature $(25\pm5)^0$C. Outside this range of acidity, the absorbance decreased (Fig. 3). For all subsequent measurements 1 mL of 0.001 mol L^{-1} sulfuric acid (pH 2.27) was added.

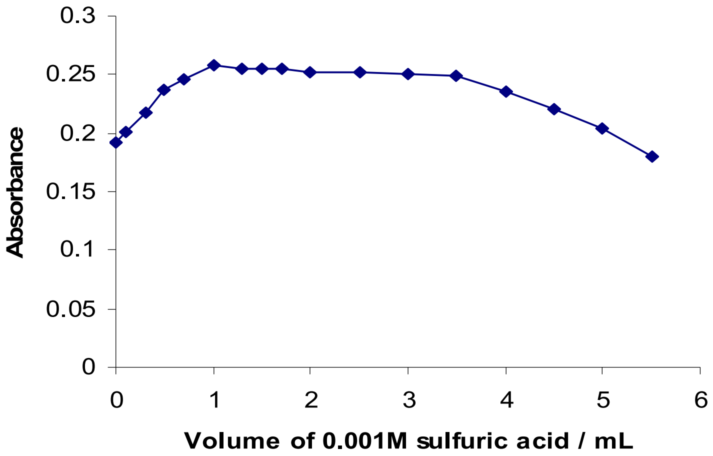


Fig 3. Effect of the acidity on the absorbance of Cu (II) - HNABH system

3.4. 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.

3.5. Effect of Reagent Concentration

Different molar excesses of HNABH were added to a fixed metal ion concentration and the absorbance was measured according to the general procedure. It was observed that a 1 mg L^{-1} of copper metal, the reagent molar ratio of 1:100 to 1:1000 produced a constant absorbance of Cu - chelate (Fig. 4). For different copper- concentration (0.5 and 1 mg L^{-1}) an identical effect of varying the reagent concentration was noticed. A greater excess were not studied. For all subsequent measurements, 1 mL of 3.9 $\times 10^{-3}$ mol L^{-1} HNABH reagent was added.

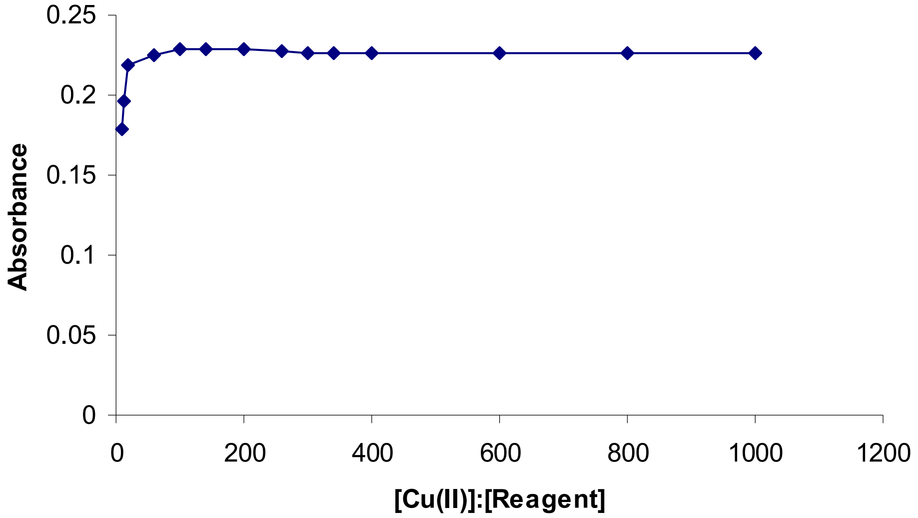


Fig 4. Effect of reagent (HNABH:Cu^{II} molar concentration ratio) on the absorbance of Cu (II) - HNABH system

3.5. 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 0.01-12.0 mg L^{-1} at 427 nm. Of the three calibration graphs one showing the limit of the linearity is given in Fig. 5. The next two are straight-line graphs passing through the origin. The molar absorptivity and the Sandell's sensitivity [31] were found to be 4.35 x 10^4 L mol^{-1} cm^{-1} and 5 ng mL^{-1} of copper (II), respectively. The selected analytical parameters obtained with the optimization experiments are summarized in Table 2.

3.6. 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 2-0% for 0.01-12.0 µg of copper in 10-mL indicating that this method is highly precise and reproducible. The detection limit (3s of the blank) and Sandell's sensitivity (concentration for 0.001 absorbance unit) for copper were found to be 1.0 ng mL^{-1} and 5 ng cm^{-2}, respectively and theoretical detection limit was 5 ng mL^{-1}. The results for total copper(II) were in good agreement with the certified values (Table 5). The reliability of our copper-chelate procedure was tested by recovery studies. The average percentage recovery obtained for the addition of copper(II) spike to some environmental water samples was quantitative, as shown in Table 6. The method was also tested by analyzing several synthetic mixtures containing copper(II) and diverse ions (Table 4). The results of biological samples analyses by the spectrophotometric method were in excellent agreement with those obtained by AAS which were verified by performing the t-test on the obtained results by two methods (Table 7). The results of speciation of Cu(I) and Cu(II) in mixture were highly reproducible (Table 9). Hence, the precision and accuracy of the method were found to be excellent.

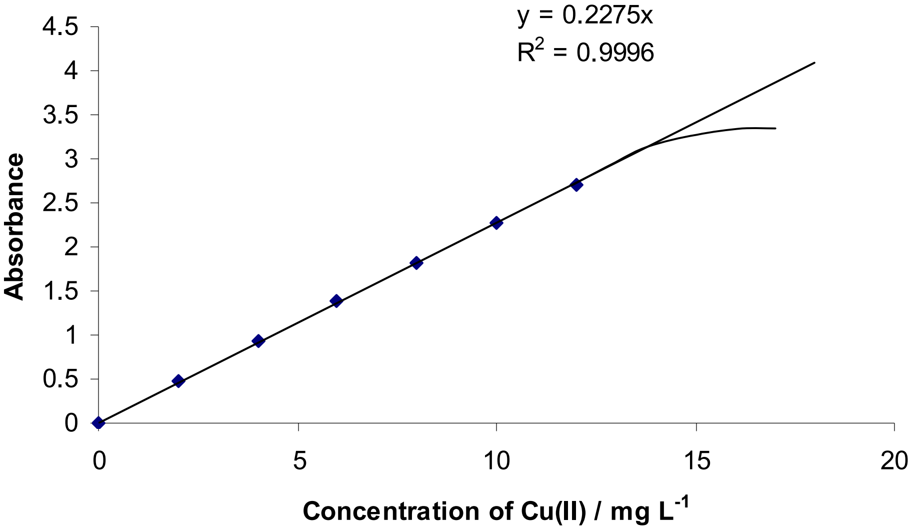


Fig 5. Calibration graph C: 1-12 mg L^{-1} of copper(II)

Table 2. Selected analytical parameters obtained with optimization experiments.

Parameters	Studied range	Selective value
Wavelength / $\lambda_{max\ (nm)}$	200-800	427
Solvent / (%)	0-70	30-70 (preferably 50)
Acidity H_2SO_4 / M	0.0001-1.0	0.0001-0.00035 (preferably 0.0001)
pH	2.66-0.076	2.58-1.21(preferably 2.27)
Time / h	0-24	1 min.-24 h (preferably 5 min)
Temperature / °C	10-70°	20-40°(preferably 25±5)
Reagent(fold molar excess, M:R)	1:10-1:1000	1:100-1:1000(preferably 1:200)
Molar absorption Coefficient / L mol^{-1} cm^{-1}	2.55×10^4– 6.15×10^4	4.35×10^4
Linear range / mg L^{-1}	0.001-100	0.01-12
Detection limit / µg L^{-1}	0.1-10	1.0
Reproducibility(%RSD)	0.00-10	0-2
Regression co-efficient	0.9985-0.9999	0.9998

3.7. 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 [32] was an absorbance value varying by more than 5% from the expected value for copper alone. As can be seen in the Table 3, a large number of ions have no significant effect on the determination of copper. The some interferences were from Al(III), Mo(VI), Fe(III), Ni(II) and V(V) ions. Interferences from these ions are preferably due to complex formation with HNABH. The greater tolerance limits for these ions can be achieved by using several masking methods. In order to eliminate the interference of Al(III), Mo(VI), Fe(III), Ni(II) and V(V) ions tartrate, thiocyanate, dimethylglyoxime and oxalate can be used as masking agents, respectively. A 10-fold excess of Ni(II) could be masked with dimethylglyoxime and a 10-fold Fe(III) could be masked with thiocyanate, a 20 fold Al(III) could be masked with tartrate, V(V) could be masked with oxalate, and Mo(VI) could be masked with tartrate. As stated above the proper masking agents may be added by while aiming at different interfering ions according to the actual composition of the sample. For this reason, the reliability of the proposed method is greatly improved and the practically is increased particularly the copper amounts in complex sample may be determined by using the proposed method. 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.

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)
Oxalate	1000	Arsenic(III + V)	100
Tartrate	1000	Tin(II)	50
Chloride	1000	Manganese(II)	100
Fluoride	100	Cerium(II)	100
Bromide	1000	Cesium(II)	100
Acetate	100	Barium	20
Thiocyanate	100	Molybdenum(VI)	10[b]
Citrate	1000	Nickel	10[c]
Phosphate	100	Vanadium(V)	10[d]
Iodide	1000	Iron(III)	10[e]
Ascorbic acid	100	Zinc	100
Nitrate	100	Strontium	100
Aluminum	20[b]	Mercury(II)	100
Azide	100	Cadmium	100
Ammonium	100	Chromium(III +VI)	100
Calcium	100	Silver	100
Sodium	100	Magnesium	100
Potassium	100	Selenium(VI)	100
Sulfate	100	Thallium (I)	100
Cobalt(II+III)	100	Iron(II)	100
Selenium (IV)	100	Pb(II)	50[b]

[a]Tolerance limit defined as ratio that causes less than 5 percent interference.
[b]with 10 mg L^{-1}, tartrate,
[c]with 50 mg L^{-1} DMG
[d]with 10 mg L^{-1} oxalate,
[e]with 100 mgL^{-1} thiocyanate.

3.8. Composition of the Absorbent Complex

Job's method [33] of continuous variation and the molar-ratio [34] method were applied to ascertain the stoichiometric composition of the complex. A Cu: HNABH (1:1) complex was indicated by both methods.

3.9. 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 of 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. 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 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.

3.10. 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 some 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
B	As in A+ Ca^{2+}(25)+Mg^{2+}(25)	0.5	0.50	100±0
		1.0	1.02	102±1
C	As in B+ Mn^{2+}(25) +Zn^{2+} (25)+ tartrate	0.5	0.49	98±1
		1.0	0.99	98±1
D	As in C+As^{3+}(25)+ Se^{6+}(25)	0.5	0.52	104±1
		1.0	1.04	104±1
E	As in D+ Hg^{2+}(25)+Cr^{3+}(25)	0.5	0.53	106±1
		1.0	1.05	105±1
F	As in E +Cd^{2+}(25)+Ce^{3+}(25)	0.5	0.54	108±2
		1.0	1.08	108±1

[a] Average of five analysis of each sample

[b] The measure of precision is the standard deviation (s).

3.11. Determination of Copper In Some Alloys, Steels and Brass

A 0.1 g amount of an alloy or steel or brass sample containing 0.18 - 70.61% of copper was accurately weighed and placed in a 50-mL Erlenmeyer flask. To it, 10-mL 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 5-mL 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)^{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 1-2-mL of 0.01% (w/v) tartrate solution. The resulting solution filtered, if necessary, through Whatman no. 40 filter paper into a 25-mL calibrated flask. The residue (silica and tungstenic acid) was washed with a small volume (5-mL) 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 (1-2-mL) of the above solution was taken into a 10-mL 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.

Table 5. Determination of copper in some certified reference materials

Sample	Certified Reference Material (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	60.6±0.3	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±0.5	1.2
3	Brass, Class-1: Pb, 0.00. Fe, 0.01. Cu, 70.61.	70.61	70.35±1	1.8
4	YSBC20a-95: Mn, 0.81, Cr, 16.30, Mo, 0.52, V, 0.24, Co, 1.45	1.35	1.32±0.3	1.5
5	GSBH-40101-96: C,1.5, Mn, 0.15 Cr, 11.63, Ni, 0.1, Mo, 0.99, V, 0.41, Co, 0.02	0.18	0.175±0.02	2.0

[a] Average of the five replicate determinations

[b] The measure of precision is the relative standard deviation (RSD).

3.12. Determination of Copper in Some Environmental Water Samples

Each filtered (with Whatman No. 40) environmental water sample (1000-mL) was evaporated nearly to dryness with a mixture of 2-mL of concentrated H_2SO_4 and 5-mL of concentrated HNO_3 to sulfur trioxide fumes in a fume cupboard following a method recommended by Greenberg et al [35]. After cooling additions of 5-mL 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 1-2-mL of a 0.01% (w/v) tartrate solution. The resulting solution was then filtered and quantitatively transferred into a 25-mL calibrated flask and made up to the mark with deionized water.

An aliquot (1-2-mL) of this preconcentrated water sample was pipetted into a 10-mL 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 [36]. The concentration of copper in natural and sea water is a few µg L^{-1} in developed countries [35]. The mean concentration of copper in natural water found in U.S. drinking water is greater than 20 µg L^{-1}[35].

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	35		
		100	135	100±0	0.00
		500	540	101±1	0.32
Well water		0	32		
		100	133	101±1	0.25
		500	535	101±1	0.21
Rain Water		0	9.5		
		100	110	100.4±1	0.21
		500	512	101±1	0.26
River water	Karnafuly (upper)	0	55		
		100	156	101±0.4	0.24
		500	560	101±1	0.29
	Halda (upper)	0	61	-	-
		100	160	99.4±0.3	0.15
		500	565	101±1	0.18
Sea water	Bay of Bengal (upper)	0	40		
		100	142	101.4±1	0.26
		500	545	101±1	0.35
	Bay of Bengal (lower)	0	45	-	-
		100	145	100±0	0.00
		500	545	101±1	0.45
Lake water	Kaptai	0	80		
		100	175	97±1	0.29
		500	585	101±1	0.37
Drain water	Cable Factory[c]	0	135		
		100	238	101.3±1	0.29
		500	640	101±1	0.25
	Paint Industry[d]	0	155	-	-
		100	260	102±2	0.56
		500	650	99±2	0.49

[a] Average of five replicate determinations.

[b] The measure precision is the relative standard deviation (s_r)

[c] Estern Cable Factory, Chittagong.

[d] Elite Paint Industry,Chittagong

3.13. Determination of copper in some biological samples

Regarding human blood (2-5-mL) and urine (20-50-mL), transferred into a 25-mL beaker. The sample was then ashed in a Muffle furnace at 500^0C for a 4 h in the presence of 1-mL concentrated nitric acid following a method recommended by Stahr [37]. Then at the following content of each beaker were cooled at room temperature, 1.5-mL 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 1-2-mL of 0.01% (w/v) tartrate solution, transferred quantitatively into a 10-mL calibrated flask and made up to the mark with deionized water.

A suitable aliquot (1-2-mL) 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 too many symptoms: depression, spiciness, paranoia, alternating moods, anxiety, panic, fearfulness, schizophrenia, phobias, etc [38]. 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 [39].

Table 7. Concentration of copper in blood and urine samples

Serial No	Sample	Copper / µg L^{-1} AAS	Copper / µg L^{-1} Proposed method[b]	Sample source[a]
1.	Blood	515±1	521±1	Wilson's diseases patient
	Urine	248±1	260.0±1	(Male)
2.	Blood	165±1	170.0±1	Hypertension (Male)
	Urine	84±2	88±2	
3.	Blood	260±1	266±1	Lung cancer
	Urine	133±2	135±2	(Female)
3.	Blood	123±1	135±2	Normal adult (Male)
	Urine	65±2	66±1	

[a]Samples were from Chittagong Medical College Hospital.
[b]Average of five replicate determinations ± s

3.14. Determination of copper in some soil samples

An air-dried homogenized soil sample (100 g) was accurately weighed and placed in a 100-mL micro-Kjeldahl flask. The sample was digested in the presence of an oxidizing agent following a method recommended by Jackson [40]. The content of flask was filtrated through Whatman No. 40 filter paper into a 25-mL calibrated flask, and neutralized with dilute ammonia in the presence of 1-2-mL of a 0.01% (w/v) tartrate solution. It was then diluted up to the mark with deionized water.

A suitable aliquot (1-2-mL) was transferred into a 10-mL 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.

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

Suitable aliquots (1-2-mL) of copper(I+II) mixtures (preferably 1:1, 1:3 and 1:5) were taken in 25-mL conical flasks. A few drops of 1 mol L^{-1} sulfuric acid and 1-3-mL of 1% (w/v) potassium permanganate solution were added to oxidize the monovalent copper, 5-mL 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 2-3-mL 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 10-

mL volumetric flask; 1-mL of 3.9×10^{-3} mol L^{-1} HNABH reagent solution was then added, followed by the addition of 1-mL of 0.001 mol L^{-1} sulfuric acid and 5mL DMF was made up to the mark with deionized water. The absorbance was measured after 1 min. at 427 nm against the reagent blank. The total copper content was calculated with the help of a calibration graph.

Table 8. Determination of copper in some surface soil samples

Serial No.	Copper / µg g^{-1} (n = 5)[a]	Sample source
S$_1$[b]	26±2[c]	Agriculture soil (Chittagong University Campus)
S$_2$	46±2	Esturine soil (Karnafuly River)
S$_3$	20±1	Marine soil (Chittagong Sea Beach)
S$_4$	115±2	Traffic soil (Kadamtali Bus Station)
S$_5$	75±2	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.

An equal aliquot of the above mentioned copper (I+II) mixture was taken into a 25-mL beaker. One mL 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 10-mL volumetric flask. Then, 1-mL of a 0.001 mol L^{-1} sulfuric acid solution and 5 mL DMF was added followed by the addition of 1-mL of 3.9×10^{-3} mol L^{-1} HNABH 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 mL^{-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 [41]. The results of a set of determination are given in Table 9.

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	0.99	0.98	0.01	0.02
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	1:3	1.00	3.00	0.99	2.98	0.01	0.02
2	1:3	1.00	3.00	0.98	2.99	0.02	0.01
3	1:3	1.00	3.00	0.99	2.98	0.01	0.02
		Mean error: Cu(II) = ±0.013; Cu(I) = ± 0.016 Standard deviation:Cu(II)= ±0.0058;Cu(I) = ±0.0058					
1	1:5	1.00	5.00	0.98	4.98	0.02	0.02
2	1:5	1.00	5.00	0.99	4.99	0.01	0.01
3	1:5	1.00	5.00	0.98	4.98	0.02	0.02
		Mean error: Cu(II) = ±0.016; Cu(I) = ± 0.016 Standard deviation:Cu(II) = ±0.0058; Cu(I) = ±0.006					

4. Conclusions

A new simple, sensitive, and inexpensive method with the copper (II)-HNABH complex was developed for the determination of copper in some real, biological, soil and environmental samples, for continuous monitoring to establish the trace levels of copper in different samples matrices. Compared with other methods in the literature (Table 1), the proposed method has several remarkable analytical characteristics:

1. the proposed method is highly with molar absorptivity of the complex of 4.35×10^4 L mol^{-1} cm^{-1}. Thus, amount of ng g^{-1} of copper can be determined without pre-concentration;
2. The proposed method is very simple, rapid, and stable. The reaction of copper (II) with HNABH is completed rapidly in 1 min at room temperature so it does not involved any stringent reaction conditions and offer the advantages of high complex stability (24h).
3. The method has added the advantage of determining individual amounts of Cu(II) and Cu(I) With suitable masking, the reaction can be made highly selective.

The proposed method using HNABH in presence of aqueous solutions not only is one of the most sensitive methods for the determination of copper but also is excellent in terms of selectivity and simplicity. Therefore, this method will be successfully applied to the monitoring of trace amounts of copper in real, environmental, biological and soil samples.

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