

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL STUDY OF METAL (II) COMPLEXES OF BARBITURIC ACID

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ABSTRACT

The complexes were synthesized by refluxing ethanolic solutions of metal (II) chlorides salts (MCl_2 , $M=Cr(II)$, $Mn(II)$ and $Fe(II)$,) and barbituric acid in the ratio 1:1. The complexes were characterized by conductivity measurement, solubility, elemental analysis and infrared spectroscopy. The percentage yields of Cr (II), Mn (II) and Fe (II), are 74.40%, 87.56%, and 78.21%, respectively. The molar conductivity measurements are within $27.2-28.0 \times 10^{-6} \text{ ohm}^{-1}\text{cm}^2\text{mol}^{-1}$ respectively. The complexes have high decomposition temperature in the range $328^\circ\text{C}-334^\circ\text{C}$. The complexes showed appreciable antibacterial activities against *Staphylococcus aureus* and *Escherichia coli* whereas ligand shows none, all the synthesized complexes are not as active as reference drug (Apron plus) on

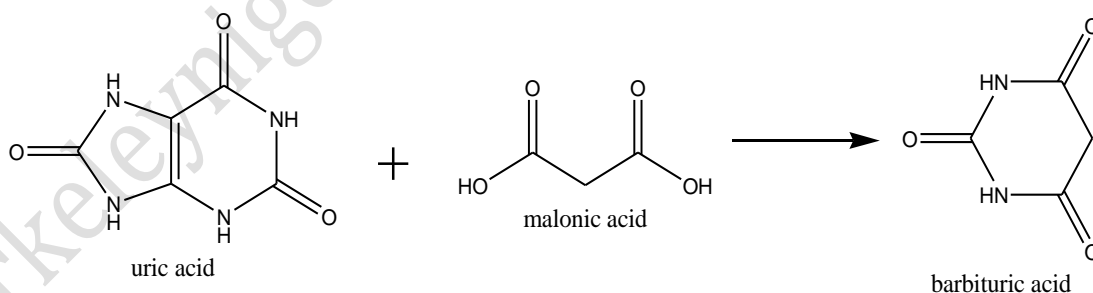
Introduction:

A Co-ordinating Complex or Metal Complex consists of a central atom or ion, which is usually metallic and is called co-ordination centre, and surrounded by molecules or ions that are in turn known as ligands. Therefore, for the formation of complex the central metal ion must have an empty orbital of suitable energy while the ligand must have a lone pair of electron. Ligand is a neutral molecule or ion that forms coordinate bond with metal ion to give a complex. A ligand must have at least one unshared pair of electron, it act as Lewis base; donating one or more electron pairs to metal ion which act as lewis acid (Laird, 2009). Ligands are classified,

Penicillium species, while Cr (II) complex showed better inhibition zone on Aspergillus specie more than the standard.

Keywords: *Refluxing, Ethanolic solutions, Metal (II) chlorides, Inhibition zone and Complexes.*

Depending on the number of donor atoms (electron pair) present, as Monodentate, bidentate, tridentate, tetradentate etc. for example water (H_2O) and ammonia (NH_3) are monodentate, while ethylenediamine (en) is bidentate. Many metal-containing compounds, especially those of transition metal are co-ordination complexes. Co-ordination complexes were known although not understood in any sense since the beginning of chemistry. The key breakthrough occurred when Alfred Werner proposed in 1893 that Co (III) bears six ligands in an octahedral geometry. His theory allows one to understand the difference between co-ordinate and ionic compound. Barbituric acid (2, 4, 6-trioxohexahydropyrimidine) (Scheme 1) was first synthesized in 1864 by German chemist Adolf von Baeyer (1835-1975) (Kauffman, 1980). Baeyer's first professional studies involved combining uric acid ($C_5H_4N_4O_3$) with malonic acid ($CH_2(COOH)_2$), from which he derived barbituric acid ($C_4H_4N_2O_3$). It was from this parent compound that a group of sedative drugs known as the barbiturates were developed.



Scheme 1: Reaction of malonic acid and uric acid to produce barbituric acid.

The study of barbituric acid and its derivatives has received a lot of attention over the years due its various applications in drugs such as anticonvulsive, hypnotic, anesthetics, cures anxiety, epilepsy, and other

psychiatric disorders. Also affect the motor and sensory functions (Ashnagar et al., 2007). However, it affect the motor and sensory functions (Fillaut, et al., 2002). Sparse transition Metal complexes of barbituric acid have been synthesized and characterized (Refat et al., 2008). Two new azo-dyes were prepared by linking 8-aminoquinoline to barbituric acid and 1, 3-dimethyl barbituric acid through diazo-coupling reactions (Gup et al., 2007). Metal complexes of these ligands were also prepared and characterized. All spectral data indicated that the azo-dyes acted as tridentate ligands. Cobalt (II), nickel (II) and copper (II) complexes of barbituric acid have also been synthesized at room temperature (Masoud et al., 1983). The complexes were characterized with the infrared and electronic spectra, as well as magnetic susceptibility measurements. Their elemental analyses suggested a range of stoichiometries 1:1, 1:2, 1:3. Their structures were also determined (Masoud et al., 1983). Furthermore, some researches disclosed that Barbituric acid derivatives are associated with a number of biological activities (Jursic, 2001). This research is aim to synthesize, characterize and investigate antimicrobial activities of Metal (II) Complexes.

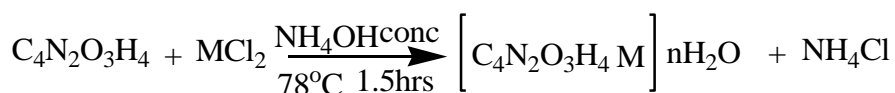
Materials and Methods

All reagents were of Analar Grade and were used without further purification. All the glass wares used in this research were thoroughly washed with detergent, rinsed with distilled water and dried in an oven. Electric balance model AB54 was used for weighing, molar conductance measurement was done using Jenway conductivity meter model 4010, and the infrared spectral analysis were recorded using FTIR 8400s Fourier transform spectrometer, elemental analysis was observed on Flash 2000 Organic Analyzer at A. B. U. Zaria. Decomposition temperatures of the complexes were obtained using Gallen Kamp melting apparatus. The *in vitro* - antibacterial and anti-fungal screening was performed by disc diffusion method in the Department of Microbiology, Bayero University, Kano.

Preparation of metal (II) barbituric acid complexes

The metal (II) barbituric acid complexes were prepared by refluxing ethanolic solutions of a metal (II) chlorides and barbituric acid in the mole

ratio 1:1, concentrated ammonium hydroxide was added at 78°C for 1½ hours (Ikotun *et al.*, 2011). The precipitate that formed was separated, washed with ethanol dried in an oven at 60°C, weighed and stored in the desiccators for three days under P₂O₅. The equation of the reaction is as follows;



Where M=Cr, Mn and Fe

Scheme2: The equation of the reaction between metal (II) chlorides and barbituric acid.

Percentage yield of the complexes

Theoretical yield of the metal (II) complexes from the balanced equation;
1mole of barbituric acid produced 1mole of the complex

1mole × 127gmol⁻¹ of barbituric acid → 1mole ×
305gmol⁻¹ of Cr (II) Complex

1g of barbituric acid → Xg of Cr (II)
complex

$$Xg \text{ of Cr (II) complex} = \frac{305gmol^{-1} \times 1g}{127gmol^{-1}} = 2.40g$$

Therefore, theoretical yield of Cr (II) complex is 2.40g

Experimental yields are obtained by taking an average of three readings recorded for each complex i.e. for Cr (II) the average weight is 1.787g.

$$\% \text{ yield} = \frac{\text{experimental weight}}{\text{theoretical weight}} \times 100$$

$$\text{e.g. \% yield of Cr (II) complex} = \frac{\text{exp}}{\text{Theo}} \times 100 = \frac{1.787}{2.40} \times 100 = 74.40\%$$

The percentages yields of the other complexes were similarly determined.

Determination of Water of Crystallization

0.2g of each complex was measured and placed in an oven at about 110°C, the change in weight are recorded, until a constant weight was attained. The percentage water of crystallization was evaluated from an expression below;

Average weight loss of chromium is 0.019g.

$$\begin{aligned} \text{Percentage H}_2\text{O of crystallization} &= \frac{\text{weight lost}}{0.2} \times 100\% \\ &= \frac{0.019}{0.2} \times 100\% = 9.5\% \end{aligned}$$

The percentages of H₂O of crystallization of other complexes were similarly determined.

Antimicrobial determination

Three different concentrations (1000µg/disc, 500µg/disc and 50µg/disc) of each synthesized complex was prepared using DMSO in different sample bottles containing 50 discs each and allowed to dry off. From each synthesized complex 0.1g was weighed and 1cm³ of DMSO was added to dissolve it in a sample bottle containing 50 discs in order to have prepared a concentration of 1000µg/ cm³ (stock solution). 0.5cm³ of the solution was fetched into another sample bottle containing 50 discs and 0.5cm³ of DMSO was added in order to have prepared 500µg/cm³. 0.1cm³ was fetched from the second solution into the third sample bottle containing 50 discs and 0.9cm³ of DMSO was added in order to obtain 50µg/cm³ concentration (Cheesbrough, 2000). A sterilized Nutrient Agar solution were poured into the petri dishes in a septical way and allowed for almost an hour for the agars to solidify in the dishes. The cultured bacteria was prepared in a 5cm³ normal saline, while the fungi were prepared in 5cm³ sterilized distilled water and standardized with McFaland standard. The swab stick was used to gently rob both the bacteria and fungi over the solidified Nutrient Agar and Saboraud Dextrose Agar contained in petri dishes respectively. Two dried discs were gently placed over each

inoculated dish in a septic way using sterilized needle. The inoculants were then put in an ovum for 24hrs (Cheesebrough, 2000).

Results and Discussion

Table 1: Physical Properties of the Metal (II) barbituric acid Complexes

Complexes	Colour	Decomposition	Percentage yield (%)
		Temperature (°C)	
Cr (II)	Dark Blue	330	74.40
Mn (II)	Light brown	328	87.56
Fe (II)	Dark Brown	334	78.21

Table 2: Solubility of Metal (II) barbituric acid Complexes

Complexes	Solubility in					
	H ₂ O	H ₂ O ₂	CH ₄ O	C ₂ H ₄ O ₂	DMSO	CHCl ₃
Cr (II)	SS	S	SS	S	S	SS
Mn (II)	SS	S	SS	S	S	SS
Fe (II)	SS	S	SS	S	S	SS

Key: S = Soluble; SS = Slightly Soluble

Table 3: Molar Conductivity of the Complexes.

Complexes	Molar Concentration moles/dm ³	Electrical conductivity $\Omega^{-1}\text{cm}^{-1}$	Molar Conductivity $\Omega^{-1}\text{cm}^2 \text{mol}^{-1}$
Cr (II)	1×10^{-3}	27.2	27.2×10^{-6}
Mn (II)	1×10^{-3}	29.0	29.0×10^{-6}
Fe (II)	1×10^{-3}	28.0	28.0×10^{-6}

Table 4: Relevant IR of the Ligand and Complexes

Complexes	$\nu(\text{O-H})$ cm ⁻¹	$\nu(\text{N-H})$ cm ⁻¹	$\nu(\text{C=O})$ cm ⁻¹	$\nu(\text{C=N})$ cm ⁻¹	$\nu(\text{O-H}_2)$ cm ⁻¹	$\nu(\text{M-N})$ cm ⁻¹	$\nu(\text{M-O})$ cm ⁻¹	$\nu(\text{H}_2\text{O})$ cm ⁻¹
Ligand H ₂ L	3532	3370	1743	1617	-	-	-	-
Cr (II)	-	3274	1619	-	-	571	421	621
Mn (II)	-	3146	1753	1597	3865	523	411	634
Fe (II)	3464	3237	1752	1598	3843	570	450	648

Table 5: Elemental Analysis of Metal (II) Barbituric acid Complexes

Complexes	ELEMENTS	N %	C%	H %
Cr(II)	Calculated	8.2239	14.1042	1.4795
	Found	8.6039	14.9605	2.0161
Mn(II)	Calculated	5.0201	8.5025	0.9000
	Found	5.8296	7.6625	0.0350
Fe(II)	Calculated	2.8285	4.8501	0.3184
	Found	1.6134	3.2509	0.1205

Table 6: Antibacterial Activity Inhibition Zones of Metal (II) Barbituric acid Complexes

Complexes	Concentrations ($\mu\text{g}/\text{disc}$)	Bacteria Inhibition Zones (mm)	
		<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
Cr (II)	1000	14	15
	500	10	8
	50	6	6
Mn (II)	1000	14	6
	500	9	6
	50	6	6
Fe (II)	1000	6	6
	500	6	6
	50	6	6
Ligand	1000	6	6
	500	6	6
	50	6	6
Control (streptomycin)	10	24	6

Table 7: Antifungal Activity Inhibition Zones of Metal (II) Barbituric acid Complexes

Complexes	Concentrations ($\mu\text{g}/\text{disk}$)	fungal Inhibition Zones (mm)	
		<i>Penicillium specie</i>	<i>Aspergillus specie</i>
Cr (II)	1000	21	23
	500	15	15
	50	13	11
Mn (II)	1000	18	6
	500	13	6

	50	6	6
Fe (II)	1000	16	18
	500	6	11
	50	6	6
Ligand	1000	6	6
	500	6	6
	50	6	6
Control(Apron plus)	10	38	20

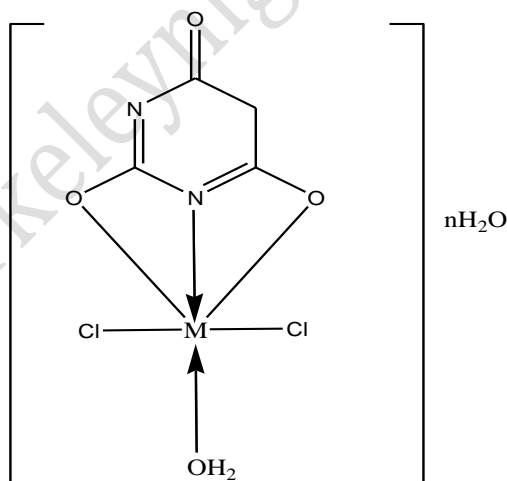
Discussion

The complexes were prepared by refluxing ethanolic solutions of barbituric acid and the corresponding metal (II) chloride. The decomposition temperature of each complex was determined and found to be in the range of 328°C-334°C, suggesting that the complexes are quite stable. The Chromium (II) complex is blue, while Iron (II) and Manganese (II) complexes are brown. The percentage yields were within the range of 74.40-87.56% as shown in the Table 1. The solubility test of the complexes was carried out in common organic solvents and water. All the complexes are slightly soluble in water, methanol and chloroform, but found to be soluble in hydrogen peroxide, acetic acid and dimethyl sulphoxide, as shown in Table 2. The molar conductivity measurement of the metal complexes in DMSO was measured and found to be at the range $27.2-29.0 \times 10^{-6} \text{ ohm}^{-1}\text{cm}^2\text{mol}^{-1}$ suggested that the complexes are non - electrolytic (Mozaffar *et al.*, 2010). The infrared measurement of the Ligand and complexes were determined. The ligand shows a strong signal at 3532cm^{-1} which is assigned to $\nu(\text{O-H})$ stretching. The strong bands at 1743 cm^{-1} is attributed to $\nu(\text{C=O})$ stretching modes, while the band at 1617cm^{-1} is assigned to $\nu(\text{C=N})$ vibrational frequencies, similar bands were also observed in the complexes, suggesting coordination of barbituric acid to the respective metals. The absence of band in the complexes in the region 3532cm^{-1} indicates in Cr (II) and Mn (II), complexes is due to deprotonations of barbituric acid before coordination to the respective metals (Aliyu and Toud, 2012). The new strong bands appeared in the ranges, $520-590\text{cm}^{-1}$ and $411-450\text{cm}^{-1}$ which are attributed to $\nu(\text{M-N})$ and $\nu(\text{M-O})$ stretching vibrations respectively, and confirming the coordination of the ligand to the respective metals (Raman *et al.*, 2001). The strong bands in the ranges $3427-3585\text{cm}^{-1}$ and $600-678\text{cm}^{-1}$ attributable to $\nu(\text{O-H})$ stretching frequencies for water of crystallization and coordinated water

in metal (II) complexes respectively (Table 4). The micro elemental analysis of C, N and H. was observed, their percentage compositions correlated with the calculated values. The result obtained is in good agreement with 1:1 metal-barbituric ratio as shown in Table 5.

The results of antibacterial activity of metal complexes, ligand and streptomycin (the standard) against a gram positive (*Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*) have been determined. The metal (II) complexes have been found to be more effective than the free ligands which agreed with Tweedy's chelation theory which predicts that chelation reduces the polarity of the metal atom mainly because of partial sharing of its positive charge with the donor groups and possible electron delocalization over the whole ring. This consequently increases the lipophilic character of the chelates, favouring its permeation through lipid layers of the bacterial membrane (Thangadura *et al*, 2001). The ligand showed no activity against bacterial strains, Mn (II) complex showed activity against *Staphylococcus aureus* only. The Cr (II) complex has moderate activity on *Staphylococcus aureus* and *Escherichia coli*. The result is in Table 6. The antifungal activity of metal complexes, ligand and apron plus (the standard) used against *Penicillium specie* and *Aspergillus specie* have been determined. The Mn (II) complex is active against *Penicillium specie* at higher concentrations, the Fe (II) complex at higher concentrations proved to be active on both fungi. The Cr (II) complex displayed better activity on both fungi even at lower concentrations. All the synthesized complexes are not as active as reference drug (Apron plus) on *Penicillium species*. The result is shown in Table 7.

The propose structure



M =Cr (II), Mn (II) and Fe (II)

Figure 1:The proposed structure of aqua barbiturito-metal (II) complex.

CONCLUSION

The metal complexes of Cr (II), Mn (II) and Fe (II), were successfully synthesized; the metal (II) barbituric acid complexes synthesized are coloured and stable. The Solubility, decomposition temperature, molar

conductance measurement, percentage of water of crystallization, elemental analysis, infrared spectra, antibacterial and antifungal properties of the ligand and metal (II) complexes were carried out. Elemental analysis of the complexes suggested 1:1 metal-ligand ratio. They are non-electrolytes from molar conductivities values of the complexes are in range of $27.2 \times 10^{-6} \text{ ohm}^{-1} \text{ cm}^2 \text{ mol}^{-1}$. $29.0 \times 10^{-6} \text{ ohm}^{-1} \text{ cm}^2 \text{ mol}^{-1}$ indicating that are non-electrolyte. Also the metal complexes showed better antibacterial and antifungal activities than the free ligand.

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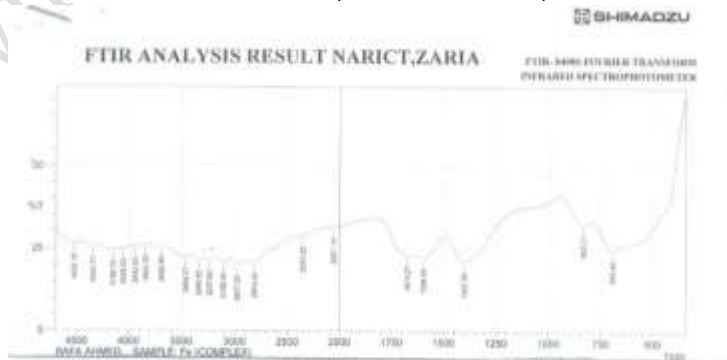
APPENDIX IA: IR Spectrum of Chromium (II) Complex



APPENDIX IB: IR Spectrum of Manganese (II) Complex



APPENDIX IC: IR Spectrum of Iron (II) Complex



APPENDIX 2A: Elemental Analysis of Chromium (II) Complex

Eager 300 Report

Page: 1 Sample: Cr/Com B A (File012)

Method Name : MUSEL/ABU Zaria
 Method File : C:\Eager for FLASH\data\Sys_data_example\Multi user Science F
 Chromatogram : File012
 Operator ID :
 Analysed : 08/01/2013 15:39 Company Name :
 Sample ID : Cr/Com B B (# 12) Printed : 08/01/2013 15:15
 Instrument N. : 2011F0138
 Analysis Type : Unknown (Area) Sample weight : .003244

Calib. method : using 'K Factors'

!!! Warning missing one or more peaks.

Element Name	%	Ret. Time	Area	BC	Area ratio	K factor
Nitrogen	8.4039	47	728438 RS		2.452185	.132683E+1
Carbon	14.9605	69	1815402 RS		1.000000	.463970E+1
Hydrogen	2.0141	199	2814213 RS		.645062	.173407E+1

APPENDIX 2B: Elemental Analysis of Manganese (II) Complex

Page: 1 Sample: Mn/Com B (File014)

Method Name : MUSEL/ABU Zaria
 Method File : C:\Eager for FLASH\data\Sys_data_example\Multi user Science F
 Chromatogram : File014
 Operator ID :
 Analysed : 08/01/2013 16:10 Company Name :
 Sample ID : Mn/Com B (# 14) Printed : 08/01/2013 16:22
 Instrument N. : 2011F0138
 Analysis Type : Unknown (Area) Sample weight : .004205

Calib. method : using 'K Factors'

!!! Warning missing one or more peaks.

Element Name	%	Ret. Time	Area	BC	Area ratio	K factor
Nitrogen	5.8296	47	593459 RS		3.033074	.132683E+1
Carbon	7.6625	70	1800005 RS		1.000000	.463970E+1
Hydrogen	0.0359	175	1215638 RS		1.480708	.173407E+1

APPENDIX 2C: Elemental Analysis of Iron (II) Complex

Eager 300 Report

Page: 1 Sample: Fe/Com B (File011)

Method Name : MUSEL/ABU Zaria
 Method File : C:\Eager for FLASH\data\Sys_data_example\Multi user Science F
 Chromatogram : File011
 Operator ID :
 Analysed : 08/01/2013 15:22 Company Name :
 Sample ID : Fe/Com B (# 11) Printed : 08/01/2013 15:37
 Instrument N. : 2011F0138
 Analysis Type : Unknown (Area) Sample weight : .004712

Calib. method : using 'K Factors'

!!! Warning missing one or more peaks.

Element Name	%	Ret. Time	Area	BC	Area ratio	K factor
Nitrogen	1.6134	47	322665 RS		1.486973	.132683E+1
Carbon	3.2509	71	802459 RS		1.000000	.463970E+1
Hydrogen	0.1205	177	1284152 RS		.624894	.173407E+1