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An application of Ag(III) complex chemiluminescence system for the determination of enoxacin in capsule and biological fluid

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Ag(III) complex chemiluminescence (CL) system was applied for the determination of enoxacin (ENX). The CL conditions of $[Ag(HIO_6)_2]^{5-}$–H_2SO_4–ENX systems without any luminescence reagent were investigated and optimized. Under the optimized conditions, the CL intensity was proportional to the concentration of ENX in the range from 6.6×10^{-5} to 3.3×10^{-3} g/L. The limit of detection ($s/n = 3$) was 2.0×10^{-5} g/L. The recovery of ENX from the spiked pharmaceutical preparations was in the range of 82.9–108% with a relative standard deviation of 1.9–3.0%. For spiked serum and urine samples the recovery of ENX was in the range of 83.7–110% with a relative standard deviation of 1.1–2.8%. The proposed method was applied successfully to the determination of the drug in capsule, serum and urine samples.

Keywords: chemiluminescence; Ag(III) complex; enoxacin; capsule; urine; serum

Introduction

Enoxacin(ENX), 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)- 1,8- naphtyridine-3-carboxylic acid, is one of the third generation quinolone antibiotics active against most Gram-negative and Gram-positive bacteria.[1,2] Therefore ENX is used in the treatment of systemic infections, including urinary tract, respiratory, gastrointestinal, and skin infections. However, enoxacin can cause side effects such as skin rash, shortness of breath, platelet and blood corpuscle decrease, and renal obstacle.[3] Quality control of ENX dosage and its monitoring in biological fluids by quick automated techniques is an important analytical task.

A variety of techniques was reported for the determination of ENX in biological samples, such as electrochemical analysis, spectrophotometry, fluorimetry, and liquid chromatography with the limit of detection (LOD) range from 2.0×10^{-6} g/L to 1.0×10^{-4} g/L.[4] Chemiluminescence (CL) is known to be a powerful analytical technique that promises high sensitivity, wide linear range and simple instrumentation and has been applied for biomedical, pharmaceutical, and clinical analysis.[5–7] However, a few CL methods are used for the analysis of ENX with different reaction systems, such as Tb^{3+}–Ce(IV)–SO_3^{2-} system,[8] ru$(phen)_3^{2+}$–Ce(IV) system,[9] luminol–H_2O_2–mmanganese-tetrasulfonatophthalocyanine system.[10] and $KMnO_4$–$NaSO_3$–Tb^{3+} system.[11] In our previous work, use of Dy^{3+}–Ce(IV)–NaS_2O_3 CL system was reported for the determination of ENX.[4] Otherwise, Ag(III) complex was used for the determination of another four fluorquinolones.[12,13] To our knowledge, there is no report using the Ag(III) complex for CL determination of ENX without any luminescence reagent. This work focuses on applying Ag(III) complex CL system for the determinations of ENX in the capsules and biological samples.

Materials and Methods

Chemicals and solution

The following reagents were used: Sodium periodate ($NaIO_4$, 99.5%) was purchased from Tianjin Kermel Chemical Reagent Company (Tianjin, China); potassium peroxydisulfate ($K_2S_2O_8$, 99.5%) was purchased from Beijing Chemical Reagent Company (Beijing, China); silver nitrate ($AgNO_3$, 99.8%) and potassium hydroxide (KOH, 82%) were purchased from Tianjin Damao Chemical Reagent Company (Tianjin, China). All chemicals were of analytical reagent grade and used without further purification. Deionized water was used throughout.

The Ag(III) complex, $[Ag(HIO_6)_2]^{5-}$, was prepared by oxidizing Ag(I) in the alkaline medium according the known method.[14] Briefly, KIO_4 (3.24 g), $AgNO_3$ (1.36 g), $Na_2S_2O_8$ (3.00 g), and KOH (8.00 g) were added to 200 mL water. The mixture was heated to boiling for about 40 min on a hot plate with constant stirring. The mixture was then cooled and the Ag(III) complex obtained. The obtained stock solution was stored in refrigeration at 0–4 °C. Under such storage conditions, it was found fairly stable for several months and Ag(III) complex solutions were freshly prepared by diluting the stock solution with deionized water before use. The complex was characterized by UV/visible spectrum, which exhibits two absorb peaks at 362 ± 1 nm and 252.6 ± 0.4 nm. The concentration of Ag(III) complex solutions was determined

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spectrophotometrically at 362 nm by use of the molar absorptivity of $\varepsilon = 1.26 \times 10^4\ M^{-1}\ cm^{-1}$.[15]

ENX was purchased from the Institute of Medicinal Biotechnology (Beijing, China). Stock standard solution of ENX (0.5 g/L) was prepared by dissolving 25.00 mg ENX in 1.5 mL of 0.1 M sodium hydroxide and diluting with deionized water to 50 mL. ENX capsules were purchased from Changtian Pharmaceutical Ltd Com. (Baoding, China) Co. 0.4 M H_2SO_4 was used in the CL reaction.

Instrumentation

The flow-injection system used for CL is an IFFM-E analysis system (Xi'an Remex Electronic Sci-Tech. Co. Ltd, Xi'an, China) which consists of two peristaltic pumps working at a constant flow rate (60 rpm) and a six-way injection valve with a sample loop (120 μL), which is automatically operated by a computer equipped operation system of IFFM-E flow injection analysis. PTFE tubing (0.8 mm i.d.) is used to connect all components in the flow system. The flow cell is a twisted glass tube, with a large surface area exposed to the adjacent photomultiplier tube (PMT, operated at −800 V). A UV-265 spectrophotometer (Shimadzu, Japan) was used to measure UV data at 266 nm for the determination of ENX in capsule in order to evaluate the accuracy of this method.

Sample treatment

An accurately weighed portion (0.2285 g) of the homogenized capsule sample (about 200 mg ENX) was dissolved with 30 mL of 0.01 M NaOH in a small beaker, then sonicated for 20 min. The solution was filtered and the residue washed several times with deionized water. Then it was transferred into a 100 mL volumetric flask and diluted to the mark with deionized water. The solution was diluted further with deionized water so that the concentration of ENX in final solution was at 10^{-5} g/L level. The content of ENX for one capsule was calculated from a previously plotted calibration graph.

The serum sample was provided by Hebei University Hospital. The protein in 1 mL volume of serum was removed by adding 4.0 mL 10% trichloroacetic acid (CCl_3COOH) in a centrifuge tube, which was shaken for 5 min, then centrifuged for 10 min at 10 000 rpm. A 0.5 mL volume of the supernatant was diluted with deionized water to a 250-mL volumetric flask, and mixed thoroughly for CL analysis and recovery test.

Urine samples were provided by two volunteers without oral administration. 1.0 g PbO_2 powder was added to 5.0 mL of urine sample and stirred for 10 min to eliminate urine acid, thiourea and ascorbic acid, etc. After centrifugation for 10 min at 10 000 rpm, the supernatant was filtrated, 0.5 mL of the sample solution was diluted with deionized water to 250 mL and mixed thoroughly for CL analysis and recovery test.

Analytical procedure

In a 10-mL calibrated flask, 0.2 mL ENX working solution and 0.2 mL H_2SO_4 were mixed. 1 mL $[Ag(HIO_6)_2]^{5-}$ (0.07 mM) was then injected into the reaction tube by a quantitative injector and the CL intensity was measured without stirring.

The FIA procedure is shown in Figure 1. The flow lines (a) and (b) were inserted into ENX and H_2SO_4 solution, respectively, and then mixed with $[Ag(HIO_6)_2]^{5-}$ from the flow lines (c) to produce CL when the injection valve was switched to the position of injection. The concentration of ENX was quantified by the peak height of the CL signals.

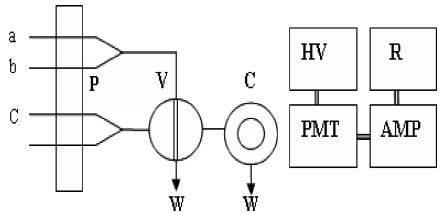

Figure 1. Schematic diagram of flow injection CL analysis system. P–peristaltic pump, V–sampling inlet valve, C–flowing cell, PMT–photomultiplier tube, AMP–amplifier, HV–high voltage, R–recorder, W–waste, a–sample solution, b–H_2SO_4 solution, c–$[Ag(HIO_6)_2]^{5-}$ solution.

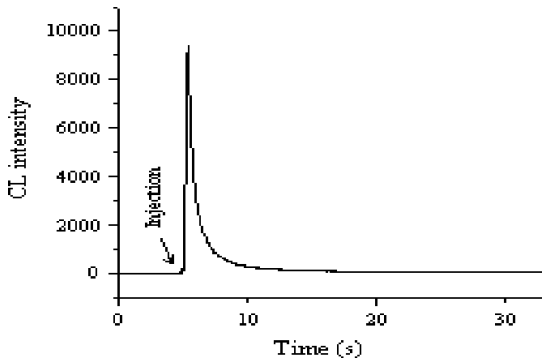

Figure 2. kinetic characteristic of the CL reaction of $[Ag(HIO_6)_2]^{5-}$–H_2SO_4–ENX system $[Ag(HIO6)_2]^{5-}$, 0.07 mM; H_2SO_4, 0.4 M; ENX, 6.8×10^{-3} g/L.

Results and Discussion

Investigation of enhanced CL system

An attempt was made to research and apply Ag(III) complex CL system. The kind of acid used in the reaction had 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 $[Ag(HIO_6)_2]^{5-}$ solution to test the effect of acidic medium on the CL signal, respectively. The results indicated that CL signal could be produced by the direct CL reaction of H_2SO_4 and Ag(III) complex. When ENX was added to the $[Ag(HIO_6)_2]^{5-}$—H_2SO_4 system, the CL signal was enhanced significantly.

The CL kinetic characteristics of the reactions of two systems were investigated in detail. The results are shown in Figure 2. It was shown that the CL reaction rate in solution was very fast, from reagent mixing to peak maximum only 0.4 s was needed for $[Ag(HIO_6)_2]^{5-}$–H_2SO_4–ENX system, and it took 14 s for the signal to return to zero again. The kinetic curve indicated the CL method was sensitive enough and suitable to perform the determination of ENX.

The CL spectra were observed by using a CL analyzer with 10 light filters in the range of 400–650 nm (Figure 3). The maximal CL peak at 460 nm was founded, which was different to that reported in the literature.[12,13] The possible mechanism of CL emission tested needs to be researched further.

Optimization of $[Ag(HIO_6)_2]^{5-}$ and H_2SO_4 concentration

The concentration of $[Ag(HIO_6)_2]^{5-}$ and H_2SO_4 was an important factor for CL emission. In this CL system, $[Ag(HIO_6)_2]^{5-}$ was used as the oxidant; its concentration not only influenced the sensitivity,

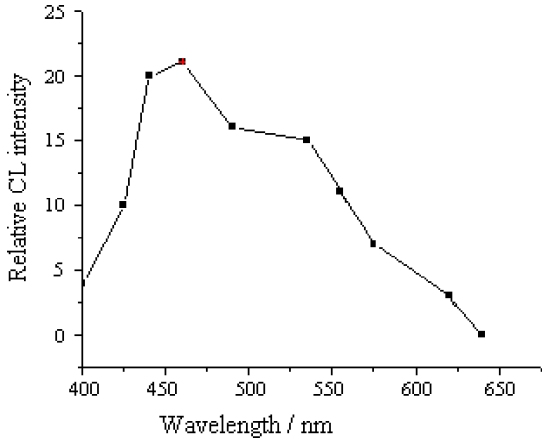

Figure 3. CL spectra of $[Ag(HIO_6)_2]^{5-}$–H_2SO_4–ENX system $[Ag(HIO_6)_2]^{5-}$, 0.07 mM; H_2SO_4, 0.4 M; ENX, 4.0×10^{-3} g/L.

but also influenced the linear range for the assay. Therefore, the dependence of the $[Ag(HIO_6)_2]^{5-}$ concentration on the CL intensity was investigated for 1.0×10^{-3} g/L ENX. The CL intensity increased remarkably with the increase of $[Ag(HIO_6)_2]^{5-}$ concentration in the range of 0.025 mM to 0.07 mM, then decreased obviously with further increase of $[Ag(HIO_6)_2]^{5-}$ concentration. So 0.07 mM $[Ag(HIO_6)_2]^{5-}$ was selected as the optimal concentration.

The concentration of H_2SO_4 used in the reaction had a very significant influence on the CL emission intensity. The CL intensity increased remarkably with the increase of concentration of H_2SO_4 in the range from 0.1 M to 0.4 M, then decreased with further increase of H_2SO_4 concentration. So 0.4 M H_2SO_4 was selected as the optimal concentration.

Effect of sample volume and flow rate

The role of sample volume and flow rate was critical; for instance, if sample volume was too small or too large, CL maximum could not be obtained. The highest emission was produced if 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 was recommended because of greater precision and economy in the use of reagents.

Analytical performance of CL systems

The interfering effects from foreign species were investigated. The tolerance content was defined as the amount of coexisting species that produced an error not exceeding ±5% in the determination of ENX. The results were listed in Table 1. The data showed that there were a few interferences. If there were interference components in real samples, by using the standard addition method the interferences would be eliminated. Since the serum sample had a higher concentration of proteins than the urine sample, trichloroacetic acid should be added to each serum sample to remove proteins before determination. However, dilution could minimize the interference in measurement of the urine samples, because these species become fewer when the sample is diluted.

Under the optimized conditions, the linearity was evaluated for ENX by using the proposed systems. The regression equations are as follows: for ENX in the range of $6.6 \times 10^{-5} - 3.3 \times 10^{-3}$ g/L, $I = 166C + 71.0$ with a correlation coefficient of 0.998.

The LOD was determined as the sample concentration that produces a peak with a height three times that of the level of baseline noise. The LOD was 2.0×10^{-5} g/L. The high assay sensitivity allowed a high-fold dilution of the samples before analysis to avoid sample matrix effects. The relative standard deviation (RSD) was found to be 2.1% for 11 determinations of 3.0×10^{-4} g/L of ENX. The LOD of the proposed method is lower than that reported by CL method.[9]

It is indicated that the proposed enhanced CL system with $[Ag(HIO_6)_2]^{5-}$–H_2SO_4 has satisfactory linearity, sensitivity and precision.

Table 1. Tolerable concentration ratios with respect to 3.67×10^{-4} g/L ENX

Substance	Tolerance concentration ratio
Polyglycol	100
Sodium benzoate	10
Amylum,	40
Dextrin	200
Glucose	8.0
Lactose	50
Ca^{2+}	7.0
Mg^{2+}	82
EDTA	200
Zn^{2+}	30
Cu^{2+}	20
Co^{2+}	30
Fe^{2+}	6.0

Table 2. Analytical results of ENX in capsule samples

Capsule	Labelled (mg/particle)	Proposed method (mg/particle*)	UV method** (mg/particle*)
1	200.0	197.7 ± 1.90	195.3 ± 0.71
2	200.0	202.4 ± 2.40	198.2 ± 0.89

* Mean ± S.D.(n = 5), ** Chinese Pharmacopoeia[16].

Sample analysis

The proposed method was applied for the determination of ENX in pharmaceutical preparations. In order to evaluate the validity of the method, the result obtained for the determination of ENX in pharmaceutical by the proposed method was compared with that obtained using the UV-method according to the Chinese Pharmacopoeia,[16] as shown in Table 2.

The results obtained by the proposed method agree with the labelled contents. Statistical analysis of the results using Student's *t*-test and the variance ratio F-test showed that there was no significant difference at $p = 0.05$ between the results obtained by this method and pharmacopoeia methods.

ENX had 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 3.0×10^{-4} g/L.[17] 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

Table 3. Determination of ENX in capsule, serum and urine samples

Sample	Content ($\times 10^{-4}$ g/L)	Added ($\times 10^{-4}$ g/L)	Found ($\times 10^{-4}$ g/L)	Recovery (%)	RSD, n = 5 (%)
Capsule 1	2.201	3.680	5.761	96.7	1.9
		11.04	14.09	108	2.9
		18.40	19.32	93.0	2.6
Capsule 2	3.112	2.208	4.942	82.9	3.0
		6.624	8.854	86.7	2.7
		11.04	13.27	92.0	2.2
Serum	*	1.208	1.049	86.8	1.8
		3.624	3.976	110	1.3
		6.040	6.656	110	2.6
Urine 1	*	1.208	1.011	83.7	2.2
		3.624	3.338	92.1	2.0
		6.040	6.161	102	1.1
Urine 2	*	2.208	2.292	104	2.4
		6.624	6.908	104	2.5
		11.04	11.42	103	2.8

* Not detected.

addition method. The recovery tests were performed to evaluate the accuracy of this method. The results are given in Table 3. The recovery of ENX was in the range of 82.9–108% with an RSD of 1.9–3.0% for two spiked capsule samples, 86.8–110% with an RSD of 1.3–2.6% for a spiked serum sample and 83.7–104% with an RSD of 1.1–2.8% for two spiked urine samples.

Conclusion

Use of Ag(III) complex chemiluminescence system is described for the determination of ENX in capsule and biological samples. There was no significant difference at $p = 0.05$ between the results obtained by this method and pharmacopoeia methods. The Ag(III)- H_2SO_4 CL system for the determination of ENX has higher sensitivity good precision and facility, with any two reagents and without any luminescence reagent. The possible mechanism of the CL emission needs to be researched further.

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