

SOLVENT- FREE SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL STUDIES OF UREA AND THIOUREA –METAL COMPLEXES

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Abstract

Solvent-based synthesis as well as Liquid Assisted Grinding (LAG) synthesis of urea and thiourea complexes is described for comparison purposes. The resulting complexes of both methods of syntheses were characterized using melting points, conductivity values, spectroscopic data (IR, UV-Visible) and antibacterial activity study. Spectral data of the complexes synthesized by LAG method was found to be similar to those synthesized by conventional synthesis. All the ligands were found to be monodentate and all the complexes exhibited tetrahedral and square-planar geometry. The LAG method was carried out by introducing a few drops of solvent. The method is faster with a better yield compared to solvent-based method. The antimicrobial study of the ligands and the complexes was carried out against Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*). The antibacterial activity study of the complexes showed that they possessed higher inhibitory activity than their parent ligands at concentration of 200 µgml⁻¹.

Keywords: Urea, Thiourea, Transition metals, Antibacterial activity, Spectroscopic studies

Introduction

Urea also known as carbamate CH₄N₂O was the first organic compound to be synthesized in the laboratory by Wöhler in 1828 (Fieser 1967). It is produced in the liver of human body and found in the urine of mammals. Replacement of oxygen atom in urea by sulfur atom produces thiourea. Thiourea is one of the simplest organic molecules containing a thioamide group, and its structures and properties have been studied extensively by various experimental and theoretical techniques (Martin & Filleux-Blanchard 1980., Ha & Peubia, 1994. and Grare *et al* 2007). The oxygen, nitrogen and sulphur donor atoms of urea and thiourea derivative provide multitude of bonding possibilities.

In the last few years, improving fertilizer design (urea) for plant nutrition is important to fertilizer producers and users, which has lead to its considerable attention for attainment of Millennium Development Goals (MDG) (Amberger, 1986). Unfortunately, the emergence of multi drug resistant bacteria strains has made the treatment of bacteria diseases more complex [5]. Also, urea and thiourea compounds have been associated with a wide range of biological activities such as antitumour and antimicrobial properties (Yildiz *et al* 2012). Synthesis of metal complexes of urea and thiourea using solvent-based methods have been reported by some researchers (Martin and Filleux-Blanchard, 1980., Calogero, 1980 and Amolegbe *et al* 2012). It was reported that urea and thiourea derivative-based complexes may likely improve the nutrient level in the soil as well as environmentally friendly (Amolegbe *et al* 2012). In view of this observation, we have synthesized new compounds of urea and thiourea metal complexes using reflux and liquid assisted grinding methods..

Materials and Methods

The materials used were products of British Drug House and were gotten from the department of Chemistry, University of Ilorin. They are urea, thiourea, ZnSO₄.7H₂O, Fe (NH₄)₂(SO₄)₂.6H₂O, H₃PO₄, methanol and distilled water. The method described by Amolegbe *et al* 2012 was adopted for the conventional synthesis while the method described by Bowmark *et al* 2008 was adopted for the liquid assisted grinding synthesis.

Conventional Synthesis of Zn(II) and Fe(II) Urea Complexes: The complexes of Zn(U)₂(SO₄) and Fe(U)(NH₄)₂(SO₄)₂(OH)₂ were prepared by mixing separately, solution of urea (5 mmol, 0.3002 g) in 10ml distilled water with each of solution of ZnSO₄.7H₂O, (2.5 mmol, 0.719 g) in 10ml of methanol and solution of

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$\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$, (5 mmol, 1.96 g) in 10ml of distilled water. The mixture was refluxed for 2 hrs and 10 ml of 0.1M H_3PO_4 was added to the refluxed mixture which was then refluxed for another 30 min. The solution was left to cool after which the precipitate was filtered, washed and kept in a dessicator.

Conventional Synthesis of Zn(II) and Fe(II) Thiourea Complexes: The complexes of $\text{Zn}(\text{Tu})(\text{SO}_4)$ and $\text{Fe}(\text{Tu})(\text{NH}_4)_2(\text{SO}_4)_2(\text{OH})_2$ were prepared by mixing separately 10ml of methanolic solution of thiourea (5 mmol, 0.3800 g) with each of solution of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, (2.5 mmol; 0.719 g) in 10ml methanol and solution of $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$, (5 mmol, 1.96 g) in 10ml distilled water. The mixture was refluxed for 2 hrs and 10 ml of 0.1M H_3PO_4 was added to the refluxed mixture which was then refluxed for another 30 min. The solution was left to cool after which the precipitate was filtered, washed and kept in a dessicator.

Liquid Assisted Grinding Synthesis of Zn(II) and Fe(II) Urea Complexes: The $[\text{Zn}(\text{U}_2)(\text{SO}_4)]$, $\text{Fe}(\text{U})(\text{NH}_4)_2(\text{SO}_4)_2(\text{OH})_2$ and $\text{Zn}(\text{Tu})(\text{SO}_4)$, $\text{Fe}(\text{Tu})(\text{NH}_4)_2(\text{SO}_4)_2(\text{OH})_2$ complexes were prepared by weighing 5 mmol, of U/Tu and 2.5 mmol of the metal salts into a mortar that had been previously washed and dried. The reactants were ground continuously with 3 drops of sulphuric acid in the mortar with pestle for 15 minutes to a fine paste.

Antibacterial Studies

The urea and thiourea complexes were screened for their antimicrobial activity against *S. aureus*, *E. coli*, and *B. subtilis* using the agar diffusion method (Grare et al 2007). The antibacterial screening of the ligands and the complexes were determined by the method described by Adomi, 2008 and Larbini, (2007).

Table: 1a Physical and analytical data of urea complexes

Complexes	Colour	Conductivity ($\mu\text{S}/\text{cm})(\times 10^{-6})$	Yield (%)	M.pt. ($^{\circ}\text{C}$)	Elemental Analysis C H N (Calc)%
$[\text{Zn}(\text{U}_2)(\text{SO}_4)]$ Solvent-based	White	5.45	78.6	210	8.41(7.66), 2.83(2.56) 19.47(17.89)
$[\text{Zn}(\text{U}_2)(\text{SO}_4)]$ LAG	White	4.10	91.3	225	7.89(7.36), 3.46(3.01), 19.01(19.45)
$[\text{Fe}(\text{U})(\text{NH}_4)_2(\text{SO}_4)_2(\text{OH}_2)]$ Solvent-based	Yellow	6.42	35.8	250	0.91(0.94), 2.62 (3.13), 4.51(4.39)
$[\text{Fe}(\text{U})(\text{NH}_4)_2(\text{SO}_4)_2(\text{OH}_2)]$ LAG	Yellow	9.34	91	250	2.66(2.56), 5.34(5.12), 15.54 (11.97)

Table 1b: Physical and analytical data of thiourea complexes

Complexes	Colour	Conductivity ($\mu\text{S}/\text{cm})(\times 10^{-6})$	Yield (%)	M.pt. ($^{\circ}\text{C}$)	Elemental Analysis C H N (Calc)%
$[\text{Zn}(\text{Tu}_2)(\text{SO}_4)]$ Solvent-based	White	5.75	73.6	240	8.60(7.36), 2.36(2.01), 19.32(17.45)
$[\text{Zn}(\text{Tu}_2)(\text{SO}_4)]$ LAG	White	3.55	92.3	229	8.01(8.00), 2.39(2.21), 18.32(18.45)
$[\text{Fe}(\text{Tu})(\text{NH}_4)_2(\text{SO}_4)_2(\text{OH}_2)]$ Solvent-based	Yellow	0.88	65.53	226	0.84(0.89), 2.62(2.13), 3.51(3.39)
$[\text{Fe}(\text{Tu})(\text{NH}_4)_2(\text{SO}_4)_2(\text{OH}_2)]$ LAG	Yellow	2.79	90.9	226	2.42(2.76), 4.34(4.05), 14.64(13.97)

Table 2a: UV-Visible spectroscopy data of urea and its complexes:

Ligand/Complex	λ (nm)	E (cm ⁻¹)	Transition/crystal field parameter
U	364 232 208	2747 4310 4808	$\pi \rightarrow \pi^*$ $n \rightarrow \sigma^*$ $\sigma \rightarrow \sigma^*$
[Zn(U ₂)(SO ₄)] Solvent-based	274 232 199	3650 4310 5025	$\pi \rightarrow \pi^*$ $n \rightarrow \sigma^*$ $\sigma \rightarrow \sigma^*$
[Zn(U ₂)(SO ₄)] LAG	364 274 232 205	2747 3650 4310 4878	MLCT $\pi \rightarrow \pi^*$ $n \rightarrow \sigma^*$ $\sigma \rightarrow \sigma^*$
[Fe(U)(NH ₄) ₂ (SO ₄) ₂ (OH ₂)] Solvent-based	463 427 283 232	2160 2342 3534 4310	MLCT $T_{2g} - E_{2g}$ $\pi \rightarrow \pi^*$ $n \rightarrow \sigma^*$
[Fe(U)(NH ₄) ₂ (SO ₄) ₂ (OH ₂)] LAG	502 484 283 252	1992 2066 3534 3968	$T_{2g} - E_{2g}$ $\pi \rightarrow \pi^*$ $n \rightarrow \sigma^*$ $\sigma \rightarrow \sigma^*$
Ligand/Complex	λ (nm)	E (cm ⁻¹)	Transition/crystal field parameter
Tu	241 193	4149 5181	$\pi \rightarrow \pi^*$ $n \rightarrow \sigma^*$
[Zn(Tu ₂)(SO ₄)] Solvent-based	277 232	3610 4310	$\pi \rightarrow \pi^*$ $n \rightarrow \sigma^*$
[Zn(Tu ₂)(SO ₄)] LAG	238	4202	$n \rightarrow \sigma^*$
[Fe(Tu)(NH ₄) ₂ (SO ₄) ₂ (OH ₂)] Solvent-based	820 271 229 199	1220 3690 4367 5025	$T_{2g} - E_{2g}$ $\pi \rightarrow \pi^*$ $n \rightarrow \sigma^*$ $\sigma \rightarrow \sigma^*$
[Fe(Tu)(NH ₄) ₂ (SO ₄) ₂ (OH ₂)] LAG	886 271 238 196	1129 3690 4202 5102	$T_{2g} - E_{2g}$ $\pi \rightarrow \pi^*$ $n \rightarrow \sigma^*$ $\sigma \rightarrow \sigma^*$

Table 3 : Some selected Infra-red spectra complexes of Urea and Thiourea complexes:

Ligand/Complex	ν (N-H) (stretching)	(C=O) (stretching)	ν (C-N) (stretching)	ν (N-H) (bending)	N(M-O)	ν (Fe-OH ₂)
U	3441 s	1681 m	1153 s	1464s	-	-
[Fe(U)(NH ₄) ₂ (SO ₄) ₂ (OH ₂)] (solvent-based)	3439 w b	1647 s	1039 s b	1577 w	472 s	-
[Fe(U)(NH ₄) ₂ (SO ₄) ₂ (OH ₂)] (LAG)	3450 w b	1660 w	1087 w b	1556 w	468 s	721 w
Tu	3385 s w	731 s w	1084 s	1618 s w	-	-
[Fe(U)(NH ₄) ₂ (SO ₄) ₂ (OH ₂)] (solvent-based)	3419 s w	781 s w	1402 s	1649 s b	472 s	617 s
[Fe(U)(NH ₄) ₂ (SO ₄) ₂ (OH ₂)] (LAG)	3477 w	767 w	1452 w	1627 s b	457 s	619 s

Antibacterial Studies of the Ligands and their Complexes

Table 4a: Antibacterial activities of urea and its complexes showing the zone of inhibition against the organisms (ppm)

LIGAND/COMPLEX	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>
U	8.0 ± 0.3	7.35 ± 0.1	5.35 ± 0.2
[Zn(U ₂)(SO ₄)] Solvent-based	22.50 ± 0.1	22.00 ± 0.3	22.00 ± 0.1
[Zn(U ₂)(SO ₄)] LAG	16.23 ± 0.3	18.10 ± 0.2	19.10 ± 0.3
[Fe(U)(NH ₄) ₂ (SO ₄) ₂ (OH ₂)] Solvent-based	23.50 ± 0.2	21.00 ± 0.2	27.10 ± 0.3
[Fe(U)(NH ₄) ₂ (SO ₄) ₂ (OH ₂)] LAG	19.23 ± 0.4	16.00 ± 0.1	24.20 ± 0.1
Standard Drug	22.20 ± 1.6	24.80 ± 0.8	21.00 ± 0.9

Table 4b: Antibacterial activities of thiourea and its complexes showing the zone of inhibition against the organisms (ppm)

LIGAND/COMPLEX	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>
Tu	2.5 ± 0.1	-	2.0 ± 0.5
[Zn(Tu ₂)(SO ₄)] Solvent-based	16.12 ± 0.2	13.00 ± 0.2	18.13 ± 0.2
[Zn(Tu ₂)(SO ₄)] Solvent-free	9.11 ± 0.1	10.00 ± 0.1	13.12 ± 0.1
[Fe(Tu)(NH ₄) ₂ (SO ₄) ₂ (OH ₂)] Solvent-based	17.00 ± 0.2	14.00 ± 0.3	18.50 ± 0.1
[Fe(Tu)(NH ₄) ₂ (SO ₄) ₂ (OH ₂)] LAG	16.00 ± 0.1	12.00 ± 0.1	17.00 ± 0.3
Standard Drug	22.20 ± 1.6	24.80 ± 0.8	21.00 ± 0.9

Table 5a: Minimum Inhibitory Concentration (MIC) of Urea Complexes (µgml⁻¹)

COMPLEX	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>
[Zn(U ₂)(SO ₄)] Solvent-based	15.00	15.00	10.50
[Zn(U ₂)(SO ₄)] LAG	20.00	25.00	20.00
[Fe(U)(NH ₄) ₂ (SO ₄) ₂ (OH ₂)] Solvent-based	12.00	14.00	10.00
[Fe(U)(NH ₄) ₂ (SO ₄) ₂ (OH ₂)] LAG	19.00	20.002	22.00

Table 5b: Minimum Inhibitory Concentration (MIC) of Thiourea Complexes (µgml⁻¹)

COMPLEX	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>
[Zn(Tu ₂)(SO ₄)] Solvent-based	20.00	25.00	20.00
[Zn(Tu ₂)(SO ₄)] LAG	24.00	30.00	25.00
[Fe(Tu)(NH ₄) ₂ (SO ₄) ₂ (OH ₂)] Solvent-based	19.00	24.00	15.00
[Fe(Tu)(NH ₄) ₂ (SO ₄) ₂ (OH ₂)] LAG	24.00	30.00	25.00

Results and Discussion

The results of the physical and spectroscopic data are presented in Table 1 to 3.

The melting points of the complexes are generally higher than that of the corresponding free ligands which suggests formation of complexes. In all cases, the LAG gave better yield than the solvent-based synthesis. The colors of the complexes formed differed from their parent ligand, e.g. from color white to pale yellow (Fe^{2+} complexes). The conductivity measurements of the ligands in water was found higher than their respective complexes which showed that the degree of ionization of the complexes are low and thus are weak electrolytes with high probability of no anions present outside the coordination sphere (Obaleye *et al.* 1997). These characteristics conformed to the previous studies of Amolegbe *et al.*, 2012.

The UV-Visible spectra is being interpreted in terms of band due to electron transfer within ligands, charge transfer from the ligand orbital to the metal atom as well as d-d transition (Obaleye *et al.*, 1997). The UV-Visible spectra of urea showed three absorption bands at 208 nm, 232 nm and 364 nm while that of thiourea showed two absorption bands at 193 nm and 241 nm. These bands have been shifted in the metal complexes due to complexation, though some bands in the complex were observed at far wavelength region. In all the complexes, a comparable absorption ($\pi \rightarrow \pi^*$) is obtained at higher wavelength (bathochromic shift). Table 3 shows the infrared spectrum of urea with a band at 727.19 cm^{-1} , which is attributed to the $\nu(\text{M}-\text{OH}_2)$ of the associated water molecules of the complex which was found to be absent in the free ligand. This result is an indicative of the coordination of oxygen of water molecule to the metal ion (Angiolini *et al.*, 2009). The spectrum of thiourea had 3385 cm^{-1} , 731 cm^{-1} and 1084 cm^{-1} which were assigned to $\nu(\text{N}-\text{H})$, $\nu(\text{C}=\text{S})$ and $\nu(\text{C}-\text{N})$ respectively. The spectra of the complexes showed similar bands as observed in the ligands, this is an evidence of the presence of the ligand in the complex. The band at 617 cm^{-1} and 619 cm^{-1} which is attributed to the $\nu(\text{M}-\text{OH}_2)$ of the associated water molecules of the complex which was found absent in the free ligand (Amolegbe *et al.*, 2012).

The antibacterial activities of the complexes were found to be higher than that of their respective ligands (Table 4 to 5). The complexes also showed a better growth inhibition than their respective ligands. This was due to the presence of metal in the complexes and their significance in inactivating bacterial enzymes. The solvent-based synthesized complexes showed a growth inhibition of the bacteria up to $10.00 \mu\text{gml}^{-1}$ while the solvent-free synthesized complexes only showed a growth inhibition of up to $20.00 \mu\text{gml}^{-1}$. The ability of the solvent-based synthesized complexes to inhibit the growth and kill the bacterial at a lower concentration than the solvent-free synthesized complexes is an indication of a broad therapeutic potential of the solvent-based synthesized complexes.

Conclusion

Urea and thiourea with their complexes of Zn(II), Fe(II), reported here are of good yield in stoichiometry ratio 1:1 at mild warming. Urea and thiourea coordinated to the metal salts through $\text{C}=\text{O}$ and $\text{C}=\text{S}$ respectively. This confirms the ability of both ligand acting as monodentate ligand in complexation as have been reported in literature (Calogero 1980). It can be seen from this study that liquid assisted grinding is quick and gives a quantitative yield without water and heat.

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