Synthesis, characterisation, DFT and biomedical investigation of new Ga(III) complex with imidazole

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A new Ga(III) complex with imidazole (IMZ) in 1:2 Ga:IMZ stoichiometric ratio has been synthesised. The complex has been characterised by spectroscopic (IR, NMR, UV-Vis.,), thermogravimetric (TG, DTG, DTA), electrophoresis and microanalytical techniques and shown to be [GaCl(IMZ)$_2$(H$_2$O)]. The geometry of [GaCl(IMZ)$_2$(H$_2$O)] was optimised in a singlet state by the DFT method with B3LYP functional. Theoretical analysis of vibration modes of this complex has been undertaken and the results were compared with the experimental data for this compound. The complex was subjected to radiolabelling with ^{67}Ga via the nuclear reaction ^{68}Zn (p, 2n) ^{67}Ga. The radiochemical yield was greater than 80%. HPLC analysis in isotonic systems revealed that this complex is highly unstable in an isotonic system where it readily dissociated. The investigated complex showed relatively high anti-bacterial activities compared with the free ligand.

Keywords: Ga(III), chloride, imidazole, complex, DFT, ^{67}Ga label

Metals offer many opportunities for designing radiopharmaceuticals by modifying the environment around the metal and allowing specific *in vivo* targeting to be incorporated into the molecule. [1-5]

The current interest in the development of chelating ligands for the complexation of Ga^{3+} has been stimulated by the use of the Ga radionuclides, ^{67}Ga and ^{68}Ga as diagnostic agents in nuclear medicine. [6-8]

As a part of an ongoing investigation into the design and tailoring of new binding groups for potential Ga radiopharmaceuticals, we report here the complexation of Ga^{3+} by imidazole. The newly prepared complex has been characterised and subjected to clinical investigation as potential diagnostic radiopharmaceutical.

Currently density functional theory (DFT) is commonly used to optimise the geometry and examine the electronic structure of metal complexes. It meets with the requirements of being accurate, easy to use and fast enough to allow study of the relatively large molecules of transition metal complexes.[9]

In the present work, geometry optimisation and IR absorption spectra of the investigated [Ga Cl(IMZ)$_2$ (H$_2$O)] complex have been calculated with DFT.

Experimental

All chemicals used were of analytical grade and were used without further purification. IR spectra were recorded as KBr pellets on a Perkin Elmer FT-IR spectrophotometer "spectrum 1000" in the range of 200–4000 cm^{-1}. ^{1}H and ^{13}C NMR spectra (400 MHz) were referenced to an external TMS and recorded on JEOL Eclipse 400 spectrometer. UV-Vis absorbance was performed on Shimadzu UV-2100 spectrophotometer. TG measurements were carried out in static air using a Netzsch STA-429 thermal analyser. The weight loss was measured from ambient temperature up to 700°C at a heating rate of 10°C/min. Electrophoresis in different pH medium was measured on Shandon-Volarn 400–100 instrument (England) using a buffer solution in different pH as electrolyte. All manipulations were carried out under argon using standard Shlenk-line and glove box techniques.

Preparation of [GaCl(IMZ)$_2$ (H$_2$O)] complex
0.176 gm (1 mmol) of GaCl$_3$ anhydrous was dissolved in dry EtOH (10 ml). The prepared solution was added dropwise with stirring to 5 ml of an aqueous solution of 0.2042 gm (3 mmol) of IMZ. Two drops of NH$_4$OH solution (6 mmol) was added to the reaction mixture to raise pH to ≈6.7.

After the reaction mixture was stirred for 2 h at room temperature, the solvent was removed in a vacuum. The residue was extracted twice with 10 ml portions of ether, then the combined extracts were filtered and the filtrate was evaporated to dryness in a vacuum.

The resulting white solid was washed twice with 3 ml portion of pure EtOH and dried. Yield (80%), m.p. (decomposition) 277°C. Anal. Found: C, 27.05; H, 3.4; N, 22.2; O, 5.5; Cl, 13.7; Ga, 28.2. Calc. for C$_6$H$_8$N$_4$OClGa: C, 28.0; H, 3.1; N, 21.7; O, 6.2; Cl, 13.8 Ga, 27.1. FTIR (KBr): 3414 s ($\nu_{OH\ str.}$ (H$_2$O)); 407 s ($\nu_{OH\ bend.}$ (H$_2$O)); 774 s ($\nu_{Ga-O\ str.}$); 624 m ($\nu_{Ga-N\ str.}$), 346 m ($\nu_{Ga-Cl\ str.}$).
^{1}H NMR (DMSO, ppm) δ 8.92–7.581 (s, 2H, IMZ), 3.1(s, H, H$_2$O). ^{13}C NMR, δ 135(C=N), 122 (C=C). UV (MeOH), λ$_{max}$ 380 (ε = 400), 268 (ε = 59), 212 (ε = 2970) nm.

Computational details

The geometry of [GaCl(IMZ)$_2$ (H$_2$O)] was optimised in a singlet state by the DFT method employing the Gaussian 98 suite of programs.[10] The complex was treated as a closed-shell system using restricted DFT wave functional, (B3LYP),[11] *i.e.* the Becke three parameters exchange functionals in combination with LYP correlation functional of Lee, Yang and Parr with the small LANL2DZ basis set for all the atoms in the molecule. No symmetry constrains were applied and only the default convergence criteria were undertaken during optimisation.

Radiolabelling experiments
Enriched zinc oxide (^{68}ZnO) was purchased from "Isotopes" Russia. Copper target plates, aluminum target holder, plastic target carrier and electroplating vessels were all fabricated at the precision machine shop attached to the cyclotron unit at King Faisal Specialist Hospital and Research Centre (KFES). All newly fabricated plates, holders/carriers and vessels were inspected.

HPLC was carried out on Jascopu-980 chromatographic systems equipped with a variable wavelength UV monitor in tandem with a Canberra flow through sodium iodide (NaI) radioactivity detector using Alltech Econosil C-18, 10∞cm column (analytical, 250 mm × 4.6 mm) in isocratic mode. The flow rate was maintained at 1 ml/min. water (A 80%) and methanol (B 20%) mixture with 0.1% KH$_2$PO$_4$ were used as mobile phase.

Gallium-67 radiolabelling
Zinc-68 target plating solution preparation: ^{68}ZnO (4.5 g) was added to a 200 ml beaker,, followed by the addition of NaCN (3.2 g), NaOH (6.8 g) and deionised water (20 ml). The solution was filtered and the filtrate was diluted to 50 ml with portion of deionised water to be ready for electroplating.

Zinc-68 target electroplating: The freshly prepared solution of zinc-68 was poured into the plating vessel. The four outputs of the current booster were connected to the cathodes by introducing the four block male plugs (copper target, 1.5 cm thickness). This was followed by the introduction of the electromagnetic stirrer in a way that the central Pt-anode wire fits into hollow stirring cylinder. The rotational speed of the stirrer is 1200 rpm for 16 s periods of time. The electrolysis starts by introducing the common output plug of the boosters into the bottom of the deposition vessel with 100 mA current. After four hours of electrolysis which is needed to produce four zinc-68 targets (425±10% each), the stirring motor was stopped and the four electroplated targets were removed and rinsed extensively with deionised water then dried, weighed and finally stored in a vacuum dessicator.

Irradiation and extraction of gallium-67 from enriched zinc-68
Gallium-67 was produced by the bombardment of enriched zinc-68 (425±10% each), (425±10%) with 26 MeV protons from the CS-30 cyclotron internal beam using the ^{68}Zn(p, 2n) ^{67}Ga nuclear process. The proton current ranged between 40 and 65μA and the irradiation time was seven hours. The irradiated targets were dissolved in HCl (20 ml, 7.5 mol dm^{-3}). This aqueous layer was extracted with diisopropyl ether (DIPE, 50 ml), then equilibrated with HCl (7.0 mol dm^{-3}). This was followed by washing the DIPE layer (organic) three times with HCl (7.0 mol dm^{-3}, 20 ml) (first wash contain 1 ml of 20% titanium chloride solution). The dried Ga-67 was transferred to a pre-weighed collection bottle. Finally 0.6 ml of the Ga-67 bulk solution was given to quality control for radionuclidic purity.

Preparation of ^{67}Ga-IMZ complex
Four screw-cap vials each containing 1 mCi of ^{67}Ga chloride (^{67}GaCl$_3$, 37 MBq) dissolved in diethyl ether were dried. To each, stoichiometric amounts of IMZ dissolved in methanol (200 μl) were added and stirred. This was followed by the addition of equal molar ratio of sodium bicarbonate. The reaction mixture was stirred and monitored by means of TLC and HPLC at different time intervals. For product purification, activity was passed through silica 'sep-pak' conditioned with diethyl ether. The 'sep-pak' was dried, washed with diethyl ether (1.5 ml) and the radioactive complex was eluted by methanol (500 μl).

Results and discussion

The investigated complex is microcrystalline and soluble in polar solvents, but insoluble in non-polar solvents. Analytical data for the complex agree with the proposed empirical formula C$_6$H$_8$N$_4$OClGa. The complex is air stable.

The investigated complex was subjected to investigated spectroscopically as described below.

The IR spectrum of the free ligand was compared with that of the Ga(III) complex [GaCl(IMZ)$_2$ (H$_2$O)] in order to ascertain the binding mode of the ligand to the metal ion. The spectrum showed disappearance of the $\nu_{NH\ str.}$ band for the free ligand which provides an evidence for the mode of attachment of the ligand to the metal. The spectrum of the complex displays strong to medium absorption bands at 472, 656, and 346 cm^{-1} attributed to $\nu_{Ga-O\ str.}$, $\nu_{Ga\ -N\ str.}$, and $\nu_{Ga-Cl\ str.}$, respectively. A sharp split band at 3414 cm^{-1} is assigned to $\nu_{OH\ str.}$ (H$_2$O) (ν_{OH} bending of coordinated water was recorded 1407 cm^{-1}).

The ^{1}H NMR of the free IMZ showed the following signals in ppm.

(s, 7.03) H$_a$ (s, 7.66)

H$_c$

H$_b$

(s, 12.3)
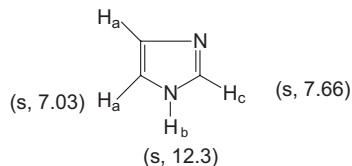
The ^{1}H NMR spectrum of the investigated complex showed that the signal due to the (N–H) group had disappeared from the spectrum of the complex, indicating the participation of this group in chelation through proton displacement. The disappearance of the (N–H) proton was also detected by exchange with D$_2$O. The other two signals showed a markedly down-field shift compared with signals of the free ligand.

The electronic absorption spectrum of the complex in 1×10^{-3}M alcoholic solution showed absorption bands in the UV region, being assigned to interaligand n–π* and π–π* transitions. The complex display a weak and broad band at 380nm being assigned to L→MCT.

Electrophoresis measurements indicated that the formal charge on the complex is zero.

Thermogravimetric analysis
Typical TG–DTA curves of the thermal decomposition of [GaCl(IMZ)$_2$(H$_2$O)] showed that the decomposition proceeds in two major steps. The first step is in the range of 200–300°C is attributed to the loss of coordinated H$_2$O molecules. This decomposition step is followed by another decomposition step in the range of 310–490°C attributed to the loss of IMZ and Cl ligands. The two decomposition steps are accompanied by two endothermic peaks as recorded in the DTA curve. The theoretically calculated weight losses for the two decomposition steps (7%, 65.8%) respectively were found to be in good agreements with the experimentally calculated values (8.8%, 63.3%). The decomposition ended with the formation of Ga$_2$O$_3$ as a solid residue.

Determination of reaction order of the decomposition
The Horowitz and Metzger equation is

$$C_s = (n)^{1/1-n}$$

where C_s is the weight fraction of the substance present at the DTG peak temperature T_s, is given as

$$C_s = (W_s - W_f)/(W_i - W_f) \qquad (1)$$

This equation was applied for the determination of the reaction order (n) of the decomposition. Here W_s stand for weight remaining at a given temperature T_s, i.e. the DTG peak temperature W_i and W_f are the initial and final weight of substance, respectively.[12] The calculated values of C_s are in range of 0.3–0.4 indicating that the decomposition follows first order kinetic for both the dehydration and the main decomposition steps.

The following Coats–Redfern equations were applied for determination of activation energy Ea and Arrhenius constant A, of the decomposition.[13]

$$\ln\left[\frac{-\ln(1-\alpha)}{T^2}\right] = \frac{-Ea}{RT} + \ln\frac{AR}{\varphi Ea} \qquad n = 1 \qquad (2)$$

$$\ln\left[\frac{-\ln(1-\alpha)}{(1-n)T^2}\right]^{1-n} = \frac{-Ea}{RT} + \ln\frac{AR}{\varphi Ea} \qquad n \neq 1 \qquad (3)$$

Where α is the fraction decomposition at time t and is given by:

$$\alpha = \frac{m_o - m_t}{m_o - m_f}$$

(m_0 is the mass at the beginning of the decomposition steps, m_t is the mass at time t, m_f is the mass at the end of the decomposition step), Ea, A, R, φ and Ts are activation energy of decomposition, Arrhenius constant, gas constant, heating rate and temperature at DTG peak, respectively. The kinetic parameters were calculated from the plots of eqns (2) and (3) versus I/T and $(T-T_s)$, respectively. The correlation coefficient r (>0.95) is computed using the least-square method for eqns (2) and (3). The activation entropy S^*, activation enthalpy H^* and the free energy of activation G^* were calculated using the following equations.

$$S^* = 2.303\left[\log\frac{Ah}{kT}\right]R \qquad (3)$$

$$H^* = E^* - RT \qquad (5)$$

$$G^* = H^* - T_5 S^* \qquad (6)$$

k and h are the Boltzman and Plank constants, respectively. The temperature T involved in the calculation was selected as the temperature at the end of the decomposition step. The calculated kinetic and thermodynamic values are reported in Table 1 for the two decomposition steps.

Table 1 Kinetics and thermodynamic parameters calculated for [GaCl(IMZ)$_2$(H$_2$O)] according to (Horowitz–Metzger) and Coat–Redforn methods

| Complex	Decomposition steps	Ea kJmol^{-1}	S^* JK^{-1}mol^{-1}	H^* kJmol^{-1}	G^* kJmol^{-1}	A s^{-1}	n
[GaCl(IMZ)$_2$(H$_2$O)]	Step I (loss of H$_2$O)	71.2 (81.8)	–264.6 (–266.1)	66.4 (77.1)	218.1 (229)	0.18 (0.15)	1
	Step II (loss of IMC, Cl)	195 (192)	–262.0	189 (185)	288	33.2	≠1

The kinetic parameters, especially Ea and S^*, are helpful in assigning the strength of the bonding of both water molecule and ligand moieties with the metal ion. The value of the major decomposition step of the studied compound *i.e.* the stage of the decomposition of ligands to volatile products are in the range 192–195 kJ/mol. which indicates that the ligand is strongly bound to the metal ion. The activation energy for the dehydration step of complexes lies in the range 71–82 kJ/mol. These values are comparable to the generally accepted values of activation energy for coordinated water.[14] The negative values indicated that the activated complexes have a more ordered structure than the reactants and that the complexation reactions are slower than normal.

Computational calculations
The optimised structure for [Ga(IMZ)$_2$Cl(H$_2$O)], which has been calculated using the B3LYP functional and LANL2DZ basis set is presented in Fig. 1. The optimised geometric parameters are listed in Table 2. The calculated bond lengths and bond angles are in good agreement with those determined experimentally using single crystal X-ray diffraction studies and reported[15,16] for related Ga complexes.

The calculated vibrational frequencies for [Ga(IMZ)$_2$Cl(H$_2$O)] in spectral range of 200–4000 cm^{-1} are presented in Table 3 together with the experimental frequencies data. The results indicated that the used DFT method and basis set for [GaCl(IMZ)$_2$(H$_2$O)] are suitable for obtaining results with satisfactory agreement with experiment. It should be mentioned that the theoretical values are usually higher than the experimental data: one has to scale the theoretical data by an optimal scaling factor. In our case quite good agreement between the theoretical and experimental data was detected.
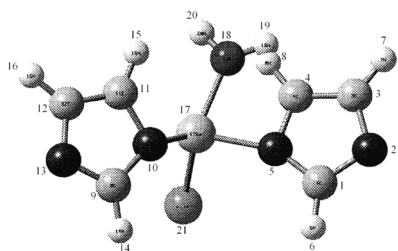
The calculated charge on the gallium atom is considerable lower than the formal charge + 3, corresponding to the 4 s 4p configuration of the central atom. It results from charge donation from Cl/H$_2$O/ and IMZ ligands. The coordinated N atoms are less negative than the uncoordinated N atoms indicating the high electron density delocalisation from the coordinated N towards the Ga atom.

The populations of the 4 s and 4p gallium orbitals are also significantly different from the formal 4 s^2 4p^1 configuration of the free gallium atom.

Stability, radiochemical investigation and biodistribution
The results of the radiochemical investigation on the ^{67}Ga–IMZ complex indicated a notable increase in the radiochemical yield when the amount of the reactants and the reaction time increase and the optimum reaction conditions resulted in quantitative yield were found to be when 30 minutes and 5 mg of IMZ were used. It is very important to note that the addition of a molar ratio of sodium carbonate is a necessary to neutralise the acidic medium. The stability of the ^{67}Ga–IMZ complex was assessed by HPLC in isotonic medium (0.9% NaCl) at ambient temperature and different intervals. Figure 4 shows the HPL chromatogram of the investigated complex. The complex is highly unstable and dissociated to free IMZ and ^{67}Ga.

It is worth mentioning that the chromatogram of [GaCl(IMZ)$_2$(H$_2$O)] using the same mobile phase revealed one major peak at approximately the same retention time as that of the labelled complex, indicating that the complex [^{67}GaCl(IMZ)$_2$(H$_2$O)] is also formed at the tracer level.

The instability of the tracer complex [^{67}GaCl(IMZ)$_2$(H$_2$O)] has hampered bioevaluation of the tracer. This finding does not necessarily mean the complex is worthless as a potential radiopharmaceutical in nuclear medicine. With more investigations, the low stability of the complex could be utilised as an advantage for labelling of leukocytes (white blood cells).

Antibacterial activity
The antibacterial activity of the free IMZ ligand and the complex were tested against various gram-positive and gram-negative bacterial cultures by using the agar diffusion method.[17] The bacterial strains

Table 2 Some selected band lengths (Å) and angles (o) for [GaCl(IMZ)$_2$(H$_2$O)]

Bond length	Å	Bond angles	(°)
Ga(17)–Cl(21)	2.2103	N(10)–Ga(17)–N(5)	121.084
Ga(17)– O(18)	2.0027	N(5)–Ga(17)–O(18)	95.3258
Ga(17)– N(10)	1.8505	N(5)–Ga(17)–Cl(21)	116.1641
Ga(17)– N(5)	1.8559	N(10)–Ga(17)–Cl(21)	114.878
		O(18)–Ga(17)–Cl(21)	103.1412
		N(10)–Ga(17)–O(18)	99.9673

Table 3 Experimental and calculated vibrational frequencies data of [GaCl(IMZ)$_2$(H$_2$O)] in the range of 200–4000 cm^{-1}

Experiment v, cm^{-1}	DFT B3LYP/ LANL2DZ v, cm^{-1}	Assignment
3414 s	3708	v_{OH} str. (H$_2$O)
656 s	676	v_{Ga-O} str.
472 m	492	v_{Ga-N} str.
346 m	369	v_{Ga-Cl} str.

Table 4 presents the atomic charge from the natural population analysis (NPA) for the investigated complex [GaCl(IMZ)$_2$(H$_2$O)].

Table 4 Atomic charge from the natural population analysis (NPA)

Atom	Charge
N(2)	–0.37460
N(5)	–0.51817
N(10)	–0.50521
N(13)	–0.36868
Ga(17)	0.72657

were grown in Mueller–Hinton agar (Merck) plates at 37°C for 18 hours, and then were diluted to a final concentration of approximately 10^6 CFU/ml. 0.1 ml of the each bacterial suspension was spread over the surface of the Mueller-Hinton agar. 10 μl of 10 mg/ml of each of the free ligand and the investigated complex was absorbed onto a sterilised filter paper disk of 6 mm diameter. The sterilised filter paper disk were dried under sterile condition and then immediately placed on the surface Mueller–Hinton agar plates after their inoculation with the test bacterium. The plates were incubated at 37°C for 24 hours and diameter (mm) of inhibition zone was recorded. The results indicated that the investigated complex has a significant activity against the gram-negative bacteria with actual inhibition zone diameter of 14 mm particularly *Escherichia coli* with no detectable impact on the gram positive bacteria.

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