

New oxorhenium (V) complex with an imidazol [NN] /hydantoin [SN] mixed ligand system, and radiochemical behavior of its oxotechnetium (V) complex analog

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ABSTRACT

A [ReO(Imz)(Hyd)(H₂O)₂OH] complex was successfully synthesized by the ligand exchange method using oxorhenium citrate and an imidazole /Hydanton mixed ligand system. Geometry optimization of complex has been carried out using DFT at the B3LYP/LANL2DZ functional in singlet state. B3LYP predicated infrared spectrum of the geometrically optimized structure using the same level of the theory and the same base set showed good agreement with experimentally observed values. The spin allowed singlet-singlet electronic transition of the [ReO(Imz)(Hyd) (H₂O)₂OH] complex was calculated with time dependent density function theory (TD-DFT) and the UV-Vis spectra has been discussed on this basis. The complex was characterized using microanalysis and IR, UV-Vis, NMR and mass spectroscopic. The technetium tracer [^{99m}TcO(Imz)(Hyd) (H₂O)₂OH] has also been synthesized by two methods using ^{99m}Tc-gluconate as a precursor or; by direct reduction. The radiochemical purity of the complex was over 95% as measured by thin layer chromatography. *In vitro* studies showed that the complex possessed good stability under physiological conditions. The partition coefficient indicated that the complex hydrophilic and the electrophoresis results showed that the complex cationic. Biodistribution in mice showed that the complex accumulated in heart uptake of 9.53 ± 3.87% ID/gm at 5 min and good retention (6.37 ± 1.21)% ID/gm at 60 min. One hour after the injection, the heart/liver, heart/lung and heart/blood radioactivity ratios were 0.46, 1.04 and 0.56, respectively. These findings indicate that the complex might be

suitable for myocardial imaging.

Keywords: Imidazole (Imz); Hydantoin (Hyd); mixed ligand; [ReO]³⁺; DFT; ^{99m}Tc-gluconate

1. INTRODUCTION

Many metallic elements play a crucial role in living systems. As a result of their tendency to easily lose electrons to form positively charged ions, metals readily interact with electron rich biological molecules such as proteins and DNA, or bind with small molecules that are vital to life, such as oxygen. Because metals are so extensively used in biological systems, it was reasonable to investigate the potential use of metal ions alone and incorporated into drugs as well as the use of coordination complexes for medicinal purposes [1]. The use of metals offers many opportunities for the design of radiopharmaceuticals through the modification of environment around the metal that allow specific *in vivo* targeting to be incorporated into the molecule. The coordination chemistry of the metal will determine the ultimate geometry and stability of the radiopharmaceutical [1].

Radiopharmaceuticals, *i.e.*, chemicals or cellular structures that include a radionuclide, are routinely used in nuclear medicine, for both diagnostic and treatment purposes. Currently, ^{99m}Tc is the most commonly employed radionuclide to SPECT (single photon emission computed tomography), because of its ideal nuclear properties (*t*_{1/2} = 6 hr, γ -particle energy 140 keV), availability and low cost [2]. The coordination chemistry of rhenium and technetium is currently attracting much attention due to the radionuclide-based application in radiopharmaceuticals. Rhenium belongs to the same group of the periodic table (VII) as technetium and exhibits similar chemical properties. Rhenium is often used as a non-radioactive alternative to technetium for struc-

tural characterization [3,4].

The mixed ligands concept and particularly the SN/N combination have been applied for the preparation of neutral oxorhenium and oxotechnetium complexes. Chemically, the synthesis of Re (V) complexes requires the presence of metal-containing chemical cores that are, stable enough to exist in aqueous solutions and versatile enough to combine with the selected ligands. An example of such a core is the oxo form $[\text{Re}^{\text{V}}\text{O}]^{3+}$, in which the +5 oxidation state of the metal is stabilized by the oxo-core [5,6]. The literature shows that a search for new radiopharmaceuticals requires studies of new oxorhenium (V) complexes with nitrogen and sulfur donor sets, as well as investigations of the chemical behavior of their $^{99\text{m}}\text{Tc}$ analogs. In this work, we report the synthesis and characterization of a novel complex of rhenium of the general type $[\text{ReO}(\text{Imz})(\text{Hyd})\text{OH}]$. We have also studied the radiochemical properties of the corresponding technetium complex, which was prepared by two methods.

Nowadays, *ab initio* computational techniques are a potent tool for research. Chemistry has changed from a purely empirical science into a discipline in which deductive theory, as represented by first-principles calculations using computers, is increasingly important for interpretation and guidance of experimental work. Recent developments in this area include the field of density functional theory which meets with the requirements of being accurate. Currently density functional theory (DFT) is commonly used for geometric optimization and electronic structure of transition metal complexes. It meets the requirements of being accurate, easy to use and fast enough to allow the study of relatively large molecules of transition metal complexes [7,8]. Geometric optimization and electronic structure of $[\text{ReO}(\text{Imz})(\text{Hyd})\text{OH}]$ has been determined on the basis of the density functional theory (DFT) calculations and additional information about binding has been obtained by NBO analysis. The accuracy of the DFT calculations can be tested by comprising the available experimental data against calculation values. In our case study the experimental data available will be the data gathered from spectroscopic measurements such as UV-Vis, FTIR and NMR data.

2. COMPUTATIONAL DETAILS

The GAUSSIAN 03 program [9] (Gaussian 03 program) was used in the calculations. The complex was treated as open shell-system and no symmetry constraints were applied. Geometrical optimization of the investigated complex was carried out with Beck's three parameter exchange functional B3LYP using the Los Alamos LANL2DZ split-valence basis set [10,11]. A harmonic vibrational analysis was performed following the

geometrical optimization of the complex at the same level of theory B3LYP and the same basis set [12]. An additional d function with exponent $a = 0.3811$ and an f function with exponent $a = 2.033$ on the rhenium atom were added [13]. Natural bond orbital (NBO) calculations were performed with the NBO code [14] included in GAUSSIAN 03. The optimized geometry for various forms of the MBT molecule is shown in **Figure 1**. The two tautomeric forms of MBT were subjected to geometry optimization at B3LYP/6-31G**.

3. EXPERIMENTAL

3.1. Materials and Methods

All the reagents used in the synthesis were commercially available and were used without further purification. The $\text{ReO}(\text{citrate})_2^-$ precursor was prepared according to the literature [15]. IR was recorded on a Perkin Elmer spectrophotometer 1000 in the spectral range 200 - 4000 cm^{-1} with sample in the form of KBr pellets. Elemental analyses were performed on a Perkin-Elmer Analyzer 2400. ^1H - ^{13}C NMR spectra were obtained in DMSO- D_6 on JEOL NMR 400 MHz. Electronic spectrum was measured on a UV-Vis Beckman Du-70 spectrophotometer in the range 190 - 700 nm in aqueous medium.

$^{99\text{m}}\text{TcO}_4^-$ in saline solution was eluted from a $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator obtained from Cis Bio International Elumatic III, B.p.32. (France). Chemical reactions were monitored by thin-layer chromatography on instant thin layer chromatography (ITLC, SG-TLC) using Whatman No.1 chromatography paper (Whatman International Ltd. UK) and Silica gel-60 F₂₅₄ TLC-aluminium sheets (Alltech). HPLC separations were carried out using a normal phase silica column (Econosil 10 μm \times 264 mm) using a dual pump (Jasco-pu-2089 plus Quaternary Gradient); methanol/ dichloromethane (1 : 1) was used as the mobile phase with a flow rate of 0.5 ml/min. A JASCO chromatography system equipped with a variable wavelength ultraviolet monitor (JASCO, UV-2075 plus, intelligent UV/Vis detector) was set up in tandem with a Canberra flow through NaI radioactivity detector (TI). Ultraviolet absorption and radiochromatogram was monitored at 254 nm. Chromatography was acquired and analyzed using BORWIN software.

Electrophoresis measurements were carried out in 0.1 M sodium borate buffer (pH8) using Whatman No.1 chromatography paper. The paper was presoaked in the same buffer for 5 minutes before spotting the radio-labeled sample at the middle of the paper. After a potential of 160 V was applied for 120 min, the chromatography paper was dried, and the distribution of radioactivity was measured in a γ -well counter. The radiochemical

purity of the prepared complex was determined by spotting on ITLC-SG strips.

The biodistribution experiments for the ^{99m}Tc complex were performed in normal female mice to ascertain the *in vivo* distribution profile of the radiotracers according to literature [16]. The animal biodistribution experiments were performed in accordance with institutional, national and international regulations governing the safe and humane use of laboratory animals in research.

Mice (BALB/C, 25 - 30 g) were injected *via* the tail vein with 0.1 ml of the radiotracer formulated in saline. Each dose contained 20 - 30 μCi of radioactivity. Animals were sacrificed at 5, 30 and 60 min post injection and the organs of interest and blood were collected, weighed, and the radioactivity was measured with an automatic gamma counter (Cobra II). The percentage of the injected dose per gram was then calculated for all tissues using a stored sample of the injection solution to estimate the total dose injected per mouse.

3.2. Synthesis of $[\text{ReO}(\text{Imz})(\text{Hyd})(\text{H}_2\text{O})_2\text{OH}]$.

NaReO_4 (1 mmol, 0.273 g) was added to a solution of SnCl_2 (1 mmol, 0.256 g) in citric acid (0.5 M, 5 ml). Hyd (1 mmol, 0.1008 g) and Imz (2 mmol, 0.136 g) in ethanol were added dropwise to the mixture. The reaction mixture was stirred at room temperature for 3 hr. The pH was adjusted to 9 with NaOH (0.5 M), and the precipitate was recrystallized in hot Methanol and acetone according to literature [17]. Attempts to make a single crystal were unsuccessful and the complex was precipitated in micro crystalline form with 98 % yield of the compound: Anal. Calc.(found) for $\text{C}_6\text{H}_7\text{N}_4\text{O}_4\text{Re}$: C, 18.7(18.9); H, 1.83(2.05); N, 14.5(14.56); Re, 48.3 (47.2)%. IR (KBr, ν/cm^{-1}): 916 (Re=O), 560(Re-N), 430(Re-O). ^1H NMR (DMSO, ppm): 10(*s*, NH), 3.8(*s*, CH_2), 7 - 7.1(*t*, CH=CH), 7.73 - 7.77(*t*, CH=N). ^{13}C NMR (DMSO, ppm): 122(C=C), 135($\underline{\text{C}}=\text{N}$), 158.8(O= $\underline{\text{C}}$ - NH), 174,171(O= $\underline{\text{C}}$ - C), 47.7(CH_2). UV-Vis (H_2O ; $\lambda_{\text{max}}[\text{nm}](\epsilon; \text{dm}^3\text{mol}^{-1}\text{cm}^{-1})$: 191(2026), 203(2058), sh 245(288). MS (*m/z*): 385[M] $^+$.

3.3. Synthesis of $[\text{ReO}(\text{Imz})(\text{Hyd})(\text{H}_2\text{O})_2\text{OH}]$

3.3.1. Direct Method

The preparation of the ^{99m}Tc -complex was achieved by addition of sodium borohydride (0.29 mmol, 11.1 mg) in freshly prepared saline to a mixture containing $^{99m}\text{TcO}_4^-$ (0.7 ml, 27 mCi) and ligands (Imz: 9.0 mg, 0.13 mmol; Hyd: 10.4 mg, 0.10 mmol) with stirring. The mixture was allowed to stand for 45 min for complete reaction and then extracted with dichloromethane (3×1.5 ml). The combined organic extracts were dried over MgSO_4 , which was filtered off. The radioactivity

content of both the aqueous and organic phase is measured separately. The organic solvent was expelled under a mild stream of nitrogen, and ethanol was added so that a final solution 30% ethanol final solution was obtained for complex according to literature [16]. The radiolabeling yield was $88\% \pm 3\%$ with 96% radiochemical purity.

3.3.2. Ligand Exchange Method

Preparation of the ^{99m}Tc complex was accomplished using ^{99m}Tc -gluconate as the precursor [16]. A vial containing a lyophilized mixture of 200 mg sodium gluconate and 0.2 mg of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ was reconstituted with 5 ml of water. A volume of 0.5 ml of this solution was mixed with $[\text{ReO}(\text{Imz})(\text{Hyd})(\text{H}_2\text{O})_2\text{OH}]$ (0.5 - 1 ml with an activity of 5 - 50 mCi (185 - 1850 MBq)). The precursor ^{99m}Tc -gluconate, (radiochemical purity > 95%) was added to a centrifuge tube containing the mixed ligands. The mixture was stirred in a vortex mixer and left to react at room temperature for 10 min. The complex was extracted with dichloromethane (3×1.5 ml) and the organic layer was dried with MgSO_4 , filtered and analyzed by ITLC and HPLC.

4. RESULTS AND DISCUSSION

The reaction between oxorhenium citrate and the imidazole/hydantoin mixed ligands system lead to the formation of $[\text{ReO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$ complex according to Scheme 1.

We found that the labeling yield of the final ^{99m}Tc complex was higher when the direct reduction method was used compared with the ligand exchange method.

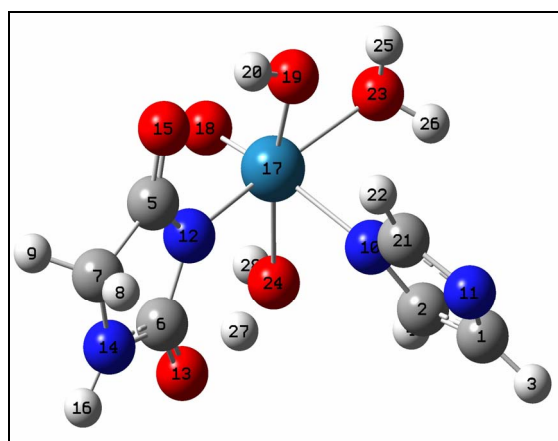
4.1. Geometry Optimization, Charge Distribution, Electronic Structure and NBO Analysis

The geometry of the $[\text{ReO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$ complex was optimized in a singlet state by the DFT method with the B3LYP function as shown in **Figure 1**. The calculated bond lengths and angles for Re=O, Re-N and Re-O are in quite good agreement with the values reported for other oxorhenium complexes of related structure as listed in **Table 1** [18].

Table 2 presents the atomic charges from the Natural Population Analysis (NPA) for $[\text{ReO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$. The calculated charge on the rhenium atom is considerably lower than the formal charge of +5. This difference is a result of significant charge donation from the oxo, OH and N-ligand donors. The charge on the oxo ligand is significantly smaller than (-2) and less negative than the charge on the OH ligand, indicating that there is higher electron density delocalization from the oxo core to the rhenium [19].

Table 1. Some geometrically optimized bond length(A°)and angles(°) for [ReO(Imz)(Hyd)OH(H₂O)₂].

Bond length (A°)		Bond angle (°)		B3LYP/LANL2DZ
Re(17)-O(18)	1.7196 (1.7071)	O(18)-Re(17)-O(19)		107.9908 (119.7782)
Re(17)-O(18)	1.9253 (1.8662)	N(12)-Re(17)-N(10)		90.2611 (102.792)
Re(17)-N(12)	2.0531 (1.9322)	N(12)-Re(17)-O(18)		100.4543 (100.260)
Re(17)-N(10)	2.0861 (1.9737)	N(10)-Re(17)-O(19)		93.4614 (120.723)
Re(17)-O(19)	1.9253 (1.862)	N(17)-Re(11)-O(12)		102.2092(102.792)
Re(17)-O(23)	2.1676 (1.9322)	N(12)-Re(17)-O(24)		90.0916 (102.792)
N(12)-C(5)	1.3998(1.398)	O(23)-Re(17)-O(24)		99.2639(100.07)
N(10)-C(2)	1.4066(1.398)	Re(17)-O(19)-H(20)		117.5155 (116.99)

**Figure 1.** The molecular structure of [ReO(Imz) (Hyd) OH(H₂O)₂].**Table 2.** Atomic charges from the natural population analysis (NPA) for [ReO(Hyd)(Imz)OH(H₂O)₂].

Atom	Atomic charge
Re(17)	1.437
N(12)	-0.475
N(10)	-0.562
O(18)	-0.413
O(19)	-0.742
C(5)	-0.126
C(2)	0.374

Table 3. The occupancies and hybridization of the calculated Re-O natural bond orbitals (NBOs) for [ReO(Hyd)(Imz)OH(H₂O)₂].

BD	Occupancy	Hybridization of NBO
Re(17) ≡ O(18)	1.95735 (0.2786)	0.5196(sd) _{Re} + 0.8544(sp) _O
	1.78553 (0.2268)	0.3809(d) _{Re} + 0.9246(p) _O
	1.78648 (0.1699)	0.4145(d) _{Re} + 0.9101(p) _O
Re(17) - O(19)	1.89301 (0.1667)	0.3359(sd) _{Re} + 0.9419(p) _O
	1.88269 (0.1662)	0.2906(d) _{Re} + 0.9569(p) _O
Re(17) - N(12)	1.89452 (0.200)	0.4653(d) _{Re} + 0.8852(p) _N

Table 3 presents the occupancy and hybridization of the calculated Re-O bond orbitals (NBOs) for the

[ReO(Imz)(Hyd) OH (H₂O)₂] complex. Three natural bond orbitals were detected for the core Re-O bond, and one bond orbital was observed for the Re-O bond of the OH ligand. The Re-O bond orbital of σ -character is polarized toward the O atoms, and the s and d-orbitals of the Re participate in the bonding. The Re-O bond orbitals of π character are significantly polarized towards the metal atom and only the d-orbitals of Re are included into the bonding.

The complex adopts an octahedral structure in which two water molecule is weakly coordinated to the Re atom, as seen from the unusual bond length of 2.1676 pm. It is worth mentioning that these calculations are performed in the gas phase. Dissolution of the investigated complex in aqueous medium could result in another H₂O molecule being coordinated to the rhenium to form a distorted octahedral structure. These results were further confirmed by measuring UV-Vis spectra of the complex in different solvents; shifts in λ_{\max} were recorded by changing the solvent, which indicates coordination of an additional solvent molecule with the Re atom to complete the octahedral geometry typically found for Re=O complexes [19].

Table 4 compares the calculated and observed vibrational modes of [ReO(Imz)(Hyd)OH(H₂O)₂]. Although one must normally scale the theoretical data by optimal scaling factors, which vary by basis set, the theoretical values are usually higher than the experimental data. **Figures 2, 3** show experimentally and theoretically calculated IR spectra of complex. In our case study, however, there was good agreement between the theoretical and experimental data.

In the IR, (**Figure 2**) the strong $\nu(\text{Re}=\text{O})$ bond of the [ReO(Imz)(Hyd)OH(H₂O)₂] complex was found in the narrow range around 910 cm⁻¹. This value is in the lower end of the range observed for a [ReO]³⁺ core, and suggests that the Re-hydroxy bond competes effectively. In many investigated studies concerning the M=O_{oxo} distances in complexes containing the O_{oxo}=M-O_{trans} fragment, they found demonstrated that when the *trans*

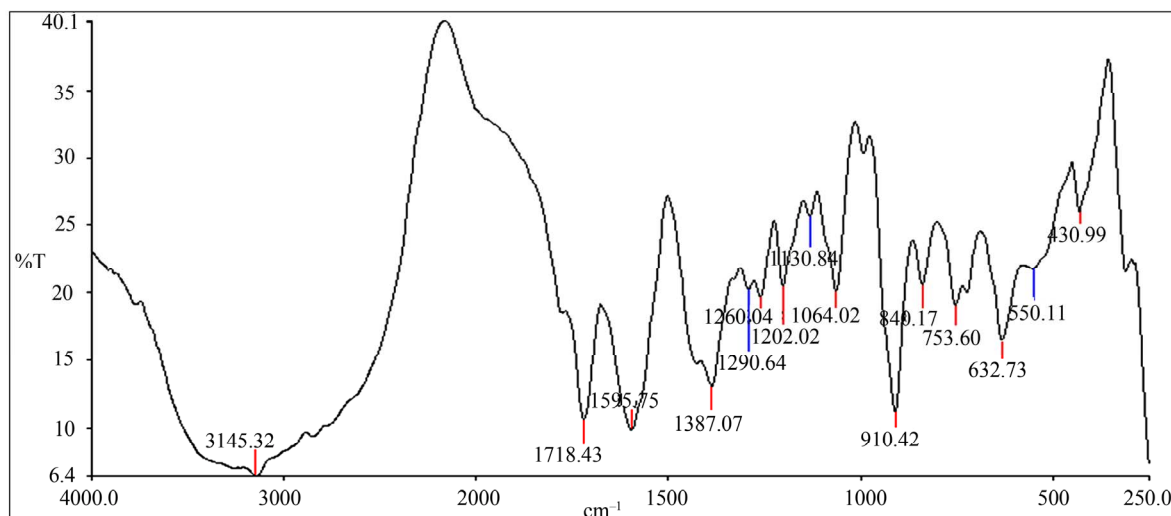


Figure 2. The IR of $[\text{ReO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$.

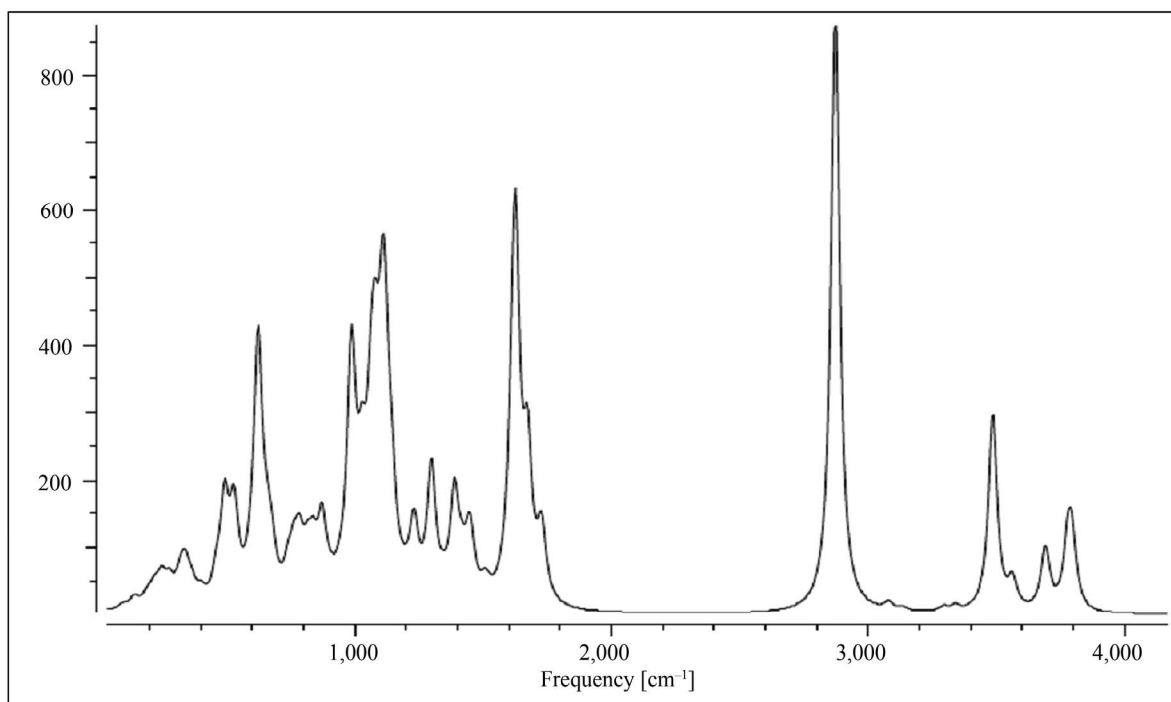


Figure 3. The Theoretical IR of $[\text{ReO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$.

Table 4. Comparison of the calculated and observed vibrational modes of $[\text{ReO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$.

Experimental	B3LYP/LAN2DZ	Assignment
560	694	ν Re-N
632	730	ν Re-N
910	988	ν Re=O
430	370	ν Re-O
1387	1460	ν OH _{bend}
3435	3494	ν OH _{str}
3145	3162	ν NH

ligand is an alcoholate RO^- anion, which is a very strong Lewis base, the $\text{M}=\text{O}_{\text{oxo}}$ distance is long, and the $\text{M}-\text{O}_{\text{trans}}$ distance is very short. The short bond distance is reflected in a high value of the $\nu(\text{M}=\text{O}_{\text{oxo}})$ stretching-mode, where $\text{M}=\text{Re}, \text{Tc}$ [20].

In Electronic spectra (**Figure 4**) the experimental and calculated electronic spectra for $[\text{ReO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$ complex using time-dependent density functional theory TD-DFT of the complex are presented in **Table 5**. The experimental absorption band at 203 and

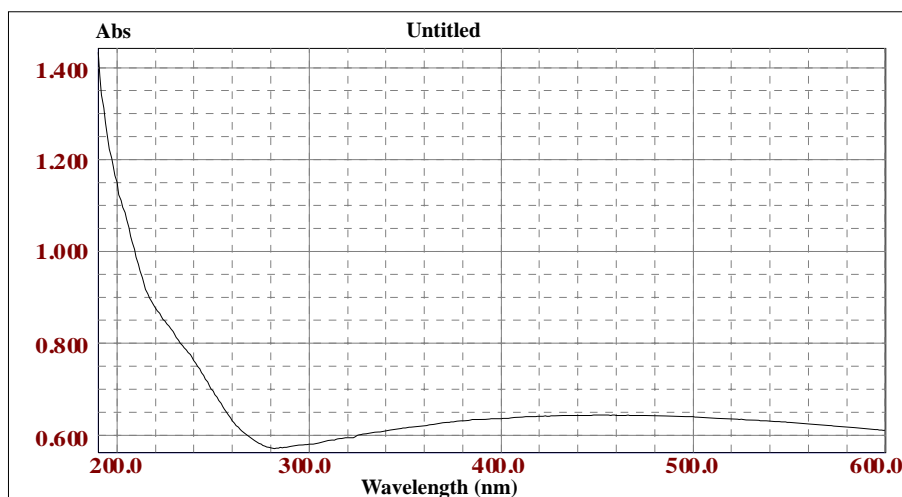


Figure 4. The UV-vis spectra of $[\text{ReO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$.

Table 5. The experimental electronic data.

Character	Experimental data in different solvent λ_{max} nm., (ϵ , $\text{dm}^3\text{mol}^{-1}\text{cm}^{-1}$)	solvent
$\pi \rightarrow \pi^*$	191(2026), 203(2058), sh 245(288)	H_2O
$n \rightarrow \pi^*$	207(333), 233(333), sh 460(280)	CH_3CN
LMCT	271(1850)	DMSO

ϵ - molar absorption coefficient.

191 nm are attributed mainly to intraligand transitions. The band recorded at 245 nm could be attributed to LMCT ligand metal charge transition from π -oxygen-bonding orbital to the d orbital of the Re [18].

In the ^1H NMR spectrum of the $[\text{ReO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$ complex (Figure 5), the Imz ring protons appear as two doublets that are shifted markedly down field relative to the free ligands from 7 ppm and 7.7 ppm to 7.13 ppm and 7.7 ppm, respectively. The NH proton signals for the Imz and NH ligands were absent in the spectrum of the complex and only a signal due to an NH of Hyd was recorded, which is shifted down field relative to the free ligand from 10 to 10.6 ppm. The ^{13}C NMR spectrum of the complex showed a signals at 174 and 158 ppm attributed to the (C = O) bond for Hyd. In addition, the spectrum showed signals assignable to the (C = N) bond at 135 ppm and the (C = C) at 122 ppm for the imz ring (Figure 6).

4.2. Quality Control of the $^{99\text{m}}\text{Tc}$ Complex.

The $[\text{ReO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$ complex can be prepared in two-steps. Quality control of the $[\text{ReO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$ complex was performed by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). The final $^{99\text{m}}\text{Tc}$ -oxo

hetero complex $[\text{ReO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$ was obtained in high yield (>88%). TLC and HPLC analyses were used to evaluate the radiochemical purity (RCP) and the stability of the complex. ITLC was performed on instant thin layer chromatography with Whatman No.1 chromatography paper and using acetone and saline as the mobile phase according to literature [16]. R_f values for $[\text{ReO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$ were around 0.6 in acetone; the other values are reported in Table 6.

The HPLC chromatograms of the $[\text{ReO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$ intermediate and $[\text{ReO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$ are shown in (Figure 7). The retention time of the final $^{99\text{m}}\text{Tc}$ -oxo complex $[\text{ReO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$ was found to be about 9.6 min, and that of $[\text{ReO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$ intermediate was 15.6 min. Their characterization was made using HPLC comparing their retention time (γ detection) with the retention time of the well-defined analogous rhenium complex (UV detection at 254 nm). The paper electrophoresis pattern of showed that the $[\text{ReO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$ complex moved to the point of cathode (-) percentage of radioactivity (96.20%), suggesting that it is a cationic complex (Figure 8).

Corroboration of structure of the $^{99\text{m}}\text{Tc}$ -complex was achieved by comparing its HPLC profiles with the corresponding to the Re-complex. Radioactivity and U.V detectors showed identical chromatographic profiles, suggesting that the same chemical structure was formed under both synthesis conditions.

In vitro stability of the complex was evaluated by measuring the RCP in ITLC at different time points. The

Table 6. The R_f values of compounds in acetone.

Compound	$[\text{ReO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$	$[\text{ReO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$ intermediate	$[\text{ReO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$ complex
R_f	0.9	0.8 - 1	0.642

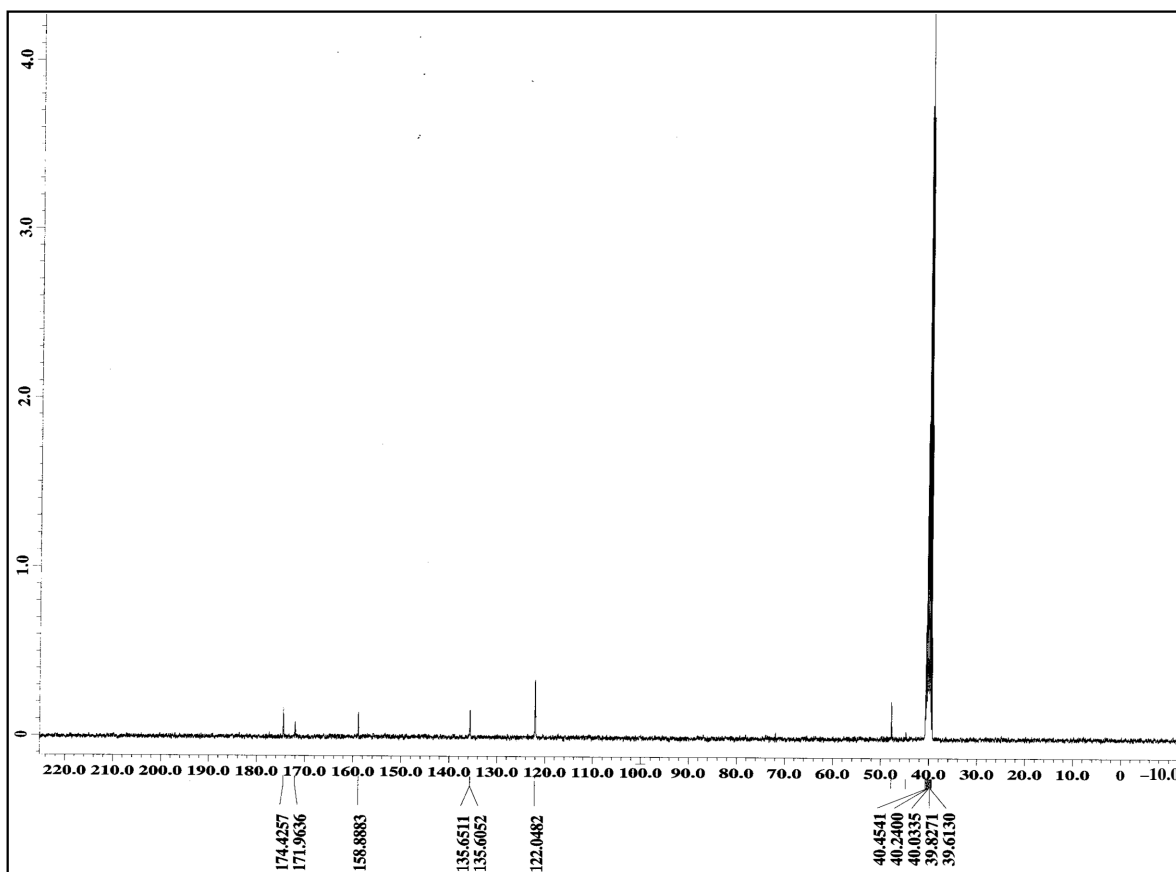


Figure 5. The ^1H NMR of $[\text{ReO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$ complex.

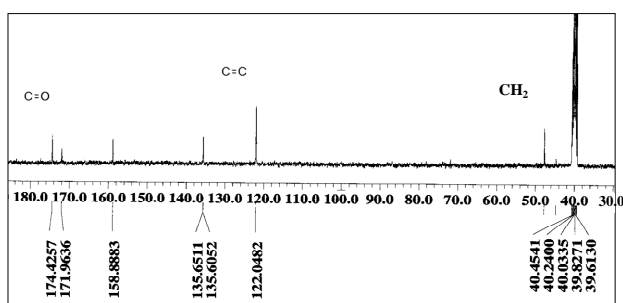


Figure 6. The ^{13}C NMR of $[\text{ReO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$.

RCP was still over 96% after 5 h, which suggested that the complex $[\text{}^{99\text{m}}\text{TcO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$ possessed great stability *in vitro*. Stability studies of the $^{99\text{m}}\text{Tc}$ complex as summarized in **Table 7**, found that the complex is stable in saline and plasma for at least 5 hours, which is an important parameter to pursue with biological studies. The lipophilicity of the compound was evaluated by determination of the partition coefficient (P) in physiological conditions (n-octanol/saline). The results obtained, were expressed as $\log P_{\text{O/W}}$ and the data were represented as the mean \pm the standard deviation is -0.142 ± 0.052 . As expected, the compound has low

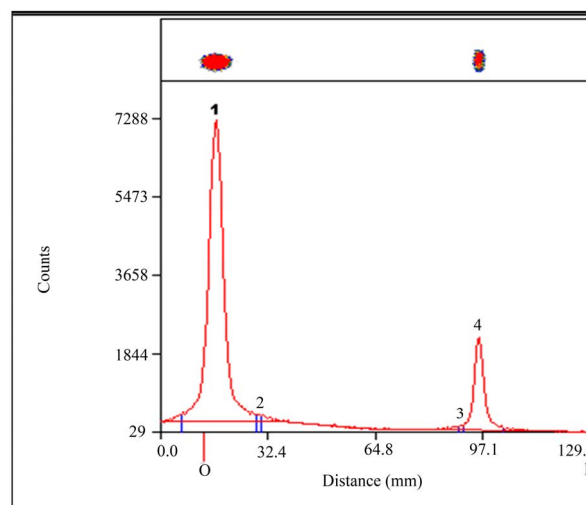


Figure 7. Chromatography ITLC of the $^{99\text{m}}\text{Tc}$ labeled compound in acetone.

lipophilicity [21] (**Figures 9** and **10**).

The animal biodistribution experiments using $[\text{}^{99\text{m}}\text{TcO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$ were performed in accordance with institutional, national and international regulations governing the safe and humane use of labo-

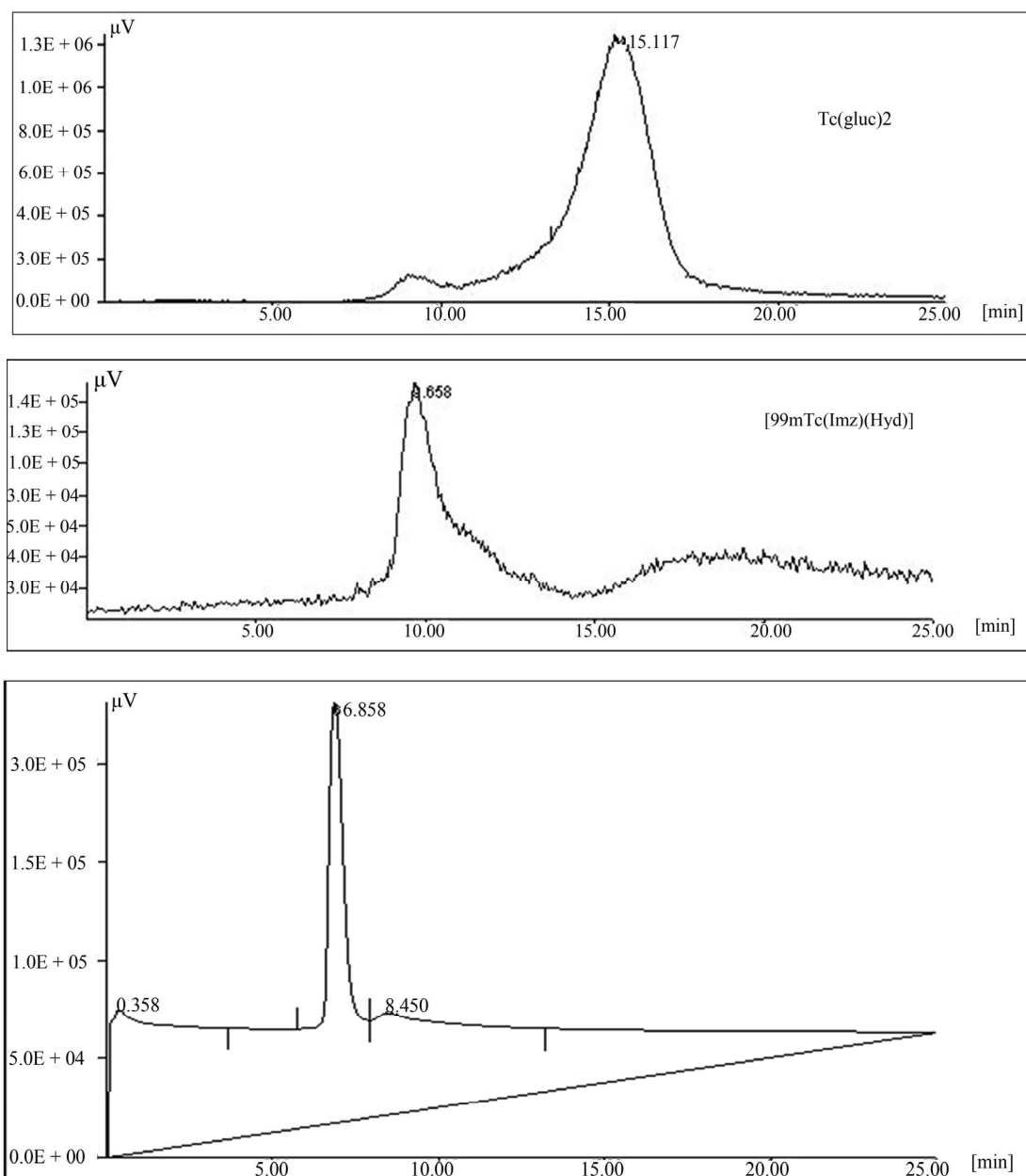


Figure 8. HPLC radio chromatogram for the $[^{99m}\text{Tc}(\text{Hyd})(\text{Imz})\text{OH}(\text{H}_2\text{O})_2]$ and $[\text{ReO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$.

ratory animals in research. In **Table 8**, one can see the initial heart uptake (9.53 ± 3.87)% ID/gm at 5 min and good retention (6.37 ± 1.21)% ID/gm at 60 min. One hour after the injection, the heart/liver, heart/lung and heart/blood radioactivity ratios were 0.46, 1.04 and 0.56, respectively. This indicates that the complex might be suitable for myocardial imaging. The radioactivity was excreted from the body *via* both the renal and the hepatobiliary systems. The ^{99m}Tc -complex shows moderate liver uptake and slow clearance, which may be attributable to the highly hydrophilic nature of the prepared complex. Hepatobiliary clearance of the complex is in-

ferable from the gradually increasing activity in the intestinal tissue 30 min p.i. It was pertinent to compare the biodistribution parameters with that of $[^{99m}\text{TcN}(\text{NOEt})_2]$ [22]. The results indicate that the myocardial uptake and critical ratios of heart non-target organ (liver, lungs and blood) observed with the present compound are much lower indicating that a structural modification is required in the design of the ligand. Increasing the polar nature of the final compound could be envisaged as a possibility to reduce the lipophilicity of the complex [23]. This can be achieved by incorporating polar substituents consisting of O, N, CO donor atoms on the ligand backbone,

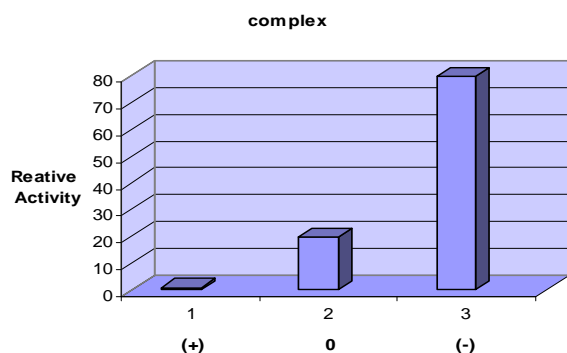


Figure 9. Electrophoresis histograms of $[^{99m}\text{Tc}(\text{Hyd})(\text{Imz})\text{OH}(\text{H}_2\text{O})_2]$; (+)Anode; (-)Cathode; (0) neutral.

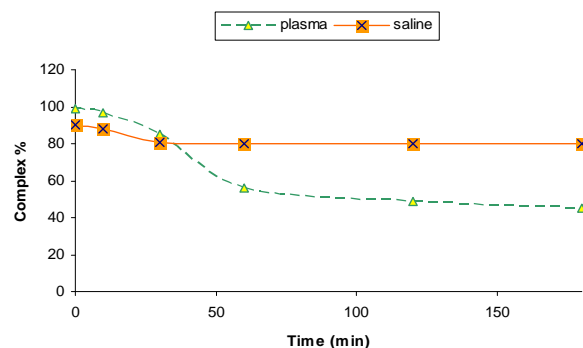


Figure 10. Stability of complex of $[^{99m}\text{Tc}(\text{Hyd})(\text{Imz})\text{OH}(\text{H}_2\text{O})_2]$ in plasma and in saline.

Table 7. The Stability of compound in Saline/Plasma.

Compound	Log P _{OW}	Saline stability (%)				Plasma stability (%)			
		10 min	30 min	1 h	3 h	10 min	30 min	1 h	3 h
	-0.142 ± 0.052	88	81	80	80	97	90	85	75

Table 8. The distribution data for Complex as %ID/gm in mice at different time intervals.

Organ	5 min	30 min	60 min
Brain	0.59 ± 0.25	0.25 ± 0.01	0.23 ± 0.09
Blood	14.49 ± 1.71	8.61 ± 1.07	11.22 ± 1.40
Liver	16.53 ± 4.08	10.94 ± 3.69	13.63 ± 3.27
Lung	9.74 ± 3.30	5.66 ± 1.65	6.08 ± 2.44
Kidney	30.43 ± 3.60	26.001 ± 3.81	31.02 ± 6.33
Intestine	6.22 ± 0.69	4.42 ± 2.26	8.30 ± 1.37
Heart	9.53 ± 3.87	7.90 ± 0.51	6.37 ± 1.21
Muscle	2.24 ± 0.23	2.22 ± 2.11	1.34 ± 0.34
Spleen	7.04 ± 1.85	1.34 ± 0.40	2.90 ± 0.48

which in turn, will increase the clearance of the complex from the blood [5]. Additionally, it has been documented that suitable modifications such as increase in the number aliphatic substituent in the molecule enhances its clearance from liver, lungs, etc. [23] due to fast metabolism [24]. A large fraction of the activity (35%) can be seen in the kidneys, which could possibly be due to the small size of the complex [15]. The activity appears to be retained for a long time in the kidney, diminishing a certain extent at 60 min, perhaps owing to the metabolism of the complex within the kidney.

5. CONCLUSIONS

The oxorhenium complex was synthesized based on the [NO] [N], mixed ligand system and fully characterized at the macroscopic level. In this complex, a novel complex $[^{99m}\text{TcO}(\text{Imz})(\text{Hyd})\text{OH}(\text{H}_2\text{O})_2]$ was formed using (Hyd) hydantoin ligand and (Imz) imidazole ligand and the $[\text{ReO}]^{+3}$ core. The chemistry was successfully transferred at the tracer level, and the corresponding

oxotechnetium-99m complex was prepared using ^{99m}Tc -gluconate and by direct reduction of pertechnetate in the presence of N,N mixed ligand. Its structure was established by comparative HPLC analysis using the oxorhenium complex as a reference. Tissue distribution studies in normal mice demonstrated.

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