



## **Biological Importance and Spectral Characterization of Zn(II) and Cd(II) Complexes of 4-Aminobenzonitrile and Azide Ligands**

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### **ABSTRACT**

Transition elements are relatively small in size and have high positive charge density which facilitates the acceptance of lone pair of electrons from the ligands. Metal ions have  $(n-1) d$  orbitals which have approximately the right type of energy to accept a lone pair of electrons. The stereochemistry of the complexes is determined by the size, charge and electronic structure of the metal ion. Position of the ligands in the Spectrochemical and Nephelauxetic series. The special occupancy of the ligands, size and polarisability of the anion,  $\pi$ -accepting ability of the ligands. The present investigation aims at the synthesis and characterization of the complexes of Zn(II) and Cd(II) with N,N-donor (4-aminobenzonitrile) along with azide ion (additional ligand). The prepared complexes will be characterized by the following physicochemical, spectral and biological studies. The antimicrobial (antibacterial and antifungal) of 4-ABN and its complexes were evaluated. Antimicrobial study revealed that the Zn(II) and Cd(II) metal complexes showed enhanced antimicrobial activity. The ligand, 4-ABN was moderately active against all the tested microbes. The microbes used were *E. coli*, *P. aeruginosa*, *S. aureus*, streptococci and *C. albicans*. The agar-well diffusion method was used for antimicrobial screening. The complexes exhibit higher antifungal activity against the fungi *C. albicans* when compared to pure 4-ABN and Azide. Cyclic voltammetric study of the metal ions in complexes studied. A comparison of infrared spectra of pure ligands and their complexes will confirm the entry of the ligands into the coordination sphere. By agar well diffusion method, the antibacterial and antifungal studies done to know the activities of the complexes against a few microorganisms. Anti-inflammatory activities determined for complex.

**Key words:** Zn(II) metal complexes, Cd(II) metal complexes, 4-ABN, antibacterial, antifungal, *E. coli*

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### **INTRODUCTION**

The ions or neutral molecules which donate a pair of electrons to the central metal atom or ion are called ligands. The bond between the central metal atom and the ligand is called coordination bond or dative bond. The metal complexes are classified as mononuclear complexes, polynuclear complexes, mixed ligand complexes, protonated complexes, mixed donor complexes, chelate complexes, etc., Cis-platin and its derivatives such as carboplatin, oxaplatin are currently used as anticancer [1]. The coordination compounds of many metal ions are involved in life process. All the transition metals from vanadium to zinc are essential for human life. The function of the metal ions depends upon their size and charge. Coordination compounds are used in chemical industries, laboratory, dyeing industry and drugs. The ruthenium (II) complexes also possess anticancer activity. The cadmium, mercury and aluminium complexes play important roles in the field of medicine. The gold compounds are also used in the treatment of arthritis [2]. Some of the coordination compounds are used as dyes and pigments. For example, metal-azide azo dyes are valuable in textile industry. Many naturally occurring compounds such as chlorophyll and hemoglobin are coordination compounds [3].

The chemistry of coordination complexes containing metal-nitrogen bonds has attracted increasing attention in current years. This is partially due to the abnormal properties of many of the complexes with nitrogen containing ligands and these pose a challenge to interpretation. In part, the interest has also been due to the relevancy of the compounds in biochemistry and uses of the complexes as antioxidants, oil additives, and coloring agents for plastics and as pesticides [4-7]. It is well known that such ligands coordinate to a metal ion in different ways in different media depending on the diversified synthetic

procedures. Complexes of nitrogen- nitrogen chelating ligands, nitrogen bridging and nitrogen semi bridging ligands with different metals have also been studied widely [8].

The 4-aminobenzonitrile is one of the N, N-donor ligand. Hence the construction of hybrid organic – inorganic coordination complexes is of high interest in crystal engineering which tries to foretell and organize the fashion molecules accumulated in the solid state[9].

Among the aminobenzonitriles, 4-aminobenzonitrile (4-ABN) is used for the induction of nitrilase activity in arthrobacter, radio protective agent and starting materials for the synthesis of biologically active compounds [10]. Consequently, 4- aminobenzonitrile ligands have attracted less consideration from both theoretical and experimental points of view due to their weak coordination sites such as cyano and amino nitrogen [11] . The 4-aminobenzonitrile can coordinate to a metal centre in monodentate (using either amino nitrogen or cyano nitrogen), bidentate or bridging (using both aminonitrogen and cyanonitrogen groups) fashion [12].

Azide ion contains three nitrogen atoms which are connected to each other. The azide ion is connected to the carbon atom of alkyl or aryl group of organic compounds but it is connected to the metal ion in inorganic compounds. It is one of the weak basic anionic chelating ligand with the pK<sub>b</sub> values of 9.28[13]. A number of strongly bound radicals can form uninegative ions, similar to halide ions or may dimerize to form molecules, similar to diatomic halogens. The univalent anions have properties similar to those of halide ions, and for this reason, are termed as pseudohalides[14]

The Zn(II) shows only +2 oxidation state, as the removal of the two electrons result in a very stable electronic configuration of [Ar] (3d<sup>10</sup>). Zn(II), a d<sup>10</sup> system forms tetrahedral and square planar complexes. Depending upon the nature of the ligand, some of the complexes are octahedral. The usual oxidation state of cadmium is +2 which will form many complexes with octahedral geometry. The stable oxidation state of cadmium is +2 but the +1 oxidation state is a rare one.

## **MATERIAL AND METHODS**

### **Physicochemical and Spectral Measurements**

The following methods were used for the characterization of the complexes prepared.

#### **Elemental Analysis**

The elemental analysis was carried out using Elemental Vario EL III CHNS Analyzer. The percentages of carbon, hydrogen, nitrogen and sulphur in the complexes were found.

#### **Metal Estimation**

A known weight of each complex was decomposed with dil. HCl and made up to a known volume in a standard measuring flask. The metal ions in the solutions were estimated volumetrically/colorimetrically following the standard procedure. The percentage of metals in each complex was determined.

#### **Molar conductance**

Conductance of the complexes in acetonitrile solution (10<sup>-3</sup>M) was measured using a digital conductivity bridge (Equiptronics EQ 664) at 30°C with a dip-type standardized platinum cell.

#### **Cyclic Voltammetry**

The cyclic voltammograms of a few complexes were taken in acetonitrile medium using Princeton make (MC-Tech, Applied Research) equipment. Tetrabutylammonium tetra fluoroborate was used as the supporting electrolyte.

#### **Magnetic susceptibility**

The magnetic moments of the paramagnetic complexes were determined using a Lakeshore -7410 Vibrating Sample Magnetometer (VSM) at room temperature.

#### **Electronic Spectra**

Using solid state diffused reflectance method, the UV-visible spectra of the complexes were obtained in the range of 200 to 800 nm using Varian Cary 5000 Spectrophotometer.

#### **IR Spectra**

The infrared spectra of the complexes were recorded using Perkin Elmer Spectrum RX1 Spectrometer in 4000-400 cm<sup>-1</sup> range with KBr pellet technique.

#### **Biological studies**

##### **Antimicrobial activities**

Antimicrobial activities of all the complexes on a few microbes were carried out by agar - well diffusion method. The prepared complexes and the ligand (4-ABN) were tested for *in vitro* antimicrobial activity by the well diffusion method using agar nutrient as the medium. The antibacterial and the antifungal activities of the ligands and their complexes were evaluated by well diffusion method against the strains, cultured on potato dextrose agar as medium. According to the typical procedure a well was made on the agar medium inoculated with the microorganisms. The well was filled with the test solution using a micropipette and the plate was incubated for 24 hours for bacteria and 72 hours for fungi at 35°C. At the

end of the period, inhibition zones formed on the medium were evaluated in millimeters (mm) and diameter.

#### Anti-inflammatory activity Inhibition of Albumen Denaturation

Method as prescribed was followed with modifications. The reaction mixture was consisting of test extracts and 1% solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount at 37°C HCl. The sample extracts were incubated at 37°C for 20 minutes and then heated to 51°C for 20 minutes after cooling the samples the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was taken as a standard drug. The experiment was performed in triplicates. Percent inhibition of protein denaturation was calculated as follows.

$$\% \text{ Inhibition} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

## RESULTS AND DISCUSSION

### Mixed Ligand Complexes of 4-ABN and Azideion

The optimum conditions were applied during the preparation. All the prepared complexes are colorless. The yields of all the complexes were fairly high. The results obtained from the elemental analysis, metal estimation, molar conductance, cyclic voltammetry, magnetic moment, electronic spectra, FT IR spectral analysis and biological activities of the complexes are discussed.

### Characterization of metalcomplexes

#### Elemental Analysis

From the elemental analytical data the molecular formulae for the complexes were determined. The values given in parentheses are theoretical, calculated on the basis of the molecular formula of each complex (Table 1).

#### Metal Estimation

The metal ions in the prepared complexes were estimated colorimetrically or volumetrically or gravimetrically using standard procedures. The percentage of metal ion in each complex is in good agreement with its formula derived from elemental analysis.

**Table 1 Elemental analysis and molar conductance**

S.No	Ligand/Complexes	Elements found (Calc) %				$\Lambda_m$ ( $\Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$ )
		C	H	N	M	
1	4-aminobenzonitrile	71.15 (71.16)	5.05 (5.11)	23.61 (23.71)	-	-
2	[Zn(4-ABN) <sub>2</sub> (N <sub>3</sub> ) <sub>2</sub> ]	53.98 (53.53)	3.59 (3.82)	38.56 (38.73)	16.19 (17.23)	42.61
3	[Cd(4-ABN) <sub>2</sub> (N <sub>3</sub> ) <sub>2</sub> ]	46.01 (46.83)	3.28 (3.40)	35.21 (35.73)	23.47 (23.13)	51.56

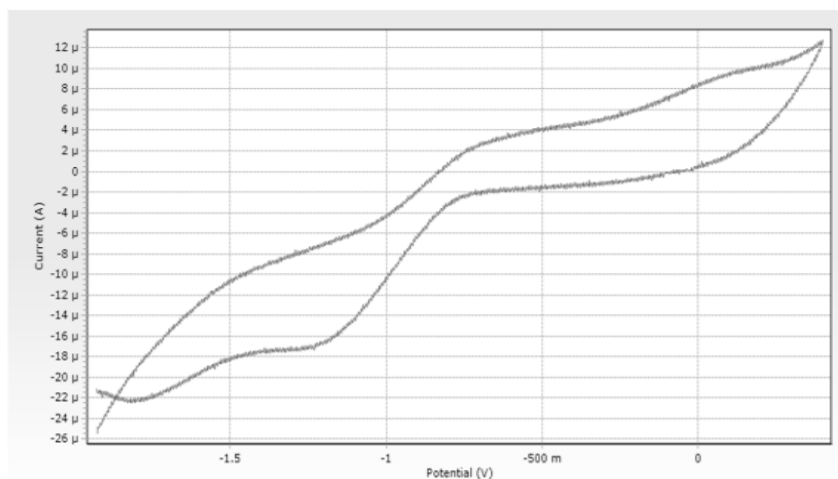
### Electrical Conductance

The molar conductance of the complexes in acetonitrile at 30°C lie in the range of 40 to 50  $\text{ohm}^{-1} \text{ cm}^2 \text{ mol}^{-1}$  and this indicates the non-electrolyte nature (1:0 type) of the complexes[15]. Comparisons of the measured molar conductance with that of a known ionic compound allows estimation of the number of ions produced in acetonitrile solution. It is predicted from the analytical and molar conductance data that the complexes may have the following general formulae  $[\text{MX}_2\text{L}_2]$  where  $\text{M}=\text{Zn(II), Cd(II)}$ ,  $\text{L}=\text{4-aminobenzonitrile}$  and  $\text{X}=\text{N}^-$  ion.

### Cyclic Voltammetry

#### Cyclic voltammetry of [Zn(4-ABN)<sub>2</sub>(N<sub>3</sub>)<sub>2</sub>]

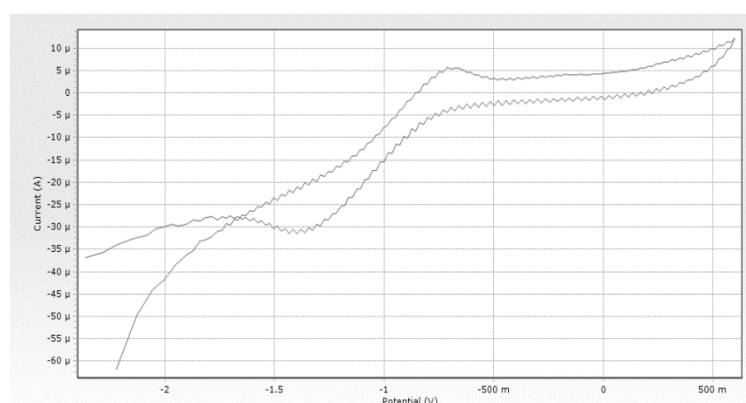
The cyclic voltammogram of [Zn(4-ABN)<sub>2</sub>(N<sub>3</sub>)<sub>2</sub>] complex shows a redox process corresponding to the formation of one electron transfer quasi reversible Zn(II)/Zn(I) couple. The cathodic peak potential is ( $E_{pc} = -1.80\text{V}$ ), anodic peak potential is ( $E_{pa} = -0.12\text{V}$ ) and the peak potential separation is  $\Delta E_p = 1.68\text{V}$ [16]. The cyclic voltammogram of [Zn(4-ABN)<sub>2</sub>(N<sub>3</sub>)<sub>2</sub>] complex shows in the figure :1



**Fig:1 Cyclic voltammogram of [Zn(4-ABN)<sub>2</sub>(N<sub>3</sub>)<sub>2</sub>]**

### Cyclic voltammetry of [Cd(4-ABN)<sub>2</sub>(N<sub>3</sub>)<sub>2</sub>]

The cyclic voltammogram of [Cd(4-ABN)<sub>2</sub>(N<sub>3</sub>)<sub>2</sub>] complex indicates the well defined redox process corresponding to the formation of the quasi reversible Cd(II)/Cd(I) couple. The cathodic peak at -1.38V versus SCE and the associated anodic peak at -0.42V correspond to the Cd(II)/Cd(I) couple. The peak potential separation ( $\Delta E_p$ ) is 0.96V indicating a quasi reversible and one electron transfer process. The increase of peak separation is due to the slow electron transfer reaction taking place at the electrode surface[17].The cyclic voltammogram of [Cd(4-ABN)<sub>2</sub>(N<sub>3</sub>)<sub>2</sub>] complex shown in the figure: 2



**Fig. 2: Cyclic voltammogram of [Cd(4-ABN)<sub>2</sub>(N<sub>3</sub>)<sub>2</sub>]**

### Magnetic Moment

The effective magnetic ( $\mu_{\text{eff}}$ ) moments observed at room temperature and magnetic moments calculated show very close to and below the normal values. The observed magnetic moments of the prepared metal complexes are shows diamagnetic nature[18].

### Electronicspectra

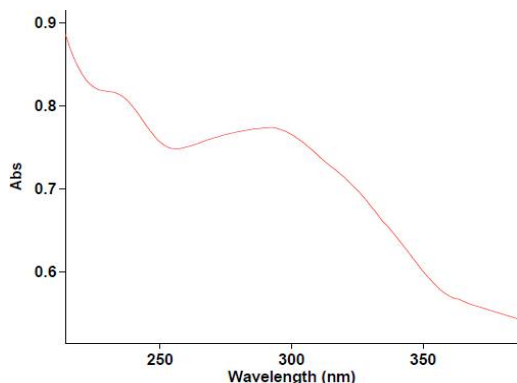
The electronic spectra of the metal complexes in the solid state diffused reflectance spectral method are discussed in this section. The electronic spectra show that the absorptions around 200–800 nm is due to ligand to metal charge transfer(LMCT) and d-d transition bands of the metal in the complexes[19].

### Zn(II)complex

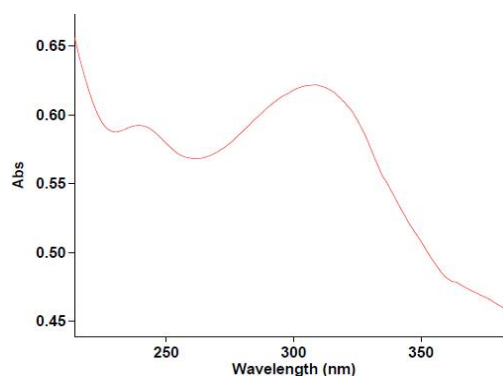
In the Zn(II) complex, the d shell is complete, and also not available for bonding. The metal is quite soft compared to the other transition metals, presumably because the d electrons do not participate in metallic bond. There is no ligand field stabilization effect in Zn<sup>2+</sup> ions because of its complete d shell. The electronic spectrum of Zn(II) complex shows absorption band at 310nm, which is described as charge transfer transition CT band[20]. The Zn(II) complex is diamagnetic and, according to empirical formula, it is proposed to have a octahedral geometry around the Zn(II) metal ion with sp<sup>3</sup>d<sup>2</sup> hybridization.

### Cd(II)complex

The Cd(II) complex is also diamagnetic nature and its d shell is complete, and is not available for bonding. The metal is relatively soft compared to the other transition metals, probably because the d electrons do not participate in metallic bond. There is no ligand field stabilization effect in Cd<sup>2+</sup> ions because of its complete d shell. The electronic spectrum of Cd(II) complex shows only one absorption band at 290 nm which is explained as charge transfer transition CT band. The Cd(II) complex is diamagnetic and, according to empirical formula octahedral geometry is proposed for this complex and sp<sup>3</sup>d<sup>2</sup> hybridization.



**Fig:3 UV- spectrum of Zn(II) complex**  
**UV- spectrum of Cd(II) complex**



**Fig:4**

### FT- IR Spectra

#### IR spectrum of 4-aminobenzonitrile

The important spectral bands of 4-aminobenzonitrile together with the provisional assignments are assigned as follows. The spectrum shows a sharp peak at 3325 cm<sup>-1</sup> which can be assigned to ν(NH<sub>2</sub>) [21]. and the stretching frequencies at 2212 cm<sup>-1</sup> can be assigned to ν(CN) group [22]. small band noticed at 2998 cm<sup>-1</sup> is due to aryl ν(CH) [23]. The spectrum also shows a peak at 1587 cm<sup>-1</sup> which can be attributed to aromatic ν(C=C) stretching frequency [24].

#### IR spectra of metal complexes

The IR spectra of the Zn(II) and Cd(II) complexes are compared with those of the free ligands in order to determine the coordination sites that may involve in chelation. The position/or the intensities of these peaks are expected to be changed upon coordination. Upon comparison, ν(NH<sub>2</sub>) stretching vibration is found in the free 4-ABN at 3325 cm<sup>-1</sup>. This band is shifted to lower wave numbers (3305-3320 cm<sup>-1</sup>) in all the complexes and the stretching frequencies of the CN group is observed near (2225-2235 cm<sup>-1</sup>) which is higher than those of the corresponding free ABN (2212 cm<sup>-1</sup>) [25]. This indicates that the 4-aminobenzonitrile coordinates to the metal ion *via*, nitrogen atoms of both amino and cyano groups (N,N-donor). The trend is in good agreement with the previous examples reported for nitrile ligands [26]. The increase in nitrile stretching frequency has been attributed to the increased bond polarity as the electronegativity of the nitrogen of the nitrile group increases on nitrogen-metal bond formation [27]. The symmetric stretching frequency of azide (N<sup>-</sup>) ion at 1344 cm<sup>-1</sup> was shifted to lower frequency range of 1319-1357 cm<sup>-1</sup> in all the complexes. The asymmetric stretching frequency of azide (N<sup>-</sup>) ion appears at 2098-2045 cm<sup>-1</sup> in all the prepared complexes [28].

The other stretching frequencies of the aryl ν(C-H), aryl ν(C=C) and the alkyl ν(C-H) values of the free ligands appear uninfluenced upon complexation suggesting non-involvement of other groups of 4-aminobenzonitrile and azide ion in coordination.

#### Biological Activities Antibacterial and Antifungal activity

The antibacterial and antifungal activities of 4-aminobenzonitrile, azide ion and their Zn(II), Cd(II) metal complexes were tested against *E.coli*, *P.aeruginosa*, *S.aureus*, *streptococci* and *C.albicans* by the well diffusion method. The test solution was prepared in the DMSO solvent. Amikacin and ketoconazole were used as standards for antibacterial and antifungal activities respectively. The results are given in the Table 2.

**Table:2 Biological activities of AAP and azide ion complexes (mm) in DMSO**

S.No	Ligad/Complex	Zone of inhibition (mm)				
		Bacterial strain				<i>C.albicans</i> (Fungal strain)
		<i>E.coli</i>	<i>P.aeruginosa</i>	<i>S.aureus</i>	<i>Streptococci</i>	
1	4-ABN	06	07	15	12	05
2	[Cd(N <sub>3</sub> ) <sub>2</sub> (4-ABN) <sub>2</sub> ]	23	18	17	23	28
3	[Hg(N <sub>3</sub> ) <sub>2</sub> (4-ABN) <sub>2</sub> ]	20	21	14	20	17

The results indicate that the [Zn(4-ABN)<sub>2</sub>(N<sub>3</sub>)<sub>2</sub>] and [Cd(4-ABN)<sub>2</sub>(N<sub>3</sub>)<sub>2</sub>] complexes are enhanced activity against the few tested microorganism. In general, all these complexes showed good biological activity against the tested microorganism. The metal complexes had a higher inhibition zone than the free ligands and this can be explained on the basis of Overtone's concepts and Tweedy's chelation theory. On chelation, the polarity of the metal ion will be reduced to greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the delocalization of π-electrons over the whole chelate ring and enhances the lipophilicity of the complexes.



**Fig:5 Antibacterial screening of Screening of [Zn(N<sub>3</sub>)<sub>2</sub>(ABN)<sub>2</sub>] complex**



**Fig: 6 Antibacterial screening of screening of [Cd(N<sub>3</sub>)<sub>2</sub>(ABN)<sub>2</sub>]complex**

### Anti-inflammation activity

#### Inhibition of Albumen Denaturation

Problems still exist in continuing pharmacological research on animals. Such as ethical issues and the lack of rationale for their use when other suitable methods are available. Henceforward, in the present study, the protein denaturation bioassay was selected for in vitro assessment of the anti-inflammatory property of complexes. The Albumen Denaturation is a well-documented cause of inflammation. Most biological proteins lose their biological functions when denatured. Production of autoantigen in certain arthritic disease is due to denaturation of protein. The mechanism of denaturation involves an alteration in electrostatic hydrogen, hydrophobic, and disulfide bonding. In the presence study, denaturation of proteins is the main cause of inflammation. As part of the examination on the mechanism of the anti-inflammatory activity, ability of the extract to inhibit protein denaturation was studied. Selected ligands were effective in inhibiting heat-induced albumin denaturation. Aspirin was used as a standard antiinflammation drug.

**Table 3 Inhibition of Albumen Denaturation**

S.No	Test	Concentration of the sample (mg/ml)	% of inhibition of the Sample	Ascorbic acid (Standard)
1	Albumin denaturation	100	22.04	45
2		200	28.43	56.25
3		300	34.67	66.2
4		400	42.04	68.02
5		500	47.52	72.05

## CONCLUSION

The present investigation aims at the synthesis and characterization of the complexes of Zn(II) and Cd(II) with N,N-donor (4-aminobenzonitrile) along with azide ion (additional ligand). The prepared complexes will be characterized by the following physicochemical, spectral and biological studies. The complexes prepared using required mole ratios of the metal nitrate with the ligands in methanol/ethanol medium by conventional heating. Elemental analysis and metal estimation will be used to arrive at molecular formulae of complexes. Complexes electrical conductance measured. Cyclic voltammetric study of the metal ions in complexes Studied. A comparison of infrared spectra of pure ligands and their complexes will confirm the entry of the ligands into the coordination sphere. By agar well diffusion method, the antibacterial and antifungal studies done to know the activities of the complexes against a few microorganisms. Anti-inflammatory activities will be determined for complex.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest in this work

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