Electronic Supplementary Material (ESI) for Analytical Methods

Study on the interaction behavior of catalase with cephalosporins by chemiluminescence with flow injection analysis

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Experimental

Reagents

All reagents were of analytical pure grade, and the water used was purified in a Milli-Q system (Millipore, Bedford, MA, USA). Luminol (Fluka, Switzerland) and CAT (Sigma) were used as received without further purification. Cephalosporins (cefradine, cefadroxil, cefazolin, cefaclor, cefuroxime, cefotaxime, ceftriaxone and cefoperazone) were purchased from National Institute for the Control of Pharmaceutical and Biological Products, China. 2.5×10^{-2} mol L^{-1} luminol was prepared by dissolving 0.44 g luminol in 100 mL of 0.1 mol L^{-1} NaOH solution in a brown calibrated flask. The stock solutions of 5.0×10^{-5} mol L^{-1} CAT and 1.0×10^{-3} mol L^{-1} cephalosporins were prepared in purified water. Working standard solutions of cephalosporins were prepared daily from the stock solutions by appropriate dilution as required. All of the stock solutions were stored at 4 °C.

Apparatus

The FI mode for the assay of cephalosporins was shown in **Fig. S1**. The FI–CL apparatus (Xi'an Remex Analysis Instrument Co. Ltd., Xi'an, China) consisted of a sampling system (IFFM-E), a CL detector (IFFS-A) and a computer. The sampling system contained two peristaltic pumps which were used to deliver the luminol and carrier streams (main pump) and CAT and sample streams (assistant pump), respectively. Polytetrafluoroethylene (PTFE) tube (1.0 mm i.d.) was used to connect all the components of the flow system. A six-way valve with a loop of 100 μL was used for quantitatively injecting luminol into carrier stream. The CL detector contained a flow cell made by coiling 15 cm of colorless glass tube (1.0 mm i.d.) into a spiral disk shape with a diameter of 2.0 cm and placed close to the photomultiplier tube (PMT). Extreme precautions were taken to ensure that the sample compartment and PMT were light tight. The CL signal produced in flow cell was detected without wavelength discrimination, and the PMT output was recorded by computer with IFFM-E client system. Fluorescence measurements were carried out on Easy life LSTM fluorescence lifetime system (PTI, USA).

General procedures

The carrier (purified water) and the solutions of luminol, CAT and cephalosporins were propelled by peristaltic pumps at a flow rate of 2.0 mL min^{-1} on each flow line. The whole flow system was washed until a stable baseline was recorded. Then 100 μL of luminol solution was injected into the carrier stream by the six-way valve and merged with CAT, which was then mixed with the cephalosporins stream. The mixed solution was delivered into the flow cell, and the emitted CL was collected by the PMT at a voltage of –700 V and recorded by the computer. The decrement of CL intensity ($\Delta I = I_0 - I_s$, where I_s and I_0 were CL signals in the presence and in the absence of cephalosporins) was measured to construct the calibration curves versus the concentrations of cephalosporins.

Results and discussion

Optimization of the experimental conditions

The effects of luminol and CAT concentrations on the CL intensity were examined over the ranges of 5.0×10^{-7} to 5.0×10^{-4} mol L^{-1} and 5.0×10^{-10} to 1.0×10^{-6} mol L^{-1}, respectively. It was found that the maximum CL intensity could be obtained when using a concentration of 2.5×10^{-5} mol L^{-1} luminol, and the CL intensity reached maximum with 2.0×10^{-7} mol L^{-1} CAT and afforded approximately constant CL intensity over 2.0×10^{-7} mol L^{-1}. Therefore, 2.5×10^{-5} mol L^{-1} luminol and 2.0×10^{-7} mol L^{-1} CAT were chosen as the optimum concentrations and used in subsequent experiments. A series of NaOH solutions with different concentrations ranging from 1.0×10^{-3} to 1.0×10^{-1} mol L^{-1} were tested and 2.5×10^{-2} mol L^{-1} NaOH was used as the optimum concentration and used in the subsequent experiments. The flow rate and the length of mixing tube had great effect on the CL intensity. A flow rate of 2.0 mL min^{-1} and the length of mixing tube of 10.0 cm were selected for this CL system with good sensitivity, precision and reproducibility.

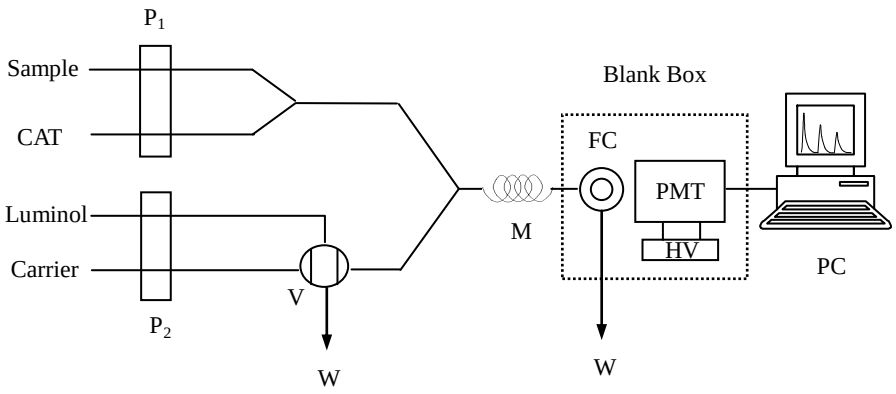


Fig. S1. Schematic diagram of the present FI–CL system.

P_1: assistant pump; P_2: main pump; M: mixing tube; V: valve; FC: flow cell; W: waste; PC: personal computer.

Table S1 The chemical structures of the studied cephalosporins.

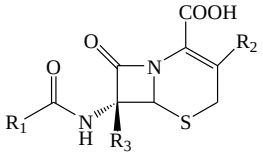
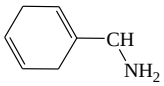
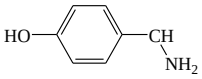
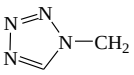
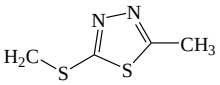
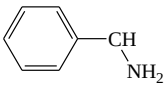
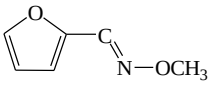
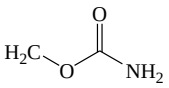
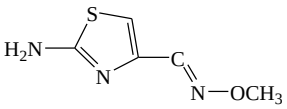
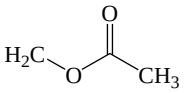
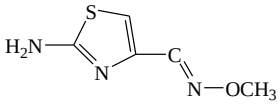
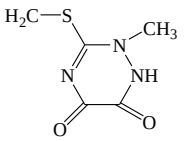
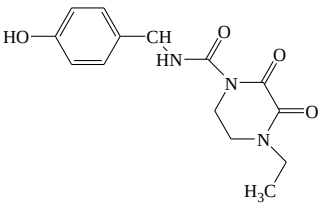
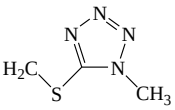
Nucleus cephalosporanic				
Generation	Drug	R_1	R_2	R_3
1st	cefradine		CH_3	H
	cefadroxil		CH_3	H
	cefazolin			H
2nd	cefaclor		Cl	H
	cefuroxime			H
	cefotaxime			H
3rd	ceftriaxone			H
	cefoperazone			H

Table S2 Comparison of different methods for determination of cefradine

Method	Linear range μg mL^{-1}	LOD μg mL^{-1}	Ref
Spectrophotometry	5.0 ~ 60.0	2.0	17
CZE	3.0 ~ 1.0×10^{3}	0.5	22
Colorimetry	2.0 ~ 10	0.8	16
HPLC–UV	0.2 ~ 30	–	20
Spectrofluorimetry	0.2 ~ 2.2	5.0×10^{-2}	18
SPE–CL	0.1 ~ 10.0	4.0×10^{-2}	23
HPLC–MS/MS	5.0×10^{-2} ~ 50	–	21
The proposed CL	3.5×10^{-5} ~ 7.0×10^{-2}	1.2×10^{-5}	–

Table S3 Calibration curves and LODs of cephalosporins

Drug	Linear equation	Linear range nmol L^{-1}	LOD nmol L^{-1}	R
cefradine	$\Delta I = 8.6LnC_{cefradine} + 17.5$	0.1–200	0.03	0.9975
cefadroxil	$\Delta I = 14.1LnC_{cefadroxil} + 27.5$	0.3–100	0.1	0.9982
cefazolin	$\Delta I = 9.3LnC_{cefazolin} + 25.3$	0.1–100	0.03	0.9961
cefaclor	$\Delta I = 6.3LnC_{cefaclor} + 20.6$	0.1–100	0.03	0.9960
cefuroxime	$\Delta I = 11.0LnC_{cefuroxine} + 28.5$	0.1–100	0.03	0.9965
cefotaxime	$\Delta I = 12.8LnC_{cefotaxime} + 28.4$	0.1–100	0.03	0.9953
ceftriaxone	$\Delta I = 14.0LnC_{ceftriaxone} + 43.7$	0.1–100	0.03	0.9959
cefoperazone	$\Delta I = 9.1LnC_{cefoperazone} + 20.8$	0.1–200	0.03	0.9972

Table S4 Results of monitoring excretive cefradine in human urine after taking cefradine capsules [a]

Time hour	Added ng mL^{-1}	Found ng mL^{-1}	Recovery %	RSD %	Cefradine in urine $M_{(mg)}$ / $V_{(mL)}$	Cefradine excretive ratio in urine %
0.5	0	4.5	106.7	2.3	50.1/90	10.0
	3.0	7.7		1.6		
1.0	0	8.9	94.0	1.5	89.2/80	17.8
	5.0	13.6		1.1		
2.0	0	10.4	101.0	1.3	123.5/95	24.7
	10.0	20.5		0.8		
3.0	0	5.3	106.0	2.0	66.0/100	13.2
	5.0	10.6		1.3		
4.0	0	3.5	96.7	2.7	37.1/85	7.4
	3.0	6.4		1.7		
5.0	0	2.2	105.0	2.9	19.5/70	3.9
	2.0	4.3		2.6		
6.0	0	1.4	95.0	3.2	9.0/50	1.8
	2.0	3.3		2.7		
7.0	0	0.4	90.0	3.5	3.9/80	0.8
	1.0	1.5		3.2		
8.0	0	–	–	–	–/75	–
	1.0	0.9		3.3		
					Total 397.8/725	Total: 79.6

[a] The average of five determinations