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Indirect Flow Injection Chemiluminescence Method for the Determination of Tetracyclines Using Cu(Ⅱ) as a Probe Ion

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Abstract: This paper reported an indirect flow injection chemiluminescence (FI-CL) method for the determination of the drugs tetracycline (TC), chlortetracycline (CTC) and oxytetracycline (OTC) using Cu(Ⅱ) as a probe ion. The CL reaction was induced on-line and after injection of the sample the negative peaks appeared as a result of complexation. The method was applied to the determination of TCs in pharmaceuticals and human urine with recoveries in the range 95~105%.

Keywords: Cu(Ⅱ) probe ion, inhibitory chemiluminescence, tetracyclines.

Tetracycline antibiotics (TCs) are active against a wide range of gram-positive and gram-negative bacteria, being extensively used in human and veterinary medicine to treat and prevent bacterial infections[1]. Among the TCs, TC, OTC and CTC are commercially available and permitted for human administration. However, the degradation products occurring in storage conditions might induce potential adverse effects. Thus, many studies have focused on the development of a rapid, sensitive and reliable method for the analysis of TCs and spectrophotometric[2,3], spectrofluorimetric[4,5], electrochemical[6] and chromatographic methods[7,8] have been well explored, further CL has attracted high attention for its high sensitivity [9-13]. However, these CL methods suffered from the complicated procedure for the preparation of standard solution and involved some instable, expensive and toxic reagents. Time-consuming derivatization of non-CL analyte is often required for normal CL detection. The best choice is indirect detection, if the analyte can suppress a CL reaction, viz., the CL intensity decreases when the analyte zone is detected. It was well known that Cu(Ⅱ) can form stable complexes with TCs[14] and the complexed Cu(Ⅱ) is a more poor catalyst than the uncomplexed form of Cu(Ⅱ)[15]. In this work, the determination of TCs was based on their complexation with Cu(Ⅱ) which effected on the emission intensity of Cu(Ⅱ)-catalyted CL reaction between luminol and H_2O_2 under certain conditions, causing an analytically proportional decrease of the CL emission. To our knowledge, this was the first application of an indirect CL method for the determination of TCs.

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Experimental

CL signal measurements were carried out with a computerized BPCL-type ultra weak CL analyzer (Institute of Biophysics, Chinese Academic of Sciences, Beijing). The flow system consisted of a IFIS peristaltic pump (Remex Electronic Instrument Limited Company, Xi'an) which delivered each solution at equal flow rate (2.0 mL/min) through flow tubes and a six-way injection valve with a 50 μL sample loop, through which sample solution was injected into the stream of the water carrier. The experimental configuration was schematically illustrated in **Figure 1**.

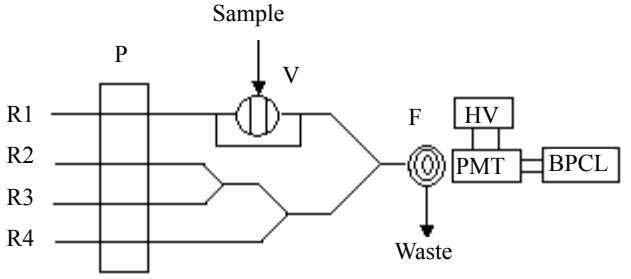
Analytical reagent-grade chemicals and ultra-pure water (Ultra-Pure Water System, Germany) were used throughout. Stock solutions of TCs (1.0×10^{-3} mol/L) were prepared from the hydrochloride of TC, CTC and OTC (purchased from Drug and Biological Products Examination Institute of China, Beijing), respectively. 0.01 mol/L luminol (Shaanxi Normal University, Xi'an) to obtain was dissolved in 0.1 mol/L NaOH solution. 30% H_2O_2 was diluted to 0.1 mol/L. The stock solution of metal ion (1.0×10^{-3} mol/L) including Cu(II), Co(II) and Cr(III) were prepared by dissolving the metal salts in water.

Results and Discussion

Some transition metal ions such as Cu(II), Co(II) and Cr(III) possess catalytic effects on the luminol-H_2O_2 reaction[16,17]. Our experiment also showed that a high and constant CL background was obtained by the CL reaction of luminol-H_2O_2 using Cu(II), Co(II) and Cr(III) as catalyst, however, when TCs samples were injected, the inhibition of CL signals was observed only using Cu(II) as catalyst. This result agreed with the literature[18]. So Cu(Ⅱ) was chosen as the catalyst.

Effects of the reaction parameter on the CL intensity were investigated. The optimum conditions were 1.0×10^{-6} mol/L Cu(II), 2.0×10^{-6} mol/L luminol, 0.1 mol/L H_2O_2, pH 11 of the luminol solution, pH 3.5 of the TCs solutions and 2.0 mL/min of the flow rate, respectively. Under the selected optimum conditions, the calibration curves for the determination of TCs were established from the inhibitory effect upon the concentration of analyte (**Table 1)**.

Figure 1 Schematic diagram of the FIA-CL manifold employed for the determination of TCs



R1, water carrier; R2, luminol solution; R3, H_2O_2 solution; R4, Cu(**II**) solution; P, peristaltic pump; V, injection valve; F, CL flow cell; PMT, photomultiplier tube; HV, negative high-voltage supply; BPCL, luminescence analyzer controlled by personal computer.

Table 1 Analytical characteristics for the determination of TCs by the proposed method

Species	Linear range (mol/L)	R^2	Detection limit (mol/L)	RSD % (n = 11)
TC	3.6×10^{-8}~1.0×10^{-5}	0.9974	5.0×10^{-9}	1.8
CTC	1.1×10^{-7}~1.0×10^{-5}	0.9950	1.0×10^{-8}	2.0
OTC	1.9×10^{-7}~1.0×10^{-5}	0.9947	2.0×10^{-8}	2.1

Table 2 Tolerable concentration level of interferents to TCs

Interferents	Tolerable level (times)
Na^+, K^+, Mg^{2+}, Cl^-, NO_3^-	>1000
CO_3^{2-}, PO_4^{3-}, SO_4^{2-}, Starch, glucose, Ca^{2+}	>100
Creatine, creatinine, Fe^{3+}	50
Oxalate, lactate, lactose, sucrose, β-cyclodextin, $H_2PO_4^-$	10
Urea, HCO_3^-, NH_4^+	25
Uric acid, citric acid, magnesium stearate, Al^{3+}	5
Ascorbic acid, Cd^{2+}, Cr^{3+}, Co^{2+}, Fe^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}	3

Table 3 Determination of TCs in some commercial formulations and human urines (n = 3)

Sample	Initially present (mol/L)	Added (mol/L)	Recovery (%)	Amount of TCs (mg/tablet)
TC tablets	3.53×10^{-6}	1.80×10^{-6}	95	251
	1.22×10^{-6}	1.80×10^{-6}	98	248
OTC tablets	4.12×10^{-6}	5.61×10^{-6}	104	251
	3.89×10^{-6}	5.61×10^{-6}	98	249
Spiked TC urine	2.10×10^{-6}	1.80×10^{-6}	110	
	4.90×10^{-6}	1.80×10^{-6}	100	
Spiked OTC urine	1.22×10^{-6}	5.61×10^{-6}	102	
	3.81×10^{-6}	5.61×10^{-6}	99	
Spiked CTC urine 1	9.36×10^{-6}	9.30×10^{-6}	99	
	6.24×10^{-6}	6.43×10^{-6}	103	
	3.12×10^{-6}	3.28×10^{-6}	105	
Spiked CTC urine 2	9.36×10^{-6}	9.56×10^{-6}	102	
	6.24×10^{-6}	6.13×10^{-6}	98	
	3.12×10^{-6}	3.13×10^{-6}	100	

The influence of the foreign species on the determination of 1.0×10^{-6} mol/L TCs was studied. The results of the tolerable concentration for interference at 5% level were listed in **Table 2**. It could be seen that the excipients and common components in tablet and human urine showed no influence on the detection of TCs.

The proposed method was applied to the analysis of TC or OTC tablets and TCs in spiked human urine samples. The results were shown in **Table 3**.

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