

# Fluorimetric determination of pipemidinic acid using the europium chelate <sup>☆</sup>

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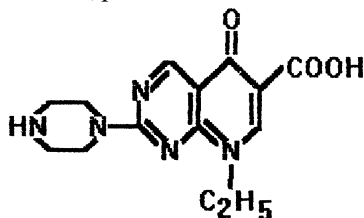
## Abstract

The sensitized luminescence of europium ion in the complexes with pipemidinic acid is investigated. It was shown that in the result of intramolecular energy transfer from ligand to lanthanide ion the luminescence intensity of the latter increases by  $10^{10}$  times. The luminescence properties of the complex were studied and the high sensitive luminescence method for the determination of pipemidinic acid has been developed. © 1999 Elsevier Science B.V. All rights reserved.

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## 1. Introduction

Pipemidinic acid (2-piperazine-5-oxo-8-ethyl-5,8-dihydropyrido(2,3-d)piri-midine-6-carboxylic acid) contains a structural  $\alpha$ -ketocarboxylic fragment that, as in the cases of nalidixic acid and fluoroquinolones, provided its antibacterial activity.



It is extensively used for the treatment systematic infections causing the necessity of its determination in various biological fluids (blood, urine, etc.).

To determine qualitatively pipemidinic acid in biological fluids the spectrofluorimetric [1] and chromatographic [2] methods have been proposed. The revealing minimum was 1 and 5  $\mu\text{g/ml}$  respectively.

Previously we have demonstrated that for determination of a number of drugs (nalidixic acid and catecholamines) the luminescence sensitization of lanthanide ions taking place in their presence can be used [3,4]. The determination of theophylline [5] and ciprofloxacin [6] by sensitized luminescence of europium ion was also described.

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The purpose of this work was the study of optimum conditions of europium ion luminescence in complexes with pipemidinic acid and development of the methods for its determination in substance and biological fluids (urine).

## 2. Experimental

### 2.1. Apparatus

The luminescence measurements were obtained on an SDL-2 spectrophotometer (Leningrad Opto-mechanical 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

The europium chloride solution with concentration of 1 mg/ml that was prepared by dissolving the europium 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 was determined by complexometric titration with Arsenazo I as the indicator. The solution of pipemidinic acid was obtained by dissolution of accurately weighed preparation in

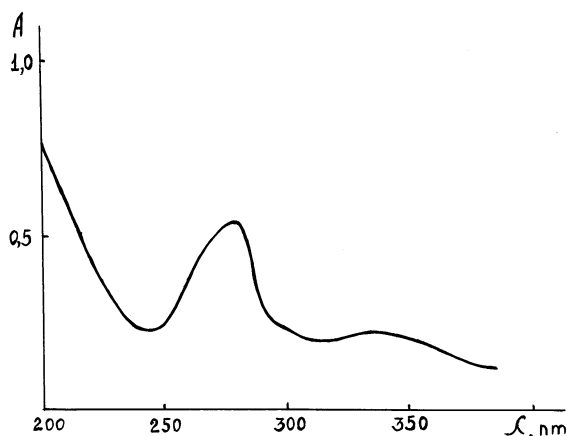


Fig. 1. Absorption spectra of pipemidinic acid ethanolic solution ( $C = 1 \times 10^{-5}$  M,  $l = 0.5$  cm).

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 Eu(III) luminescence in complex with pipemidinic acid the assays were prepared as following: the europium chloride solution was added to the solution of pipemidinic acid, adjusted required pH value, added the ethanol to the volume of 10 ml and recorded the luminescence intensity of solutions at 612 nm.

The content of pipemidinic acid was calculated by the method of additives using the formula:  $C_x = CI_x / (I_{x+ad} - I_x)$ , where  $I_x$  and  $I_{x+ad}$  are the luminescence intensity of the assay and assay with additive, and  $C$  the content of additive.

The triplet levels of the ligand were calculated from its phosphorescence spectra at 77 K.

## 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 275 nm is 90 000 giving the possibility for effective absorption of light energy. The energy of triplet level of a reagent is equal to  $22\,350\text{ cm}^{-1}$ . One can be supposed that the excitation energy transfer to the energetic level  $^5D_2$  ( $21\,500\text{ cm}^{-1}$ ) of lanthanide following the non-radiative deactivation to the first excited state  $^5D_0$  ( $17\,300\text{ cm}^{-1}$ ) takes place in the compound of Eu(III) with mentioned ligand. The band at 612 nm ( $^5D_0 \rightarrow ^7F_2$  transition) is the most intensive (Fig. 2).

### 3.2. Influence of pH

The complexation of Eu(III) with the ligand occurs in a wide range of pH values 3–9 (Fig. 3). The most luminescence intensity ( $I_{lum}$ ) of the com-

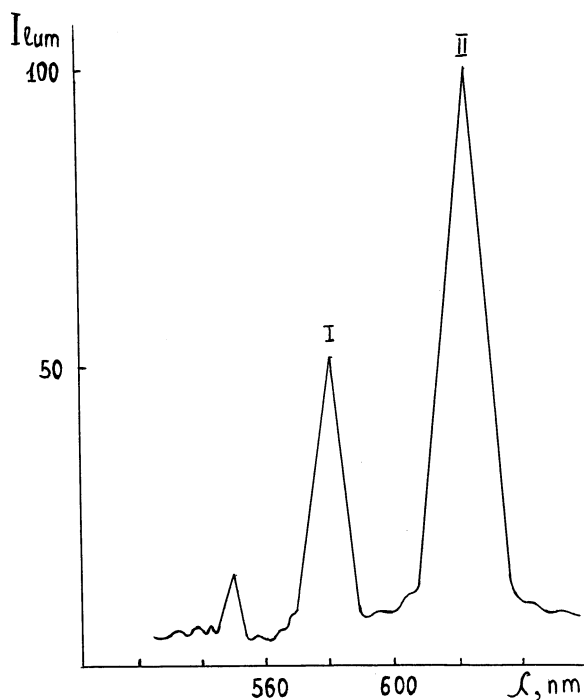


Fig. 2. Luminescence spectrum of Eu(III) in the complex with pipemidinic acid. Transitions: I,  ${}^5D_0 \rightarrow {}^7F_1$ ;  ${}^5D_0 \rightarrow {}^7F_2$ ;  $C_{Eu} = 1 \times 10^{-5}$  M

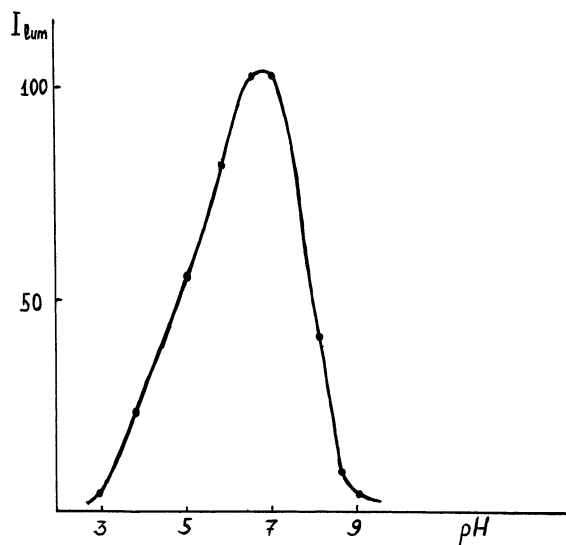


Fig. 3. Dependence of  $I_{lum}$  of Eu(III) in the complex with pipemidinic acid on pH of solution ( $C_{Eu} = 1 \times 10^{-5}$  M).

plex is observed at pH 7.0–7.5. Upon the lesser pH values (in acid solutions) 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 europium hydroxide is observed.

### 3.3. Influence of the solvent

The  $I_{lum}$  depends on the solvent nature presented in solution. The most  $I_{lum}$  is typical for the aqueous ethanolic solutions (Table 1). The acetone decreases the  $I_{lum}$  by 40% and dimethylformamide and dimethylsulphoxide that are the strong complexing agents, can participate in competitive interaction with the lanthanide ion, crowing out the molecules of pipemidinic acid from the inner coordination sphere of the complex, reduce the luminescence intensity in the larger degree.

Since the most  $I_{lum}$  of europium is observed in aqueous ethanolic solutions, the dependence of  $I_{lum}$  of Eu in complex on amount of ethanol was studied (Fig. 4). The maximal  $I_{lum}$  is achieved at 95% content of ethanol in solution.

One can be supposed that at large amounts of ethanol  $I_{lum}$  will be increase, however, we introduced the ethanolic solutions of pipemidinic acid, aqueous solutions of europium chloride and urothropine the total volume of which was 5% (v), therefore, to obtain the solutions with larger content of ethanol was impossible.

$I_{lum}$  of Eu(III) in the complex with pipemidinic acid depends on acid concentration in solution allowing to use this property for determination of

Table 1  
Dependence of  $I_{lum}$  of europium(III) in complex with pipemidinic acid on kind of the solvent

Solvent, 50%	$I_{lum}$ , %
Water	3.5
Methanol	4.0
Ethanol	100.0
Isopropanol	80.0
Acetone	60.0
Dimethylsulfoxide	26.0
Dimethylformamide	38.0

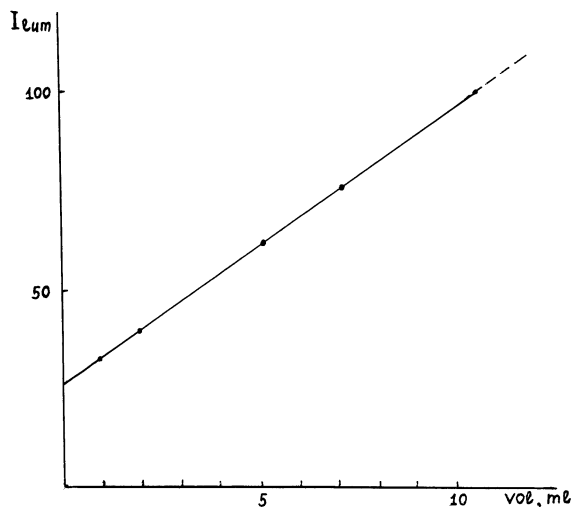


Fig. 4. Dependence of  $I_{lum}$  of Eu(III) in the complex with pipemidinic acid on amount of ethanol ( $C_{Eu} = 1 \times 10^{-5}$  M).

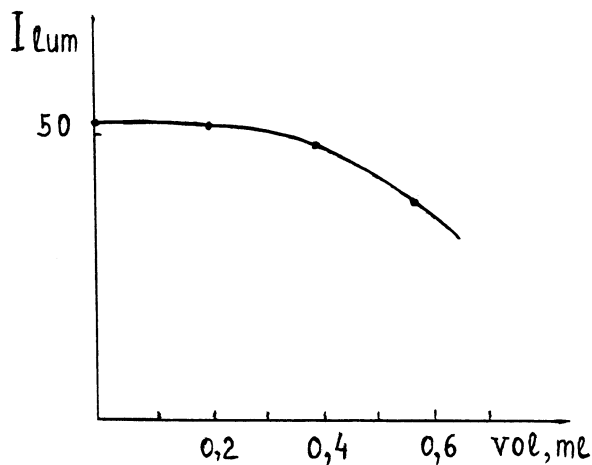


Fig. 5. Influence of the urea on  $I_{lum}$  of Eu(III) in the complex with pipemidinic acid.  $C_{urea} = 25$  mg/ml.

allowing to use this property for determination of the latter. A linear dependence between  $I_{lum}$  of Eu(III) and content of pipemidinic acid in solution is maintained in a range of concentrations  $2.5 \times 10^{-3}$ – $2.5$   $\mu\text{g/ml}$ . The calibration plot crosses the origin of coordinates and its slope is  $45^\circ$ . The low limit of determined contents found by  $3\sigma$ -criterion is  $2.0 \times 10^{-3}$   $\mu\text{g/ml}$  for the substance and  $0.1$   $\mu\text{g/ml}$  for the urine solutions.

### 3.4. Influence of various components of biological fluid

To examine the possibility of determination of pipemidinic acid in urine, the influence of urea, sodium chloride, sulphates, phosphates and oxalates that are the main urine components, on  $I_{lum}$  of europium in the complex with pipemidinic acid. As can be seen from Fig. 5, the introduction into solution 0.1-g urea virtually does not reduce the  $I_{lum}$  of europium ion. The presence in solution 0.033 g of NaCl slightly (by 10%) increases the luminescence intensity, at the larger concentration of NaCl in solution the  $I_{lum}$  of Eu(III) ion is reduced by 20% (Fig. 6). The oxalates also insignificantly decrease (by 20%) the  $I_{lum}$  of Eu(III) ion upon their content in solution 0.6 mg (Fig. 7(I)). The phosphates significantly quench the luminescence. The presence in solution 0.03 g of  $\text{PO}_4^{3-}$  ion virtually completely quench the luminescence of Eu(III) (Fig. 7(II)). The  $\text{SO}_4^{2-}$  ions at amounts of 28.8 mg increase the  $I_{lum}$  by the order value, at its larger contents in the solution  $I_{lum}$  is slightly reduced (Fig. 8).

### 3.5. Analytical performance

Thus, the conducted study demonstrated the possibility for determination of pipemidinic acid in urine. Upon the oral dose of 200 mg of pipemi-

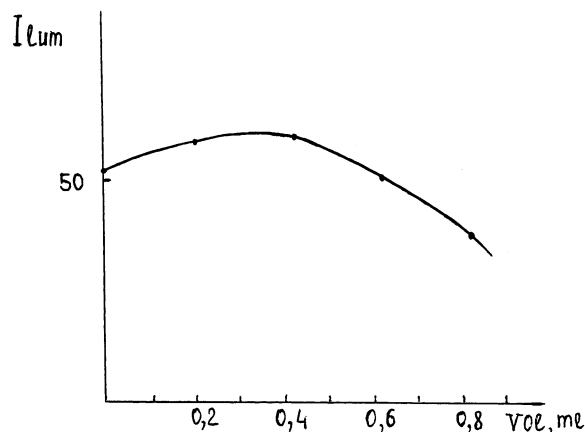


Fig. 6. Influence of NaCl on  $I_{lum}$  of Eu(III) in the complex with pipemidinic acid.  $C_{NaCl} = 116$  mg/ml.

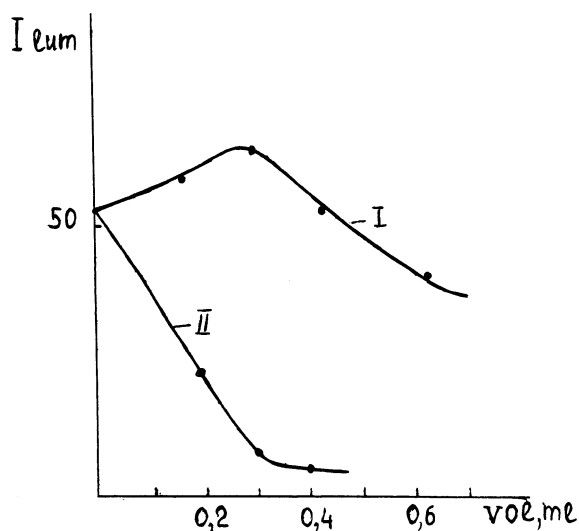


Fig. 7. Influence of oxalates (I) and phosphates (II) NaCl on  $I_{lum}$  of Eu(III) in the complex with pipemidinic acid.  $C_1 = 1$  mg/ml;  $C_{II} = 100$  mg/ml.

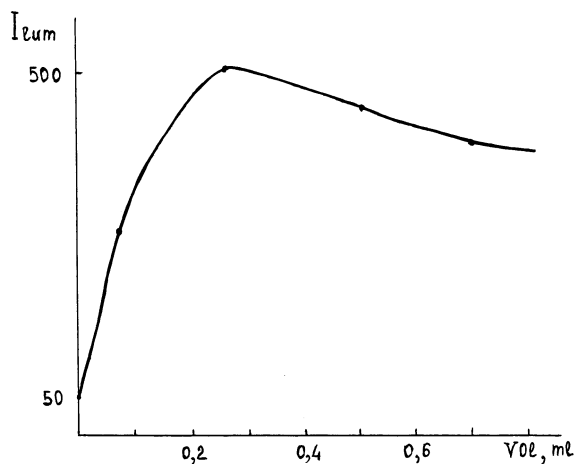


Fig. 8. Influence of sulfate ion on  $I_{lum}$  of Eu(III) in the complex with pipemidinic acid.  $C_{sulfate} = 96$  mg/ml.

dinic acid by healthy volunteers, after 4 h its concentration in the urine was 48 mg. The determination was performed as following. 0.5 ml of urine was taken away and 8 ml of ethanol, 1 ml of

europium chloride solution with concentration of 1 mg/ml, 0.1 ml of 40% solution of urothropine were added and the volume of solution was adjusted to 10 ml. The solution was stirred and  $I_{lum}$  of europium ion was recorded at 612 nm. The content of pipemidinic acid was calculated by the method of additives. At  $n = 5$  and  $P = 0.95$  the value of RSD was 4.3%.

Accuracy and precision of the method were checked by the 'added-found' procedure and the static treatment of data of pipemidinic acid determination in the substance (Table 2).

The proposed method is a simpler and more sensitive compared with known chromatographic one [2]. Developed by us method is more specific and exceeds in sensitivity by two order of value compared with spectrofluorimetric method [1] based on registration of own luminescence of pipemidinic acid enhanced by aluminium.

#### 4. Conclusions

The highly sensitive method for determination of pipemidinic acid (PA) in substance and biological fluids (urine) using the luminescence sensitization of Eu(III) ion has been developed. The influence of the main components of urine (urea, phosphates, oxalates, sulfates, chlorides and sodium ions) on the luminescence intensity of europium ion was studied. The low limit of determining contents of PA is  $2 \times 10^{-3}$   $\mu\text{g/ml}$  in substance and 0.1  $\mu\text{g/ml}$  in urine.

Table 2

Results of the determination of pipemidinic acid substance in model solutions ( $\mu\text{g/ml}$ ) ( $n = 5$ ;  $P = 0.95$ )

Added	Found	$S_r$
0.005	0.0051	0.066
0.010	0.0105	0.060
0.020	0.0201	0.013
0.040	0.0401	0.009

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