J Fluoresc (2012) 22:779–788
DOI 10.1007/s10895-011-1013-1

ORIGINAL PAPER

Spectrofluorimetric Assessment of Chlorzoxazone and Ibuprofen in Pharmaceutical Formulations by using Eu-Tetracycline HCl Optical Sensor Doped in Sol–Gel Matrix

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Received: 20 July 2011 / Accepted: 20 October 2011 / Published online: 9 November 2011

Abstract A novel, simple, sensitive and selective spectrofluorimetric method was developed for the determination of trace amounts of chlorzoxazone and Ibuprofen in pharmaceutical tablets using optical sensor Eu-Tetracycline HCl doped in sol–gel matrix. The chlorzoxazone or Ibuprofen can remarkably enhance the luminescence intensity of Eu-Tetracycline HCl complex doped in a sol–gel matrix in dimethylformamide (DMF) at pH 9.7 and 6.3, respectively, λ_{ex}=400 nm. The enhancing of luminescence intensity peak of Eu-Tetracycline HCl complex at 617 nm is proportional to the concentration of chlorzoxazone or Ibuprofen a result that suggested profitable application as a simple optical sensor for chlorzoxazone or Ibuprofen assessment. The dynamic ranges found for the determination of chlorzoxazone and Ibuprofen concentration are 5×10^{-9}–1×10^{-4} and 1×10^{-8}–7×10^{-5} mol L^{-1}, and the limit of detection (LOD) and quantitation limit of detection (LOQ) are 3.1×10^{-10} , 9.6×10^{-10} and 5.6×10^{-10}, 1.7×10^{-9} mol L^{-1}, respectively.

Keywords Chlorzoxazone · Ibuprofen · Luminescence intensity · Optical sensor · Europium-tetracycline HCl complex

Introduction

Ibuprofen [(R,S)-α-methyl-4-(2-methylpropyl) benzeneacetic acid] is a non-steroidal anti-inflammatory drug with anti-inflammatory, analgesic and antipyretic properties. Ibuprofen is extensively used in the treatment of many diseases like rheumatoid arthritis, degenerative joint disease, ankylosing spondylits and acute gouty arthritis [1]. Several analytical methods have been reported for the determination of Ibuprofen in pharmaceutical preparations including spectrophotometry [2–4], spectrofluorimetry [5, 6], polarography [7], conductometry [8], high-performance liquid chromatography [9–12], capillary electrophoresis [13, 14], infrared spectrometry [15], supercritical fluid chromatography [16] and proton magnetic-resonance spectroscopy [17].

Chlorzoxazone (CZX) is a centrally acting skeletal muscle relaxant that is used in the treatment of muscle spasms [18]. Several HPLC methods have been reported for the analysis of CZX in biological fluids [19–26]. In humans, CZX exists in the unchanged form in plasma but is not generally found (<1% of a dose) in urine. The assay methods reported previously are only for the measurement of CZX in plasma [19, 25]. A reversed-phase HPLC is the only method that determine both CZX and Ibuprofen in one pharmaceutical formulation [27]. However, most of these techniques are time-consuming, involving the use of organic solvents or requiring expensive and sophisticated instruments. A common limitation of the other methods reported is insufficient sensitivity for low dose of CZX pharmacokinetic studies [21–23]. Due to the inherent low temperature process, sol –gel technology has acquired great popularity in the field of optical sensors [24–28]. The driving force for these attempts is that, the sol – gel chemistry provides a relatively simple way to incorporate recognition species in a stable host environment. The sol–gel technology provides a unique means to prepare inorganic and organic–inorganic hybrid material for use in sensing devices. The simple doping of the sol–gel solution with the desired compound is the most popular technique for immobilization because of its generality, simplicity and retention of the properties of the compound in the immobilized state. A recent literature on the analytical

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applications of the europium(III) ion has revealed no study on the use of this species in sol- gel for the simulteanous measuring of the concentration of CZX and Ibuprofen in the pharmaceutical samples. In the present work we introduce a novel technique in which the optical sensor Eu-tetracycline complex doped in sol–gel matrix was used in the determination of CZX and Ibuprofen in pharmaceutical samples. The novelty of this technique lies in the use of the optical sensor Eu-tetracycline complex doped in sol–gel matrix in the determination of CZX and Ibuprofen at a high pH in which we overcame the difficulty of working at high pH and hence avoid the formation of insoluble hydroxides of the lanthanides in alkaline solutions.

Experimental

Chemicals and Reagents

All chemicals used were analytical-reagents of the highest grade. Pure standards of CZX and Ibuprofen were either purchased from Sigma or supplied by the National Organization for Drug Control and Research (Cairo, Egypt) Fig. 1. Pharmaceutical preparations: Mark-fast (Marcyrl Co.), Myofen (EVA Co.) and Profenzone (Adwia Co.) tablets containing 250 mg of CZX and 200 mg of Ibuprofen per tablet were purchased from local market. Europium chloride (99.99%) and Tetracycline HCl were purchased from Aldrich.

Distilled water and pure grade solvents from (Aldrich) were used for the preparation of all solutions and during all determinations. Stock solutions of CZX and Ibuprofen (1×10^{-3} mol L^{-1}) were prepared and dissolved in ethanol and stored at 4 °C when not in use. The working standard solution of (1×10^{-4} mol L^{-1}) was prepared by appropriate dilution with DMF.

A Eu^{3+} ion stock solution (1×10^{-2} mol L^{-1}) was prepared by dissolving $EuCl_3$ with a small amount of ethanol in 100 ml measuring flask, then diluted to the mark with ethanol.

Tetracycline HCl stock solution (1×10^{-2} mol L^{-1}) was prepared and dissolved in ethanol and stored at 4 °C when not in use.

Acetate buffer 0.2 mol L^{-1} (pH3.6–5.6) was prepared by mixing appropriate volume of 0.2 mol L^{-1} acetic acid with 0.2 mol L^{-1} sodium acetate. Borate buffers (pH 6–8.5) were prepared by mixing appropriate volumes of 0.2 mol L^{-1} boric acid with 0.2 mol L^{-1} sodium hydroxide. Phosphate buffer (pH 8.8–11.0) was prepared by mixing appropriate volume of 0.2 mol L^{-1} K_2HPO_4 with 0.1 mol L^{-1} sodium hydroxide. Sodium hydroxide (BDH, UK), 0.1 mol L^{-1} aqueous solution.

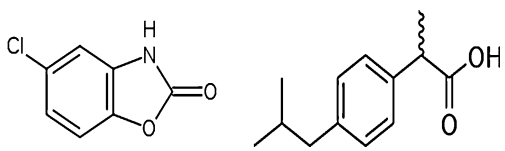

Fig. 1 Structure of CZX and Ibuprofen

Apparatus

All fluorescence measurements were carried out on Shimadzu RF5301 Spectrofluorophotometer in the range (290–750 nm). The absorption spectra were recorded with a Unicam UV-Visible double-beam spectrophotometer from Helios Company. It employs a Tungsten filament light source and a Deuterium lamp, which have a continuous spectrum in the ultraviolet region. The spectrophotometer is equipped with a temperature-controller cell holder. All pH measurements were made with a pHs-JAN-WAY 3040 ion analyzer.

General Procedure

a. Preparation of lanthanide complex Eu^{3+}-Tetracycline HCl doped in sol–gel matrix

The complex of Eu^{3+} ion with the tetracycline HCl was prepared by mixing the tetracycline HCl at concentration of 4×10^{-4} mol L^{-1} with 2×10^{-4} mol L^{-1} of $EuCl_3\cdot6H_2O$ in a molar ratio of 2:1 in spectral grade dry ethanol at room temperature. A pale pink precipitate was obtained and was separated from the solution by filtration. A hydrous complex was obtained from the solid by washing with ether and drying. Mixture consisting of TEOS (tetraethoxysilane), C_2H_5OH and H_2O in a molar ratio of 1:5:1 was refluxed for 1 h to give precursor sol solutions, using a few drops of diluted HCl solution as a catalyst. Subsequently, appropriate amount of the complex and the precursor solutions were mixed and stirred together for 15 min until the mixture became homogeneous. The obtained complex-dispersed sol solution was casted into polystyrene cup and kept at 25 °C in air for 2 weeks then heating at 100–500 °C for 24 h to give solidified and transparent composite sample [24].

b. Preparation of CZX and Ibuprofen solutions

To 10 mL clean and sterilized measuring flasks, the standard solutions of CZX and Ibuprofen were prepared by different additions of 1×10^{-3} mol L^{-1} CZX and Ibuprofen stock solution to give the following concentrations of CZX and Ibuprofen, 1×10^{-4} to 1×10^{-9} mol/ L. The solutions were diluted to the mark with DMF at room temperature. The above solutions were used for subsequent measurements of absorption and emission spectra as well as the effect of pH and solvents. The luminescence intensities were measured at $\lambda_{ex}/\lambda_{em}$=400/ 617 nm.

c. Calibration curve

After the preparation of the different standard solutions of CZX and Ibuprofen in DMF as described above, the optical sensor Eu^{3+}-Tetracycline HCl doped in sol–gel matrix was immersed in each standard solution of CZX and Ibuprofen in the cell of the spectrofluorimetric device, then the luminescence spectrum was measured at the selected excitation wavelength. The optical sensor was rinsed after each measurement using DMF Figs. 2 and 3.

d. Determination of CZX and Ibuprofen in pharmaceutical tablets

Ten tablets of each Mark-fast and Myofen and Profenzone were individually carefully ground to finely divided powders. Accurate weights equivalent to 25 mg Mark-fast or Myofen or Profenzone were each accurately transferred to 50 ml beaker and dissolved in DMF and the solutions were left to stand for about 10–15 min then filtered up using 12 mm filter papers and transferred to 100 mL volumetric flasks, then the filtrate was divided into two parts. 2.2 mL of borate buffer was added to the first part(Ibuprofen, pH=6.3) and 2.8 mL of phosphate buffer was added to the second part (Chlorzoxazone, pH=9.7) then completed to the mark with DMF to give the test solution. The optical sensor Eu^{3+} -tetracycline HCl was immersed in each test solution. The luminescence intensity of the test solution was measured before and after addition of Eu^{3+} -tetracycline HCl optical sensor. The change in the luminescence intensity was used for determination of CZX or Ibuprofen. The concentration of the drug was determined by using 9 concentrations for each sample from the corresponding calibration graph.

Results and Discussions

Spectral Characteristics

Absorption Spectra

The absorption spectra of 1-Tetracycline HCl, 2-CZX, 3-Ibuprofen, 4-Eu-Tetracycline HCl, 5-CZX-Eu-Tetracycline HCl and 6-Ibuprofen-Eu-Tetracycline HCl in sol–gel matrix are shown in Fig. 4. The spectra 1, 2 and 3 contain two bands at 352 and 276 nm attributed to n-π and π-π transitions. Comparing spectrum 4 with 1 after the addition of Eu^{3+} to Tetracycline HCl in sol–gel matrix, a red shift was observed for the two bands by 8 nm. Comparing spectrum 4 with 5 and 6 after the addition of CZX and Ibuprofen to Eu^{3+}-Tetracycline HCl in sol–gel matrix, a blue shift was observed by 32 and 24 nm, respectively. The absorbance was also enhanced, which indicates that CZX and Ibuprofen can bind with the complex of Eu-Tetracycline HCl.

Emission and Excitation Spectra

The excitation spectrum of the complex Eu^{3+}- Tetracycline HCl (spectrum 1), as well as the emission spectra of CZX and Ibuprofen (spectra 2 and 3), and that of Eu^{3+} ion in sol–gel

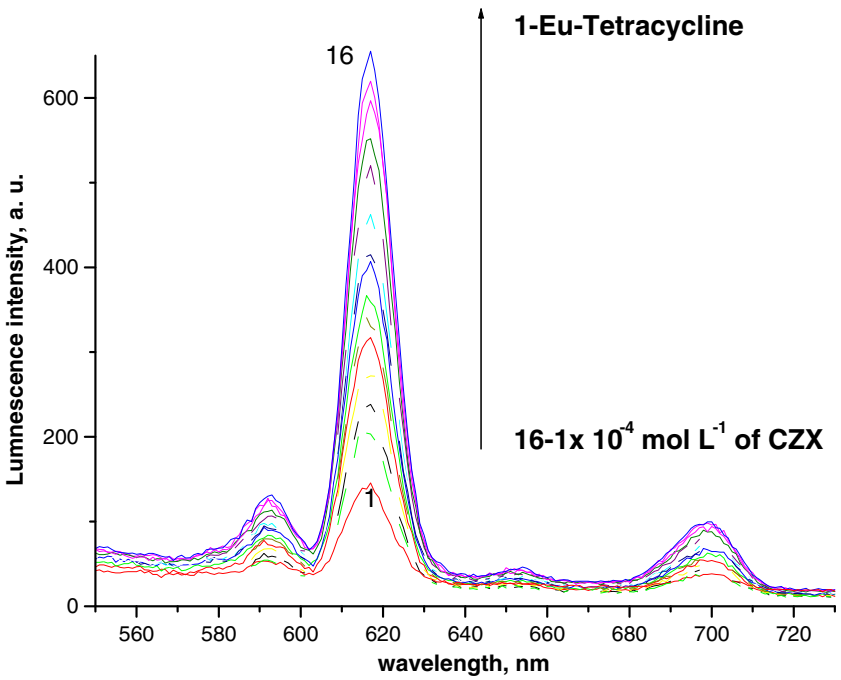

Fig. 2 Luminescence emission spectra of Eu-Tteracycline HCl doped in sol–gel matrix in the presence of different concentrations of CZX in DMF at λ_{ex}=400 nm and pH 9.7

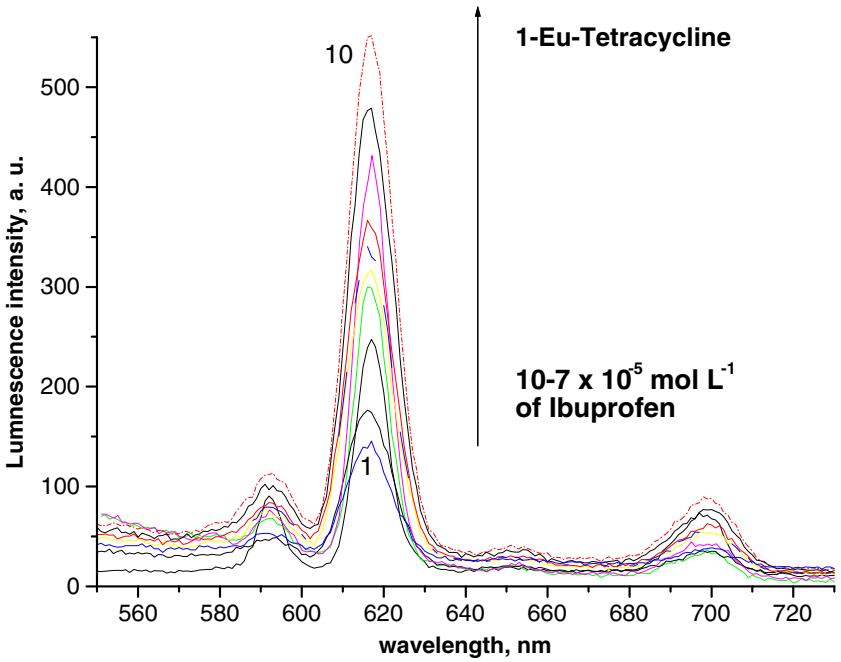

Fig. 3 Luminescence emission spectra of Eu-Tteracycline HCl doped in sol–gel matrix in the presence of different concentrations of Ibuprofen in DMF at λ_{ex}=400 nm and pH 6.3

(spectrum 4) and those of Eu^{3+}- Tetracycline HCl, CZX-Eu-Tetracycline HCl and Ibuprofen-Eu-Tetracycline HCl in sol–gel matrix (spectra 5, 6 and 7) are shown in Fig. 5. From spectrum 4 in Fig. 5, it can be seen that Eu^{3+} ion in sol–gel matrix has two very weak peaks. Comparing spectra (2 and 3) with (5 and 6) in Fig. 5, after the addition of CZX and Ibuprofen into the Eu^{3+}- Tetracycline HCl in sol–gel matrix, show that CZX and Ibuprofen can form a complex with Eu^{3+}- Tetracycline HCl and the characteristic peaks of Eu^{3+} ion appear at ($^5D_0 \rightarrow {}^7F_0$=580 nm, $^5D_0 \rightarrow {}^7F_1$=593 nm, $^5D_0 \rightarrow {}^7F_2$=6 17 nm, $^5D_0 \rightarrow {}^7F_3$=653 nm and $^5D_0 \rightarrow {}^7F_{4,5}$=693 ,704 nm).

Comparing spectra 6 and 7 with 5 in Fig. 5, it can be seen that the characteristic peak of Eu^{3+} at 617 nm has remarkably been enhanced after the addition of CZX and Ibuprofen, which indicates that CZX and Ibuprofen effectively enhance the energy of CZX-Eu-Tetracycline HCl and Ibuprofen-Eu-Tetracycline HCl complexes. The mechanism of the energy transfer from DC and CZX or Ibubrofen to Eu^{3+} takes place by the energy transfer from the triplet excited state of DC and CZX or Ibuprofen to the excited emitted state ($^5D_0$) of Eu^{3+} ion [29, 30].

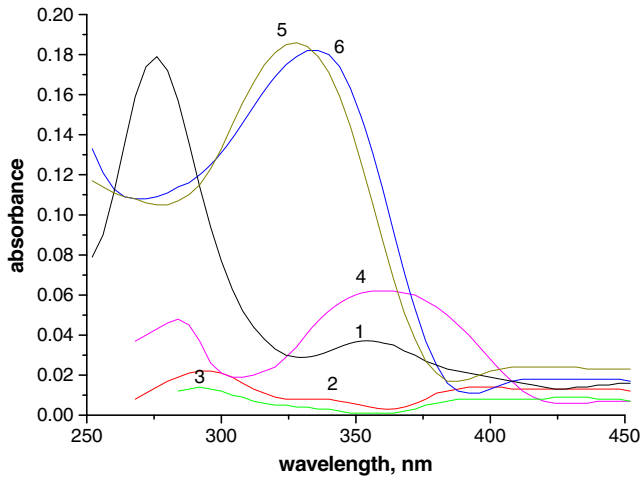

Fig. 4 The absorption spectra of 1–4×10^{-4} mol/L Tetracycline HCl, 2–5×10^{-4} mol/L CZX, 3–5×10^{-4} mol/L Ibuprofen, 4-Eu-Tetracycline HCl, 5-CZX-Eu-Tetracycline HCl and 6-Ibuprofen-Eu-Tetracycline HCl in sol–gel matrix

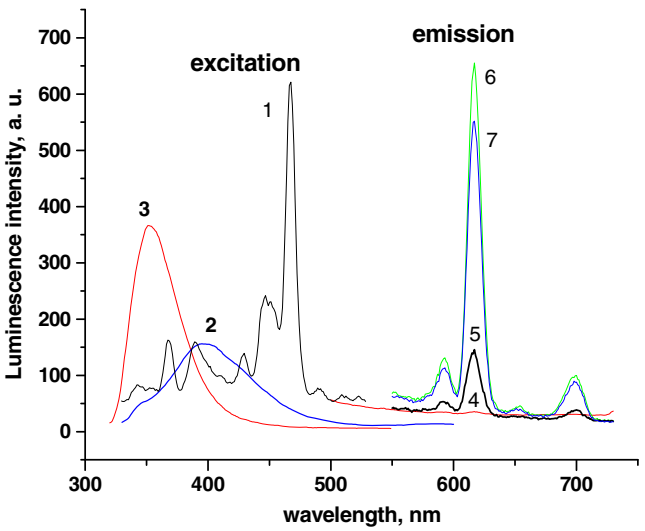

Fig. 5 The excitation spectrum of the complex Eu^{3+}- Tetracycline HCl (spectrum 1), the emission spectra of 2-CZX, 3-Ibuprofen, 4- Eu^{3+}, 5-Eu^{3+}- Tetracycline HCl, 6-CZX-Eu-Tetracycline HCl and 7-Ibuprofen-Eu-Tetracycline HCl in sol–gel matrix

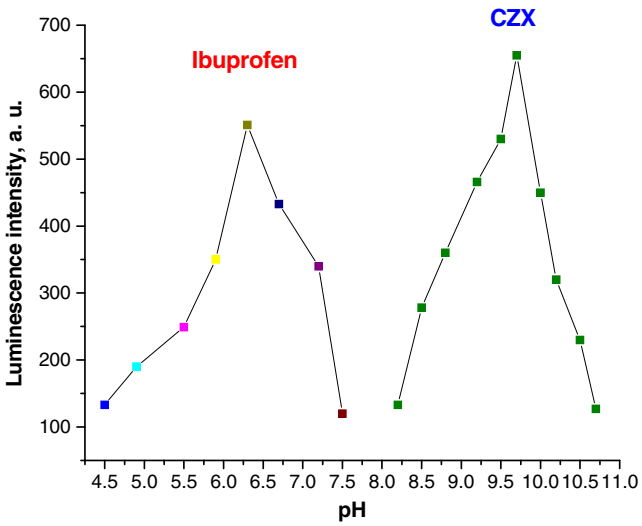

Fig. 6 Luminescence emission spectra of Eu-Tetracycline HCl in sol–gel matrix in the presence of 5×10^{-4} mol/L of both CZX and Ibuprofen at different pH at λ_{ex} =400 nm

Effect of Experimental Variables

Effect of the Amount of CZX and Ibuprofen

The influence of the amount of CZX and Ibuprofen on the luminescence intensities of the Eu-Tetracycline HCl doped in the sol–gel matrix were studied. The luminescence intensity of Eu-Tetracycline HCl complex was increased upon increasing the concentration of CZX and Ibuprofen till 5×10^{-4} mol L^{-1} then became constant. The experimental results showed that the luminescence intensity reached maxima and remained constant when CZX and Ibuprofen concentrations are 5×10^{-4} mol L^{-1} in the DMF preparations.

Effect of the Amount of Eu^{3+}

The influence of the amount of Eu^{3+} ion on the luminescence intensities of Eu-Tetracycline HCl in sol–gel matrix was studied under the conditions established above. The luminescence intensity of Eu-Tetracycline HCl complex at 617 nm increased upon increasing the concentration of Eu^{3+} up to 2×10^{-4} mol L^{-1} then became constant. When the concentration of Eu^{3+} ion was 2×10^{-4} mol L^{-1}, the composition ratio for the Eu^{3+} to Tetracycline HCl for the Tetracycline HCl – Eu^{3+} system was 1: 2. Thus, 2×10^{-4} mol L^{-1} Eu^{3+} ion concentration was used for further analysis in the sol–gel matrix.

Effect of the Amount of Tetracycline HCl

The influence of the amount of Tetracycline HCl on the luminescence intensities of Eu-Tetracycline HCl in sol–gel matrix was studied under the conditions established above. The luminescence intensity of Eu-Tetracycline HCl complex at 617 nm increased upon increasing the concentration of Tetracycline HCl up to 4×10^{-4} mol L^{-1} then became constant. When the concentration of Tetracycline HCl was 4×10^{-4} mol L^{-1}, the composition ratio for the Eu^{3+} to Tetracycline HCl for the Eu^{3+}-Tetracycline HCl system was 1:2. Thus, 4×10^{-4} mol L^{-1} Tetracycline HCl concentration was used for further analysis in the sol–gel matrix.

Effect of pH

The influence of pH on the fluorescence intensity of the studied drugs was studied using different buffers covering the whole pH range, e.g. acetate buffer over the pH range of 3.6–5.6, borate buffer over the pH range 6–8.5 and phosphate buffer over the pH range 8.8–11. The fluorescence intensity of CZX remained constant with the increase of pH from 3.6 up to pH 7. Fluorescence intensity decreased at pH 7.5, then it increased from pH 8.6 to 9.7, after which it decreased gradually up to pH 11, Fig. 6. As for Ibuprofen, increasing the pH values resulted in a gradual increase in the fluorescence intensity up to 6.3,

Table 1 Freeze–thaw stability of CXZ + Ibuprofen in pharmaceutical tablets (n=3)

Drug	Normal concentration × 10^{-7} mol L^{-1})	Found Average recovery ±S.D. × 10^{-7} mol L^{-1}			R.S.D. (%)
		0 day	15 days	30 days	
Mark-fast (250 mg CXZ+200 mg Ibuprofen) Marcyrl Co.	0.1	0.1	0.101	0.102	0.38
	1.0	1.0	1.01	1.02	
	1.5	1.5	1.5	1.502	
Myofen (250 mg CXZ+200 mg Ibuprofen) EVA Co.	0.1	0.1	0.102	0.102	0.85
	1.0	1.01	1.03	1.03	
	1.5	1.50	1.501	1.502	
Profenazone (250 mg CXZ+200 mg Ibuprofen) Adwia Co.	0.1	0.1	0.101	0.102	0.72
	1.0	1.01	1.02	1.03	
	1.5	1.5	1.5	1.502	

Table 2 Results of analysis of tablets by the proposed method and statistical comparison of the results with the reference method

Tablet brand name	Nominal amount, Added ($_{\times}10^{-8}$ mol L^{-1})	[b]Found (Percent of label claim ±SD)				
		Reading	[a]Average Found ($_{\times}10^{-8}$ mol L^{-1})	B.P. (LC)	Proposed method	
					Students t and F values	Average recovery ±R.S.D. (%)
Mark-fast (250 mg CXZ+ 200 mg Ibuprofen) Marcyrl Co.	100	100.02, 99.89, 100.01	99.97	98±0.2	t=0.65, F=6.36	102±0.66
	10.0	9.87,10.05,10.01	9.97		t=0.52, F=4.1	
	1.0	1.06,1.08,0.99	1.04		t=0.97, F=7.9	
Myofen (250 mg CXZ+200 mg Ibuprofen) EVA Co.	100	99.94,100.04,100.01	99.99	99.5±0.15	t=0.33, F=8.6	99±0.75
	10.0	10.02,10.02,10.05	10.03		t=1.29, F=14	
	1.0	0.97,1.0,0.95	0.97		t=1.26, F=13.3	
Profenazone (250 mg CXZ+ 200 mg Ibuprofen) Adwia Co.	100	100.01, 99.90, 100.04	99.98	99±0.2	t=0.45, F=6.9	98±0.41
	10.0	10.07,10.02,10.01	10.03		t=1.01, F=15.3	
	1.0	1.02,1.03,0.87	0.97		t=0.54, F=4.4	

[a] each reading was repeated four times (average was taking for three reading by four analysts)

[b] Average of four determinations

Tabulated t value at the 95% confidence level is 4.303. Tabulated F value at the 95% confidence level is 19

then decreased up to pH 9.50, this attributed to the different charged formula of CZX and Ibuprofen in the different pH ranges. Therefore, the presence of highly emissive species at pH 6.3 and 9.7 in case of CZX and Ibuprofen with the optical sensor leads a good energy transfer from this species to the optical sensor, Fig. 6.

Effect of Solvent

The influence of the solvent on the luminescence intensity of the Eu-Tetracycline HCl complex in sol–gel matrix was studied under the conditions established above. The results show the enhanced emission of CZX or Ibuprofen in DMF. This can be attributed to the formation of anhydrous solvates of CZX or Ibuprofen introducing solvent molecules in the first coordination sphere of CZX or Ibuprofen leads to the enhancement of the intensity of all transitions of Eu-Tetracycline HCl complex ($^5D_0 \rightarrow ^7F_0$=580 nm, $^5D_0 \rightarrow ^7F_1$=593 nm, $^5D_0 \rightarrow ^7F_2$=6 17 nm, $^5D_0 \rightarrow ^7F_3$= 653 nm and $^5D_0 \rightarrow ^7F_{4,5}$=693 ,704 nm) especially $^5D_0 \rightarrow ^7F_2$ transition in Eu^{3+}.

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+} ternary complex. Also, the luminescence intensity for the complex in DMF solution was stronger than in ethanol as hydroxy solvent. This may be due to vibrational energy transfer to the solvent molecules. It is well know 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 O-H oscillators [24, 31–33].

Stability Studies

The processed pharmaceutical tablet samples (10, 100 and 150 n mol L^{-1}) treated as sample preparation were kept at room temperature for 24 h and then the stability was determined. The freeze–thaw stability was determined after three repeated freezing and thawing cycles in day 0, 15 and 30, Table 1.

No significant loss of CZX and Ibuprofen (0.75 % R.S.D) was observed after storage of pharmaceutical tablets (0.41% R.S.D) at room temperature for at least 24 h, Table 2. Pharmaceutical tablet samples were stable over at least three freeze–thaw cycles, Table 1, indicating that the pharmaceutical tablets can be frozen and thawed at least three times prior to analysis (0.85 % R.S.D).

Table 3 Sensitivity and regression parameters for optical sensor

Parameter	CZX	Ibuprofen
λ_{em}, nm	617	617
Linear range, mol L^{-1}	5×10^{-9}–1×10^{-4}	1×10^{-8}–7×10^{-5}
Limit of detection (LOD), mol L^{-1}	3.1×10^{-10}	5.6×10^{-10}
Limit of quantification (LOQ), mol L^{-1}	9.6×10^{-10}	1.7×10^{-9}
Regression equation, Y[a]		
Intercept (a)	14.8	109.8
Slope (b)	124.5	76.6
Standard deviation	12.9	13.2
Variance (Sa^2)	167.7	174.2
Regression coefficient (r)	0.99	0.99

[a] Y = a + bX, Where Y is luminescence intensity, X is concentration in n mol L^{-1} , a is intercept, b is slope

Table 4 Comparison of spectrofluorimetric technique with some existing methods for the determination of CZX and Ibuprofen

Method	Linear range	Detection limit	Reference
Spectrofluorimetric method (ibuprofen)	2–73 mg L^{-1}	100 mg L^{-1}	[8]
Capillary zone electrophoresis (ibuprofen)	2–500 mg mL^{-1}	500 mg mL^{-1}	[35]
Potentiometric method (ibuprofen)	10^{-8}–10^{-3} mol L^{-1}	8.0×10^{-9} mol L^{-1}	[36]
High-performance liquid chromatography (CZX)	100–3000 ng/mL	3500 ng/mL	[37]
Reversed-phase HPLC method (ibuprofen and CZX)	2–10 μg /mL	50, 20 ng/mL	[38]
Optical sensor Eu-tetracycline HCl doped in sol–gel	5×10^{-9}–1×10^{-4} (CZX) and 1×10^{-8}–7×10^{-5} (Ibuprofen)mol L^{-1}	3.1×10^{-10} , 9.6×10^{-10} mol L^{-1}	Present work

Analytical Performance

Method Validation

Analytical parameters of the proposed method A linear correlation was found between luminescence intensity of Eu^{3+}- Tetracycline HCl complex at λ_{em}=617 nm and logarithium(concentration) of CZX and Ibuprofen in the ranges given in Table 3. The thirteen-points and eight-points 10^5 to 5 n mol L^{-1} and 7×10^4 to 10 n mol L^{-1} for CZX and Ibuprofen, respectively, calibration curves were obtained by plotting the peak intensity of Eu^{3+}-Tetracycline HCl at λ_{em}=617 nm versus the log(concentration) of CZX and Ibuprofen 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 = log concentration in n mol mL^{-1}). Regression analysis of luminescence intensity data using the least square method was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) and the values are presented in Table 3. The limit of detection (LOD) and quantitation limit of detection (LOQ) were calculated according to ICH guidelines [34] 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 3. The low value of LOD indicates the high sensitivity of the proposed method if compared by other previous methods Table 4.

Accuracy and precision of the method To compute the accuracy and precision, the assays described under "general procedures" 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 concentration levels of analyte. The results of this study are summarized in Table 5. The percentage relative standard deviation (%RSD) values were ≤0.1% (intra-day) and ≤0.13% (inter-day) indicating high precision of the method. Accuracy was evaluated as percentage relative error (RE) between the measured mean

Table 5 Evaluation of intra-day and inter-day accuracy and precision

Method	(CXZ +Ibuprofen) taken[a]	Intra-day accuracy and precision (n=3)			Inter-day accuracy and precision (n=3)		
		(CXZ + Ibuprofen) Average Found[a]±CL	%RE	%RSD	(CXZ + Ibuprofen) average found[a] ± CL	%RE	%RSD
Mark-fast (250 mg CXZ+200 mg Ibuprofen) Marcyrl Co.	3.0	2.93±0.11	2.30	0.10	2.92±0.25	2.60	0.13
	6.0	5.95±0.25	0.83	0.08	5.93±0.32	1.16	0.10
	9.0	8.89±0.17	1.20	0.07	8.85±0.22	1.66	0.09
Myofen (250 mg CXZ+200 mg Ibuprofen) EVA Co.	3.0	3.03±0.15	1.00	0.05	3.04±0.22	1.33	0.09
	6.0	5.97±0.10	0.50	0.04	5.96±0.20	0.66	0.08
	9.0	8.94±0.10	0.66	0.04	8.92±0.17	0.88	0.07
Profenazone (250 mg CXZ+200 mg Ibuprofen) Adwia Co.	3.0	2.91±0.17	3.00	0.10	2.90±0.22	3.30	0.12
	6.0	6.02±0.15	0.33	0.06	6.05±0.22	0.83	0.09
	9.0	9.09±0.25	1.00	0.05	9.10±0.30	1.10	0.07

[a] The values are mulitiplied by 10^{-7} mol L^{-1} for method

%RE. Percent relative error, %RSD. relative standard deviation and CL. Confidence limits were calculated from: CL = ± tS/√n. (The tabulated value of t is 4.303, at the 95% confidence level; S = standard deviation and n = number of measurements)

Table 6 Method robustness and ruggedness expressed as intermediate precision (% RSD)

Method	(CXZ+Ibuprofen) taken[a]	Robustness			Ruggedness
		Parameter altered			Inter-analysts, (%RSD) (n=3)
		Concentration of Eu^{3+*}(%RSD)	Concentration of Tetarcycline HCl[b](%RSD)	Reaction time[b] (%RSD)	
Mark-fast (250 mg CXZ+200 mg Ibuprofen) Marcyrl Co.	6.0	1.08	2.62	0.48	1.25
Myofen (250 mg CXZ+200 mg Ibuprofen) EVA Co.	8.0	0.88	2.12	0.28	1.05
Profenazone (250 mg CXZ+200 mg Ibuprofen) Adwia Co.	10.0	0.62	1.07	0.44	0.89

[a] The values are muiltiplied by 10^{-7} mol L^{-1}. [b] Concentrations of Eu^{3+} were 2, 5 and 6×10^{-4} mol L^{-1}; and the concentrations of Tetracycline HCl were 5, 8 and 10×10^{-4} mol L^{-1}. [c] The reaction times studied were 19, 20 and 21 min

concentrations and the taken concentrations of MCP. Bias $\{\text{bias}\% = [(\text{Concentration found} - \text{known concentration}) \times 100/\text{known concentration}]\}$ was calculated at each concentration and these results are also presented in Table 5. Percent relative error (%RE) values of ≤3.3% 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 (200 mg), starch (200 mg), lactose (20 mg), calcium carbonate (50 mg), calcium dihydrogen orthophosphate (20 mg), methyl cellulose (40 mg), sodium alginate (50 mg) and magnesium stearate (80 mg) was extracted with water and the 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 CZX and Ibuprofen in a synthetic mixture. To the placebo blank of similar composition, different amounts of CZX and Ibuprofen of different products were 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. Five ml of the resulting solution was assayed (n=3) by proposed method which yielded a % recovery of 99.00± 0.60 for tablets, Table 2. The results demonstrated the accuracy as well as the precision of the proposed methods. These results are in agreement with those of the placebo blank analysis with respect to selectivity.

Robustness and ruggedness The robustness of the method was evaluated by making small incremental changes in the concentration of Eu^{3+}, Tetracycline HCl and contact time, and the effect of the changes was studied on luminescence intensity of the optical sensor. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as % RSD (≤ 2.62%). The method ruggedness was expressed as the RSD of the same procedure applied by three different analysts. The inter-analysts RSD were within 1.25% for the same Tetracycline

Table 7 Results of recovery study using standard addition method

Proposed method				
Tablet studied	(CXZ + Ibuprofen) in tablet extract, mg	Pure (CXZ +Ibuprofen) added, mg	Total (CXZ + Ibuprofen) found, mg	Pure (CXZ + Ibuprofen) recovered (Percent ± SD*)
Mark-fast (250 mg CXZ+200 mg Ibuprofen) Marcyrl Co.	22.0	1.0	22.95	97.30±0.55
	22.0	2.0	23.45	101.88±0.75
	22.0	3.0	25.11	106.82±0.70
Myofen (250 mg CXZ+200 mg Ibuprofen) EVA Co.	20.0	1.0	21.15	99.00±0.75
	20.0	2.0	21.45	97.00±0.80
	20.0	3.0	22.91	101.00±0.70
Profenazone (250 mg CXZ+200 mg Ibuprofen) Adwia Co.	25.0	1.0	25.46	96.3±0.47
	25.0	2.0	27.15	98.0±0.33
	25.0	3.0	27.73	99.7±0.43

HCl concentrations ranged from 0.89 to 1.25% suggesting that the developed method was rugged. The results are shown in Table 6.

Application to formulations The proposed method was applied to the determination of CZX and Ibuprofen in three representative tablets. The results in Table 2 show that the method is successful for the determination of CZX and Ibuprofen and the inactive species in the dosage forms did not interfere. The results obtained (Table 2) were statistically compared with the official British Pharmacopoeia [B.P] method [39]. The average recovery and R.S.D for the tablet in our method were found to be (99.0% and 0.60%). Data obtained by B. P method showing average recovery 99.90% and R.S.D 0.2% were also presented for comparison and show a good correlation with those obtained by the proposed method. The results obtained by the proposed method agreed well with those of the reference method and with the label claim. When the results were statistically compared with those of the reference method by applying the Student's *t*-test for accuracy and F-test for precision on the tablets the calculated Student's t- value and F-value [40] at 95% confidence level did not exceed the tabulated values of (0.97, 7.9), (1.29, 14) and (1.0, 15.5), for two degrees of freedom. Hence, no significant difference exists between the proposed methods and the reference method with respect to accuracy and precision Table 2.

Recovery study To further assess the accuracy of the methods, recovery experiments were performed by applying the standard-addition technique. The recovery was assessed by determining the agreement between the measured standard concentration and the added known concentration to the sample. The test was done by spiking the pre-analysed tablet powder with pure CZX and Ibuprofen with three different levels (20, 22 and 25 mg) of the content present in the tablet powder (taken) and the total was found by the proposed method. Each test was repeated three times. In all cases, the recovery percentage values ranged between 106.82 and 96.2% with relative standard deviation in the range 0.33–0.80% for tablets. Closeness of the results to 100% showed the fairly good accuracy of the methods. The results are shown in Table 7.

Conclusion

The Eu^{3+}-Tetracycline complex doped in sol–gel matrix has high sensitive and characteristic peaks in the presence of CZX and Ibuprofen. The intensities of these peaks are enhanced by increasing the concentration of CZX and Ibuprofen, due to energy transfer from CZX and Ibuprofen to the Europium ion. It can be used for determination of CZX and Ibuprofen in pharmaceutical preparations with high accuracy.

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