

Analytical and Biological Evaluation of Two Schiff's Bases: Spectrophotometric Analysis of Copper (II) in Water and Soil Samples

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Abstract

Two novel ligands, (*E*)-*N*'-(2-hydroxy-5-nitrobenzlidene) isonicotinoylhydrazone (HNBISNH)/2-(4-fluorobenzlideneamino)benzenethiol (FBBT) were synthesized and used for the quantification of copper (II) in various water and soil samples. HNBISNH/FBBT interacts with copper (II) to form 1:1 [L:M] orange /brick red color complexes in presence of phosphate buffer of pH 3.7/4.5 which increases the sensitivity of the complexes. Thus formed colored complex followed the Beer's law up to 1.6 and 1.9 mg L⁻¹ with an optimum concentration range over 0.11-1.3 mg L⁻¹ and 0.10-1.7 mg L⁻¹, the slope of Ringbom's plot are found to be 0.3500 and 0.3300 for copper(II)-HNBISNH and copper(II)-FBBT complexes, respectively. The molar absorptivity and Sandell's sensitivity of copper (II)-HNBISNH and copper(II)-FBBT complexes were calculated and found to be 4.91×10⁴ L mol⁻¹ cm⁻¹, 6.1×10⁴ L mol⁻¹ and 0.0010 μg cm⁻² and 0.0014 μg cm⁻² respectively. The detection limits was calculated and found to be 0.043 μg L⁻¹ and 0.036 μg L⁻¹ with HNBISNH and FBBT respectively. Various optimum conditions such as effect of pH, reagent concentration, accuracy, precision and reproducibility were investigated to improve the sensitive for the present method. The detailed study of various excipients confirmed the high selectivity of the method. The proposed method was applied to the determination of copper (II) in different water & soil samples obtained results were in good agreement with atomic absorption spectrometric method (AAS).

Keywords: (*E*)-*N*'-(2-hydroxy-5-nitrobenzlidene) isonicotinoyl hydrazone (HNBISNH); 2-(4-fluorobenzlideneamino) benzenethiol (FBBT); Copper (II); Spectrophotometry; Biological activity; Environmental samples

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]. Heterocyclic azo dyes have been used as chromophores in spectrophotometric determination of copper (II) ions. These azo dyes are 2-amino-5-bromopyridylazo resorcinol [4], 1-[pyridyl 2-azo]-naphthol (PAN) [5,6], and its derivatives are 1-(2-thiazolylazo)-2-naphthol [7], 1-(5-bromo-2-pyridylazo)-2-naphthol-6-sulphonic acid [8], 2,6-bis(4-sulfo-1-hydroxy-2-naphthylazo) pyridine [9], 4-(1H-pyrazolo 3,4-d)pyrimidin-4-ylazo benzene-1,3-diol [10], 4-(p-Nitrophenylazo)-2-amino-3-pyridinol [11], 1-(2-quinolyazo)-2,4,5-trihydroxybenzene [12], bromosulphonazo III [13] and alizarin red S [14]. These dyes are highly sensitive but lack of the selectivity. The coordination chemistry of hydrazones is an intensive area of study of numerous transition metals. Hydrazones have been studied as a group of most useful spectrophotometric reagents. The shortcomings of hydrazones were the lack of selectivity for metal ions, therefore much work has been devoted to the development of masking agents for use of hydrazones. Among these some of the hydrazones are 2,2'-dipyridyl-2-pyridylhydrazone [15] and 2-pyridine carboxaldehyde isonicotinyl hydrazone [16]. Many techniques such as Atomic Absorption Spectrophotometry [17], *Inductively Coupled Plasma Atomic Emission Spectroscopy* [18], Voltammetry [19] and Spectrophotometry [20,21] have been reported for the determination of copper (II).

The aim of the present work was to provide a facile, sensitive and rapid non-extractive spectrophotometric method for the determination of trace amounts of copper (II) in different sample of environmental importance. In this study, new analytical reagents (*E*)-*N*'-(2-hydroxy-5-nitrobenzlidene) isonicotinoyl hydrazone (HNBISNH) and 2-(4-fluorobenzlidene amino) benzenethiol (FBBT) was successfully synthesized for the determination of copper (II) in environmental samples.

Experimental

Instrumentation

A HITACHI model U 3400 UV VIS NIR Spectrophotometer with 10 mm stopped glass cells was used. An ELICO model Li-129 was used for all pH measurements.

Reagents

All reagents used were of analytical reagent grade and solutions were prepared with deionized distilled water unless mentioned. A phosphate buffer solution prepared by adding: 50 mL of 0.25M disodium hydrogen orthophosphate (SD fine Chemicals, India) dissolved in 20 mL of 0.1M phosphoric acid (SD fine Chemicals, India) and pH was adjusted to 4.0/4.7 with 0.25 M disodium hydrogen orthophosphate solution and diluted to 100 mL in a volumetric flask.

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Received January 27, 2014; Accepted March 10, 2014; Published March 12, 2014

Citation: Natesh Kumar B, Kanchi S, Bisetty K, Vijaya Jyothi NV (2014) Analytical and Biological Evaluation of Two Schiff's Bases: Spectrophotometric Analysis of Copper (II) in Water and Soil Samples. J Environ Anal Chem 1: 106. doi:10.4172/10.4172/2380-2391.1000106

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Synthesis of ligands

Preparation of (E)-N¹-(2-hydroxy-5-nitrobenzylidene)isonicotinoyl hydrazone (HNBISNH): Accurately weighed 1.2g of 2-hydroxy-5-nitrobenzaldehyde and 0.822g of isonicotinoyl hydrazide were transferred into a 50 mL round bottom flask. Ethyl alcohol was used as solvent and the condensation process was carried out about 2 h at 60°C. The yellow colored product of HNBISNH was formed and further subjected to evaporate the solvent and collected the pure solid compound with yield about 1.98g (86%). The melting point of HNBISNH was found to be in the range of 214-216°C with solubility in DMF. The preparation pathway of color forming ligand was shown in Scheme 1a.

Preparation of 2-(4-fluorobenzylideneamino) benzenethiol (FBBT): The physical state of 2-aminobenethiol was semi solid while 4-fluorobenzaldehyde was colorless oily liquid. Both reactants density values were taken into consideration for the synthesis of product. Since those two reactants are nearly liquids accurately measured 0.48 mL of 2-aminobenethiol and 0.48 ml of 4-fluorobenzaldehyde were transferred into a 50 mL round bottomed flask and methanol was used as a solvent. Reflux the reaction mixture for about 1h at 60°C. Pale yellow colored oily product was formed and further subjected rotor to expel the excess of solvent and obtained the pure product. This product on long standing in ambient temperature converted to yellow solid. The melting point was in the range of 202-204°C with solubility in hexane. The reaction procedure of color forming ligand was shown in Scheme 1b.

Preparation of standard solutions

2.86 g of HNBISNH and 2.31 g of FBBT was dissolved in deionized distilled water and diluted to 100 mL to get 0.1 M of stock solution. Working solution of HNBISNH and FBBT were prepared by appropriate dilution and the solutions were stable for several months. A stock solution of copper (II) (1.0 mg mL⁻¹) was prepared by dissolution of 0.250 g copper sulphate pentahydrate (SD fine Chemicals, India) in 100 mL standard flask and further dilutions as required were employed.

General procedure

In the first step an aliquot containing not more than 1.0–100 µg mL⁻¹ of copper (II) was transferred into 25 mL calibrated flask, 3.0 mL of 0.005 M HNBISNH and 5 mL of phosphate solution with pH 4.0 solution were added successively and the mixture was diluted up to the mark with deionized distilled water. The first step was repeated for second ligand, FBBT as well. The appearance of the orange and brick red color chromophores was instantaneous and absorbance was measured at 480 nm and 520 nm against reagent blank solution. The reagent blank was prepared in a similar manner without copper (II). All measurements were carried out at ambient temperature. The copper(II) content in an unknown sample was determined using a calibration graph.

Procedure for evaluation of biological activity of complexes

To a methanolic solution of copper (II) sulphate, a hot methanolic solution of the HNBISNH/FBBT ligand was added slowly with stirring. The mixture was refluxed on a hot water bath. It was concentrated under reduced pressure to two-third the original volume and cooled. The solid that separated out was filtered, washed with water, hot methanol and ether and was vacuum dried to obtain solid complexes.

Antibacterial activity of the two synthesized complex compounds was determined with gram positive organisms (*Bacillus subtilis* and

Staphylococcus aureus) and gram negative organisms (*Escherichia coli* and *Pseudomonas aeruginosa*) using disc diffusion method [22]. The Muller-Hinton agar was rigorously tested for the composition and pH. Further, the depth of the agar in the plate was a factor to be considered in the disc diffusion method. The test plates were prepared by 0.5 mL of standard inoculums pipetted into a sterile petri plate; 20 mL of melted agar medium was then added to 10 cm petri plate and mixed well by gently swirling on the table top. The seeded plates are allowed to solidify and 10 µg mL⁻¹ of complex compounds was impregnated on sterilized filter paper disc was carefully transferred in the agar plates. The chemical diffuses from the disc into the agar and place the chemical only around the disc. The solubility of the chemical and its molecular size was determined using the chemical infiltration size around the disc. Standard disc of Gentamycin 10 µg (antibacterial agent) was served as positive controls for antimicrobial activity on the agar plates. The plates were incubated at 37°C for 24 h around the disc opaque area indicates that the compound, which diffused into the agar from the disc, which inhibits the growth of the organism. The inhibition Zone (IZ) was recorded with the help of scale, and compared with control containing standard antibiotic Gentamycin disc. The experiment was triplicated and the mean values are presented along with the standard deviation. Minimum Inhibitory Concentrations were determined by the Vollekova and Usman two fold dilution method [23,24].

Sample preparation

Procedure for water samples: One liter of the water sample collected from various sites outside the Tirupati city were acidified with hydrochloric acid and filtered with a 0.45 µm filter. The samples were pre-heated and evaporated to 250 mL. The copper (II) contents were determined according to aforesaid procedures. The obtained results were compared with standard atomic absorption method in terms of student's 't'-test and variance ratio 'f'-test.

Procedure for soil samples: Soil samples collected from industrial areas surrounding Tirupati city were accurately weighed (2.0 g) in a 100 mL beaker. The content was digested as per to the reported method, [25] then 10 mL concentrated HNO₃ and 2.0 mL of 70% HClO₄ (v/v) was added and heated for 1 h. The mixture was filtered through a Whatman No. 40 filter paper into a 250 mL calibrated flask and its pH was adjusted to desired value and diluted to mark with deionised water. In all of real and synthetic amount of copper (II) ion was found by standard addition method.

Results and Discussion

Absorption spectra

Under the optimal experimental condition, the absorption spectra of HNBISNH/FBBT and copper (II)-HNBISNH/FBBT complexes were scanned. The absorption maximum of reagent blanks HNBISNH/FBBT, were measured at 370 nm and 395 nm, whereas copper (II)-HNBISNH/FBBT complexes gave an absorption peak at 460 nm and 530 nm. The difference (bathochromic shift) of the two peaks was 90 nm and 145 nm, and could be obviously distinguished. Thus, the absorption peaks at 460 nm and 530 nm were chosen as the determination of wave length for copper (II)-HNBISNH/FBBT complexes as illustrated in Figure 1.

Effect of pH

pH studies were carried out on the peak height of copper (II)-HNBISNH/FBBT complexes at various concentrations (0.001–0.10M) by fixing 0.005M HNBISNH and FBBT concentration. The pH of

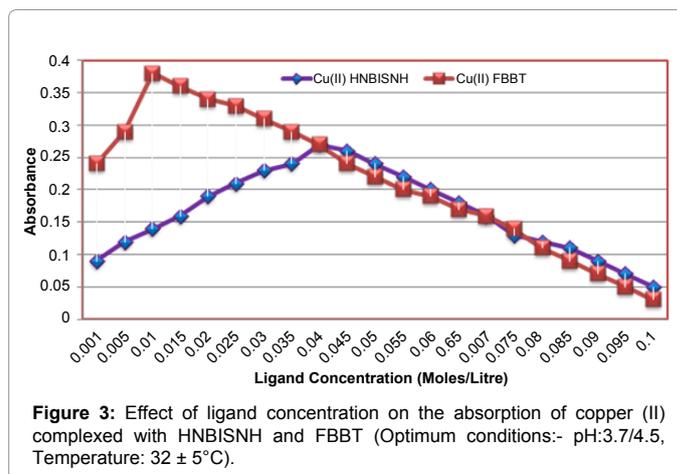
phosphate buffer was changed over a range of 2.0-7.0 and the peak height were measured for each concentration level of copper (II). At all concentration levels of copper (II)-HNBISNH/FBBT complexes, maximum absorbance were found between pH 3.0 and 5.0. Therefore, a pH 3.7 for HNBISNH and 4.5 for FBBT of phosphate buffer system was chosen throughout the study as shown in Figure 2.

Effect of ligand concentration on absorbance

The effect of concentrations of both ligands (HNBISNH and FBBT), on the peak height was investigated at pH 3.7 and 4.5 in a phosphate buffer system. The concentrations of HNBISNH and FBBT was varied over the range 0.001– 0.10 M. Maximum peak height was obtained at a concentration of 0.04 M for HNBISNH and 0.01 M FBBT as color developing reagents for lower concentration level of copper (II) in the sample solution. Further, increase in HNBISNH and FBBT concentration, slight decrease in the peak height was observed. Hence, 0.04 M for HNBISNH and 0.01 M FBBT was optimized for all further studies as represented in Figure 3.

Effect of time and temperature on absorbance

The reaction was instantaneous for the both complexes, but these systems attained maximum and constant absorbance just after the dilution to volume (25 mL in volumetric flask) at room temperature



(32 ± 5°C) and remained stable for 30 h and 60 h for copper (II)-HNBISNH/FBBT complexes, respectively.

Stability of the chromophoric system

After mixing all the components, the absorbance related to both complexes reached its maximum in less than 1 min at ambient temperature and remain stable for 30 h [copper (II)-HNBISNH] and 60h [copper (II)-FBBT] in aqueous solution. Due to stability of the complex and clarity of complex solution, the extraction step was omitted to reduce usage of environmental hazardous solvents, which made the method eco-friendly.

Composition of the complexes

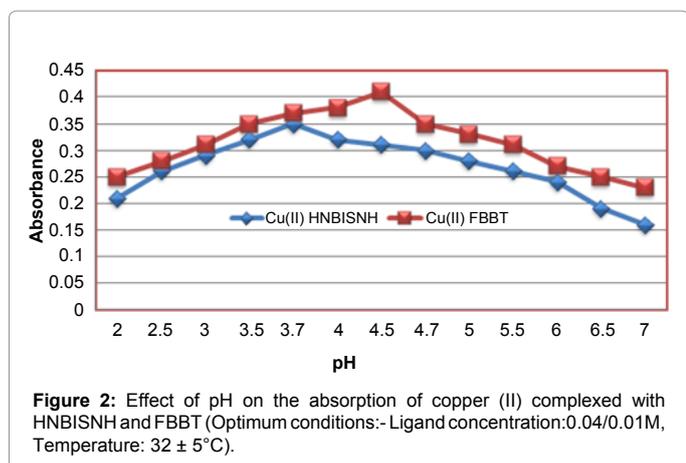
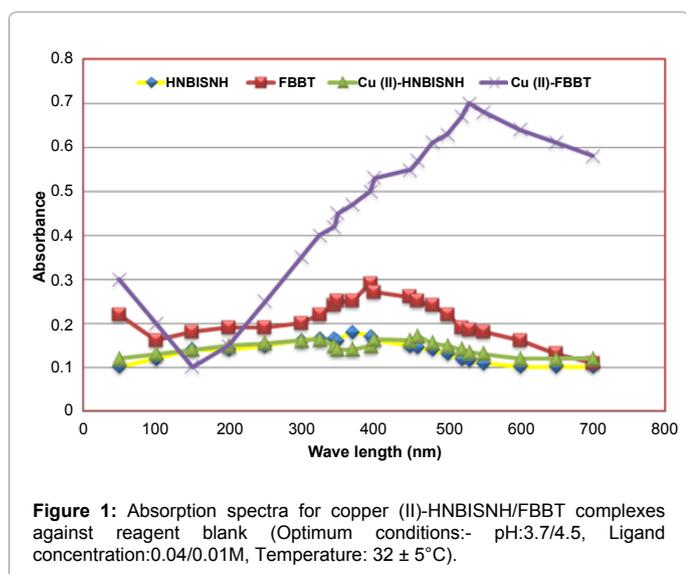
The stoichiometry of the complexes was identified using the molar ratio at the optimum conditions discussed above. Figures 4a and 4b show that maximum absorption was found to be at molar ratio 1.0 by varying the concentrations of HNBISNH and FBBT, representing the interaction of one ligand molecule with metal in the complex formation. Accordingly, the results indicated that the stoichiometric ratio was (1:1) [M:L]. The composition of the both complexes [copper (II)-HNBISNH/FBBT] were determined using Job's continuous variation method indicates the formation of 1:1 (M:L) complexes.

Ringbom plot's for copper (II)-HNBISNH/FBBT complexes

Ringbom's plot is the standard protocol adopted to know the optimum range of concentration for chromophoric system that obeys Beer's law. The plots were drawn between $\log C$ of copper (II) and $(1-T)$ (where T is the transmittance). The plots were sigmoid shape with a linear segment at intermediate absorbance values 0.1–1.0, 0.2–1.6 and concentration values 1.20–3.1 $\mu\text{g mL}^{-1}$ and 1.22–3.4 $\mu\text{g mL}^{-1}$ for copper (II)-HNBISNH and copper (II)-FBBT respectively. The slope of Ringbom's plot from Figure 5 are found to be 0.3500 for copper (II)-HNBISNH and 0.3300 for copper (II)-FBBT complexes. Hence, the ratio between the relative error in concentration and photometric errors are 2.50, 2.20 for found to be concentration of 0.0250, 0.0220 having 1.0% photometric error.

Sensitivity and calibration curves

The complexes obey Beer's law up to 1.7 and 2.0 mg L^{-1} with an optimum concentration range between 0.11 - 1.4 mg L^{-1} and 0.10 - 1.8 mg L^{-1} for copper (II)-HNBISNH/FBBT systems respectively. The molar absorptivity of complexes at 460 and 530 nm and at pH 3.7 and 4.5 was calculated $4.91 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ and $6.1 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$



respectively. Sandell's sensitivity for both complexes were found to be $0.0010 \mu\text{g cm}^{-2}$ and $0.0014 \mu\text{g cm}^{-2}$ for copper (II)-HNBISNH/FBBT systems respectively. The correlation coefficient (γ) for the complexes of copper (II)-HNBISNH/FBBT experimental data was 0.9996 and 0.9998 respectively. The composition of the both complexes [copper (II)-HNBISNH/FBBT] were determined using Job's continuous variation method indicates the formation of 1:1 (M:L) complexes. The stability constants corresponding to copper (II)-HNBISNH/FBBT was determined and found to be 5.9×10^{15} and 6.7×10^{15} (1:1, M:L) respectively. The calibration graphs were obtained at the optimum working conditions as shown in Figures 6a and 6b.

Effect of excipients

To understand the selectivity of the present method using two ligands (HNBISNH/FBBT), the effect of potentially interfering species on the copper (II) determination was studied and carried out by adding a known concentration of excipients to copper (II) solution of $0.2 \mu\text{g mL}^{-1}$. Several anions and cations were studied in detail. Table 2 summarizes the tolerance limits of interfering ions in the determination of $0.2 \mu\text{g mL}^{-1}$ copper (II). The tolerance limit was taken as the amount causing an error of $\pm 2\%$ at the peak height.

Method evaluation

The proposed method was critically evaluated with regard to reproducibility, accuracy, and detection limit for analysis of copper (II) in various environmental samples.

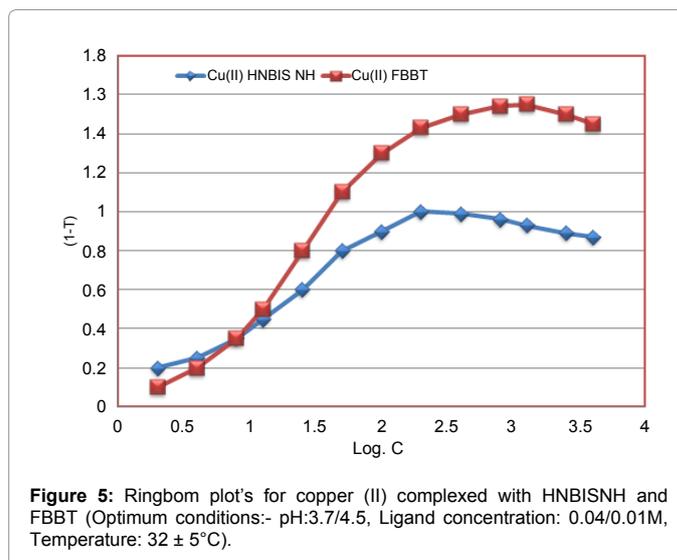


Figure 5: Ringbom plot's for copper (II) complexed with HNBISNH and FBBT (Optimum conditions:- pH:3.7/4.5, Ligand concentration: 0.04/0.01M, Temperature: $32 \pm 5^\circ\text{C}$).

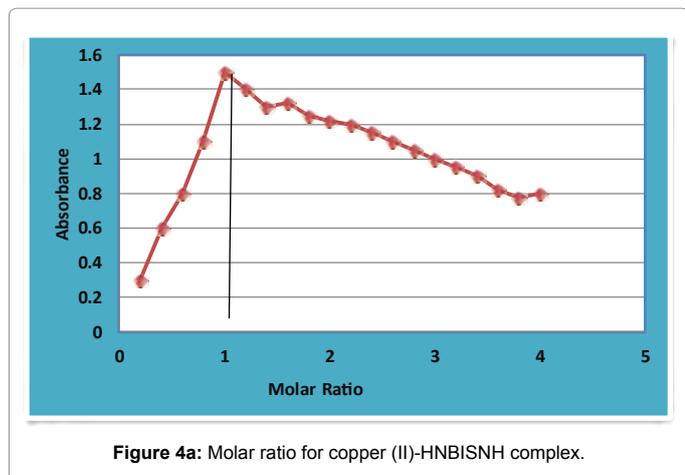


Figure 4a: Molar ratio for copper (II)-HNBISNH complex.

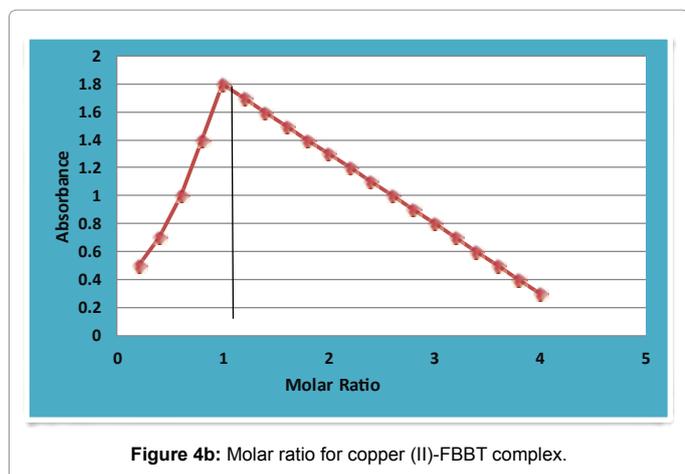


Figure 4b: Molar ratio for copper (II)-FBBT complex.

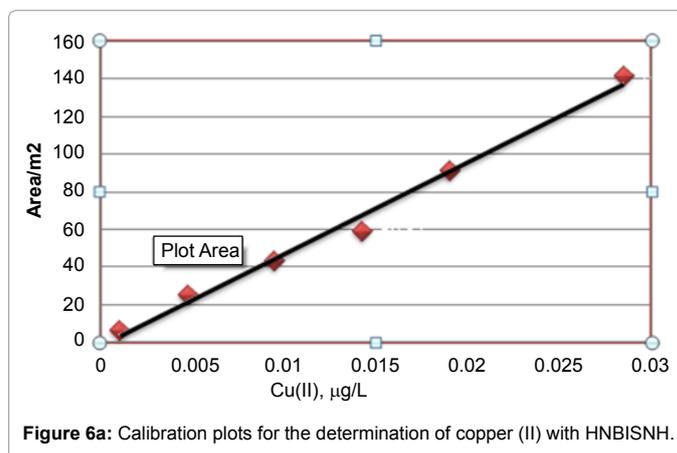


Figure 6a: Calibration plots for the determination of copper (II) with HNBISNH.

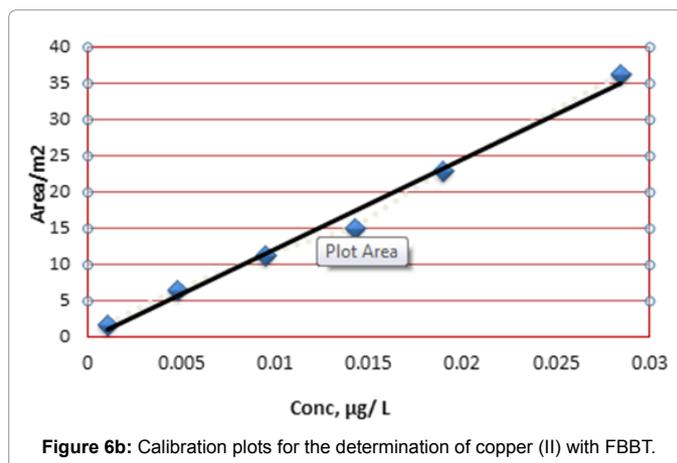


Figure 6b: Calibration plots for the determination of copper (II) with FBBT.

Reproducibility

To test the reproducibility of the present method, four repetitive analyses of each sample was studied. The RSD (%) values for copper (II)-HNBISNH/FBBT were found to be 0.600 and 0.598 (Table 1). A % SD values obtained from the present method ranges 0.42–1.95 as summarized in Tables 3 and 4.

Optical Characteristics	HNBISNH	FBBT
	Values	Values
Color	Orange	Brick Red
λ_{max} [nm]	460	530
Stability	30 h	60 h
Beer's law range [mg L ⁻¹]	0.11 - 1.4	0.10 - 1.8
Molar absorptivity [L mol ⁻¹ cm ⁻¹]	4.91×10 ⁴	6.1×10 ⁴
Sandell_s sensitivity [µg cm ⁻²]	0.0010	0.0014
Regression equation (Y ^b)		
Slope ^a	0.1715	0.1728
Intercept ^b	0.0163	0.0152
Correlation coefficient [r]	0.9994	0.9998
Relative standard deviation [%] ^c	0.600	0.598
Range of error (95% confidence level)	± 1.186	± 1.044
Detection limit [µg L ⁻¹]	0.043	0.036
% Error	0.101	0.108

^aExperiments performed under optimized conditions (see text),

^bY = ax + b, where x is the concentration of analyte in µg ml⁻¹,

^cn = 4

Table 1: Optical characteristics for the analysis of copper (II) with HNBIFBBT using spectrophotometer.

Tolerance limit/g L ⁻¹	Interfering ions
>50000	K(I),Na(II), Ca(II),Mg(II), Al(III), Cr(III)Cl-,Br-,PO ₃ ³⁻ , CO ₃ ³⁻ ,SO ₄ ²⁻
>10000	Zn(II), Hg(II), NO ₃
>5000	Pb(II), Cd(II)
>800	Co(II),Ni(II), Fe(II),Fe(III), Mn(II)

^aCan be masked up to 10000g L⁻¹ by the addition of 2 mL of EDTA, thiosulfate, cyanide.

^bCan be masked up to 5000 g L⁻¹ by the addition of 2 ml of 2 % citrate solution.

^cCan be masked upto 800 g L⁻¹ by the addition of 1 % thiocyanate

Table 2: Tolerance limits of interfering ions on the determination of 0.2 µg mL⁻¹ copper (II) ion.

Samples	Present Method					ASS Method	
	Cu (II) [µg L ⁻¹]						
	Added	Found	Recovery ± SD ^d	t-Test ^e	F-Test ^f	Found	Recovery ± SD ^d
Water samples							
SWS	2.0	1.98	97.50 ± 0.74	6.40	2.30	1.97	98.50 ± 0.72
NWS	-	0.24	-	-	-	0.24	-
SWS	3.0	2.94	98.46 ± 0.44	2.38	0.74	2.96	98.66 ± 0.50
NWS	-	0.21	-	-	-	0.22	-
SWS	4.0	3.92	98.15 ± 0.42	1.82	0.96	3.95	98.75 ± 0.60
NWS	-	0.17	-	-	-	0.18	-
SWS	5.0	4.96	99.55 ± 0.60	3.50	0.94	4.98	99.60 ± 0.55
Soil samples							
SSS	2.0	1.91	95.00 ± 1.26	3.17	0.64	1.92	95.50 ± 1.28
NSS	-	0.29	-	-	-	0.29	-
SSS	3.0	2.88	96.22 ± 0.46	4.34	1.32	2.90	97.10 ± 0.64
NSS	-	0.10	-	-	-	0.10	-
SSS	4.0	3.90	97.50 ± 1.66	2.66	0.87	3.92	98.00 ± 0.98
NSS	-	0.32	-	-	-	0.32	-
SSS	5.0	4.94	98.80 ± 0.54	1.98	0.40	4.95	99.00 ± 0.89

^aSamples collected around Amaraja Batteries

^bIndustrial Estate, Renigunta

^cChandragiri,

^dn=4

^eP≥0.000284

^fP= 0.059

Table 3: Analytical data for the determination of copper (II) with HNBISNH in various water and soil samples.

Samples	Present Method					ASS Method	
	Cu (II) [µg L ⁻¹]						
	Added	Found	Recovery ± SD ^d	t-Test ^e	F-Test ^f	Found	Recovery ± SD ^d
Water samples							
SWS	2.0	1.96	94.0 ± 0.66	7.00	2.69	1.97	99.00 ± 0.99
NWS	-	0.23	-	-	-	0.24	-
SWS	3.0	2.94	99.30 ± 0.32	2.66	0.731	2.96	99.33 ± 0.84
NWS	-	0.20	-	-	-	0.22	-
SWS	4.0	3.97	99.25 ± 0.68	1.90	1.02	3.98	99.50 ± 0.58
NWS	-	0.20	-	-	-	0.21	-
SWS	5.0	4.98	99.60 ± 0.80	3.85	0.15	4.99	99.80 ± 0.42
Soil samples							
SSS	2.0	1.94	98.10 ± 1.65	2.96	0.40	1.95	98.50 ± 1.30
NSS	-	0.31	-	-	-	0.31	-
SSS	3.0	2.95	98.23 ± 0.70	3.00	1.16	2.95	98.73 ± 0.64
NSS	-	0.24	-	-	-	0.25	-
SSS	4.0	3.95	99.10 ± 1.46	2.22	0.89	3.96	99.45 ± 1.98
NSS	-	0.30	-	-	-	0.30	-
SSS	5.0	4.99	99.45 ± 0.77	1.88	1.05	4.98	99.40 ± 0.90

^aSamples collected around Amaraja Batteries,

^bIndustrial Estate, Renigunta,

^cChandragiri,

^dn=4,

^eP≥0.000284,

^fP= 0.05

Table 4: Analytical data for the determination of copper(II) with FBBT in various water and soil samples.

Accuracy

The accuracy of the proposed method was evaluated by comparing the results with those obtained by the AAS method. The results showed in Tables 3 and 4 reveals that the good correlation between the two methods indicative of present method was almost sensitive to that of AAS method.

Detection limit

Under optimized conditions the detection limit for determination of copper (II) by using present method (signal to noise ratio = 2) was 0.043 µg L⁻¹ and 0.036 µg L⁻¹ with HNBISNH and FBBT respectively.

Analytical applications

The present method was applied to the determination of copper (II) in different water and soil samples using new synthesis ligands, HNBISNH/FBBT. This method was comparable with the standard AAS method in terms of students 't' test and 'f' test as shown in Tables 3 and 4. The analytical data summarized in Tables 3 and 4 suggest that the percentage of copper (II) recovery from water and soil samples ranges from 96.00 to 99.80% which is more reliable and sensitive than the other methods. The recovery percentage of the copper (II) represented in the Tables 3 and 4 indicates that their order in various environmental systems under study are as follows:

Water samples with FBBT > Water samples with HNBISNH

Soil samples with FBBT > Soil samples with HNBISNH

It is evident from the previous data that the proposed method was simple, rapid and sensitive for the determination of copper (II) in different samples of environmental importance. The results

showed that the calculated values (Tables 3 and 4) did not exceed the theoretical values. Therefore, there is no significant difference between the proposed and the standard method, indicating that the developed method is as accurate and precise as the standard AAS method.

Biological activity of the complexes

Antibacterial activity of copper (II)-HNBISNH/FBBT synthesized complexes were studied against the gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) at a concentration of $10\mu\text{g mL}^{-1}$ by disc diffusion method. The antibacterial activity was more effective with copper (II)-FBBT synthesized complex with 23.2 mm to 16.5 mm Inhibition Zone than copper (II)-HNBISNH synthesized complex with an inhibition zone of 17.5 to 14.2 mm; the antibacterial activity of both the complexes was more when compared to that of the control Gentamycin – Standard with 9.33 mm to 12.26 mm Inhibition Zone. In this experiment, copper (II)-HNBISNH synthesized complex, *S.aureus* shows more activity with an inhibition zone of 17.5 mm followed by *P.aeruginosa* (16.4 mm), *E.coli* (15.2 mm) and *B.subtilis* (14.2 mm). In case of copper (II)-FBBT synthesized complex, *B.subtilis* shows effective antibacterial activity with an inhibition zone of 23.2 mm; *S.aureus* 22.5 mm and *Escherichia coli* 18.3 mm; followed by *P.aeruginosa* 16.5 mm. Therefore, the antibacterial activity of the synthesized complexes of copper (II)-HNBISNH, copper (II)-FBBT and control was in the order of copper (II)-FBBT complex > copper (II)-HNBISNH complex > Control as shown in Table 5. Both the ligands show Minimum Inhibitory Concentrations at very low concentrations against all pathogens ranging between 0.078 to 1.25 mg. The least concentration was against *Bacillus subtilis* with copper (II)-FBBT synthesized complex all the results are shown in Figure 7. Therefore the present findings may also be open a new search for the complexes for use in bacterial diseases.

Organism	Antimicrobial activity (IZ diameter in mm) 10 μg / disc		Control 10 μg / disc
	Cu(II)-HNBISNH complex*	Cu (II)-FBBT complex*	Gentamycin
<i>Bacillus subtilis</i>	14.2 \pm 0.14	23.2 \pm 0.09	11.76 \pm 0.555
<i>Staphylococcus aureus</i>	17.5 \pm 0.16	22.5 \pm 0.04	9.33 \pm 0.471
<i>Escherichia coli</i>	15.2 \pm 0.16	18.3 \pm 0.12	9.33 \pm 0.235
<i>Pseudomonas aeruginosa</i>	16.4 \pm 0.16	16.5 \pm 0.16	12.26 \pm 0.188

IZ = inhibition zone, *Standard Deviation, \pm Mean of Triplicates

Table 5: Results of the antibacterial activity of the synthesized complexes in mm.

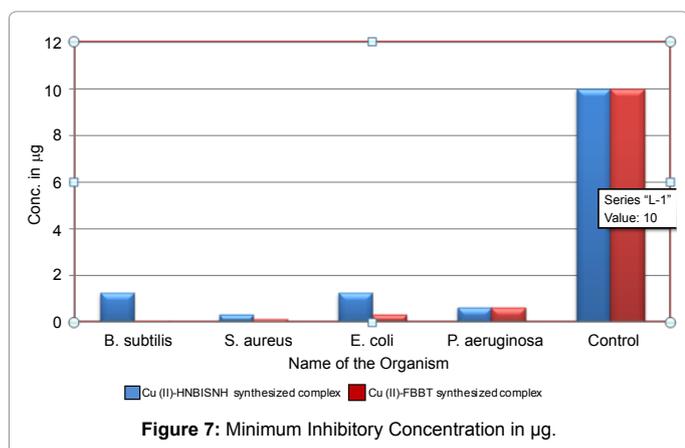


Figure 7: Minimum Inhibitory Concentration in μg .

Conclusion

In this present investigation, evaluation of two ligands, HNBISNH and FBBT were successfully study the analytical performance (complex affinity) experimentally. Due to the high complexing ability of the ligands, the developed analytical method/applications for the determination of copper (II) become facile, sensitive, rapid and selective. This non-extractive spectrophotometric method is eco-friendly and effectively applied to analyze copper (II) in different water and soil samples. Moreover, antibacterial activity copper (II)-FBBT synthesized complex was greater than that of copper (II)-HNBISNH. This antibacterial activity facilitates the biological importance of the synthesized ligands to control diseases effectively.

Acknowledgements

BNK is grateful for the financial support provided by the University Grant Commission, New Delhi, India in the form of JRF-BSR fellowship to carry out this work. One of the authors is highly thankful to the Department of Botany, S.V. University, India for biological evaluation of the compounds and complexes.

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