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Gatifloxacin Assessment by the Enhancement of the Green Emission of Optical Sensor Tb^{3+} Doped In Sol-Gel Matrix

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THE EFFICIENCY of excited-state interaction between Tb^{3+} doped in sol- gel matrix and the industrial product Gatifloxacin (GFX) has been studied in different solvent and pHs. A high luminescence intensity peak at 545 nm of terbium- Gatifloxacin complex at λ_{ex} = 340 nm in acetonitrile was obtained. The photophysical properties of the green emissive Tb^{3+} complex doped in sol-gel matrix have been elucidated, the terbium was used as optical sensor for the assessment of Gatifloxacin in the pharmaceutical tablets and serum samples at pH 8.0 and λ_{ex} = 340 nm with a concentration range of 5.0×10^{-9} - 1.0×10^{-6} mol L^{-1} for Gatifloxacin, correlation coefficient of 0.99 and detection limit of 1.65×10^{-9} mol L^{-1}.

Keywords: Gatifloxacin, Terbium (III), Enhancing , Luminescence, Optical sensor , Sol-Gel.

Introduction

Gatifloxacin,1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxyli acid sesquihydrate ($C_{19}H_{22}FN_3O_4$ 1.5 H_2O), is a synthetic broad-spectrum fluoroquinolone antibacterial agent with a 3-methylpiperazinyl-side chain at position 7 and a methoxy group at position 8 of the quinolone ring [1] (Fig. 1). It is active against Gram -ve and Gram +ve organisms, including anaerobes and is indicated for the treatment of acute bacterial exacerbation of chronic bronchitis, acute sinusitis, and complicated and uncomplicated urinary tract infections due to *Escherichia coli, Klebsiella pneumoniae*, or *Proteus mirabilis* [2].

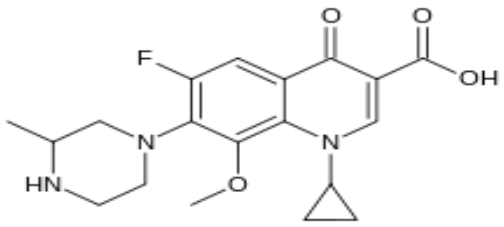

Fig. 1. Structure of Gatifloxacin.

In humans, they are used to treat an extensive range of diseases, including urinary, respiratory and gastrointestinal tract infections [3]. The analysis of Gatifloxacin 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)-sensitised luminescence [4], capillary electrophoresis [5- 7] or immunoaffinity chromatography [8], have also been applied. Last generation LC-MS-(MS) equipment has also been used [9-11], 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 single and various combinations of Gatifloxacin in biological fluids, foods, environmental samples and pharmaceutical preparations using either UV or fluorescence as the detection method [12-29]. In this work, Gatifloxacin concentration was determined by the complexation with the Tb^{3+} ion doped in sol-gel matrix and the possibility of the enhancement of the Tb^{3+} luminescence sensitized by (GFX) was established and investigated.

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Experimental

Materials

Pure standard Gatifloxacin supplied by the National Organization for Drug Control and Research (Giza, Egypt). Pharmaceutical preparation (Tequin) containing 200 mg of Gatifloxacin produced by Bristol-Myers Squibb.

Reagents

All chemicals used are of analytical grade and pure solvents were purchased from Aldrich. A stock solution of (GFX) (1.0 x 10^{-2} mol L^{-1}) was freshly prepared by dissolving 0.093 g in 25 mL pure Ethanol. More diluted solution (3.0 x 10^{-4} mol L^{-1}) was prepared by appropriate dilution with acetonitrile. Stock and working solutions are stored at 20ºC when are not in use.

A Tb^{3+} ion stock solution (1.0 x 10^{-2} mol L^{-1}) was prepared by dissolving 0.109 g $Tb(NO_3)_3$. $5H_2O$ (delivered from Aldrich- 99.99%) in small amount of ethanol in 25 mL measuring flask, then dilute to the mark with the same solvent. The working solution of Tb^{3+} ion of 1.0 ×10^{-4} mol L^{-1} was obtained by appropriate dilution with acetonitrile. The pH of the working solutions was adjusted to 8 by using 0.1 mol L^{-1} of 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 (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 using a 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 Tb- (GFX) complex-Doped in sol gel

i. The sol matrix was prepared according to earlier reported work [30-47] as follow: A mixture consisting of tetraethoxysilane (TEOS), ethanol and water in 1: 5:1 molar ratio was stirred for 15 min.
ii. 0.102 g of the prepared complex (Tb3+: GFX, 1:3 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 Tb-(GFX) complex-Doped in sol gel in a molar ratio of 0.3 mL of 1x10^{-2} mol L^{-1} (GFX) solution and 0.1 mL of 1.0 x10^{-2} mol L^{-1} Tb^{3+} solution to give 3.0 x 10^{-4} mol L^{-1} of (GFX) and 1.0 x10^{-4} mol L^{-1} of Tb^{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 λ_{ex}/ λ_{em} = 340/545nm. The calibration curve was sett 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 Tb^{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 Tb^{3+} doped in the sol gel, then The luminescence intensity was measured at $\lambda_{ex}/\lambda_{em}$ =340/545nm.

Determination of Gatifloxacin in pharmaceutical preparations

Five tablets of pharmaceutical formulation Tequin 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.

Preparation of serum samples

3.0 mL of citrate solution was added to 4.0mL plasma and the solution was centrifuged for 15.0min at 4000 rpm to remove all proteins. After decantation, 1.0 mL of the serum was added to 2.0 mL of 1.0 X 10^{-7} mol L^{-1} GFX at pH 8.0, then this solution was added to the thin film nano of the optical sensor in the 1.0 cm cell, and The luminescence intensity was measured at $\lambda_{ex}/\lambda_{em}$ =340/545nm.

Result and Discussion

Absorption and Emission Spectra

The absorption spectra of (GFX) and Tb^{3+}-

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

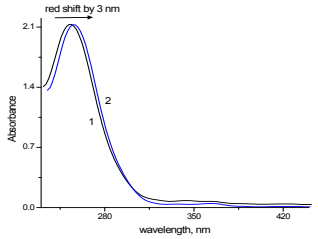

Fig. 2. Absorption spectrum of (1)-2 x 10^{-4} mol/L Gatifloxacine doped in sol-gel matrix (2)- 1 x 10^{-4} mol/L Gatifloxacine with 1x 10^{-4} mol/L Tb^{3+} doped in sol-gel matrix in acetonitrile.

The emission spectra of Tb^{3+}– (GFX) complex doped in sol-gel matrix in different concentrations of (GFX) are shown in Fig. 3. After the addition of different concentrations of (GFX) into the Tb^{3+} ion doped in sol-gel matrix in acetonitrile, the intensity of the characteristic peak at 545 nm of Tb^{3+} was enhanced indicating that (GFX) can form a complex with Tb^{3+} ion. The characteristic peaks of Tb^{3+} ion appear at ($^5D_4 \rightarrow {}^7F_6$ = 490 nm, $^5D_4 \rightarrow {}^7F_5$ = 545 nm, $^5D_4 \rightarrow {}^7F_4$ = 590 nm, $^5D_4 \rightarrow {}^7F_3$= 620 nm and $^5D_4 \rightarrow {}^7F_2$ = 650 nm), [35, 40, 42].

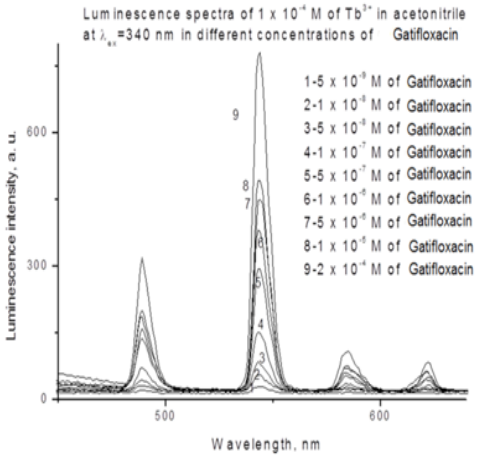

Fig. 3. Luminescence emission spectra of 1x 10^{-4} mol/L Tb^{3+} in the presence of different concentrations of Gatifloxacine doped in sol-gel matrix in acetonitrile and pH 8.0.

Effect of experimental variables

Effect of the amount of (GFX) and Tb^{3+}

The ion titration revealed that the complex formed M : L (1 : 3) for Tb : (GFX), respectively, doped in sol-gel matrix 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. 4)[43-45].

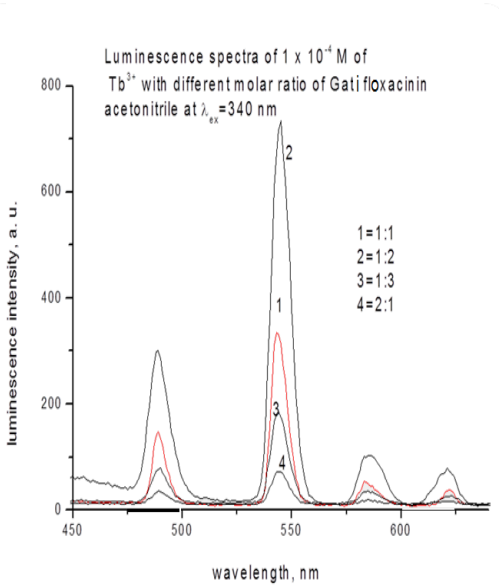

Fig. 4. Molar ratio between Tb^{3+} and Gatifloxacine doped in sol-gel matrix in acetonitrile at λ_{ex}=340 nm.

Effect of pH

The pH of the medium has a great effect on the luminescence intensity of the Tb-GFX complex. The luminescence intensity of the Tb^{3+} - GFX at different pH ranged from 2 to 10 using 0.1 M of HCl and /or NH4OH was tested. The results obtained show that the maximum luminescence intensity is obtained at pH 8.0. Therefore, in the subsequent work, the pH of the tested solution was adjusted by 0.1 mol L-1 of HCl and /or NH_4OH to pH 8.0 before each measurement.

Linearity and validation parameters

linearity and range

A linear correlation was found between luminescence intensity of (GFX)– Tb^{3+} complex at λ_{em} = 545 nm and concentration of (GFX) in the ranges given in Table 1. The six-points (5 x 10^{-9} to 1.2 x 10^{-6} mol L 1) calibration curve was obtained by plotting the peak intensity of Tb^{3+} at

λ_{em} = 545 nm versus the concentration of (GFX) and the graph was described by the regression equation: Y = a + bX
(Where Y = luminescence intensity of the optical sensor at λ_{em} = 545 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.

Detection and quantification limits

The limit of detection (LOD) and quantification (LOQ) calculated according to ICH guidelines [48] using the formulae : LOD = 3.3 S/b, 1.6 x 10^{-9} and LOQ = 10 S/b, 4.8 x 10^{-9} (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 methods [4-23].

TABLE 1. Sensitivity and regression parameters for photo probe.

Parameter	GFLX
λ_{em}, nm	545
Linear rang, mol L^{-1}	$5x10^{-9}$ to $1.2x10^{-6}$
Limit of detection (LOD),molL^{-1}	$1.6x10^{-9}$
Limit of quantification (LOQ), molL^{-1}	$4.8x10^{-9}$
Intercept (a)	84.5
Slope (b)$x10^{9}$	1.3
Standard deviation	6.4
Variance (Sa^2)	4.96
Regression Coefficient	0.99

Accuracy and precision

The results demonstrated that the proposed method is more accurate as well as more precise. These results complement the findings of the placebo blank analysis with respect to selectivity. To compute the accuracy and precision, the assays were repeated three times within the day to determine the repeatability (intra-day precision) and three times on different days to determine the intermediate precision (inter-day precision) of the method. These assays were performed for three levels of the analyte. The results of this study are summarized in Table 2. The percentage relative standard deviation (%RSD) values were ≤ 0.21-0.21% (intra-day), ≤ 0.14-0.22% (inter-day) for drug and (%RSD) values were ≤ 0.16-1.66 % (intra-day), ≤ 0.19-0.36% (inter-day) for serum samples, respectively, the inter-day values indicating high precision of the method. Accuracy was evaluated as percentage relative error (RE) between the measured mean concentrations and the taken concentrations of GFX. Bias {bias % = [(Concentration found - known concentration) x 100 / known concentration] was calculated at each concentration and these results are also presented in Table 2. Percent relative error (% RE) values of ≤ 0.14-5.00 and 0.16-0.66 % for the drug and serum samples, respectively, demonstrates the high accuracy of the proposed method.

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 (GFX) in a synthetic mixture. To the placebo blank of similar composition, different amount of (GFX) of pharmaceutical formulation of tablet Tequin was added, homogenized and the solution of the synthetic mixture was prepared as describe under "analysis of dosage forms". The filtrate was collected in a 100-mL flask. 1.0, 2.0 and 4.0 mL of the resulting solution was assayed (n=9) by proposed method which yielded % 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 precision for optical sensor Tb-GFX doped in sol-gel matrix .

Sample	Actual GFX found X 10^{-7} mol/L	Intra-day precision (readings, n=3)			Inter-day precision (readings, n=3)		
		GFX Average Found ± CL	% RE	% RSD	GFX average found CL	% RE	% RSD
Gaticin 200 mg	3.0	3.01 ± 0.12	3.40	0.12	2.95± 0.14	6.89	0. 14
	4.0	3.9 ± 0.25	5.00	0.15	4.1 ± 0.24	2.50	0. 16
	5.0	4.9 ± 0.10	2.50	0.21	5.11 ± 0.12	5.10	0.22
Serum	3.0	3.05 ± 0.21	1.66	0.37	3.15 ± 0.18	5.00	0. 36
	6.0	6.01 ± 0.14	0.16	0.26	6.11 ± 0.15	1.83	0. 27
	9.0	9.06 ± 0.13	0.66	0.62	9.11 ± 0.15	1.22	0. 19

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 = $[(\text{average N- value 1})^2 + (\text{average N- value 2})^2 + (\text{average N – value 3})^2]^{1/2}$. 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 .

Conclusion

The Tb^{3+} ion doped in sol-gel matrix in acetonitrile has high sensitive and characteristic peaks in the presence of (GFX). The proposed method for the determination of (GFX) offers simple, rapid and sensitive method for the analysis of (GFX) in acetonitrile and pH 8.0 with a linear range of $1.2 \times 10^{-6} – 5.0 \times 10^{-9}$ mol L^{-1} and detection limit of 1.6×10^{-9} mol L^{-1}. The developed optical sensor is selective, accurate and attractive for routine control analysis of the drug.

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تقدير الجاتيفلوكساسين باستخدام زيادة كثافة الضوء الاخضر المنبعث من ايون التربيوم المغموس في وسط الصول جيل كمجس ضوئي

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يشمل تحضير مجس ضوئي جديد من عنصر التربيم في وسط السول جل لتقييم جاتيفلوكساسين في أقراص الأدوية و عينات الدم . و تعتمد الطريقة على عملية انتقال الطاقة من مركب جاتيفلواكساسين لعنصر التربيم عند و λex= ٣٤٠ nm (pH ٨,٠ = nm) مما يؤدي الي زيادة طيف الانبعاث الفلوروسيني الخاص بعنصر التربيم و تستخدم هذه الزيادة في عملية تقدير مادة جاتيفلوكساسين في الادوية و عينات الدم. ونستطيع باستخدام الحساس الضوئي في تعيين معامل التحديد = ١,٦٥ x ١٠-٩ $mol\ L$-١