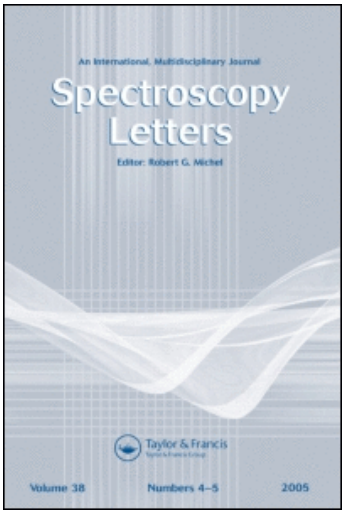

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Comparative Study on Three Different Systems of Chemiluminescence Flow-Injection Determination of Leucogen

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Abstract: The effects of three systems on the chemiluminescence (CL) intensity have been studied in this paper, such as leucogen–potassium permanganate–rhodamine B, leucogen–cerium (IV)–rhodamine B, and leucogen–luminol–hydrogen peroxide (called system 1, system 2, and system 3, respectively). The mechanism of these reactions is also discussed. Surfactant (CTMAB) has a remarkably sensitive effect on these systems mentioned above. Therefore, three new flow injection chemiluminescence methods for the determination of leucogen have been established. For system 1, the linear range is 8.0×10^{-8} to $4.0 \times 10^{-5}\,g\,mL^{-1}$, with limits of detection $2 \times 10^{-8}\,g\,mL^{-1}$; the relative standard deviation is 2.5% ($n = 11$, $Cs = 4.0 \times 10^{-6}\,g\,mL^{-1}$). For system 2, the linear range is 1.0×10^{-8} to $5.0 \times 10^{-6}\,g\,mL^{-1}$, with limits of detection $3 \times 10^{-9}\,g\,mL^{-1}$; the relative standard deviation is 5.1% ($n = 11$, $Cs = 1.0 \times 10^{-6}\,g\,mL^{-1}$). For system 3, the linear range is 4.0×10^{-8} to $2.0 \times 10^{-6}\,g\,mL^{-1}$, with limits of detection $1 \times 10^{-8}\,g\,mL^{-1}$; the relative standard deviation is 1.3% ($n = 11$, $Cs = 1.0 \times 10^{-7}\,g\,mL^{-1}$). Compared with the three methods above, system 3 is confirmed as the best method. This method has been applied to the determination of leucogen with satisfactory results.

Keywords: Chemiluminescence, CTMAB, flow-injection, hydrogen peroxide, leucogen, luminol

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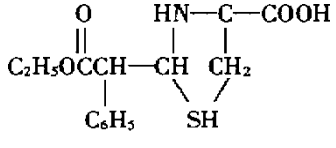
INTRODUCTION

Leucogen, known as 2-(α-phenyl-α-ethoxy-carbonyl-methyl) thiazolidine-4-carboxylic acid in chemistry, is a chemical drug that can resist anemia and promote leukocyte hyperplasia and has been widely used for the treatment of diseases caused by all kinds of factors, such as leukocyte decrease, anemia, and thrombocyte decrease, and it is also the ideal leukocyte hyperplasia drug for tumor patients after radiotherapy and chemotherapy.[1] Its structure is shown in Scheme 1.

So far, there are several methods for the determination of leucogen, such as first derivative spectrophotometry,[2] ultraviolet spectrophotometry,[3] flow injection spectrophotometry,[4] indirect atomic absorption spectrometry,[5] and single-sweep oscillopolarography.[6] The linear ranges in literature[2–6] are 5.00×10^{-5} to 1.25×10^{-4} g mL^{-1}, 1.0×10^{-5} to 1.0×10^{-4} g mL^{-1}, 1.0×10^{-5} to 2.0×10^{-4} g mL^{-1}, 1.0×10^{-5} to 1.0×10^{-4} g mL^{-1}, and 4.0×10^{-7} to 6.0×10^{-6} g mL^{-1}, respectively. However, these methods mentioned above have narrow linear range and low sensitivity and are not suitable for the analysis of drug. Is there a method of higher sensitivity? The method of flow injection chemiluminescence has many merits of high sensitivity, wide linear range, and simple operation.[7–11] Therefore, three new flow injection chemiluminescence methods for the determination of leucogen have been studied. System 1 was the system of leucogen–potassium permanganate–rhodamine B, in this research; it was found that potassium permanganate could oxidize the decomposed product of leucogen in acid media to generate chemiluminescence signals; rhodamine B improved the intensity of this system sharply. The flow injection chemiluminescence method for the determination of leucogen had been established in combination with the technique of the flow injection. The operation of this method was simple. The linear range of this stable and repeatable system was 8.0×10^{-8} to 4.0×10^{-5} g mL^{-1}, with limits of detection 2.0×10^{-8} g mL^{-1} and the relative standard deviation (RSD) was 2.5% ($n = 11$, $Cs = 4.0 \times 10^{-6}$ g mL^{-1}). Based on the facts above, it was observed that this method had wider linear range. However, its sensibility was not high. System 2 was the system of leucogen–cerium (IV)–rhodamine B; results of this research also indicated that cerium (IV) could oxidize the decomposed product of leucogen in acid media to generate chemiluminescence signals. Rhodamine B and cetyltrimethyl ammonium bromide (CTMAB) improved the intensity of this system



Scheme 1. Structure of leucogen.

sharply. Another flow injection chemiluminescence method for the determination of leucogen had been established in combination with the technique of the flow injection. This method had higher sensitivity [the limit of detection (LOD) was $3 \times 10^{-9}\,g\,mL^{-1}$] and wider linear range (1.0×10^{-8} to $5.0 \times 10^{-6}\,g\,mL^{-1}$). However, this system was not as stable as the leucogen–potassium permanganate–rhodamine B system, more factors could affect the chemiluminescence intensity, the conditions of operation were different to control, and the RSD was 5.1% ($n = 11$, $Cs = 1.0 \times 10^{-6}\,g\,mL^{-1}$). System 3 was the system of leucogen–luminol–hydrogen peroxide; the subsequent experiment showed that hydrogen peroxide could oxidize the decomposed product of leucogen in alkaline media to form oxide, and the formed oxide could oxidize luminol to generate chemiluminescence signals. When surfactant (CTMAB) was added to the system, it could enhance the chemiluminescence intensity remarkably. Based on the facts above, the third new method for the determination of leucogen with flow injection chemiluminescence had been established. The linear range of this method was 4.0×10^{-8} to $2.0 \times 10^{-6}\,g\,mL^{-1}$ with the limit of detection (LOD) of $1 \times 10^{-8}\,g\,mL^{-1}$. The relative standard deviation was 1.3% ($n = 11$, $Cs = 1.0 \times 10^{-7}\,g\,mL^{-1}$). This method had many merits of higher sensitivity, wider linear range, and more stable system; what's more, leucogen could be determinated directly without decomposition. Compared with the three methods above, system 3 is confirmed as the best method. This method had been applied to the determination of leucogen with satisfactory results.

MATERIALS AND METHODS

Apparatus and Reagents

A IFFL-DD flow injection chemiluminescence detector (Ruike Company, Xi'an, China) and a SP-1105 UV–Vis spectrophotometer (Shanghai Spectrum Instrument Plant, Shanghai, China) were also used. All chemical reagents were analytical reagent (A.R.) grade, and the water used was prepared by double sub-boiling distillation.

Method 1

Leucogen standard stored solution($2.0 \times 10^{-4}\,g\,L^{-1}$): 10.0 mg of leucogen (sub-refined) was weighed accurately, transferred to a beaker, dissolved and diluted to 50 mL of volumetric flask with 0.5‰ NaOH. The solution was put on the table in room temperature for 6 hr then gradually diluted to required concentration with water before being used. $0.01\,mol\,L^{-1}$ potassium permanganate stored solution, $0.015\,mmol\,L^{-1}$ rhodamine B (The Third Reagent Factory of Shanghai, China) stored solution (gradually

diluted to required concentration with water before being used), and 0.5% NaOH solution were prepared.

Method 2

Leucogen standard stored solution(1.0×10^{-4} g L^{-1}): 10.0 mg of leucogen (sub-refined) was weighed accurately, transferred to a beaker, dissolved by 8 mL of 2.0 mol L^{-1} NaOH and diluted to 100 mL of volumetric flask with 2.0 mol L^{-1}NaOH. The solution was put on the table in room temperature for 10 hr then gradually diluted to required concentration with water before being used. 20.0 mmol L^{-1} Ce(IV) stored solution: 2.741 g $(NH_4)_2Ce(NO_3)$ was weighed accurately and diluted to 250 mL of volumetric flask with 0.050 mol L^{-1} H_2SO_4. 1.0 mmol L^{-1} rhodamine B stored solution was prepared in 0.010 mmol $L^{-1}H_2SO_4$ used as medium. All stored solutions mentioned above were gradually diluted to required concentration with water before being used.

Method 3

Leucogen standard stored solution (1.0×10^{-4} g mL^{-1}):10.0 mg of leucogen (standard sample) was weighed accurately, transferred to a beaker and dissolved by 5 mL of *N*,*N*-dimethylformamide, then diluted to 100 mL of volumetric flask with water. The solution was gradually diluted to required concentration with water before being used. Luminol stored solution (20 mmol L^{-1}): 0.1772 g of luminol was weighed accurately and dissolved in the NaOH–$NaHCO_3$ buffer solution (prepared by 0.200 g NaOH and 0.420 g $NaHCO_3$), then diluted to 50 mL of volumetric flask with water, finally diluted to 2.0×10^{-4}mol L^{-1} before being used. H_2O_2 working solution (8.0×10^{-3} mol L^{-1}) and NaOH solution (0.1 mol L^{-1}) were also prepared. In addition, 1 mmol L^{-1} EDTA was added into each working solution mentioned above in order to avoid metalic ion perturbation.

Experimental Method

Figure 1 shows the schematic diagram of the FI-CL analyzer for the determination of leucogen. The FI system consists of two peristaltic pumps and a six-way valve. One pump delivered sample at a flow rate of 30R/min, the other delivered luminol solution and H_2O_2 solution at a flow rate of 45R/min. The distance between the valve and the flow cell was 10 cm. PTFE tubing (0.8 mm i.d.) was used to connect all components in the flow system. Sample solution (50 μL) was injected into the carrier stream through the six-way injection valve. The CL emission signal was measured by an IFFM-D CL detector produced by Ruike Company (Xi'an, China), which can be used to determinate the containment of leucogen. Then the results

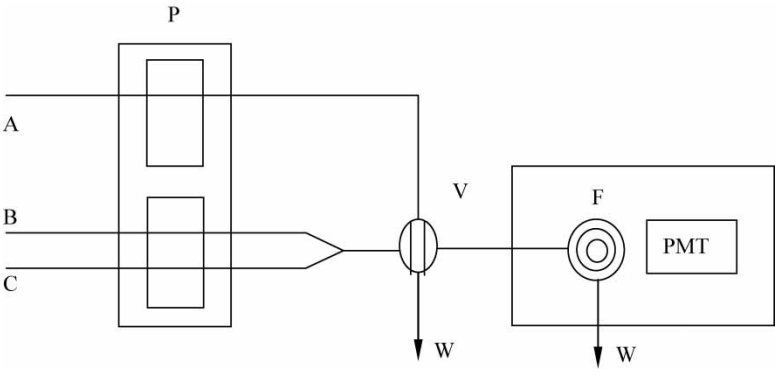


Figure 1. Schematic diagram of flow injection chemiluminescence analysis.

compared with those of UV detection were obtained by using a SP-1105s UV spectrophotometer.

Method 1

B. Rhodamine B solution
C. Potassium permanganate solution

Method 2

B. Rhodamine B solution
C. Ce(IV) solution

Method 3

B. Luminol solution
C. H_2O_2 solution

RESULTS AND DISCUSSION

Selecting Analytical Parameters

For the quick process of chemiluminescence, the signal was greater when high pump speed was used. In the flow injection analysis, high pump speed did not need more volume of reagents and sample. What is more, it reduced the washing time and improved the sampling frequency relatively.

Method 1

When the concentration of potassium permanganate solution was 4.0×10^{-4} mol L^{-1}, rhodamine B solution was 0.015 mmol L^{-1}, and leucogen solution was 4.0×10^{-6} g mL^{-1}, the effects of analytical parameters on the chemiluminescence intensity were examined. Results showed that the signal/background ratio reached the maximum when the corresponding analytical parameters were as follows: main pump speed was at a flow rate of 40R/min, and auxiliary pump speed was at a flow rate of 50R/min, the internal diameter of infusion manage was 0.8 mm, the sampling tube was 10 cm in length (sample volume: 50 μL), and the distance between the valve and the flow cell was 10 cm.

Method 2

When the concentration of cerium (IV) solution was 1.0×10^{-5} mol L^{-1}, rhodamine B solution was 0.010 mmol L^{-1}, and leucogen solution was 1.0×10^{-6} g mL^{-1}, the effect of analytical parameters on the chemiluminescence intensity were examined. Results showed that the signal/background ratio reached the maximum when the corresponding analytical parameters were as follows: main pump speed was at a flow rate of 20R/min, and auxiliary pump speed was at a flow rate of 40R/min, the internal diameter of infusion manage was 0.8 mm, the sampling tube was 10 cm in length (sample volume: 50 μL), and the distance between the valve and the flow cell was 10 cm.

Method 3

When the concentration of hydrogen peroxide solution was 8.0×10^{-3} mol L^{-1}, luminol solution was 2.0×10^{-4} mol L^{-1}, and leucogen solution was 5.0×10^{-7} g mL^{-1}, the effects of analytical parameters on the chemiluminescence intensity were examined. Results showed that the signal/background ratio reached the maximum when the corresponding analytical parameters were as follows: main pump speed was at a flow rate of 30R/min, and auxiliary pump speed was at a flow rate of 45R/min, the internal diameter of infusion manage was 0.8 mm, the sampling tube was 10 cm in length (sample volume: 50 μL), and the distance between the valve and the flow cell was 10 cm.

Optimization of Reaction Condition

Method 1

Leucogen cannot be dissolved in water, but it can be dissolved in NaOH solution (20.0 mg of leucogen was weighed accurately and dissolved

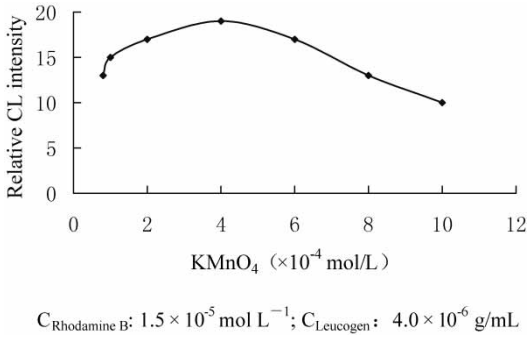


Figure 2. Effect of $KMnO_4$ concentration on CL intensity.

by 0.5‰ NaOH solution, then diluted to 100 mL of volumetric flask with NaOH solution). However, potassium permanganate must react with the decomposed product of leucogen in acid media to generate chemiluminescence signals. Rhodamine B improved the intensity of this system sharply. Results showed that the optimum conditions were as follows: the concentration of H_2SO_4 was $0.05\,mol\,L^{-1}$(Fig. 1), potassium permanganate solution was $4.0 \times 10^{-4} mol\,L^{-1}$ (Fig. 2), and rhodamine B solution was $0.015\,mol\,L^{-1}$ (Fig. 3).

Method 2

Leucogen cannot be dissolved in water, but it can be dissolved and decomposed in NaOH solution. However, cerium (IV) must react with the

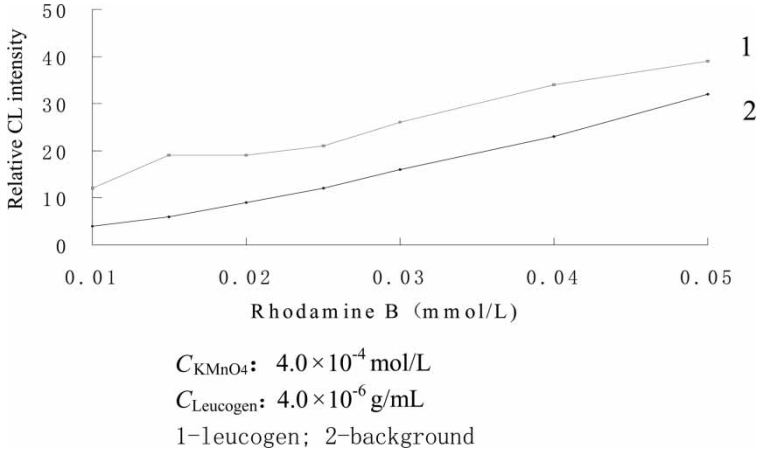


Figure 3. Effect of rhodamine B concentration on CL intensity.

Table 1. Effects of different alkaline conditions on CL intensity (the signal/background ratio) of leucogen [concentration of cerium(IV): 1.0 mmol L^{-1}; concentration of rhodamine B: 0.01 mmol L^{-1}]

2.0 mol L^{-1} NaOH Volume (mL)	Alkaline hydrolysis time (hr)					
	2	4	6	8	10	12
	Signal/background ratio					
2	8.0	11.25	8.2	9.6	9.7	9.6
6	10.5	13.25	10.0	11.2	11.0	11.0
7	9.0	10.4	12.4	11.8	12.8	13.0
8	12.8	14.0	14.6	14.8	16.8	17.2
9	12.8	14.0	15.0	14.8	15.0	15.4
10	11.0	15.5	11.0	14.0	15.33	15.33

Note: In the system of leucogen–cerium (IV)–rhodamine B, the concentration of CTMAB is 0.2%, and the concentration of leucogen is 1.0×10^{-6} g mL^{-1}.

decomposed product of leucogen in acid media to generate chemiluminescence signals. Rhodamine B improved the intensity of this system sharply. Results indicated that different decomposed conditions would affect the chemiluminescence intensity (Table 1). The optimum conditions of this system were as follows: the concentration of H_2SO_4 was 0.05 mol L^{-1}, cerium (IV) solution was 1.0×10^{-5} mol L^{-1} (Fig. 4), and rhodamine B solution was 0.015 mol L^{-1} (Fig. 5).

The experimental figures above indicate (1) the longer the decomposing time was, the higher chemiluminescence intensity of the decomposed product of leucogen in alkaline media was. But the subsequent experiment

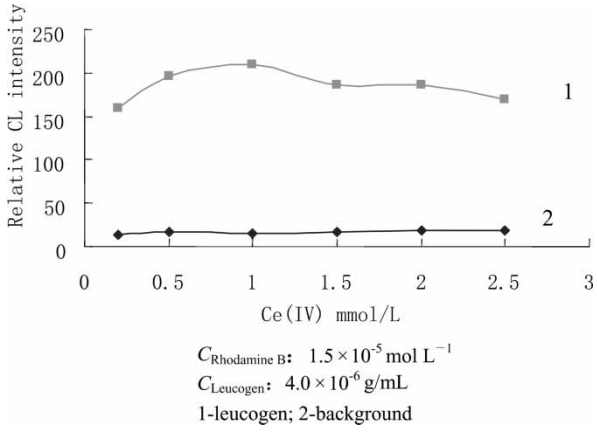


Figure 4. Effect of Ce(IV) concentration on CL intensity.

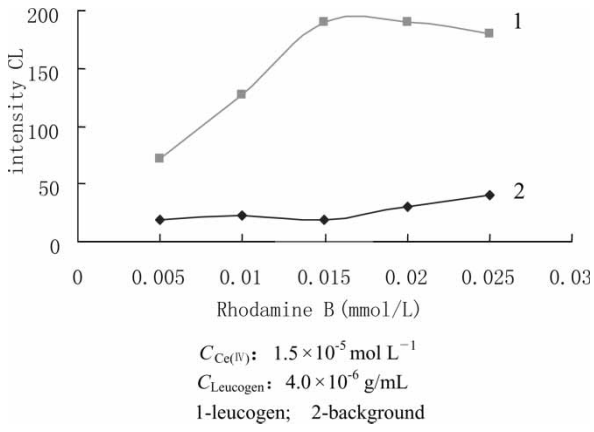


Figure 5. Effect of rhodamine B concentration on CL intensity.

showed that the system was not stable when the time exceeded 10 hr. (2) The chemiluminescence intensity of leucogen was varied by adding 2 mol L^{-1} NaOH solution, and the intensity reached the highest when 8–9 mL 2 mol L^{-1} NaOH was added.

Therefore, the conclusion was that the system of leucogen–cerium (IV)–rhodamine B was not suitable for the determination of leucogen for its low stability and harsh experimental condition, though the sensitivity was very high and linear range was quite wide.

Method 3

Leucogen can be dissolved in *N,N*-dimethylformamide solution, and luminol–hydrogen peroxide could react with the solution of leucogen–*N,N*-dimethylformamide in alkaline media to generate chemiluminescence signals directly. And CTMAB improved the intensity of this system sharply. The optimum conditions of this system were as follows: the concentration of luminol was 0.20 mmol L^{-1} (Fig. 6), hydrogen peroxide solution was 8.0×10^{-3} mol L^{-1}, NaOH solution was 0.104 mol L^{-1} (Fig. 7), and CTMAB was 0.1–0.2‰.

Working Curve, Accuracy and Limit of Detection

Method 1

Under the optimum conditions described above, when the concentration of leucogen was within the range of 8.0×10^{-8} to 4.0×10^{-5} g mL^{-1}, the CL intensity had a good linear correlation with the concentration of leucogen,

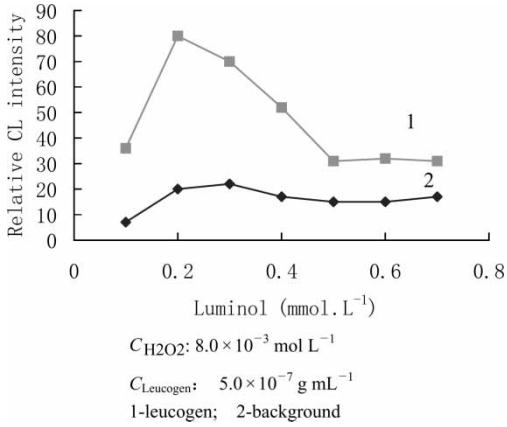


Figure 6. Effect of luminol concentration on CL intensity.

with a LD of 2.0×10^{-8} g mL^{-1} (3σ). The relative standard deviation was 2.5% (n = 11, Cs = 4.0×10^{-6} g mL^{-1}). In order to improve the accuracy, the regulated curve was depicted according to the amount concentration of leucogen. The parameters of regulated curve are listed in Table 2.

Method 2

Under the optimum conditions described above, when the concentration of leucogen was within the range of 1.0×10^{-8} to 5.0×10^{-5} g mL^{-1}, the CL intensity had a good linear correlation with the concentration of leucogen, with a LOD of 3.0×10^{-9} g mL^{-1} (3σ). The relative standard deviation

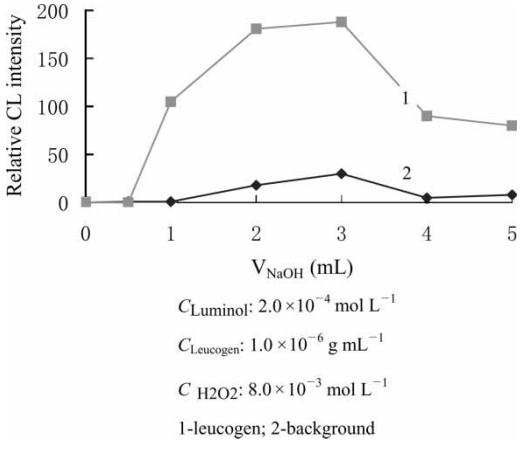


Figure 7. Effect of NaOH concentration on CL intensity.

Table 2. Linear range and regressive equations of the calibration curves

Linear range (g/mL)	Reg. eq. $y = b + ax$	Correlation coefficient	H.V. (V)
$8.0 \times 10^{-8} \sim 1.0 \times 10^{-6}$	$y = 0.0726 + 0.9903x$ (unit of x is 10^{-7} g/mL)	0.9998	600
$1.0 \times 10^{-6} \sim 4.0 \times 10^{-5}$	$y = 10.381 + 2.0362x$ (unit of x is 10^{-6} g/mL)	0.9974	600

was 5.1% (n = 11, Cs = 1.0×10^{-6} g mL^{-1}). The regression equation could be expressed as $Y = 11.689 + 50.202X$ (unit of X: $\times 10^{-6}$ g mL^{-1}), r = 0.9922.

Method 3

Under the optimum conditions described above, when the concentration of leucogen was within the range of 4.0×10^{-8} to 2.0×10^{-6} g mL^{-1}(n = 7), the CL intensity had a good linear correlation with the concentration of leucogen. The regression equation could be expressed as $Y = -3.45612 + 78.65857X$ (unit of X: $\times 10^{-6}$ g mL^{-1}), r = 0.9993. And the relative standard deviation was 1.3% according to eleven-fold measurements for sample containing 1.0×10^{-7} g mL^{-1} leucogen, with a LOD of 1×10^{-8} g mL^{-1} (3σ).

Interferences Experiment

Method 1

For 1.0×10^{-6} g mL^{-1} leucogen solution, the tolerance (multiple) of coexistent chemicals of inorganic ions and organic substance were as follows: starch (1000), glucose, hard fatty acid, magnesium, and dextrin (500); K^+, Na^+, Co^{2+}, Cd^{2+}, Al^{3+}, Fe^{3+}(500); Zn^{2+} (100); Pb^{2+}, NH_4^+, SO_4^{2-}, CO_3^{2-}, PO_4^{3-}, Cl^-, NO_3^-, SO_3^{2-}, Ac^-(200).The tolerance (multiple) of positive interferences were as follows: Ca^{2+}, Br^-, Sr^{2+}, Bi^{3+} and Mg^{2+} (more than 10). The tolerance (multiple) of negative interferences were as follows: Sn^{4+}, Hg^{2+} (more than 50).

Method 2

For 1.0×10^{-6} g mL^{-1} leucogen solution, the tolerance (multiple) of coexistent chemicals of inorganic ions and organic substance were as follows: starch (1000); glucose and dextrin (500); K^+, Na^+, Ca^{2+}, Al^{3+},

SO_4^{2-}, Cl^-, NO_3^- (500); Ni^{2+} (400). The tolerance (multiple) of positive interferences were as follows: Mg^{2+} (50). The tolerance (multiple) of negative interferences were as follows: Pb^{2+}, Mn^{2+}, Fe^{3+}, Ag^+, Cu^{2+} (50).

Method 3

For the sample containing $5.0 \times 10^{-7}\,g\,mL^{-1}$ leucogen, the tolerance (multiple) of coexistent chemicals of inorganic ions and organic substance were as follows: starch, glucose, hard fatty acid, magnesium, and dextrin (500); Cl^- (500); K^+, Na^+, SO_4^{2-}, and NO_3^- (1000); Ni^{2+}, NH_4^+, Ca^{2+}, Cu^{2+}, and Zn^{2+} (20); Mn^{2+} (10); Al^{3+}, CO_3^{2-} (5). The tolerance (multiple) of positive interferences were as follows: Cr^{3+} (5). The tolerance (multiple) of negative interferences were as follows: Hg^{2+} (20), Fe^{3+} (5). Considering the sample used in this paper was medical tablet, we concluded that common additives had no interference effect on this CL method for the determination of leucogen.

Effect of Surfactants

In order to improve the CL intensity and the detection sensitivity further, the effects of some surfactants upon the CL intensity were examined, such as CTMAB, Tween-80, Triton-X-100, polyoxyethylene-400, polyvinyl alcohol, β-CD, ferrocene, dodecyl benzene sulfonic acid sodium salt, and so on.

Method 1

Results showed that CTMAB, Tween-80, and Triton-X-100 had better sensitization effect on the CL intensity of this system when the concentration of leucogen, H_2SO_4, potassium permanganate, and rhodamine B solution was $4.0 \times 10^{-6}\,g/mL$, $0.05\,mol\,L^{-1}$, $4.0 \times 10^{-4}\,mol\,L^{-1}$, and $0.015\,mol\,L^{-1}$, respectively. However, the effects from Tween-80 and Triton X-100 were more obvious (Fig. 8).

Method 2

Results showed that 0.05‰ CTMAB had better sensitization effect on the CL intensity of this system when the concentration of leucogen, H_2SO_4, cerium (IV), and rhodamine B solution was $1.0 \times 10^{-6}\,g/mL$, $0.05\,mol\,L^{-1}$, $1.0 \times 10^{-5}\,mol\,L^{-1}$, and $0.015\,mol\,L^{-1}$, respectively (Fig. 9).

Method 3

Results showed that $0.1 \sim 0.2$‰ CTMAB had better sensitization effect on the CL intensity of this system when the concentration of leucogenm, luminol,

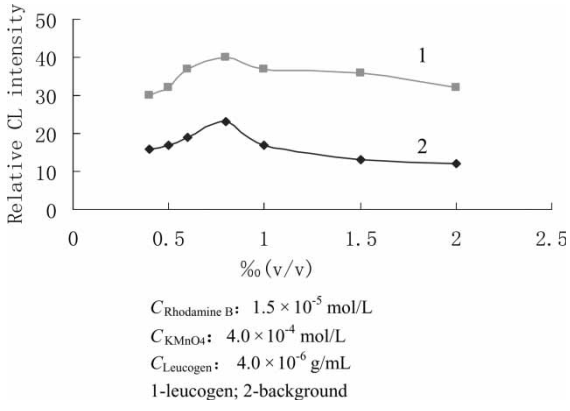


Figure 8. Effect of (1) Tween-80 and (2) Triton X-100 concentration on the CL.

hydrogen peroxide, and NaOH solution was $5.0 \times 10^{-7}\,g\,mL^{-1}$, $0.20\,mmol\,L^{-1}$, $8.0 \times 10^{-3}\,mol\,L^{-1}$, and $0.104\,mol\,L^{-1}$, respectively. So CTMAB was chosen as the activating reagent for further work (Fig. 10).

It was reported that the quantum yield of luminol of the CL would be increased sharply(about 10–100 times) in the micelle of cationic surfactant.[12] CTMAB was a kind of cationic surfactant. The micelle formed from CTMAB had better sensitization effect on the CL intensity.

Contrast of the Methods

The effects of three systems on the chemiluminescence (CL) intensity have been studied in this paper, such as leucogen–potassium

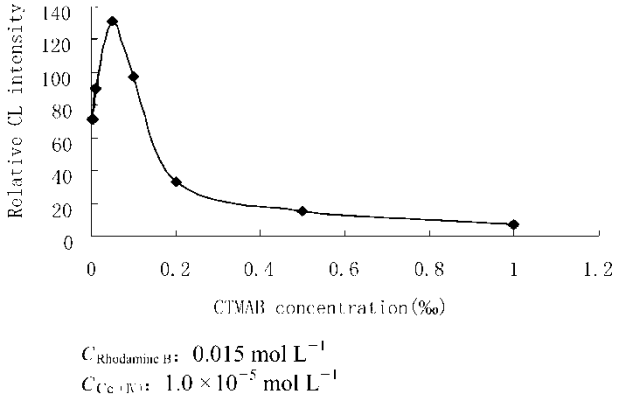


Figure 9. Effect of CTMAB concentration on the CL intensity.

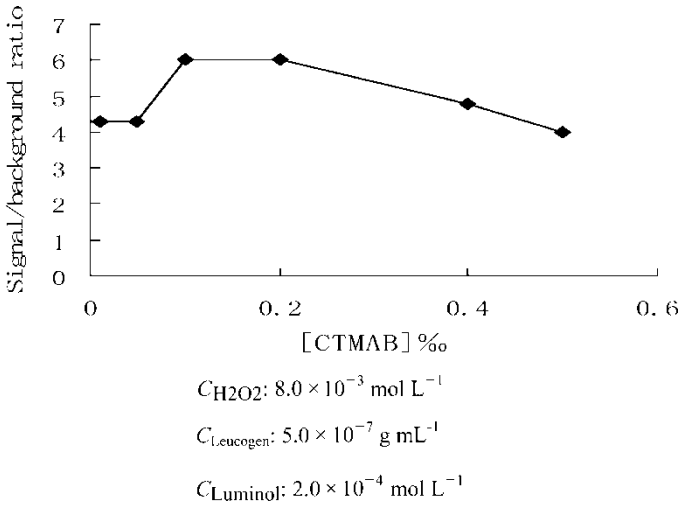


Figure 10. Effect of CTMAB concentration on the CL intensity.

permanganate–rhodamine B, leucogen–cerium (IV)–rhodamine B, and leucogen–luminol–hydrogen peroxide. The advantage and disadvantage of the three methods were analyzed and listed in this paper. For system 1, it was more stable and had wider linear range; however, its sensibility was not high; for system 2, the sensibility of this system was high, the linear range was wider, but the stability was not good, the conditions of reaction were not easy to control (Table 1), what's more, there were more iron interferences. For system 3, the linear range was wider; it was more stable, with higher sensibility, and the operation was simpler than the two methods mentioned above, it was possible to carry out online determination. Therefore, the conclusion was that the system of leucogen–luminol–hydrogen peroxide (system 3) was suitable for the determination of leucogen. This method has been applied to the determination of leucogen with satisfactory results.

Table 3. Analytical results of samples

Samples	Present method (mg/tablet)	RSD (%, n = 5)	Spectrophotometry method (mg/tablet)	Nominal content (mg/tablet)
1	10.4	2.9	10.59	10.0
2	10.0	4.0	9.60	10.0

Note: Sample 1 was purchased from Gibell Pharmaceutcal Co., Ltd. (Jiangsu, China; H32025443), and sample 2 was from First Pharmaceutical Factory (Shenyang, China; H21022575).

Table 4. Analytical Results of leucogen in samples (standard addition method)

Sample	Found (μg/mL)	Added (μg/mL)	Total found (μg/mL)	Recovery (%)	RSD (%, n = 5)
1	0.500	0.500	0.996	99.2	5.1
2	0.100	1.000	1.141	104.1	2.0
3	0.200	1.000	1.193	99.3	3.6

Sample Analysis

Twenty pieces of leucogen tablets (from Gibell Pharmaceutcal Limited Company, Jiangsu, China) were weighed accurately, ground to powder, and mixed homogeneously. 2.22822 g leucogen (gross weight) was obtained, then 1/10 of the weight (0.22822 g in theory) was expected, but actually 0.22286 g sample was obtained. 10 mL of *N,N*-dimethylformamide was added to dissolve it; finally the solution was diluted to 200 mL after suction. Thus, the solution containing 1.0×10^{-4} g mL^{-1} leucogen and 5% *N,N*-dimethylformamide was prepared.

Twenty pieces of leucogen tablets (from First Pharmaceutical Factory, Shenyang, China) were weighed accurately and treated in the same way as the former sample. 1.80737 g leucogen (gross weight) was obtained, then 1/10 of the weight (0.18073 g in theory) was expected, but actually 0.18074 g sample was obtained. Then the powder was also dissolved by 10 mL of *N,N*-dimethylformamide, finally diluted to 200 mL after suction. Thus, the solution containing 1.0×10^{-4} g mL^{-1} leucogen and 5% *N,N*-dimethylformamide was prepared.

Certain amount of above solution was measured accurately and diluted to 1000 times as test solution. According to the experimental procedure, the sample was analyzed with flow injection chemiluminescence method. The results are listed in Table 3 in contrast with those of the spectrophometry method.[3] In addition, the sample was also analyzed with standard addition method, and the results are listed in Table 4.

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