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Application of f–f luminescence of terbium ion for determination of non-steroidal anti-inflammatory drug-niflumic acid

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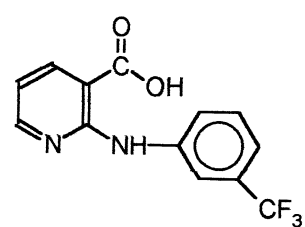
Abstract

A simple, rapid and sensitive luminescence method for determination of niflumic acid (NFA) is described. The method is based on the intramolecular energy transfer from niflumic acid to terbium ion (Tb^{3+}) in the presence of trioctylphosphine oxide (TOPO). Optimum conditions for the formation of the NFA–Tb^{3+}–TOPO ternary complex have been investigated. The calibration graph is linear over the range 0.002–0.02 μg ml^{-1}. The relative standard deviation is close to 4%. The recoveries obtained by applying the method to the analysis of urine ranged from 94–102%. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Sensitized luminescence; Niflumic acid; Terbium (III) ion; Drug analysis

1. Introduction

Niflumic (2-[3-(trifluoromethyl)amino]nicotinic acid) acid (NFA) is organic fluoro — containing carboxylic acid used in preparation ' Donalgin' as the active substance. In medicine NFA used as non-steroidal anti-inflammatory, analgetic drug [1].



The extensive application of this preparation in medical practice necessitates its determination in various biological fluids (serum, urine, etc.). Several sensitive HPLC and gas-liquid chromatography methods have been reported for the determination of niflumic acid in human serum and plasma [2,3].

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The analytical application of lanthanide-sensitized luminescence has a great interest. The main advantages of lanthanide chelates in fluorescence spectrometry include large Stokes shifts, narrow emission bands and long fluorescence lifetimes [4]. The strong ion emission of these complexes as a result of the intramolecular energy transfer process from the ligand to the lanthanide ion.

Previously, we have demonstrated that for determination of a number of drugs (nalidicxic, mefenamic, pipemidinic, thiaprophenic acids, fluoroquinolones and catecholamines) the luminescence sensitization of lanthanide ions taking place in their presence can be used [5–10].

The determination of theophilini [11], fluoroquinolones [12–15], antibiotics [16,17], steroids [18] by lanthanide-sensitized luminescence was also described.

The purpose of this work was a detailed study on the sensitization of terbium ion luminescence by niflumic acid and the development of a sensitive method for its determination in urine samples.

2. Experimental

2.1. Apparatus

The luminescence measurements were obtained on an SDL-2 spectrofluorimeter (Leningrad Optomechanical Association, St. Petersburg, Russia). Xenon lamp was used as an excitation source. The pH values of solutions were measured using an OP-211/1 laboratory digital pH-meter (Radelkis, Budapest, Hungary).

2.2. Reagents

Niflumic acid was kindly donated by pharmaceutical company. All other chemicals used were from Merck (Germany). Double-distilled water was used to prepare all aqueous solutions. The terbium chloride solution with concentration of 1×10^{-1} M that was prepared by dissolving the terbium oxide (99.99%) in hydrochloric acid (1:1) the excess of which was evaporated to wet residue and diluted with distilled water. The metal concentration were determined by complexometric titration with Arsenazo I as the indicator. The solution of niflumic acid was obtained by dissolution of accurately weighed preparation in ethanol. The solution of trioctylphosphinoxide (TOPO) at a concentracion of 1×10^{-2} M was prepared by dissolution of an accurately weighed substance in ethanol. The pH of solutions was maintained at 7.0–7.5 with 40% aqueous solution of urothropine.

2.3. Methods

To choice of optimal conditions for Tb(III) luminescence in complex with niflumic acid the assays were prepared as following: the terbium chloride solution was added to the solution of niflumic acid, added the ethanol solution of TOPO, adjusted required pH value, then added the water to the volume of 10 ml and recorded the luminescence intensity (I_{lum}) of solution at $\lambda_{em} =$ 545 nm, $\lambda_{ex} = 365$ nm.

The content of niflumic acid was calculated by the usual in analytical practice method of additives [19] using the formula: $C_x = C \times I_x/(I_{x+ad} - I_x)$, where I_x and I_{x+ad} are the luminescence intensity of the assay and assay with additive, C — the content of additive.

The triplet level of the ligand was calculated from its phosphorescence spectra with yttrium at 77K.

3. Results and discussion

3.1. Optical characteristics of the ligand and complex

The absorption spectra of ethanolic solution of the ligand considered is characterized by the presence of two bands in ultraviolet region (Fig. 1). The molar extinction coefficient for the band at 270 nm is 9600 giving the possibility for effective absorption of light energy. The energy of triplet level of a reagent is equal to 20 900 cm^{-1}. One can be supposed that the excitation energy transfer to the energetic level $^5D_4$ (20 500 cm^{-1}) of terbium. The band at 545 nm ($^5D_4 \rightarrow {}^7F_5$ transi-

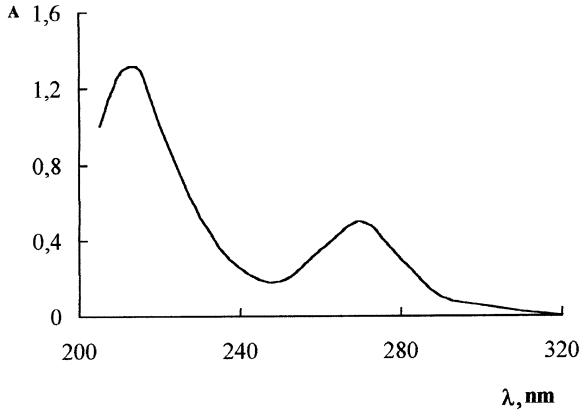

Fig. 1. Absorption spectra of niflumic acid ethanolic solution ($C_{NFA} = 5 \times 10^{-5}$ M; $l = 1$ cm).

tion) is the most intensive (Fig. 2). The native fluorescence of the NFA molecule is drastically decreased with increasing concentration of terbium (Fig. 3), that also confirms the effective energy transfer between the NFA and terbium ion.

3.2. Influence of TOPO and terbium concentration

The presence of TOPO (synergetic agent) is significant increase the $I_{lum.}$ Tb(III) in complex with NFA. The dependence of I_{lum} on the concentracion of TOPO is shown in Fig. 4. The maximum luminescence was observed at 2.5×10^{-3} M

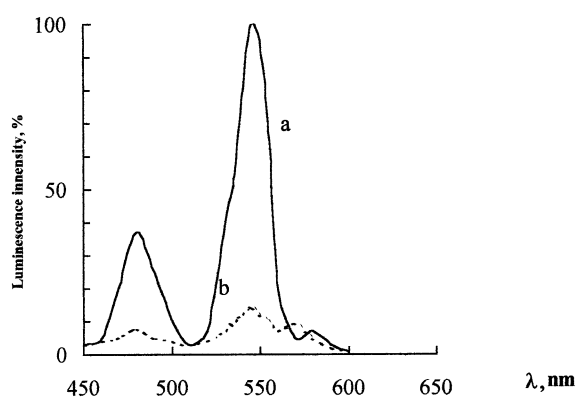

Fig. 2. Emission spectra of Tb^{3+} sensitized by NFA : (a) in the presence of TOPO. (b) in the absence of TOPO ($C_{Tb} = 1 \times 10^{-5}$ M; $C_{NFA} = 1 \times 10^{-4}$ M).

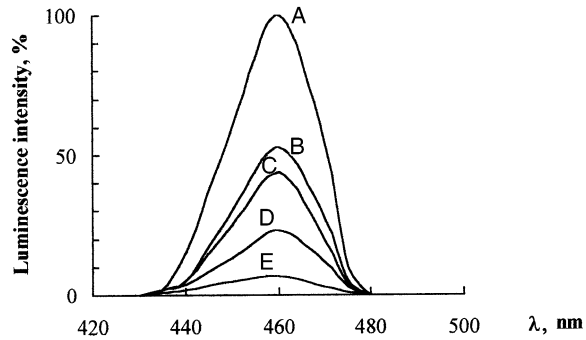

Fig. 3. Emission spectra of niflumic acid ($C_{NFA} = 1 \times 10^{-3}$ M) as a function of the terbium concentration : (a) in the absence of Tb^{3+}. (b) $C_{Tb} = 1 \times 10^{-7}$ M. (c) $C_{Tb} = 1 \times 10^{-6}$ M. (d) $C_{Tb} = 5 \times 10^{-6}$ M. (e) $C_{Tb} = 1 \times 10^{-5}$ M.

of TOPO. It could be supposed that in presence of TOPO, there is an increase in the structure microregulation and rigidity of the compound formed.

From the data of I_{lum} for the complex the ratio of component NFA:Tb^{3+}:TOPO was established equal to 2:1:1 by the continuos variations method.

The effect of terbium concentration on the analytical signal for NFA–Tb^{3+}–TOPO ternary complex was studied. As can be seen from Fig. 5 the maximum luminescence is observed at a terbium concentration above 1×10^{-3}M. A terbium concentration 2×10^{-3} M was selected for the measurements.

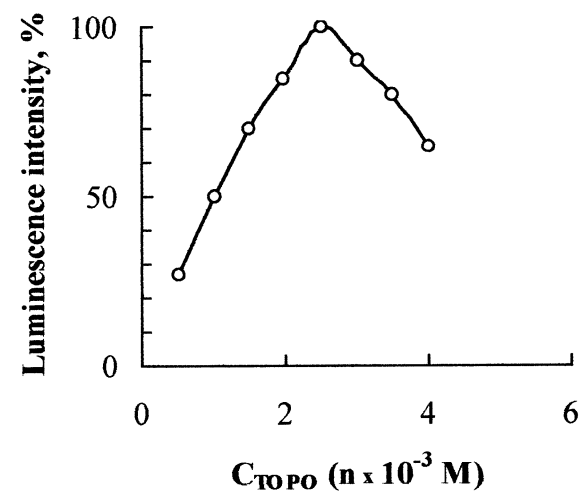

Fig. 4. Dependence of I_{lum} of Tb^{3+} in complex with NFA on the TOPO concentration ($C_{Tb} = 1 \times 10^{-3}$ M; $C_{NFA} = 1 \times 10^{-2}$ M).

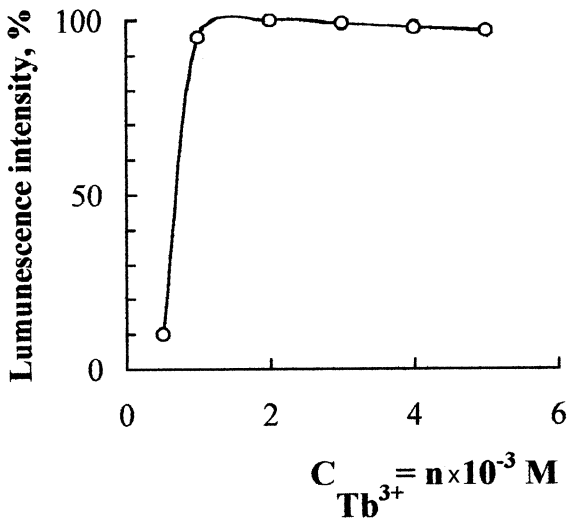

Fig. 5. Effect of terbium concentration on the luminescence intensity of NFA–Tb^{3+}–TOPO ternary complex.

3.3. Influence of pH

The complexation of Tb(III) with the ligand occurs in a wide range of pH values 2–10 (Fig. 6). The most luminescence intensity (I_{lum}) of the ternary complex is observed at pH 7.0–7.5. Upon the lesser pH values (in acid solutions, $pH < 2$) the complex, evidently, does not form or the degree of its formation is very low. In alkaline solutions ($pH > 8$) the decomposition of complexes with forming the terbium hydroxide is observed. The 40% aqueous solution of urothropine of pH 7.5 at a volume of 0.4 ml was found to be suitable for the measurements.

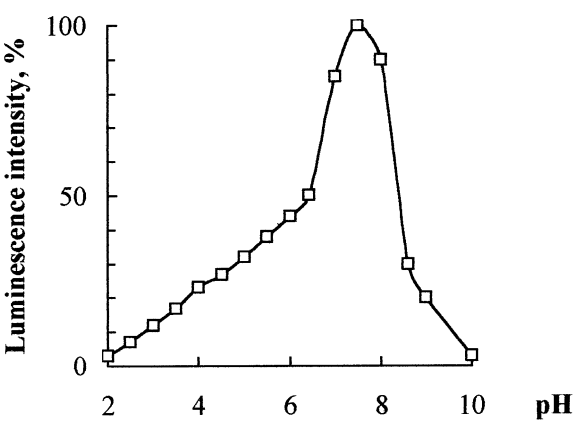

Fig. 6. Dependence of I_{lum} of Tb^{3+} in the ternary complex NFA–Tb^{3+}–TOPO on pH of solution.

3.4. Analytical performance for urine samples by some healthy volunteers

The influence of the main componets of urine on I_{lum} of the terbium complex was studied. It was established that I_{lum} decreases by 10% in the presence of 0.03 g of NaCl, 20% — 0.6 mg of oxalates, 60% — 0.03 g of phosphates, 15% — 7.5 mg of urea, the sulphates in amount of 30 mg do not affect the luminescence. The elimination of hindering influence of the mentioned components can be accomplished in the conditions of the method of additives. Under optimized conditions NFA final concentration and relative luminescence intensity were linearly over the range 0.002–0.2 μg ml^{-1}. Pearson's correlation coefficient for the calibration graph was 0.9994. The limit of detection (LOD) by proposed method is 1×10^{-3} μg ml^{-1}. The determination was performed in the following manner. Two hours after the oral dose of one tablet of the preparation 'Donalgin', contained 250 mg of niflumic acid, the portion 100 ml of urine was collected (three healthy volunteers). For the analysis 0.5 ml of the urine was selected.

0.5 ml of the urine to be investigated was placed into the each of three test-tubes, then the additives of niflumic acid stock solution (1 mg ml^{-1}) were added in such amounts that the luminescence intensity of the assay with additives increases by two and four times, respectively. Two milliliter of terbium chloride solution (1×10^{-2} M) and 0.25 ml of TOPO ethanolic solution (1×10^{-2} M), 0.4 ml of 40% solution of urothropine were added to all the test-tubes and the volume was adjusted to 10 ml. The assays were stirred and after 5 min the I_{lum} was recorded at 545 nm. The content of niflumic acid was calculated by the additives method using the above formula. This method applied to three urine samples (Table 1). The relative standard deviation (RSD) were 3.8–4.3% ($n = 5$; $P = 0.95$).

Table 1
Results of the determination of the niflumic acid in some urine samples

Sample	Concentration (μg ml^{-1}) of NFA	RSD, %
1	0.080	3.8
2	0.070	4.3
3	0.085	3.5

Table 2
Results of the determination of the niflumic acid in model urine samples

Sample	Concentration (μg ml^{-1}) of NFA		Recovery (%)	RSD (%)
	Added	Found		
1	0.0050	0.0049	94.0	3.95
	0.0100	0.0099	98.0	2.70
	0.0500	0.0508	101.6	1.45
2	0.0050	0.0051	102.0	3.80
	0.0100	0.0094	94.4	2.95
	0.0500	0.0502	100.4	1.33

3.5. Analytical performance for model urine solutions

The accuracy and precision of the method were checked in the model urine solutions by the 'added-found' procedure [20] by means of the statistical treatment of determination data (Table 2). For this, 0.5 ml of the urine was placed into the test-tubes and the different contents of niflumic acid and all the necessary reagents (as mentioned above) were introduced, the volume was adjusted to 10 ml and the luminescence spectra were recorded. The niflumic acid content was calculated by the additive method.

4. Conclusions

The highly sensitive method for determination of niflumic acid in biological fluid (urine) using the luminescence sensitization of Tb^{3+} ion has been developed. The influence of the main components of urine (urea, phosphates, oxalates, sulphates, chlorides and sodium ions) on the luminescence intensity of terbium ion was studied. The limit of detection of NFA is 1×10^{-3} μg ml^{-1} in urine. The proposed chemical system could also been exploited as a post-column detection system for the HPLC determination of this drug.

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