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Kinetics of lanthanide (III) complexes of EDTA-$(His)_2$: - An important molecule for MR and chelation therapy

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ABSTRACT

EDTA-bis (amide) analogue has been synthesized and evaluated as an important agent for chelation therapy. The stability and protonation constants of the complexes formed between the ligand [EDTA-$(His)_2$] and Gd^{3+}, Eu^{3+}, and Cu^{2+} have been determined by pH potentiometry (Gd^{3+}, Eu^{3+}) and spectrophotometry (Cu^{2+}) at 25 °C and at constant ionic strength maintained by 0.10 M KCl. The kinetic inertness of Gd [EDTA-$(His)_2$] was characterized by the rates of exchange reactions with Cu^{2+} , Zn^{2+} and Eu^{3+}.

***Keywords: EDTA, Spectrophotometry, Protonation constants and Stability constants*.**

I. INTRODUCTION

Complexes of trivalent lanthanide cations are currently employed in several areas of chemistry, material science, biology and medicine. The large and increasing interest for these compounds is explained by the rich variety of magnetic, optical and radiochemical properties associated with the different metal ions of the lanthanide series that can be partly modulated by controlling the stereochemical and electronic properties of their complexes. At the same time, the chemical and structural properties of the lanthanide complexes typically show only minor variations across the series as a function of the atomic number, thus simplifying their characterization.

In recent years several natural as well as non natural amino acids have been conjugated with different chelating agent for labeling with either gamma radiation-emitting radio nuclides or positron-emitting radio nuclides for the diagnosis of different diseases [1-5]. DTPA (Diethlenetriamine- N, N, N'N"N''-pentaacetic acid) is a well known chelating agent and used as a contrast agent after complexation with Gd^{3+} ions [6]. Similarly complexes of Eu^{3+} and Tb^{3+} with DTPA and its derivative are used as optical imaging agents. The detail kinetics and mechanisms of the formation of the lanthanide complexes of DOTA and DOTA derivatives have been studied in detail [7-15]. The Gd^{3+} complexes of these chelating agents used as MRI contrast agents are particularly important, because its excretion from the body is relatively slow and they may participate in exchange reactions with endogenous metal ions and ligands in body fluids. The toxicity of contrast agents strongly depends on the extent of such exchange reactions.

Histidine is basic amino acids, which contained a heterocyclic ring. This amino acid is the precursors for various neurotransmitters. The various biological properties of these basic amino acids prompted us, to conjugate them

with a very efficient chelating agent like DTPA, EDTA for the complexation with lanthanide metal ions and their kinetic and thermodynamic studies for understanding the capabilities of these molecules utilized for various applications.

II. MATERIALS AND METHOD

All chemicals used in Kinetic and potentiometric study are of analytical grade and purchased from Sigma-Aldrich and Merck. All the solvents were used after distillation. The concentration of $GdCl_3$, $EuCl_3$, $CuCl_2$, and $ZnCl_2$ stock solutions was determined by complexometric titration with standardized disodium-EDTA solution. The concentration of the DTPA, and EDTA solutions were determined by potentiometric titrations in the presence and absence of a 35-fold excess of Ca^{2+}. The pH potentiometric titrations were done with standardized 0.2 N NaOH.

TLC was run on the silicagel coated aluminum sheets (silica gel 60 F_{254}, E Merck, Germany) and visualized under UV light. FTIR spectra were recorded on the FT-IR Perking Elmer Spectrum BX Spectrophotometer with KBr discs. NMR spectra were measured in D_2O by Bruker 400 MHz system. EI-MS spectra were recorded on a JEOL SX102/DA (KV 10 mA) instrument.

III. PREPARATION OF LIGAND:-

Ethylenediamine dianhydrid has been prepared according to references [16]. The product was obtained as a white solid (93%). The final ligand EDTA-$(His)_2$ was synthesized with some modification of the previous reported method [17].

0.1 mol)of EDTA dianhydride was dissolved in 50 mL of DMF. A 10% excess of L-Histidine (0.22 mol) dissolved in DMF was added drop wise and the mixture was stirred overnight at ambient temperature until the colour of the solution changed to light brown. The pH of the reaction mixture was adjusted to 8 by using triethylamine. The reaction mixture stirred for additional 14 h at 60^{o}C under nitrogen atmosphere. To the reaction mixture, 100 mL of diethyl ether was added and left to stand for 12 h. After filtration and drying in vacuo, EDTA-$(His)_2$ was obtained as a brown solid (yield: 85.0%). The complete scheme is given as **S1**.

IV. FORMATION KINETICS OF M^{N+}[EDTA-$(HIS)_2$] COMPLEXES :

The kinetics of formation of M^{n+} [EDTA-$(His)_2$] complexes has been studied for two lanthanide ions, Gd^{3+} and $Eu^{3+.}$ The formation is slow enough to be followed by classical UV-Vis spectrophotometry. For Eu complex the formation was studied in buffered solutions by monitoring the pH decrease with bromocresol green as an indicator. In the presence of Eu^{3+} excess, the complex formation is a pseudo-first-order process. The formation reactions were investigated at different pHs with varying concentration of the gadolinium ion to obtain k_{obs} values with a saturation curve against Eu^{3+} concentration. Saturation kinetics can be mechanistically defined as to rapid formation of an intermediate that rearranges to the product in a slow, rate controlling process.

The formation kinetics of copper complex was studied by UV-Vis spectrophotometry. **Figs. 1 and 2** show the pseudo-first-order rate constants, k_{obs}, for the reactions with $Eu^{3+}$and Cu^{2+} respectively.

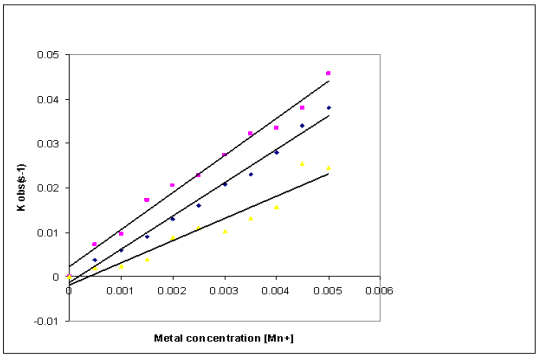

Fig 1 : Rates of Formation of [Eu (EDTA(His)$_2$] at 25°C in 0.1 M KCL
[EDTA(His)$_2$] = 2.0 X 10^{-4} M. The pH values are 3.5 (yellow),4.5 (Blue) and 5.6 (Red).

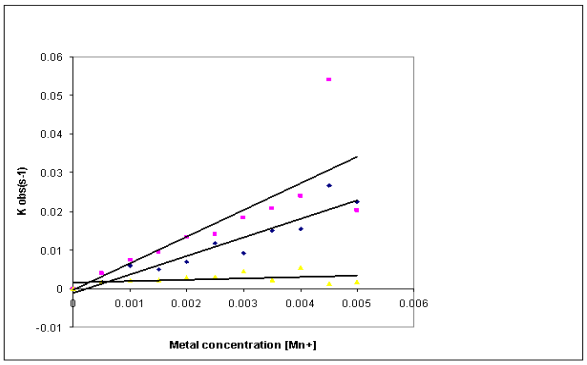

Fig 2 : Rates of Formation of [Cu (EDTA(His)$_2$] at 25°C in 0.1 M KCL
[(EDTA(His)$_2$] = 2.0 X 10^{-4} M. The pH values are 3.5 (Yellow),4.5 (Blue) and 5.6 (Red).

V. EQUILIBRIUM MEASUREMENTS

All the equilibrium measurements of reaction kinetics were made at constant ionic strength maintained by 0.10M KCl at 25°C. To determine the protonation constants of EDTA, its histidine analogue, and DTPA two or three parallel pH potentiometric titrations were carried out with 0.2 M NaOH in 0.005 **M** ligand solutions **(Table 1).** The log K_i^H values were calculated with the use of about 200 volume base/pH data points. The stability constants at equilibrium state characterizing the complex formation between the ligands and metal ions were determined by direct pH potentiometric titration (0.002 to 0.004 molar M^{n+} and 0.002 molar ligand solutions) **(Table 2**).

The stability constants for Cu complexes in two different ratio 1:1 and 1:2 were determined by spectrophotometry, with the use of competition reactions taking place between DTPA and EDTA analogue for Cu^{2+} in the pH range 6.5–7.5. The concentration of Cu^{2+} in the samples (5 samples for each derivative) was 0.002 M and that of EDTA analogue was 0.002 M or 0.005 M. In order to reach equilibrium, the ligand with metal ions were kept at 50 °C for three weeks and then at 25 °C for another two weeks. The molar absorptivity of Cu^{2+} and the Cu complexes were determined in 1.0, 2.0, 3.0, 4.0, and 5 mM solutions. Spectrophotometric measurements were made between 500 and 750 nm at 10 wavelengths. The pH potentiometric titrations were carried out with a Tiamo 2.0 titration workstation with the use of a Metrohm-6.0233.100 combined electrode. The titrated solutions (10 mL) were maintained at 25 °C by thermostat. The ligand and metal ion solution were stirred with a magnetic stirrer and to avoid the effect of CO_2, N_2 gas was bubbled through the solutions. The titrations were carried out in the pH range 2.0–12.5. For the calibration of the pH meter, potassium hydrogen phthalate (pH = 4.000) and borax buffers were used.

Table 1: Protonation constants of the ligands EDTA, DOTA and [EDTA (His)$_2$] at 25 °C.

	EDTA	DOTA	EDTA (His)$_2$	EDTA (His)$_2$
	0.1 M KCl	0.1 M KCl	0.1 M KCl	0.1 M $(CH_3)_4NNO_3$
$\log K_1^H$	10.52	11.05	9.95	10.05
$\log K_2^H$	8.56	9.20	8.20	8.30
$\log K_3^H$	4.80	4.15	4.60	4.65
$\log K_4^H$	2.25	3.10	2.65	2.19

Table 2. Equilibrium constants characterizing the complex formation between the ligands EDTA, DOTA and EDTA (His)$_2$ and the metal ions Gd^{3+} and Zn^{2+} (0.10 M KCl, 25 °C).

	DOTA	EDTA	EDTA (His)$_2$
	0.1 M KCl	0.1 M KCl	0.1 M KCl
$\log K_{GdL}$	23.5	20.2	19.5
$\log K_{EuL}$	24.7	18.5	18.3
$\log K_{ZnL}$	18.1	16.1	15.5
$\log K_{Zn2L}$	--	8.4	6.9
$\log K_{CuL}$	22.2	21.2	18.5
$\log K_{Cu2L}$	--	6.5	5.4

V. KINETIC MEASUREMENTS

The rates of the metal-exchange reactions of gadolinium complex with Cu^{2+} , Zn^{2+} and Eu^{3+} were studied by spectrophotometry, following the formation of the Eu^{3+}, Cu^{2+} and Zn^{2+}complexes at 285, 330 and 345 nm, respectively, with spectrophotometer. The concentration of EDTA analogue was taken in the range of 10^{-4} to 10^{-5} M in experiments with Eu^{3+} , Cu^{2+} and Zn^{2+} respectively, while the concentration of metal ions was 10–40 times higher, respectively, in order to guarantee pseudo-first-order conditions. The results are shown in **(Table 3).**

Table 3. Rate constants characterizing the metal-exchange reactions of [Gd EDTA (His)$_2$] with Eu^{3+},Cu^{2+}, and Zn^{2+} (25 °C).

	Gd DTPA (Me-Trp)$_{2\ in}$ (0.10 M KCl)		
	Eu^{3+}	Cu^{2+}	Zn^{2+}
k_0 (s^{-1})	$(3.5 \pm 1.1) \times 10^{-6}$	–	–
k_1 ($M^{-1}s^{-1}$)	0.52 ± 0.1	–	–
k_2 ($M^{-2}s^{-1}$)	$(5.1 \pm 0.2) \times 10^{4}$	–	–
k_3 ($M^{-1}s^{-1}$)	$(4.0 \pm 0.5) \times 10^{-4}$	0.54 ± 0.06	$(2.90 \pm 0.15) \times 10^{-2}$
k_4^M ($M^{-2}s^{-1}$)	65 ± 10	$(2.05 \pm 0.03) \times 10^{4}$	128.7 ± 5.0

The temperature was maintained at 25 °C and the ionic strength of the system was kept constant (0.10 M KCl). For maintaining a constant pH value, 1,4-dimethylpiperazine (pH range 3.3–4.2), *N*-methylpiperazine (pH range 4.2–5.2), and piperazine (5.2 < pH < 6) buffers (0.02 M) were used.

VI. ANIMAL MODELS FOR BIOMEDICAL IMAGING.

Animal protocols have been approved by Institutional Animal ethics Committee. New Zealand Rabbits 2-3 Kg and BALB/c 22-28 g were used for blood clearance, and imaging and biodistribution studies. Rabbits were housed under conditions of controlled temperature of 22 ± 2^0C and normal diet. Radio labeling of the compounds was done by following the procedure described in the literature [18-19**].** It was performed by taking 100 µl of 0.03 nM solution of the compounds dissolved in DMSO and taken in a shielded vial. Further 60 µl of 1x10 $^{-2}$ M $SnCl_2$.$2H_2O$ (dissolved in N_2 Purged 1ml 10% acetic acid) was added followed by freshly eluted saline solution of sodium pertechnetate ($NaTcO_4$) (74 MBq, 100 ml). The pH of the reaction mixture was adjusted to 6.5 with 0.1 M $NaHCO_3$ solution. The vial was allowed to incubate for 20-30 minutes at room temperature. Labeling of the compounds, radiochemical purity as well as R_f of the ^{99m}Tc based complex was determined by ITLC-SG strips using 0.9 % NaCl aqueous solution (saline) as developing solvent and simultaneously in acetone and PAW (Pyridine, acetic acid and water in 3:5:1.5 ratio). Each ITLC was cut in 0.1 cm segments and counts of each segment were taken.

Albino mice strain (A) (taken in triplicate set) was used for the tissue distribution studies. Animal handling and experimentation were carried out as per the guidelines of the Institutional Animal Ethics Committee. An equal dose of 10 µCi of labeled test compound was injected in mice through tail vein of each animal. At different time intervals mice were sacrificed, blood was collected and different tissue and organs were dissected and analyzed. The radioactivity was measured in a gamma counter. The actual amount of radioactivity administered to each animal was calculated by subtracting the activity left in the tail from the activity injected. Radioactivity accumulated in each organ was expressed as percentage administered dose per gram of tissue. Total volume of the blood was calculated as 7% of the body weight. Scintigraphic imaging was taken to confirm the distribution patteren of compound **(Fig 3).**

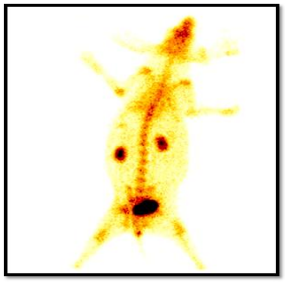

Fig 3 : Scintigraphic Imaging of [EDTA (His)$_2$] in mice

VII. RESULT AND DISCUSSION

The exchange reactions between the complexes GdL and the metal ions Eu^{3+}, Cu^{2+} and Zn^{2+} are as follows:

$GdL+M^{n+} \rightleftharpoons ML+Gd^{3+}$ **(1)**

In the presence of an excess of the exchanging ion, the trans metallation process may assumed as pseudo-first-order process and the rate of reactions can be expressed with Equation (2), in which k_{obs} is a pseudo-first-order rate constant and [GdL] is the total concentration of the complex.

$$d[\mathbf{GdL}]/dt = k_{\mathbf{obs}}[\mathbf{GdL}] \qquad (2)$$

The rates of the transmetallation reactions have been studied at different concentrations of the exchanging ions in the pH range 3.0–5.5.

The stability constants of the Gd^{3+} and Eu^{3+} complexes (K_{LnL}) are very similar (log K_{GdL}=19.5; log K_{EuL}=18.3). Accordingly, in the presence of a 10–40-fold excess of Eu^{3+}, reaction proceeds practically quantitatively. The stability constants for Ln(III) complexes of studied ligand lower than of parent EDTA this is due to steric nature as well as reduction of carboxylic group at appropriate distance but the selection of specific amino acid derivative gives more specificity in biomedical applications. The stability constants of the Cu^{2+} and Zn^{2+} complexes formed with the ligand are lower than the log K_{GdL} values. In the presence of a Cu^{2+} or Zn^{2+} excess, however, reaction proceeds practically completely because, similarly to EDTA, the octadentate ligand form dinuclear complexes with Cu^{2+} and Zn^{2+}. In order to calculate the stability constants of the complexes, the protonation constants of the ligands DOTA, EDTA and EDTA $(His)_2$ were first determined at an ionic strength of 0.10 M KCl by pH potentiometric titration. The equilibrium constants, which characterizes the formation of the EDTA $(His)_2$ complexes of Cu^{2+} and Zn^{2+}were determined by pH potentiometry at 1:1 and 2:1 metal-to-ligand concentration ratios. The equilibrium constants obtained are presented in **Table 2.**

The most important aspect for clinical use of contrast agents is safety. The Gd^{3+} complexes are supposed to be acute toxic if long-term residual of Gd^{3+} remains in the body. It is generally accepted that the toxicity of MR contrast agents depends primarily on the concentration of free Gd^{3+} released by the dissociation of its lanthanide complexes. The extent of dissociation depends on the selectivity of the ligand for Gd^{3+} over the endogenous metals (such as Zn^{2+}) and on the kinetic stabilities of the complexes. However, the role of the selectivity in the toxicity also depends on the rates of dissociation of the Gd^{3+} complexes in the body fluids. If the rate of dissociation of this complex is much lower than the rate of excretion of the complex from the body ($t_{1/2}$=1.5 h), then the system is far from equilibrium and the extent of dissociation is determined by the inertness of the complex. In order to obtain information on the rates of dissociation of the complex [Gd(EDTA **$(His)_2$**)]$^{2-}$, the kinetics of the exchange reaction were studied in 0.10 M KCl solution at 25 °C.The rate constants characterizing the exchange reactions between [Gd(EDTA **$(His)_2$**)]$^{2-}$, and Eu^{3+} were calculated by fitting the k_d values (**Figure 1)** to Equation . In the fitting procedure, a fixed value of K_{GdLH} was used, which was determined by pH potentiometry. The rate constants and the K_{GdLM} values obtained are presented in **Table 3**, where similar rate constants, characterizing the reaction of [Gd(EDTA **$(His)_2$**)]$^{2-}$, are also shown.

In the Eu^{3+} exchange, at pH <5 the rate decreases with increasing concentration of the exchanging ion. At physiological pH, the kinetic inertness of [Gd(EDTA **$(His)_2$**)]$^{2-}$, is more inert than [Gd(EDTA **$(His)_2$**)]$^{2-}$, the most commonly used MRI contrast agent ($t_{1/2}$ = 127 h).High kinetic stability is an important requirement for the Gd complexes used as contrast enhancement agents in magnetic resonance imaging.

Biodistribution of the radio complexes is an important phenomenon to study because it gives an idea about its excretory metabolic pathway and *in vivo* distribution of the radio complex drug. Accumulation of low amount of

radioactivity in the stomach precludes the presence of free pertechnetate, indicative of the *in vivo* stability of radiotracers. The percentage distribution of drug in various organs of mice is shown as percentage of injected dose per organ or tissue at different time interval. Initially the drug was localized in the brain, heart and liver, but with the passage of time the activity in kidney was amplified, while in intestine there was negligible increase in activity. This suggests that the major route of excretion of activity is through kidneys. Also with the passage of time, there was an increase in the accumulation of activity in urinary bladder. Besides this, there was retention of radioactivity in liver for considerable period, indicating that metabolism of drugs probably takes place in liver, but the excretion of drugs and metabolites is mainly through kidney. The scintigraphic images confirm the nature of compound for specific biomedical imaging.

VIII. CONCLUSION

The work represented here demonstrates that, the synthetic way for the preparation of the EDTA **$(His)_2$**, derivatives is easy and convenient. Further, biodistribution studies of the compounds showed rapid and persistent accumulation of radioactivity in neuronal area with an excellent signal to background ration. Others tissues studies including muscle, lungs, heart, and liver showed relatively low uptake of radioactivity. The thermodynamic and kinetic study confirms its applicability as imaging agent. Additional investigation to increase their specificity and to improve the pharmacokinetics performance of these new derivatives may result in potent drugs, become available for commercial exploitation

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