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Short communication

Terbium chelates for fluorescence immunoassays[1]

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Abstract

The sensitization of terbium(III) ion luminescence in the presence of 1-ethyl-1,4-dihydro-6,7-methylenedioxy-4-oxo-quinoline-3-carboxylic (oxolinic) acid was studied. The terbium label is bound to the antibody with diethylenetriamine-pentaacetic acid anhydride (DTPAA). Optimum luminescence intensity is observed at pH 7.5 and the luminescence significantly increases in the presence of cationic surfactant, cetyltrimethylammonium bromide. The sensitivity of Tb(III) detection is 5×10^{-14} mol l^{-1}. This luminescence system is proposed for time-resolved fluoroimmunoassay.

Keywords: Terbium complex; Sensitized luminescence; Fluoroimmunoassay; Fluororescence enhancement solution

1. Introduction

In the last years the lanthanide immunofluorescence assay (LIFA) attracts the increasing attention of investigators. For indication the antigen–antibody complex the lanthanide labels, europium, terbium, samarium ions are used. The label is introduced into the composition of indicator antibody using the complexone. To determine the amount of indicator immunoglobuline molecules entering into the composition of immunocomplex, the complex of polycarboxilic acid is destroyed by acidifying to pH 3.2 and the lanthanide ion is influenced upon luminescence sensitizer [1,2] entering into the composition of enhancing solution. The most effective luminescence sensitizer are β-diketones the complexes of which are well-known and used for labeling the biologically active antibodies [1–5]. The heterocyclic aromatic acids are also effective. In particular, 4,7-bis(chlorosulfophenyl)-1,10-phenanthroline-2,9-dicarboxilic acid is applied for immune determination of cortisol in the blood serum [6]. The method for determination of protein traces using the terbium chelates with *p*-aminosalicilic acid as a label was described [7,8]. In these determinations the detection limits (DL) are very low, for instance, DL for immunoglibuline is ~ 0.1 μg ml^{-1} [4] for protein 10^{-11} mol l^{-1} [7].

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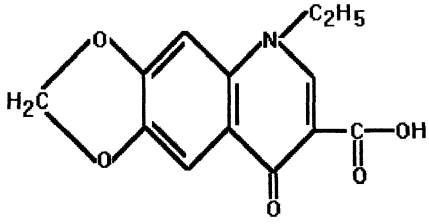
This work presents the study of possibility of using the 4-oxoquinoline derivative, 1-ethyl-1,4-dihydro-6,7-ethylenedioxy-4-oxoquinoline-3-carboxylic (oxolinic) acid (L), as a sensitizer of Tb(III) ion luminescence in the composition of enhancing solution.



The optimization for the component rations of enhancing solution was also performed. Early the compounds of this class have not been described in the literature.

2. Experimental

2.1. Apparatus

Absorption spectra of ligand were registered on a Lambda-9-spectrophotometer (Perkin–Elmer). The luminescence measurements were obtained on an SDL-2 spectrophotometer (Leningrad Optomechanical Association, St. Petersburg, Russia). Xenon lamp was used as an excitation source. The Arcus 1230 fluorimeter (LKB-Wellac) was also used. The pH values of solutions were measured using an OP-211/1 laboratory digital pH-meter (Radelkis, Budapest, Hungary).

2.2. Materials and methods

All reagents were of analytical-reagent grade (Chemical Reagent Plant, Shostka, Ukraine). Stock standard terbium chloride solution of 0.1 mol l^{-1} was prepared by dissolving the exact mass of annealed preliminary terbium oxide in HCl followed by evaporation of the excess of HCl.

Metal concentration were determined by complexometric titration with Arsenazo I as the indicator. Aqueous solutions of oxolinic acid (1×10^{-3} mol l^{-1}) and cetyltrimethylamonium bromide (0.05%) were obtained by dissolution of accurately weighed preparation in twice distilled water.

To estimate the possibility of energy transfer from ligand molecule to the Tb(III) ion the absorption spectrum of the ligand was obtained and its triplet level was determined.

The absorption spectra of oxolinic acid were recorded in water solutions at concentration 1×10^{-4} mol l^{-1} and length of absorbing layer, $l = 1$ cm.

The triplet level of ligand was calculated from phosphorescence spectra of yttrium complex with oxolinic acid at 77 K, the concentrations of yttrium and acid were 1×10^{-5} and 5×10^{-4} mol l^{-1}, respectively.

The luminescence excitation spectra of terbium complexes with oxolinic acid were obtained at concentrations of terbium and ligand 1×10^{-5} and 5×10^{-5} mol l^{-1}, respectively.

The optimal conditions of complexation were determined using the solutions containing 1×10^{-5} mol l^{-1} of terbium(III). The different pH values in solutions were achieved by addition of various amounts of HCl and NH_4OH.

The studies of surfactant influence was performed at terbium, oxolinic acid and surfactant concentrations 1×10^{-5}, 5×10^{-5} mol l^{-1} and 0.05%, respectively.

A total of 0.2 ml of human IgG was added to 1 mg diethylenetriaminepentaacetic dianhydride (DTPAA) and neutralized to pH 7 with diluted NaOH solution. After 10 min, 0.3 ml of $TbCl_3$ was added to DTPAA-IgG solution. Thirty minutes later, conjugates were purified using a 1.0×20 cm Sephadax G50 column with 0.05 mol l^{-1} Tris–HCl buffer (pH 7), containing 9 g l^{-1} NaCl and 0.05% NaN_3 as the eluting agent.

3. Results and discussion

3.1. Optical characteristics of ligand and complex

The absorption spectrum of oxolinic acid solution is characterized by the presence in UV spectral region two bands at 320 nm and 263 nm (Fig.

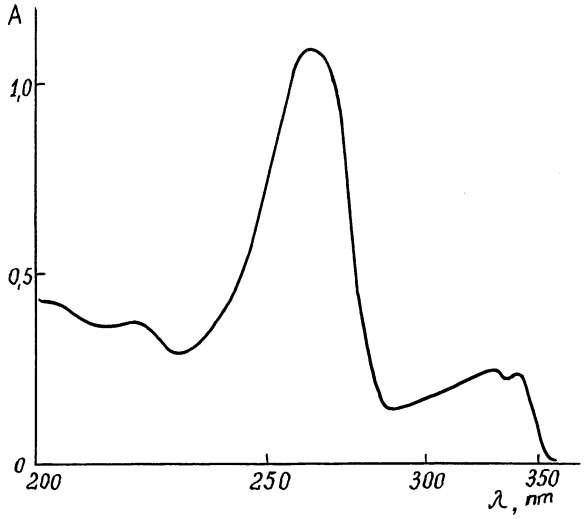


Fig. 1. Absorption spectrum of oxolinic acid solution. $C = 1 \times 10^{-4}$ mol l^{-1}.

1). The extinction coefficient of the short-wave band is 54500. This makes possible the effective absorption of light energy by the ligand molecule. The energy of triplet level, 21000 cm^{-1} exceed considerably the energy of the first excited level ($^5D_4$, 20500 cm^{-1}) of Tb(III) causing the effective energy transfer to this ion. Owing to that the terbium ion intensively emits the luminescence in solution (Fig. 2) in a range of 520–560 nm ($^5D_4 \rightarrow {}^7F_5$ transition) with maximum at 545 nm. The excitation band situates in a range of 300–360 nm with maximum at 300 nm (Fig. 2).

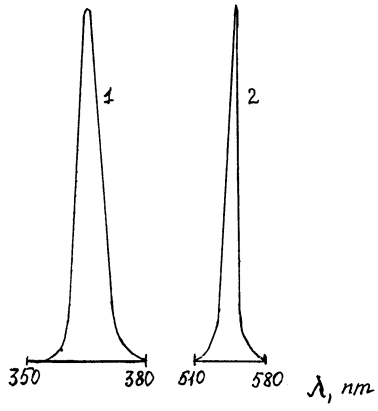


Fig. 2. Excitation (1) and luminescence (2) spectra of Tb(III) ion in complex with oxolinic acid. $C_{Tb} = 1 \times 10^{-5}$ mol l^{-1}.

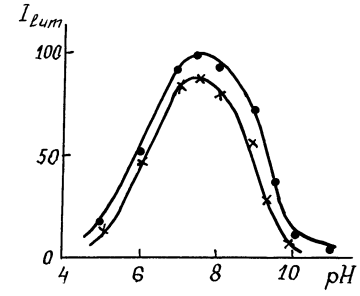


Fig. 3. Dependence of luminescence intensity of terbium ion in complex with oxolinic acid on pH of solution (○, in the absence of DTPA; ×, in the presence of DTPA). $C_{Tb} = 1 \times 10^{-5}$ mol l^{-1}.

3.2. Optimal conditions of the complexation

Maximum luminescence is observed at pH 7.5 (Fig. 3). At the lesser pH values (in acid solutions) the complex, probably, does not form or degree of its formation is very small and in alkaline solutions (at pH > 9) decomposition of the complex with a formation of terbium hydroxide is observed. Further the optimal value of pH 7.5 in solutions was achieved using 40% urothropine solution (0.2 ml).

To obtain the optimum luminescence intensity (I_{lum}) for Tb(III) in solution the five-fold excess of oxolinic acid is required (Fig. 4).

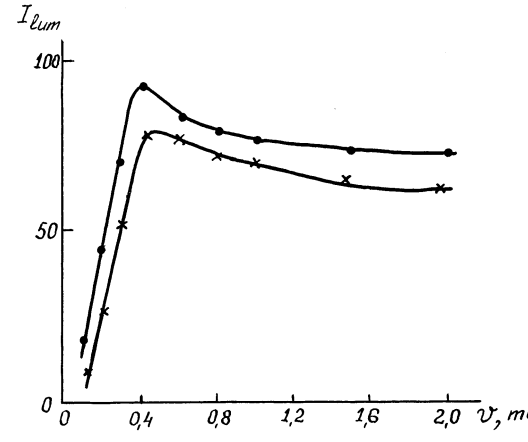


Fig. 4. Dependence of luminescence intensity of terbium ion in complex with oxolinic acid on ligand amount (○, in the absence of DTPA; ×, in the presence of DTPA). $C_{Tb} = 1 \times 10^{-5}$ mol l^{-1}.

3.3. Influence of surfactants

Since the measurements of luminescence in LIFA method are performed in micellar solutions, the influence of different surfactants on luminescence intensity of terbium complex was studied. Upon that, it was found that anionic surfactants (laurylsulfate) and neutral ones (Triton X-100, Tween-80) decrease I_{lum} of Tb(III) in solution. Only cationic surfactant, cetyltrimethylamonium bromide (CTA(Br)), increases the I_{lum} of Tb(III) by three times that is due the hydrophobic action of surfactant.

3.4. Influence of complexone

Since the label is introduced into the composition of indicator antibody by means of complexones that, on one hand, strongly bind the lanthanide ion, and the other hand, are able to attach covalently to the immunoglobuline molecules, it is of interest to follow by what manner the luminescence properties of terbium complexes will be changed in the presence of copmlexone, in particular, diethylenetriaminepentaacetic acid (DTPA) extensively using at the present time in LIFA [2]. The introduction of DTPA in solution at the concentration ratio $C_{Tb}:C_{DTPA} = 1:1$ reduces I_{lum} of Tb(III) by 15–20% only indicating that this complex can be used in LIFA.

3.5. Detection limit of terbium

The Tb-L-CTA(Br) system in the presence of urothropine was applied to the determination of trace amounts of terbium in solution. The detection limit was calculated to be 5×10^{-14} mol l^{-1} of terbium. The calibration graph was linear up to 1×10^{-13} mol l^{-1} of terbium.

Analogous result was obtained using the immunoglobuline labeling by the terbium ions as a source of terbium ions. The enhancing solution using in this work was prepared with 0.4 ml oxolinic acid (1×10^{-3} mol l^{-1}), 1 ml of CTA(Br) (0.05%) and 0.2 ml of urothropine (40%) and diluted with distilled water to 10 ml. Before fluorescence intensities were measured, the solutions were stabilized for 15 min. Accuracy and precision of the Tb(III) ions detection in solutions were examine by the statistic treatment of the determination data. At $n = 5$ and $P = 0.95$ ($C_{Tb} = 1 \times 10^{-9}$ mol l^{-1}) the value of mean standard deviation, S_r, is 0.05–0.09.

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