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Spectrophotometric Determination of Cadmium(II) in Water and Soil Samples Using Schiff's Bases

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A simple and rapid method was developed with the two novel Schiff's base ligands, (E)-N'-(2-hydroxy-5-nitrobenzylidene)isonicotinoylhydrazine and 2-(4-fluoro benzylideneamino)benzenethiol for monitoring the cadmium(II) in different water and soil samples. The two ligands react with cadmium(II) at pH 4.9/5.7 to form pale yellow/pale brown complexes with stoichiometric ratios of 1:1 (M:L). The complexes obeyed Beer's law in the range of 2.0 and 2.5 mg L⁻¹ with an excellent linearity in terms of the correlation coefficient of 0.99. The molar absorptivity and Sandell's sensitivity of the complex systems were found to be 3.68×10^4 , 4.32×10^4 L mol⁻¹ cm⁻¹ and 0.00298, 0.0034 µg cm⁻², respectively. The limit of detection for cadmium(II) was noted as 0.042 and 0.063 µg L⁻¹, respectively for these ligands. Furthermore, *in vitro* antimicrobial activities of both ligands and their complexes were successfully examined and reported.

Keywords: Schiff's base, Cadmium(II), Spectrophotometric determination, Water and soil samples, Biological activity.

INTRODUCTION

The widespread use of industrial technology in most cases results in the continuous increase in pollution, so that a great effort has been devoted to minimizing these hazardous pollutants. Cadmium is known as a prevalent toxic metal occurring naturally in the environment. People effected with cadmium contact through air, food and water show major health risks such as kidney, liver and lung failures. In addition to this, major damage was noticed to the cardiovascular, immune and reproductive systems [1,2]. Cadmium is efficiently retained in the kidney (half-life of 10-30 years) and the concentration is proportional to that in urine [3].

Thus monitoring of trace amount of Cd(II) in the environmental matrices is crucial. Previously numerous chelating agents such as derivatives of sulphonic acid [4], luminol [5], resorcinol [6], naphthol [7], amino phenols [8], amino benzene [9], quinolinol [10] hydroxy quinoline [11] and thiosemicarbazones derivatives [12-15] has been reported for the analysis of Cd(II) in different environmental matrices by using different analytical instruments. In addition to these reported ligands, the proposed complexing agents like the derivatives of isonicotinoyl hydrazide and benzene thiol compounds contain strong complexing ability due to the presence of electron donors (nitrogen and sulphur atoms) apart from its widely known medicinal and agricultural applications.

So far various analytical techniques have been reported for the determination of Cd(II) in real matrices such as voltammetry [16], electro chemical methods [17,18], atomic absorption spectroscopy (AAS) [19,20], inductively coupled plasma atomic emission spectroscopy (ICP-AES) [21] and inductively coupled plasma optical emission spectroscopy (ICP-OES) [22]. Usually, such techniques involves amendment steps and are more expensive to quantify [23]. Among those, spectrophotometric methods are cost effective and easy to handle with comparable sensitivity. Thus in this study, spectrophotometric techniques was preferred for routine analysis of Cd(II) in different water and soil samples. In the presence of acetate buffer, Cd(II) forms pale yellow/pale brown colour complex with (E)-N'-(2-hydroxy-5-nitrobenzylidene)isonicotinoylhydrazine (HNBISNH) and 2-(4-fluoro benzylideneamino)benzenethiol (FBBT) at pH 4.9 and 5.7, respectively. Various optimal parameters such as ligand concentration, effect of pH, reaction time and the effect of temperature was monitored to improve the sensitivity of the method. The results obtained were in good agreement with the flame atomic absorption spectroscopic (FAAS) method and it also offers advantages like reliability and reproducibility in addition to its simplicity, instant colour development and less interference.

EXPERIMENTAL

Systronics, UV-visible double beam spectrophotometer-117 (Ahmedabad, India). AA-6300, Shimadzu, FAAS

instrument (Tokyo, Japan). Perkin-Elmer, Infrared double beam spectrometer and An ELICO model Li-129, pH meter.

Analytical reagent grade chemicals were used throughout the experiment (SD Fine Chemicals, Mumbai, India) and all the standards were prepared with deionized water. Stock solutions (0.1 mol L^{-1}) were made by dissolving 2.86 and 2.31 g of (E)-N'-(2-hydroxy-5-nitrobenzylidene)isonicotinoylhydrazide (HNBISNH) and 2-(4-fluorobenzylideneamino)benzenethiol (FBBT) in 100 mL deionized water and on further dilution working standard solutions were prepared. 3.928 g of $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ was dissolved in 100 mL deionized water containing a few drops of concentrated sulphuric acid to prepare the stock solution and standardized with standard methods. Solutions of alkali metal salts (1 %) and various metal salts (0.1 %) were used to study the excipients effect.

Synthesis and characterization of HNBISNH and FBBT ligands: The two Schiff's base ligands HNBISNH and FBBT were prepared as per reported literature [24]. The procedure, synthesis of HNBISNH, accurately weighed 1.2 g of 2-hydroxy-5-nitrobenzaldehyde and 0.822 g of isonicotinoylhydrazide were weighed and dissolved in ethyl alcohol and refluxed for condensation in a round bottomed flask for about 2 h at 60°C . The yellow coloured solid of HNBISNH ligand was formed and further subjected to evaporation to give 1.98 g of yield. FT-IR spectrum of shows bands at 3450 cm^{-1} (O-H) symmetric, 3298 cm^{-1} (NH), 1664 cm^{-1} (C=O), 1643 cm^{-1} (NO_2) asymmetric, 1581 cm^{-1} (C=N) azomethine, 1550 cm^{-1} (NO_2) symmetric and 1242 cm^{-1} (C-O) stretchings.

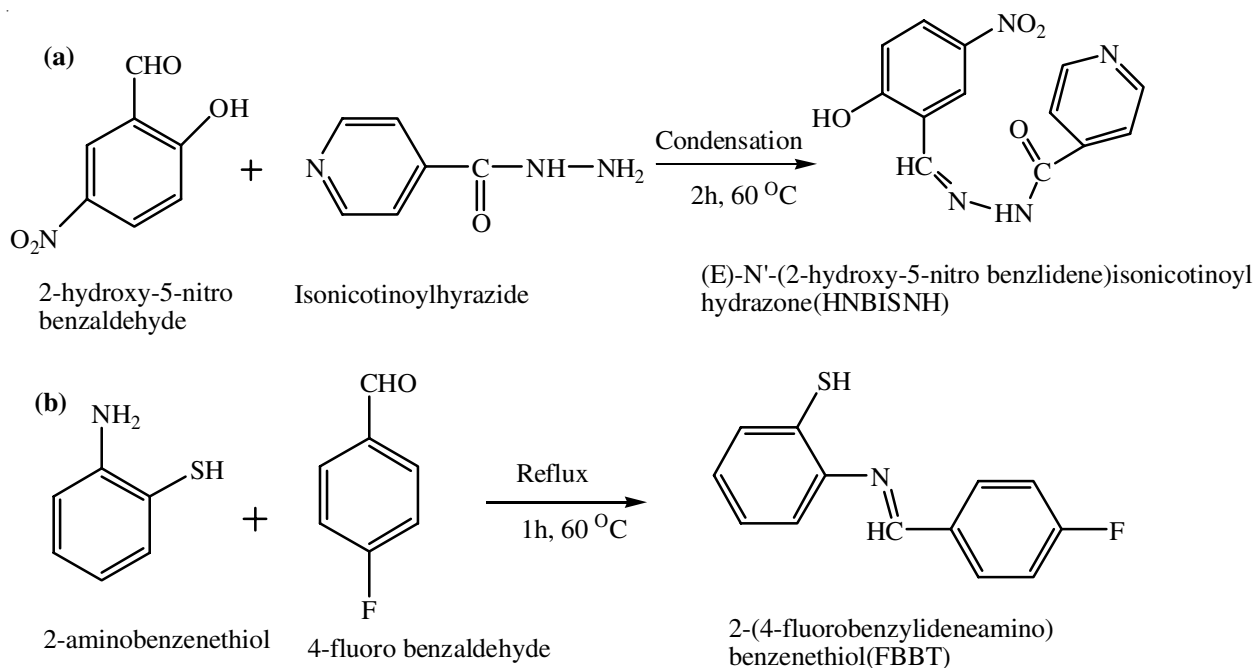
The FBBT was prepared by accurately measurement of 0.48 mL of 2-aminobenzenethiol and 0.48 mL of 4-fluorobenzaldehyde, which were then dissolved in dimethyl formamide and the contents were refluxed in a round bottomed flask for about 1h at 60°C . A pale yellow oily product was formed and evaporated at 160°C for 0.5 h. On standing for 24 h at ambient temperature, the product converted to a yellow

solid 1.89 g of yield. The FTIR spectrum shows at 3055 cm^{-1} (C-H) aromatic, 2550 cm^{-1} (S-H), 1602 cm^{-1} (C=N) azomethine, 1409 cm^{-1} (C-F) and 1296 cm^{-1} (C-N) stretchings. The structure of HNBISNH and FBBT were confirmed by FT-IR spectral data. The schematic reactions are shown in **Scheme-Ia** and **Ib**.

Synthesis of Cd(II)-HNBISNH/FBBT complexes: The HNBISNH/FBBT ligands were separately dissolved in ethanol and then aqueous cadmium nitrate tetrahydrate solution was added slowly with constant stirring. The resulting mixture was then refluxed on a condenser for about 0.5 h. The solid formed was separated out, filtered, washed with deionized water, followed by ether and dried in the oven to obtain Cd(II)-HNBISNH/FBBT complexes.

General procedure: 2 mL of $5 \times 10^{-3} \text{ M}$ HNBISNH and FBBT was separately decanted into a 10 mL standard flask, to this 4 mL of acetate buffer solution with pH 4.9/5.7 were added. An aliquot having $1.0\text{--}100 \mu\text{g mL}^{-1}$ of Cd(II) was successively added and the contents was diluted up to the mark with deionized water. The formed pale yellow and pale brown coloured chromophores absorbance were measured at 400 to 570 nm against the blank solution. The blank was prepared in the same way except the addition of Cd(II). The whole experiment was conducted at room temperature. The unknown sample strength was calculated by using a calibrated graph.

Antimicrobial activity of HNBISNH/FBBT and Cd(II)-HNBISNH/FBBT complexes were carried out with *Bacillus subtilis* and *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* strains using agar well diffusion method. Nutrient agar was inoculated with the selected microorganisms by spreading the bacterial inoculums in the media. Wells (9 mm diameter) were punched in the agar and filled with ligands and metal complexes at various concentrations (50, 100, 150 and $200 \mu\text{g mL}^{-1}$). Control wells containing pure ligands and standard antibiotic (positive control) viz.,



Scheme-Ia and Ib: Preparation pathway for (E)-N'-(2-hydroxy-5-nitrobenzylidene) isonicotinoyl hydrazide (HNBISNH) and 2-(4-fluoro benzylideneamino)benzenethiol (FBBT)

ampicillin, nystatin. The plates were incubated at $37 \pm 2^\circ\text{C}$ for 24 h for bacterial activity. The antimicrobial activity was assessed by measuring the zone of inhibition in mm for the respective drug. The experiment was done in triplicates and the mean values are presented along with the standard deviation [25].

Real sample preparation: The tap water and industrial wastewater samples were collected in 1 L polypropylene bottles from various industrial areas nearby the temple town of Tirupati, India. Each water sample was primarily acidified with 5 mol L^{-1} nitric acid and then filtered over a $0.45\text{ }\mu\text{m}$ filter. The samples were pre-heated at 100°C for about 1.5 h and then vanished to 250 mL for analysis.

2 g of homogeneous soil samples were weighed and digested with aqua regia on hot plate at 100°C for 1 h, till the clear transparent solution was obtained, after evaporation of silica residues from the sample, the solution was cooled to room temperature and filtered through a $0.45\text{ }\mu\text{m}$ pore size membrane filter into a 50 mL beaker. The pH of the solution was adjusted to the desired value. The standard addition method was employed to quantify the Cd(II) in spiked and real samples.

RESULTS AND DISCUSSION

Method optimization: The absorption spectrum of HNBISNH/FBBT and Cd(II)-HNBISNH/FBBT complexes were scanned with UV-visible spectrophotometer. The absorption maximum of reagent blanks was measured at 370 and 395 nm, whereas Cd(II)-HNBISNH/FBBT complexes gave an absorption peak at 460 and 520 nm, respectively. Hence the wavelength for maximum absorption was fixed at 460 and 520 nm for all the subsequent studies as illustrated in Fig. 1a. The influence of pH on the chromophoric system was studied

ranging from 2-7. The absorbance *versus* pH graph (Fig. 1b) shows maximum and constant absorbance for Cd(II)-HNBISNH/FBBT were obtained at pH 4.9 and 5.7, respectively. The effect of the ligand concentration (HNBISNH and FBBT) at optimum pH (4.9/5.7) was studied with solutions containing $50\text{ }\mu\text{g Cd(II)}$ in an acetate buffer system. The concentrations of HNBISNH and FBBT varied from $0.001\text{--}0.10\text{ mol L}^{-1}$. The maximum absorbance for HNBISNH and FBBT were obtained at 0.03 mol L^{-1} and 0.05 mol L^{-1} , respectively (Fig. 1c). The reaction was rapid for both complexes [Cd(II)-HNBISNH/FBBT], but the chromophoric system achieved maximum absorbance at ambient temperature ($32 \pm 5^\circ\text{C}$).

Stability and composition of the chromophoric system:

The absorbance of Cd(II)-HNBISNH/FBBT complexes were measured at different intervals to ascertain the time stability of the complexes. It was observed that colour development was instantaneous ($< 1\text{ min}$) and remained stable for 24 and 48 h with an average absorbance of 416 nm (SD %: 0.61) and 517 nm (SD %: 0.24) in aqueous solutions. Due to the stability and clarity of complex solutions, the extraction step was omitted to minimize the environmental hazardous solvents in this experiment. The stoichiometry of the complexes was established using the molar ratio method at the optimum conditions. The results showed that maximum absorption was at a molar ratio of 1.0 for HNBISNH/FBBT concentration, representing the interaction of one metal with the ligand molecule in the complex formation. Accordingly, the results indicated that the stoichiometric ratio was 1:1 (M:L).

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

Ringbom's plot is the standard protocol adopted to know the optimum range of concentration for a chromophoric system that obeys Beer's law. The plots were drawn between $\log C$ of

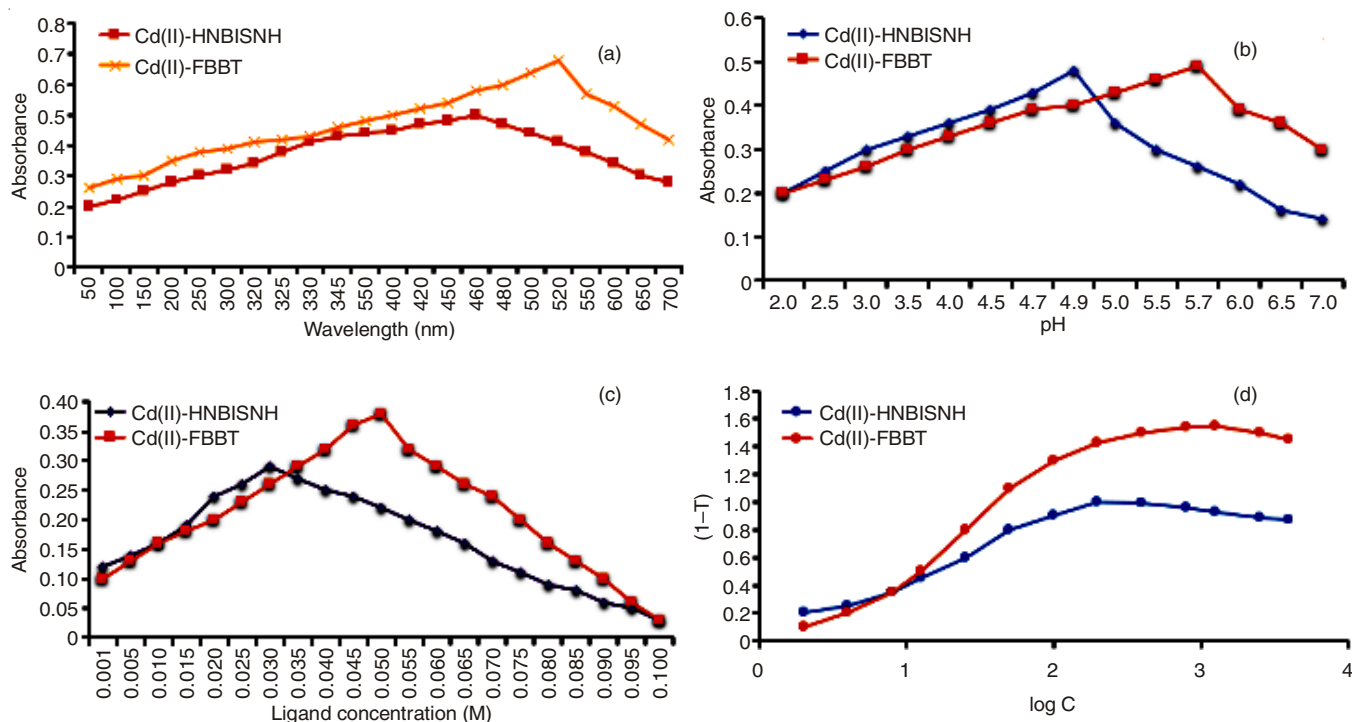


Fig. 1. (a) Absorption spectra of Cd(II)-HNBISNH/FBBT complex (b) Effect of pH for Cd(II)-HNBISNH/FBBT complex (c) Effect of ligand concentration for Cd(II)-HNBISNH/FBBT complex (d) Ringbom plot for Cd(II)-HNBISNH/FBBT (Optimum conditions: pH: 4.9/5.7, Ligand concentration: $0.03/0.05\text{ mol L}^{-1}$, Temperature: $32 \pm 5^\circ\text{C}$)

Cd(II) and (1-T) (where T is the transmittance). The plots were sigmoid shaped with a linear segment at intermediate absorbance values 0.1-1.0, 0.2-1.6 and concentration values 1.25-3.3 and 1.29-3.6 $\mu\text{g mL}^{-1}$ for cadmium(II)-HNBISNH and cadmium(II)-FBBT, respectively. From Fig. 1d the slopes were calculated and found to be 0.1554 and 0.1721 for Cd(II)-HNBISNH/FBBT complexes, respectively. Hence, the ratio between the relative error in concentration and photometric errors are 2.00, 1.80 for found to be concentration of 0.0200, 0.018 having 1.0 % photometric error.

Figures of merit: Cd(II)-HNBISNH/FBBT complexes obeyed Beer's law up to 2.0 and 2.5 mg L^{-1} with an optimum concentration range between 0.287-3.84 and 0.324-4.21 mg L^{-1} . The molar absorptivity of complexes was calculated to be 3.68×10^4 and $4.32 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ at 460 and 520, respectively. Sandell's sensitivity were found to be 0.0029 and 0.0034 $\mu\text{g cm}^{-2}$ with a correlation coefficient (r^2) 0.9982 and 0.9991. The stability constants found to be 4.5×10^{15} and 6.2×10^{15} , respectively. The optical characteristics for Cd(II)-HNBISNH/FBBT complexes are presented in Table-1.

Optical characteristics	HNBISNH	FBBT
Colour	Pale yellow	Pale brown
λ_{max} (nm)	385	413
pH range (optimum)	4.9	5.7
Stability (h)	24	48
Beer's law range (mg L^{-1})	0.287-3.84	0.324-4.21
Molar absorptivity ($\text{L mol}^{-1} \text{ cm}^{-1}$)	3.68×10^4	4.32×10^4
Sandell's sensitivity ($\mu\text{g cm}^{-2}$)	0.0029	0.0034
Regression equation (Y^b) Slope ^a	0.1554	0.1721
Intercept ^b	0.0178	0.0173
Correlation coefficient (r^2)	0.9982	0.9991
Relative standard deviation (%) ^c	0.321	0.410
Range of error (95 % confidence level)	± 1.94	± 1.092
Detection limit ($\mu\text{g L}^{-1}$)	0.042	0.063
% Error	0.092	0.098

^aExperiments performed under optimized conditions (see text), ^b $Y = ax + b$, where x is the concentration of analyte in $\mu\text{g mL}^{-1}$, ^c $n = 4$

Effect of excipients: The effect of excipients on the determination of Cd(II) using HNBISNH/FBBT was studied under optimal conditions. The results presented in Table-2 indicate that most of the common ions does not interfere with the Cd(II) determination. Therefore, the selectivity of this method is fairly satisfactory and the tolerance limits of interfering ions in the determination of 0.2 $\mu\text{g mL}^{-1}$ Cd(II) is taken as the amount causing an error of $\pm 2\%$ at the peak height.

Biological activity of the complexes: The effective antibacterial activity of both Cd(II)-HNBISNH/FBBT complexes were noticed by Gram-negative bacteria *Pseudomonas aeruginosa* showing 17.1 ± 0.20 with Cd(II) HNBISNH and 23.0 ± 0.08 with Cd(II) FBBT complexes followed by *E. coli*, *B. subtilis* and *S. aureus*. A comparison was made for the free ligands 8.3 ± 0.21 (HNBISNH), 8.5 ± 0.12 (FBBT) and with the control ampicillin 10.8 ± 0.08 . Moreover both Cd(II)-HNBISNH/FBBT complexes show substantially more effective antibacterial activity than that of the standard drug ampicillin

TABLE-2
TOLERANCE LIMIT OF EXCIPIENTS FOR
DETERMINATION OF Cd(II) (0.20 μg)
WITH HNBISNH AND FBBT

Excipients	Tolerance limit ($\mu\text{g mL}^{-1}$)	Excipients	Tolerance limit ($\mu\text{g mL}^{-1}$)
K^{2+}	1.00	Ni^{2+}	0.04
Mg^{2+}	0.50	Na^+	0.20
Ca^{2+}	0.50	SO_4^{2-}	0.05
Cl^-	0.20	Cr^{3+}	0.15
Al^{3+}	0.08	Fe^{3+}	0.30
Cd^{2+}	0.05	NO_3	0.10
Co^{2+}	0.05	Pb^{2+}	0.01
Zn^{2+}	0.05	—	—

(Table-4). However, the individual ligands have less activity when compared to their metal complexes. It is evident that Cd(II) FBBT complex shows good antibacterial activity than Cd(II) HNBISNH complex. Hence the present findings may also open a new search for the complexes investigated to cure the bacterial diseases.

Method evaluation: The developed method was evaluated in terms of reproducibility, accuracy and detection limits. To test the reproducibility of present method, four repetitive analyses of each sample was studied. The RSD (%) values for Cd(II)-HNBISNH/FBBT were found to be 0.566 and 0.542 and the SD (%) ranged from 0.36-1.86 as summarized in Table-3. The accuracy was evaluated comparing the obtained results with FAAS method. The results (Table-3) revealed that the good correlation between the two methods, confirms the sensitivity of present method over the FAAS method. Under optimized conditions the detection limits (signal to noise ratio = 2) were found to be 0.042 and 0.063 $\mu\text{g L}^{-1}$.

Analytical applications: The present method was successfully applied for the determination of Cd(II) in different water and soil samples. This method was comparable with the standard FAAS method in terms of students 't' test and 'F' test (Table-3). The analytical data summarized in Table-3 suggest that the percentage of Cd(II) recovery from water and soil samples ranges from 96.00 to 99.80 % which is more reliable and sensitive than the other methods. According to WHO (1993) the recommended dietary allowances for a 60 kg adult are reported as Cd 60 mg/d person [26,27]. It is evident from the previous data that the proposed method is simple, rapid and sensitive for the determination of Cd(II) in different samples of environmental importance. The results showed that the calculated values 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 FAAS method.

Conclusion

In the present investigation a simple, rapid, sensitive and reproducible method was proposed for the determination of Cd(II) using HNBISNH and FBBT in an aqueous medium. The ligands were found to be sensitive when compared to the earlier reagents reported in literature. The selectivity of the ligands was improved by the use of masking agents to suppress the excipients. The results of the proposed method are comparable with FAAS. Additionally, antibacterial activity

TABLE-3
ANALYTICAL DATA FOR THE DETERMINATION OF Cd(II) IN VARIOUS WATER AND SOIL SAMPLES

Samples	HNBISNH						
	Present method					FAAS method	
	Cd(II) (µg L ⁻¹)					Found	Recovery ± SD ^d
	Added	Found	Recovery ± SD ^d	t-Test ^e	F-Test ^f		
Water samples							
SWS ^a	2.0	1.90	96.39 ± 0.26	6.23	2.22	1.89	96.85 ± 0.34
NWS ^a	—	0.18	—	—	—	0.19	—
SWS ^b	3.0	2.84	97.46 ± 0.32	2.23	0.56	2.86	97.72 ± 0.26
NWS ^b	—	0.20	—	—	—	0.21	—
SWS ^c	4.0	3.85	97.79 ± 0.24	1.62	0.88	3.92	98.56 ± 0.65
NWS ^c	—	0.19	—	—	—	0.20	—
SWS	5.0	4.88	98.21 ± 0.35	3.36	0.89	4.88	99.60 ± 0.36
Soil samples							
SSS ^a	2.0	1.94	96.69 ± 1.02	3.12	0.58	1.94	76.86 ± 1.12
NSS ^a	—	0.28	—	—	—	0.29	—
SSS ^b	3.0	2.92	97.03 ± 0.55	4.28	1.31	2.93	97.78 ± 0.56
NSS ^b	—	0.10	—	—	—	0.11	—
SSS ^c	4.0	3.92	96.29 ± 1.23	2.58	0.86	3.94	98.05 ± 1.22
NSS ^c	—	0.31	—	—	—	0.32	—
SSS	5.0	4.96	98.63 ± 0.23	1.96	0.40	4.97	99.10 ± 0.20
FBBT							
Water samples							
SWS ^a	2.0	1.89	98.89 ± 0.23	7.00	2.78	1.90	98.89 ± 0.42
NWS ^a	—	0.22	—	—	—	0.22	—
SWS ^b	3.0	2.85	99.12 ± 0.72	2.33	0.69	2.56	99.22 ± 0.36
NWS ^b	—	0.21	—	—	—	0.22	—
SWS ^c	4.0	3.88	99.33 ± 0.36	1.76	0.88	3.89	99.36 ± 0.72
NWS ^c	—	0.19	—	—	—	0.19	—
SWS	5.0	4.85	99.65 ± 0.42	3.52	0.98	4.86	99.65 ± 0.42
Soil samples							
SSS ^a	2.0	1.90	98.12 ± 1.26	3.00	0.48	1.91	98.26 ± 1.34
NSS ^a	—	0.25	—	—	—	0.29	—
SSS ^b	3.0	2.92	98.54 ± 0.62	4.02	1.12	2.93	98.62 ± 0.30
NSS ^b	—	0.24	—	—	—	0.24	—
SSS ^c	4.0	3.90	99.05 ± 1.46	2.22	0.78	3.91	99.15 ± 1.32
NSS ^c	—	0.29	—	—	—	0.28	—
SSS	5.0	4.92	99.56 ± 0.57	1.88	0.96	4.94	99.62 ± 0.76

^aSamples collected around Amaraja Batteries, ^bIndustrial Estate, Renigunta, ^cChandragiri, ^dn = 4, ^eP $\geq$ 0.000284, ^fP = 0.059; SWS: Sewage water sample; NWS: Normal water sample; SSS: Sewage soil sample; NSS: Normal soil sample.

TABLE-4
ANTIBACTERIAL ACTIVITY OF THE HNBISNH/FBBT AND Cd(II)-HNBISNH/FBBT AGAINST CONTROL (AMPICILLIN)

Ligand/complex	Organisms	Inhibition zone (mm)			
		50 $\mu\text{g/mL}$	100 $\mu\text{g/mL}$	150 $\mu\text{g/mL}$	200 $\mu\text{g/mL}$
Cd(II)-HNBISNH	<i>S. aureus</i>	8.5 $\pm$ 0.08	9.2 $\pm$ 0.09	11.0 $\pm$ 0.08	12.7 $\pm$ 0.12
	<i>B. subtilis</i>	9.8 $\pm$ 0.08	10.7 $\pm$ 0.12	12.8 $\pm$ 0.14	14.4 $\pm$ 0.41
	<i>E. coli</i>	12.7 $\pm$ 0.09	14.0 $\pm$ 0.08	15.7 $\pm$ 0.04	16.9 $\pm$ 0.40
	<i>P. aeruginosa</i>	12.5 $\pm$ 0.09	14.3 $\pm$ 0.12	15.4 $\pm$ 0.14	17.1 $\pm$ 0.20
Cd(II)-FBBT	<i>S. aureus</i>	11.4 $\pm$ 0.08	12.7 $\pm$ 0.12	13.8 $\pm$ 0.04	14.5 $\pm$ 0.08
	<i>B. subtilis</i>	13.8 $\pm$ 0.04	19.5 $\pm$ 0.04	20.0 $\pm$ 0.04	21.8 $\pm$ 0.08
	<i>E. coli</i>	19.0 $\pm$ 0.12	19.5 $\pm$ 0.04	20.0 $\pm$ 0.04	21.8 $\pm$ 0.08
	<i>P. aeruginosa</i>	18.8 $\pm$ 0.09	20.8 $\pm$ 0.09	21.9 $\pm$ 0.04	23.0 $\pm$ 0.08
HNBISNH	<i>S. aureus</i>	7.1 $\pm$ 0.08	7.4 $\pm$ 0.16	8.0 $\pm$ 0.16	9.2 $\pm$ 0.16
	<i>B. subtilis</i>	6.3 $\pm$ 0.21	7.2 $\pm$ 0.09	7.5 $\pm$ 0.12	8.6 $\pm$ 0.16
	<i>E. coli</i>	5.9 $\pm$ 0.16	7.0 $\pm$ 0.14	7.6 $\pm$ 0.18	8.6 $\pm$ 0.16
	<i>P. aeruginosa</i>	6.7 $\pm$ 0.09	7.6 $\pm$ 0.16	7.6 $\pm$ 0.12	8.3 $\pm$ 0.21
FBBT	<i>S. aureus</i>	7.1 $\pm$ 0.20	7.6 $\pm$ 0.14	8.4 $\pm$ 0.04	8.8 $\pm$ 0.08
	<i>B. subtilis</i>	7.5 $\pm$ 0.12	7.6 $\pm$ 0.20	8.2 $\pm$ 0.16	8.3 $\pm$ 0.04
	<i>E. coli</i>	7.2 $\pm$ 0.18	7.8 $\pm$ 0.08	8.3 $\pm$ 0.08	8.1 $\pm$ 0.08
	<i>P. aeruginosa</i>	7.7 $\pm$ 0.21	7.8 $\pm$ 0.40	8.3 $\pm$ 0.21	8.5 $\pm$ 0.12
Control (Ampicillin)	<i>S. aureus</i>	9.7 $\pm$ 0.08	9.6 $\pm$ 0.12	10.2 $\pm$ 0.09	10.4 $\pm$ 0.12
	<i>B. subtilis</i>	11.3 $\pm$ 0.08	11.4 $\pm$ 0.08	11.7 $\pm$ 0.16	11.8 $\pm$ 0.08
	<i>E. coli</i>	12.4 $\pm$ 0.12	12.5 $\pm$ 0.16	12.8 $\pm$ 0.08	13.2 $\pm$ 0.16
	<i>P. aeruginosa</i>	10.3 $\pm$ 0.08	10.5 $\pm$ 0.12	10.8 $\pm$ 0.08	10.8 $\pm$ 0.08

of Cd(II)-FBBT complex was greater than that of Cd(II)-HNBISNH. This antimicrobial activity facilitates the biological importance of the synthesized ligands to control diseases effectively.

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