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Experiments
Експерименти в природните науки

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITIES OF PYRAZOLYL COPPER(II) NITRATE COMPLEXES

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Abstract. Three pyrazolyl copper(II) complexes vz. diaqua-bis(3-ferrocenyl-1H-pyrazole)-dinitrato-copper(II) **(C1)**, diaqua-(3-ferrocenylpyrazolyl-1-methylene pyridine)-dinitrato-copper(II) **(C2)** and diaqua-bis(3-phenyl-1H-pyrazole)-dinitrato-copper(II) nitrito **(C3)** have been synthesized and characterized by IR spectroscopy, UV-vis, thermogravimetric analysis, melting point determination and magnetic measurements. The complexes were obtained in low to high yields and the combination of various techniques suggests that these complexes show an octahedral geometry with coordination via two nitrogen atoms from the pyrazolyl ligand, two oxygen atoms from water molecules and two oxygen atoms from the nitrate group. All complexes are thermally stable in the temperature range of 80-100^{o}C. The antimicrobial activities were screened using a range of gram negative and gram positive bacteria and fungi. It was observed that all the complexes have anti-microbial activities against studied microbes.

Keywords: pyrazole, copper, nitrate, anti-microbial activities

Introduction

Nitrogen donor ligands such as amines, imines, pyridyl, imidazoles and pyrazoles are good ligands for coordination with transition metal ions. In particular, pyrazolyl complexes with transition metals are well documented (Herchel et al., 2016; Moreno-Lara et al., 2015; Das et al., 2015; Soria et al., 2014; Guerreo et al., 2015). Usually the coordination is done via the *N*(2) nitrogen atom of the five membered pyrazole ring which comprises two nitrogen atoms and three contiguous carbons (Mighell & Reimann, 1967).

The substituents around the pyrazole ring might be small entities like protons but could also be larger groups such as substituted phenyls or ferrocenyl moieties (Zsoldos-Mady et al., 2009).

Copper(II) complexes with pyrazoles and other nitrogen donor ligands might be tetracoordinated (Zats et al., 2011), pentacoordinated (Indra et al., 2011) but mostly octahedral especially when a nitrate group is part of the structure (Giles et al., 2015;

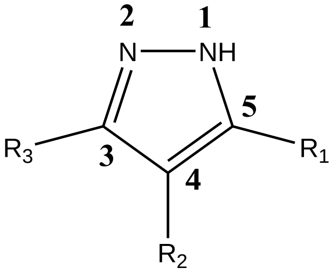


Fig. 1. General structure of a pyrazole compound showing the numbering of the ring

Shaw et al., 2004; Karmakar et al., 2007). The octahedral geometry is sometimes distorted from the ideal geometry and this can be seen in the electronic spectra of the compounds (Devi & Batra, 2015).

Pyrazolyl copper (II) complexes have catalytic applications (Shobani et al., 2016) but also various biological activities (El-Bindary et al., 2016). It is well established that pyrazolyl compounds have generally good anti-cancer (Reddy et al., 2015), anti-inflammatory (Alegaon et al., 2014) and anti-microbial activities (Wang et al., 2015) to name a few. On the other hand, Cu^{2+} is reported to have activities against common pathogens (Liu et al., 2013; Yang et al., 2012). When a pyrazolyl ligand coordinates to copper, a synergic effect emerges with highly enhanced anti-microbial activities (Saha et al., 2014). This effect has been explained by various researchers as a result of chelating effect which increases the lipophilicity of the metal center after coordination to the ligand. The lipophilicity enhances the biological properties by increased penetration of the biocidal agent in the cell membrane which is mostly made of layers of lipidic structures (Liu et al., 2013; Tweedy, 1964; Murukan & Mohanan, 2007).

In this study, we report on the synthesis, characterization and anti-microbial activities of some pyrazolyl copper(II) complexes against some gram negative and gram positive microbes.

Experimental

Instrumentation

Infrared spectra were recorded on a Perkin Elmer FT-IR 2000 spectrometer in 4000-500 cm^{-1} range (University of Botswana, Indian Institute of Science, Bangalore). Magnetic susceptibility was measured at the University of Botswana using Johnson Matthey Alfa magnetic susceptibility balance. Thermogravimetric analysis (TGA) was performed using a Mettler Toledo TGA/SDTA 851^e instrument (Indian Institute of Science, Bangalore) between 20-1000°C at a heating rate of 10°C/min under nitrogen atmosphere. Solid state UV-vis spectra were recorded on a Perkin Elmer UV/Vis/NIR Spectrometer Lambda 750 (Indian Institute of Science, Bangalore). Melting points were determined using STUART SMP3 version 5.0 apparatus (University of Botswana). The antibacterial activities were measured in the Biological Sciences Department (University of Botswana).

Chemicals

The chemicals were purchased from Sigma-Aldrich and used as received. Solvents were dried and distilled using conventional methods. Toluene was dried using sodium wires and distilled in the presence of benzophenone. Ligands L1(3-ferrocenyl-1H-pyrazole) and L2 (3-ferrocenylpyrazolyl-1-methylenepyridine) were synthesized according to previously reported procedures (Niedenzu et al., 1991; Obuah et al., 2014). Ligand L3 (3-phenyl-1H-pyrazole) was purchased from Sigma-Aldrich and used without any further purification.

Synthetic experimental procedures

Synthesis of diaqua-bis(3-ferrocenyl-1H-pyrazole)-dinitrato-copper(II) ***(C1)***

To a 0.200 g (0.794 mmol) solution of 3-ferrocenyl-1H-pyrazole in methanol was added 0.096 g (0.397 mmol) of $Cu(NO_3)_2.3H_2O$. The color of the solution immediately changed to dark brown. The reaction was allowed to proceed at room temperature for about 18 hours after which the solvent was removed to afford the desired product.

Yield: 90 % m. p. > 250°C

Synthesis of diaqua-(3-ferrocenylpyrazolyl-1-methylene pyridine)-dinitrato-copper(II) ***(C2)***

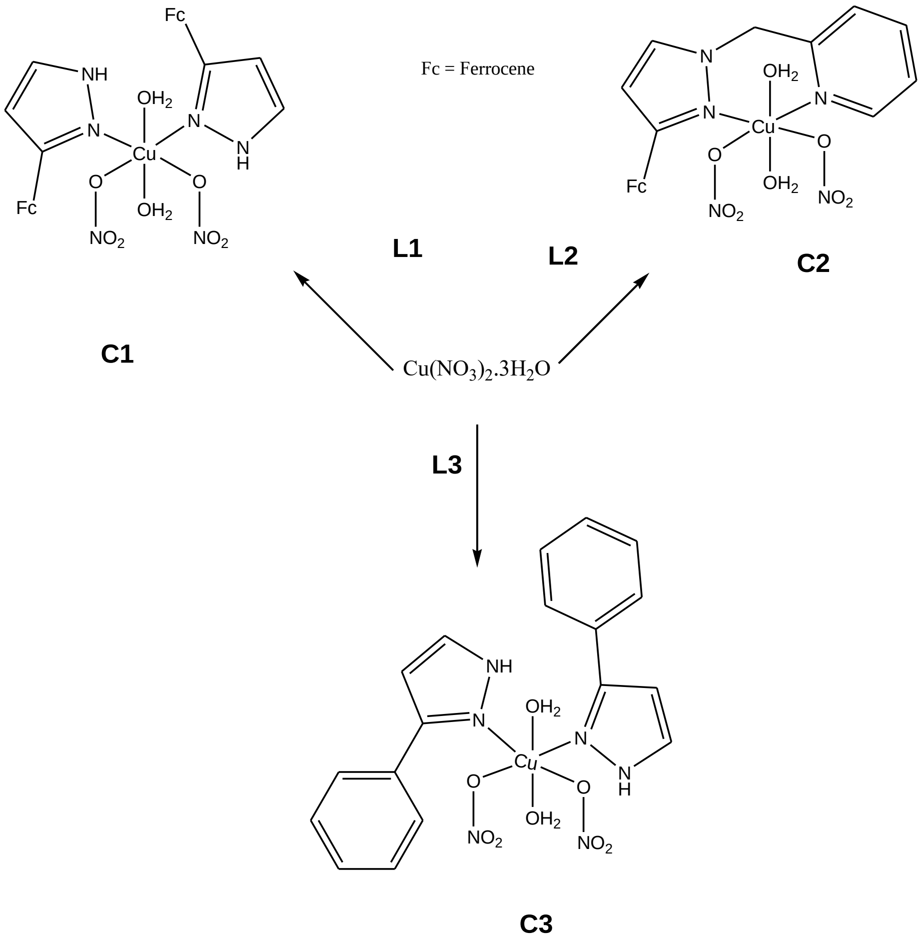
To a 0.150 g (0.437 mmol) solution of 3-ferrocenylpyrazolyl-1H-methelenepyridine in methanol was added 0.106 g (0.437 mmol) of $Cu(NO_3)_2.3H_2O$. The color of the solution immediately changed to dark brown. The reaction was allowed to proceed at room temperature overnight. The solvent was removed to afford the desired product.

Yield: 60 % m. p. = 117-120°C

Synthesis of diaqua-bis(3-phenyl-1H-pyrazole)-dinitrato-copper(II) nitrito ***(C3)***

To a 0.300 g (2.08 mmol) solution of 3-phenyl-1H-pyrazole in methanol was added 0.251 g (1.04 mmol) of $Cu(NO_3)_2.3H_2O$. The reaction was allowed to proceed at room temperature for about 18 hours after which the solvent was removed to afford the desired product.

Yield: 40 % m. p. = 178-180°C



Scheme 1. Synthesis of copper(II) complexes

Antimicrobial activities determination

Copper (II) complexes (C1-C3) were tested for antibacterial and antifungal activity using Agar disc diffusion method. The microorganisms were grown overnight at 37°C in 20 mL of Müller-Hinton broth (Oxoid). The cultures were adjusted with sterile saline solution to obtain turbidity comparable to that of McFarland no. 5 standard (1.0 x 10^8) CFU/ml. 90 mm Petri dishes (Merck, South Africa) containing 12 mL of sterilized Müller-Hinton agar (Oxoid) were inoculated with these microbial suspensions. Sterile Whatman No.1 (6 mm) discs papers were individually placed on the surface of the seeded agar plates and 10µl of the complexes was applied to the filter paper disk. The plates were incubated at 37 °C for 24 h and the diameter of the resulting zones of inhibition (mm) of growth was measured. All tests were performed in triplicates. Kanamycin (600 µg/mL) was used as a control.

Results and discussions

IR spectroscopy

The C=N bond vibration frequencies were easily observed in the 1500-1600 cm^{-1} region. For complex C1, this vibration was seen at 1595 cm^{-1}, 1608 cm^{-1} in complex C2 and 1567 cm^{-1} in complex C3. In complexes C1, C2 and C3, the ρ(HOH) frequencies are observed at 804 cm^{-1}, 766 cm^{-1} and 758 cm^{-1} respectively. This is characteristic of coordinated water in octahedral copper(II) complexes (Nakagawa & Shimanouchi. 1964). A typical IR spectrum of Complex 2 is shown in Fig. 2.

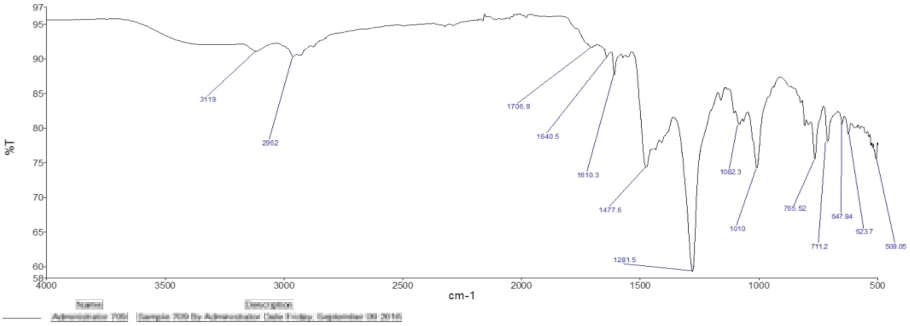


Fig. 2. IR spectrum for complex C2

Thermogravimetric analysis (TGA)

Thermogravimetric analyses revealed that all the three complexes are not stable to high temperatures as they start decomposition at temperatures 80-100°C and their thermograms are quite similar. The first decomposition step is the loss of one water molecule followed immediately by the loss of a second water molecule. This suggests that the structures of C1-C3 contain two coordinated water molecules. The continuous thermal decomposition of the complexes then follows but does not complete until 1000°C, the limit of the instrument used during these experiments. Therefore, it was not possible for us to confirm the final decomposition residue. The TGA graphs for C1-C3 complexes are shown in Figs. 3-5.

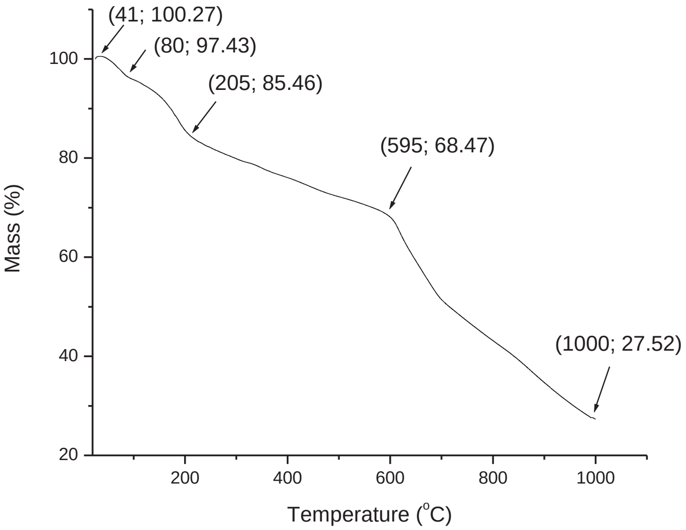


Fig. 3. TGA graph for complex C1

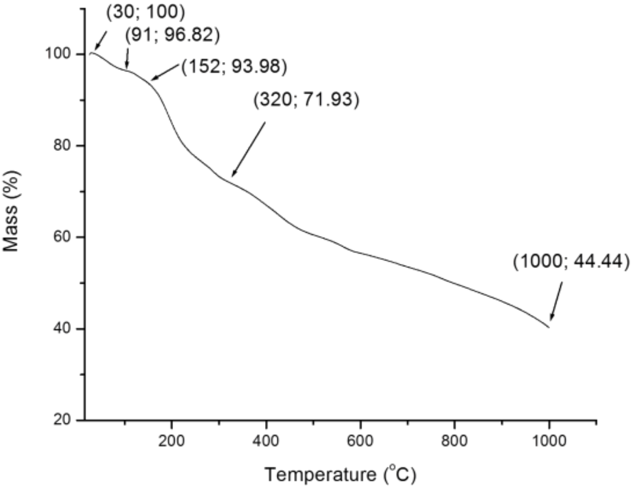


Fig. 4. TGA graph for complex C2

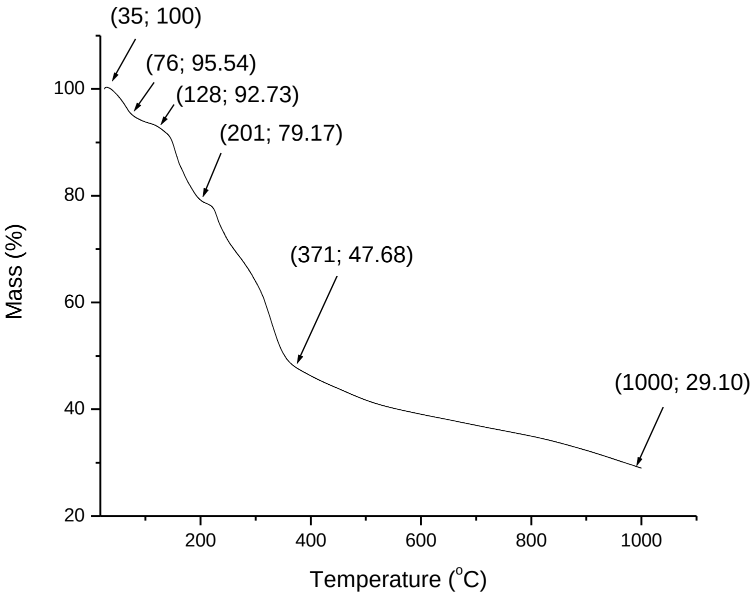


Fig. 5. TGA graph for complex C3

UV-vis

The UV-vis spectra of complexes C1-C3 were recorded as neat samples. A broad band is observed in the region 731-759 nm for all the complexes. This band is characteristic of d-d transition in Cu^{2+} (d^9), $^2E_g \rightarrow {}^2T_{2g}$. The broadness of this band suggests a distorted octahedral geometry. Furthermore, intra-ligand transfer bands are observed in 200 nm region, while the ligand to metal charge transfer (LMCT) bands are seen in 500 nm region except for C3 where the intraligand transfer band is seen at 421 nm and LMCT at 600 nm. These results are in accordance with some other copper(II) complexes reported in the literature (Devi & Batra, 2015).

Table 1 summarizes the UV-vis bands for the three complexes and their electronic spectra are shown in Figs. 6-8.

Complex	Intra-ligand transfer (nm)	LMCT (nm)	d-d transitions (nm)
C1	210	515	757
C2	211	502	731
C3	421	600	759

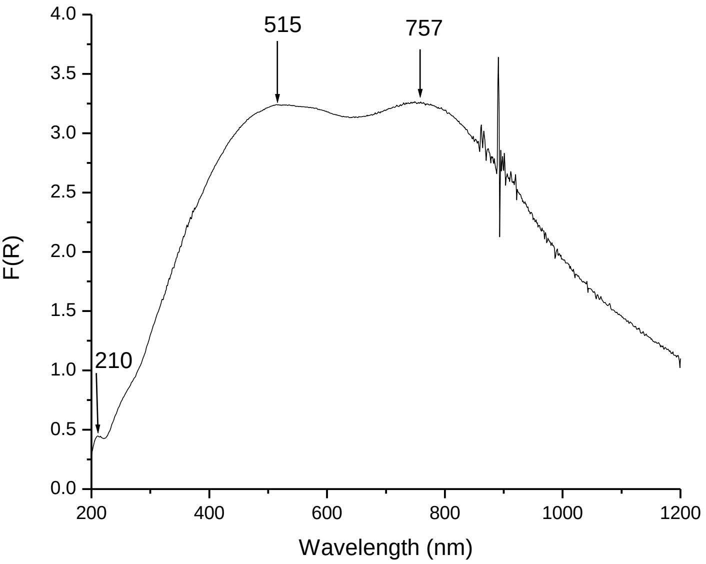


Fig. 6. Electronic spectrum of complex C1

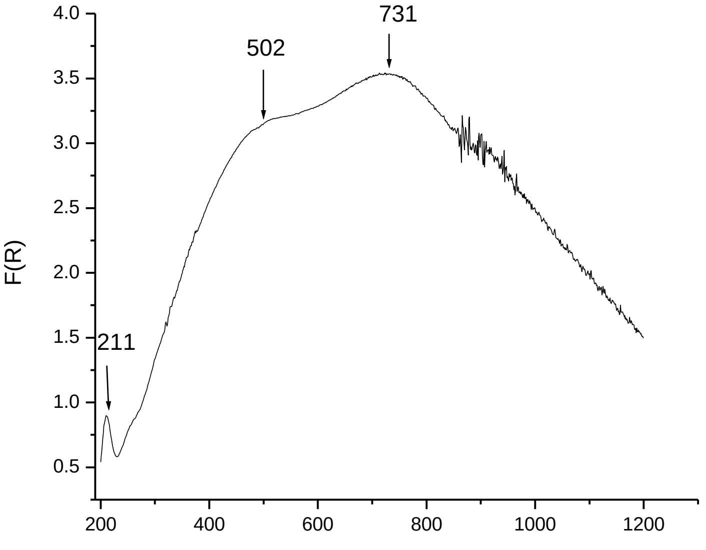


Fig. 7. Electronic spectrum for complex C2

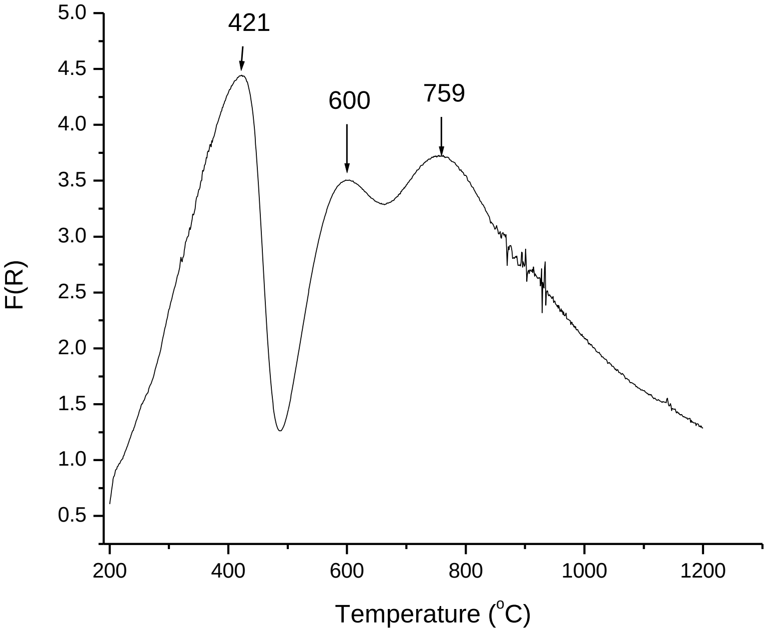


Fig. 8. Electronic spectrum for complex C3

Magnetic measurements

The value of μ_{eff} for the copper(II) complexes are in the 1.73-2.02 BM range corresponding to one unpaired electron, hence paramagnetic complexes (Figgis & Lewis, 1960). The value of the magnetic moments also suggests that the complexes have an octahedral geometry (Devi & Batra, 2015).

Table 2. Magnetic measurements for complexes C1-C3

Complex	Temp K	χ x 10^{-6}cgs	χ_M x $^{10-6}$cgs	$\chi_{M'}$ x 10^{-6}cgs	μeffB M
C1	295 2.532	1841.62	1949.22	2.14	
C2	291	3.416	1939.00	2068.24	2.19
C3	300	2.758	1412.08	1451.76	1.86

Antimicrobial activities

Complexes C1-C3 were screened for biological activities against the following bacteria: *Staphylococcus Aureus* (gram positive), *Micrococcus Luteus* (gram positive), *Escherichia Coli* (gram negative), *Pseudomonas Aeruginosa* (gram negative), *Klebsiella Pneunomiae* (gram negative) and two fungi: *Candida albicans*, *Aspergillus Niger*. Kanamycin (600 µg/mL) was used as a control. All the three copper complexes showed activities against all the strains. Complex C3 displayed the same activity as compared to the control antibiotic. Furthermore, complexes C1-C3 have good activities against *Aspergillus Niger*, *Escherichia Coli* and *Micrococcus Luteus*. This behavior is in accordance with previous reports on the good biological activities of copper complexes (El-Bindary et al., 2016). *Klebsiella Pneunomiae* showed very high resistance against all the complexes. Detailed results are condensed in Table 3.

Table 3. Diameter of zone of inhibition (mm) for complexes C1-C3

Compound	Organism						
	S. Aureus	*K.Pneumonia*	*E. Coli*	*M. Luteus*	*P. Aerogenosa*	*C. Albicans*	*A. Niger*
Control	17	11	16	12	4	16	10
C1	7	1	5	6	3	9	6
C2	8	0	5	7	3	9	7
C3	8	2	6	7	4	9	7

Conclusion

Pyrazolyl complexes of copper(II) have been successfully synthesized and characterized by various techniques including spectroscopy and thermal analysis. The combination of structural information from all these methods suggests that the pyrazolyl copper(II) complexes studied have an octahedral geometry with two coordinated water molecules. The other coordination sites are satisfied by two oxygen atoms from two nitrate groups and two nitrogen atoms from the pyrazolyl ligands. The synthesized complexes showed activities against a range of gram negative, gram negative bacteria and some fungi.

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