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Solid-Phase Luminescence Determination of Ciprofloxacin and Norfloxacin in Biological Fluids

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A simple, rapid, and sensitive luminescence test method for the determination of ciprofloxacin (CIP) and norfloxacin (NOR) has been described. The method is based on the intramolecular energy transfer from organic acid to terbium (Tb^{3+}) ion. Luminescence of terbium (III) complex with CIP (NOR), sorbed on the zeolite has been studied. Under optimized conditions the detection limit is 1 μg/mL in urine and human plasma.

KEY WORDS: Sensitized luminescence; ciprofloxacin; norfloxacin; zeolite; terbium (III) ion; drug analysis.

INTRODUCTION

The test methods of solid-phase spectroscopy provided the registration of analytical signal directly on the solid matrix are extensively used in analysis [1]. The test methods are rapid, easy, and inexpensive, can be performed without difficulties, and do not require unavailable devices and reagents. Zeolites with ion-exchanging properties are widely used as solid phase [2].

The fluorocontaining quinoline carbonic acids, the group to which NOR and CIP belong, play a great role in the treatment of diseases caused by various bacteria. The antibacterial activity of 6-fluoro substituted 7-piperazine-1-yl-quinolones is considerable higher than that of their non-fluorinated analogues. NOR (1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carbonic acid) and CIP (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carbonic acid), as well as other fluoroquinolones, possess wide antibacterial amplitude of action and are effective against microorganisms that are unresponsive to most antibiotics and sulfanilamides.

The analytical application of lanthanide-sensitized luminescence has great interest. The main advantages of lanthanide chelates in fluorescence spectrometry include large Stokes' shifts, narrow emission bands, and a long fluorescence lifetime [3]. The strong ion emission of these complexes is the result of the intramolecular energy transfer process from the ligand to the lanthanide ion.

Previously, authors have demonstrated that for the determination of these drugs in solutions the luminescence sensitization of Tb^{3+} ion taking place in their presence can be used [4–6].

This work is aimed at detailed study of the sensitization of Tb^{3+} ion luminescence by CIP and NOR on the zeolite of CaA-type and development of a simple and rapid test method for their determination in urine and human plasma samples.

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EXPERIMENTAL

Apparatus

The luminescence measurements were obtained on an SDL-2 spectrofluorimeter (Leningrad Opto-mechanical Association, St. Petersburg, Russia). A 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).

Reagents

CIP and NOR were kindly donated by a 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 a concentration of 1×10^{-1}M 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 was determined by complexometric titration with Arsenazo I as the indicator. The stock solutions of CIP and NOR (1mg/ml; 0,1 mol/l) were obtained by dissolution of accurately weighed preparation in ethanol. The pH of solutions was maintained at 6–7 with a 40% aqueous solution of urothropine. The zeolyte of CaA mark was preliminary sited through the sieve with the aperture diameter of 0,1 mm. The zeolite fraction with a particle size less than 0,1 mm was washed with hydrochloric acid for 2 h, then the stirred, washed with distilled water to neutral reaction, and dried at a temperature of 100°C for 2 h.

Methods

For optimal conditions of the sorbates luminescence, the assays were prepared as follows. The preliminary sorbtion of Tb^{3+} ions was performed on the zeolite. To obtain the complex compound, the zeolite was treated with CIP (NOR) diluted stock solutions at an appropriate pH value. Then the zeolite was filtered off, washed with aqueous-ethanolic mixture (1:1), and dried at a temperature of 80°C for 2 h. Luminescence of sorbates was recorded at λ_{em} = 545 nm, λ_{ex} = 365 n*M*. The sorbtion was performed in static conditions.

RESULTS AND DISCUSSION

Influence of the Sorption Time and Terbium Concentration

The luminescence intensity (I_{lum}) of the sorbates depends on the sorption time and concentration of Tb (III) ion in sorbates. The Tb^{3+} were introduced into the cavities of zeolite through the ionic exchange.

The sorption time of Tb (III) ion was studies as follows: 0.5 ml of 0.1 mol/L solution of Tb (III) chloride was introduced into the glass; the pH of solution was adjusted to 2–3, 60 mg of zeolite was added, the solution was diluted with water, and the solution was stirred for different times (30 min, 1, 2, 3, 4, 5 and 7 h). Then the zeolite was filtered out, washed with aqueous-ethanolic mixture (1:1), and dried. Dried zeolite was treated by CIP (NOR) solution, filtered again, washed, and dried. The luminescence intensity in sorbates is presented in Fig. 1. As can be seen, the optimum sorption time of Tb^{3+} from solution is 5 h. The luminescence intensity in sorbates depends on amount of Tb^{3+} (III) ions introduced into the zeolite cavities. The highest I_{lum} in sorbates is observed at the concentration of Tb^{3+} (III) 0.1 mol/L.

Influence of pH

The dependence of I_{lum} of Tb (III) in sorbate on the pH of aqueous phase from which the sorption of complex was performed was studied. It was shown that the I_{lum} of Tb (III) is observed in a range of pH 3–10, with a maximum pH of 6 (CIP) and 7 (NOR), corresponding to the pH value for optimum complex formation in solution. The 40% aqueous solution of urothropine at a volume of 0,2 ml was found to be suitable for the measurements.

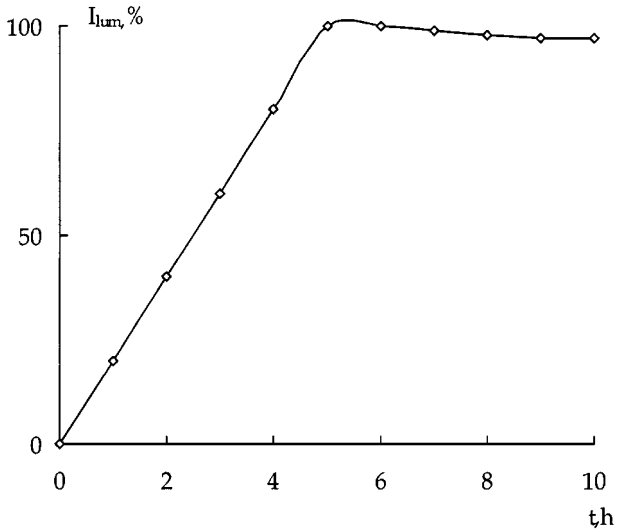

Fig. 1. Dependence of I_{lum} of Tb (III) in sorbates of the complex on sorption time of Tb ions on zeolite. $C_{Tb} = 1 \times 10^{-2}$ mol/L; $C_{CIP} = 1.5 \times 10^{-3}$ mol/L.

Influence of the Ligand Sorption Time

It was established that 15 min of stirring the zeolite with CIP (NOR) solution is enough to obtain the highest I_{lum} of Tb (III) in sorbates (Fig. 2).

Influence of Solvents

It was established that increase the I_{lum} of Tb (III) in sorbate (approximately by 5 times) was observed when the sorbate on zeolite was prepared from dimethyl sulfoxide (DMSO) solution. It is evident that the quenching action of OH-bonds of water molecules residing in the zeolite cavities was eliminated in the presence of the solvents.

The thermic stability is important parameter for these sorbates. Therefore, the Tb sorbates were under the temperature action for 2 h at 50°C, 100°C, and 200°C. It was found that the I_{lum} of sorbates was constant upon the heating to 50°C and 100°C, at 200°C I_{lum} reduced by 50%.

Analysis of Model Urine and Human Plasma Samples

Under optimized sorption conditions, the analyte final concentration and relative luminescence intensity were linear over the range of 5–100 μg/ml. The limit of detection, defined as the concentration corresponding to a signal equal to three times the S.D. of the blank, was 1 μg/ml.

The accuracy and precision of the test method were checked on the model urine and human plasma samples at three different concentrations by the «added-found» procedure, as well as by means of the statistical treatment of determination data (Table I). With this purpose, 0.5 ml of the model urine (human plasma) samples spiked with CIP (NOR) to be analyzed was dissolved in 5 ml of DMSO. 0.2 ml of 40% solution of urothropine was added to reach the optimal pH value, then this mixture was sorbed on the zeolite modified by Tb ions, and sorption in optimal conditions was performed. For preparation of the scale of etalonic samples the same procedure was performed and the calibration carve was drawn. I_{lum} was recorded at 545 nm. The content of CIP (NOR) was found from calibration curve. The relative standard deviation (RSD) was 3.8–6.5% (n = 5; P = 0.95).

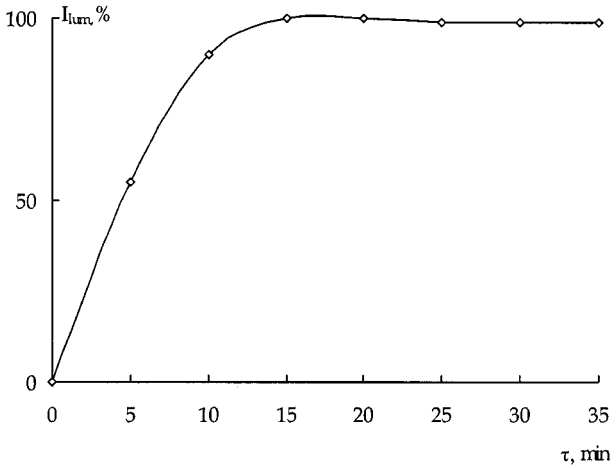

Fig. 2. Dependence of I_{lum} of Tb (III) in sorbate of complex on the sorption time of ligands. $C_{Tb} = 1 \times 10^{-2}$ mol/L; $C_{CIP} = 1.5 \times 10^{-3}$ mol/L.

Table I. Results of the Determination of CIP and NOR in Model Urine and Human Plasma Samples ($p = 0{,}95$; $n = 5$).

	Urine			Human plasma		
Analyte	Addad (μg/ml)	Found (μg/ml)	RSD (%)	Addad (μg/ml)	Found (μg/ml)	RSD (%)
CIP	5,0	5,1	6,5	20,0	19,9	6,1
	10,0	9,9	5,3	30,0	29,8	5,7
	50,0	50,1	4,8	50,0	49,9	4,5
NOR	5,0	4,9	5,9	20,0	19,8	6,2
	10,0	9,9	4,2	30,0	29,9	5,3
	50,0	49,9	3,8	50,0	50,0	3,9

CONCLUSIONS

The luminescence properties of Tb (III) sorbates with CIP (NOR) on zeolite of CaA type were studied. It was shown that the luminescence intensity of sorbates depends on the sorption time of Tb (III) ion and its complex with the above mentioned ligands on the zeolite, on Tb (III) ion concentrations, the acidity of the solution and the nature of solvents. Detection limits (1 μg/ml) obtained were higher approximately in order of value, compared to those described previously (0,13–0,3 μg/ml) in works (4–6). However, our methods can be used for the determination of the investigated drugs in biological fluids directly, without preliminary isolation of protein, which simplifies analysis.

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