Synthesis, characterization of metal complexes of 2-furoic 9-anthracenecarboxy hydrazone and 1,10 phenonthroline with cobalt(III) salt and their biological applications.

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Abstract. A new complex of Cobalt was isolated with 2-furoic hydrazide , 9-Anthracarboxaldehyde and 1,10phenonthroline and characterized by spectroscopic methods. The results show that the ligand is coordinated by the azomethine nitrogen and carboxyl oxygen ,has a general structure of the type [Co(L)Cl$_2$(phen)]. The structure of Cobalt(II) complex was optimized and theoretical data show good agreement with the experimental results. The cytotoxic activity was evaluated in a chronic myelogenous leukemia cell line, which revealed that the complex presented antimicrobial activity more efficient than ampicillin, chloranfenicol and kanamicyn.

Keywords: Cobalt complexes, cell culture, hydrazones, DFT studies, antibacterial activity.

1.INTRODUCTION

The hydrazide properties are of major interest due to their biological activities. Hydrazides have been demonstrated to possess, among other, antibacterial, antifungal, and antitumoral activities. For example, isonicotinic acid hydrazide (isoniazid) exhibited high in vivo inhibitory activity towards M. tuberculosis H37Rv.1 The formation of metallic complexes plays an important role in the growth of their biological activity1–4 and, therefore, many complexes of hydrazides have been synthesized and characterized.5–7. Some of these metallic complexes also exhibit good fungicide, antitumoral and antibacterial activity.8−11. As example, platinum complexes containing hydrazides derived from benzoic acid showed strong growth inhibitory effect in leukemia cells in vitro, not verified with the free ligands.9 These observations encouraged us to synthesize new cobalt complexes as possible antitumor and antibacterial agents. In this work, we report the synthesis and characterization of a new cobalt(II) compound with 2-furoic hydrazide 9-anthracenecarboxaldeyhde and 1,10 phenonthroline. The compound was characterized by ^{1} H NMR, IR, UV-Vis, The cytotoxic and antibacterial activity of the synthesized compound was evaluated.

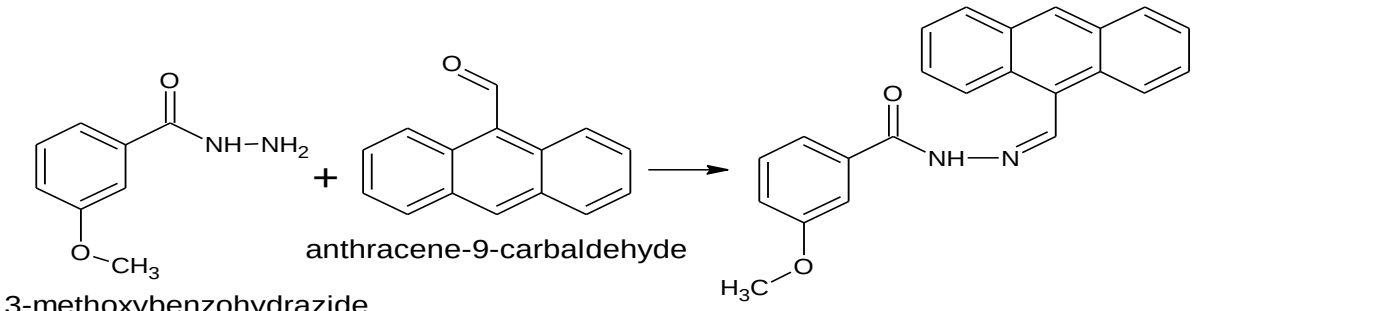
2.EXPERIMENTAL

Physical Measurements; Conductivity study was carried out with a Control dynamic conductivity meter using a cell of constant 1.0 cm^{-1} , Elemental analyses were performed at the Analytical Centre of the IISC, using a Perkin-Elmer 2400 CHN Elemental Analyser. IR spectra were registered in KBr pellets on a Bruker 1003-3610 alpha-P FTIR spectrometer. A spectrophotometer UV Perkin Elmer lamda 650 was used for UV and visible absorption measurements. NMR spectra were obtained using a Bruker DPX 400 MHz spectrometer with tetramethylsilane as an internal standard.

Cells and Culture; The K562 cell line was purchased from the Bio Cell Bank. This cell line was established from pleural effusion of a 55 year-old female with chronic myelogenous leukemia in terminal blast crisis. Cells were cultured in RPMI 1640 (Sigma Chemical Co.) medium supplemented with 10 % fetal calf serum at 37 °C in a humidified 5 % CO_2 atmosphere. Cultures grow exponentially from 105 cells mL^{-1} to about 8×105 cells mL^{-1} in three days. Cell viability was checked by Trypan Blue exclusion. The cell number was determined by Coulter counter analysis. The cytotoxic effect was evaluated by incubating 1×105 cells mL^{-1} in the absence and the presence of a range of concentrations of tested compounds for 72 h. Afterwards, cells were counted and the concentration required to inhibit cellular growth by 50 %, the IC50, was calculated. Stock solutions of the compounds were prepared in DMSO (2 %) and H_2O. Antimicrobial Activity An oxaciclin-resistant Escherichia coli strain and a methicilin-resistant Staphylococcus aureus strain were used to evaluate the antimicrobial activity of the complex. Both strains were isolated. 100 µL of a suspension of each strain previously cultured at 37 °C in LB (NaCl 10 g L^{-1} ; Yeast extract 5 g L^{-1} ; Triptone 10 g L^{-1}) were used to obtain an OD600 = 0,35 LB culture. The complex was added to each culture and the bacterial growth was monitored by optical density observation at 0; 6; 12; 24; 48; and 72 hours. The complex was tested at the concentrations of 100 µg mL^{-1} ; 200 µg mL^{-1} ; 300 µg mL^{-1} ; 500 µg mL^{-1} and 1000 µg mL^{-1} . Each test was replicated 4 times.

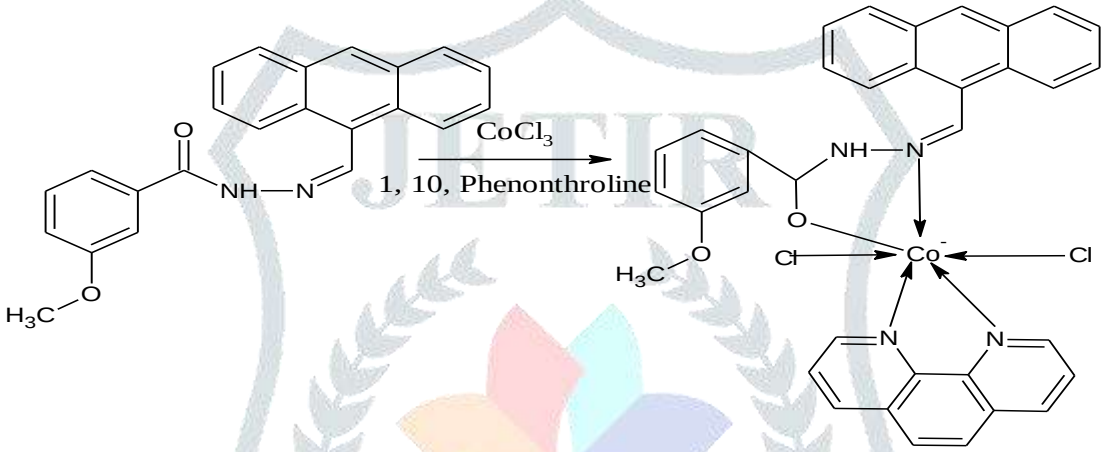
Starting Materials ;The reagents $CoCl_3$ 9-Anthracenecarbaxaldehyde and 2-furoic hydrazide and 1,10 phenonthroline are commercially available (Sigma Aldrich). All other reagents chemicals were of analytical grade, purchased from different sources, and used without further purification.

Preparation of the ligand; About 0.266 g of 9-Anthracenecarbaxaldehyde was added to 10 ml of methanol and 0.126 g of 2-Furoic hydrazide was dissolved in 10 ml of methanol the reaction mixture was refluxed for four hours in a round bottom flask deep yellow colored ligand was formed.which is filtered washed with methanol and yield is noted. Yield; 65% colour; Deep yellow.



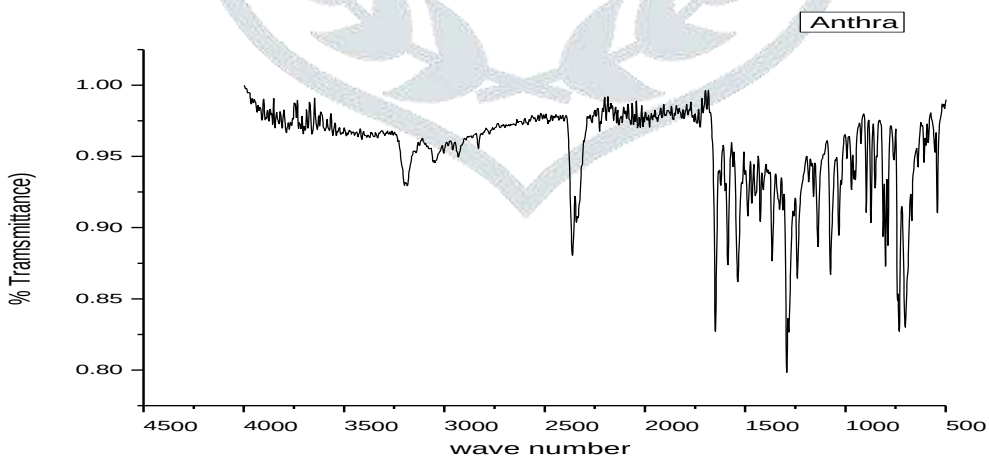
Preparation of the Complex

0.165 g of $CoCl_3$ (0.5 mmol) was added to 5 mL of an aqueous solution of the ligand (1.0 mmol) and the mixture was stirred for 24 h. 0.180 g of 1,10 phenonthroline was added and stirred for 24 hrs. The solid formed was separated by filtration, washed methanol, and dried under vacuum. Yield: 31 %. Color: Yellow.

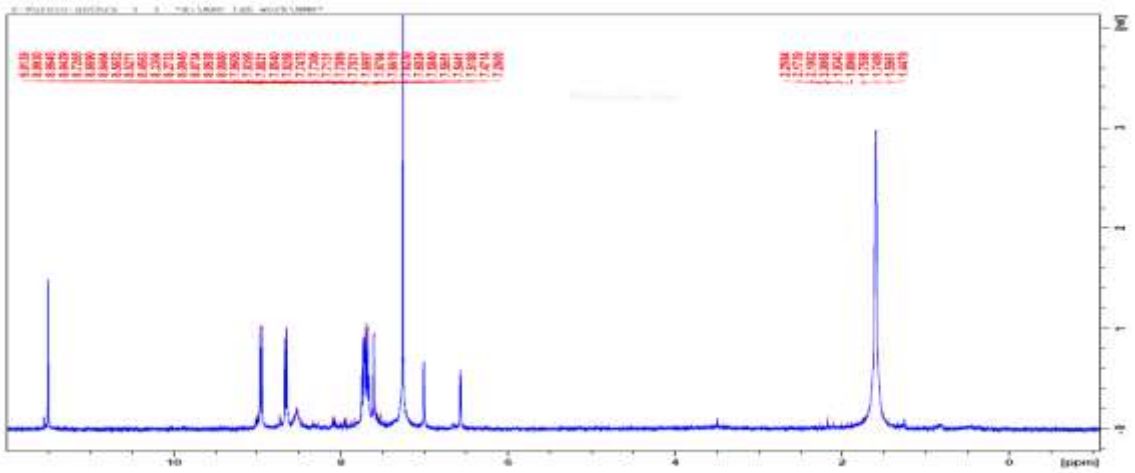


3.RESULTS AND DISCUSSION

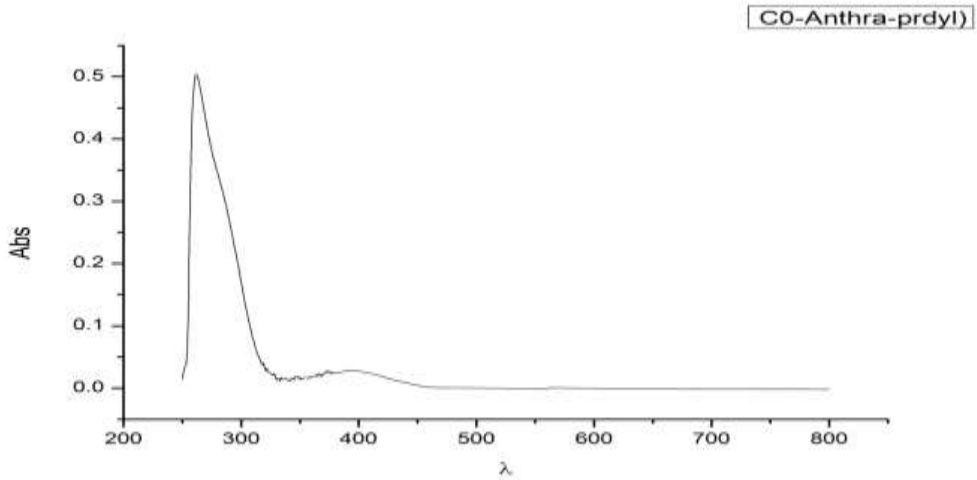
The ligand and the complex was characterized by elemental analysis, IR, UV-Vis and ^{1}H NMR. In this work, the ligand acts as a bidentate The Cobalt complex is soluble in organic solvents such as DMSO and DMF. The chemical structures of the ligand and its complex are presented in Figure . The results of the elemental analysis (C, N, and H) are in accordance with the proposed structure. The freshly prepared DMF solution of the complex exhibited molar electric conductivity value far below that of the 1 : 1 standard electrolyte indicating that the complex is not charged.



IR spectrum of the cobalt complex



NMR spectrum of the complex



UV-Vis spectrum of the ligand

Table 1. Elemental analysis and mass spectrometry of the ligand and complexes.

Ligand/complex	C%	H%	N %	Metal	Conductivity	Magnetism
[Ligand]	76.47(77.30)	4.43(4.35)	8.91(8.30)	----------	5-10	0.00
[Co(L)(phen.)Cl_2]	69.31(69.70)	4.15(4.20)	10.12(10.20)	1061(10.24)	10-20	1.98BM

IR Spectra; The IR spectrum of the free ligand was performed just for comparison to the corresponding complex isolated. The characteristic absorptions in the 3400 to 3032 cm^{-1} NH),ν region were observed, corresponding to (CH). The spectrum also exhibited bands(νNH), and (ν at 1657 and 1633 cm−1 C=O) and(ν, assigned to (C=C), respectively. ν In the IR spectrum of the complex, the bands due (C=N) were found to be shifted towards lower wave numbers(ν (Figure IR). This shift can be attributed to participation of the N group in the coordination to the cobalt ion. In addition, in the IR spectrum of the (M−N) appeared at ν complex, a new peak assigned to 541 cm^{-1} , indicating formation of the M−N bond .Another observation is that the carbonyl group appears at a lower wave number of the ligand, therefore, we cannot rule out an involvement of this group in the coordination to the metallic ion. The ^{1}H NMR spectra of the complex and the ligand were recorded in d6-DMSO. In the spectra of the free ligand, the NH protons 9.3. The signal of the NH proton was much less affected. IR spectra (640–440cm^{-1}) of (in red) and its complex. The experimental UV-visible absorption spectrum of the ligand exhibit two bands centered at 260 nm and 401 nm .Regarding the assignment of the electronic spectrum of the cobalt (II) complex the theoretical results confirm that the experimental band at 255 nm is assignable to intraligand π→π* transitions, while the band centered at 401 nm is assigned to metalto-ligand charge-transfer.

Cytotoxic Activity; The cytotoxic activity of the ligand and its complex was examined on K562 cells. In IC50 values obtained for cisplatin, the free ligand and its complex are shown for the sake of comparison. The complex inhibits the growth of K562 cells and, its activity is 2-fold higher than of the corresponding free ligand. However, in comparison to cisplatin or carboplatin, the effect of the complex is much lower. Antibacterial Activity Antimicrobial activity against two bacterial species belonged to main bacterial group was tested: the Gram positive Staphylococcus aureus, and the Gram-negative Escherichia coli. The results showed that the complex presented antimicrobial activity against S. aureus from g mL^{-1}μ concentrations of 300 On the other hand, this activity was not observed against E. coli. Thus, the complex presented antimicrobial activity against Gram-positive, but was not effective against Gram-negative bacteria. However, more detailed studies have done to evaluate the action spectrum of this complex against Gram-positive bacteria. The antimicrobial activity of complex against S. aureus was compared to ampicillin,28 chloranfenicol and kanamicyn.29,30 Staphylococcus aureus growth after 6 hours of ligand and complex addition at 0,

100, 200, 300, 500, 700, and, g mL^{-1}µ1000 . (B) Escherichia coli after 6 hours of ligand and complex addition at 0, 100, 200, 300, 500, 700, and, g mL−1µ1000 . (C) Staphylococcus aureus growth after ligand, complex, chloranfenicol, kanamicyn, and, ampicillin. Binder: g mL^{-1}µ300 g mL^{-1}µ; Complex: 300 g mL^{-1}µ; Chloranfenicol: 100 g mL^{-1}µ; Kanamicyn: 100 ; Control: S. aureus culture. g mL^{-1}µ(D) Staphylococcus aureus growth after binder, complex, chloranfenicol, kanamicyn, and, ampicillin at 100 . 206 G. D. de Souza et al., A New Complex of Palladium(II) with 2-Furoic Hydrazide Croat. Chem. Acta 86 (2013) 201. At 300 µg mL^{-1} , the complex presented antimicrobial activity more efficient than ampicillin, chloranfenicol and kanamicyn, and this activity was observed to 72 hours . However, when the complex was used at 100 µg mL^{-1} (the same concentration of the antimicrobials) the antimicrobial activity was observed only after 24 hours . The results suggested that the complex presented a stable antimicrobial activity against Gram-positive bacteria at 300 µg mL^{-1} .

4.CONCLUSION

A new complex containing ligand and cobalt complex was prepared and characterized by spectroscopic techniques. The spectroscopic and theoretical techniques show that the ligand is coordinated to the cobalt by the basic nitrogen of N group carboxyl oxygen. The biological studies showed that the complex has a poor cytotoxic activity against K562 cell line, but it is active against Gram-positive bacteria.

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