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# Comparative studies on the metallurgical and biological activity of peroxy complexes of molybdenum (VI) and uranium (VI)

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**Abstract:** New peroxy complexes of molybdenum (VI) and uranium (IV) have been prepared. The complexes were characterized on the basis of elemental analyses, conductivity measurements, magnetic measurements, infrared spectral studies and by reactions with allyl alcohol, triphenylphosphine and triphenylarsine. The complexes of U (VI) showed strong activity against both the gram positive and gram negative bacteria than the other complexes indicating the higher zone of inhibition. The present findings of MIC experiment showed that the complex 3 of U (VI) was more potent against all the bacteria tested than the other complex. Results showed that the complex 2 of Mo (VI) exhibits more toxic to brine shrimp compared to other complexes of Mo (VI) and U (VI) indicating the lower values of LC<sub>50</sub> and LC<sub>99</sub> for both the exposure 16h and 36h.

**Keywords:** Peroxy Complexes, Mo (VI), U (VI), Metallurgical, Biological Activity, Toxicity

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## 1. Introduction

Peroxy complexes of transition metals have received special importance due to their role in a variety of industrial, pharmaceutical and biological processes [1]. They are widely used for catalytic oxidation in organic and biochemistry reaction [2], e.g. in the oxidation of thioanisole [3], methyl benzene [4], tertiary amines, alkenes, alcohols [5], bromide [6] and also in olefin epoxidation [7]. They also act as isomerization catalysis for some allylic alcohols [8] and have been applied in the bleaching processes [9]. Peroxy complexes of molybdenum (VI) and Uranium (VI) have been known for a long time [10]. A variety of peroxy molybdates coordinated with nitrogen and oxygen donors have been characterized structurally [11] and studied in solution and solid state [12]. Polynuclear molybdenum (VI) peroxy complexes containing amino acids have been synthesized and spectroscopically characterized [13]. Metal complexes of aroylhydrazones have broad application in biological processes such as in the treatment of tumors, tuberculosis, leprosy [14] and metal disorders. These are also known to act as herbicides, insecticides and acaricides [15]. The biological activity has been attributed [16] to the complex forming abilities of the ligands with metal ions present in the cells and involvement of molybdenum in molybdenum oxo-transferase enzymes [17].

Peroxy complexes of uranium (VI) have been attracting interest as oxidants in organic synthesis [18]. As uranium somewhat resembles the group VIB elements, it was of interest to discover whether it would form analogous peroxy complexes which contain organic moieties.

It has been reported that the cytotoxicity of the metal complex could be used as potential prodrugs in conjunction with the known anti cancer compounds [19]. More specifically, by binding a known anti tumor agent as the dissociating ligands, it has capability of using a transition metal as a delivery system for anti tumor agents. Another incentive for the development of these types of systems is that upon cleavage of the pharmacologically active ligand, delivery of a cytotoxic metal species also occurs [20, 21].

In view of the importance of peroxy complexes, this report deals a comparative study on the peroxy complexes of molybdenum (VI) and uranium (VI) with particular references to their effect on the antimicrobial and toxicological activity.

## 2. Materials and Methods

General methods for the preparation of the complexes of the type  $[M(O)(O_2)_2.amH.L_2]$  and  $[U(O)(O_2)_2.amH.L_2]$  where,  $M=Mo(VI)$ ,  $amH=$  amino acids, such as leucine;  $L_2=$  ligands such as pyridine.

The molybdc acid (1.5 gm, 0.01 mol) in H<sub>2</sub>O<sub>2</sub> (30%, 30-50 ml) was heated and filtered to obtain a clear solution. Solution of the ligand such as pyradine was dissolved in ethanol (30-50 ml). The solution of other ligands of amino acids such leucine; phenylalanine dissolved in water with slowly constant stirring. These above three solutions were mixed carefully with slowly constant stirring and reduced the volume to 20 ml. The yellow precipitate of the complex was observed immediately. The product was isolated and washed with water and ethanol. The complex was dried *in vacuo* over P<sub>2</sub>O<sub>5</sub> in vacuum desiccators.

A solution of amH phenylalanine (0.330g, 0.002mol) or leucine (0.2623g, 0.002mol) in water (20 ml) was added with stirring to a solution of Uranyl nitrate (1.005g, 0.002 mol) in water (20 ml). A solution of 'L' (0.01 mol) in ethanol was then added with continuous stirring to the above mixture followed by the addition of 30% H<sub>2</sub>O<sub>2</sub> (2 ml). The precipitate appeared, which was filtered, washed several times successively with ethanol. It was then dried and stored in *Vacuo* over P<sub>4</sub>O<sub>10</sub>.

### 2.1. Catalytic Reaction of the Compound 3 with Allyl Alcohol

Allyl alcohol (0.30 mol) was dissolved in dioxane (25 ml) and 0.5 g of compound 8 or 11 was added followed by 30% H<sub>2</sub>O<sub>2</sub> solution (25 ml). The mixture was kept under reflux at 90°C for 24 hours. The reaction mixture was then filtered and the filtrate was distilled at 19 mm Hg pressure. The product collected at 175-180°C was glycerol (50% yield). The glycerol was identified as its tribenzoyl ester derivative, m.p. 70°C (, m.p. 69°C).

### 2.2. Reaction of the Compound 4 with Triphenylarsine

Triphenylarsine (0.90g, 0.005 mol) in THF (50ml) added to a suspension of above compounds in the same solvents (50ml). The mixture was refluxed for 60 hours. The progress of the reaction was monitored using TLC, indicates that triphenylarsine was converted completely into triphenylarsine oxide. The reaction mixture was filtered and the residue was stored. Evaporation of the filtrate yielded the product, m.p.(m.p. 190-192°C).

The present complexes were characterized by IR, UV, magnetic moment, melting point, conductivity measurement.

### 2.3. Antimicrobial Assay

The disc diffusion method for *in vitro* antimicrobial assay was used for screening primary selection of the compounds as therapeutic agent [22, 23]. Disc diffusion method is highly effective for rapidly growing microorganisms and the activities of the test compounds are expressed by measuring the diameter of the zone on inhibition. Generally the more susceptible the organism the bigger is the zone of inhibition. The method is essentially a qualitative or semi quantitative test indicating sensitivity or resistance of microorganisms to the test materials as well as bacterostatic or bactericidal activity of a compound [24]. The standard test microorganisms were collected from the Molecular Genetics Laboratory, Department of Pharmacy, Rajshahi University.

All the complexes were tested against the pathogenic fungi *viz. Aspergillus niger, A. fumigatus, and A. flarus* as a concentration of 200 µg/disc for each. The antimicrobial activity was determined after 72 h of incubation at room temperature (30°C). The media used in these respects were nutrient agar (DIFCO) for antibacterial assay and potato dextrose agar for antifungal assay. The experiment was performed in duplicate to minimize errors.

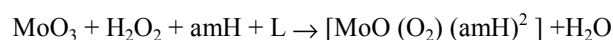
### 2.4. Cytotoxicity Bioassy

The bioactive compounds could be used against brine shrimp as lethality bioassay [25], which determines cytotoxicity as well as a wide range of pharmacological activities e.g. anticancer, antiviral, pesticidal etc. of the compounds. In the present investigation, *in vivo* lethality test was carried out against the brine shrimp nauplii eggs (*Ariemia salina* L.). Eggs were placed on one side of a small tank divided by a net containing 3.8% NaCl solution for hatching. In other side of the tank, a light source was placed in order to attract the nauplii. After two days of hatching period the nauplii were ready for the experiment as described previously. Three mg of the complexes were accurately measured and dissolved in 0.6 ml of DMSO to get a concentration of 5mg/ml. From the stock solutions, 10, 20, 40, 80 and 160 µl were placed in 5 different vials making the volume up to 5 ml.

The brine shrimp nauplii 10 in number were then placed in each vial. For the control test of each vial, one vial containing the same volume of DMSO plus water up to 5 ml was used. After 24h of incubation, the vials were observed using a magnifying glass and the number of survivors in each vial were counted and noted. From this data, the percentage of mortality of the nauplii was calculated for each concentration and LC<sub>50</sub> and LC<sub>99</sub> values were determined using probit analysis.

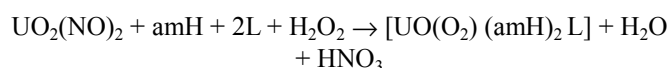
## 3. Results and Discussion

The formation of the complexes can be shown by the following reaction of molybdc acid:



Where, M = Mo(VI); amH= phenylalanine and leucine, L = pyridine.

The complexes were prepared from the reaction of the Uranyl nitrate and it may be represented as follows:



Where, M=U(VI) amH= phenylalanine and leucine; L= pyridine.

The analytical data and their physical properties of the complexes for both the metals are given in Figure 1. The analytical data are in good agreement with the proposed empirical formulae of the present complexes for both Mo(VI) and U(VI). Their structures have been proposed on the basis of conductivity and magnetic measurements and electronic

spectral data (Tables 1 and 2).

The molar conductance of  $10^{-3}$  M solutions of the complexes in DMSO was measured at 30°C. The molar conductance values (Table 1) indicate all the complexes are non-electrolytes in DMF revealing that the anions are covalently bonded in all the cases.

Salient features of the IR spectra of the complexes for both Mo(VI) and U(VI) are summarized in Table 3. The complexes 1, 2, 3, and 4 display band 1652, 1619, 1617 and 1653  $\text{cm}^{-1}$  respectively due to  $\nu(\text{C}=\text{O})$  and they showed significantly lower than that of free ligand, indicating the coordination of amino acid through its carboxylate anions. The disappearance of the  $\nu(\text{O}-\text{H})$  mode observed in the free amino acid molecule clearly indicate the loss of protons from O-H group upon coordination, revealing that acids are dinegative bidentate ligand coordinating through the carboxylate anion.

All complexes showed  $\nu(\text{N}-\text{H})$  bands from 3104 to 3390  $\text{cm}^{-1}$ , which are significantly lower than the free ligand (amino base) bands. It clearly suggests the coordination of amino groups through nitrogen atoms of amino base. Further more, the metal peroxo grouping gives rise to three IR active vibrational modes. These are mainly O-O stretching ( $\nu_1$ ), the symmetric M-O stretch ( $\nu_2$ ) and the anti-symmetric M-O stretch ( $\nu_3$ ). The characteristic  $\nu_1(\text{O}-\text{O})$  mode of the complexes appeared at 832-915  $\text{cm}^{-1}$ , indicating that the dioxygen moieties are bonded on “side-on” fashion with the Mo(VI) and U(VI). In the complex No. 1, the  $\nu_3$  and  $\nu_2$  modes appeared at 624 to 516  $\text{cm}^{-1}$  respectively. The  $\nu(\text{M}=\text{O})$  bands in the all complexes appeared at 976, 978, 905 and 977  $\text{cm}^{-1}$  respectively.

The in-plane and out-of-plane ring deformation modes of heterocyclic amines undergo positive shifts in mixed ligand complexes confirming their coordination through nitrogen. The presence of metal nitrogen bonding in the complexes is evident from the appearance of  $\nu(\text{M}-\text{N})$  modes in the spectra of the complexes [26, 27].

The observed values of effective magnetic moment ( $\mu_{\text{eff}}$ ) of the complexes of room temperature are given in Table 1. The magnetic moment values of dioxomolybdenum (VI) complexes (-0.261 to 0.450 B.M.) and dioxouranium (VI) (0.410 to 0.644 B.M.) indicated that these complexes were diamagnetic in nature suggesting that there were no changes in the oxidation states of the metal ions upon complexation. The electronic spectral data of the complexes A and B showed bands at 266-376 nm region due to the charge transfers (Table 3).

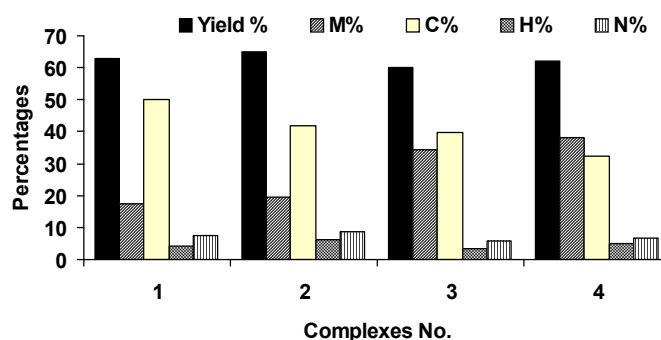


Fig. 1. Analytical data and physical properties of Mo(VI) and U (VI) complexes (1)  $[\text{MoO}(\text{O}_2)(\text{pha})_2(\text{py})]$ , (2)  $[\text{MoO}(\text{O}_2)(\text{leu})_2(\text{py})]$ , (3)  $[\text{UO}(\text{O}_2)(\text{pha})_2(\text{py})]$ , (4)  $[\text{UO}(\text{O}_2)(\text{leu})_2(\text{py})]$ .

Table 1. Physical properties of Mo(VI) and U(VI) complexes.

Complex No.	Complexes	Colour	Melting point ( $\pm 0.5^\circ\text{C}$ )	Molar conductance $\Omega^{-1}\text{cm}^2\text{Mole}^{-1}$	Magnetic moment $\mu_{\text{eff}}$ (B.M.)
1	$[\text{MoO}(\text{O}_2)(\text{pha})_2(\text{py})]$	Light yellow	180	6.4	0.450
2	$[\text{MoO}(\text{O}_2)(\text{leu})_2(\text{py})]$	Yellow	172	5.6	-0.261
3	$[\text{UO}(\text{O}_2)(\text{pha})_2(\text{py})]$	Yellow	165	7.9	0.410
4	$[\text{UO}(\text{O}_2)(\text{leu})_2(\text{py})]$	Light yellow	197	6.2	0.644

Table 2. IR spectral data of Mo(VI) and U(VI) complexes.

Complex No.	$\nu(\text{N}-\text{H})$ $\text{cm}^{-1}$	$\nu(\text{C}=\text{O})$ $\text{cm}^{-1}$	$\nu(\text{C}-\text{O})$ $\text{cm}^{-1}$	$\nu(\text{M}=\text{O})$ $\text{cm}^{-1}$	$\nu(\text{M}-\text{N})$ $\text{cm}^{-1}$	$\nu_1(\text{O}-\text{O})$ $\text{cm}^{-1}$	$\nu_3(\text{M} \begin{smallmatrix} \diagup \text{O} \\ \diagdown \text{O} \end{smallmatrix})$ $\text{cm}^{-1}$	$\nu_2(\text{M} \begin{smallmatrix} \diagup \text{O} \\ \diagdown \text{O} \end{smallmatrix})$ $\text{cm}^{-1}$
1	3390 br	1652 w	1594 vs	976 s	421 w	915 vs	624 vs	516 vs
2	3390 br	1619 w	1573 s	978 s	457 w	845 s	-	-
3	3105 br	1617 m	1559 w	905 s	464 m	832 m	-	-
4	3200 br	1653 w	1659 s	927 vs	423 w	882 s	600 w	536 m

Related band intensities are denoted by vs, s, m, w and br representing very strong, strong, medium, weak and broad band respectively.

Table 3. Electronic spectral data of Mo(VI) and U(VI) complexes.

Complex No.	Complexes	$\lambda_{\text{max}}$ (nm)
1	$[\text{MoO}(\text{O}_2)(\text{pha})_2(\text{py})]$	375
2	$[\text{MoO}(\text{O}_2)(\text{leu})_2(\text{py})]$	320
3	$[\text{UO}(\text{O}_2)(\text{pha})_2(\text{py})]$	266, 370
4	$[\text{UO}(\text{O}_2)(\text{leu})_2(\text{py})]$	275

### 3.1. Antimicrobial Activity

Antimicrobial activities of the test samples are expressed by measuring the zone of inhibition observed around the area.

The results revealed that the complexes are more microbial toxic than the free metal ions or ligands. All the complexes of metals under investigations for molybdenum (VI) and uranium (VI) showed more or less activities against the four pathogenic bacteria tested. From the zone of inhibition, it is observed that all the complexes of molybdenum (VI) and uranium (VI) exhibited greater susceptibilities towards all the bacteria used (Table 4). The results also revealed that the

complex 3 and 4 of uranium (VI) showed strong activity against both the Gram positive and Gram negative bacteria than the complexes of molybdenum (VI) indicating the higher zone of inhibition (Fig 2 and 3) (Table 4).

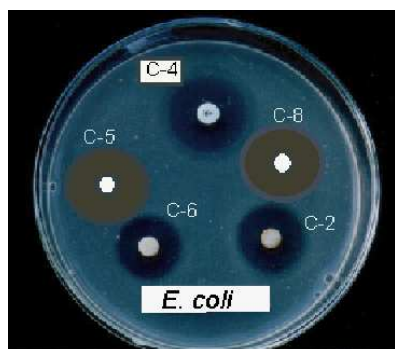


Fig. 2. Photographic representation of zone of inhibition of Mo(VI) complexes against *E. coli*.

The results of the antifungal activity of the complexes are

recorded in Tables 5. From the zone of inhibition, it is observed that all the complexes of molybdenum (VI) and uranium (VI) showed significant activity towards all the fungi used. The highest antifungal activity was shown in the complex 1 and 2 against *A. fumigatus* and complex 4 against *A. niger* (13mm) while the complex 3 of U(VI) showed lowest activity against *A. fumigatus* (10mm).



Fig. 3. Photographic representation of zone of inhibition of U(VI) complexes against *S. shiga*.

Table 4. The MIC values of the complexes of Mo(VI) and U(VI) against bacteria *P. auriginosa*, *S.-β-haemolyticus*, *E. coli*, *B. subtilis*.

Complex No.	Complexes	Minimum inhibition concentrations (µg/ml)			
		<i>P. auriginosa</i> (-ve)	<i>S.-β-haemolyticus</i> (+ve)	<i>E. coli</i> (-ve)	<i>B. subtilis</i> (-ve)
1	[MoO(O <sub>2</sub> )(pha) <sub>2</sub> (py)]	32	32	32	32
2	[MoO(O <sub>2</sub> )(leu) <sub>2</sub> (py)]	32	32	16	32
3	[UO(O <sub>2</sub> )(pha) <sub>2</sub> (py)]	64	64	64	64
4	[UO(O <sub>2</sub> )(leu) <sub>2</sub> (py)]	32	64	32	32

Table 5. Antifungal activity of the complexes of Mo(VI) and U(VI) against *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*.

Complex No.	Complexes	Diameter of zone inhibition (mm) 200 µg/disc		
		<i>A. niger</i>	<i>A. fumigatus</i>	<i>A. flavus</i>
1	[MoO(O <sub>2</sub> )(pha) <sub>2</sub> (py)]	11	13	12
2	[MoO(O <sub>2</sub> )(leu) <sub>2</sub> (py)]	12	13	12
3	[UO(O <sub>2</sub> )(pha) <sub>2</sub> (py)]	11	10	11
4	[UO(O <sub>2</sub> )(leu) <sub>2</sub> (py)]	12	11	13

### 3.2. Cytotoxicity Activity

The mortality rate of brine shrimp nauplii was found to be increased with the increase of concentration for all the complexes (Table 6). The results showed that the concentration 160 µL/mL caused 100 % mortality in brine

shrimp for all the complexes of Mo (VI) and U (VI) at the exposures of 16 and 36h. Results showed that the complex 2 of Mo (VI) exhibits more toxic to brine shrimp compared to all complexes indicating the lower values of LC<sub>50</sub> for both the exposure 16h and 36h (Table 6).

Table 6. Brine shrimp lethality bioassay for Mo (VI) and U(VI) complexes.

Complex No.	Complexes	Exposure 16 h		Exposure 36 h	
		LC <sub>50</sub> µg/mL	LC <sub>99</sub> µg/mL	LC <sub>50</sub> µg/mL	LC <sub>99</sub> µg/mL
1	[MoO(O <sub>2</sub> )(pha) <sub>2</sub> (py)]	19.32	121.01	9.34	57.91
2	[MoO(O <sub>2</sub> )(leu) <sub>2</sub> (py)]	15.69	109.21	6.47	47.20
3	[UO(O <sub>2</sub> )(pha) <sub>2</sub> (py)]	52.60	1048.5	11.93	275.77
4	[UO(O <sub>2</sub> )(leu) <sub>2</sub> (py)]	233.89	2068.0	13.38	67.41

## 4. Conclusion

It is concluded that the analytical data were in good agreement with the proposed empirical formulae of both the complexes. The molar conductance values indicated all the complexes of Mo(VI) and U(VI) are non-electrolytes in DMF revealing that the anions are covalently bonded in all the cases. The complexes of U (VI) showed strong activity against both

the gram positive and gram negative bacteria than the other complexes indicating the higher zone of inhibition. The present findings of MIC experiment showed that the complex 3 of U (VI) was more potent against all the bacteria tested than the other complex. Results showed that the complex 2 of Mo (VI) exhibits more toxic to brine shrimp compared to other complexes of Mo (VI) and U (VI) indicating the lower values of LC<sub>50</sub> and LC<sub>99</sub> for both the exposure 16h and 36h.

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