J Fluoresc (2011) 21:1739–1748
DOI 10.1007/s10895-011-0869-4

ORIGINAL PAPER

Spectrofluorimetric Assessment of Doxycycline Hydrochloride in Pharmaceutical Tablets and Serum Sample Based on the Enhancement of the Luminescence Intensity of the Optical Sensor Sm^{3+} Ion

M. S. Attia · W. H. Mahmoud · M. N. Ramsis · L. H. Khalil · A. M. Othman · S. G. Hashem · M. S. Mostafa

Received: 25 October 2010 / Accepted: 17 February 2011 / Published online: 2 March 2011

Abstract A simple and sensitive spectrofluorimetric method for determination of trace amount of doxycycline hydrochloride (DC) in pharmaceutical tablets and serum samples was developed. In ammonia buffer solution of pH 8.9 the doxycycline hydrochloride can remarkably enhance the luminescence intensity of the Sm^{3+} ion in Sm^{3+}- DC complex at λ_{ex}=400 nm. The produced luminescence intensity of Sm^{3+}- DC complex in DMSO is in proportion to the concentration of DC and used as optical sensor for its determination. The dynamic range for the determination of DC is 1×10^{-8} – 5×10^{-6} mol L^{-1} and in case of quantum yield calculations is 7×10^{-9} – 5×10^{-6} mol L^{-1} with detection limit of 6.5×10^{-10} mol L^{-1}. The enhancement mechanism of the luminescence intensity in the Sm^{3+}- DC system has been also discussed. A comparison with other spectrofluorimetric methods for tetracycline derivatives in which Eu^{3+} ion is used instead of Sm^{3+} ion is also studied.

M. S. Attia (✉) · M. N. Ramsis · L. H. Khalil · S. G. Hashem · M. S. Mostafa
Department of Chemistry, Faculty of Science,
Ain Shams University,
Cairo, Egypt
e-mail: Mohamed_sam@yahoo.com

A. M. Othman
Department of environmental biotechnology,
Genetic Engineering and Biotechnology Research Institute,
(GEBRI), Minofiea University,
Sadat City, Egypt

W. H. Mahmoud
Chemistry Department, Faculty of Applied Science,
Taibah University,
Al-Madinah, Al-Munawarah, Kingdom of Saudi Arabia

Keywords Doxycycline · Sm^{3+}- doxycycline complex · Optical sensor · Luminescence intensity

Introduction

Doxycycline Fig. 1 is a tetracycline antibiotic obtained by modification of the oxytetracycline molecule. It has a broad spectrum of activity against a wide variety of microorganisms, including aerobic and anaerobic Gram-positive and Gram-negative bacteria, chlamydiae, rickettsiae and mycoplasmas, and it exerts a bacteriostatic effect by inhibiting protein synthesis. Doxycycline has been successfully used for more than 40 years in certain respiratory, skin, soft tissue and genitourinary infections [1–3]. In veterinary medicine, doxycycline is used to treat infections in several animal species, such as ehrlichiosis or respiratory tract diseases in dogs, pneumonia in cattle and pigs, and colibacillosis and psittacosis in poultry. A major advantage of doxycycline, compared to other members of the tetracycline family, is a high lipophilicity, which will increase its distribution and tissue penetration, plus prolonge its half-life, all of which contribute to its enhanced antimicrobial activity [1, 4, 5]. In addition, this drug has limited adverse effects and is relatively inexpensive. Consequently, there has been a growing interest in using doxycycline in veterinary clinical practice.

Many HPLC methods have been used to determine tetracyclines in pharmaceutical formulations using for example silica gel [6, 7], porous graphitic carbon [8], silica-based and polymer based HPLC column stationary phases [9–17]. Most of these methods are time consuming and complex, some are very expensive and some even

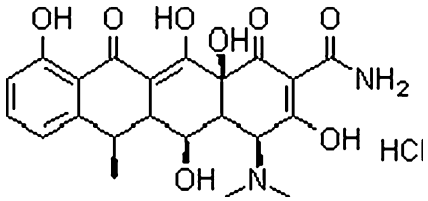

Fig. 1 Chemical structure of doxycycline hydrochloride

unable to separate tetracyclines from their degradation products. Several methods have been proposed for the analysis of doxycycline both in pharmaceutical preparations and biological samples, such as spectrophotometry [18–20], fluorimetry [21, 22] and phosphorimetry [23], thin-layer chromatography [24–26], LC [27–29], flow injection analysis [30, 31], and capillary electrophoresis [32]. Doxycycline-optosensors [33, 34] and doxycycline selective membrane electrodes [35–37] have been also reported.

In our previous work the lanthanide ions were used as optical sensor in solution [38] or in sol–gel [39–41]. In this work, a comparison with other spectrofluorimetric techniques in which the Eu^{3+} ion was used instead of Sm^{3+} ion [42–44] and the selectivity of Sm^{3+} towards DC over other tetracycline derivatives was discussed. Where the complexation between the DC as a ligand for Sm^{3+} ion and the possibility of the enhancement of the Sm^{3+} luminescence sensitized by DC was studied and investigated.

Experimental results show that Sm^{3+} ion in Sm^{3+}- DC complex has high sensitivity and selectivity characteristic peaks and can be used for determination of DC in DMSO and at pH 8.9.

Also, this method is simple, relatively free from interference of coexisting substances and can be successfully applied to the determination of DC in pharmaceutical preparations and in serum samples with satisfactory results. At 400 nm the luminescence intensity can be greatly enhanced in DMSO and the mechanism of enhanced luminescence intensity was discussed.

Experimental

Reagents

All chemicals used are of analytical-reagent or higher grade. Distilled water and pure grade solvents from (Aldrich) were used for the preparation of all solutions and during the determinations. Stock solutions of different tetracyclines and doxycycline hydrochloride DC (1×10^{-2} mol L^{-1}) were directly prepared and dissolved in ethanol. The working standard solutions of (1×10^{-4} mol L^{-1}) were freshly prepared by appropriate dilution with dimethylsulphoxide (DMSO). All stocking and working solutions given above were stored at 0–4 °C when not in use.

Sm^{3+} and Eu^{3+} ions stock solutions (1×10^{-2} mol L^{-1}) were prepared by dissolving $Sm(NO_3)_3$ or $Eu(NO_3)_3$ (delivered from Aldrich, 99.99%) with a small amount of ethanol in 100 ml measuring flask, then diluting to the mark with ethanol. The working solutions of Sm $(NO_3)_3$ and Eu $(NO_3)_3$ of concentrations 1×10^{-4} mol L^{-1} were obtained by appropriate dilution of the stock solution with DMSO. NH_4OH/NH_4Cl buffer was used to obtain pH 8.9 during measurements.

Ammonia buffer of various pH(8–10) were prepared by half filling a 1 L volumetric flask with distilled water and adding 214 g of NH_4Cl. Under a hood, add 270 mL of concentrated NH_4OH, swirl the flask gently to dissolve the solid, and allow to cool. Fill the flask to the mark with distilled water and mix well several times to obtain the solution.

Apparatus

All luminescence measurements are carried out on Shimadzu RF5301 (PC) spectrofluorophotometer in the range (290–750 nm). The absorption spectra are recorded with a Unicam UV–Visible double-beam spectrophotometer from Helios Company. It employs a Tungsten filament light source and a Deuterium lamp, which has a continuous spectrum in the ultraviolet region. The spectrophotometer is equipped with a temperature-controller cell holder. All pH measurements are made with a pHs-JAN-WAY 3040 ion analyzer. (All measurements are measured at Photoenergy Center, Faculty of Science, Ain Shams Univ.).

General Procedure

To 10 ml clean and sterilized measuring flasks, the prepared solutions were added in the following order: 0.2 mL (1×10^{-2} mol L^{-1}) DC solution, 0.1 mL (1×10^{-2} mol L^{-1}) Sm^{3+} solution and 1.0 ml buffer solution to give 2×10^{-4} mol L^{-1} of DC and 1×10^{-4} mol L^{-1} of Sm^{3+}. The mixture was diluted to the mark with DMSO at room temperature. The above method was used for the subsequent measurements of absorption, emission spectra, effect of pH and solvents.

The luminescence intensity was measured at $\lambda_{ex}/\lambda_{em}$= 400/645 nm. Luminescence quantum yield (Φ_L) determinations in different solvents were obtained using the following Eq., [45].

$$\Phi_L = [(F_{(\acute{u})}\ A^{o}_{\lambda e}\ n^2) / (F^{o}_{(\acute{u})}\ A_{\lambda e}\ n_o^2)]\ \Phi_f^{o}$$

Where, $A^{o}_{\lambda e}$, $F^{o}_{(\acute{u})}$, n^2_o and Φ^{o}_f are the absorbance at the exciting wavelength, the area under the emission spectrum,

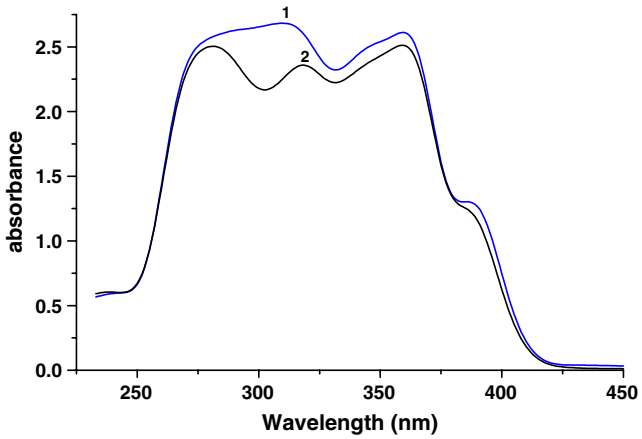

Fig. 2 Absorption spectra of (1)-2×10^{-4} mol L^{-1} of DC in DMSO (2)- 2×10^{-4} mol L^{-1} of DC –in the presence of 1×10^{-4} mol L^{-1} of Sm^{3+} in DMSO

the refractive index of the solvent (rhodamine101 in ethanol $n=1.329$) and quantum yield (1 for rhodamine101 in ethanol) of the reference, respectively. $A_{\lambda e}$, $F^{o}_{(\acute{\upsilon})}$, n^2 and Φ_L are the absorbance at the exciting wavelength, the area under the emission spectrum, the refractive index of the solvent and quantum yield of the unknown, respectively.

Determination of Doxycycline Hydrochloride in Pharmaceutical Preparations

The contents of one capsule of the drug formulations (Vibramycin 100 mg/capsule or Doxymycin 100 mg/capsule or Telexine 50 mg/capsule) were accurately transferred to 50 ml beaker and dissolved in ammonia buffer of pH 8.9 and DMSO then transferred to 100 mL volumetric flask and completed to the mark with DMSO to give the test solution containing 100 mg/100 mL for Vibramycin or Doxymycin and 50 mg/100 mL for Telexine drugs. The concentration of the drug was determined by repeating the method three times for three concentrations of each sample from the corresponding calibration graph.

Determination of Doxycycline Hydrochloride in Serum Solution

3 mL of trichloroacetic acid were added to 1.0 mL serum of a real healthy donor and the solution was centrifuged for 15 min at 4000 r/min to remove proteins, then 100 micron of the serum was added to Sm^{3+} ion in 10 mL measuring flask and completed to the mark with DMSO and at pH 8.9.

The luminescence intensity of the test solution was measured before and after addition of 1.0 mL of previously prepared serum solution. The change in the luminescence intensity was used for determination of DC in serum sample.

Results & Discussions

Spectral Characteristics of Sm^{3+}-DC Complex

The absorption spectrum of 2×10^{-4} mol L^{-1} of doxycycline hydrochloride in DMSO shows two bands in UV at 309 and 359 nm and a shoulder at 385 nm, with molar absorptive coefficient ($\varepsilon=26830$, 26120, and 13020 $M^{-1}cm^{-1}$), respectively. Upon addition of Sm^{3+} ion to the DC solution a red shift was observed in the three bands by 3, 1 and 3 nm respectively, as shown in Fig. 2. And also it gives the characteristic emission spectrum of the Sm^{3+} ion containing different transition bands ($^4G_{5/2}\rightarrow{}^6H_{5/2}$= 564 nm, $^6H_{7/2}$=599 nm, $^6H_{9/2}$=643 nm, $^6H_{11/2}$=707 nm). Moreover, the intensity of the emission band at 643 nm of Sm^{3+} was enhanced in presence of different addition of DC (Fig. 3). The ion titration revealed that the complex formed is in the ratio M: L (1:2), which indicates that the metal may coordinate to the ligand from different coordination sites.

The Effect of Experimental Conditions

The Effect of pH

The pH of the medium has great effect on the luminescence intensity of the system. The experimental results showed that the luminescence intensity reached a maximum at pH 8.9. Therefore pH 8.9 was selected for further investigation using NH_4Cl–$NH_3{\cdot}H_2O$ buffer solution. When the volume of buffer solution added was 0.12 mL the luminescence intensity reached maximum.

The remarkable effect of pH occurring in the pH range of 8.0–10.0 was probably due to the cause of lactone

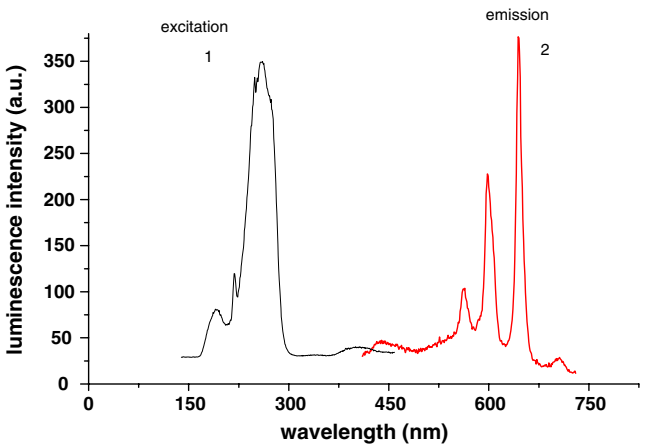

Fig. 3 Luminescence emission and excitation spectra of 1×10^{-4} mol L^{-1} of Sm^{3+} in the presence of 2×10^{-4} mol L^{-1} of DC in DMSO at $\lambda_{ex}/\lambda_{em}$=400/645 nm

isomerism of doxycycline molecule which is the favored formula for binding to Sm^{3+} ion in DMSO. Below this range of pH the doxycycline is an amphiprotic compound. In addition, dehydration of doxycycline takes place easily in a strongly acidic solution. It was also observed that the color of DC changed with increase of pH and/or time to yellowish brown [46].

The Effect of the Addition Order of Reagents

Adding the reagents in different orders has a great influence on the luminescence intensity. The experimental results indicate that the optimum luminescence intensity was obtained when solutions were added in the following order: DC, Sm^{3+} and buffer. So this order was chosen in all experiments.

The Effect of the Amount of DC and Sm^{3+}

The influence of the amount of DC on the luminescence intensity of the Sm^{3+} solution was studied (Fig. 4). The enhanced luminescent intensity increased at first and then remained constant with the increasing amount of DC. The experimental results showed that the luminescence intensity reached maximum and remained constant when 0.2 mL of 1.0×10^{-2} mol L^{-1} of DC solution was added. Therefore, this concentration was used for further study. Also the influence of the amount of Sm^{3+} ion on the luminescence intensity of the solution containing 2.0×10^{-4} mol L^{-1} of DC was studied under the conditions established above. The enhanced luminescent intensity increased with the increasing concentration of Sm^{3+} ion up to 1×10^{-4} mol L^{-1} and then decreased. When the concentration of Sm^{3+} ion was 1.0×10^{-4} mol L^{-1}, the composition ratio for the Sm^{3+} to DC in the Sm^{3+}-DC system was 1:2 Thus 1.0×10^{-4} mol L^{-1} Sm^{3+} ion (0.1 mL of Sm^{3+} ion) was used for further study

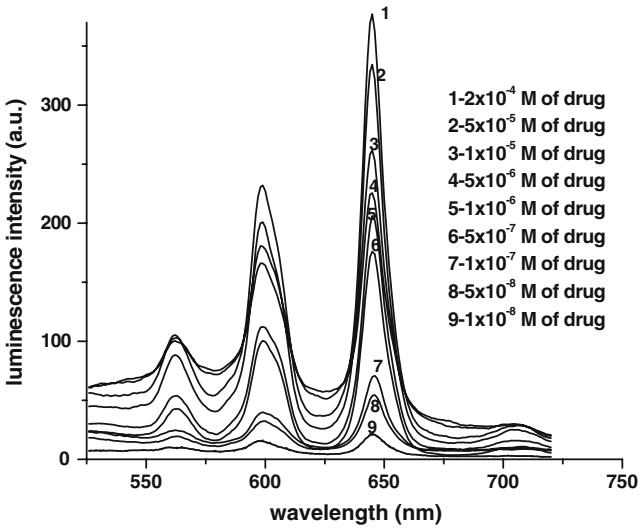

Fig. 4 Luminescence spectra of 1×10^{-4} mol L^{-1} of Sm^{3+} in the presence of different molar concentration of DC in DMSO at λ_{ex}=400 nm

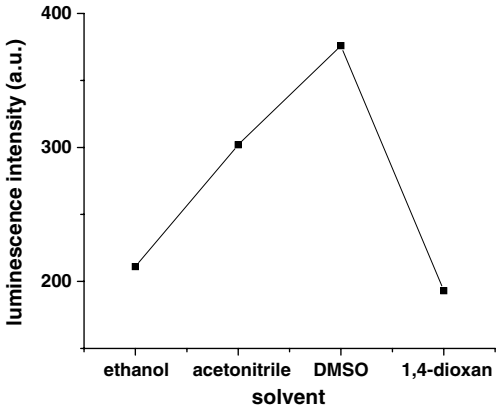

Fig. 5 Relationship between the intensity of luminescence and different solvents of 1×10^{-4} mol L^{-1} of Sm^{3+} in the presence of 2×10^{-4} mol L^{-1} of DC at λ_{ex}=400 nm

The Effect of Solvent

The influence of the solvent on the luminescence intensities of the solutions containing 2.0×10^{-4} mol L^{-1} of DC and 1.0×10^{-4} mol L^{-1} Sm^{3+} was studied under the conditions established above. The results show the enhanced emission of Sm^{3+}-DC in DMSO and CH_3CN as shown in Fig. 5.

This can be attributed to the formation of anhydrous solvates of Sm^{3+}-DC complex introducing solvent molecules in the first coordination sphere of Sm^{3+}-DC leading to the enhancement of the intensity of all transitions ($^4G_{5/2}\rightarrow{}^6H_{5/2}$= 564 nm, $^6H_{7/2}$=599 nm, $^6H_{9/2}$=643 nm, $^6H_{11/2}$=707 nm) especially $^4G_{5/2}\rightarrow{}^6H_{9/2}$ transition in Sm^{3+}.

By increasing the radiative rate, Sm^{3+} excited states will become less sensitive to deactivation processes, ultimately resulting in a more efficiently emissive Sm^{3+} complex [47]. Also, the luminescence intensities for the complexes in DMSO and CH_3CN solutions are 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

Table 1 Quantum yield values of 1×10^{-4} mol L^{-1} of Sm^{3+} in the presence of 2×10^{-4} mol L^{-1} of DC in different solvents, Φ (±5%)

Solvent	Quantum yield
Ethanol	0.01
Acetonitrile	0.04
DMSO	0.09
1,4-Dioxan	0.01

Table 2 Evaluation of intra-day and inter-day accuracy and precision

Sample	DC extracted[a]	Intra-day accuracy and precision (n=3)			Inter-day accuracy and precision (n=3)		
		DC Average Found ± CL[b]	%RE[c]	%RSD[d]	DC average found ± CL	%RE	%RSD
Doxymycine, (100 mg), Nile Co. Egypt	2.0	2.02±3.30	1.00	2.29	2.04±3.31	2.00	2.13
	4.0	3.95±2.72	1.25	2.23	4.05±2.68	1.25	2.12
	6.0	6.09±2.55	1.50	2.19	6.12±2.26	2.00	2.11
Vibramycine, (100 mg), Pfizer Co. Egypt	2.0	2.07±3.43	3.50	2.26	1.99±2.96	2.00	2.11
	4.0	4.02±2.80	0.50	2.21	4.04±2.83	1.00	2.06
	6.0	5.99±2.36	0.13	2.15	6.03±2.43	0.38	2.02
Tolexine, 50 mg, Alkan Pharma, Co. Egypt	5.0	5.07±5.56	1.40	2.36	4.89±5.64	2.20	2.31
	10.0	10.12±5.29	1.20	2.21	10.14±5.52	1.40	2.26
	20.0	19.69±5.19	1.55	2.05	20.43±5.37	2.15	2.12
Serum sample	10.0	10.25±3.48	2.50	2.36	9.89±4.45	1.10	2.71
	20.0	20.44±3.20	2.20	2.21	20.54±3.63	2.70	2.46
	30.0	30.30±2.91	1.00	2.13	30.43±3.43	1.43	2.22

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

[b] 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 and n = number of measurements

[c] % RE. Percent relative error

[d] % RSD. relative standard deviation

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 [48], Table 1 lists the values of the luminescence quantum yield in a variety of solvents.

Analytical Application

Linear Range and Limit of Detection

The six-point (10, 25, 50, 100, 200, 400 n mol L^{-1}) calibration curve was obtained by plotting the peak intensity at λ= 645 nm of Sm^{3+} ion on y axis against 1/quantum yield (x) of Sm^{3+} -DC complex. The six point (10, 25, 50, 100, 200, 400 n mol L^{-1}) calibration curve was obtained by plotting the peak intensity at λ=645 nm of Sm^{3+} against concentration of doxycycline. The concentrations of calibrated standards were analyzed and the linearity was evaluated by comparing the correlation coefficient (r) between theoretical and back-calculated concentrations of calibrated standard samples and the graph was described by the regression equation:

$$Y = a + bX$$

(where Y=luminescence intensity of the optical sensor at λ_{em}=645 nm; a = intercept; b = slope and X = concentration

Table 3 Method robustness and ruggedness expressed as intermediate precision (% RSD)

sample	DC extracted[a]	Robustness			Ruggedness
		Parameter altered			Inter-analysts, (%RSD) (n=3)
		Sm^{3+} Conc. (%RSD)	pH[b] (%RSD)	Reaction time[c]	
Doxymycine, (100 mg), Nile Co. Egypt	5.0	2.68	2.62	0.38	2.35
Vibramycine, (100 mg), Pfizer Co. Egypt	5.0	2.62	2.87	0.26	2.49
Tolexine, 50 mg, Alkan Pharma, Co. Egypt	10.0	2.48	2.21	0.60	2.14
Serum sample	10.0	2.78	2.55	0.66	2.05

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

[b] Concentrations of Sm^{3+} were 2, 5 and 6×10^{-5} mol L^{-1}; and the values of pH were 9.2, 9.4 and 9.8

[c] The reaction times studied were 10, 12 and 15 min

Table 4 Results of recovery study using standard addition method

Drug	Extracted ($\times 10^{-7}$ mol L^{-1})	Found ($\times 10^{-7}$ mol L^{-1})	Average[a]	Average recovery ± R.S.D. (%)	B.P. (LC)
Doxymycine, (100 mg), Nile Co. Egypt	0.5	0.52, 0.49, 0.51	0.50	100.1±2.66	97.5±2.0
	1.0	0.97, 1.05, 1.03	1.01		
	1.5	1.51, 1.48, 1.49	1.49		
Vibramycine, (100 mg), Pfizer Co. Egypt	0.5	0.54, 0.49, 0.51	0.51	101.6±2.86	98.5±1 .4
	1.0	1.02,1.03,1.05	1.03		
	1.5	1.52, 1.46, 1.51	1.50		
Tolexine, 50 mg, Alkan Pharma, Co. Egypt	0.5	0.44, 0.48, 0.52	0.48	98.3±4.50	98.0±2.4
	1.0	0.96, 0.98, 1.03	0.99		
	1.5	1.49,1.53,1.49	1.50		
Serum sample	0.5	0.49,0.53,0.52	0.51	99.2±2.67	99.3±1.4
	1.0	0.95, 0.99, 0.98	0.97		
	1.5	1.51, 1.45, 1.48	1.48		

[a] Average of nine measurements

in ng mL^{-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). The limit of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines [49] 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). Under the experimental conditions, there is a linear relationship between $1/\Phi$ of Sm^{3+} -DC complex and DC concentration in the range of 7×10^{-9} to 5×10^{-6} mol L^{-1} with a correlation coefficient of 0.999. The regression equation is $1/\Phi=3.9\times10^{8}\times$ Concentration (mol L^{-1}) + 31.5.

The dynamic range for the determination of DC based on the luminescence intensity was 1×10^{-8} to 5×10^{-6} mol L^{-1}. Limits of detection (LOD) and quantitation (LOQ) were calculated at pH 8.9 to be 6.5×10^{-10} and 2.2×10^{-9} mol L^{-1}, respectively. The low value of LOD indicate the high sensitivity of the proposed method.

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 levels of analyte. The results of this study are summarized in Table 2. The percentage relative standard deviation (% RSD) values were ≤2.48% (intra-day) and ≤2.46% (inter-day) indicating high precision of the method. Accuracy was evaluated as percentage relative error (RE) between the measured mean concentrations and the taken concentrations of melamine. Bias {bias% = [(Concentration found - known concentration) × 100/known concentration]} was calculated at each concentration and these results are also presented in Table 2. Percent relative error (%RE) values of ≤3.5% demonstrates the high accuracy of the proposed method.

Table 5 Comparison of spectrofluorimetric methods for the determination of DC

Method	Linear range (mol L^{-1})	Detection limit (mol L^{-1})	References
Spectrophotometry	1.7×10^{-7} – 1.7×10^{-6}	1.28×10^{-8}	[18]
Ion selective electrode	5.0×10^{-6} – 3.2×10^{-3}	2.4×10^{-6}	[35]
Potentiometry	1.0×10^{-5} – 1.0×10^{-2}	4.0×10^{-6}	[36]
HPLC	0.1×10^{-6} – 1.5×10^{-6}	0.04×10^{-6}	[10]
Flow injection with pulsed amperometric detection	1.0×10^{-6} – 0.1×10^{-3}	2.3×10^{-6}	[30]
Spectrofluorimetry	1.89×10^{-7} – 8.61×10^{-6}	7×10^{-8}	[21]
	2.0×10^{-8} – 1.0×10^{-5}	2.0×10^{-8}	[22]
Optical sensor	7.0×10^{-9} – 5.0×10^{-6}[a]	6.5×10^{-9}	Present work
	1.0×10^{-8} – 5.0×10^{-6} [b]		

[a] In the case of quantum yield calculations

[b] In the case of luminescence intensity calculations

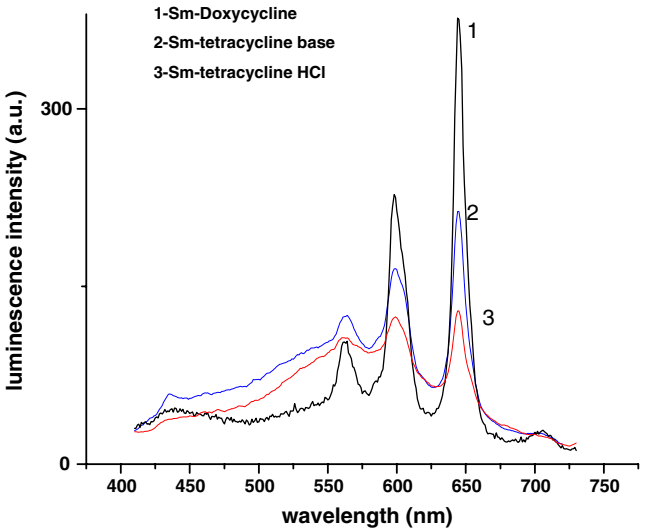

Fig. 6 Luminescence spectra of 2×10^{-4} mol L^{-1} of different Tetracycline with 1×10^{-4} mol L^{-1} of Sm^{3+} in DMSO at λ_{ex}=400 nm

Selectivity

The proposed method was tested for selectivity by placebo blank and synthetic mixture analysis. A placebo blank containing : starch (300 mg), lactose (30 mg), calcium carbonate (50 mg), calcium dihydrogen orthophosphate (20 mg), methylcellulose (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 DC in a synthetic mixture. To the placebo blank of similar composition, different amounts of DC were added, homogenized and the solution of the synthetic mixture was prepared as done under "analysis of samples". 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 99.30±2.65 and 98.60±2.85 for pharmaceutical tablets and serum sample, respectively. The results demonstrated the accuracy as well as the precision of the proposed method. These results complement the findings of the placebo blank analysis with respect to selectivity.

Robustness and Ruggedness

The robustness of the methods was evaluated by making small incremental changes in the concentration of Sm^{3+}, pH and contact time, and the effect of the changes was studied on luminescence intensity of the optical sensor (Table 3). The changes had negligible influence on the results as revealed by small intermediate precision values expressed as% RSD (≤ 2.87%). Method ruggedness was expressed as the RSD% of the same procedure applied by three different analysts. The inter-analysts RSD were within 2.41% for the same DC concentrations ranged from 2.05 to 2.49% suggesting that the developed method was rugged. The results are shown in Table 3.

Application to Samples

The developed method was applied to the determination of DC in pharmaceutical preparations as shown in Table 4. For the assay of DC, the samples must be diluted appropriately within the linear range of determination of DC and the sample solution was analyzed by the method developed above, using the standard calibration method. The average

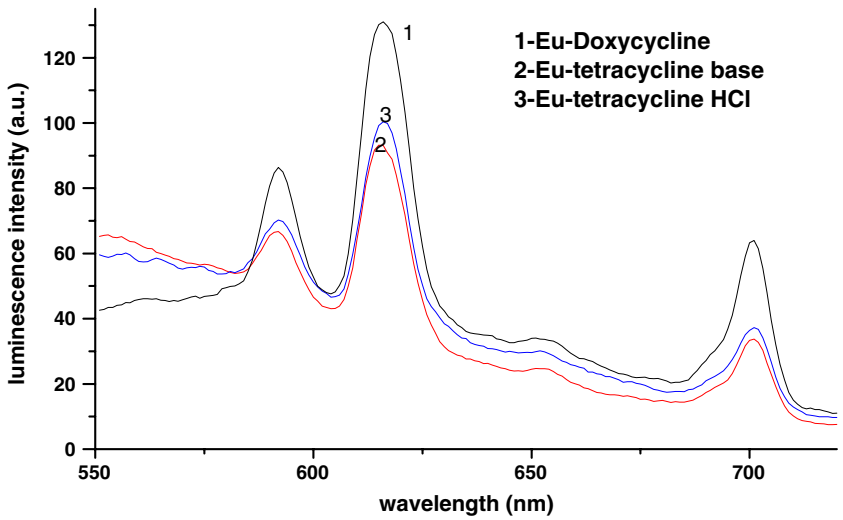

Fig. 7 Luminescence spectra of 2×10^{-4} mol L^{-1} of different Tetracycline with 1×10^{-4} mol L^{-1} of Eu^{3+} in DMSO at λ_{ex}=400

Table 6 The intensity ratio of the hypersensitive band to magnetic dipole band in case of Sm^{3+} and Eu^{3+} ions in the presence of different tetracyclines

Complex	$I_{H9/2}{}^{6}/I_{H7/2}{}^{6}$ ratio	$I_{F2}{}^{7}/I_{F1}{}^{7}$ ratio	Band width at 645 nm and 618 nm of Eu^{3+}	Height of band at 645 nm and 618 nm of Eu^{3+}
Sm^{3+}-doxycycline	1.62	–	55	376
Sm^{3+}-tetracycline –base	1.30	–	98	213
Sm^{3+}-tetracycline –HCl	1.05	–	141	129
Eu^{3+}-doxycycline	–	1.50	37	130
Eu $^{3+}$-tetracycline –base	–	1.40	75	100
Eu $^{3+}$-tetracycline –HCl	–	1.42	74	93

recovery and relative standard deviation (R.S.D) were (99.3% and 3.38%) respectively. Data obtained by Liquid Chromatography method of British Pharmacopoeia [50] (average recovery 98.5% and R.S.D 1.6%) were also presented for comparison and show a good correlation with those obtained by the proposed method. The developed method can be easily performed and afforded good precision and accuracy when applied to determination of DC in pharmaceutical preparations.

The developed method was also applied to the determination of DC in human serum sample. The experimental results in Table 4 show that an average recovery of 99.2% with relative standard of 2.67%, which indicates that the developed method can be easily performed and affords good precision and accuracy when applied to human serum sample.

By comparison with some existing methods, as shown in Table 5 the present method has the advantages in the following terms: high sensitivity, good stability and wide linear range.

Comparison Between Sm^{3+} and Eu^{3+} Ions Emission Spectra in the Presence of Tetracycline Derivatives

The higher intensity of the emission bands of Sm^{3+} in comparison with Eu^{3+} in the presence of DC and different

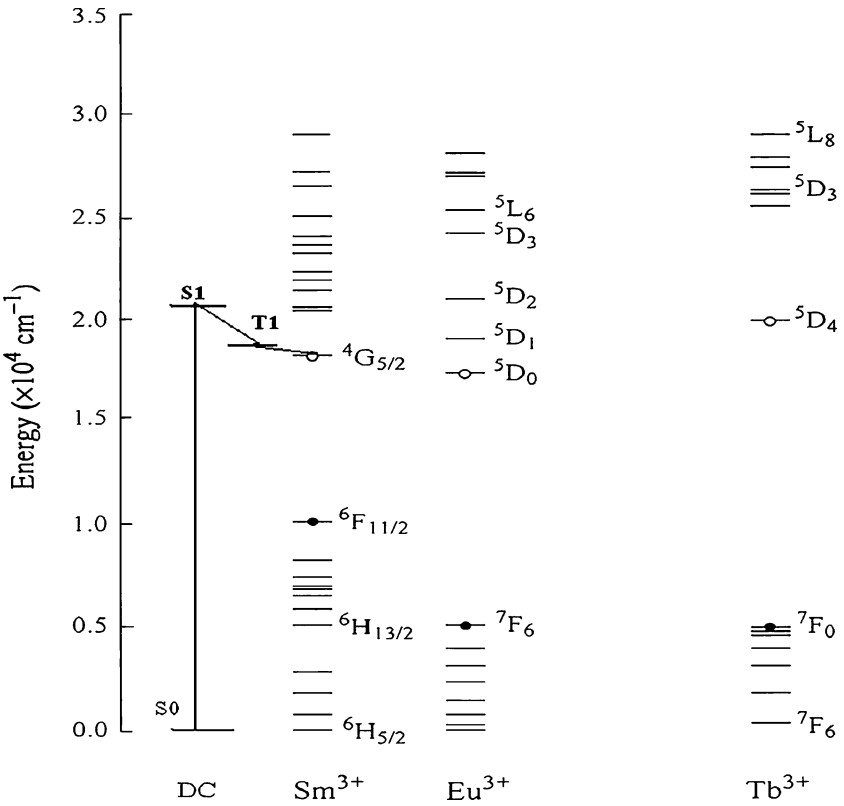

Fig. 8 The Sm^{3+} emitting state is very near from the triplet state of the drug therefore very efficient energy transfer takes place in case $Sm^{3+} > Eu^{3+}$ but in case Tb^{3+} the emitting state is higher than the triplet state of the drug therefore there is no energy transfer in case Tb^{3+}

tetracyclines (tetracycline base and tetracycline HCl) as in Figs. 6 and 7, show that the higher selectivity of Sm^{3+} to DC over other tetracycline. The comparison of the ratio between the intensity of the emission bands $^4G_{5/2} \rightarrow {}^6H_{9/2}$ at 643 nm and $^4G_{5/2} \rightarrow {}^6H_{7/2}$ at 599 nm and the ratio between the intensity of the emission bands $^5D_0 \rightarrow {}^7F_2$ at 617 nm and $^5D_0 \rightarrow {}^7F_1$ at 589 nm as incase of Eu^{3+} showed that the higher ratio was in case of Sm^{3+} and DC which means that the binding of Sm^{3+} ion to DC is stronger than other tetracyclines and stronger than Eu^{3+} ion with DC and other tetracyclines.

Where the highest relative intensities of $^4G_{5/2} \rightarrow {}^6H_{9/2}$ at 643 nm, $^5D_0 \rightarrow {}^7F_2$ at 617 nm exhibit magnetic dipole forbidden and electric-dipole allowed (hypersensitive transitions) [51], therefore the Sm^{3+} ion is more selective and more sensitive to DC over tetracycline derivatives and more selective and more sensitive over Eu^{3+} ion to DC and other tetracyclines (Figs. 6 and 7 and Table 6).

This can also be explained by Fig. 8 which indicates that the perfect energy transfer takes place from triplet state of DC to the excited state energy level of Sm^{3+} over Eu^{3+} but in case of Tb^{3+} no energy transfer can take place from DC to Tb^{3+} ion because the excited state energy level of Tb^{3+} is higher in energy than the triplet state of the DC.

Conclusion

The proposed method for determination of doxycycline hydrochloride offers simple, rapid and sensitive method for the analysis of doxycycline hydrochloride over concentration range of 7×10^{-9} – 5×10^{-6} mol L^{-1} in the case of quantum yield calculations and 1×10^{-8} – 5×10^{-6} mol L^{-1} in the case of luminescence intensity calculations and detection limit of 6.5×10^{-10} mol L^{-1}. The developed optical sensor is selective, accurate and attractive for routine control analysis of the drug.

The characteristic peaks of Sm^{3+} ion in Sm^{3+}-DC complex show high sensitivity and selectivity when compared with other peaks of tetracycline derivatives and can be used for the determination of DC in DMSO solvent and buffer solution of pH 8.9. The proposed method can therefore be successfully used for determination of doxycycline hydrochloride in pharmaceutical preparations and in serum samples.

References

1. Aronson AL (1980) J Am Vet Med Assoc 176:1061–1106
2. Cunha BA, Sibley CM, Ristuccia AM (1982) Ther Drug Monit 4:115–135
3. Riond JL, Riviere JE (1988) Vet Hum Toxicol 30:431–444
4. Shaw DH, Rubin SI (1986) J Am Vet Med Assoc 189:808–810
5. Riviere JE, Spoo JW (2001) Tetracycline antibiotics. In: Adams HR (ed) Veterinary pharmacology and therapeutics, 8th edn. Iowa State University Press, Ames, pp 828–840
6. Naidong W, Verresen K, Busson R, Roets E, Hoogmartens J (1991) J Pharm Biomed Anal 9:717–723
7. Monser L, Darghouth F (2000) J Pharm Biomed Anal 23:353
8. Croubels S, Vermeersch H, De Backer P, Santos MDF, Remon JP, Van Peteghem C (1998) J Chromatogr B 708:145
9. Kazemifard AG, Moore DE (1997) J Pharm Biomed Anal 16:689
10. Santos MD, Vermeersch H, Remon JP, Schelkens M, De Backer P, Ducatelle R, Haesebrouck F (1996) J Chromatogr B 682:301
11. Vienneau DS, Kindberg CG (1997) J Pharm Biomed Anal 16:111
12. Carson MC, Ngoh MA, Hadley SW (1998) J Chromatogr B 712:113
13. Nouws JFM, Loeffen G, Schouten J, Van Egmound H, Keukens H, Stegeman H (1998) J Dairy Sci 81:2341
14. Bryan PD, Stewart JT (1994) J Pharm Biomed Anal 12:675
15. Bryan PD, Stewart JT (1993) J Pharm Biomed Anal 11:971
16. Naidong W, Verresen K, Roets E, Hoogmartens J (1991) J Chromatogr 596:61
17. Hoogmartens J, Khan NH, Vanderhaeghe H, van der Leeden AL, Oosterbaan M, Veld-Tulp GL, Plugge W, van der Vlies C, Mialann D, Melamed R, Miller JHMB (1989) J Pharm Biomed Anal 7:607
18. Saha U, Sen AK, Das TK, Bhowal SK (1990) Talanta 37:1193
19. Lopez Paz JL, Martinez Calatayud J (1993) J Pharm Biomed Anal 11:1093
20. Espinosa-Mansilla A, Salinas F, De Orbe Paya I (1995) Anal Chim Acta 313:103
21. Salinas F, Munoz de la Pena A, Duran Meras I (1990) Anal Lett 23:863
22. Hu N, Wang D-Y, Zhao Y-B, Xu J-G (1996) Fenxi Shiyan Shi 15:46
23. Xie HZ, Dong CA, Jin WJ, Wei YS, Liu CS, Zhang SS, Zhou BL (1996) Anal Chim Acta 319:239
24. Naidong W, Greelen S, Roets E, Hoogmartens J (1990) J Pharm Biomed Anal 8:891
25. Naidong W, Hua S, Roets E, Hoogmartens J (1994) J Planar Chromatogr Mod TLC 7:297
26. Xie HZ, Dong CA, Fen YL, Liu CS (1997) Anal Lett 30:79–90
27. Bryan PD, Stewart JT (1994) J Pharm Biomed Anal 12:675
28. Croubeks S, Vermeersch H, De Backeer P, Ssantos MDF, Remon JP, van Peteyhem C (1998) J Chromatogr B 708:145
29. Farin D, Piva G, Gozlan I, Kitzes R (1998) Chromatographia 47:547
30. Izquierdo P, Gomez Hens A, Perez Bendito D (1994) Anal Lett 27:2303
31. Oungpipat W, Southwell-Keely P, Alexander PW (1995) Analyst 120:1959
32. Van Schepdael A, Kibaya R, Roets E, Hoogmartens J (1995) Chromatographia 41:367
33. Gong ZL, Zhang ZJ (1997) Anal Chim Acta 351:205
34. Liu WH, Wang Y, Tang JH, Shen GL, Yu RQ (1998) Analyst 123:365
35. Shoukry AF, Badawy SS (1987) Microchem J 36:107
36. Yao SZ, Xiao J, Nie LH (1988) Acta Pharm Sin 23:12
37. Novak-Pekli M, Nagy A, Nagy G (1995) Magy Kem Foly 101:12–16, cited from Analytical Abstracts, 57 (9): 9G39
38. Attia MS (2009) Spectrochim Acta Part A 74:972–976
39. Attia MS (2010) J Pharm Biomed Anal 51:7–11
40. Attia MS, Othman AM, Aboaly MM, Abdel-Mottaleb MSA (2010) Anal Chem 82(14):6230–6236
41. Attia MS, Aboaly MM (2010) Talanta 82:76–82
42. Files LA, Hirschy LM, Winefordner JD (1985) J Pharm Biomed Anal 100:95

43. Jee RD (1995) Analyst 120:2867
44. Arnaud N, George J (2001) Analyst 126:694
45. Millar JN (1981) Standards in fluorescence spectrometry. Chapman and Hall Ltd, USA, p 44
46. Li ZH (1993) Yaowu Huaxie (Chinese), 3rd edn. Chinese People Hygene's Publishing House, Beijing, p 423
47. Attia MS, Bakir E, Abdel-aziz AA, Abdel-mottaleb MSA (2011) Talanta 84:27–33
48. Attia MS, Othman AM, Elraghi E, Aboul-Enein HY (2011) J Fluoresc. doi:10.1007/s10895-010-0764-4
49. International Conference on Hormonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R 1), Complementary Guideline on Methodology dated 06 November 1996, incorporated in November 2005, London
50. British Pharmacopoeia (1999) vol. II, Her Majesty's Stationary Office, London, pp 2705
51. Cybinska, Legendziewicz J, Trush J, Reisfeld V, Saraidarov R (2008) J Alloys Compd 451:94