

Spectrophotometric Method for Determination and Biological Activity of Trace Amount of Cd(II) by bis(2-((pyridin-2-ylimino)methyl)phenyl)-4,4'-(diazene-1,2-diyl)dibenzoate

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Abstract: A heterocyclic Schiff base ligand bis(2-((pyridin-2-ylimino)methyl)phenyl)-4,4'-(diazene-1,2-diyl)dibenzoate (BPMPD) and its cadmium complex have been synthesized and characterized through UV-Visible, FTIR, ¹HNMR and elemental analysis. The absorbance of forming complexes at λ_{max} of 488 nm, molar absorptivity ($L \cdot mol^{-1} \cdot cm^{-1}$) is 3.488×10^4 , detection limit ($\mu g/ml$) is (0.010066396) and stability constant is 1.7×10^7 . For determination of Cd(II) (0.1-4 ppm) at pH=8. Sequence of addition, conductivity measurements, the effect of Interferences and biological activity were also studied. This method applied to the determination in Cd(II) in water samples and the results compare with a flame atomic absorption spectrophotometer.

Keywords: Schiff base, cadmium complex, absorbance, water samples and flame atomic absorption spectrophotometer.

1. Introduction

Schiff base ligands are theoretically accomplished by forming stable complexes with different metal ions [1]. Because of the simplistic production of Schiff bases, various ligands of diverse structure types have been synthesized, it's can put up dissimilar metal centers involving various coordination modes by this means agree to popular synthesis of homo and hetero metallic complexes with different stereochemistry [2]. Currently, Schiff bases are appealing to chemists as they are known to be medically significant and are used to propose pharmaceutical compounds [3]. It has been informed that the biologically active compounds display greater activity when managed as metal complexes than as free organic compound [4]. The biomedical possessions of free organic molecule upon chelation with right metal ion led to the carrying out of metal complexes for several biomedical applications as antipyretic [5], therapeutically active, possessing analgesic [6], cytotoxic [7], anti-inflammatory [8], antiviral [9], antitubercular activity [10]. Since the above details in view, a heterocyclic Schiff base ligand (BPMPD) and its complex with Cd(II) has been synthesized and characterized.

2. Experimental

2.1 Materials and Measurements

All chemicals of highest purity were gotten from Sigma-Aldrich. Spectrophotometric measurements were through with Shimadzu / Japan UV-Visible-1650Pc double beam. The FTIR measurements were through in Shimadzu 8400 Series Japan. ¹HNMR spectrum of the ligand was Mercury Plus 300MHz NMR spectrometer in DMSO-*d*₆. CE440 CHN/O/S Elemental Analyser was made by EAI. Atomic Absorption Spectrophotometer Flame Model Shimadzu/Japan AA-7000F. The pH measurements were made with a "HANNA pH meter H19841-5 Instrument. Electric molar conductivity measurements were made at

room temperature using an Alpha digital conductivity model-UK 9300.

2.2. Synthesis of ligand BPMPD

Synthesis of ligand BPMPD was began from the starting material 4-nitrobenzoic acid as shown in Scheme 1, to synthesis of 4,4'-(diazene-1,2-diyl)dibenzoic acid was made as per literature [11] method, which give bis(2-formylphenyl)4,4'-(diazene-1,2-diyl)dibenzoate. Then, take from this product (2 mmol) was condensed with 2-aminopyridine (4 mmol) in methanol solution (100 ml). The mixture was refluxed for 10 hours. A light orange color precipitates were found, which were filtered and washed with methanol followed by ether and dried over anhydrous CaCl₂. The simple product was recrystallized from hot methanol, which gave pure ligand of bis(2-((pyridin-2-ylimino)methyl)phenyl)-4,4'-(diazene-1,2-diyl)dibenzoate (BPMPD) with a yield of 41%, calculated from the starting compound 4-nitrobenzoic acid. Cadmium complex was prepared by adding (1×10^{-3} M) of BPMPD solution in methanol, was drop wise to a stirred solution of Cd(II) (4 ppm) at room temperature and then, the mixture was refluxed for five hours. The precipitate, light green, was filtered and washed with water, followed by methanol, ether and then dried in the vacuum, yield in %, Cd-BPMPD = 40.

the compound in distilled water completing the volume to 100 ml in a volumetric flask.

2.4. Procedure

To get highest absorbance of complex made, it is necessary to develop optimum conditions of forming this complex, which include, the selection of the suitable wavelength (λ_{\max}), effect of pH values, effect of time, effect of sequence of addition and effect of interferences of ions. The general procedure was summarized by taking (0.1 – 4 ppm) of Cd(II) ions with (2 ml) of 1×10^{-3} M of BPMPD then the volume was completed to 10 ml. After 5 min, the absorbance of forming complexes was measured at λ_{\max} of 488nm .

3. Results and Discussion

3.1. Synthesis of ligand BPMPD

In the FTIR spectrum of BPMPD, the stretching vibrational band due to ν C=N band at 1640 cm^{-1} of imine was disappeared and appearance of characteristics imine ν C=O at 1690 cm^{-1} of dialdehyde was observed. A sharp band due to ν C=O of the ester groups was observed at 1734 cm^{-1} . Another band obtained at 2896 cm^{-1} was attributed to ν C-H of the azomethine groups. These observations confirm the condensation of dialdehyde with the 2-aminopyridine, primary amine. Two bands for in-plane and out of plane pyridine ring deformation were observed at 624 and 421 cm^{-1} , respectively. The cadmium complex showed a sharp band in the region of 1532 cm^{-1} which is attributed to the ν C=N of azomethine groups, which was shifted towards lower frequency compared to the free ligand band representative the coordination of azomethine nitrogens in the complex. A negative shift in the frequency of ν C=O of the ester groups 1618 cm^{-1} in the spectra of corresponding cadmium complex indicate the coordination of carbonyl oxygens of the ligand to the central cadmium ion. The shift in the bands due to in plane and out of plane deformation of pyridine 661 - 442 cm^{-1} in case of cadmium complex indicate the coordination of pyridine nitrogens. In the fingerprint region, the advent of new bands in cadmium chelates at ~ 559 , ~ 446 and $\sim 331 \text{ cm}^{-1}$ may be attributed to the ν Cd-O, ν Cd -N and ν Cd -N(pyridine), respectively.

In ^1H NMR spectrum of BPMPD by using (DMSO-*d*₆), the presence of (2H, s, HC=N) for azomethine group is characterized as a singlet at $\delta = 8.74$ ppm. At $\delta = 8.51$ ppm (8H, m, Pyridine ring), where at $\delta = 7.56$ ppm for (8H, m, C₆H₄) aromatic protons linked to the azomethine group. At $\delta = 8.37$ ppm for (8H, m, C₆H₄) azobenzene protons.

Elemental analysis (%): Anal. Calc. for C₃₈H₂₆N₆O₄: C, 72.37; H, 4.16; N, 13.33; O 10.15 and found: C, 72.33; H, 4.11; N, 13.29 ; O 10.12.

3.2 Absorption Spectra

The electronic spectra of BPMPD and its complex with Cd(II) are given λ_{\max} wavelengths were fixed in (Fig .1) shows the absorbance λ_{\max} at 431 and 488nm respectively. It is flawless that giving to the red shift that occurred in λ_{\max}



Scheme 1, Synthesis of BPMPD and Cd-BPMPD Complex

2.3. Solutions

A stock standard cadmium solution (100 ppm) was prepared by dissolving 0.0237 gm of (CH₃COO)₂Cd.2H₂O, the volume was completed to 100 ml with distilled water. Solution of ligand BPMPD (1×10^{-3} M) was prepared by dissolving (0.0630 gm) and complete the volume to 100 ml with absolute methanol. Foreign ions solutions (100 ppm), these solutions were prepared by dissolving an amount of

show the stable complexes are formed immediately, π \rightarrow π^* transition within the azomethine group [12].

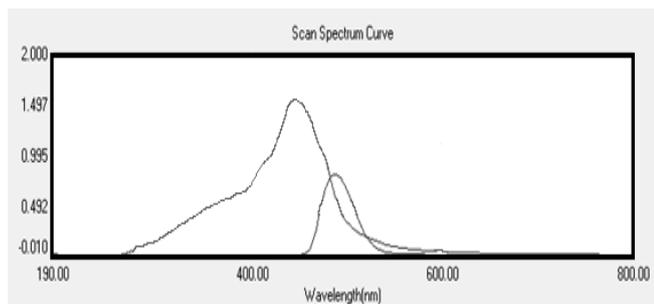


Figure 1: The absorbance λ_{max} of BPMPD and Cd- BPMPD Complex

3.3 Calibration Curve

A linear calibration curve for Cd-BPMPD complex was obtained, (Fig. 2) that Beers law is obeyed over the concentration range of (0.1-4 ppm) Cd(II) with R^2 equal to (0.9982). The results of investigative performance are summarized in Table 1.

3.4 The Effect of pH

The influence of pH value on the absorbance of Cd-BPMPD complex was studied at different pH (Fig. 3) by using of HCl and NaOH solutions (pH 2-10). It was found that the highest absorbance at pH 8, because of the formation of the anionic form of BPMPD, which can simply react with Cd(II) to form complex [13].

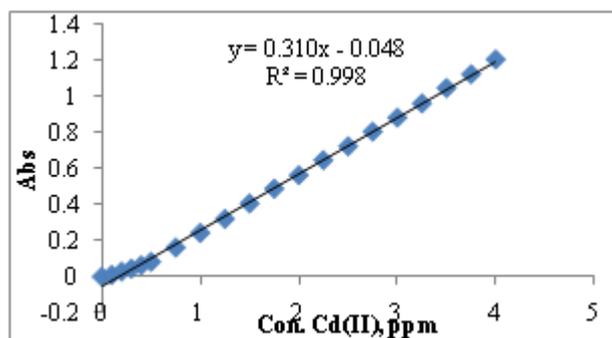


Figure 2: Calibration Curve for Cd-BPMPD Complex

Table 1: Analytical characteristics of Cd-BPMPD Complex

Characteristic	Cd(II)
Absorption maxima (λ_{max}) nm	488
Bee's law range ($\mu\text{g/ml}$)	0.1-4
Molar absorptivity ($\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$)	3.488×10^4
Sandell's sensitivity (μgcm^{-2})	2.869×10^{-8}
variance	6.66667×10^{-7}
Detection Limit ($\mu\text{g/ml}$)	0.010066396
% R.S.D	0.33554654
standard deviation	0.000816497
% Error	0.136986301
% Recovery	99.8630137
Stability constant	1.7×10^7

for six determinations

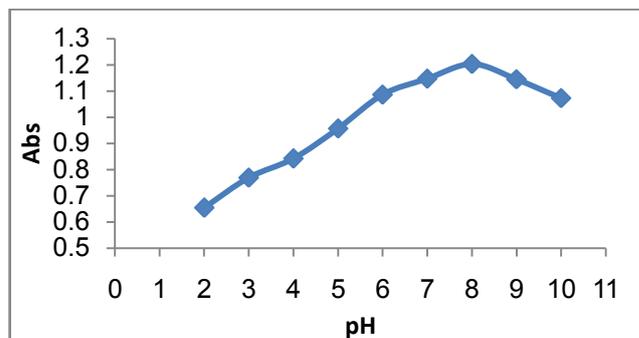


Figure 3: The Effect of pH on Absorbance of Cd-BPMPD complex

3.5. The Effect of Time

The stability of Cd-BPMPD complex with time was showed in (Fig. 4), from the data obtained it was created that the highest absorbance reached at 5 min and leftovers constant up 24 hrs.

3.6. The Effect of BPMPD Volume

Various volume of BPMPD (0.5-5 ml) of 1×10^{-3} M were added to a fixed amount of Cd(II) of 4 ppm (2 ml). It's found sufficient to change the color to it's higher intensity with a minimum blank value, the volume obtained 2 ml. (Fig. 5) show the effect of BPMPD volume with Cd(II) ions.

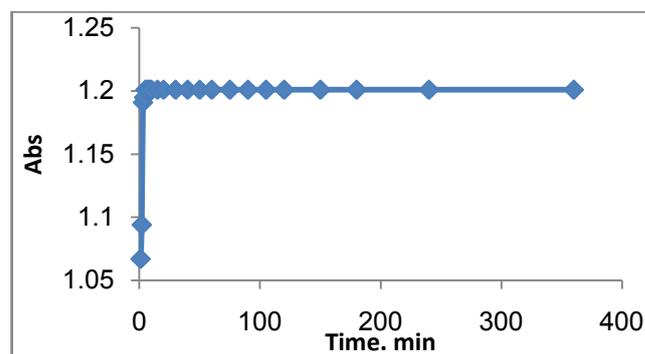


Figure 4: The Effect of Time on Absorbance of Cd-BPMPD complex

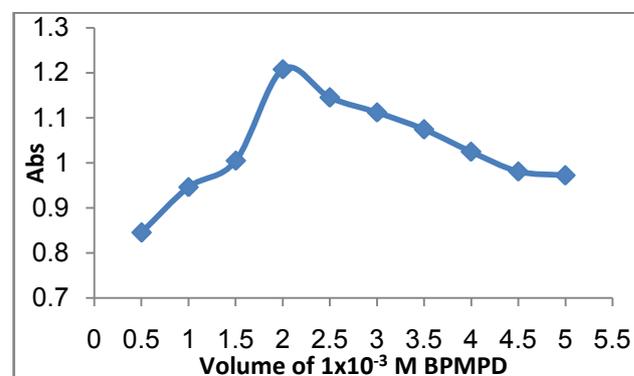


Figure 5: The Effect of BPMPD Volume with Cd(II).

3.7. Sequence of Addition

To obtain optimum results, the order of addition of BPMPD ligand must be followed as given under the method, if not a

loss in color intensity and stability were observed. Which are: [Cd(II) + BPMPD + pH] or [Cd(II)+ pH + BPMPD]. It is found that the first one offers more absorbance.

3.8. Conductivity Measurements

The Cd-BPMPD complex are insoluble in common organic solvents, but are soluble in methanol, DMSO and DMF, permitted of the molar conductivity at room temperature, the molar conductance values are 270.4, 254.7 and 247 ohm⁻¹cm²mol⁻¹, respectively.

3.9. Effect of Interferences

It was essential to investigate the effect of concomitant ions on the determination of cadmium according to this process. The results of intervention studies and the conditions of the research are shown in Table 2. Most of the ions have no interfering effect under the point out conditions. Hg(II), Co(II), Zn(II) and Ag(I) ions have the positive interference result on the absorbance of cadmium. While, Ge(IV), Pb(II), Cu(II) and Sb(III), have the negative interference result on the absorbance of cadmium in the similar conditions.

Relative absorbance

$$= \frac{\text{Absorbance of 1 ppm cadmium + foreign ion 100 ppm}}{\text{Absorbance of 1 ppm cadmium}}$$

Table 2: The Effect of Interference Ions on Determination of 1 ppm Cadmium in the Presence of 100 Fold of Interferences Ions

Ions	Relative absorbance*	Ions	Relative absorbance	Ions	Relative absorbance
Al(III)	1.00	Bi(III)	1.00	Sr(II)	1.00
Ag(I)	1.32	Se(IV)	1.00	Br ⁻	1.00
Sb(III)	0.94	Sn(II)	1.00	Cl ⁻	1.00
Pb(II)	0.91	Zn(II)	1.18	I ⁻	1.00
Ba(II)	1.00	Co(II)	1.19	F ⁻	1.00
Cu(II)	0.95	Ge(IV)	0.93	NO ₃ ⁻	1.00
Fe(III)	1.00	Hg(II)	1.28	PO ₄ ³⁻	1.00
Na(I)	1.00	Mg(II)	1.00	SO ₄ ²⁻	1.00

*The results are the average of triplicate measurements.

3.10. Biological Activity

Two kinds of bacteria *Escherichia coli* (*E. coli*) (gram negative) and *Staphylococcus aureus* (gram positive), and two kinds of fungal *Candida albicans* and *Aspergillus niger*. This type that used to investigate BPMPD ligand and Cd-BPMPD complex by adopting the serial dilution method [14]. To determine (MIC) minimum inhibitory concentration values, the sets of two fold serial dilution of the test compounds were prepared by serial dilution method. The results in (Fig. 6) reveal that BPMPD ligand and Cd-BPMPD complex showed antibacterial and antifungal activities. It was experiential that there was an appreciable increase in the antimicrobial activities in case of Cd-BPMPD complex compared to the ligand, BPMPD.

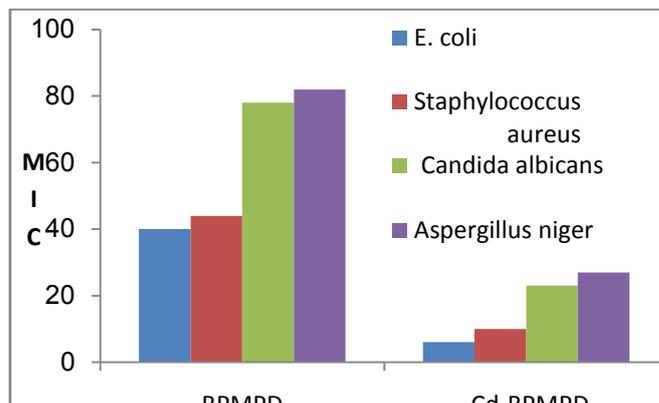


Figure 6: Biological activity of BPMPD and Cd-BPMPD

3.11. Determination of Cadmium in Water Samples

In view of the application of this method, and using AAS, to the determination of Cd(II) in water, the ability to recover Cd(II) from different samples was investigated. For this purpose, standard solutions containing different quantities of Cd(II) were added to water samples and the resulting materials were prepared as described under Experimental. The results of these analyses are summarized in Table 3.

Table 3: Determination of cadmium in water samples

Sample	Added / ppm	ASS/ Found/ ppm	This method/ found/ ppm	%Recovery
Tap water	0	0	0	-
	5	4.99	4.98	99.6
	10	9.99	9.98	99.8
Rain water	0	0	0	-
	5	4.98	4.97	99.4
	10	9.98	9.96	99.6
River water	0	0.94	0.91	-
	5	5.98	5.92	118.4
	10	11.01	10.98	109.8
Waste water	0	4.24	4.22	-
	5	9.32	9.30	186.0
	10	14.96	14.91	149.1

4. Conclusion

The present study shows The ligand BPMPD acts as a hexadentate ligand and formed a stable complex with Cd(II). Beers law is obeyed over the concentration range of (0.1-4 ppm) Cd(II) with R² equal to (0.9982). The most of the ions have no interfering effect under the point out conditions, except Hg(II), Co(II), Zn(II), Ag(I), Ge(IV), Pb(II), Cu(II) and Sb(III). It was experiential that there was an appreciable increase in the antimicrobial activities in case of Cd-BPMPD complex compared to the ligand, BPMPD.

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