Procedure

An aliquot of copper(II) stock solution (~20 mg of Cu) containing 1–2 g of tartaric acid was rendered ammoniacal by adding (1 : 1) ammonia and heated to ~ 80°C and the reagent in slight excess over the molar ratio was added. A dark green complex first formed eventually changed to light yellow on being digested for 30–40 min., over a water-bath. It was filtered hot, through sintered glass crucible (G–4), washed with hot water and dried to constant weight at 120–130°C. The copper content was computed from the formula $Cu(C_{11}H_{11}N_4S)$. In the case of alloys, the samples were dissolved in hot HNO_3 (1 :1) the SnO_2, if any, was filtered off and the filtrate used for the estimation of copper. In the case of ores the samples were dissolved in aqua regia and the excess of the acid evaporated. The residue was extracted with dil. HCl, the silica filtered off and the solution was used for the estimation of copper.

Results and Discussion

Composition and properties of the complex

Analytical results reported elsewhere[1] show that copper forms 1 :1 (metal to ligand) complex. The dark green complex initially formed is presumed to be Cu(II) complex which gets reduced to the stable light yellow Cu(I) complex. The complex is insoluble in hot water and dilute ammonia but is readily soluble in mineral acids. It is sparingly soluble in ethanol and acetone. The TGA study shows no weight loss upto ~270°C, thus indicating good thermal stability of the complex.

Effect of diverse ions

Various metal ions were added to study their interference. The metal ions which show no interference in ammoniacal tartrate medium in the range of 20–40 mg include As(III), Sn(II), Sb(III), Pb(II), Bi(III), Fe(III), Cr(III), Al(III), Mo(VI), Zn(II), Co(II), Ni(II) and Mg(II). In the case of Fe(III), more than twice the quantity of the reagent was needed, due perhaps to the reduction of Fe(III). However large amounts of Fe(III) cause interference. Ag(I) and Tl(I) also, interfere as they too form their respective insoluble complexes. Anions like acetate, citrate, tartrate oxalate, sulphate, nitrate and chloride do not interfere. But EDTA prevents precipitaion of copper as it is a better complexing agent.

Accuracy of the method

The relative error in the range 10–40 mg of copper is ±0·26% and standard deviation at 40 mg. level is 0·2%. The results of analyses of copper alloys, ore and complexes (Table I) indicate that the method is accurate and reproducible,

TABLE I

Determination of Copper in ores, alloys and complexes

Sample	Cu present %	Cu found%
Chalcopyrites	2·02	2·09
Copper ore concentrate	23·93	24·33
Brass	64·46	63·94
Bronze	83·24	83·51
Duralumin	4·04	4·04
German silver	65·00	64·58
$Cu(C_{11}H_{11}N_4S)$	21·55	21·84
$Cu(C_3H_5N_4S)$	33·00	33·12
$Cu(C_3H_2F_3N_4S)$	25·76	25·80

The outstanding merits of the method include high selectivity of the reagent for copper, non-interference by most metal ions, low relative errors, high reproducibility, low conversion factor, rapidity of estimation and ease of reduction of Cu(II) to Cu(I) without the use of extraneous chemicals.

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Department of Chemistry,
Karnataka Regional
Engineering College,
Srinivasanagar 574 157,
February 25, 1980.

MAHESH GOUDAR,
R. V. GADAG.
M. R. GAJENDRAGAD.*

* For correspondence.

1. Gadag, R. V. and Gajendragad, M. R., *Curr. Sci.*, 1979, 48 (19), 839.

LUTETIUM(III) MYOGLOBIN: INTERACTION OF Lu(III) MESOPORPHYRIN IX WITH APOMYOGLOBIN

THE trivalent lanthanides, namely, terbium and europium, are the most frequently used fluorescence probes for both proteins and nucleic acids[1]. They exhibit sharp emission spectra due to intramolecular energy transfer from the electronic states of organic residues of proteins and nucleic acids to the 4*f* energy levels of the lanthanide ions. Recently, the trivalent lute-

tium porphyrin complexes show moderately strong porphyrin fluorescence and phosphorescence[2,3]. In this paper we describe the first report on detection, synthesis, and spectral properties, namely, absorption and fluorescence spectroscopy, of lutetium(III) myoglobin.

The hydroxy derivative of lutetium(III) mesoporphyrin IX [Lu(III)–MP–IX] was prepared by the following method: The anhydrous lutetium trichloride (500 mg) and mesoporphyrin IX dimethyl ester (100mg) were heated in melt of imidazole (3 g) at 210°–220° C for 3 hours[4]. The reaction product was dispersed in water, filtered, and washed with water to get dimethyl ester of Lu(III)-MP–IX. This ester was hydrolyzed by refluxing it in 30% potassium hydroxide in methanol[5]. The Lu(III)–MP–IX was further purified on a silica gel (grade V) column equilibrated with methanol. The fraction of Lu(III)–MP-IX was collected, filtered, concentrated, and stored at 5° C.

The lutetium(III) myoglobin [Lu(III)–Mb] was prepared by mixing the Lu(III)–MP-IX (1·2 moles) in a small quantity of pyridine with apomyoglobin (1 mole) in 0·1 M Tris–HCl abuffer of pH 8. This mixture was passed through a long Sephadex G–25 column equilibrated with 10 mM potassium phosphate buffer of pH 7·2. This column was eluted with the same buffer and the major fraction was collected. The Lu(III)–Mb was further concentrated in the collodian bag (Schleichler and Schuell, UH 100/10) and the final solution was stored at 5° C.

The complex formation between Lu(III)-MP–IX and apomyoglobin in 1 : 1 molar ratio was investigated by difference absorption spectroscopy. The difference spectrum of Lu(III)–MP–IX and apomyoglobin in molar ratio of 1 : 1 against the same concentration of Lu(III)–MP–IX shows a positive peak at 403 nm and a negative peak at 395 nm shown in Fig. 1. The red shift of the Soret peak in the Lu(III)–MP–IX protein complex as compared with its value in Lu(III)–MP–IX was used to determine the stoichiometric binding of Lu(III)-MP–IX with apomyoglobin. Thus, the stoichiometric titration of Lu(III) MP–IX with apomyoglobin was carried out at 403 nm and the plot of changes of optical density against molar ratios of Lu(III)–MP IX and apomyoglobin shows 1 : 1 complex formation.

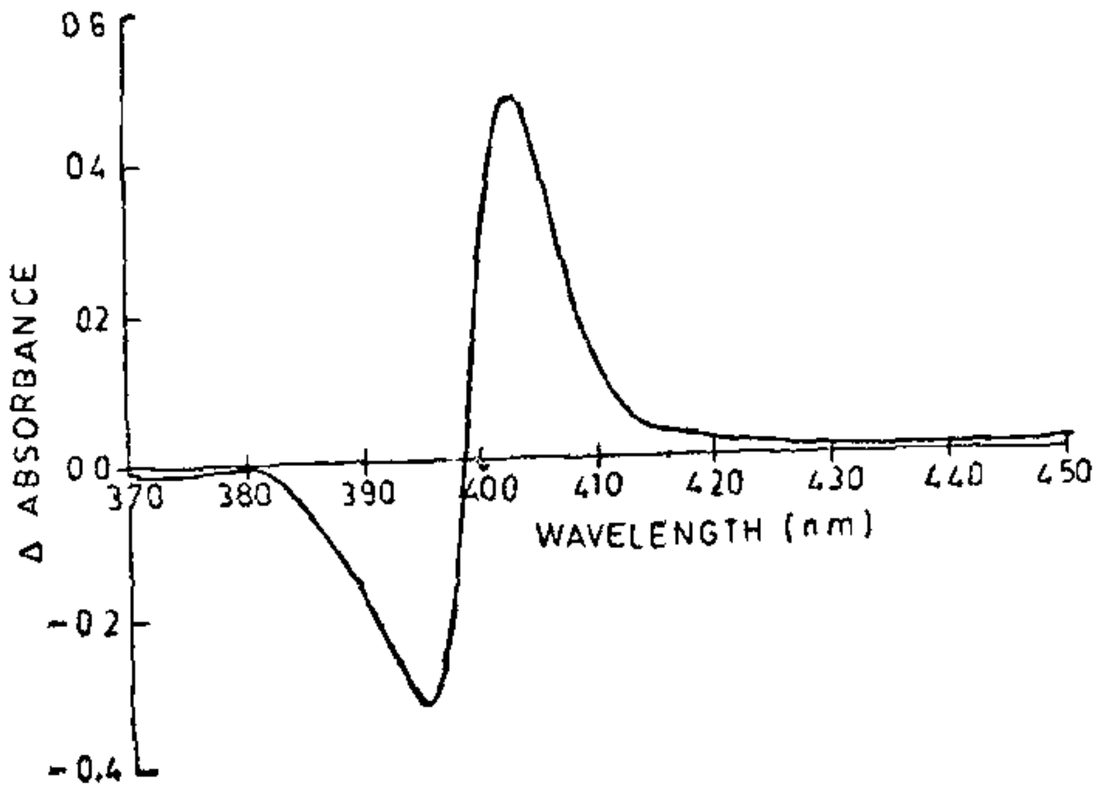

Fig. 1. Difference spectrum of Lu(III)–MP–IX (3·35 μM) + apomyoglobin (3·35 μM) against Lu(III)-MP IX (3·35 μM) in 0·1 M Tris HCl buffer of pH 8.

The visible absorption maxima of Lu(III) MP–IX and Lu(III)–Mb in 0·1 M Tris-HCl buffer of pH 8 are given in Table I. The Soret peak of Lu(III)–Mb is about 6 nm red shifted as compared its value of Lu(III)–MP–IX. The fluorescence spectra of Lu(III)–MP–IX and Lu(III)–Mb are given in Table I.

TABLE I

Visible absorption and fluorescence emission maxima of Lu(III)–MP–IX and Lu(III)–Mb in 0·1 M Tris-HCl buffer of pH 8[a,b]

Compound	Soret or B (0, 0)[2]	α or Q (0, 0)[2]	β or Q (1, 0)[2]
Lu(III)–MP–IX	396·5 with weak shoulder (222)[c]	569 (12·2)	532 (8·8)
Lu(III)–Mb	402·5 (287)	570·5 (21·8)	533·5 (15)
	$\lambda_{excitation}$	Q (0, 0)[2]	Q (0, 1)[2]
Lu(III)–MP–IX	400	573	612
Lu(III)–Mb	400	574·5	616

[a] Concentrations of Lu(III)–MP–IX and Lu(III)–Mb are in the range 3 to 5 μM.

[b] Wavelength in nm.

[c] The values in paranthesis are millimolar extinction coefficients ϵ_{mM} (mM^{-1} cm^{-1}).

The site of binding of Lu(III) MP–IX in Lu(III)–Mb was established by displacing the Lu(III)–MP–IX from Lu(III)–Mb with hemin. A difference spectrum of a mixture of Lu(III)–Mb (3·35 μM) and hemin (3·35 μM) against Lu(III)–Mb (3·35 μM) and hemin (3·35 μM) are shown in Fig. 2. The spectrum shows two positive peaks at 395 and 411·5 and one negative peak at 402 nm. This shows that metmyoglobin [Fe(III)–Mb] is formed by displacing Lu(III)–MP–IX from the protein complex with hemin. Thus, this result supports the binding of Lu(III) MP–IX in the heme pocket of Lu(III) Mb.

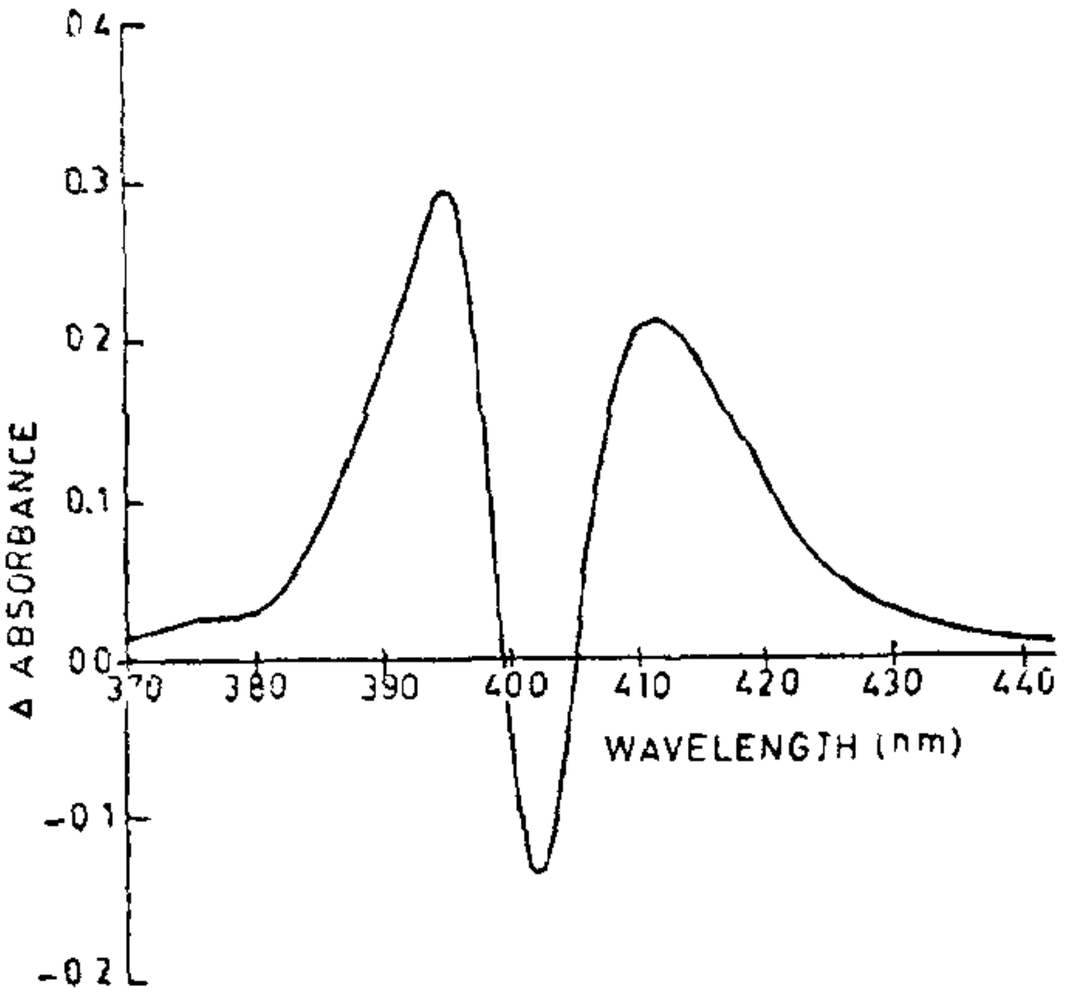

FIG. 2. Difference spectrum of Lu(III)-Mb (3·35μM) + hemin (3·35μM) against Lu(III)-Mb (3·35μM) and hemin (3·35μM) in 0·1 M Tris-HCl buffer of pH 8.

In order to establish the structure of Lu(III)–Mb, the fluorescence emission spectra of fixed concentration of apomyoglobin in presence of various concentrations of Lu(III)–MP–IX were measured and shown in Fig. 3. The fluorescence of apomyoglobin is

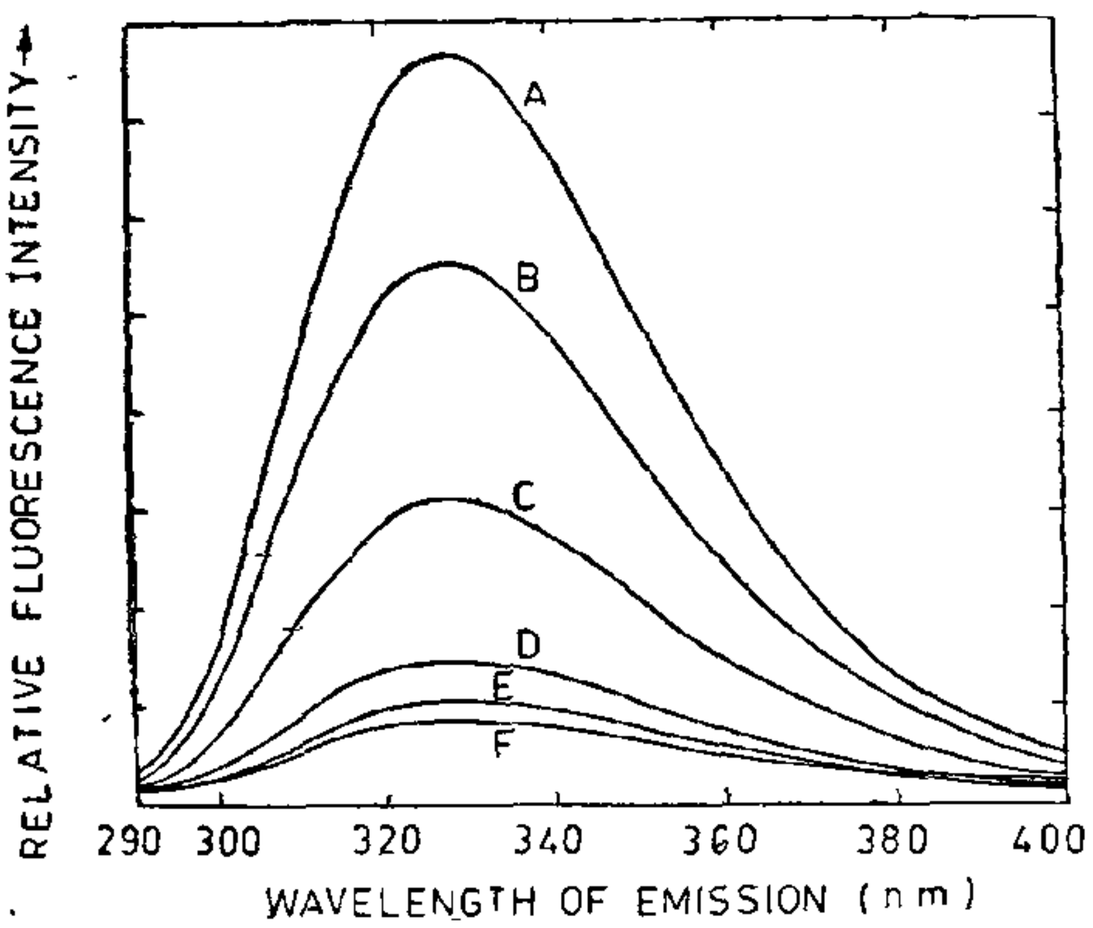

FIG. 3. Fluorescence emission spectra of apomyoglobin (2·45μM) in presence of 0·00μM (A), 0·63μM (B), 1·47μM (C), 2·31μM (D), 3·15μM (E), Lu(III)–MP–IX and 2·45μM (F) sperm whale metmyoglobin in 0·1 M Tris-HCl buffer of pH 8. Excitation wavelength was at 280 nm.

quenched with an increase in the concentration of Lu(III)-MP–IX. The fluorescence of protein is very weak when molar ratio of Lu(III)–MP–IX and apomyoglobin is 1·3 to 1. Thus, all apomyoglobin is complexed in the presence of slight excess of Lu(III)–MP–IX. The amplitude of fluorescence in apomyoglobin is due to tryptophan and tyrosine[7]. The intensity of fluorescence of tryptophan and tyrosine depends upon the degree of quenching mediated by interaction with the metal porphyrin. The Lu(III)–MP–IX effectively quenches the fluorescence of tryptophan and tyrosine residues in the protein complex because of its central position and the overlap of its absorption spectrum with emission spectra of tryptophan and tyrosine. The protein fluorescence of Lu(III)–Mb is comparable with sperm whale metmyoglobin. Therefore they must have the same mean separation of the tryptophan and tyrosine fluorophores from metal porphyrin and thus have the same structures.[8]

Department of Chemistry, T. S. SRIVASTAVA.
Indian Institute of Technology,
Powai, Bombay 400 076,
March 3, 1980.

1. Nieber, E., *Structure and Bonding*, ed. by J. D. Dunitz *et al.*, Spring Verlag, Berlin, 1975, **22**, 1.
2. Gouterman, M., *The Porphyrins*, ed. by D. Dolphin, Academic Press, New York, 1978, **3**, Part A, 1.
3. Srivastava, T. S., Hershberg, R. and Yonetani, T., *Biophys. J.*, 1975, **15**, 146*a*.
4. —, *Bioinorg. Chem.*, 1978, **8**, 61.
5. Smith, K. M. (ed.), *Porphyrins and Metalloporphyrins*, Elsevier, Amsterdam, 1975, p. 796.
6. Teale, F. W. J., *Biochim. Biophys. Acta*, 1959, **35**, 543.
7. Weber, G. and Teale, F. W. J., *Disc. Faraday Soc.*, 1959, **27**, 134.
8. Schechter, A. N. and Epstein, C. J., *J. Mol. Biol.*, 1968, **35**, 567.

PREPARATION AND CHARACTERIZATION OF COPPER(II) NITRATE COMPLEXES WITH NICOTINIC ACID AND RELATED LIGANDS

NICOTINIC acid (NA), nicotinamide (NICA) and isonicotinamide (INA)—pyridine derivatives with a carboxyl or amido group in the ring—are important constituents of coenzymes which participate in various oxidation-reductions of physiological importance. Metal complexes of biologically important ligands are often more effective than the free ligands. This study describes the coordination compounds formed by the interaction of NA, NICA and INA with copper(II) nitrate which are likely to exhibit significant biological effects. Molar conductance, magnetic susceptibility, electronic and i.r. spectral studies down to 200 cm^{-1} have been carried out to elucidate