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Dysprosium-sensitized chemiluminescence system for the determination of enoxacin in pharmaceutical preparations and biological fluids with flow-injection sampling

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A novel trivalence dysprosium(Dy^{3+})-sensitized chemiluminescence method was developed for the first time for the determination of enoxacin (ENX) using flow-injection sampling based on the chemiluminescence (CL) associated with the reaction of the Dy^{3+}-cerium(Ce(IV))-$S_2O_3^{2-}$-ENX system and the Dy^{3+}-MnO_4^- $S_2O_3^{2-}$-ENX system. The analytical conditions for CL emission were investigated and optimized. The relationship between the CL intensity of ENX and its concentration has good linearity, with a correlation coefficient of 0.9984–0.9994. The limit of detection (LOD, 3σ) was 0.20 ng/mL for the Dy^{3+}-ENX-$S_2O_3^{2-}$-Ce(IV)-H_2SO_4 system and 0.22 ng/mL for the Dy^{3+}-ENX-$S_2O_3^{2-}$-MnO_4^--HNO_3 system. The relative standard deviation (RSD, $n = 11$) was 1.8% for 11 determinations of 60 ng/mL ENX. The proposed method was applied to the analysis of ENX in injections, serum and urine samples with a recovery of 98%–105%. A possible mechanism for this sensitized CL reaction is discussed by comparing the CL spectra with the fluorescence emission spectra. The proposed method represents a wide linear range, high sensitivity and accuracy, and can be used for the routine determination of ENX in pharmaceutical preparations and biological fluids.

Keywords: biological fluids; dysprosium-sensitized chemiluminescence; enoxacin; pharmaceutical analysis

Introduction

Enoxacin (ENX), 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphtyridine-3-carboxylic acid, is a new addition to the class of 4-quinolone antibacterial drugs. It has a broad spectrum of *in vitro* antibacterial activity and is particularly potent against Gram-negative organisms and staphylococci.[1,2] The mechanism of this effect is based on the inhibition of the DNA-gyrase of bacteria and RNA and protein synthesis. ENX has been used in the treatment of systemic infections including urinary tract, respiratory, gastro-intestinal and skin infections. The quality control of ENX dosage and its monitoring in biological fluids using quick automated techniques is of importance.

The *Chinese Pharmacopoeia* describes an UV spectrophotometric method for the determination of ENX.[3] A variety of techniques have been reported for the determination of ENX in pharmaceutical preparations and environmental and biological samples, such as electrochemical analysis,[4] spectrophotometry,[5] fluorimetry,[6–11] liquid chromatography(LC),[12–23] capillary electrophoresis (CE),[24,25] and chemiluminescence (CL).[26,27] (Table 1).

Among these methods, high-performance liquid chromatography (HPLC) was used widely for the simultaneous determination of fluoroquinolones (FQNs) because of its good separation performance. Molecularly imprinted matrix solid-phase dispersion (MI-MSPD),[16] ultrasonic-assisted extraction,[17] and accelerated solvent-extraction procedures were coupled with HPLC[28] to increase detectability. Solid-phase microextraction (SPME)–LC–MS/MS was used for the determination of five fluoroquinolones including ENX in river waters and 11 (fluoro)quinolones in swine kidneys with lower LOD.[22,23] Like HPLC, CE was used for the analysis of several quinolones including ENX in pig plasma and porcine tissue.[24,25] Capillary electrophoresis and HPLC have the advantage of high separation capability suitable for multicomponent determination, but there are some problems, including the complexes of the procedures and complicated lower sensitivity.

Chemiluminescence methods have the advantages of simplicity, rapidity and high sensitivity and have been used for the analysis of pharmaceutical compounds. A CL method based on the effect of terbium(III) on the CL reaction of potassium permanganate and sodium sulfite has been described for the determination of ofloxacin and ENX in dosage forms and urine samples.[26] $Ru(bipy)_3^{2+}$ has been used as an enhancer for the determination of ENX giving an LOD of 21 ng/mL.[27] Several researches on enhanced CL for potassium permanganate system has been reported.[29] A review of the CL reactions in the systems containing lanthanide ions as emitters and different chemical compounds has been presented.[30]

Our objective was to develop a new CL detection system for the determination of fluoroquinolones. We found that Dy^{3+} has a sensitizing effect on the CL of some fluoroquinolone drugs in Ce(IV)-$Na_2S_2O_3$ and $KMnO_4$-$Na_2S_2O_3$ systems. A novel Dy^{3+}

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Table 1. The abstract of analytical methods for the determination of ENX

Analyte	Matrix	Method	Linear range (ng/mL)	LOD (ng/mL)	Reference Number
ENX	Urine, serum	Electroanalysis	5.1–5100	2.0	[4]
ENX	Pharmaceutical preparation	Spectrophotometry	2,000–20,000 for bromophenol blue	0.84	[5]
ENX	Human plasma	Fluorimetry		100	[6]
Tree FQNs	Urine	Fluorimetry	up to 300 for ENX	10 for ENX	[7]
ENX	Serum, urine	Fluorimetry	100–13000	2.0	[8]
ENX	Pharmaceutical formulation	Fluorimetry	64–32000	9.6	[9]
Four FQNs	Pharmaceutical preparations	Spectrofluorimetry	28.5–700 for NX	8.5 for ENX	[10]
ENX		Fluorimetry	1.6–640	0.9	[11]
Tree FQNs	Blood, serum	HPLC[a]		0.02 ng per 20 μL injection for ENX	[12]
Four FQNs	Urine, serum	HPLC		7.3 for ENX	[13]
ENX, 4-oxo-ENX	Plasma, prostatic tissue	HPLC	20–1000 for Plasma,	10 for ENX	[14]
Two FQNs	Serum	Micellar-LC	100–5000	25 for ENX	[15]
Six FQNs	Serum	MIMSD[b] -HPLC	50–100000		[16]
Ten quinolones	Soil	HPLC		1000 ng/g	[17]
Four FQNs	Urine, serum	HPLC		15 for ENX	[18]
Four FQNs	Urine, serum	HPLC		7.3 for ENX	[19]
ENX, ciprofloxacin	Serum	Micellar LC	100–5000	25 for ENX	[20]
ENX, theophylline, ciprofloxacin	Plasma, saliva	HPLC	50–10000 for ENX		[21]
Five FQNs	River water	SPME–LC–MS/MS[c]	0.1–10	0.07–0.29	[22]
Eleven FQNs	Swine kidney	LC – MS/MS		<25 ng/g	[23]
Ten quinolones	Pig plasma	SPE[d]-CE	500–20000	1100–2400	[24]
Seven FQNs	Porcine tissue	CE	500–100000	2 3 ng/g for ENX	[25]
ENX, ofloxacin	Dosage form, urine	CL	0.26–3200 for ENX	0.077 for ENX	[26]
ENX	Pharmaceutical formulation, human serum	CL	640–64060	21	[27]

[a] High-performance liquid chromatography
[b] molecularly imprinted matrix solid-phase dispersion,
[c] solid-phase microextraction-liquid chromatography-tandem mass spectrometry,
[d] solid-phase extraction

sensitized CL detection method combined with flow injection sampling was successfully applied for the determination of ENX in injections, serum and urine samples.

Materials and Methods

Chemicals

All chemicals were of analytical reagent grade and deionized water was used throughout. A stock standard solution (500 μg/mL) of ENX (Institute of Medical Biotechnology, Beijing, China) was prepared by dissolving 25.00 mg ENX in 1.5 ml 0.1 M sodium hydroxide and diluting with deionized water to 50 mL. More dilute solutions were freshly prepared by diluting the stock solution with deionized water. Enoxacin injection samples were provided by Yuanda Pharmaceuticals Co., Ltd (Wuhan, China). A stock solution of the Dy^{3+} (10 mM) was prepared by dissolving 373 mg Dy_2O_3 in 15.0 mL HCl (11.6 M) at 95 °C, evaporating the solution until it was almost dry, then diluting it to 100 mL with deionized water. A stock solution of Ce(IV) (10 mM) was prepared by dissolving 0.6686g $Ce(NH_4)_2SO_4 \cdot 4H_2O$ in 4 mL (v/v) sulfuric acid and diluting to 100 mL. Stock $KMnO_4$ solutions (50 mM) and a $Na_2S_2O_3$ solutions (2 mM) were prepared daily and diluted as required.

Apparatus

The MPI-B flow injection analysis system (Xi'an Remex Electronic Science-tech Company, China) used in this sample, automatically operated by a computer, consists of two peristaltic pumps working at a constant flow rate (30 rpm) and a six-way injection valve with a sample loop (120 μL). Polytetrafluoroethylene (PTFE) tubing (0.8 mm i.d.) was used to connect all components in the flow system. The Ce(IV) (or MnO_4^-) solution and the Dy^{3+} solution were mixed through a three-way pipe and the mixture flowed into a flow cell. A mixture of sample and $Na_2S_2O_3$ solution was injected from a sample valve. An analyzer recorded the signal from the CL reaction. A UV-265 spectrophotometer (Shimadzu, Japan) was used to

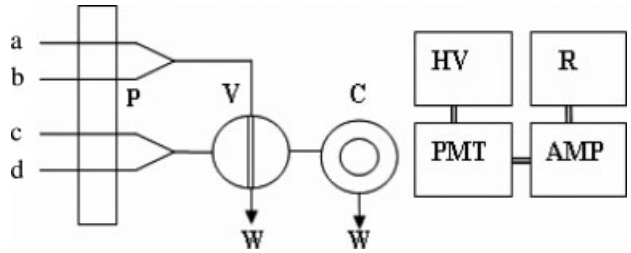

Figure 1. Schematic diagram of flow injection CL analysis system. P – peristaltic pump; V – sampling inlet valve; C – flow cell; PMT – photomultiplier tube; AMP – amplifier; HV – high voltage; R – recorder; W – waste; a – ENX solution; b – $Na_2S_2O_3$ solution; c – Ce(IV) (or MnO_4^-) solution; d – Dy^{3+} solution.

record UV spectra for the determination of ENX. Fluorescence spectra were recorded with RF-5301PC spectrofluorophotometer (Shimadzu, Japan). The flow-injection CL analysis system for the determination of ENX is shown in Figure 1.

Sample preparation

The injection sample of ENX was five bottles of ENX vials randomly prepared from the same batch. The working solutions were directly diluted with deionized water.

Human serum was taken from the Hebei University Hospital, and urine samples were kindly provided by a healthy volunteer. A 1 mL volume of serum sample was deproteinized by adding 4.0 mL 10% trichloroacetic acid (CCl_3COOH) in a tube, which was then centrifuged at 2610 g-force for 15 min. The supernatant was diluted with deionized water as to make a concentration of ENX in the linear part of the assay range. No further pre-treatment was required for urine samples.

Procedure

As shown in Figure 1, all solutions were continuously pumped through the system. A 120 μL ENX solution and $Na_2S_2O_3$ solution were injected into a mixed stream of Ce(IV) (or MnO_4^-) and Dy^{3+} solutions. The mixed solution was transferred into the CL flow cell where an intensive CL signal was immediately observed. The signal from the CL reaction was recorded. Calibration graphs were constructed by plotting the intensity (peak height) of the CL signal versus the concentration of analyte.

Results and Discussion

Effect of sample volume and flow rate

The role of sample volume and flow rate is critical; for example, if the sample volume was too small or too large, a CL maximum could not be obtained. The highest emission was observed when the injected sample volume was 120 μL. The CL intensity increased with increasing flow rate. However, a flow rate of 3.0 mL/min for all solutions is recommended because of greater precision and economy in the use of reagents.

Effect of sensitizers

The four systems, Ce(IV)-$S_2O_3^{2-}$, Ce(IV)-$S_2O_3^{2-}$-ENX, MnO_4^--$S_2O_3^{2-}$ and MnO_4^--$S_2O_3^{2-}$-ENX, could produce only weak CL emission. The effect of various fluorescence compounds, such as rhodamine 6G, rhodamine B, eosin and fluorescein, on CL emission was investigated. No enhancing effect was observed. Based on the potential fluorescence properties of lanthanide ions,[31] lanthanum (La^{3+}) and lutetium (Lu^{3+}) (no fluorescence), gadolinium (Gd^{3+}) (weak fluorescence), samarium (Sm^{3+}), europium (Eu^{3+}) and dysprosium (Dy^{3+}) (strong fluorescence) and praseodymium (Pr^{3+}), neodymium (Nd^{3+}), holmium (Ho^{3+}), erbium (Er^{3+}), thulium (Tm^{3+}) and ytterbium (Yb^{3+}) (low fluorescence efficiency), respectively, for the CL systems of Ce(IV)-$S_2O_3^{2-}$-ENX and MnO_4^--$S_2O_3^{2-}$-ENX. The experimental results indicated that only Dy^{3+} obviously enhanced the CL signal of Ce(IV)-$S_2O_3^{2-}$-ENX and MnO_4^--$S_2O_3^{2-}$-ENX systems.

The effect of Dy^{3+} concentration on the CL intensity was studied in the range 100 μM to 1.1 mM, as shown in Figure 2. The effect of Dy^{3+} concentration on the CL intensity for the MnO_4^--$S_2O_3^{2-}$ ENX system was more obvious than that for the Ce(IV)-$S_2O_3^{2-}$ ENX system. The maximum CL intensity was obtained when the Dy^{3+} concentration was 1 mM for Ce(IV)-$S_2O_3^{2-}$-ENX and 200 μM for MnO_4^--$S_2O_3^{2-}$-ENX. From the cheaper and sensitive point of view, the Dy^{3+} working concentration was fixed at 600 μM for Ce(IV)-$S_2O_3^{2-}$-ENX and 200 μM for MnO_4^--$S_2O_3^{2-}$-ENX for further works.

Effect of acid medium

The nature and the concentration of the acid used in the reaction have a very significant influence on the CL emission intensity. Therefore, several acids, such as HCl, H_2SO_4, HNO_3, H_3PO_4 and $H_6P_4O_{13}$ were added to the Ce(IV) and the $KMnO_4$ solutions to test the effect of the acid on the CL signal. The highest stable emission was observed from H_2SO_4-treated Ce(IV) solutions and HNO_3-treated MnO_4^- solutions. Hence, H_2SO_4 and HNO_3 were chosen for the Dy^{3+}-Ce(IV)-$S_2O_3^{2-}$-ENX system and the Dy^{3+}-MnO_4^--$S_2O_3^{2-}$-ENX system, respectively. The effect of acid concentration on the CL intensity was investigated in the range of 5 μM to 100 μM range, as shown in Figure 3. The optimal concentrations of acid medium were 60 μM H_2SO_4 and 10 μM HNO_3.

Effect of oxidant concentration

In the Dy^{3+}-Ce(IV)-$S_2O_3^{2-}$-ENX system and the Dy^{3+}-MnO_4^--$S_2O_3^{2-}$-ENX system, Ce(IV) and MnO_4^-, respectively, were used as

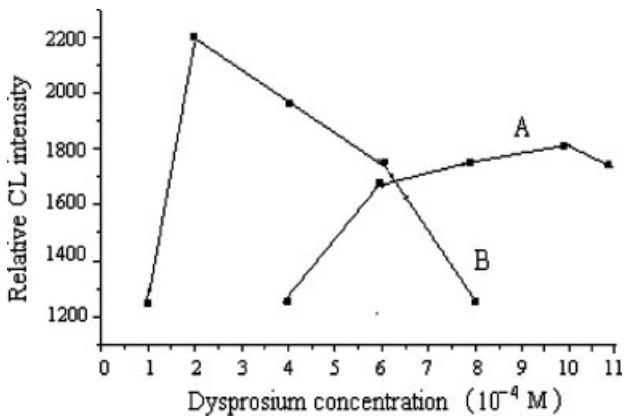

Figure 2. Effect of Dy^{3+} concentration on relative CL intensity for Ce(IV)-$S_2O_3^{2-}$ ENX-H_2SO_4(A) and MnO_4^--$S_2O_3^{2-}$ ENX-HNO_3(B). A: Ce(IV): 750 μM; $Na_2S_2O_3$: 100 μM; H_2SO_4: 60 μM; ENX: 1 μg/mL. B: $KMnO_4$: 75 μM; $Na_2S_2O_3$: 100 μM; HNO_3: 10 μM; ENX: 1 μg/mL.

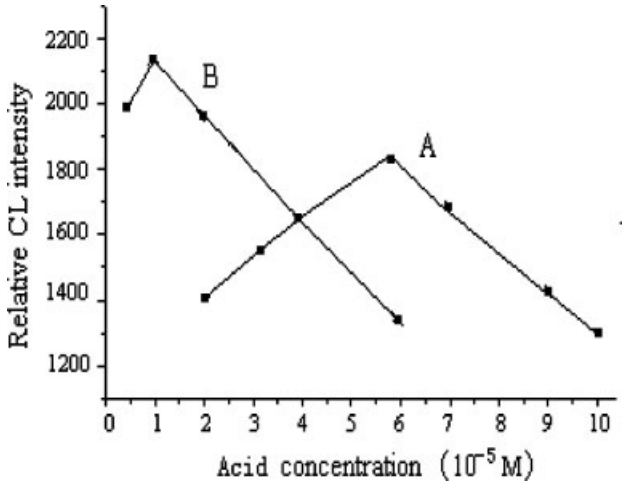

Figure 3. Effect of acid concentration on relative CL intensity for Dy^{3+}-Ce(IV)-$S_2O_3^{2-}$ ENX-H_2SO_4(A) and Dy^{3+}-MnO_4^--$S_2O_3^{2-}$ ENX-HNO_3(B). A: Dy^{3+}: 600 μM; Ce(IV): 750 μM; $Na_2S_2O_3$: 100 μM; ENX: 1 μg/mL. B: Dy^{3+}: 200 μM; $KMnO_4$: 75 μM; $Na_2S_2O_3$: 100 μM; ENX: 1 μg/mL.

the oxidants. The concentration not only had an effect on the sensitivity but also influenced the linear range for the assay. The dependence of the oxidant concentration on the CL intensity was investigated for 1.0 μg/mL ENX. The results showed that the maximum CL intensity was obtained when the oxidant concentration was 750 μM, which was used for further investigation.

Effect of sodium thiosulfate concentration

The effect of sodium thiosulfate concentration on CL intensity was examined over the range of 25 μM to 250 μM for Dy^{3+}-Ce(IV)- $S_2O_3^{2-}$-ENX and Dy^{3+}-MnO_4^--$S_2O_3^{2-}$-ENX systems. When the concentration of sodium thiosulfate was in the range of 25 μM–100 μM, CL intensity increased with the increase of sodium thiosulfate. However, CL intensity decreased with sodium thiosulfate concentration greater than 1×10^{-4} M. Therefore, a 1.0×10^{-4} M sodium thiosulfate was adopted for further work.

Interference studies

The influence of some common excipients used in drugs, metal ions in human body and several organic compounds on the CL intensity was investigated for the determination of ENX. Tolerance content was defined as the amount of foreign species that produced an error not exceeding ±5% in the determination of 0.4 ng/mL ENX. The tolerance content for Dy^{3+}-Ce(IV)-$S_2O_3^{2-}$-ENX system was as follows: 100-fold lactose, sucrose, starch, dextrin, galactose, glucose, fructose, EDTA, 25-fold sodium benzoate, polyglycol, 15-fold sodium citrate, 5-fold ascorbic acid, 100-fold Zn^{2+}, Ba^{2+}, Ca^{2+}, 50-fold Ni^{2+}, Mg^{2+}, 25-fold Co^{2+}, 10-fold Fe^{3+} and equal amounts Fe^{2+}. The tolerance content for Dy^{3+}-MnO_4^--$S_2O_3^{2-}$-ENX system was as follows: 50-fold sucrose, lactose, fructose, 25-fold dextrin, galactose, 10-fold starch, glucose, EDTA, polyglycol, 5-fold sodium benzoate and sodium citrate, and 50-fold Zn^{2+}, Ni^{2+} and Ca^{2+}.

Kinetic characteristics of the CL reaction

The kinetic characteristics of the CL reactions for the two systems were studied in detail. It was found that the reaction rate in solution was very fast – only 3 s and 2.5 s were needed from reagent mixing to a peak maximum appearing, and it took 9.5 s and 9 s for the signal to return to zero again for the Dy^{3+}- ENX-$S_2O_3^{2-}$-Ce(IV)-H_2SO_4 system and the Dy^{3+}-ENX-$S_2O_3^{2-}$-MnO_4^--HNO_3 system, respectively.

Performance of the system for ENX measurements

Under optimum conditions, described above, the linearity of the calibration graph was investigated in the range from 1.0 to 10 000 ng/mL. The calibration graph of ENX was divided into four plots in order to improve the accuracy of measurement. The experimental results are listed in Table 2.

The two proposed CL systems have good linearity. For the Dy^{3+}-ENX-$S_2O_3^{2-}$-Ce(IV)-H_2SO_4 system and the Dy^{3+}-ENX-$S_2O_3^{2-}$-MnO_4^--HNO_3 system, the LOD (3σ) for the first equation was 0.20 ng/mL and 0.22 ng/mL, respectively. This is much lower than that for electroanalysis,[4] spectrophotometry,[5] fluorimetry,[6–11] and LC,[12–15,18–21] CE,[24,25] and CL.[27] The relative standard deviation (RSD) for this method was 1.8% for 11 determinations of 60 ng/mL ENX.

Sample analysis

To evaluate the validity of the proposed method for the determination of ENX in pharmaceutical preparation, recovery was investigated on samples to which known amounts of ENX were added. The results are given in Table 3.

The proposed method was applied for the determination of ENX in the injections. The results agree well compared to all UV-method published in the Chinese Pharmacopoeia; as displayed in Table four.[3] There was no significant difference between the labeled contents and the results obtained by the proposed method.

Table 2. The regression equations for CL detection of ENX

CL system	Regression equation	Correlation coefficient	Linearity range (ng/mL)
Dy^{3+}-ENX-$S_2O_3^{2-}$ - Ce(IV)-H_2SO_4	$I = 1.2C + 10.0$	0.998	1.0–10
	$I = 6.6C + 15.8$	0.999	10–100
	$I = 68.4C + 13.8$	0.999	100–1000
	$I = 579.1C + 217.9$	0.999	1000–10000
Dy^{3+}-ENX-$S_2O_3^{2-}$-MnO_4^--HNO_3	$I = 6.5C + 50.0$	0.999	1.0–10
	$I = 19.2C + 98.4$	0.999	10–100
	$I = 256.4C + 6.6$	0.999	100–1000
	$I = 590.9C + 2134.4$	0.998	1000–8000

Table 3. Recovery experiments

CL system	Content (ng/mL)	Added (ng/mL)	Found (ng/mL)	Recovery n = 7 (%)
Dy^{3+}-ENX-$S_2O_3^{2-}$-Ce(IV)-H_2SO_4	0.036	0.01	0.046	100
		0.03	0.067	103
		0.05	0.085	98.0
	0.239	0.20	0.433	97.0
		0.40	0.648	102
		0.60	0.863	104
Dy^{3+}-ENX-$S_2O_3^{2-}$-MnO_4^--HNO_3	0.028	0.01	0.038	100
		0.03	0.058	100
		0.05	0.079	102
	0.235	0.20	0.432	98.5
		0.40	0.640	101
		0.60	0.866	105

Table 4. Determination results of ENX in the injections

Batch number	Labeled (mg/100 mL)	Proposed method[a] (mg/100 mL)	RSD[a] %	Proposed method[b] (mg/100 mL)	RSD[b] (%)	UV method (mg/100 mL)
050 101	200	206	1.8	208	2.0	206
041 201	200	205	1.7	204	1.7	203

[a] Dy^{3+}-ENX-$S_2O_3^{2-}$-Ce(IV)-H_2SO_4 system, n = 7;
[b] Dy^{3+}-ENX-$S_2O_3^{2-}$-MnO_4^--HNO_3 system, n = 7

Table 5. Determination of spiked serum and urine samples

Sample	Added (ng/mL)	Found (ng/mL)	Recovery (%)	RSD n = 5 (%)
Serum	0.06	0.059	98.3	1.7
	0.60	0.613	102.2	1.8
Urine	0.04	0.039	97.5	1.6
	0.40	0.385	96.3	1.8

Enoxacin has been found in body tissues, blood, serum and urine a few hours after oral administration. A single oral dose of 400 mg gave peak serum level of 0.3 mg/L.[6] In order to bring the sample concentration of the drug within our assay working range of determination, the serum sample was diluted appropriately. The standard addition method was used to avoid matrix effects. The urine samples were diluted properly and analyzed by the standard addition method. The results obtained with Dy^{3+}-ENX-$S_2O_3^{2-}$-Ce(IV)-H_2SO_4 system are given in Table 5. Recovery was in the range of 98.3%–102%. The relative standard deviation was 1.8% for five determinations of spiked serum and urine samples.

CL mechanism

The oxidation of SO_3^{2-} with Ce(IV) or MnO_4^- in acid solution is a well-known CL reaction. The CL emission was attributed to the formation of excited SO_2^* species which emit during relaxation.[32] However, the CL intensity was very weak because of the low fluorescence efficiency of SO_2^*. By introducing a fluorophore whose absorption falls in the emission range of the excited sulfur dioxide (300 nm – 450 nm),[33] the CL intensity can be enhanced through energy transfer from SO_2^* to the fluorophore.[34] $Na_2S_2O_3$ in an acidic medium reacts to produce HSO_3^-. Nevertheless, when Dy^{3+} alone or ENX alone was added to either of the CL systems, Ce(IV)-$S_2O_3^{2-}$ and MnO_4^--$S_2O_3^{2-}$, no notable increase in the CL intensity could be observed. However, when Dy^{3+} and ENX were added together to the CL systems Ce(IV)-$Na_2S_2O_3$ and MnO_4^--$Na_2S_2O_3$, the CL intensity was greatly enhanced.

In order to gain a better understanding of the nature of the CL enhancement, the fluorescence emission spectra of the two systems are same, as shown in Figure 4.

The native fluorescence emission of ENX shows a broad peak at 448 nm. When mixing with Dy^{3+}, this broad emission band decreases considerably in intensity with the appearance of two sharp emission peaks at 482 nm and 578 nm, corresponding to the transitions of the $Dy^{3+4}F_{9/2} \rightarrow {}^6H_{15/2}$ and $^4F_{9/2} \rightarrow {}^6H_{13/2}$,

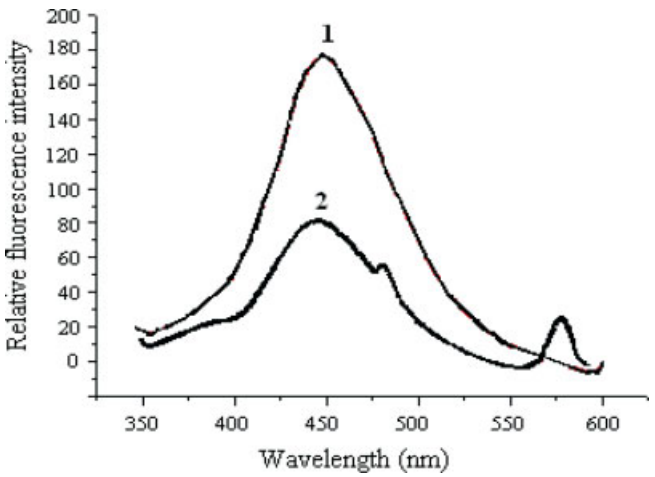

Figure 4. Fluorescence emission spectra of ENX (B) and Dy^{3+}-ENX(C). λ_{ex} = 303 nm: Dy^{3+}: 200 μM, ENX: 0.2 μg/mL; flow rate: 3.0 mL/min.

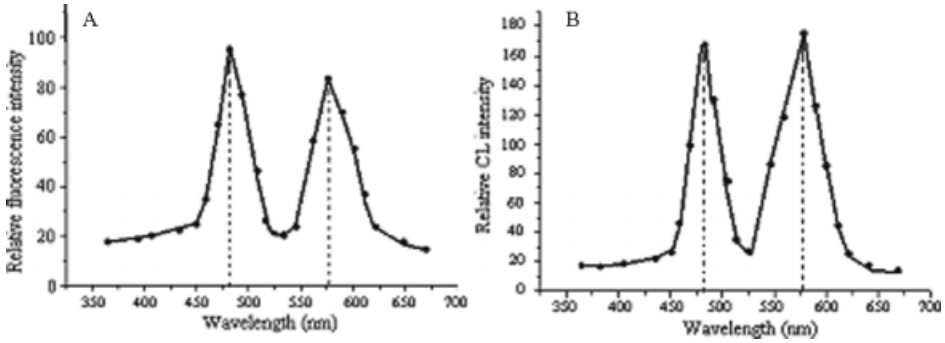

Figure 5. CL spectra of Dy^{3+}-ENX -Ce(IV)-$Na_2S_2O_3$-H_2SO_4 system (A) and Dy^{3+}-ENX- $KMnO_4$-$Na_2S_2O_3$-HNO_3 system(B). A: Ce(IV): 750 μM; $Na_2S_2O_3$: 100 μM; Dy^{3+}: 6 μM; H_2SO_4: 60 μM: ENX: 1 μg/mL. B: $KMnO_4$: 75 μM; $Na_2S_2O_3$: 100 μM; Dy^{3+}: 200 μM; HNO_3: 10 μM; ENX: 1 μg/mL.

respectively. The formation of a Dy^{3+}-ENX complex[35] is implied with intramolecular energy transfer between ENX and the Dy^{3+}.[36] The CL spectra of the two systems are shown in Figure 5.

Enoxacin fluorescence is absent, replaced by chemically sensitized emission features located at 482 nm and 578 nm, which are characteristic of the Dy^{3+} fluorescence spectrum.[37] Clearly the excited Dy^{3+} ion is the emitter, and there must be energy transfers in the two systems. The following possible mechanism is proposed. Dy^{3+} forms a chelate with ENX; an intermolecular energy transfer takes place from SO_2^* to the ligand (ENX) in the (Dy^{3+}-ENX) complex, and Dy^{3+}-ENX* is produced. Then, through intramolecular energy transfer from ENX* to Dy^{3+}, Dy^{3+*}-, ENX is formed, followed by the narrow characteristic emission of Dy^{3+*}. The mechanism stated above can be expressed chemically as follows:

$$S_2O_3^{2-} + H^+ \rightarrow HSO_3^-$$

$$Ce(IV) + HSO_3^- \rightarrow Ce(III) + HSO_3^* \text{ or } MnO_4^- + HSO_3^- \rightarrow MnO_4^{2-} + HSO_3^*$$

$$2\,HSO_3^* \rightarrow S_2O_6^{2-} + 2\,H^+$$

$$S_2O_6^{2-} \rightarrow SO_4^{2-} + SO_2^*$$

$$SO_2^* + [Dy^{3+}\text{-ENX}] \rightarrow SO_2 + [Dy\text{-ENX}^*]^{3+}$$

$$[Dy\text{-ENX}^*]^{3+} \rightarrow [Dy^*\text{-ENX}]^{3+}$$

$$[Dy^*\text{-ENX}]^{3+} \rightarrow [Dy\text{-ENX}]^{3+} + h\nu$$

Conclusion

This paper, for the first time, reports two CL systems using Dy^{3+} as a sensitizer for the determination of ENX. The CL spectra are from the narrow characteristic emission lines of Dy^{3+} at 482 and 578 nm ($^4F_9 \rightarrow {}^6H_{15/2}$, $^4F_9 \rightarrow {}^6H_{13/2}$) through an energy transfer from the excited SO_2^* to the analyte, followed by intramolecular energy transfer from the analyte* to Dy^{3+}. The proposed method has high detectability and wider linear range, and can be used for the routine determination of ENX in pharmaceutical preparations and biological fluids.

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