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Synthesis, characterization, chromatographic and antimicrobial studies of transition metal complexes of cobalt(II) and iron(III)

ABSTRACT

Metal complexes Cobalt(II) and iron(III) were synthesized by reacting metal nitrate with alprazolam. During this study, The elemental analyses of complexes confine to the stoichiometry of the type $[(L)M(NO_3)_2]$, $[(L)M(NO_3)_3]$ where $M = \text{Cobalt(II)}$ and Iron(III) and $L = \text{alprazolam}$. Alprazolam's IR bands shift to confirm metal ion coordination. Complexes of metal ions were found to be bidentate and tridentate with "N(1) and N(4)" positions on the ligand. This study examined alprazolam and its complexes for their antimicrobial properties against selected bacteria and fungi. Streptomycin and Nyastatin were used as standard drugs for antimicrobial and antifungal studies, respectively. These complexes are bioactive as the complexes of Iron(III) and Cobalt(II) ions of alprazolam are more effective against selected bacterial and fungal species than the drug Alprazolam. The increased chelating tendency of metal complexes results in enhanced activity and these inhibit more of bacterial and fungal growth than ligand alprazolam.

Keywords: -Metal complexes, infra-red, alprazolam, and antimicrobial and bioactive

1. INTRODUCTION

Alprazolam (APZ) belongs to the benzodiazepine and tranquilizer drugs family. It is a hypnotic drug, anxiety disorders are treated with this medication [1]. Benzodiazepines are drugs derived from diazepam and benzene. It has been reported that 1,4-benzodiazepine complexes with transition metal ions possess anticancer and biological properties [2-4]. Complexes of benzodiazepine drugs showed higher antimicrobial activity as compared to free ligands [5]. It is imperative that during complex formation the ligand molecule occupies all coordination sites of the metal ion, with the solvent molecule occupying vacant sites [6]. Alprazolam complexes are more active than their ligands. The complexes of the benzodiazepine drug nitrazepam etc. in which the ligand acts as an anion were reported [7]. APZ used as a ligand in this study, has the following structure (Fig. 1).

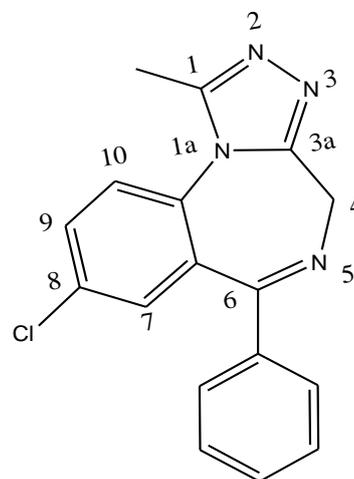


Figure 1. Alprazolam (APZ)

This study examines, the synthesis and characterization of Cobalt and Iron complexes based on elemental analysis and spectral analysis and chromatographic study. The coordination of Iron and Cobalt(II) with Alprazolam and these complexes showed improved antimicrobial activity in this study. The formation of alprazolam complexes influences its pharmacological effects by modifying drug release kinetics and receptor binding affinity. The complexation of drugs with

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polymeric carriers can facilitate controlled drug release, minimizing the side effects of sedation and dependence[8].The antimicrobial activities of complexes of Iron (III) and Cobalt(II)are reported in the reviewed literature [9]. The antimicrobial study explains the application of metal complexes.

2. EXPERIMENTAL

Analytical reagents (E. Merck) are used in experimental analysis.A stock solution of $[\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}]$ and $[\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}]$ in double distilled water and these solutions were standardized by using standard methods [10].A solution of cobalt nitrate and iron nitrate was treated with nitric acid to assess hydrolysis.In ethanol, alprazolam ligand solution was prepared. An equimolar amount of drug alprazolam and metal nitrates of $[\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}]$ and $[\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}]$

were mixed in 1:1 ratio then precipitation took place which confirms the complex formation.In order to remove soluble impurities from complexes, the precipitate was washed with hot water and then washed with $\text{C}_2\text{H}_5\text{OH}$.By using Job's method[10], the stoichiometry of metal and ligand was determined, which is also confirmed by the elemental analysis and the results of experimental and calculated values were approximately the same which are listed in(Table 2).A gravimetric method is used in elemental analysis of metal complexes to determine chlorine content.

The precipitate of metal complexes was dried in an oven at about 100°C and stored in desiccators.The purityof metal complexes was checked by TLC[11] by calculating the Rf factor. Rf values are listed in(Table1).

Table 1. Retention factor (Rf) values of different complexes

Solvent	Complex of metal ions	Retention factor value	Sample Impurities
Ethanol: Benzene 80:20	Cobalt(II)-APZ complexes	0.73	Invisible
	Iron(III) – APZ complexes	0.78	Invisible

The Rf values of both Cobalt(II) and iron(III)complexes are 0.73 and 0.78 respectively. Present results confirm no impurities in the sample of metal complexes.

2.1. Antimicrobial study

The antimicrobial activity of the ligand APZ and its complexes have been carried out against some selected bacterial species and antifungal activity against selected fungal species by using the "Filter paper disc method andbroth serial dilution method" [12]. To obtain 1000 ppm solution, a calculated amount of DMF (dimethylformamide) 0.5 ml was dissolved with sterilized distilled water and added to the metal complexes of alprazolam. A stock solution of different concentrations (250 ppm, 500 ppm, 1000 ppm) was prepared by further dilution.These solutions are named stock solutions. Some standard antibacterial and antifungal drugs were used for comparisonand verification of the activity of compounds.

2.2. Infra-red and Elemental Analysis

IR spectra of the APZ and their complexes in the KBr matrix were recorded on Perkin –Elmer 842- IR spectrophotometer. C, H, and N were estimated by using an elemental analyzer at CDRI, Lucknow.

3. RESULT AND DISCUSSIONS

Alprazolam ligand molar formula is $\text{C}_{16}\text{H}_{11}\text{ClN}_4$, and its complexes with Fe(III) and Co(II) is $[(\text{C}_{16}\text{H}_{11}\text{ClN}_4)\text{Fe}(\text{NO}_3)_3]$ and $[(\text{C}_{16}\text{H}_{11}\text{ClN}_4)\text{Co}(\text{NO}_3)_2]$ as explained in (table 2). Various types of elements, such as carbon, hydrogen, and nitrogen, were analyzed to determine) and Iron(III).A Coblt(II) complex has a molar mass of 491.73 and iron(III) complex has a molar mass of 550.64.Quantitative yields of complexes were obtained are colored and stable at room temperature. They are both stable and non-hygroscopic.

Table 2. Elemental and physical data of ligand and their complexes

Compounds	Molecular Mass	Analysis of elements found [calculated]%					Colour	M.P.
		Carbon	Hydrogen	Nitrogen	Chlorine	Metal(M)		
$[\text{C}_{17}\text{H}_{13}\text{ClN}_4]$	308.8	[66.06] 67.02	[4.21] 3.50	[18.13] 19.50	[11.5] 10.20	--	White	228.5
APZ $\text{Fe}(\text{NO}_3)_3$	550.64	[37.05] 38.15	[2.36] 1.43	[17.79] 18.16	(6.45) 7.23	[10.14] 11.12	Brick Red	327
APZ $\text{Co}(\text{NO}_3)_2$	491.73	[41.49] 40.12	[2.64] 2.18	[17.08] 16.52	[7.21] 6.98	[11.98] 10.50	Brown	345

Table 3. The IR frequencies of ligand and their complexes

Compounds	$\nu(>C=N)$	$\nu(\overset{\text{1a}}{\text{C}}-\text{N}-)$	$\nu(\text{C}_6\text{H}_5)$	$\nu(\text{Cl})$	$\nu(>\text{CH}_2)$	$\nu(\text{CH}_3)$	$\nu(\text{M-N})$	$\nu(\text{NO}_3)$	$\nu(\text{H}_2\text{O})$
Alprazolam(APZ)	1628	1280	1600	740	2960	1355	-	-	-
Fe-APZ Complex	1615	1259	1611	756	2962	1353	500 and 490	1370, 920 and 860	-
Co-APZ Complex	1614	1264	1608	760	2965	1360	493 and 341	1371 and 891	-

Table 3 shows a band of ligands at 1628 cm^{-1} which was shifting to 1614 and 1615 cm^{-1} in Cobalt(II) and iron(III) complexes, respectively. A stretching vibration is observed in the double bond between carbon and nitrogen in the IR region, between 1610 and 1640 cm^{-1} . In the present research paper, the band observed at 1628 cm^{-1} in infrared (IR) spectroscopy is characteristic of the C=N stretching vibration in azomethine compounds. This represents that the "azomethine nitrogen" N(5) atom of the benzodiazepine ring is coordinated with the metal ion in complexes.

The complexes of Cobalt and Iron ions exhibit vibration modes:

$$\left[\nu(\overset{\text{1a}}{\text{C}}-\text{N}-) \right]$$

at 1264 and 1259 cm^{-1} , respectively, showing lower shifting than ligand [1280 cm^{-1}], this shows that Nitrogen (1a) contributes to the formation of complexes. The IR spectrum of alprazolam shows the bands associated with the vibrational mode " $\nu(-\text{C}_6\text{H}_5)$, $\nu(-\text{Cl})$, $\nu(\text{CH}_2)$, $\nu(\text{CH}_3)$ " appear at 1600, 740, 2960, 1355 cm^{-1} as a result, complex formation has shown small positive shifts. In the Cobalt(II) complex, bands at 493 and 341 cm^{-1} and in the iron(III) complex, 500 and 490 cm^{-1} , suggest a (M-

N) linkage [13]. There are strong bands at 1371 cm^{-1} and 891 cm^{-1} in the cobalt(II) complex and 1370, 920, and 860 cm^{-1} in the iron(III) complex, suggesting the presence of monodentate nitrate groups in existing complexes [14]. Finally, infrared studies support the formation of complexes between alprazolam ligands and Iron(III) and Cobalt(II) metal ions. Fig. 3, 4 and 5 show the IR spectra of APZ and complexes. APZ and complexes can be summarized as follows in Fig. 2, 3, 4, 5, 6, 7 and 8.

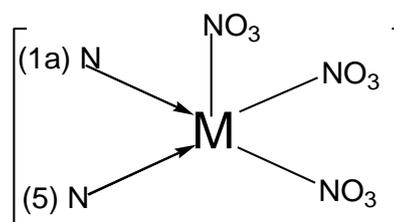


Figure 2. Metal complex (trivalent), M= Iron

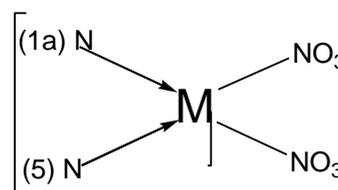


Figure 3. Metal complex (bivalent), M= Cobalt

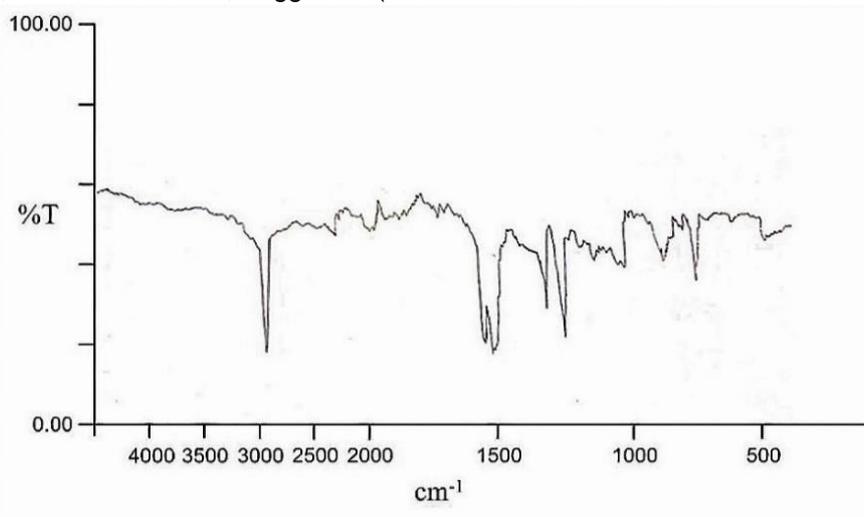


Figure 4. Infra-red spectrum of APZ

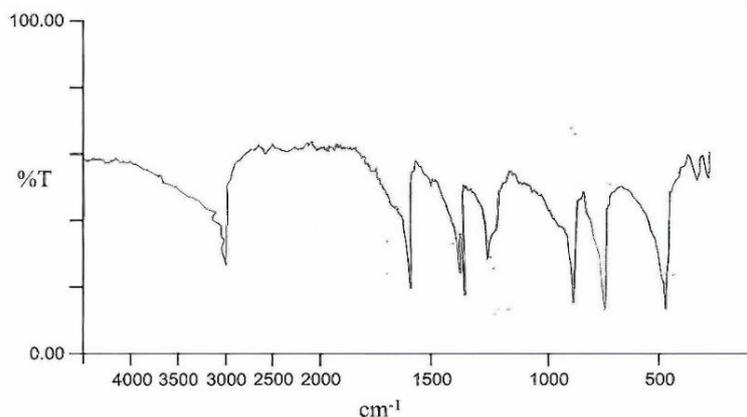


Figure 5. Infra-red spectrum of Cobalt(II) complex

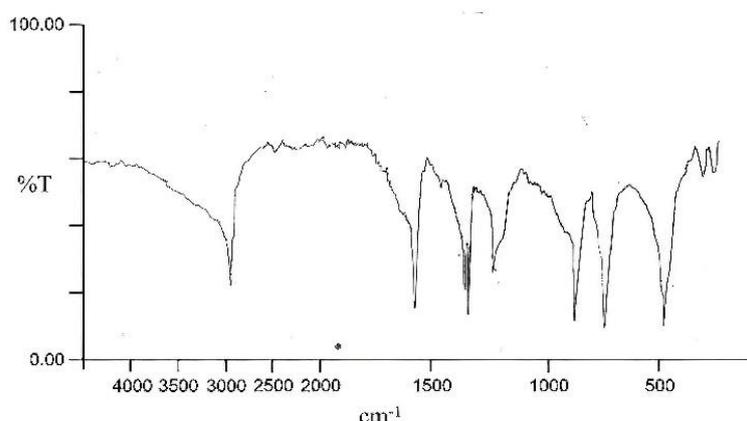


Figure 6. Infra-red spectrum of Iron (III) complex

It is evident from the results that the antimicrobial and antifungal activity of APZ complexes is higher than the ligand alprazolam and activity enhancement can be explained by chelation theory. However, it is known that chelating tends to make the Schiff base act as a more powerful and effective antibacterial agent, therefore inhibiting the growth of bacteria and fungi more than the original APZ ligand. This research confirms that the complexes of alprazolam showed much activity at a lower concentration and selected microbial species have been placed for incubation at $24 \pm 1^\circ\text{C}$ for 20 hours. Experimental results of antimicrobial studies are represented in table no. 4.

3.1. Antimicrobial studies of ligand and complexes

Antimicrobial activity of Cobalt and Iron complexes, metallic nitrates and used ligand were conducted on fungal species and bacterial species in this study [15-19]. Selected species of fungi—“*Fusarium species*, *A. flavous*, *A. niger*, *P. triticena*” and bacteria are “*S. aureus*, *S. typhi*, *B. subtilis*, *E. coli*”. The percentage inhibition was calculated by measuring the diameter of the microbial colony in the control plate and test plate by using the formula $\% \text{inhibition} = \frac{[(C-T)/C] \times 100}{C}$

100]” C is the diameter of the microbial colony in the control plate in mm and T is the diameter of the microbial colony in the treated (test) plate. *Streptomycin* and *Nyastatin* were used as standard drugs for antimicrobial and antifungal studies respectively for comparison under similar conditions.

The results of the present antimicrobial study were presented as a (Table 4) which indicated that the inhibited zone at 500 ppm showed the best results. Comparative studies were conducted using standard drugs with metal complexes. According to the results of table no. 4, the complexes of Iron(III) and Cobalt(II) ions of alprazolam are more effective against selected bacterial and fungal species than the drug Alprazolam itself. In Fig. 7 and 8 the Graphs are plotted between the percentage zone of inhibition and compounds used in antimicrobial studies at 500 ppm. In graph 7, the zone of inhibition against bacteria is plotted; in graph 8, the zone of inhibition against fungi is plotted. Metal complexes inhibit bacterial and fungal growth much more than the ligand alprazolam, which is shown in graphs 7 and 8. The increased chelating tendency of metal complexes results in enhanced activity

and this fact has been proven by many researchers [20-21]. As a result of the chelating tendency of metal complexes, complexes are stronger inhibitors of bacteria and fungi than APZ. Metal complexes are more active due to their increased chelating tendency and lipo-soluble nature [22-23]. When chelation occurs, the metal ion's polarity significantly decreases due to the interaction between its positive charge and the donor groups of the ligand. This interaction involves orbital overlap and partial charge sharing.

Additionally, chelation increases the delocalization of π -electrons across the entire chelate ring, which in turn enhances the complex's lipophilicity. The higher lipophilicity allows the complexes to penetrate lipid membranes more effectively, preventing metals from binding to key enzyme sites in microorganisms [24]. These metal complexes interfere with the cellular respiration process, preventing protein synthesis and ultimately inhibiting the organism's growth [25].

Table 4. Results of antimicrobial activity of APZ and complexes

Compounds	Zone of Inhibition against bacteria (mm).				Zone of inhibition against fungi (mm).			
Alprazolam (APZ)	-	-	-	-	-	-	-	-
Fe(NO ₃) ₃	-	-	-	-	-	-	-	-
Co(NO ₃) ₂	-	-	-	-	-	-	-	-
DMF	-	-	-	-	-	-	-	-
Fe APZ(NO ₃) ₃	27.55	33.00	34.50	37.55	34.00	39.17	35.00	35.10
Co APZ(NO ₃) ₂	28.57	34.50	35.44	39.00	35.56	39.87	39.14	35.56
Streptomycin	31.00	35.00	37.00	36.00	-	-	-	-
Nyastatin	-	-	-	-	35.00	41.00	42.00	37.00

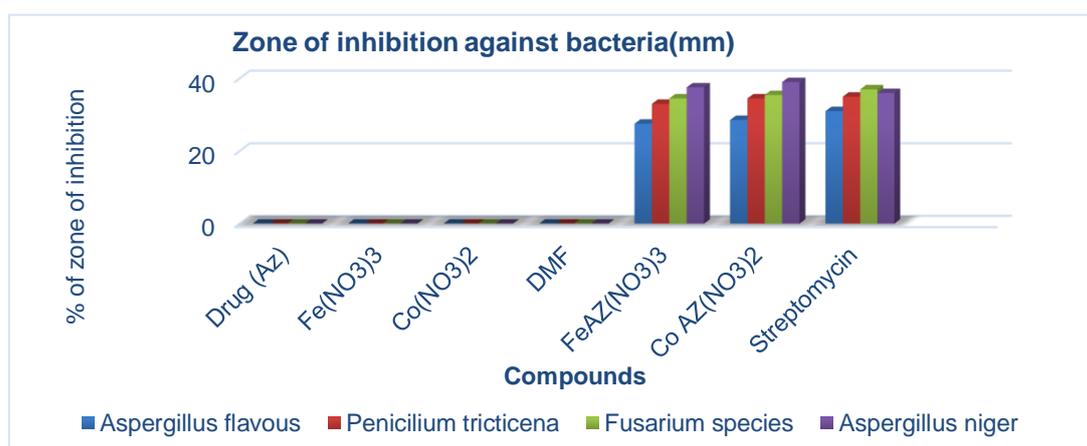


Figure 7. Zone of inhibition against bacteria(mm)

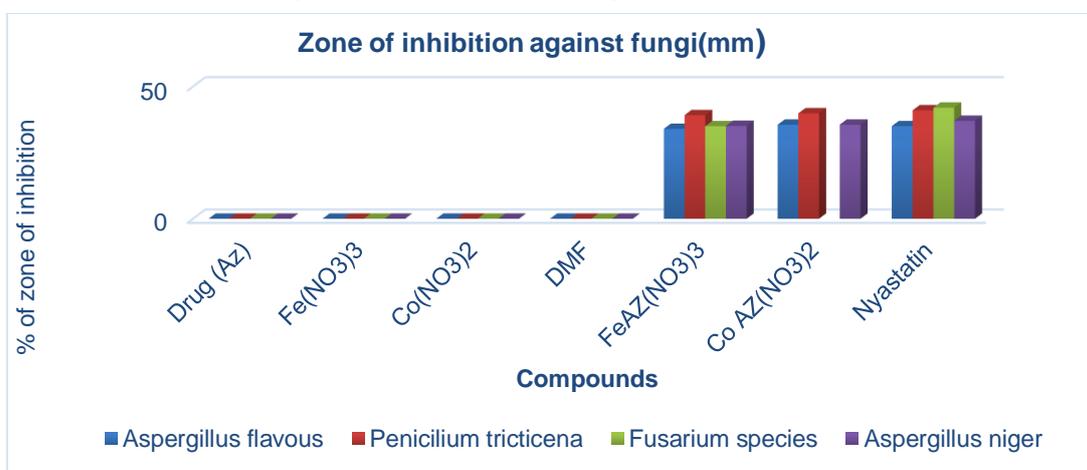


Figure 8. Zone of inhibition against fungi(mm)

4. CONCLUSION

In this study, iron(III) and Cobalt(II) complexes with alprazolam ligand were synthesized, IR studies, chromatographic as well as antibacterial and antifungal studies. By elemental analysis, the molecular weights, molecular formulae, and melting points of complexes of metal ions iron(III) and Cobalt(II) have been determined and compound purity is confirmed by TLC. IR studies confirm the complexation of Iron(III) and Cobalt(II) with APZ. The Fe(III) complex exhibited a bacterial inhibition zone of 37.55 mm, while the Co(II) complex showed a slightly larger zone of 39.00 mm. Against fungal strains, the inhibition zones measured 39.17 mm for the Fe(III) complex and 39.87 mm for the Co(II) complex. This research shows that the complexes of Cobalt(II) and Iron(III) are more effective on bacteria and fungi than APZ alone.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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IZVOD

SINTEZA, KARAKTERIZACIJA, HROMATOGRAFSKA I ANTIMIKROBNA ISTRAŽIVANJA KOMPLEKSA PRELAZNIH METALA KOBALT(II) I GVOŽĐA(III)

Metalni kompleksi kobalt(II) i gvožđe(III) su sintetizovani reakcijom metalnog nitrata sa alprazolamom. Tokom ove studije, elementarne analize kompleksa su ograničene na stehiometriju tipa $[(L)M(NO_3)_2]$, $[(L)M(NO_3)_3]$ gde je M= kobalt(II) i gvožđe(III) i L= alprazolam. Alprazolamovi IR pojasevi se pomeraju da bi se potvrdila koordinacija metalnih jona. Utvrđeno je da su kompleksi metalnih jona bidentatni i tridentatni sa „N(1) i N(4)” pozicijama na ligandu. Ova studija je ispitivala alprazolam i njegove komplekse na njihova antimikrobna svojstva protiv odabranih bakterija i gljivica. Streptomycin i niastatin su korišćeni kao standardni lekovi za antimikrobne i antigljivične studije. Kao kompleksne studije I, odnosno antifungalne, su kompleksne studije I. III. Joni kobalta(II) alprazolama su efikasniji protiv odabranih bakterijskih i gljivičnih vrsta od leka Alprazolam. Povećana sklonost heliranju metalnih kompleksa dovodi do pojačane aktivnosti i one više inhibiraju rast bakterija i gljivica nego ligand alprazolam.

Ključne reči: Metalni kompleksi, infracrveni, alprazolam, antimikrobni i bioaktivni

Naučni rad

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