Accepted Manuscript

An efficient, cost effective, sensing behaviour liquid-liquid extraction and spectrophotometric determination of copper(II) incorporated with 4-(4'chlorobenzylideneimino)-3-methyl-5-mercapto-1, 2, 4-triazole: Analysis of food samples, leafy vegetables, fertilizers and environmental samples



Umesh B. Barache, Abdul B. Shaikh, Tukaram N. Lokhande, Ganesh S. Kamble, Mansing A. Anuse, Shashikant H. Gaikwad

| S1386-1425(17)30683-2 |
|---|
| doi: 10.1016/j.saa.2017.08.054 |
| SAA 15411 |
| Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy |
| 30 March 2017 |
| 15 August 2017 |
| 19 August 2017 |
| |

Please cite this article as: Umesh B. Barache, Abdul B. Shaikh, Tukaram N. Lokhande, Ganesh S. Kamble, Mansing A. Anuse, Shashikant H. Gaikwad, An efficient, cost effective, sensing behaviour liquid-liquid extraction and spectrophotometric determination of copper(II) incorporated with 4-(4'-chlorobenzylideneimino)-3-methyl-5-mercapto-1, 2, 4-triazole: Analysis of food samples, leafy vegetables, fertilizers and environmental samples, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* (2017), doi: 10.1016/j.saa.2017.08.054

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

An efficient, cost effective, sensing behaviour liquid-liquid extraction and spectrophotometric determination of copper(II) incorporated with 4-(4'- chlorobenzylideneimino)-3-methyl-5-mercapto-1, 2, 4-triazole: Analysis of food samples, leafy vegetables, fertilizers and environmental samples

Umesh B. Barache^a, Abdul B. Shaikh^a, Tukaram N. Lokhande^a, Ganesh S. Kamble^{b,c}, Mansing A. Anuse^b, Shashikant H. Gaikwad^{a*}

^aChemistry Research Laboratory, Department of Chemistry, Shri Shivaji Mahavidyalaya, Barshi, 413411, Maharashtra, India. ^bAnalytical Chemistry Research Laboratory, Department of Chemistry, Shivaji University, Kolhapur, 416004, Maharashtra, India. ^cKolhapur Institute of Technology's, College of Engineering, Kolhapur, 416234, Maharashtra, India.

Abstract

The aim of the present work is to develop an efficient, simple and selective moreover cost-effective method for the extractive spectrophotometric determination of copper(II) by using the Schiff base 4-(4'-chlorobenzylideneimino)-3-methyl-5-mercapto-1, 2, 4-triazole [CBIMMT]. This chromogenic reagent forms a yellow coloured complex with copper(II) in acetate buffer at pH 4.2. The copper(II) complex with ligand is instantly extracted into chloroform and shows a maximum absorbance at 414 nm which remains stable for more than 48 h. The composition of extracted complex is found to be 1:2 [copper(II) : reagent] which was ascertained using Job's method of continuous variation, mole ratio method and slope ratio method. Under optimal conditions, the copper(II) complex in chloroform adheres to Beer's law up to 17.5 μ g mL⁻¹ of copper(II). The optimum concentration range obtained from Ringbom's plot is from 5 μ g mL⁻¹ to 17.5 μ g mL⁻¹. The molar absorptivity, Sandell's

sensitivity and enrichment factor of the extracted copper(II) chelate are 0.33813 x 10⁴ L mol⁻¹ cm⁻¹, 0.01996 µg cm⁻² and 2.49 respectively. In the extraction of copper(II), several affecting factors including the solution pH, ligand concentration, equilibrium time, effect of foreign ions are optimized. The interfering effects of various cations and anions were also studied and use of masking agents enhances the selectivity of the method. The chromogenic sulphur containing reagent, 4-(4'-chlorobenzylideneimino)-3-methyl-5-mercapto-1, 2, 4-triazole has been synthesized in a single step with high purity and yield. The synthesized reagent has been successfully applied first time for determination of copper(II). The reagent forms stable chelate with copper(II) in buffer medium instantly and quantitatively extracted in chloroform within a minute. The method is successfully applied for the determination of copper(II) in various synthetic mixtures, complexes, fertilizers, environmental samples such as food samples, leafy vegetables, and water samples. The results are compared with those obtained with a reference procedure. Good agreement was attained. All the obtained results are indicative of a convenient, fast method for the extraction and quantification of micro levels of copper(II) from various environmental matrices without use of sophisticated instrumentation and procedure. The method showed a relative standard deviation of 0.42%.

Keywords: CBIMMT; Copper(II); Environmental samples; Food samples; Spectrophotometric determination.

Abbreviations:

CBIMMT: 4-(4'-Chlorobenzylideneimino)-3-methyl-5-mercapto-1, 2, 4-triazole

RSD: Relative standard deviation

UV-Vis.: Ultraviolet visible

AAS: Atomic absorption spectrophotometer

2

LOD: Limit of detection

LOQ: Limit of quantification.

*Corresponding author: rasayanshg@gmail.com, rasayanshg1@gmail.com (S. H. Gaikwad)

1. Introduction

Copper is one of the very essential element that plays a vital role in the biological systems. Copper occurs naturally in many vegetables, meat and grains. In biological systems, copper counter acts the toxicity of zinc suggesting Cu–Zn antagonism [1]. Copper and its salts are more toxic to some lower organism(s) than to human beings. It turns toxic at higher level due to its accumulation in cell membranes and prevents transport across the wall of the cell [2]. Hereditary disease such as Wilson's disease occurs due to excess amounts of copper in the normal human body. It causes fatal consequences like liver damage. Due to elevated levels of copper, violent tendencies are observed in males that may leads to depression and schizophrenia [3].

As a pollutant, copper is of mainly concern because of its high degree of toxicity. There are several toxic copper species which include $Cu(OH)^+$, $Cu_2(OH)_2^{2+}$ and $CuCO_3$. However, there is no doubt, that Cu^{2+} ion, which are present in various aqueous solutions, are considered to be poisonous due to dissolved copper species. Excess of copper in water is not only harmful to human beings but also to many aquatic organisms [4]. It acts as a main cofactor in many enzymes and plays a key role in protein and cell division [5]. It may cause generalized cancer in the laboratory animals and has been linked epidemiologically with certain human cancers [6]. Most serious diseases are cirrhosis and skin cancer which causes gradually weaken bone structure and cause the diminution of stature that ultimately leads to total collapse of the entire skeletal systems of humans. Higher toxicity of copper towards marine and fresh water species is also observed [7].

An increase in copper concentrations in the environment results from industrial and domestic waste discharge, disposal of mining washings, refineries into the water streams which consequently enter in several food materials. World Health Organization (WHO) guidelines recommend the permissible limit of copper in drinking water is 2.0 mg L^{-1} [8].

Recently a variety of analytical advanced methods requiring high-priced instrumentation are reported to be dealing with the determination of copper(II) from various matrices at trace level. These methods include: neutron activation analysis [9], X-ray fluorescence [10], pulse polarography [11], inductively coupled plasma emission spectrophotometry (ICP-ES) [12], graphite furnace atomic absorption spectrophotometry (GF-AAS) [13], flame atomic absorption spectrometry (FAAS) [14-16]. Although these analytical techniques are highly sensitive and selective, they are quite expensive for the developing countries and require well qualified laboratory personnel and superior laboratory equipment. Moreover, they might not be always available in all routine analytical laboratories. Many of the reported methods have some short comings such as the availability of sophisticated instruments, time-consuming operation, tedious work, multi-step procedures for pre-concentration of the analyte and low selectivity with higher interference problems. The reported methods [9-12] have demerits with respect to their high cost and the instruments used in day-to-day analysis. GF-AAS [13] often lacks its sensitivity due to the sublimation at high temperature. The other reported sophisticated and time consuming method is catalytic kinetic effect of copper on the oxidation of cysteine by hexacyanoferrate(III) [17]. In contrast, spectrophotometry is well-known by its quick analysis ability, low cost and simplicity in its operation. Due to these advantages, a number of spectrophotometric methods have been developed to determine trace amount of copper(II).

Recently many advanced solvent extraction techniques has been developed for the determination of metal ions. Some potentially applied method includes solid phase extraction

(SPE) [14-16,18-25] and dispersive liquid-liquid microextraction (DLLME) [26,27], single drop microextraction (SDME) [28], solidified floating organic drop microextraction (SFODME) [29], hollow fiber liquid-phase microextraction (HF-LPME) [30], cloud point extraction (CPE) [31] and classical liquid-liquid extraction (LLE) methods. But these methods also have their disadvantages. In SPE and DLLME microextraction methods, the determination methods are more complex, time consuming and involve multiple steps for pre-concentration of analyte which leads to occurrence of errors in the determination. SFODME has the restricted choice of extracting solvents because only a small number of organic solvents have melting points nearer to room temperature. Another problem with this technique is the overlapping of solvent peaks with those of the analytes in the chromatograms. SDME is limited by rate of stirring which causes dislodgment of drop. HF-LPME technique requires pre-conditioning of the membrane and problem with memory effects when membranes are reused. In CPE method, the extracted analyte cannot be injected directly as the surfactants (rich phase) has more viscosity. Due to these disadvantages, the better approach is to use the classical liquid-liquid extraction (LLE) methods which are simple, quick, selective, economical and sensitive as well.

It is well-known that sulphur containing ligands and some biomolecules [32,33] form strong complexes with copper(II), as copper(II) belongs to the class of soft acids, whereas ligands containing donating sulphur atom belongs to soft bases. Soft acids always prefer to bind the soft bases [34]. The thiol and phenyl thiosemicarbazones are important sulphur and nitrogen that contain chromogenic reagents where copper(II) forms stable complexes with them and can be extracted into suitable organic solvents. The literature survey revealed that only a few thiol and phenyl thiosemicarbazones were employed for extractive determination of copper(II) [35-38]. A comparison of a few selected spectrophotometric determination methods of copper(II), their spectral characteristics and drawbacks are summarized in Table 1

[1,3,39-47], but these reported reagents to have disadvantages, such as maximum absorbance in UV region [3], some require the use of surfactants for full colour development [39,43], synergic extraction [42], heating of aqueous phase [43], longer shaking time [45] and they are less sensitive [46]. In the present paper, we have introduced a novel chromogenic reagent, 4-(4'-chlorobenzylideneimino)-3-methyl-5-mercapto-1,2,4-triazole [CBIMMT] and have developed a new, simple, rapid, selective and reproducible extractive spectrophotometric method for the determination of copper(II) in synthetic mixtures, copper(II) complexes, water samples, vegetable samples, food samples, fertilizer samples and separation from binary mixtures. The chromogenic reagent CBIMMT in chloroform instantaneously forms vellow coloured complex with copper(II). An extracted Cu(II)-CBIMMT complex in chloroform shows the maximum absorbance in visible region. The present method does not require the heating of aqueous phase, the use of surfactant and any synergent for complete complexation and no use of any sophisticated instruments.

2. Experimental

2.1 Apparatus

All absorbance measurements are taken with Elico digital UV-VIS spectrophotometer (model-SL-159) by using 1cm quartz cells. All the pH measurements are made with Equiptronics digital pH meter (model EQ-615). Calibrated glass wares are used throughout the experimental procedure. Glass wares are cleaned by soaking them in acidified solutions of potassium dichromate which was followed by washing them with soap water and rinsing two times with distilled water.

2.2 Standard copper(II) solution, buffer solution and solution of various cations and anions

Standard stock solution of copper(II) with the concentration of 1.0 mg mL⁻¹ is prepared by dissolving a 3.928 g portion of copper sulphate pentahydrate (CuSO₄.5H₂O, s.d. fine chemicals) in water. The stock solution is then standardized by well-known established

method [48]. The working solution of copper(II) is prepared by suitable dilution of a standard stock solution with doubly distilled water. The acetate buffer of pH 4.2 is prepared by mixing appropriate volume of 0.2 mol L⁻¹ acetic acid and 0.2 mol L⁻¹ sodium acetate solutions [49] and pH of this buffer solution is confirmed by using a pH meter. All chemicals and various organic solvents used are of analytical reagent grade and are used without any further purification. The solutions of various cations and anions are prepared by dissolving calculated quantities of corresponding salts in either water or dilute hydrochloric acid. Various synthetic mixtures containing copper(II) are prepared by combination with different metal ions in fixed composition. Doubly distilled water was used throughout all the experiments.

2.3 Synthesis and characterization of 4-(4'-chlorobenzylideneimino)-3-methyl-5-mercapto-1, 2,4-triazole [CBIMMT]

The chromogenic reagent, CBIMMT is synthesized in the laboratory by simple condensation reaction of 3-methyl-5-mercapto-1,2,4-triazole (2.6 g, 0.02mol L^{-1}) with 4-chlorobenzaldehyde (2.8 g, 0.02mol L^{-1})[50]. A reaction mixture of 3-methyl-5-mercapto-1, 2, 4-triazole and 4-chlorobenzaldehyde in ethyl alcohol, containing about 3 drops of glacial acetic acid is refluxed for about 4 h (scheme 1).



Scheme – 1 Synthesis of CBIMMT.

The product obtained after reaction is filtered and recrystallized from hot ethyl alcohol. On cooling, faint yellowish needles of CBIMMT are obtained. The purity of the

CBIMMT is checked by melting point and by thin layer chromatographic method. The structure is confirmed by IR and NMR spectra. A reagent solution which has concentration of 0.025 mol L^{-1} is prepared by dissolving 0.631 g of CBIMMT in chloroform and is diluted up to 100 mL in a calibrated volumetric flask. The fresh reagent solution is used it is whenever required.

2.4 Environmental and synthetic samples preparations

2.4.1 Determination of copper(II) in various binary and ternary synthetic mixtures

The binary and ternary synthetic mixtures containing $10 \ \mu g \ mL^{-1}$ of copper(II) with known amounts of other associated metal ions at higher concentrations are prepared in laboratory, and the recovery experiments are performed. The interfering metal ions are masked with suitable masking agents and the copper(II) is extracted by using recommended procedure.

2.4.2 Water samples

Various water samples from different locations such as municipal potable water, borewell water, lake water (Ganesh lake, Barshi, Solapur district, M S, India) and dam water (Ujani dam, Solapur district, MS, India) are collected in clean and dry polythene bottles. All water samples are filtered through Whatman filter paper No.40. Then 1000 mL of each filtered water sample is taken and evaporated nearly it dries on hot plate with a mixture of 2 mL of concentrated H₂SO₄ and 5 mL of concentrated HNO₃. After dryness, it is cooled and further 5 mL of concentrated HNO₃ is added and heated up to dense white fumes continue or until the solution becomes much colourless. The resultant solution is cooled and NH₄OH is added for neutralization of acid in presence of 1 mL 0.01% tartarate solution. The solution was then filtered and transferred to 100 mL volumetric flask and diluted up to the mark with distilled water. The procedure is carried out to determine copper(II) content as per recommended procedure.

2.4.3 Food samples

The copper(II) content from various food samples are determined by the present method. Food samples are collected from the local market of Barshi city (Solapur district, Maharashtra) and crushed in a mortar. A known amount (5 g) of dried food sample is taken into a silica crucible. This is followed by strong heating to oxidize organic matter at about 80°C for 2 h. Then the sample is dissolved in 5 mL of aqua regia solution and evaporated on hot plate near to dryness. The residue is treated with concentrated HClO4 and subjected to moist dryness. The resultant residue is boiled with water, subjected to filtration by the use of ordinary filter paper and washed with hot distilled water. The filtrate is taken in 25 mL volumetric flask and diluted up to the mark with distilled water. From this solution an aliquot of 5 mL is taken and the content of copper(II) is determined by the present method and further it is confirmed by atomic absorption spectrophotometer.

2.4.4 Leafy vegetables samples

The proposed method is also used for the determination of copper(II) from some green leafy vegetable samples collected from nearby vegetable market of Barshi city (Solapur district, Maharashtra). 25 g of green leafy vegetable is taken, cleaned and dried in an oven for protection from contamination of minerals. After drying the samples are crushed in a mortar. A calculated weight (5 g) of ash of each sample is taken in a dry silica crucible and is subjected to strong heating for oxidation of organic matter at about 80°C for 2 h. To the resultant ash, 15 mL of 1 mol L⁻¹ hydrochloric acid is added and heated up to dryness on a hot plate. Then the contents are boiled with water and filtered through ordinary filter paper and washed with hot water. The filtrate is collected in 25 mL volumetric flask and diluted with distilled water up to the mark. 5 mL aliquot from this solution is taken and the content of copper(II) is determined by the recommended procedure and are further confirmed by atomic absorption spectrophotometer.

2.4.5 Micronutrient fertilizer samples

Further the present method is also applied for the determination of copper(II) in various micronutrient fertilizer samples. The samples are collected from a fertilizer market. Known amount of sample (3 mL) is evaporated on hot plate near to moist dryness with 5 mL concentrated HNO₃ and 10 mL concentrated HCl mixture. This procedure is repeated two to three times and heated up to dense white fumes till the solution becomes clear. The resultant solution is cooled and filtered by Whatman filter paper No. 1. Then it is transferred to 100 mL volumetric flask and diluted up to the mark with distilled water. From this, aliquot of 1 mL is taken and determined by the recommended procedure. These results are compared with the results obtained by using atomic absorption spectrophotometer.

2.4.6 Copper(II) from complexes

The application of the present procedure are also studied to determine the copper(II) in various synthetic complex compounds. Copper(II) complexes with salicylaldoxime, 4-amino-5-mercapto-3-methyl-1,2,4-triazole [51,52], 4-(2'-nitrorobenzylideneimino)-3-methyl-5-mercapto-1,2,4-triazole [53] and ammonia are prepared in a laboratory and purified as per the reported procedure [54]. The calculated quantity of the complex is carefully decomposed with aqua regia and evaporated up to moist dryness. The residue is then dissolved in distilled water and made up to 100 mL with distilled water. The stock solution is then standardized gravimetrically by the salicylaldoximate method and volumetrically by the iodometric method [54]. Aliquots of this solution are used for the determination of copper(II) as per the recommended procedure.

2.4.7 Separation of copper(II) from binary mixtures

The separation of copper(II) from other commonly associated metal ions like Pd(II), Se(IV), Te(IV), Bi(III), Co(II), Au(III), Fe(III), Ag(I) and Ni(II) using 4-(4'-chlorobezylideneimino)-3- methyl-5- mercapro-1, 2, 4-triazole is achieved. Among these, all

the added metal ions remain quantitatively in the aqueous phase from which they are determined by spectrophotometry using reported standard methods [50, 55-62]. The metal ions such as Pd(II), Au(III), Ag(I) and Ni(II) are interfered in the determination method due to their co-extraction with copper(II). These metal ions are separated from copper(II) by masking them with suitable masking agent. These masked metal ions from the aqueous phase are de-masked with $HClO_4$ and determined spectrophotometrically by using the standard methods.

2.5 Recommended procedure

An aliquot of the sample solution containing 25-175 µg mL⁻¹ of copper(II) solution is diluted with acetate buffer having pH 4.2 solutions up to the mark in 25 mL calibrated volumetric flask. The solution is then transferred into 125 mL separatory funnel, followed by the addition of 10 mL 0.025 mol L⁻¹ CBIMMT in chloroform. The two phases are allowed to equilibrate for 1 min. The yellow-coloured organic extract is collected over anhydrous sodium sulphate to remove the traces of water if present. The organic extract is then transferred into 10 mL calibrated volumetric flask and further diluted up to the mark with the chloroform, if required. The absorbance of the extracted complex is measured at λ_{max} 414 nm against reagent blank prepared in the similar manner without copper(II). A calibration curve is obtained and the unknown amount of copper(II) is determined from the same.

2.6 Optimization of the experimental variables

To determine the optimum conditions for maximum extraction recovery of copper(II), several analytical parameters are assessed, using a 1 mL standard solution containing 100 μ g of copper(II) ions. A univariate optimization procedure is then undertaken; i.e. varying one parameter at a time while keeping the others constant.

3. Results and discussions

3.1 Absorption spectra and spectral characteristics of the complex

In this work, UV–VIS spectrophotometry is used to accomplish the determination. In order to carry out the quantification analysis, the maximum wavelength of absorption is to be found out. Copper(II) forms yellow coloured complex with 4-(4'-chlorobezylideneimino)-3-methyl-5-mercapro-1,2,4-triazole and extracted into chloroform at pH 4.2. The yellow coloured complex in organic phase shows maximum absorbance at λ_{max} 414 nm and is stable for more than 48 h. The absorbance of reagent at 414 nm wavelength is found to be too low (Fig. 1). For the effective extraction of copper(II), the optimum conditions are established by studying various parameters, such as effect of pH, a reagent concentration, the choice of organic solvent, an equilibration time. The spectral characteristics of Cu(II)-CBIMMT complex in chloroform are mentioned in the table 2.

3.2 Effect of pH

In the extraction of metal ions, the extraction efficiencies of metal complex are closely related to the pH of the system. Therefore, we have investigated the effect pH on the development of coloured complex of Cu(II)-CBIMMT by varying the pH of copper(II) solution ranging from 1 to 10 before an addition of the organic phase. The pH of copper(II) solution is adjusted by 0.1 mol L⁻¹ HCl and 0.1 mol L⁻¹ NaOH solutions. The effect of pH on the absorbance values of extracted Cu(II)-CBIMMT complex are measured at λ_{max} 414 nm against reagent blank prepared in similar manner without taking copper(II). The effect of pH on the formation of Cu(II)-CBIMMT complex is also investigated by using series of acetate buffer solutions. It is observed that the absorbance of Cu(II)-CBIMMT complex obtained with buffer solution is in accordance with that one obtained with pH, which is adjusted by HCl and NaOH solutions. Fig. 2 shows the maximum absorbance of Cu(II)-CBIMMT

complex is observed in the pH range of 3.8 to 4.4. Therefore, acetate buffer of pH 4.2 is selected conveniently for all the further studies.

3.3 Effect of organic solvents

Solvent plays a crucial intrinsic role in solvation of copper(II) species with ligand. For this purpose, the influence of various organic solvents are examined for the effective extraction of copper(II) with CBIMMT. Solutions of CBIMMT (0.025 mol L⁻¹) is prepared in various organic solvents and the effect of solvents on the absorbance value of Cu(II)-CBIMMT complex at 414 nm is examined. It is also observed that the absorbance values at λ_{max} 414 nm increased in the order ethyl acetate < MIBK < dichloromethane < isoamyl alcohol < 1, 2-dichloroethane < carbon tetrachloride < benzene < chloroform. Among all these organic solvents, chloroform is selected for all further experiments as it showed maximum absorbance at 414 nm as compared to other solvents (Fig. 3).

3.4 Effect of CBIMMT concentration

It is necessary to study the effect of reagent concentration, as the concentration of ligand has a direct effect on the complexation reaction and extraction as well. The effect of reagent concentration is investigated on the formation of complex species of copper(II) at pH 4.2 by measuring absorbance at 414 nm of solution containing 10 μ g mL⁻¹ of copper(II). The reagents in chloroform are added to the copper(II) solution maintained at pH 4.2 by using acetate buffer. It is observed that 0.008 mol L⁻¹ of CBIMMT in chloroform was sufficient for complete complexation of copper(II) (Fig. 4). Further it is observed that there is no adverse effect of excess of reagent. In the present investigation, to ensure the complete complexation of copper(II), we recommend 0.025 mol L⁻¹ of CBIMMT in chloroform for a further study.

3.5 Stability of Cu(II)-CBIMMT complex

The absorbance values of the Cu(II)-CBIMMT complex is measured at different time intervals at λ_{max} 414 nm to ascertain the stability of the complex at room temperature. It is

observed that the colour of the Cu(II)-CBIMMT complex remains almost constant for more than 48 h.

3.6 Effect of shaking time

Equilibration of organic phase with aqueous phase plays a very important role in extraction procedures. Minimum extraction time is often necessary for investigation of trace amounts of metal ions with high efficiency. Here, the results indicate that the optimum shaking time of 1 min is determined for Cu(II)-CBIMMT complex by varying the shaking time from 0.5 to 15 min for full colour development as is shown in Fig 5. It is found that, the colour formation was quite quick. Prolonged shaking has no any adverse effect on the absorbance of Cu(II)-CBIMMT complex. Hence, shaking time of 1 min is selected for equilibration in subsequent experiments. Complexation reaction of copper(II) with 0.025 mol L^{-1} of CBIMMT in chloroform is rapid and the colour developed is found to be stable for more than 48 h.

3.7 Analytical figures of merit

The performance of the present method was evaluated for the quantification of copper(II). Under optimal experimental conditions, the system follows Beer's law (Fig. 6) up to 17.5 µg mL⁻¹ of copper(II). Optimum concentration range of copper(II) is determined by Ringbom's plot. The curve is sigmoid with linear segment at intermediate corresponds to 5.0 to 17.5 µg mL⁻¹ concentration values as for copper(II). The limit of detection and quantification defined for copper(II) as LOD = $3.3 \times \sigma/k$, where k = slope of calibration plot and σ = standard deviation and LOQ = $10 \times \sigma/k$ are 0.6 and $1.8 \mu g mL^{-1}$, respectively. The relative standard deviation (% R.S.D.) for five replicates measurements of $10 \mu g mL^{-1}$ of copper(II) was 0.41%. The regression equation can be expressed as y = 0.050x - 0.007, with the correlation coefficient R²=0.999. The regression equation shows value of intercept is closer to the zero indicating good sensitivity. The molar absorptivity for the Cu(II)-CBIMMT

complex in chloroform as calculated from Beer's law over the range studied is 0.3381×10^4 L mol⁻¹cm⁻¹ and the Sandell's sensitivity is 0.01996 µg cm⁻². The enrichment factor of the present method is 2.49. These results indicate that the developed method has good level of precision and hence is found suitable for the analysis of the copper(II) from various samples. The precision and accuracy data of the method is shown in the Table 2. The values of molar absorptivity and Sandell's sensitivity indicate that the method is sensitive [63].

3.8 Stoichiometry of the Complex

The extraction of copper(II) by chelating extractant has been investigated at pH 4.2. In order to apply liquid-liquid extraction system for the separation of copper(II) from environmental samples and real samples, it is important to investigate the composition of complex. Job's method of continuous variation, the mole ratio method and log-log method are applied to ascertain the stoichiometry composition of the Cu(II)-CBIMMT complex.

3.8.1 Job's continuous variation method

Equimolar (0.001573 mol L⁻¹, 0.001179 mol L⁻¹) solutions of copper(II) and reagent, CBIMMT is prepared. Copper(II) solution is taken and added to this acetate buffer having pH 4.2 in a total volume of 25 mL in volumetric flask and transferred it into 125 mL separatory funnel. The reagent, CBIMMT was mixed in complementary proportions, containing varying amount of copper(II) and is shaken for 1 min in a separatory funnel and then it is extracted. The absorbance of an organic extract is measured at 414 nm against the reagent blank prepared in similar way without taking copper(II). The plot of absorbance versus mole fraction (M/M+L) (Fig. 8) indicates that the stoichiometry of complex is 1:2 (copper(II) : CBIMMT).

3.8.2 Mole ratio method

Different aliquots of copper(II) solution with the concentration 0.001573 mol L⁻¹ having pH 4.2 with acetate buffer is taken. These are equilibrated with 5 mL CBIMMT

having equimolar concentration i.e. $0.001573 \text{ mol } L^{-1}$. To each of these solutions, the required volume (5 mL) of chloroform is added so as to keep the final volume of organic phase to 10 mL. The absorbance of the organic phase obtained is recorded at 414 nm. The same procedure was followed for $0.001179 \text{ mol } L^{-1}$ for both copper(II) and CBIMMT. The graph of absorbance versus the mole ratio, M/L shows the stoichiometry of complex is 1:2 (copper(II) : CBIMMT) (Fig. 9).

3.8.3 Log-log plot method

In this method, amount of copper(II) is kept constant and acetate buffer of pH 5.6 is added to made the volume 25 mL. The 10 mL of reagent having various concentrations (0.0001mol L⁻¹ to 0.025 mol L⁻¹) is added to it and equilibrated for 1 min as per recommended procedure mentioned above. The absorbance of yellow coloured organic phases is recorded at 414 nm against reagent blank. The composition of extracted Cu(II)-CBIMMT complex is confirmed by plotting graph of Log $D_{[Cu(II)]}$ against Log $C_{[CBIMMT]}$ (Fig.10). The plot is linear with the slope 1.6 at pH 5.6, confirming the composition of extracted Cu(II)-CBIMMT complex is 1:2.

On the basis of the results obtained, it can be concluded that, the extracted coloured complex is formed by combining the two ions of reagent and one ion of copper(II), resulted into formation of 1:2 neutral species. Based on this investigation, the plausible mechanism for the formation of Cu(II) complex is given in scheme 2.



Scheme - 2 Mechanism for formation of the Cu(II)-CBIMMT complex.

4. Analytical applications:

Effect of diverse ions

In order to check the selectivity of the method, excess of potentially interfering ions relevant to industrial effluent and present in the environment have been studied by measuring absorbance of the Cu(II)-CBIMMT complex in chloroform. The effect of foreign ions on the determination of copper(II) is investigated by adding the known amount of these diverse ion solutions to a standard copper(II) solution and by comparing the final absorbance with the standard. The results presented in the Table 3. The tolerance limit of the ions does not cause deviation of more that $\pm 2\%$ in absorbance. The interference of various cations is removed by using suitable masking agents and thus the method becomes more selective.

In order to survey the credibility of the suggested method, it is successfully applied to the determination of copper(II) in a series of synthetic mixtures of various compositions and also in a number of environmental samples such as food samples, leafy vegetables and water samples. The method is also applied to the determination of copper(II) from the real samples such as complexes and the fertilizers. In the synthetic mixtures analysis, the recovery of copper(II) is found to be quantitative and the %R.S.D. of the analysis is satisfactory (Table 4). In view of the unknown composition of environmental water samples, the same equivalent portions of each such sample are analyzed for copper(II) content; the recoveries in both the spiked (standard addition calibration used for quantization) and the unspiked samples are in excellent agreement (Table 5). Data reports that the municipal water, bore well water and the lake water, the amount of copper (II) lies well within the limit as recommended by WHO [8]. In dam water, the amount of copper is a little more, it may be due to the industrial waste from the nearby industrial area around the dam. The food samples and the leafy vegetables also contain copper(II) (Tables 6 and 7). In case of fertilizer samples analysis, the amount of

copper(II) found is in excellent agreement with that of given by the manufacturer (Table 8). Furthermore to check the reliability of the present method, these samples are analyzed by AAS method for the copper(II) content. Then the method is applied for the analysis of the copper(II) from its complexes, the % of cooper(II) is in good agreement with that of the reported methods (Table 9). And finally, the suitability of the method is examined by applying it to the separation and the determination of the copper(II) from various binary mixtures. It is found that the recovery of copper(II) and of added metal ions is quantitative as shown in the Table 10.

5. Conclusion

This paper proposes a very effective method for rapid micro level single step extraction and spectrophotometric quantification of copper(II) by using CBIMMT as a metallochromic ligand and has been proved to be a selective spectrophotometric reagent. As compared to the other methods, this method is simple, reproducible and it requires less time for separation and determination of copper(II). Furthermore, we claim that the proposed method is quick and of low-cost if compared with other spectrophotometric methods. Due to the following important features, the developed method can be used for micro level quantification of copper(II) from the real samples.

- i) The recommended procedure requires single step extraction. The yellow coloured complex remains stable for more than 48 h.
- ii) Complexation reaction takes place at room temperature.
- iii) Complexing chromogenic reagent, CBIMMT can be synthesized easily with high yield and purity.
- iv) The method has good tolerance towards many potentially interfering ions.
- v) The present method is reliable and the results obtained show that it is in good agreement with reported methods.

Acknowledgement

Authors are highly thankful to Principal Dr. P. R. Thorat for providing necessary facilities. Authors are also thankful to Dr. V. M. Gurame for valuable suggestions during this work and Mr. K. K. Sathe for technical and grammatical support.

> School of the second se

References

- R.A. Nalawade, A.M. Nalawade, G.S. Kamble, M.A. Anuse, Rapid, synergistic extractive spectrophotometric determination of copper(II) by using sensitive chromogenic reagent N", N"-bis[(E)-5 (4-fluorophenyl) methylidene] thiocarbonohydrazide, Spectrochim. Acta A 146 (2015) 297-306.
 Doi: 10.1016/j.saa.2015.02.022
- M.D. Frahani, F. Shemirani, N.F. Ramandi, M. Gharehbaghi, Ionic liquid as a ferrofluid carrier for dispersive solid phase extraction of copper from food samples, Food Anal. Methods 8 (2015) 1979-1989.

Doi: 10.1007/s12161-014-0082-6

[3] S. Prasad, T. Halafihi, Development and validation of catalytic kinetic spectrophotometric method for determination of Cu(II), Microchim. Acta 142 (2003) 237-244.

Doi: 10.1007/s00604-003-0023-3

- [4] J. Karthikeyan, P.P. Naik, A.N. Shetty, A rapid extractive spectrophotometric determination of copper(II) in environmental samples, alloys, complexes and pharmaceutical samples using 4-[N,N(dimethyl)amino]benzaldehyde thiosemicarbazones, Environ. Monit. Assess. 176 (2011) 419-426.
 Doi: 10.1007/s10661-010-1593-2
- [5] F. Shamsa, M.B. Tehrani, H. Mehravar, E. Mohammadi, Spectrophotometric
 Determination of Cu²⁺ and Monitoring of Hg²⁺ and Ni²⁺ in some iranian vegetables
 Using 6-(2Naphthyl)-2, 3-Dihydro-as-triazine-3-thione, Iranian J. Pharma. Res. 12 (2013) 9-13.

Doi : PMC3813219

- [6] J.E. Fergusson, The toxicity of heavy element to human beings in : "The Heavy Elements: Chemistry, Environmental Impact and Health Effects", Pergamon Press, Oxford, 1989, p.548.
- [7] B. Venugopal, T.D. Luckey, "Metal Toxicity in Mammals-2", Plemum Press, New York, 1979, p.76.
- [8] Guidelines for drinking water quality 3rd ed. Incorporating the first and second addenda, World Health Organization, Geneva, 2008 Vol. 1, 335-336
- [9] A.M. Amlani, Z.R. Turel, Substoichiometric determination of copper by neutron activation analysis, J. Radioanal. and nuclear chem., 144 (1999) 27-33.
 Doi: 10.1007/BF02164896
- [10] K.E. Daugherty, R.J. Robinson, J.I. Mueller, X-ray fluorescence spectrometric determination of the copper(II) and mercury(II) complexes of 6-chloro, 2-methoxy-9thiolacridine, Anal. Chem., 36 (1964) 1098-1100.

Doi: 10.1021/ac60212a043

- [11] M.H. Pournaghi Azhar, H. Dastangoo, Extraction and polarography of copper (II)bis(acetylacetone) ethylenediamine in dichloromethane: use of differential pulse method for the determination of copper in steel, Microchem. J. 64 (2000) 187-194.
 Doi : 10.1016/S0026-265X(00)00012-6
- Y. Liu, P. Liang, L. Guo, Nanometer titanium dioxide immobilized on silica gel as sorbent for preconcentration of metal ions prior to their determination by inductively coupled plasma atomic emission spectrometry, Talanta 68 (2005) 25-30.
 Doi : 10.1016/j.talanta.2005.04.035
- [13] W.S. Zhong, T. Ren, L.J. Zhao, Determination of lead, cadmium, chromium, copper and nickel in chinese tea with high resolution continuum source graphite furnace atomic absorption spectrometry, J. Food and Drug Anal., 24 (2016) 46-55.

Doi: 10.1016/j.jfda.2015.04.010

- [14] M. Behbahani, Y. Bide, M. Salarian, M. Niknezhad, S. Bagheri, A. Bagheri, M.R. Nabid, The use of tetragonal star-like polyaniline nanostructures for efficient solid phase extraction and trace detection of Pb(II) and Cu(II) in agricultural products, sea foods and water samples, Food Chem. 158 (2014) 14-19.
- [15] H. Ebrahimzadeh, M. Behbahani, Y. Yamini, L. Adlnasab, A.A. Asgharinezhad, Optimization of Cu(II)-ion imprinted nanoparticles for trace monitoring of copper in water and fish samples using a Box-Behnken design, Reactive Functional Polymers 73 (2013) 23-29.

Doi: 10.1016/j.reactfunctpolym.2012.10.006

- M. Behbahani, M. Salarian, M.M. Amini, O. Sadeghi, A. Bagheri, S. Bagheri, Application of a new functionalized nanoporous silica for simultaneous trace separation and determination of Cd(II), Cu(II), Ni(II) and Pb(II) in food and agricultural products, Food Anal. Methods 6 (2013) 1320-1329.
 Doi: 10.1007/s12161-012-9545-9
- [17] S. Prasad, Kinetic method for determination of nanogram amounts of copper(II) by its catalytic effect on hexacynoferrate(III)-citric acid indicator reaction, Anal. Chim. Acta 540 (2005) 173-180.
 Doi : 10.1016/j.aca.2005.03.011
- [18] S. Mane, S. Ponrathnam, N. Chavan, Selective solid-phase extraction of metal for water decontamination, J. Appl. Polymer. Sci. 42849 (2016) 1-11.
 Doi : 10.1002/app.42849
- [19] S. Tokahoglu, V. Yilmaz, S. Kartal, Solid phase extraction of Cu(II), Ni(II), Pb(II),
 Cd(II) and Mn(II) ions with 1-(2-thiazolylazo)-2-napthol loaded amberlite XAD 1180, Environ. Monit. Assess 152 (2009) 369-377.

Doi: 10.1007/s10661-008-0322-6

- [20] V. Zarezade, A. Aliakbari, M. Es'haghi, M.M. Amini, M. Behbahani, F. Omidi, G. Hesam, Application of a new nanoporous sorbent for extraction and pre-concentration of lead and copper ions, Int. J. Environ. Anal. Chem. 97 (2017) 383-397.
 Doi: 10.1080/03067319.2017.1315638
- [21] M. Behbahani, Y. Bide, S. Bagheri, M. Salarian, F. Omidi, M.R. Nabid, A pH responsive nanogel composed of magnetite, silica and poly(4-vinylpyridine) for extraction of Cd(II), Cu(II), Ni(II) and Pb(II), Microchim. Acta 183 (2016) 111-121. Doi: 10.1007/s00604-015-1603-8
- [22] M. Behbahani, J. Abolhasani, M.M. Amini, O. Sadeghi, F. Omidi, A. Bagheri, M. Salarian, Application of mercapto ordered carbohydrate-derived porous carbons for trace detection of cadmium and copper ions in agricultural products, Food Chem. 173 (2015) 1207-1212.

Doi: 10.1016/j.foodchem.2014.10.101

[23] M. Behbahani, A.A. Akbari, M.M Amini, A. Bagheri, Synthesis and characterization of pyridine-functionalized magnetic mesoporous silica and its application for preconcentration and trace detection of lead and copper ions in fuel products, Anal. Methods 6 (2014) 8785-8792.

Doi: 10.1039/c4ay01692k

[24] M. Behbahani, F. Najafi, M.M. Amini, O. Sadeghi, A. Bagheri, P.G. Hassanlou, Solid phase extraction using nanoporous MCM-41 modified with 3,4-dihydroxybenzaldehyde for simultaneous preconcentration and removal of gold(III), palladium(II), copper(II) and silver(I), J. Ind. Engg. Chem. 20 (2014) 2248-2255. Doi: 10.1016/j.jiec.2013.09.057

[25] A. Bagheri, M. Behbahani, M.M. Amini, O. Sadeghi, M. Taghizade, L. Baghayi, M. Salarian, Simultaneous separation and determination of trace amounts of Cd(II) and Cu(II) in environmental samples using novel diphenylcarbazide modified nanoporous silica, Talanta 89 (2012) 455-461.

Doi: 10.1016/j.talanta.2011.12.062

- [26] C. Karadas, D. Kara, Dispersive liquid-liquid microextraction based on solidification of floating organic drop for preconcentration and determination of trace amounts of copper by flame atomic absorption spectrometry, Food Chem. 220 (2017) 242-248.
 Doi : 10.1016/j.foodchem.2016.09.005
- [27] X. Wen, Q. Yang, Z. Yan, Q. Deng, Determination of cadmium and copper in water and food samples by dispersive liquid-liquid microextraction combined with UV-vis spectrophotometry, Microchem. J. 97 (2011) 249-254.
 Doi : 10.1016/j.microc.2010.09.010
- [28] Z. Fan, W. Zhou, Dithizone–chloroform single drop microextraction system combined with electrothermal atomic absorption spectrometry using Ir as permanent modifier for the determination of Cd in water and biological samples, Spectrochim. Acta B 61 (2006) 870-874.

Doi: 10.1016/j.sab.2006.05.011

- [29] S. Dadfarnia, A.M.H. Shabani, E. Kamranzadeh, Separation/preconcentration and determination of cadmium ions by solidification of floating organic drop microextraction and FI-AAS, Talanta 79 (2009) 1061-1065. Doi : 10.1016/j.talanta.2009.02.004
- [30] C. Zeng, X. Wen, Z. Tan, P. Cai, X. Hou, Hollow fiber supported liquid membrane extraction for ultrasensitive determination of trace lead by portable tungsten coil electrothermal atomic absorption spectrometry, Microchem. J. 96 (2010) 238-242.

Doi: 10.1016/j.microc.2010.03.008

- [31] X. Wen, P. Wu, L. Chen, X. Hou, Determination of cadmium in rice and water by tungsten coil electrothermal vaporization-atomic fluorescence spectrometry and tungsten coil electrothermal atomic absorption spectrometry after cloud point extraction, Anal. Chim. Acta 650 (2009) 33-38.
- [32] E. Yilmaz, I. Ocsoy, N. Ozdemir, M. Soylak, Bovine serum albumin-Cu(II) hybrid nanoflowers: An effective adsorbent for solid phase extraction and slurry sampling flame atomic absorption spectrometric analysis of cadmium and lead in water, hair, food and cigarette samples, Anal. Chim. Acta 906 (2016) 110-117. Doi: 10.1016/j.aca.2015.12.001
- [33] R. Haddad, E. Yousif, A. Ahmed, Synthesis and characterization of transition metal complexes of 4-Amino-5-pyridyl-4H-1,2,4-triazole-3-thiol, Springer Plus 2 (2013) 510-515.

Doi: 10.1186/2193-1801-2-510

- [34] R.G. Pearson, J. Am. Chem. Soc. 85 (22) (1963) 3533–3539.
- [35] V.A. Jadhav, A.G. Vandre, 8-Methoxy-2-chloroquinoline-3-carbaldehyde thiosemicarbazone as an analytical reagent for cobalt(II) and copper(II), J. Ind. Chem. Soc. 72 (1995) 747-749.
- [36] M. Tongsen, L. Menf, H. Cengde, L. Kuaizhi, L. Zhenghao, Z .Paxuesuebav, Spectrophotometric study on copper(II) with pyruvaldehyde (NN'-dibutyl) bisthiosemicarbazone, Ziar Keruebau, 31 (1999) 70-72.
- [37] P. Lopez-de-Alba, L.L. Martiney, A. Hernandez, Spectrophotometric determination of copper in medical formulation: wheat bran and drinking water, Bol. Soc. Chil. Quim. 44 (1999) 469-477.

Doi: 10.4067/S0366-16441999000400011.

- [38] R.S. Lokhande, S.V. Poman, H. R. Kapadi, Extractive spectrophotometric determination of Cu (II) with 4-chloro-iso-nitrosoacetophenonethiosemicarbazone, Asian J. Chem. 13 (2001) 1222-1224.
- [39] B. Ranganath, D. Rajesh, L.K. Ravindranath, P.V. Ramana, Direct and derivative spectrophotometric determination of copper(II) using esomeprazole, World J. Pharma. Res. 4 (2015) 1046-1060.
- [40] A.S. Hankare, V.D. Barhate, Development of extractive spectrophotometric determination of copper(II) using [N (O Hydroxybenzylidene)-4-methylaniline]
 (NOHBMA) as an analytical reagent, Int. J. Curr. Pharm. Res. 6 (1) (2014) 30-33.
- [41] M.J. Desai, K.K. Desai, Spectrophotometric determination of copper(II) and iron(III) with 2,4-dihydroxy-5-bromoacetophenone thiosemicarbazones, Asian J. Chem. 11 (1999) 1313-1316.
- [42] G.S. Kamble, S.S. Kolekar, M.A. Anuse, Synergistic extraction and spectrophotometric determination of copper(II) using1-(2',4'-dinitro aminophenyl)-4,4,6-trimethyl-1,4-dihydropyrimidine-2-thiol: Analysis of alloys, pharmaceuticals and biological samples, Spectrochim. Acta Part A 78 (2011) 1455–1466. Doi : 10.1016/j.saa.2011.01.027
- [43] A. Kumar, P. Sharma, L.K. Chandel, B.K. Kala and S.J. Kunsogi-Mate, Synergistic solvent extraction of copper, cobalt, rhodium and iridium into 1, 2-dichloroethane at trace level by newly synthesized 25,26,27,28, tetrahydroxy-5,11,17,23-tetra-[4-(Nhydroxyl-3-phenylprop-2-enimidamido) phenylazo] calyx[4] arene, J. Incl. Phenom. Macrocycl. Chem., 62 (2008) 285–292.
- [44] D. Fu, D. Yuan, Spectrophotometric determination of trace copper in water samples with thiomichlersketone, Spectrochim. Acta A 66 (2007) 434–436.
 Doi : 10.1016/j.saa.2006.03.018

- [45] K.A. Rao, S. Shivramaiah, K.S. Subudhi, D. Sreevani, Y. Umamaheswari, G.V. Haritha, Determination of copper in water, vegetables, foodstuffs and pharmaceuticals by direct and derivative spectrophotometry, Chem. Sci.Trans.1 (2012) 590-603.
 Doi : 10.7598/cst2012.241
- [46] K. Thipyapong, C. Suksai, Spectrophotometric determination of copper(II) using diamine-dioxime derivative, Bull. Korean Chem. Soc. 24 (2003) 1767-1770.
 Doi : 10.5012/bkcs.2003.24.12.1767
- [47] A.B. Shaikh, U.B. Barache, M.A. Anuse, S.H. Gaikwad, 4-(4'nitrobenzylideneimino)-3-methyl-5-mercapto-1, 2,4-triazole, a new chromogenic reagent for extractive spectrophotometric determination of copper (II) in pharmaceutical and alloy samples, S. Afr. J. Chem. 69 (2016) 157–165. Doi: 10.17159/0379-4350/2016/v69a19
- [48] A.I. Vogel, A Text Book of Quantitative Inorganic Analysis, third ed. Longmans, London, 1975.
- [49] S.P. Colowick, M.O. Kaplan, Methods in Enzymology, Academic press inc. publishers, New York, 1955.
- [50] S.H. Gaikwad, M.A. Anuse, Extraction spectrophotometric determination of micro amounts of palladium(II) in catalysts, Ind. J.Chem., 44A (2005) 1625-1630.
 Doi : http://nopr.niscair.res.in/handle/123456789/18089
- [51] R.V. Gadag, M.R. Gajendragad, Synthesis of copper-triazole complex, Ind. J. Chem. 16(A) (1978) 703-706.
- [52] R. Gopalan, V. Ramlingam, Concise coordination chemistry, Vikas publishing house, India, 2003.

- [53] S.A. Deodware, D.J. Sathe, P.B. Chaudhari, T.N. Lokhande, S.H. Gaikwad, Development and molecular modeling of Co(II), Ni(II) and Cu(II) complexes as a high acting anti breast cancer agents, Arab. J. Chem. 10 (2017) 262-272.
 Doi : 10.1016/j.arabjc.2016.09.024
- [54] A.I. Vogel, A Text Book of Qualitative Inorganic Analysis, third ed. Longmann, London, 1964.
- [55] G.B. Kolekar, M.A. Anuse, Extractive spectrophotometric determination of selenium(IV) using 1-(4'-bromophenyl)-4,4,6-trimethyl-1,4-dihydropyridine-2-thiol, Res. J. Chem. Environ. 2 (1998) 9-15.
- [56] G.B. Kolekar, M.A. Anuse, Extraction, separation and spectrophotometric determination of tellurium(IV) with 1-(4-bromophenyl)-4,4,6-trimethyl-1,4-dihydropyrimidine-2-thiol, Bull. Chem. Soc. Jpn. 71 (1998) 859–866.
 Doi : 10.1246/bcsj.71.859
- [57] S.H. Gaikwad, S.V. Mahamuni, M.A. Anuse, Extractive spectrophotometric determination of bismuth(III) in alloy samples using 1-amino-4,4,6-trimethyl (1H,4H) pyrimidine-2-thiol, Ind. J. Chem. Tech. 12 (2005) 365-368.
 Doi : http://nopr.niscair.res.in/handle/123456789/8644
- [58] G.S. Kamble, A.A. Ghare, S.S. Kolekar, S.H. Han, M.A. Anuse, Development of an reliable analytical method for synergistic extractive spectrophotometric determination of cobalt(II) from alloys and nano composite samples by using chromogenic chelating ligand, Spectrochim. Acta A 84 (2011) 117-124.

Doi : 10.1016/j.saa.2011.09.015

[59] G.S. Kamble, S.S. Kolekar, S.H. Han, M.A. Anuse, Synergistic liquid–liquid extractive spectrophotometric determination of gold(III) using 1-(2',4'-dinitro

aminophenyl)-4,4,6-trimethyl-1,4-dihydropyrimidine-2-thiol,Talanta 81 (2010) 1088-1095.

Doi : 10.1016/j.talanta.2010.02.002

- [60] Z. Marczenko, Spectrophotometric Determination of Elements, Ellis Horwood Limited, Chichester, 1976.
- [61] G.S. Kamble, A.P. Gaikwad, B.N. Kokare, S.S. Kolekar, S.H. Han, M.A. Anuse, Rapid and sensitive synergistic extraction and spectrophotometric determination of silver(I) using 1-(2',4'-dinitro aminophenyl)-4,4,6-trimethyl-1,4-dihydropyrimidine-2-thiol: Analysis of real samples, Ind and Engg. Chem. Res. 50 (2011) 11270-11279. Doi : 10.1021/ie200812w
- [62] G.S Kamble, S.S. Joshi, A.N. Kokare, S.B. Zanje, S.S. Kolekar, A.V. Ghule, S.H. Gaikwad, M.A. Anuse, A sensing behavior synergistic liquid liquid extraction and spectrophotometric determination of nickel(II) by using 1-(2',4'-dinitro aminophenyl)-4,4,6-trimethyl-1,4-dihydropyrimidine-2-thiol: analysis of foundry and electroless nickel plating of waste water, Sep. Sci. and tech.(In-press). Doi : 10.1080/01496395.2016.1255229
- [63] E.B. Sandell, Colorimetric determination of traces of metals, third ed., Inter science, New York (1959) 97.



Fig.1. Absorbance curve for [Cu(II)-CBIMMT] complex

Copper(II) = 10.0 μ g mL⁻¹; CBIMMT = 10 mL 0.025 mol L⁻¹ in chloroform; pH = 4.2 acetate buffer; shaking time = 1 min; $\lambda_{max} = 414$ nm



Fig.2. Effect of pH on extraction of [Cu(II)-CBIMMT] complex

Copper(II) = 10.0 μ g mL⁻¹; CBIMMT = 10 mL 0.025 mol L⁻¹ in chloroform; shaking time = 1 min; $\lambda_{max} = 414$ nm



Fig.3. Effect of organic solvents on extraction of Cu(II)-CBIMMT complex

Copper(II) = 10.0 μ g mL⁻¹; CBIMMT = 10 mL 0.025 mol L⁻¹; pH = 4.2 acetate buffer; shaking time = 1 min; $\lambda_{max} = 414$ nm



Fig.4. Effect of reagent concentration on extraction of [Cu(II)-CBIMMT] complex Copper(II) = 10.0 μ g mL⁻¹; CBIMMT = 10 mL 0.0001-0.03 mol L⁻¹ in chloroform; pH = 4.2 acetate buffer; shaking time = 1 min; $\lambda_{max} = 414$ nm



Fig.5. Effect of shaking time on extraction of [Cu(II)-CBIMMT] complex Copper(II) = 10.0 μ g mL⁻¹; CBIMMT = 10 mL 0.025 mol L⁻¹ in chloroform; pH = 4.2 acetate buffer; shaking time = 0.5-15 min; $\lambda_{max} = 414$ nm



Fig.6. Validity of Beer's law for [Cu(II)-CBIMMT] complex

Copper (II) = 2.5- 20.0 μ g mL⁻¹; CBIMMT = 10 mL 0.025 mol L⁻¹ in chloroform; pH = 4.2 acetate buffer; shaking time = 1 min; λ_{max} = 414 nm



Fig.7. Ringbom's plot for [Cu(II)-CBIMMT] complex

Copper(II) = 2.5- 20.0 μ g mL⁻¹; CBIMMT = 10 mL 0.025 mol L⁻¹ in chloroform; pH = 4.2 acetate buffer; shaking time = 1 min; λ_{max} = 414 nm





CBIMMT] complex in chloroform

 $[M] = [L] = (I) \ 0.001573 \ mol \ L^{-1} \ (II) \ 0.001179 \ mol \ L^{-1} \ pH = 4.2 \ acetate \ buffer; \ shaking$ time = 1 min; $\lambda_{max} = 414 \ nm$



Fig.9. Mole ratio method

1) Copper(II) = 0.5–9.0 mL of 0.001573 mol L⁻¹; CBIMMT]= 5.0 mL of 0.001573 mol L⁻¹ in chloroform ; 2) Copper(II) = 0.5–9.0 mL of 0.0011179 mol L⁻¹; CBIMMT = 5.0 mL of 0.001179 mol L⁻¹ in chloroform; pH = 4.2 acetate buffer; shaking time = 1 min; λ_{max} = 414 nm



Fig.10. Log log plot method

Copper(II) = 10.0 µg mL⁻¹; CBIMMT = 10 mL 0.0001-0.025 mol L⁻¹ in chloroform; pH = 5.6 acetate buffer; shaking time = 1 min; λ_{max} = 414 nm.

Control Manuscraft

Table 1 Comparison of the present method with other reported reagents for the determination of copper(II).

| Name of the reagent | λmax | pH range | Beer's range in ppm | Molar absorptivity (Lmol ⁻¹ cm ⁻¹) | M:L | Remark | Ref. No. |
|--|-------------|--------------|---------------------------|---|-----------|--|-------------|
| N",N"-Bis[(E)-(4-fluorophenyl) methylidene]thiocarbonohydrazide | 375 | 2.5- 5.0 | 2.0-14.0 | 0.42545x10 ⁵ | 1:1:2 | Use of synergent, no use of any buffer | 1 |
| Chloro(phenyl) glyoxime | 290 | 4.0 | - | 0.8×10^4 | - 7 | Absorption in UV region | 3 |
| Esomeprazole | 390 | 3 | 0.5-10 | 4 | \hat{O} | Requires surfactant | 39 |
| [N-(o-Hydroxybenzylidene)-4- methyl aniline](NOHBMA) | 510 | 5.8- 6.8 | 0.1-10 | 890 | 1:2 | Less sensitive | 40 |
| 2,4-Dihydroxy-5- bromoacetophenonethiosemicarbaz one | 420 | 3.0- 6.5 | 12.7 | 0.145x10 ⁴ | 1:1 | Poor sensitivity and selectivity | 41 |
| 1-(2',4'-Dinitroaminophenyl)- 4,4,6-trimethyl-1,4- dihydropyrimidine-2-thiol | 445, 645 | 8.7- 10.5 | 10.0-80.0 | 0.877x10 ³ | 1:2:2 | Use of synergent | 42 |
| 25,26,27,28,Tetrahydroxy- 5,11,17,23-tetra-[4-(N-hydroxyl-3- phenyldrop-2- enimidamido)phenylazo]calyx[4]ar ene | 432 | acidic | 5-10 | 0.96x10 ⁻⁴ | 1:1:1 | Requires synergent and one hour heating | 43 |
| Thiomichlersketone (TMK) | 500 | 1-6 | 0-15 | 5.7x10 ⁴ | 1:4 | Buffer solution is required to adjust pH and use of surfactant | 44 |
| 5-[Alpha-methyl-3-hydroxy benzylidenerhodanine] | 430 | 5.5 | 0.05-13.0 | 0.6027x10 ⁴ | 1:2 | 10 minutes of shaking time | 45 |
| Meso-3,6,6,9-Tetramethyl-4,8- diazaundecane-2,10- dionedioximeormeso- HexaMethylPropyleneAmineOxim e(meso-HMPAO) | 497 | 9 | 0.5-3.70 | 338 | 1:1 | Less sensitive | 46 |
| 4-(4'-Nitrrobenzylideneimino)-3- methyl-5-mercapto-1, 2, 4-triazole. (NBIMMT) | 470 | 6.2 | 4.75- 16.13 | 2.825×10 ³ | 1:2 | Use of phosphate buffer | 47 |
| 4-(4'-Chlorobenzylideneimino)-3- methyl-5-mercapto-1, 2, 4-triazole. (CBIMMT) | 414 | 4.0- 4.2 | 2.5-17.5 | 0.3381×10 ⁴ | 1:2 | Simple, selective and rapid | PM |

PM = Present method

Table 2 Spectral characteristics and precision data of [Cu(II)-CBIMMT] complex.

| Parameters | Optimized parameters |
|---------------------------------------|---|
| Solvent | Chloroform(10 mL) |
| λ_{\max} | 414 nm |
| Buffer | Acetate buffer of pH 4.2 |
| Equilibration time | 1 min |
| CBIMMT concentration | $0.025 \text{ mol } L^{-1}$ |
| Stability of complex | >48 h |
| Beer's law range | Up to 17.5 μ g mL ⁻¹ |
| Ringbom's optimum concentration range | 5.0- 17.5 μg mL ⁻¹ |
| Molar absorptivity | $0.3381 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ |
| Sandell's sensitivity | $0.01996 \mu g \mathrm{cm}^{-2}$ |
| Stoichiometry (M:L) | 1:2 |
| Slope | 0.050 |
| Intercept | -0.0047 |
| Correlation coefficient (r) | 0.999 |
| Limit of detection, LOD | 0.6 $\mu g m L^{-1}$ |
| Limit of quantification, LOQ | 1.8 $\mu g m L^{-1}$ |
| Relative Standard Deviation (%) | 0.41 |
| Enrichment Factor (EF) | 2.49 |
| <pre></pre> | |

Table 3 Effect of diverse ions for the determination of copper(II) with CBIMMT.
 Cu(II) = 100 μ g mL⁻¹, pH = 4.2 acetate buffer, CBIMMT = 10 mL 0.025 mol L⁻¹ in chloroform, Shaking time = 1 min, $\lambda_{max} = 414$ nm _

| Diverse ion | Added as | Amount tolerated, |
|----------------------|--|-------------------|
| | | (mg) |
| Ni(II) ^a | Ni (NO ₃) ₂ . 6H ₂ O | 2.5 |
| V(V) | V_2O_5 | 5.0 |
| Hg(II) ^a | HgCl ₂ | 2.0 |
| Co(II) | CoCl ₂ .6H ₂ O | 10.0 |
| Mg(II) | MgCl ₂ | 20.0 |
| Ba(II) | BaCl _{2.} 2H ₂ O | 20.0 |
| Ca(II) | CaCl ₂ | 20.0 |
| W(VI) | Na ₂ WO ₄ | 1.0 |
| Fe(III) | FeCl ₃ | 20.0 |
| Sb(III) | SbCl ₃ | 10.0 |
| Pb(II) | Pb (NO ₃) ₂ | 5.0 |
| Zr(IV) | ZrCl ₄ | 1.0 |
| Ti(II) | TiCl ₂ | 1.0 |
| Fe(II) | FeSO ₄ | 10.0 |
| Mn(II) | MnCl ₂ .4H ₂ O | 10.0 |
| Zn(II) | ZnCl ₂ | 20.0 |
| Mo(VI) | (NH ₄) ₂ Mo ₇ O ₂₄ .2H ₂ O | 0.25 |
| Te(IV) | Na ₂ TeO ₃ | 1.0 |
| Ru(III) | RuCl ₃ .6H ₂ O | 0.5 |
| Cr(VI) | $K_2Cr_2O_7$ | 20.0 |
| Au(III) ^a | HAuCl ₄ .4H ₂ O | 0.5 |
| Pd(II) ^a | PdCl ₂ | 0.5 |
| $Ag(I)^{b}$ | AgNO ₃ | 5.0 |
| Sn(II) ^c | SnCl ₂ . 2H ₂ O | 20.0 |
| Cd(II) ^d | CdCl ₂ . 2H ₂ O | 5.0 |
| Al(III) | AlCl ₃ | 0.5 |
| Os(VIII) | OsO ₄ | 0.5 |
| Bi(III) | Bi(NO ₃) ₃ | 1.0 |
| Ir(III) | IrCl ₃ | 0.5 |
| Se(IV) | SeO_2 | 0.15 |
| Pt(IV) | PtCl ₄ | 0.5 |
| Bromide | KBr | 100 |
| Thiocyanate | NH4SCN | 100 |
| Acetate | Sod. acetate | 100 |
| Iodide | KI | 100 |
| Succinate | Succinic acid | 50 |
| Tartarate | Sod. Pot. tartarate | 100 |
| Sulphate | Na_2SO_4 | 100 |
| Thiourea | Thiourea | 20 |
| Nitrate | NaNO ₃ | 100 |
| Thiosulphate | $Na_2S_2O_3$ | 10 |
| Flouride | NaF | 2 |

^a Masked with 50 mg thiocyanate, ^b Masked with 100 mg iodide, ^c Masked with 50 mg Succinate, ^d Masked with 50 mg thiosulphate.

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

Cu(II) = 100 μ g mL⁻¹, pH = 4.2 acetate buffer, CBIMMT = 10 mL 0.025 mol L⁻¹ in chloroform, Shaking time = 1 min, $\lambda_{max} = 414$ nm

| Composition (µg) | Relative Recovery ^a | R.S.D. , |
|---|--------------------------------|-----------------|
| Cu(II),100; Zn(II),300 | 99.8 | 0.17 |
| Cu(II),100; Fe(III),300 | 99.6 | 0.17 |
| Cu(II),100; Cd(II),300 | 99.6 | 0.20 |
| Cu(II),100;Sn(II),300 | 99.6 | 0.17 |
| Cu(II),100; Bi(III),300 | 99.6 | 0.11 |
| Cu(II), 100; Fe(III), 200; Ni(II), 200 | 99.8 | 0.22 |
| Cu(II), 100; Pb(II), 500;Sn(II), 500 | 99.4 | 0.17 |
| Cu(II), 100; Zn(II), 500; Pb(II), 500 | 99.6 | 0.77 |
| Cu(II), 100; Zn(II), 500; Cd(II), 500 | 99.4 | 0.17 |
| Cu(II), 100; Ag(I), 100 ^b ;Co(II), 300 | 99.6 | 0.22 |
| Cu(II), 100; Bi(III), 300; Cd(II), 500 | 99.4 | 0.63 |
| Cu(II), 100; Bi(II), 500; Cr(VI), 300 | 99.6 | 0.17 |

^aAverage of five replicates, ^bMasked with 50 mg iodide.

Table 5 Determination of copper(II) in various water samples

 $pH=4.2 \mbox{ acetate buffer, CBIMMT}=10 \mbox{ mL } 0.025 \mbox{ mol } L^{-1} \mbox{ in } \mbox{ chloroform, Shaking} \label{eq:phi}$ time = 1 min, λ_{max} = 414 nm

| Water | | | Relative | R. S. | D., % |
|------------|----------------------------------|--------------------|------------------|---------------------------------------|--|
| sample | Copper(II) mg L ⁻¹ | | Recovery, (%) | Intra-day precision (<i>n</i> =5) | Inter day precision (<i>n</i> =15) |
| | Added | Found ^a | | | |
| Municipal | - | 0.6552 | - | 1.35 | 1.57 |
| Water | 5 | 5.6529 | 100.5 | 0.20 | 1.88 |
| Lake water | - | 1.5471 | - | 0.58 | 1.93 |
| | 5 | 6.5483 | 99.9 | 0.21 | 1.27 |
| Dam water | - | 2.4663 | - | 0.23 | 1.95 |
| | 5 | 7.4601 | 100.2 | 0.29 | 1.29 |
| Borewell | - | 1.1904 | - | 1.50 | 1.78 |
| water | 5 | 6.1947 | 99.7 | 0.57 | 1.01 |
| Distilled | - | N.D. | - | <u> </u> | - |
| water | 5 | 4.9970 | 99.4 | 0.12 | 1.98 |

^aAverage of five replicates, N.D. = Not detected.

Table 6 Determination of copper(II) from various food samples

pH = 4.2 acetate buffer, CBIMMT = 10 mL 0.025 mol $L^{\text{-1}}$ in chloroform, Shaking time = 1 min, λ_{max} = 414 nm

| | | R.S.D. , (%) | | | | | | |
|---|--|---------------------------------|----------------------------------|----------------------------|--|--|--|--|
| Food sample with their botanical name | Amount of Cu(II) found ^a (mg/kg), By present method | Intra-day precision (n=5) | Inter day precision (n=15) | Confidence limit,α=0.95 | | | | |
| Pea nut (Arachis <i>hypogaea</i>) | 5.6101 (5.6003) | 3.63 | 3.85 | 0.88 | | | | |
| Garlic (Allium <i>sativum</i>) | 8.8232 (8.8302) | 1.92 | 2.06 | 1.25 | | | | |
| Sesame (Sesema <i>indicum</i>) | 6.6105 (6.6112) | 3.03 | 3.46 | 0.91 | | | | |

^aAverage of five replicates, values found by AAS method are shown in the parenthesis.

K K

 Table 7 Determination of copper(II) from leafy vegetable samples

pH = 4.2 acetate buffer, CBIMMT = 10 mL 0.025 mol $L^{\text{-1}}$ in chloroform, Shaking time = 1 min, λ_{max} = 414 nm

| Vogotoblo comple | Amount of Cu(II) | R.S.D. | , (%) | |
|---|---|---|-------|-----------------------------|
| with their botanical name | found ^a (mg/kg), By present method | Intra-dayInterdayprecisionprecision(n=5)(n=5) | | Confidence limit, α=0.95 |
| Palak (Spinacia <i>oleracea</i>) | 2.4010 (2.4125) | 1.53 | 1.90 | 0.40 |
| Ambadi (Hibiscus <i>cannabinus</i>) | 21.8402 (21.7918) | 2.01 | 2.42 | 3.00 |
| Lal ambadi (Hibiscus <i>sabdariffa</i>) | 19.2472 (19.2630) | 3.88 | 3.96 | 2.66 |
| Sea Hibiscus (Hibiscus <i>tilaceus</i>) | 8.2250 (8.2402) | 3.37 | 3.66 | 1.16 |

^aAverage of five replicates, values found by AAS method are shown in the parenthesis.

Table 8 Determination of copper (II) from micronutrient fertilizer samples

pH = 4.2 acetate buffer, CBIMMT = 10 mL 0.025 mol L⁻¹ in chloroform, Shaking

time = 1 min, $\lambda_{max} = 414$ nm

| Name of the fertilizer sample | Composition (%) given by Manufacturer | Certified % values of Cu(II) | Amount of Cu(II) found ^a (%), | R.S.D. , % | | Confidence limit, α=0.95 |
|-------------------------------|---|------------------------------------|--|---------------------------------|----------------------------------|--------------------------------|
| | | | by present method | Intra-day precision (n=5) | Inter day precision (n=15) | |
| UB Supermix | Fe, 2.5; Mn, 1.0; Zn, 3.0; Mo 0.1; B, 0.5 | 1.0 | 0.9820 (0.9920) | 0.85 | 1.60 | 4.87 |
| Vardaan | Fe, 2.5; Mn, 1.0; Zn, 3.0; Mo 0.1; B, 0.5 | 1.0 | 1.0220 (1.0104) | 0.53 | 1.22 | 4.75 |
| Real | Fe, 2.5; Mn, 1.0; Zn, 3.0; Mo 0.1; B, 0.5 | 1.0 | 1.0087 (1.0100) | 0.44 | 1.46 | 4.76 |

^aAverage of five replicates, values found by AAS method are shown in the parenthesis.

Table 9 Analysis of copper(II) from complexes

pH=4.2 acetate buffer, CBIMMT = 10 mL 0.025 mol $L^{\text{-1}}$ in chloroform, Shaking time = 1 min, $\lambda_{max}\,{=}\,414$ nm

| Complex sample | Amount of Cu(II) in % | | Relative Recovery, | R. S. D., % |
|--|-----------------------|--------------------|-----------------------|-------------|
| - | Present ^a | Found ^b | (%) | |
| °Cu(C7H6O2N)2 | 18.93 | 18.91 | 99.9 | 0.06 |
| ^d Cu(C3H6N4S) | 32.81 | 32.78 | 99.9 | 0.07 |
| ^e Cu(C20H16N10S2O4) | 10.81 | 10.80 | 99.9 | 0.08 |
| ^f [Cu(NH ₃) ₄]SO ₄ .H ₂ O | 25.88 | 25.82 | 99.8 | 0.08 |

^aEstimated by salicylaldoximate method and iodometric method.

^bAverage of five replicates.

^cCopper complexes of Salicylaldoxime.

^dCopper complexes of 4-amino-5-mercapto-3-methyl-1,2,4-triazole.

^eCopper complexes of 4-(2'-nitrobenzylideneimino)-3-methyl-5-mercapto-1,2,4-triazole.

^fhydrated Tetraammine copper(II) sulphate.

Table 10 Separation and determination of copper(II) from binary synthetic mixtures pH = 4.2 acetate buffer, CBIMMT = 10 mL 0.025 mol L⁻¹ in chloroform, Shaking time = 1 min, $\lambda_{max} = 414$ nm

| Metal | Amount taken | Relative | R.S.D. Chromogenic | | Ref. |
|----------------------|--------------|---------------------------|--------------------|---------------------|------|
| ions | (µg) | recovery ^c (%) | (%) | ligand | |
| Cu(II) | 100 | 99.6 | 0.58 | CBIMMT | - |
| Pd(II) ^a | 100 | 99.4 | 0.51 | FIMMT | 50 |
| Cu(II) | 100 | 99.4 | 0.79 | CBIMMT | - |
| Se(IV) | 300 | 99.3 | 0.83 | 4'-bromo PTPT | 55 |
| Cu(II) | 100 | 98.2 | 1.22 | CBIMMT | - |
| Te(IV) | 100 | 98.7 | 1.26 | 4'-bromo PTPT | 56 |
| Cu(II) | 100 | 99.8 | 1.14 | CBIMMT | - |
| Bi(III) | 100 | 99.5 | 0.57 | 1-amino TPT | 57 |
| Cu(II) | 100 | 98.6 | 1.47 | CBIMMT | - |
| Co(II) | 100 | 98.6 | 2.39 | 2',4'-dinitro APTPT | 58 |
| Cu(II) | 100 | 99.2 | 1.72 | CBIMMT | - |
| Au(III) ^a | 100 | 98.2 | 0.78 | 2',4'-dinitro APTPT | 59 |
| Cu(II) | 100 | 98.6 | 0.58 | CBIMMT | - |
| Fe(III) | 100 | 99.2 | 0.51 | Thiocyanate | 60 |
| Cu(II) | 100 | 98.6 | 0.58 | CBIMMT | - |
| $Ag(I)^{b}$ | 100 | 99.4 | 0.51 | 2',4'-dinitro APTPT | 61 |
| Cu(II) | 100 | 98.6 | 0.58 | CBIMMT | - |
| Ni(II) ^a | 300 | 99.6 | 0.58 | 2',4'-dinitro APTPT | 62 |

^a Masked with 25 mg thiocyanate, ^b Masked with 25 mg iodide

^cAverage of five replicates.

Graphical Abstract

For

An efficient, cost effective, sensing behaviour liquid-liquid extraction and spectrophotometric determination of copper(II) incorporated with 4-(4'- chlorobenzylideneimino)-3-methyl-5-mercapto-1, 2, 4-triazole: Analysis of food samples, leafy vegetables, fertilizers and environmental samples



Research Highlights

Of

An efficient, cost effective, sensing behaviour liquid-liquid extraction and spectrophotometric determination of copper(II) incorporated with 4-(4'-chlorobenzylideneimino)-3-methyl-5-mercapto-1, 2, 4-triazole: Analysis of food samples, leafy vegetables, fertilizers and environmental samples

- Chromogenic reagent CBIMMT shows excellent sensing behavior with copper(II) in buffer medium.
- Coloured copper(II) complex extracted in single step within one minute and exhibits maximum absorbance in visible region, 414 nm.
- The method does not require synergent, surfactant and heating of aqueous phase to develop the colour of the complex.
- > No need of any sophisticated instrumentation and tedious procedure.
- The method is proved to be reliable for analysis of various samples such as water, fertilizer, food, leafy vegetable and permits separation and quantification of copper(II) from associated metal ions.