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Chemiluminescence Flow Sensor for Berberine with Immobilized Reagents

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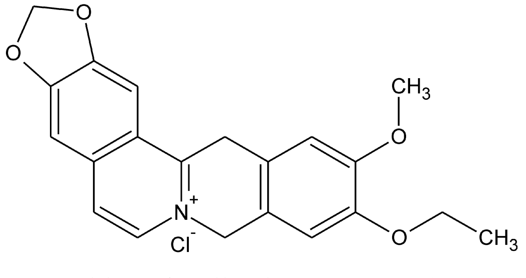
Abstract—A sensitive chemiluminescence (CL) sensor for berberine combined with flow-injection (FI) technology is described. The analytical reagents involved in the CL reaction, including luminol and potassium ferricyanide, were both immobilized on an anion-exchange resin column. While a volume of sodium phosphate was passed through the column, the two CL reagents were eluted from the resin and then mixed with a berberine stream under alkaline conditions. By means of the fast oxidation reaction between berberine and potassium ferricyanide, potassium ferrocyanide was generated, which then inhibited the CL reaction of luminol and potassium ferricyanide. The decreased CL intensity was correlated with the berberine concentration in the range from 0.05 to 300 ng mL^{-1} with a relative standard deviation of less than 4.3%, and the limit of detection was 0.02 ng mL^{-1} at a flow rate of 2.0 mL min^{-1}. It was shown that the flow sensor could greatly improve the selectivity and sensitivity for determination of berberine. The determination of analyte could be performed in 2 min, including sampling and washing and be stable for over 300 analyses. Some interference and additives in preparations were tested. Two herbal medicines commonly found in compound prescriptions, rutin and baicalin, were studied preliminarily. The method has been applied for the determination of berberine in pharmaceutical preparations successfully. © 2001 Elsevier Science Ltd. All rights reserved.

Introduction

Berberine, a quaternary ammonium salt, is widely used as medicines for stomach and bowel ailment and for internal use in the form of tablets, injections and liquids because of its sterilizing powers. In previous work, most of the procedures proposed for the extraction-spectrophotometric determination of berberine are based on the formation of ion-association complexes with anionic dyes.[1–7] Some analytical methods for the determination of berberine have been reported, including liquid chromatography,[8–19] extraction-AES,[20] electrochemical analysis,[21–25] and capillary electrophoresis,[26,27] extraction-fluorophotometry.[28–30] Suto et al.[19] proposed a new procedure for the determination berberine using supercritical-fluid chromatography extraction, but the method suffers low sensitivity and relatively complex device. Wang[31] proposed an optical fiber sensor for berberine based on fluorescence quenching of 2-(4-diphenylyl)-5-phenylbenzoxazole, and the linear range was 0.6–24 μM. However, for the above described methods the linear ranges of the calibration graphs were narrow, the sensitive and selective were not satisfaction, and also need extraction procedure.



A new sensitive chemiluminescence (CL) sensor for berberine combined flow-injection (FI) technology was presented in this paper. The CL flow sensor for berberine based on the inhibition of the intensity of CL from the ferricyanide–luminol system, and the CL reagents, luminol and potassium ferricyanide, used in this sensor, were both immobilized on Amberlyst A-27 anion-exchange resin. Through injection of 200 μL sodium phosphate, the reagents on the anion-exchange resins were eluted and in the presence of berberine, the CL

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intensity was decreased, by which berberine could be sensed, and the CL intensity was quantified by signal height.

Experimental

Reagents

All reagents used were of analytical grade; doubly distilled water was used for the preparation of solutions and all dilutions. Berberine, rutin, baicalin and gallic acid were obtained from Shaanxi Institute for Drug Control. The stock standard solution of berberine was dissolved to 0.2 mg mL^{-1} with ethanol and was stored at 4 °C in a brown flask of 50 mL. Working standards were prepared daily from the stock solution by appropriate dilution. Stock standard luminol solution, 2.5×10^{-2} mol L^{-1}, was prepared by dissolving 4.40 g of luminol (Fluka, Biochemika) in 1l of 0.5 mol L^{-1} NaOH solution. The stock solution of $K_3Fe(CN)_6$ was 1.0×10^{-2} mol Ll^{-1} prepared by dissolving 3.29 g $K_3Fe(CN)_6$ in a flask of 100 mL. Other solutions were Na_3PO_4 (1.0×10^{-2} mol L^{-1}) and KOH (1.0 mol L^{-1}). Amberlyst A-27 anion exchange resin (20+ to 50− mesh) purchased from Rohm and Haas Co. was used for reagent immobilization.

Preparation of immobilized reagents

Amberlyst A-27 (1.0 g) was shaken with 50 mL of 0.06 mol L^{-1} luminol or 0.02 mol L^{-1} potassium ferricyanide for 72 h, then the resin was filtered, washed with doubly-distilled water and dry-stored. The most convenient method to determine the amounts of luminol and potassium ferricyanide immobilized was to measure the losses of these reagents from the immobilization solutions. The concentration was detected at 360 nm for luminol and at 420 nm for ferricyanide by UV–vis. The amounts of luminol and potassium ferricyanide immobilized were 2.2 and 1.0 mmol g^{-1} resin, respectively.

Apparatus

The flow injection system used in this work is shown in Figure 1. A peristaltic pump was used to deliver all flow streams at a flow rate of 2.0 mL min^{-1} on each flow line. PTFE tubing (0.8 mm i.d.) was used between all components in the flow system. 200 µL sodium phosphate solution was injected by a six-way valve through the anion-exchange column. The eluted luminol and potassium ferricyanide merged and reacted with the sample stream in a mixing tubing of 3 cm in length to generate potassium ferrocyanide and then the resulting stream merged with a 0.2 mol L^{-1} potassium hydroxide stream just prior to reaching CL cell. the CL signal was transduced to an R-456 photomultiplier tube (PMT) and recorded with an XWT-206 recorder (Shanghai Dahua Instrument and Meter Plant).

Procedures

Flow lines were inserted into KOH solution, eluant, carrier and sample solution at a flow rate of 2.0 mL min^{-1} on each flow line, respectively. The pump was started to wash the whole flow system until a stable baseline was recorded, then 200 µL of eluant solution sodium phosphate was injected into the carrier stream, luminol and potassium ferricyanide were eluted quantitatively, and which was then mixed with the sample stream, the mixed solution was passed through the CL cell and the light emitted from the CL reaction was detected by PMT. the concentration of berberine was quantified by decreased CL intensity, $\Delta I = I_0 - I_s$, where I_0 and I_s were CL signals in the absence and in the presence of berberine, respectively. Each standard or sample solution was measured over three times.

Results and Discussion

Time profile of CL reaction

The CL kinetic curve of the CL reaction of luminol and ferricyanide was tested with a static system. It was found that the rate of the reaction in solution was not fast; from the reagent mixing to the peak maximum 26 s was need and it took 200 s for the signal to extinguished zero again, as illustrated in Figure 2. Experiments also showed that the CL intensity decreased sharply in the presence of berberine.

Selection of eluant

Anions with different eluting abilities were injected 200 µL through the resin column and released different amounts of luminol and potassium ferricyanide, thus affecting CL intensity. The results are shown in Table 1. It was found that sodium phosphate was the better eluant with the maximum CL intensity emission. Therefore, sodium phosphate was chosen for subsequent work.

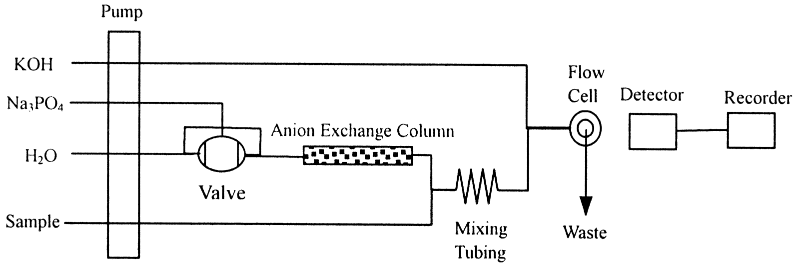

Figure 1. F1 manifold for the determination of berberine.

Effect of molar ratio of immobilized luminol and ferricyanide

To examine the influence of the mixing ratio, resins (0.18 g) with different mixing ratios were packed into a glass column with an internal diameter of 5 mm and total volume of about 0.5 mL. By injection of sodium phosphate (1.0×10^{-3} mol L^{-1}) at a fixed volume of 200 µL, different amounts of luminol and ferricyanide were eluted from the resins and caused a CL intensity in alkaline medium, which was shown in Table 2, and the best signal-to-noise ratio was found with molar ratio of 1:1 (luminol to ferricyanide).

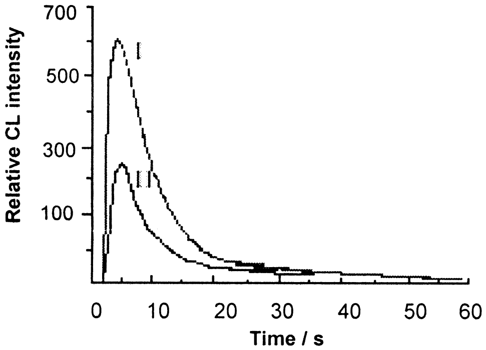

Figure 2. CL kinetic curve of Lu-$K_3Fe(CN)_6$: (i) in the absence of berberine; (ii) in the presence of 10 ng mL^{-1} berberine.

Table 1. Character of eluants for berberine determination[a]

Types of CL intensity[b]	Relative CL intensity				
	NaCl	NaAc	$NaNO_3$	Na_2SO_4	Na_3PO_4
i	58	55	65	69	76
ii	35	37	31	30	25
iii	23	18	36	40	51

[a] The concentration of each eluant was 1.0×10^{-4} mol L^{-1}. The relative CL intensity corresponds to the normalized maximum light intensity.

[b] (i) CL intensity in the absence of berberine; (ii) CL intensity in the presence of berberine of 10 ng mL^{-1}; (iii) decreased CL intensity.

Table 2. Effect of molar ratio between immobilized luminol and ferricyanide[a]

Molar ratio (luminol/ferricyanide)	Relative CL intensities		Decreased CL intensity
	Blank[b]	Signal[c]	
3:1	141	121	20
2:1	133	97	36
1:1	149	91	58
1:2	101	73	28
1:3	100	75	25
1:4	92	71	21

[a] The concentration of each eluant was 1.0×10^{-3} mol L^{-1}. The relative CL intesity corresponds to the normalized maximum light intensity.

[b] CL intensity in the absence of berberine.

[c] CL intensity in the presence of 10 ng mL^{-1} berberine.

Effect of eluant concentration

Various concentrations of sodium phosphate were injected through the anion-exchange resin column with immobilized luminol and potassium ferricyanide. The results obtained clearly demonstrated that CL intensity enhanced with the increasing of eluant concentration (Fig. 3). To obtain long lifetime and high CL intensity, 1.0×10^{-3} mol L^{-1} sodium phosphate was used for the present system. In this case, the column with immobilized CL reagents could be used more than 300 times.

Effect of acidity of sample

The effect of sample acidity was examined and the results were illustrated in Figure 4. The decreased CL intensity varied with pH, and the better signal-to-noise ratio was found with pH 6.

Effect of potassium hydroxide concentration

The CL reaction of luminol and potassium ferricyanide proceeds under a alkaline condition. The effect of the KOH concentration was examined and the results were illustrated in Figure 5. The CL intensity and the decreased CL intensity varied significantly with KOH concentration. To maximize the sensitivity, a KOH concentration of 0.2 M was chosen as optimum.

Effect of length of mixing tubing and flow rate

To improve the efficiency of the oxidation-reduction reaction between potassium ferricyanide and berberine,

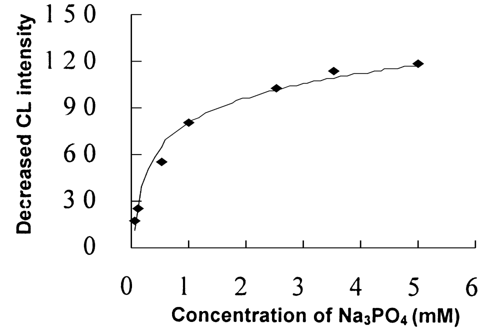

Figure 3. Effect of sodium phosphate (concentration of berberine 10 ng mL^{-1}).

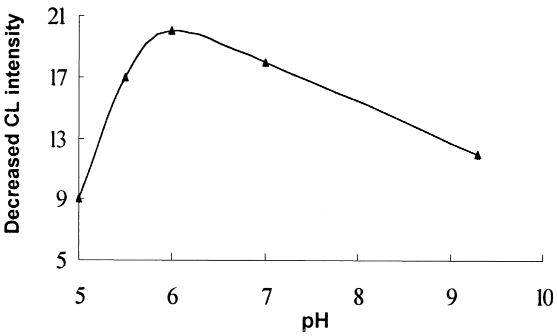

Figure 4. Effect of sample acidity concentration of berberine 10 ng mL^{-1}.

an mixing tubing was used in this system and its length was tested from 3.0 to 8.0 cm as Figure 6. shown. It was found that an optical length was 3.0 cm, from which a high decreased CL intensity was recorded. A pump rate was an important factor for the determination of berberine. It was discovered that the CL intensity increased with the increasing of pump rate. A pump rate of 2.0 mL min^{-1} was chosen for subsequent work considering the stability of instrument and low reagent consumption.

Performance of the system for berberine measurements

The calibration curve for berberine was obtained under the optimized conditions. It was found that the decreased CL intensity was linear with the logarithm of berberine concentration. As Figure 7 shows, the linear range is from 0.05 to 300 ng ml^{-1} and the regression equation is $\Delta I = 15.97\ln C_{\text{berberine}} + 36.11$, $\gamma = 0.9946$. The relative standard deviation of seven determinations was 4.3, 4.0 and 2.2% with a berberine concentration of 0.1, 1.0 and 250 ng mL^{-1}, respectively. The limit of detection (3σ, which was calculated according to IUPAC recommendations) was 0.02 ng mL^{-1}.

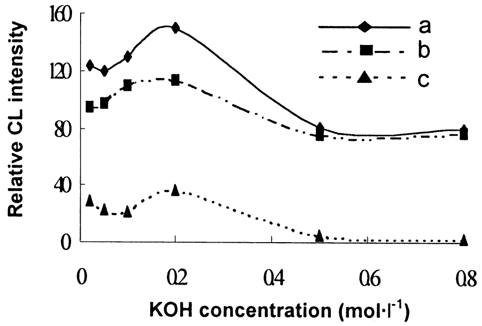

Figure 5. Effect of KOH concentration with 10 ng mL^{-1} berberine: (a) luminol-$k_3Fe(CN)_6$ system (I_0); (b) luminol- $K_3Fe(CN)_6$-berberine system (I_x); (c) decreased CL intensity ($\Delta I = I_0 - I_x$).

Interference study

The effect of foreign species was tested by analyzing a standard solution of berberine (0.10 μg mL^{-1}), to which increasing amounts of interfering species were added and the tolerable limit of a foreign species was taken if it caused a relative error of less than 5%. The tolerable ratio of some interfering inorganic ions and organic compounds was listed in Table 3. As the table shows, the main interference was caused by the metal ions such as Cu^{2+}, Fe^{3+}, Fe^{2+} and Mn^{2+}, and they could be masked by adding excess amount of EDTA.

Application

Following the procedure described in Experimental (the procedures), the proposed method was applied for the determination of berberine in pharmaceutical preparations. Two different tablets were weighed, grounded to a fine powder and mixed. A sample equivalent to approximately 100 mg of berberine was weighed accurately, transferred into a 250 mL calibrated flask and made up to volume with water, filtered and the clear filtrate was used for determination. An appropriate volume of the sample was diluted further with water so that the concentration of berberine in the final solution was within the working range (0.05–300 ng mL^{-1}). Recovery studies were also performed on each of the analyzed samples by adding a known amount of berberine to the sample before the recommended treatment.

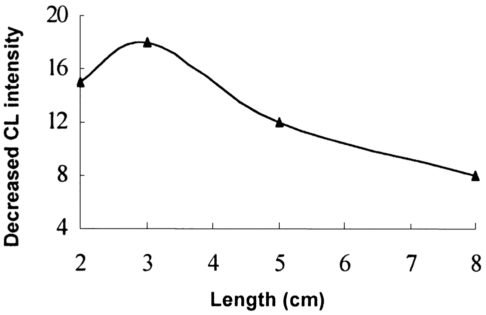

Figure 6. Effect of mixing tubing length (concentration of berberine 10 ng mL^{-1}).

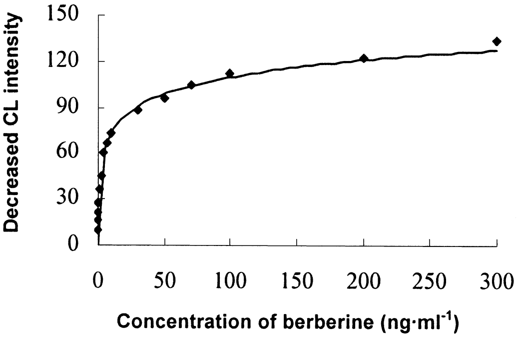

Figure 7. Calibration curve for the determination of berberine.

Table 3. Tolerable concentration with respect to berberine for some interfering species (<5% error)

Species	Tolerable concentration (μg mL^{-1})	Species	Tolerable concentration (μg mL^{-1})	Species	Tolerable concentration (μg mL^{-1})
Pb^{2+}	21	S^{2-}	3.2	Ethanol	920
Zn^{2+}	6.5	Cr^{6+}	52	Sulfosalicylic acid	380
Mg^{2+}	12	Critic acid	9.6	Solium benzosulfonate	270
Ni^{2+}	30	Glucose	18	Phenobarbital	460
Ca^{2+}	4.0	Sucrose	170	Ethylphydroxybenzoate	1000
Fe^{3+}, Fe^{2+}, Mn^{2+}	5.6×10^{-3}	Chlestrol	3.8	Potassium oxalate	1700
Cu^{2+}	6.4×10^{-2}	Methanol	800	Malic acid	100

Table 4. Results of berberine in different pharmaceutical preparations

Sample batch no.	Proposed method[a]					UV–vis
	Found ($ng\,mL^{-1}$)	Added ($ng\,mL^{-1}$)	Total ($ng\,mL^{-1}$)	Recovery (%)	Content ($mg\,tab^{-1}$)	Content ($mg\,tab^{-1}$)
980702	68.6	100	164.3	95.7	101	97
980905	91.8	165	263.9	104.3	138	136

[a]The average of three determinations.

Table 5. Calibration graph of three herbal medicine

Species	Concentration ($ng\,mL^{-1}$)	Working curves	Correlation coefficient γ	RSD (%)	Limit of detection ($ng\,mL^{-1}$) (3σ)
Berberine	0.05–300	$\Delta I = 16.0\ \ln C + 36.1$	0.9946	3.5 ($n=5$)	0.02
Rutin	1.0–400	$\Delta I = 54.9\ \ln C - 3.27$	0.9911	2.42 ($n=2$)	0.4
Baicalin	30.0–450	$\Delta I = 0.150C + 13.6$	0.9952	1.99 ($n=5$)	10.0

Table 4 summarized some of the results obtained, and which agreed well with those obtained by UV–vis method.[6]

Conclusions

The present CL flow system with immobilized reagents is advantageous not only for reducing reagent and analyte consumption, but also for operational convenience and instrumental simplification. The proposed method can be used to determine berberine at levels as low as 50 $pg\,mL^{-1}$ without the need for any pre-concentration step. Flavonoid and baicalin easily found in herbal medicine were studied preliminarily. Rutin, a flavonoid glucoside known as vitamin P, also showed an inhibitive effect under the optimal conditions, but it has a rather narrow range and a higher limit of detection (Table 5). Similar does baicalin perform. By diluting to the proper concentration range, the interference of both in some herbal medicine could be eliminated. Additives commonly found in formulations do not interfere and two herbal medicines commonly found in compound berberine prescriptions were studied preliminarily. Compared with other methods for the determination of berberine, the proposed method is sensitive, rapid, accurate, simple and higher selectivity. These advantages provide encouragement for further work in clinical research and routine control determination of berberine in pharmaceuticals.

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