

Highly Sensitive Eu³⁺ Doped in Sol-Gel Matrix Optical Sensor for The Assessment of Ciprofloxacin in Different Real Samples

M. S. Attia*, A. O. Youssef, A. M. Ismael, R. Gaafer, A. Adel, A. Twfik, A. Wafeey, H. G. Afify, A. Sayed

Chemistry Department, Faculty of Science, Ain Shams University, Abbassia, Cairo, Egypt.

THE EFFICIENCY of the excited-state interaction between Eu³⁺ doped in sol-gel matrix and the industrial product ciprofloxacin (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 Eu³⁺ 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-Cyclopropyl-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.

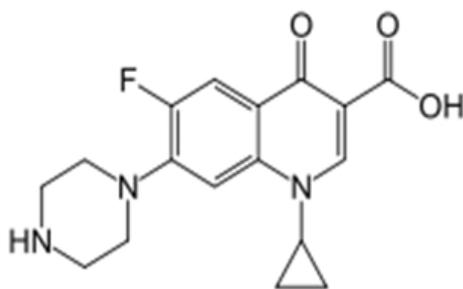


Fig. 1. Structure of Ciprofloxacin

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

*Corresponding author: Mohamed_sam@yahoo.com. Mobile: + 2 0122 98 67 311

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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) ($1.0 \times 10^{-3} \text{ mol L}^{-1}$) was freshly prepared by dissolving 0.093 g in 25 ml pure Ethanol. More diluted solution ($2.0 \times 10^{-4} \text{ mol L}^{-1}$) was prepared by appropriate dilution with acetonitrile. Stock and working solutions are stored at 4°C when are not in use.

A stock solution of Eu^{3+} ion ($1.0 \times 10^{-2} \text{ mol L}^{-1}$) was prepared by dissolving 0.0109 g $\text{Eu}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ (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 $1.0 \times 10^{-4} \text{ mol L}^{-1}$ 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 $0.1 \text{ mol L}^{-1} \text{ NH}_4\text{OH}/\text{HCl}$ 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 Thermo UV-Visible double-beam spectrophotometer. The pH was measured with pHs-JAN-WAY 3330 research pH meter. The separation of protein from samples *Egypt. J. Chem.* **61**, No. 1 (2018)

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°C in air for 2 weeks. The produced cast was heated at $100\text{-}150^\circ\text{C}$ 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 $1 \times 10^{-2} \text{ mol L}^{-1}$ (CFX) solution and 0.1 mL of $1.0 \times 10^{-2} \text{ mol L}^{-1} \text{ Eu}^{3+}$ solution to give $3.0 \times 10^{-4} \text{ mol L}^{-1}$ of (CFX) and $1.0 \times 10^{-4} \text{ mol L}^{-1}$ 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 $\lambda_{\text{ex}}/\lambda_{\text{em}} = 365/617 \text{ nm}$. The calibration curve was set up by measuring the luminescence intensity of one strip (0.8 cm x 0.8 cm x 2.0 cm) of $1.0 \times 10^{-4} \text{ mol L}^{-1}$ 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 acetonitrile at pH 8.0 was added to the optical sensor Eu^{3+} doped in the sol gel, then The luminescence intensity was measured at $\lambda_{\text{ex}}/\lambda_{\text{em}} = 365/617 \text{ nm}$.

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 μ L of the serum was added to 0.1 mL of Eu³⁺ 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 Eu³⁺ 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 Eu³⁺-(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 Eu³⁺ 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 Eu³⁺ ion.

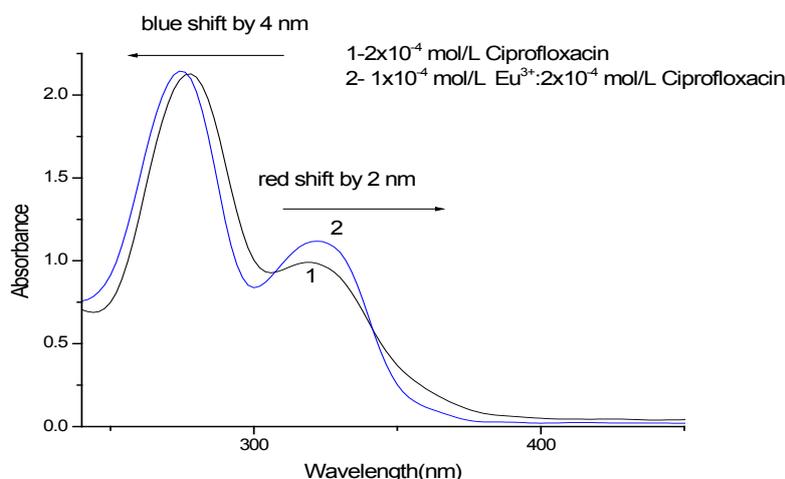


Fig. 2. Absorption spectrum of (1)- 2×10^{-4} mol L⁻¹ ciprofloxacin (2)- 2×10^{-4} mol L⁻¹ ciprofloxacin with 1×10^{-4} mol L⁻¹ Eu³⁺ doped in sol-gel matrix.

*Effect of experimental conditions on the optical properties of (CFX) and Eu³⁺ doped in sol gel matrix**Effect of the amount of (CFX) and Eu³⁺*

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 Eu³⁺ was studied under the conditions established above. The results show the enhanced emission of Eu³⁺-(CFX) doped in sol-gel matrix in acetonitrile. This can be attributed to the formation of anhydrous solvates of Eu³⁺-(CFX) complex introducing solvent molecules in the first coordination sphere of Eu³⁺-(CFX) leads to the enhancement of the intensity of all transitions (⁵D₀ → ⁷F₁ = 590 nm, ⁵D₀ → ⁷F₂ = 617 nm, ⁵D₀ → ⁷F₃ = 652 nm, ⁵D₀ → ⁷F₄ = 695 nm and ⁵D₀ → ⁷F₅ = 705 nm). Especially ⁵D₀ → ⁷F₂ transition in Eu³⁺ (Fig.4), [34-36].

By increasing the radiative rate, Eu³⁺ excited states will become less sensitive to deactivation processes, ultimately resulting in a more efficiently emissive Eu³⁺ 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

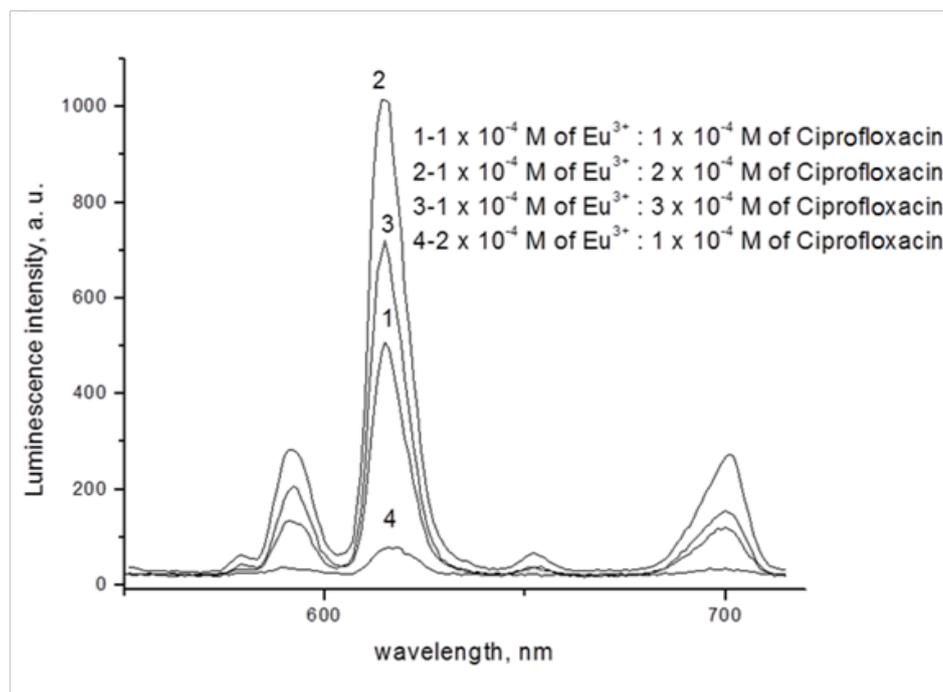


Fig. 3. Molar ratio between Eu^{3+} and ciprofloxacin doped in sol-gel matrix at $\lambda_{\text{ex}}=365$ nm. Effect of solvent

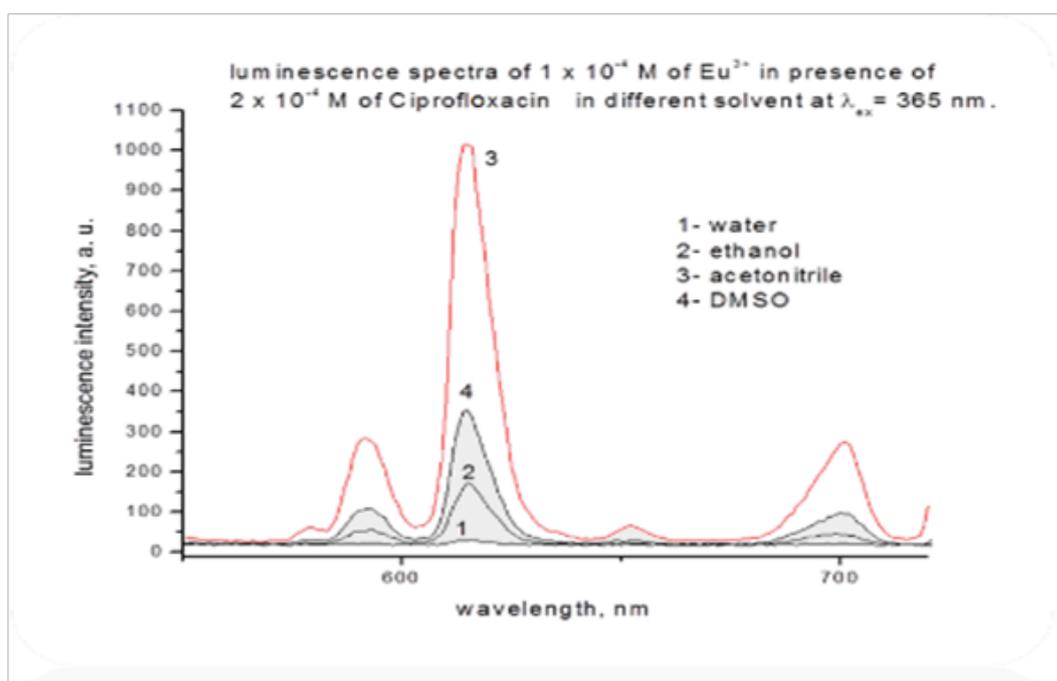


Fig. 4. Luminescence emission spectra of 1×10^{-4} mol L^{-1} Eu^{3+} in the presence of 2×10^{-4} mol L^{-1} of ciprofloxacin doped in sol-gel matrix at pH=6.0 in different solvent.

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 NH₄OH 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 Eu³⁺ 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 Eu³⁺ ion doped in sol-gel matrix, the intensity of the characteristic peak at 617 nm of Eu³⁺ was enhanced indicating that (CFX) can form a complex with Eu³⁺ ion. The characteristic peaks of Eu³⁺ ion appear at (⁵D₀ → ⁷F₁ = 590 nm, ⁵D₀ → ⁷F₂ = 617 nm, ⁵D₀ → ⁷F₃ = 652 nm, ⁵D₀ → ⁷F₄ = 695 nm and ⁵D₀ → ⁷F₅ = 705 nm).

Analytical performance.

Analytical parameter of optical sensor method

A linear correlation was found between luminescence intensity of Eu³⁺ doped in sol-

gel matrix at $\lambda_{em} = 617$ 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 Eu³⁺ at $\lambda_{em} = 617$ 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 $\lambda_{em} = 617$ 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

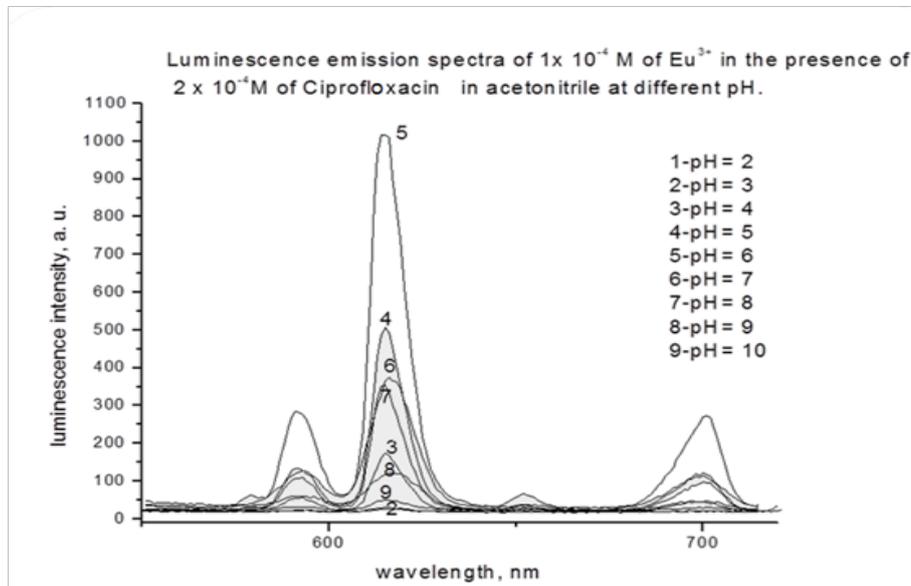


Fig. 5. Luminescence emission spectra of 1×10^{-4} mol L⁻¹ Eu³⁺ in the presence of 2×10^{-4} mol L⁻¹ of ciprofloxacin doped in sol-gel matrix at different pHs.

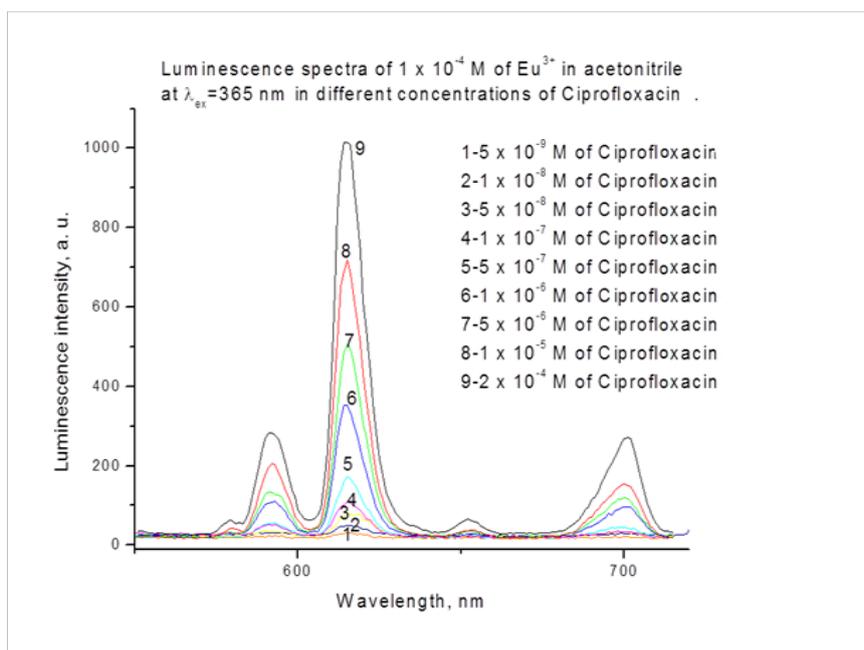


Fig. 6. Luminescence emission spectra of 1×10^{-4} mol L⁻¹ Eu^{3+} doped in sol-gel matrix in the presence of different concentrations of ciprofloxacin in acetonitrile and pH= 6.0.

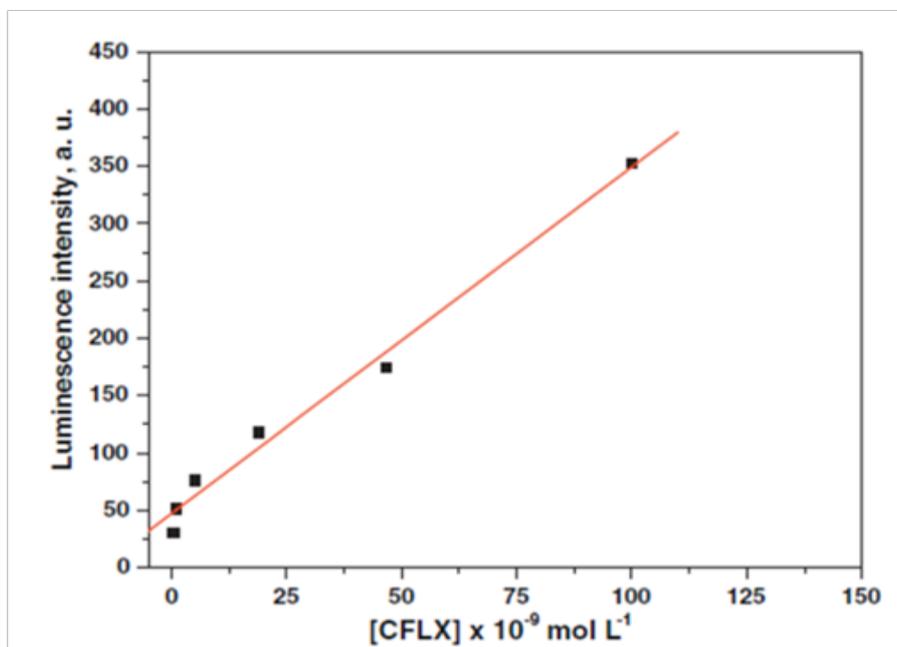


Fig. 7. Linear relationship between luminescence intensity of Eu^{3+} doped in sol-gel matrix at $\lambda_{\text{em}} = 617$ nm and concentrations of ciprofloxacin.

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	0.21 ± 0.07	5.00	0.17	0.21 ± 0.08	5.00	0.26
	0.28	0.29 ± 0.05	3.50	0.16	0.29 ± 0.06	3.50	0.17
	0.40	0.39 ± 0.09	5.00	0.12	0.41 ± 0.09	5.00	0.13
serum	0.20	0.205 ± 0.07	2.38	0.17	0.22 ± 0.08	4.70	0.26
	0.30	0.32 ± 0.05	4.70	0.16	0.30 ± 0.07	4.70	0.17
	0.50	0.51 ± 0.09	5.20	0.12	0.48 ± 0.09	5.20	0.14

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

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 Eu³⁺ 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

محمد سعيد عطيه*، احمد عثمان يوسف، احمد اسماعيل، رامي جعفر، اسماء عادل، احمد توفيق، احمد وفاني، هشام جمال عفيفي، احمد سيد
قسم الكيمياء، كلية العلوم، جامعة عين شمس، العباسية، القاهرة، مصر.

تم دراسة تأثير المذيبات المختلفة و الاس الهيدروجيني علي التفاعل بين كفاءة المثارة لايون اليوربيم المغموس في وسط السول جيل و مادة السيروفلوكساسين. فقد وجد ان كثافة الحزمة الضوئية عند طول موجي 617 نانومتر و باثارة بواسطة طول موجي 365 نانومتر في مذيب الالاسيتونيتريل تزداد بكثافة عالية. و بدراسة الخواص الفيزيوضوئية لايون اليوربيم المغموس في وسط السول جيل وجد انه يمكن استخدامه كمجس ضوئي فعال لتعيين مركب السيروفلوكساسين في عينات الادوية و الدم المختلفة.