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SYNTHESIS AND PHYSICOCHEMICAL CHARACTERIZATION OF LANTHANIDE (Ln^{3+}) COMPLEXES : EQUILIBRIUM, KINETIC AND LUMINESCENCE PROPERTY

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ABSTRACT

EDTA-bis (amide) derivative bearing catecholamine groups that mimic side chains of naturally occurring amino acids such as phenylalanine, tyrosine or dopamine was synthesized and characterized by various spectroscopic techniques. The physicochemical studies have been done to calculate the various thermodynamic and kinetic parameters for the synthesized poly-amino carboxylate ligand. The two protonation constant (pka's= 3.460 and 6.722) of the prepared ligand and stability constants (log $K_{ML's}$ formed with Eu^{3+}, Gd^{3+}, Ce^{3+}, Lu^{3+} , Sm^{3+} and Tb^{3+} were determined by potentiometric titration using 0.1 M Me_4NOH as non-aqueous base. The formation kinetics of [Eu-EDTA-$(TA)_2$], [Ce-EDTA$(TA)_2$], and [Tb-EDTA-$(TA)_2$] was studied spectrophotometrically .The Europium (III) complex of the ligand EDTA-bis(tyramide) [EDTA-$(TA)_2$] was synthesized and evaluated as an optical imaging agent. The excitation and emission of the compound were recorded at the wavelength 275 nm and 310 nm, 610 nm respectively which shows efficacy of the ligand as an optical imaging agent. The binding affinity of EDTA$(TA)_2$ to HSA was evaluated by molecular modeling studies using HSA protein (PDB 1E78). It shows that EDTA$(TA)_2$ can bind with HSA by H-bonding and hydrophobic interaction effectively, so that it can transported at a specific site to show optical imaging behaviour.

Keywords : The Europium (III) Complex, EDTA-bis(tyramide) ([EDTA$(TA)_2$])

I. INTRODUCTION

The synthesis of metal based pharmaceuticals by labeling biomolecules with metal or radioactive metal ions has seen an important growth over the last years. The biomolecules employed are able to target particular kind of cells with high selectivity and affinity. The labeling of these biomolecules with a metal ion allows their delivery to specific sites of the human body. Depending upon the selected metal ion, these species have found application in medicine as diagnostic or therapeutic agents. In diagnosis, compounds containing paramagnetic ion e.g. Gd^{3+}, Mn^{3+}, are widely used as contrast agents in magnetic resonance imaging (MRI) and Eu^{3+}, Tb^{3+} used as optical imaging agents. Optical imaging with fluorescent probes has very promising and useful features for the biological studies and to develop new probes for physiological studies[1]. It can be considered a preliminary and complementary technique to studies with radiolabeled probes. Its use, however, should not be limited to animals, as it is now, but expanded to humans for the study of superficial are as such as imaging of cutaneous

lesions, endoscopic, and intra operative use. The design of responsive cellular probes for use in optical imaging remains a key challenge for biology and medicine. It has been appreciated that lanthanide(III) complexes (Ln = Sm, Eu, Tb, Yb) have attractive properties as optical probes[2]. They possess large Stokes shifts (big separation between absorption and emission wavelengths) and long emission lifetime (range 1 μs to 5 ms) that allow the use of time-gated acquisition methods to enhance signal/noise, minimizing interference from light scattering or autofluoroscence.

EDTA is an efficient chelating agent known to stable complexes with lanthanides (Gd^{3+} ,Eu^{3+}, Tb^{3+}). EDTA can be conveniently conjugated to other molecules by conversion of the carboxylic group into a carboxamide via EDTA-bisanhydride. In most of its complexes it acts as a hexadentate ligand,using both nitrogen atoms and the four carboxylate groups.The conjugation of EDTA to other molecules may be exploited in three main ways: (i) using one (or more) of the carboxylic groups, (ii) attaching a residue to one of the α positions to the carboxylic groups, (iii) inserting a specific group in the ethylenediamine backbone[3]. Metal ion complexes of EDTA conjugates have numerous uses in medicine including as heavy metal decorporation agents, mimics for metalloenzymes studies and as contrast agent or optical imaging agent.

Human serum albumin (HSA) is the principal extracellular protein of the circulatory system, and accounts for about 60% of the total plasma proteins. In addition, HSA plays key roles in the transport, distribution and metabolism of many endogenous and exogenous ligands, such as fatty acids, steroid hormones, metabolites and an extraordinarily broad range of drugs. Thus HSA has been frequently selected as the model protein for investigating the protein folding and drug-binding mechanism.

In this study, we synthesized EDTA-bis(tyramide) ligand and its complexes with Eu^{3+} , Tb^{3+} and Gd^{3+} and evaluated these complexes for imaging applications. The thermodynamic stability and kinetic inertness of lanthanide complexes of the ligand were studied potentiometrically and spectrophotometrically respectively. Furthermore, molecular modeling studies was done to understand binding affinity of the ligand with HSA.

II. MATERIALS AND METHODS

EDTA-bisanhydride, tyramine, triethylamine, dimethylsulphoxide, water, chloroform, DPPH, ascorbic acid and methanol, purchased from SIGMA-ALDRICH Co, USA.TLC on aluminum plates coated with silica gel 1160, F254 (Merck, Germany). For the equilibrium measurements, the chemicals used in the experiments were of the highest analytical grade. The metal salts,$EuCl_3$ anhydrous, $GdCl_3.6H_2O$, $CeCl_3.6H_2O$, $LuCl_3.6H_2O$, $SmCl_3.6H_2O$ and $TbCl_3.6H_2O$ were purchased from ALDRICH(99.9%).Stock solutions of the individual metal cations were prepared by dissolving $LnCl_3$ hydrate in water, and their concentration was determined by complexometric titration with standardized Na_2H_2EDTA and xylenol orange as indicator.^{99m}Tc was procured from Regional Centre for Radiopharmaceuticals (Northern Region),Board of Radiation and Isotope Technology (BRIT), Department of Atomic Energy, India.

III. EXPERIMENTAL SECTION

Synthesis of ligand EDTA-bis(tyramide) ([EDTA(TA)$_2$]) .the chelate was synthesized by using the modified protocol[4] : 1g EDTA-bisanhydride (1eq) was reacted with 1.1243g tyramine hydrochloride(2.1eq) in DMSO in the presence of triethylamine at 60^0c under inert atmosphere of nitrogen. After 16 hrs reaction mixture was

cooled at room temperature, filtered and solvent was evaporated under reduced pressure to give crude oily sticky liquid. The compound was purified by $CHCl_3$:H_2O extraction and H_2O layer was evaporated to give brown colored powdered product.

Yield: 2.01g, 97.17% ^{1}HNMR(400 MHz,CDCl3): δ = 7.06 (dd, 4H, ArH), 6.9 (dd, 4H, ArH), . ^{13}C NMR (100 MHz, CDCl3): δ = 171.85 (CONH), 156.38 (COOH), 129 (aromatic carbon), 127.12 (aromatic carbon),115.39 (aromatic carbon), 56.7, 50.89, 48.25, 46.97, 34.18, 32.41,

Calculated mass [M^+] = 530.

Synthesis of Ln complexes of the ligand [EDTA(TA)$_2$] : The Eu, Tb and Gd complexes of the ligand [EDTA(TA)$_2$] was synthesized by the modified protocol : 500mg TE (1eq) was dissolved in a 20 ml H_2O in a three neck RB flask and aqueous solution of 1.41g $LnCl_3.6H_2O$ (1.5 eq) was added dropwise with stirring into the RB flask. pH of the reaction mixture was maintained at 6-7 throughout the reaction by adding 0.01M NaOH(aq) dropwise. After addition RM was stirred at RT for 72 hrs. RM was filtered and solvent was evaporated under reduced pressure and the solid product was lyophilized for 24 hrs.

Yield : 641.5mg (100%), mass spectra : [M^++H^+] = 681 ([Eu-EDTA(TA)$_2$]) 690 ([Tb-EDTA(TA)$_2$]) , and 684 ([Gd-EDTA(TA)$_2$])

Equilibrium measurements : The protonation constants of [EDTA(TA)$_2$] were determined with pH-potentiometry by titrating 0.1mM [EDTA(TA)$_2$] solution with standerlized tetrabutylammonium hydroxide (TBAOH) solution using a metrohm Dosimat 713 pH meter equipped with a Metrohm glass electrode , 800 Dosino autoburet. Potentiometric titration were carried out at 25^{0}C .Titration were performed in the pH range 1.5-12.5 for protonation constant.The stability constant of [EDTA(TA)$_2$] with Eu(III) , Gd(III) , Ce(III) , Sm(III) , Lu(III) , and Tb(III) were determined under same condition for the acidity constant {[EDTA(TA)$_2$] (0.1mM) , $LnCl_3$(0.1mM)}. The protonation and stability constant were evaluated from the titration data using the program *TIAMO* 2.0 .

Kinetic study : The formation rates of Ce[EDTA(TA)$_2$]$^+$, Gd[EDTA(TA)$_2$]$^+$, and Eu[EDTA(TA)$_2$]$^+$ were studied at 25^{0}C and 0.1 M KCl ionic strength by direct spectrophotometry for Ce^{3+} and by an indicator method[5] for Gd^{3+} and Eu^{3+}, on a Perkin-Elmer Lamda. In the indicator method bromocresol green was used and the pH was allowed to change 0.05-0.1 unit in slightly buffered solution. The measurements were performed in quartz cuvette in a cuvette holder capable of being thermostated. The metal concentration between 0.01-0.001 M while the concentration of [EDTA(TA)$_2$] 0.0001 M was used in a buffer and its concentration was determined in each experiment.

Fluorescence study : The fluorescence intensity and wavelength of [EDTA(TA)$_2$] was studied at 25^{0}C for different dilutions of the ligand between concentration 0.8mM-.01mM , on a Spectra Max M2, molecular devices from Spinco Laboratorypvt. Ltd. By using the software *Softmax Pro*. The measurements were performed in quartz cuvette in a cuvette holder cabable of being thermostated.

IV. RESULTS

Synthesis of [EDTA(TA)$_2$] : To achieve our aim to develop a chelating agent which can bind with lanthanides and able to show an optical imaging property EDTA-bis(tyramide) (3) was synthesized by using edta-

bisanhydride(1) and tyramine(2) as shown in scheme 1. The reaction was carried out at 60^0C for 16 hrs and the progress of the reaction was monitored by TLC and compound was obtained in 97.17% yield.

V. DISCUSSION

EDTA-bis(tyramide) ligand was synthesized in this study, has six coordinating groups, two free carboxylic pendant arms, two nitrogen and two amide groups. EDTA-bis(tyramide) have been studied for advantageous optical and MR imaging properties when complexed with Eu^{3+} and Gd^{3+} respectively. Such complexes can therefore be applied for bimodal imaging applications providing identical biodistribution of the MRI and optical probes, thus straight forward merging of the MR and optical images. EDTA-bistyramine studied for its ability to form chelate with lanthanide ions and their application as an optical imaging agent via fluorescence study. The ligand was excited at 275 nm depending upon its excitation maxima, the emission intensities were recorded at 310 and 600 nm. These bands shows good emission maxima to study the fluorescent behaviour of the ligand. Since the presence of tyramine molecule which is a decarboxylated product of tyrosine, and the tyrosine is known for its fluorescent behaviour, the ligand EDTA-bis(tyramide) shows appreciable fluorescent property.The lanthanide complexes formed with these tyramine-based ligands integrate several positive features. They are trishydrated and possess good thermodynamic stability and good selectivity for lanthanides versus endogenous cations.

VI. CONCLUSION

EDTA-based ligand in this literature, was successfully synthesized by a modified protocol and characterized by spectroscopic techniques. EDTA-bis(tyramide) ligand was complexed with Eu^{3+}, Tb^{3+} and Gd^{3+} and therefore be applied for bimodal imaging applications providing identical biodistribution of the MRI and optical probes, thus straight forward merging of the MR and optical images. The spectrophotometric studies of the ligand shows λex = 275 nm and λem = 310 nm, 600 nm shows appreciable fluorescent property. Synthesized ligand possess good thermodynamic stability, kinetic inertness and good selectivity for lanthanides versus endogenous cations. Computational study of the ligand shows its effective binding with HSA protein.

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