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Highly Sensitive Eu^{3+} Doped in Sol-Gel Matrix Optical Sensor for The Assessment of Ciprofloxacin in Different Real Samples

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THE EFFICIENCY of the excited-state interaction between Eu^{3+} doped in sol- gel matrix and the industrial product ciprofloxacin of (CFX) has been studied in different solvents and pHs. A high luminescence intensity peak at 617 nm of europium- ciprofloxacin complex at λ_{ex}=365 nm in acetonitrile was obtained. The photophysical properties of the red emissive Eu^{3+} complex doped in sol-gel matrix have been elucidated, the europium was used as optical sensor for the assessment of ciprofloxacin in the pharmaceutical tablets and serum samples at pH 8.0 and λ_{ex} = 365 nm with a concentration range of 5.0×10^{-9} - 1.0×10^{-6} mol L^{-1} for ciprofloxacin, correlation coefficient of 0.987 and detection limit of 1.65×10^{-9} mol L^{-1}.

Keywords: Ciprofloxacin; Europium (III); Enhancing; Luminescence; Optical sensor; Sol-Gel.

Introduction

Ciprofloxacin, (CFX) (Fig. 1) 1-Cyclopro Eu-(CFX) doped in sol gel matrixl-7-(4-ethyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-3-quinolone carboxylic acid and fluoroquinolones are synthetic antibiotics whose action is based on their anti-DNA activity.

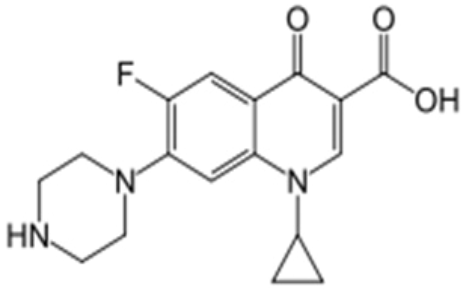

Fig. 1. Structure of Ciprofloxacin

Ciprofloxacin is an antibiotic that is useful against a wide variety of infections in human. Since Nalidixic acid was discovered [1], a number of structure modifications to the quinolone nucleus has been performed to increase antimicrobial activity and to enhance the pharmacokinetic performance of this drug. Fluoroquinolones are quinolones with a fluorine atom at the position 6 of the quinolone Naphthyridine or Benzoaxazine ring systems, and belong to the second generation of quinolones. They are characterized by their greater effectiveness against bacterial activity [2], and are used in both human and veterinary medicine. In humans, they are used to treat an extensive range of diseases, including Urinary, Respiratory and Gastrointestinal tract infections [3]. The analysis of Ciprofloxacin has traditionally been performed using microbiological methods. However, this technique is time-consuming and offers poor precision and specificity. Other non-routine techniques, such as terbium (III)-sensitized luminescence [4], capillary electrophoresis [5] or immune-affinity chromatography [6], have also been applied. Last generation LC-MS-(MS) equipments have also been used [7,8], although this equipment is very expensive and only a few laboratories can afford such instrumentation. High performance liquid chromatography (HPLC) has become an important tool for the analysis of

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single and various combinations of Ciprofloxacin in biological fluids, foods, environmental samples and pharmaceutical preparations using either UV or fluorescence as the detection method [9, 10].In this work, ciprofloxacin(CFX) concentration was determined by the complexation between (CFX) as a ligand and the Eu^{3+} ion and the possibility of the enhancement of the Eu^{3+} luminescence sensitized by (CFX) was established and investigated. The absorption and emission spectra of (CFX) and (CFX)−Eu^{3+} complex were measured in acetonitrile at pH 6.0. This method is simple, accurate and can successfully be applied to the determination of (CFX) in pharmaceutical preparation and in serum samples with remarkably satisfactory results.

Experimental

Materials

Pure standard Ciprofloxacin supplied by the National Organization for Drug Control and Research (Giza, Egypt). Pharmaceutical preparation of ciprofloxacin tablet (Ciprofar) 500 mg produced by Bayer Pharmaceutical Co., USA.

Reagents

All chemicals used are of analytical grade and pure solvents were purchased from Aldrich. A stock solution of ciprofloxacin (CFX) (1.0×10^{-3} mol L^{-1}) was freshly prepared by dissolving 0.093 g in 25 ml pure Ethanol. More diluted solution (2.0×10^{-4} mol L^{-1}) was prepared by appropriate dilution with acetonitrile. Stock and working solutions are stored at 4ºC when are not in use.

A stock solution of Eu^{3+} ion (1.0×10^{-2} mol L^{-1}) was prepared by dissolving 0.0109 g $Eu(NO_3)_3.5H_2O$ (delivered from Aldrich- 99.99%) in small amount of ethanol in 25 ml measuring flask, then dilute to the mark with ethanol. The working solution of Eu^{3+} ion of 1.0×10^{-4}mol L^{-1} was obtained by appropriate dilution with acetonitrile. The pH = 6.0 was adjusted by using 0.7 ml of 0.1 mol L^{-1} of 0.1 molL^{-1} NH_4OH/HCl solution.

Apparatus

All luminescence measurements were recorded with a Meslo- PN (222-263000) z Thermo Scientific Lumina fluorescence Spectrometer in the range of (190 – 900 nm). The optical absorption of the samples was measured in the range of 220 –750 nm with Thremo UV-Visible double-beam spectrophotometer. The pH was measured with pHs-JAN-WAY 3330 research pH meter. The separation of protein from samples was carried out by centrifuging of sample for 15 min at 3000 rpm.

Synthesis of Eu- (CFX) complex-Doped in sol gel

i. The sol matrix was prepared according to earlier reported work [11-33] as follow: A mixture consisting of tetraethoxysilane (TEOS), ethanol and water in 1: 5:1 molar ratio was stirred for 15 min.

ii. 0.11 g of the prepared complex (Eu^{3+}: CFX, 1:2 molar ratio) dissolved in ethanol is added to the sol solution and refluxed for 1 hour to give the precursor sol solution in the presence of few drops of 0.1 mol/L HCl solution as catalyst.

iii. Finally, The developed complex-dispersed sol solution was casted into polystyrene cup with diameters (2 cm, 0.8 cm, 0.8 cm) and kept at 25 ºC in air for 2 weeks. The produced cast was heated at 100-150 ºC for 24 hours to give solidified and transparent composite sample.

General procedure

One strip (0.8 cm x 0.8 cm x 2.0 cm) of Eu-(CFX) complex-Doped in sol gel in a molar ratio of 0.3 mL of $1x10^{-2}$ mol L^{-1} (CFX) solution and 0.1 mL of $1.0 x10^{-2}$ mol L^{-1} Eu^{3+} solution to give $3.0 x10^{-4}$ mol L^{-1} of (CFX) and $1.0 x10^{-4}$ mol L^{-1} of Eu^{3+} was placed in the 1 cm cell of the spectrofluorometer, then 2 mL of acetonitrile was added. The above procedure was used for the subsequent measurements of absorption, emission spectra and effect of pH and solvents. The luminescence intensity was measured at $\lambda_{ex}/\lambda_{em}$ =365/617 nm. The calibration curve was setted up by measuring the luminescence intensity of one strip (0.8 cm x 0.8 cm x 2.0 cm) of $1.0 x 10^{-4}$ mol L^{-1} of Eu^{3+} doped in sol gel in 1 cm cell of the spectrofluorometer, then 2.0 mL of the different concentration of GFX in acetonitile at pH 8.0 was added to the optical sensor Eu^{3+} doped in the sol gel, then The luminescence intensity was measured at $\lambda_{ex}/\lambda_{em}$ =365/617 nm.

Determination of ciprofloxacin in pharmaceutical preparations

Five tablets of pharmaceutical formulation (Ciprofar) were carefully weighed and ground to finely divided powders. Accurate weights equivalent to 1.5 mg was dissolved in 50 mL acetonitrile and mixed well and filtered up using 12 mm filter papers. The concentration of the drug was determined by using different concentrations from the corresponding calibration graph.

Determination of ciprofloxacin in serum solution

3 mL of trichloro-acetic-acid was added to 1.0 mL serum of a real health volunteers and the solution was centrifuged for 15 min at 4000 rpm to remove proteins, then 100 µL of the serum was added to 0.1 mL of Eu^{3+} ion stock solution (1.0×10^{-2} mol L^{-1}) in 10 mL measuring flask and complete to the mark with acetonitrile and the pH was adjusted to 6.0. The luminescence intensity of the test solution was measured before and after addition of Eu^{3+} optical sensor. The change in the luminescence intensity was used for determination of ciprofloxacin in serum sample.

Result and Discussion

Absorption Spectra

The absorption spectra of (CFX) and Eu^{3+}-(CFX) complex doped in sol-gel matrix are shown in Fig. 2. Comparing the spectrum of the (CFX) with its spectrum after the addition of Eu^{3+} ion into (CFX) doped in sol-gel matrix, a blue shift was observed and the absorbance is also enhanced which indicates that (CFX) can form a complex with Eu^{3+} ion.

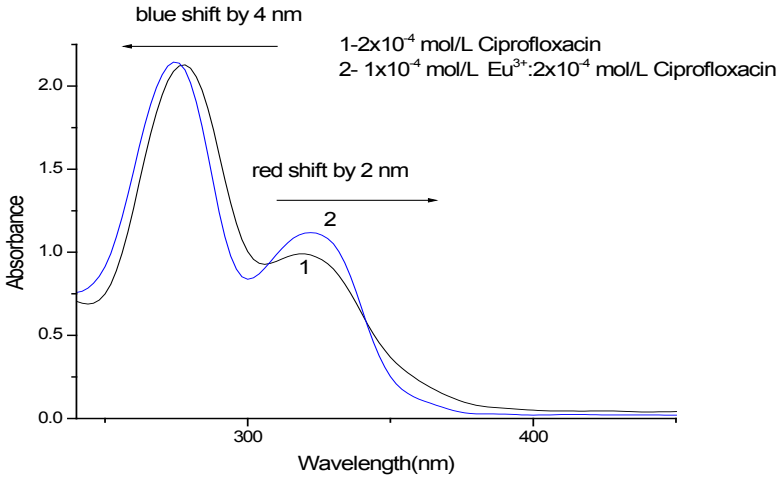

Fig. 2. Absorption spectrum of (1)-2x10-4 mol L-1 ciprofloxacin (2)- 2x10-4 mol L-1 ciprofloxacin with 1x 10-4 mol L-1 Eu3+ doped in sol-gel matrix.

Effect of experimental conditions on the optical properties of (CFX) and Eu^{3+} doped in sol gel matrix

Effect of the amount of (CFX) and Eu^{3+}

The ion titration revealed that the complex formed M : L (1 : 2) for Eu and (CFX), which indicates that the metal may coordinate to the ligand from different coordination sites and not only through oxygen of the ketone ring, but the more preferred coordination sites are the (O) of the ketone group (Fig.3), [33].

Effect of solvent

The influence of the solvent on the luminescence intensities of the sol-gel matrix doped 2.0×10^{-4} mol L^{-1} of (CFX) and 1.0×10^{-4} mol L^{-1} Eu^{3+} was studied under the conditions established above. The results show the enhanced emission of Eu^{3+}-(CFX) doped in sol-gel matrix in acetonitrile. This can be attributed to the formation of anhydrous solvates of Eu^{3+}-(CFX) complex introducing solvent molecules in the first coordination sphere of Eu^{3+}-(CFX) leads to the enhancement of the intensity of all transitions ($^5D_0 \rightarrow {}^7F_1$ =590 nm, $^5D_0 \rightarrow {}^7F_2$ =617 nm, $^5D_0 \rightarrow {}^7F_3$ = 652 nm, $^5D_0 \rightarrow {}^7F_4$=695 nm and $^5D_0 \rightarrow {}^7F_5$ = 705 nm). Especially $^5D_0 \rightarrow {}^7F_2$ transition in Eu^{3+} (Fig.4), [34-36].

By increasing the radiative rate, Eu^{3+} excited states will become less sensitive to deactivation processes, ultimately resulting in a more efficiently emissive Eu^{3+} complex. Also, the luminescence intensities for the complexes in acetonitrile solutions are stronger than in ethanol as hydroxy solvent. This may be due to vibrational energy transfer to the solvent molecules. It is well known that the excited state of the lanthanide ions is efficiently quenched by interactions with high-energy vibrations like O-H groups thereby the luminescence of this complex in –OH containing solvents can be quenched easily because of the

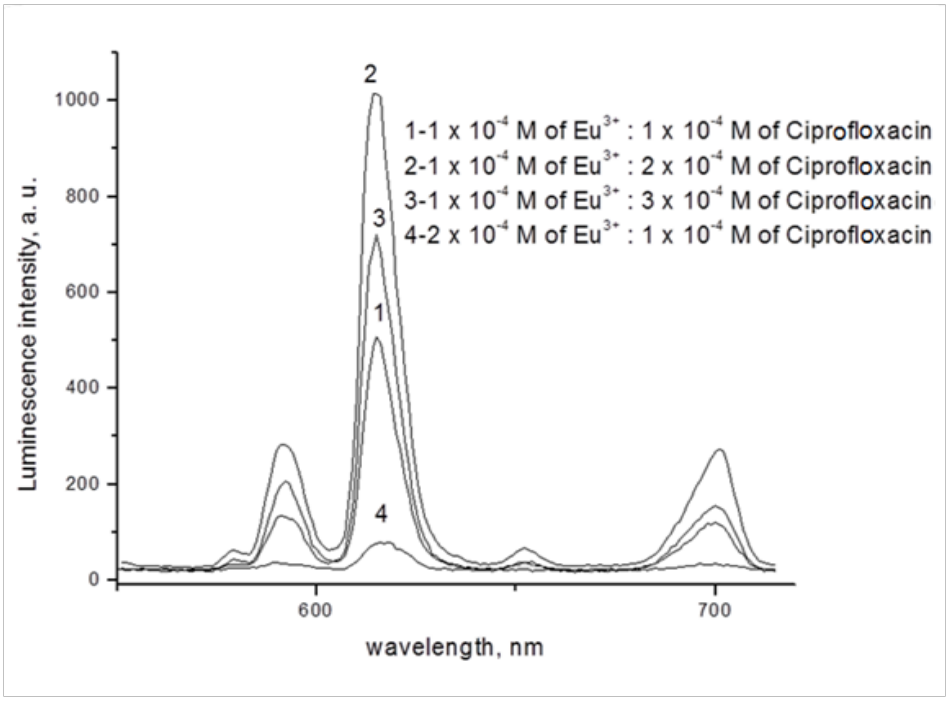

Fig. 3. Molar ratio between Eu^{3+} and ciprofloxacin doped in sol-gel matrix at λ_{ex}=365 nm. Effect of solvent

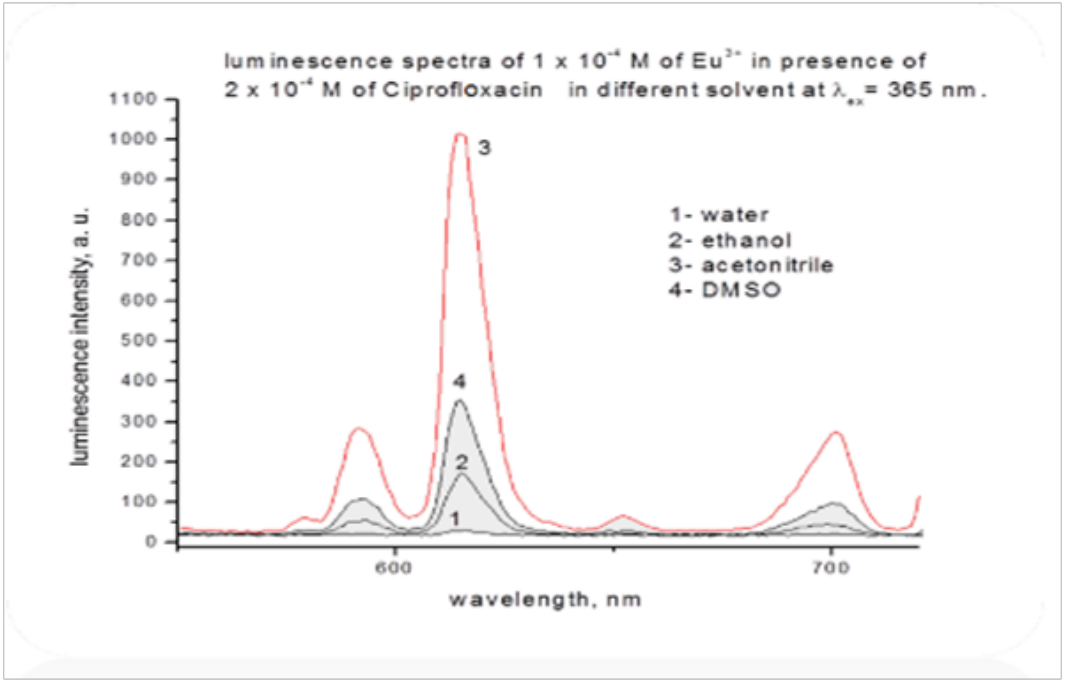

Fig. 4. Luminescence emission spectra of 1x 10^{-4} mol L^{-1} Eu^{3+} in the presence of 2x 10^{-4} mol L^{-1} of ciprofloxacin doped in sol-gel matrix at pH=6.0 in different solvent.

O-H oscillators [33-36].

Effect of pH

The pH of the medium has a great effect on the luminescence intensity of the Eu-(CFX) doped in sol-gel matrix. The pH has been adjusted using NH_4OH and HCl solutions. The optimum pH value where the peak at 617 nm has the highest intensity was obtained at pH = 6.0, Fig. (5).

Emission spectra.

The emission spectra of Eu^{3+} doped in sol-gel matrix in different concentrations of (CFX) in acetonitrile are shown in Fig. (6). After the addition of different concentrations of (CFX) into the Eu^{3+} ion doped in sol-gel matrix, the intensity of the characteristic peak at 617 nm of Eu^{3+} was enhanced indicating that (CFX) can form a complex with Eu^{3+} ion. The characteristic peaks of Eu^{3+} ion appear at ($^5D_0 \rightarrow {}^7F_1$ =590 nm, $^5D_0 \rightarrow {}^7F_2$ =617 nm, $^5D_0 \rightarrow {}^7F_3$= 652 nm, $^5D_0 \rightarrow {}^7F_4$=695 nm and $^5D_0 \rightarrow {}^7F_5$ = 705 nm).

Analytical performance.

Analytical parameter of optical sensor method

A linear correlation was found between luminescence intensity of Eu^{3+} doped in sol-gel matrix at λ_{em} = 617 nm and concentration of (CFX) in the ranges given in Table (1) The six-points (10^5–1.0 n mol L^1) calibration curve was obtained by plotting the peak intensity of Eu^{3+} at λ_{em} = 617 nm versus the concentration of (CFX) and the graph was described by the regression equation:

$$Y = a + bX$$

(where Y = luminescence intensity of the optical sensor at λ_{em} = 617 nm; a = intercept; b = slope and X = concentration in mol L^{-1}). Regression analysis of luminescence intensity data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) and the values were presented in Table (1). The limit of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines [37] using the formulae:

LOD = 3.3 S/b and LOQ = 10 S/b, (where S is the standard deviation of blank luminescence intensity values, and b is the slope of the calibration plot) are also presented in Table (1). The low value of LOD indicates the high sensitivity of the proposed method when compared by other

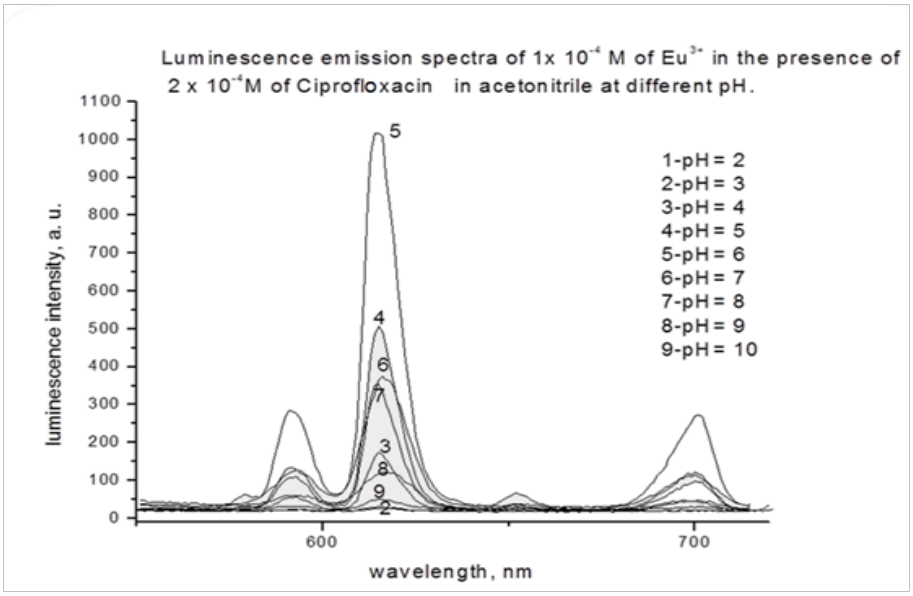

Fig. 5. Luminescence emission spectra of 1x 10-4 mol L-1 Eu3+ in the presence of 2 x 10-4 mol L-1 of ciprofloxacin doped in sol-gel matrix at different pHs.

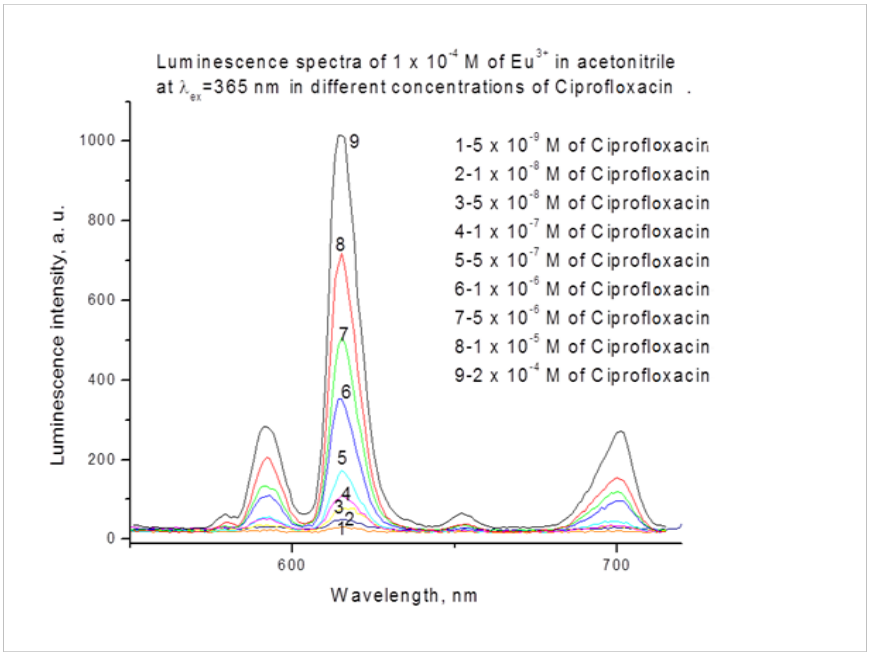

Fig. 6. Luminescence emission spectra of 1x 10-4 mol L-1 Eu3+ doped in sol-gel matrix in the presence of different concentrations of ciprofloxacin in acetonitrile and pH= 6.0.

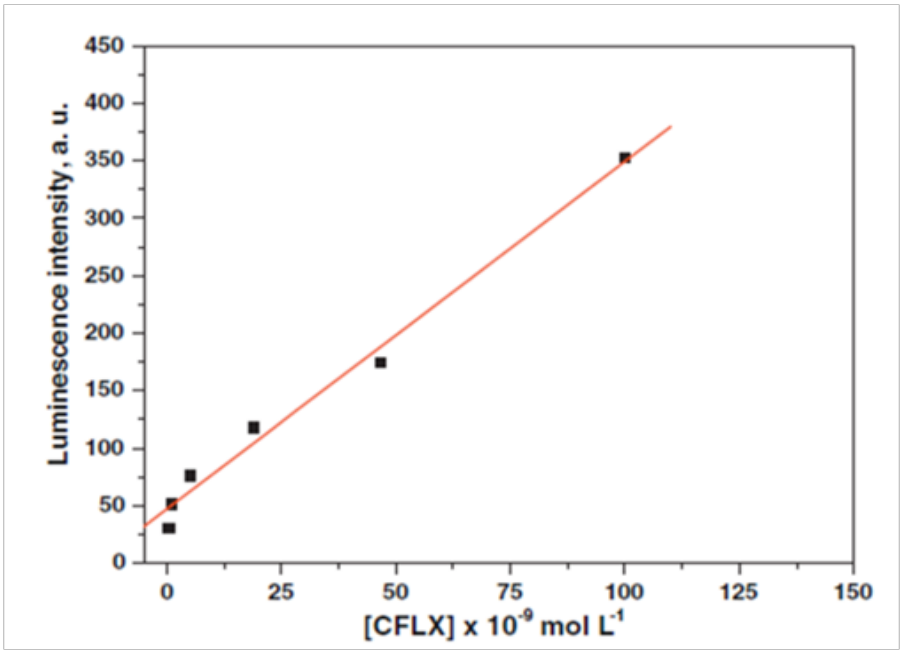

Fig. 7. Linear relationship between luminescence intensity of Eu3+ doped in sol-gel matrix at λem = 617 nm and concentrations of ciprofloxacin.

methods [4-10].

TABLE 1. Sensitivity and regression parameters for photo Eu-(CFX) probe.

Parameter	CFX
λ_{em}, nm	617
Linear rang, mol L^{-1}	5.0 x 10^{-9} to 1.0x10^{-6}
Limit of detection(LOD),molL^{-1}	3.3x 10^{-9}
Limit of quantification(LOQ),molL^{-1}	1.2 x10^{-8}
Intercept(a)	60.4
Slope(b)x10^{9}	4.3
Standard deviation	0.2
Variance(Sa^2)	0.04
Regression Coefficient	0.987

Selectivity

The proposed method was tested for selectivity by placebo blank and synthetic mixture analysis. A placebo blank containing talc (250 mg), starch (300 mg), lactose (30 mg), calcium carbonate (50 mg), calcium dihydrogen orthophosphate (20 mg), methyl cellulose (40 mg), sodium alginate (70 mg) and magnesium stearate (100 mg) was extracted with water and solution made as described under "analysis of dosage forms". A convenient aliquot of solution was subjected to analysis according to the recommended procedures. In the method of analysis, there was no interference by the inactive ingredients.

A separate test was performed by applying the proposed method to the determination of (CFX) in a synthetic mixture. To the placebo blank of similar composition, different amount of (CFX) of pharmaceutical formulation of tablet (Ciprofar) was added, homogenized and the solution of the synthetic mixture was prepared as done under "analysis of dosage forms". The filtrate was collected in a 100-mL flask. 2.5, 5 and 7.5 mL of the resulting solution was assayed (n= 9) by proposed method which yielded a % average recovery of 100.4 ± 1.13, and 98.6 ± 0.5 for tablet and serum samples, respectively (Table 2)

TABLE 2. Evaluation of intra-day and inter-day accuracy and precision of Eu-CFX photo probe.

sample	Actual CFX found * X 10^{-6} mol/L	Intra-day accuracy and precision (n=3)			Inter-day accuracy and precision (n=3)		
		CFX Average Found ±CL	%RE	%RSD	CFX average found*±CL	%RE	%RSD
Ciprofar 500 mg	0.20 0.28 0.40	0.21 ± 0.07 0.29 ± 0.05 0.39 ± 0.09	5.00 3.50 5.00	0.17 0.16 0.12	0.21 ± 0.08 0.29 ± 0.06 0.41 ± 0.09	5.00 3.50 5.00	0. 26 0. 17 0. 13
serum	0.20 0.30 0.50	0.205 ± 0.07 0.32 ± 0.05 0.51 ± 0.09	2.38 4.70 5.20	0.17 0.16 0.12	0.22 ± 0.08 0.30 ± 0.07 0.48 ± 0.09	4.70 4.70 5.20	0. 26 0. 17 0. 14

CL. Confidence limits were calculated from: CL = ± tS/$(n)^{½}$. The tabulated value of t is 4.303, at the 95% confidence level. S = standard deviation = [(average- value 1)^2 + (average- value 2)^2 + (average – value 3)^2]$^{1/2}$. N = number of measurements. %RE. Percent relative error. = [(concentration proposed –concentration known)/(concentration known)]

x 100. %RSD. relative standard deviation.= [S/(average measurement)] x 100 .

The results demonstrated the accuracy as well as the precision of the proposed methods. These results complement the findings of the placebo blank analysis with respect to selectivity.

Application to formulations

The proposed method was applied to the determination of (CFX) in one representative pharmaceutical formulation tablet (Ciprofar) 500 mg was purchased from USA and containing other inactive ingredients and in serum sample of the health volunteers. The results in Table (2) show that the method is successful for the determination of (CFX) and that the excipients in the dosage forms did not interfere. The results obtained Table (2) were statistically compared with the official British Pharmacopoeia [B.P] method [30]. The average recovery and R.S.D for the tablet, serum and urine sample in proposed method were (99.5 ± 2.13, and 102.1 ± 1.5) respectively. Data obtained by B. P method average recovery 99.7% and 99.7 for the tablet and serum samples respectively.

Conclusion

The Eu^{3+} ion doped in sol-gel matrix has high sensitive and characteristic peaks in the presence of (CFX). The proposed method for the determination of (CFX) offers simple, rapid and sensitive method for the analysis of (CFX) in acetonitrile and pH 6.0 with a linear range of 1.0 x 10^{-9} – 5.0 x 10^{-6}mol L^{-1} and detection limit of 3.3 x 10^{-9} mol L^{-1}. The developed optical sensor is selective, accurate and attractive for routine control analysis of the drug.

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مجس ضوئي دقيق من ايون اليوربيم مغموس في وسط السول جيل لتعيين مركب السبرفلوكساسين في عينات حقيقية مختلفة

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تم دراسة تاثير المذبيات المختلفة و الاس الهيدروجيني علي التفاعل بين كفاءة الحالة المثارة لايون اليوربيم المغموس في وسط السول جيل و مادة السبروفلوكساسين. فقد وجد ان كثافة الحزمة الضوئية عند طول موجي ٦١٧ نانومتر و باثارة بواسطة طول موجي ٣٦٥ نانومتر في مذيب الاسيتونيتريل تزداد بكثافة عالية. و بدراسة الخواص الفيزوضوئية لايون اليوربيم المغموس في وسط السول جيل وجد انه يمكن استخدمه كمجس ضوئي فعال لتعيين مركب السبروفلوكساسين في عينات الادوية و الدم المختلفة.