Research article

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Flow-injection chemiluminescence method for the determination of chloramphenicol based on luminol–sodium periodate order-transform second-chemiluminescence reaction

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ABSTRACT: A new chemiluminescence (CL) reaction was observed when chloramphenicol solution was injected into the mixture after the end of the reaction of alkaline luminol and sodium periodate or sodium periodate was injected into the reaction mixture of chloramphenicol and alkaline luminol. This reaction is described as an order-transform second-chemiluminescence (OTSCL) reaction. The OTSCL method combined with a flow-injection technique was applied to the determination of chloramphenicol. The optimum conditions for the order-transform second-chemiluminescence emission were investigated. A mechanism for OTSCL has been proposed on the basis of the chemiluminescence kinetic characteristics, the UV-visible spectra and the chemiluminescent spectra. Under optimal experimental conditions, the CL response is proportional to the concentration of chloramphenicol over the range 5.0×10^{-7}–5.0×10^{-5} mol/L with a correlation coefficient of 0.9969 and a detection limit of 6.0×10^{-8} mol/L (3σ). The relative standard deviation (RSD) for 11 repeated determinations of 5.0×10^{-6} mol/L chloramphenicol is 1.7%. The method has been applied to the determination of chloramphenicol in pharmaceutical samples with satisfactory results. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: order-transform second-chemiluminescence; chloramphenicol determination; flow-injection analysis; luminol; sodium periodate

Introduction

Chemiluminescence (CL) has been widely used for analysis due to its high sensitivity, wide dynamic range and simple instrumentation (1). It has been exploited with a wide range of applications in different fields, such as biotechnology, pharmacology, molecular biology and environmental chemistry (2–4). Luminol is one of the most popular CL reagents because of its excellent CL characteristics. The luminol CL system is based on the reaction of luminol (or its derivative) with hydrogen peroxide (or another oxidant) in an alkaline medium in the presence of a catalyst, in which luminol is oxidized to produce the excited 3-aminophthalate (3-aminophthalate*), then 3-aminophthalate* emits light in the wavelength range of 380–470 nm ($\lambda_{max} = 425$ nm) (5,6). The intensity of the light emitted in this reaction is directly proportional to the concentrations of luminol, catalyst and oxidant, so any of these three species may be quantified. The luminol CL reaction has been used successfully for detecting many inorganic and organic compounds (7–9).

The periodate–luminol reaction is a classical CL reaction and has been extensively investigated by many researchers(10) and it can be enhanced by many compounds. The proposed enhancement mechanism of the luminol–periodate–enhancer CL system is that these compounds are first oxidized to produce reactive oxygen species, such as $O_2^{\bullet-}$,·OH, H_2O_2 and $^1O_2$. The oxidation reaction of luminol is accelerated by the reactive oxygen species, thus, the CL emission is enhanced (11–14).

It has been found that injecting certain substances into reaction mixtures after the completion of CL reactions, such as the luminol–potassium periodate, *N*-chlorosuccinimide-alkaline dichlorofluorescein reaction, potassium permanganate–fluorescein and the potassium permanganate–luminol reactions, can cause a new CL reaction. This phenomenon has been named 'second chemiluminescence' or 'post-chemiluminescence' and the corresponding CL reaction as a second or post-chemiluminescence reaction (15–19).

In our recent investigations, we observed a new CL phenomenon. A CL signal was detected when chloramphenicol solution was injected into the mixture after the end of the reaction of alkaline luminol and sodium periodate or sodium periodate was injected into the reaction mixture of chloramphenicol and alkaline luminol. We describe this new CL phenomenon as the order-transform second-chemiluminescence (OTSCL) emission (with respect to the CL emission of the alkaline luminol–sodium periodate system and the CL emission of chloramphenicol–alkaline luminol system). The possible mechanism of this OTSCL reaction

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was based on studies of the CL kinetics, the UV-visible spectra and the CL spectra. Moreover, the OTSCL method combined with a flow-injection technique was applied to the determination of chloramphenicol.

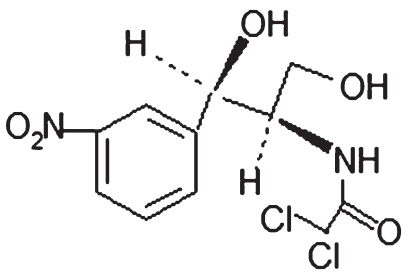
Experimental

Reagents

Chloramphenicol was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Scheme 1 shows the chemical structures of chloramphenicol. Luminol was obtained from Fluka (Biochemika) and used without further purification. A 1.0×10^{-2} mol/L stock solution was prepared by dissolving luminol in 0.10 mol/L sodium hydroxide solution. Working solutions of luminol were prepared by dilution of the stock solution. $NaIO_4$ (Shanghai Run Jie Chemical Reagent Co. Ltd, China) was used as received. All solutions were prepared from analytical reagent grade materials in double-distilled water.

Apparatus

The flow-injection analysis CL system used in this work is shown in Fig. 1. Two pumps of the Luminescence Analyzer (IFFM-E, Remex Electronic Instrument Co. Ltd, Xi'an, China) were used to deliver fluids. Polytetrafluoroethylene (PTFE) tubing (0.8 mm i.d.) was used to connect all components in the flow system. The flow cell was a 10 cm length of spiral glass tubing (2.0 mm i.d.). The CL signal was detected by the photomultiplier tube (PMT; CR-105, Hamamatsu, Beijing, China; operated at −700 V) placed near the flow cell and was recorded with a computer equipped with an A/D card.



Scheme 1. Structure of chloramphenicol.

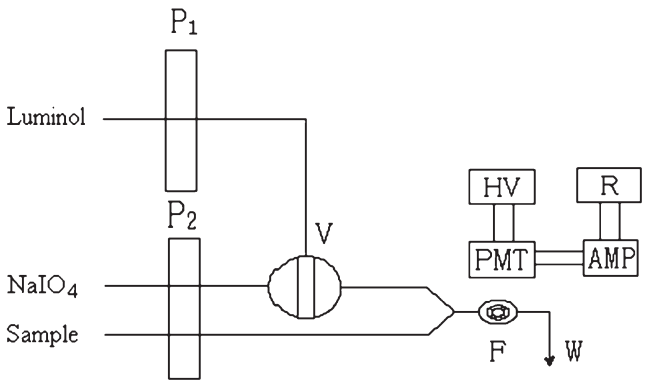


Figure 1. Schematic diagram of the CL–FIA system for the determination of chloramphenicol. P_1 and P_2, peristaltic pumps; V, six-way injection valve; F, flow cell; W, waste solution; HV, high voltage; PMT, photomultiplier tube; AMP, amplitude; R, recorder.

The CL spectrum was obtained with a series interference filters by the static method. The filters were inserted between the sample cuvette and the photomultiplier tube (PMT). Absorption spectra were acquired using a 1102 UV spectrofluorimeter (Shanghai Tian Mei Scientific instrument Co. Ltd, Shanghai).

Procedures

As shown in Fig. 1, luminol solutions were injected into the carrier stream (sodium periodate solution) through a six-way injection valve, and then mixed with sample solution/standard solution by the Y-shaped element in front of the flow cell to produce CL. The flow rate was set at 2.5 mL/min for all lines. The CL signal was detected by the PMT placed near the flow cell and was recorded with a computer equipped with an A/D card.

Results and discussion

Possible reaction mechanism

The CL kinetic characteristics of the CL reactions were examined using the static measuring system of the IFFM-E multifunction CL analyser. In both CL mode, 1.0 mL sodium periodate (5.0×10^{-5} mol/L) was injected into 0.5 mL alkaline luminol solution (1.0×10^{-4} mol/L) to produce CL emission (the first CL emission), and the signal returned to baseline after about 17.0 s, which showed that the first CL reaction had finished; 1.0 mL chloramphenicol (1.0×10^{-4} mol/L) was injected into the above reaction mixture, and a new CL emission (the second CL emission) appeared (as shown in Fig. 2). Under the same conditions, no CL signal was detected by using the blank solution (H_2O) instead of the chloramphenicol solution. In addition, Fig. 3 shows the kinetic characteristics of the luminol–chloramphenicol reaction and the luminol–chloramphenicol–sodium periodate reaction in alkaline medium. When1.0 mL chloramphenicol solution (1.0×10^{-4} mol/L) was injected into 1.0 mL luminol solution (1.0×10^{-4} mol/L), a CL reaction occured (Fig. 3a, the first CL emission). When the signal went back to baseline after about 80.0 s, which showed that the CL reaction had finished; 1.0 mL sodium periodate solution (5.0×10^{-5} mol/L) was then injected into the reaction mixture solution; in the same way, a new CL emission appeared (Fig. 3b, the second CL emission). The CL

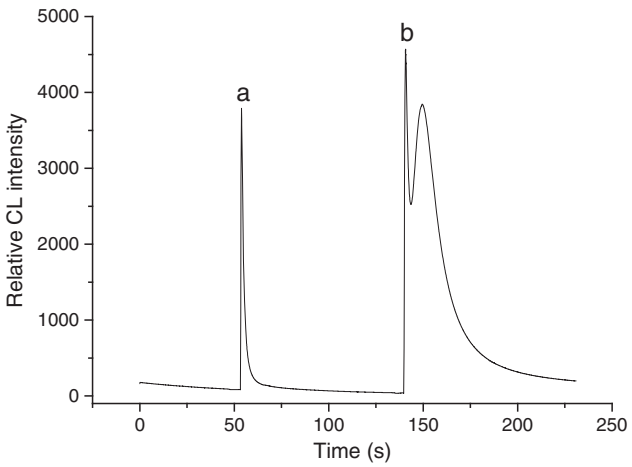


Figure 2. Kinetic curves of chemiluminescence: (a) CL emission of luminol and sodium periodate; (b) CL emission of chloramphenicol and mixture of luminol and sodium periodate.

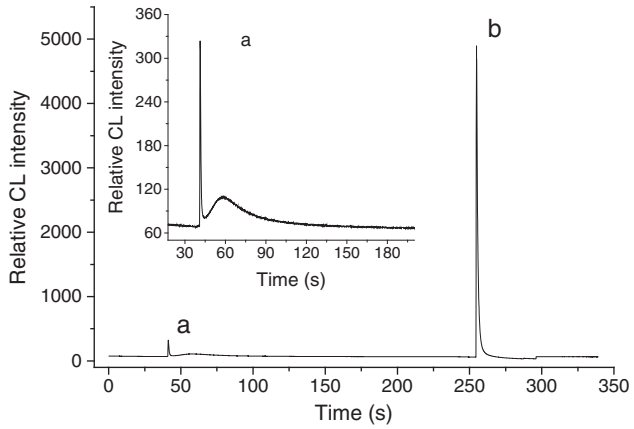


Figure 3. Kinetic curves of chemiluminescence: (a) CL emission of luminol and chloramphenicol; (b) CL emission of sodium periodate and a mixture of luminol and chloramphenicol; (insert) enlarged detail of (a).

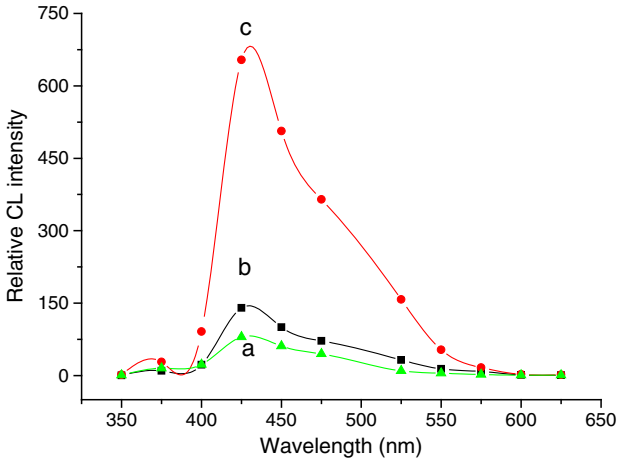


Figure 5. CL spectra: (a) chloramphenicol–luminol–NaOH; (b) luminol–NaOH–sodium periodate; (c) chloramphenicol–luminol–NaOH-sodium periodate.

reaction occurred immediately after mixing sodium periodate with the solution containing alkaline luminol and chloramphenicol, and reached a maximum within 1.0 s. The CL signal declined to baseline after approximately 20.0 s. On the other hand, in the absence of luminol, a small amount of chloramphenicol solution was injected into alkaline sodium periodate and no CL emission was observed.

The UV-visible absorption spectra of luminol–NaOH, chloramphenicol and the luminol–NaOH–chloramphenicol system were scanned using a Tianmei-1102 UV-visible spectrophotometer (Fig. 4). It can be seen that alkaline luminol had two absorption peaks at 300 and 346 nm (Fig. 4a), and chloramphenicol had one peak at about 278 nm (Fig. 4b). The addition of chloramphenicol to alkaline luminol revealed that there was a reaction between chloramphenicol and alkaline luminol, because the intensity of the peaks at about 278 nm and 300 nm decreased (Fig. 4c); meanwhile, a new absorption peak with the absorption wavelength at 290 nm was observed in the absorption spectra.

The CL spectra of the CL reactions were drawn (Fig. 5) by placing and exchanging the interference filters (350–625 nm) before the signal window of the IFFM-E multifunction CL analyser. All CL spectra have the same maximum wavelength at 425 nm (Fig. 5). It is well known that 3-aminophthalate is the luminophor of the luminol–periodate system, and the maximum emission of the CL reaction is at 425 nm (20,21). So it is easily seen that the luminophor for the CL reaction of luminol–NaOH–chloramphenicol and luminol–NaOH–sodium periodate–chloramphenicol is still 3-aminophthalate.

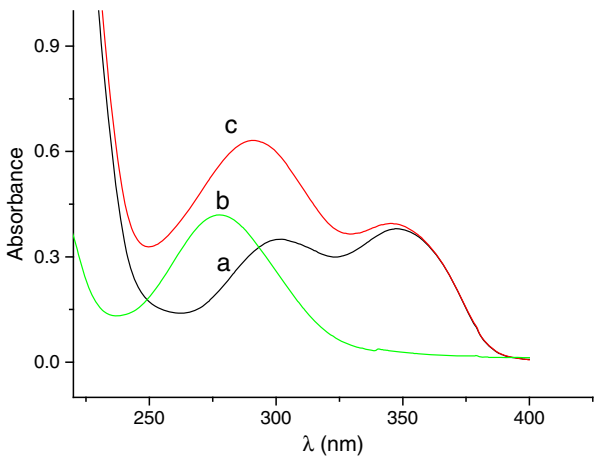


Figure 4. UV-visible absorption spectra: (a) 5.0×10^{-5} mol/L luminol + 8.0×10^{-2} mol/L NaOH; (b) 1.0×10^{-5} mol/L chloramphenicol; (c) 5.0×10^{-5} mol/L luminol + 8.0×10^{-2} mol/L NaOH + 1.0×10^{-5} mol/L chloramphenicol.

A mixture of 0.5 mL alkaline luminol (5.0×10^{-5} mol/L) and 1.0 mL sodium periodate (5.0×10^{-4} mol/L) was prepared and stored in the dark for 6 h in order for all of luminol to be oxidized. Then chloramphenicol solution was injected into the reaction mixture and no CL emission was observed. In such a reaction mixture the luminol concentration was almost zero, and thus its possible contribution to the second CL emission should be negligible. The results showed that the alkaline luminol–sodium periodate–chloramphenicol CL emission was dependent on luminol.

After deoxygenation with N_2, the alkaline luminol–chloramphenicol experiments were repeated and the results showed that the intensity of CL was not significantly changed. This phenomenon indicated that dissolved oxygen was not involved in the alkaline luminol and chloramphenicol reaction.

According to the above experiments and discussion, the possible CL mechanism of the OTSCL emission is suggested to be as follows. Luminol is first oxidized by $NaIO_4$ or chloramphenicol to 3-aminophthalate* in an alkaline medium (steps 1 and 2). Then, 3-aminophthalate* emits light to produce the first CL emission (step 5). Another reactant unknown complex$^{\bullet}$ formed during the chloramphenicol-alkaline luminol reaction can produce unknown complex* (step 3). The unknown complex* can transfer its energy to the 3-aminophthalate ion (an excellent fluorescent compound) to produce CL emission (step 4). This translation of unknown complex$^{\bullet}$ to unknown complex* is rarer and slower, so there are two peaks in the CL kinetic characteristics (Fig. 3 insert). In the alkaline luminol–sodium periodate–chloramphenicol system the iodide [IO_4^- or IO_3^- (product of reduced IO_4^-)] reacts with the unknown complex$^{\bullet}$ to produce unknown complex*, which transfers its energy to 3-aminophthalate ion (a strongly fluorescent compound) and produces the OTSCL emission (steps 6, 8 and 9). Meanwhile, IO_4^- or IO_3^- can also catalyse the slower translation of two unknown complex$^{\bullet}$ radicals to unknown

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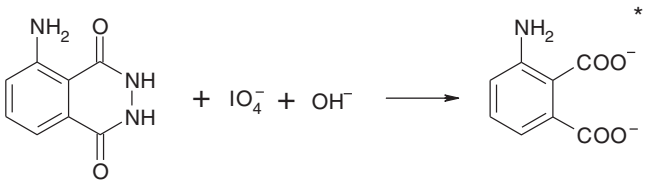
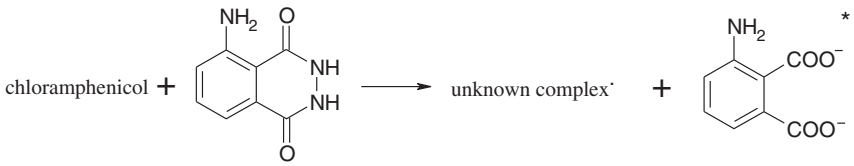
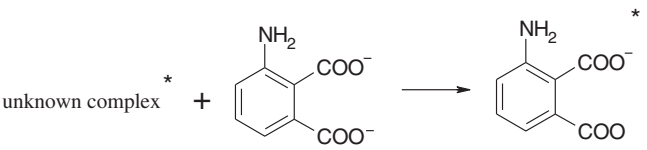
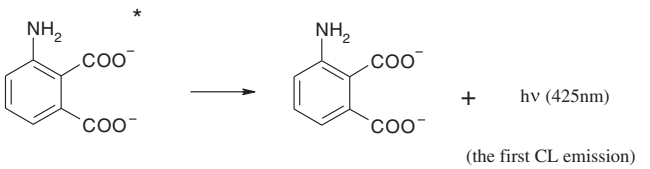
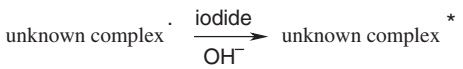
complex* (step 7). So, there are two peaks in Fig. 2b. In our experiments, the first one was applied for quantitative analysis. The possible CL mechanism of the reaction can be simply described as:

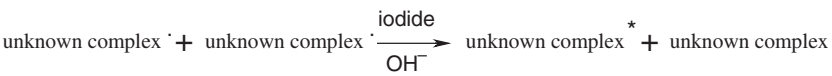


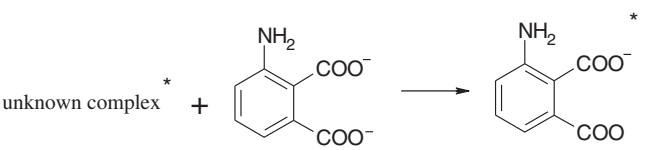


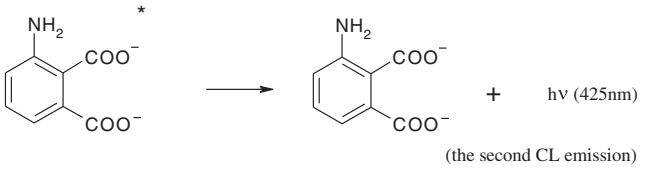












Optimization of experimental variables

The optimized conditions include the length of the mixing tube, the alkalinity of the reaction medium and the concentrations of

luminol and sodium periodate. All of these studies were performed using 5.0×10^{-6} mol/L chloramphenicol standard solution and a PMT voltage of 700 V.

Sodium periodate reacts with luminol in alkaline media. In the experiments, several solutions, such as NaOH, $NaHCO_3$–Na_2CO_3, $Na_2B_4O_7$–NaOH and NH_3–NH_4Cl, were studied. The results showed that the strong and stable OTSCL signal was obtained in NaOH. The CL emission intensity of 5.0×10^{-6} mol/L chloramphenicol–1.0×10^{-4} mol/L luminol–5.0×10^{-5} mol/L sodium periodate system in the presence of NaOH in the range 2.0×10^{-2} mol/L–2.0×10^{-1} mol/L was studied. It was found that 8.0×10^{-2} mol/L NaOH was the optimum reaction medium and this was chosen for further work.

The effect of luminol concentration on CL intensity was examined in the range 1.0×10^{-6}–3.0×10^{-4} mol/L (8.0×10^{-2} mol/L NaOH, 5.0×10^{-6} mol/L chloramphenicol and 5.0×10^{-5} mol/L sodium periodate) (Fig. 6). The CL intensity increased with increasing luminol concentration and then reached a maximum value at the luminol concentration of 1.0×10^{-4} mol/L. Higher concentrations resulted in a decrease of the emission intensity. Therefore, 1.0×10^{-4} mol/L luminol was used for subsequent work.

The concentration of $NaIO_4$ solution was examined in the range 5.0×10^{-6}–1.2×10^{-4} mol/L. The results showed that when $NaIO_4$ concentration was 5.0×10^{-5} mol/L, the CL reaction reached the maximum CL intensity.

It is critical that the CL reaction between sodium periodate and luminol is sufficient before initiating the second CL reaction. So, in the flow system (Fig. 1), a mixing tube (0.8 mm i.d.) was connected between the injection valve and the Y-piece. If the mixing tube was too short, the luminol and sodium periodate reaction was not complete and the background was too high; if the tube was too long, the second CL signal was too weak. The length of the mixing tube was examined in the range 5–50 cm when the flow rate of each solution was fixed at 2.5 mL/min (per tube). The signal:noise (S:N) ratio rapidly increased with increasing length of the mixing tube, probably due to the production of 3-aminophthalate ion. A 20 cm mixing tube of luminol and sodium periodate was found to be optimal in this flow system.

In flow injection analysis, the flow rate of each reagent stream is generally an important parameter. The solutions of 1.0×10^{-4} mol/L luminol in 8.0×10^{-2} mol/L NaOH and 5.0×10^{-5} mol/L sodium periodate were introduced into the manifold at equal flow rates and the CL emission was continuously recorded as the baseline. The intensity of background emission was relevant to the flow rate. The signal intensity increased with increasing flow rate, as was expected from the increased mixing rate. However, a high flow rate led to excessive consumption of reagents and sample solutions but little gain in CL intensity and an unstable CL signal. At a flow rate of 2.5 mL/min, the determination of chloramphenicol could be performed in 20 s, giving a sample measurement frequency of about 180 injections (60 samples)/h. Thus, the flow rate of 2.5 mL/min was selected as an appropriate condition, considering both good analytical precision and lower solution consumption.

Analytical characteristics of chloramphenicol

Under the selected conditions given above, CL intensity response to chloramphenicol concentration was linear in the range 5.0×10^{-7}–5.0×10^{-5} mol/L, with a correlation coefficient of 0.9969. The detection limit was 6.0×10^{-8} mol/L, which was calculated as the amount of chloramphenicol required to yield a net peak three times the standard deviation (SD) of the background signal (3σ). A complete analysis performed with a RSD was 1.7% ($n=11$). The typical response of CL intensity to 5.0×10^{-6} mol/L chloramphenicol is shown in Fig. 7. A complete analysis, including sampling and washing, could be performed in 20 s.

Interferences

Under the chosen conditions and using the flow system depicted in Fig. 1, the interference effect of common ions and several compounds commonly used as excipients were assessed. Samples containing chloramphenicol at a fixed concentration of 1.0×10^{-6} mol/L and increasing concentration of the interferences were analysed by the method. The

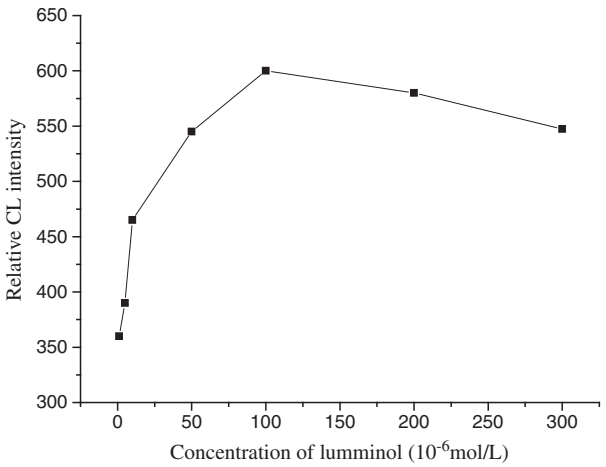


Figure 6. Effect of luminol concentration on OTSCL intensity of 5.0×10^{-5}mol/L sodium periodate + 8.0×10^{-2} mol/L NaOH + 5.0×10^{-6} mol/L chloramphenicol.

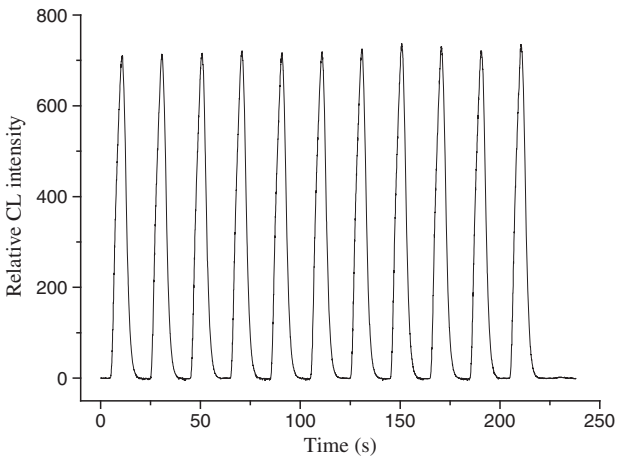


Figure 7. Typical recorder outputs for 11 measurments of 5.0×10^{-6} mol/L chloramphenicol standard solution.

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tolerable limit of a foreign species was set at a relative error of < 5.0%.

The obtained results in Table 1 showed that, under the optimized conditions, some ions and the studied excipients at concentrations usually found in capsules did not interfere with the determination of chloramphenicol. Therefore, this method can be proposed for the determination of chloramphenicol in pharmaceutical preparations.

Application

The concentrations of chloramphenicol in injections as a pharmaceutical preparation were determined using the proposed method. The sample was diluted appropriately with water prior to measurement, so that the concentration of chloramphenicol was in the linear response range. The results are shown in Table 2. The *t*-test assumes that there was no significant difference between the labelled value and the measurement results at a confidence level of 95%. The proposed method was evaluated by comparison of the results obtained with this method with those obtained in the analysis of the same samples by UV (Table 3) [$F = 4.00 < F = 6.39$ ($p = 0.95$ in a standard table of significance)]. $T = 0.378 < T = 2.31$ ($p = 0.95$ in a standard table of significance). Hence, at the 0.05 level the two means are *not* significantly different. The results suggest that the proposed method can be satisfactorily used for the determination of chloramphenicol in real samples.

Table 1. Tolerance to different substances in the determination of 1.0×10^{-6} mol/L chloramphenicol

Species added	Concentration ratio to chloramphenicol
Na^+, K^+, Mg^{2+}, Ca^{2+}, SO_4^{2-}, Cl^-	100
Starch, sucrose, glucose, fructose, ethanol	100
Zn^{2+}, Al^{3+}	50
Uric acid	20

Table 2. Determination of etimicin in injections

Sample	Labelled value	Proposed method[a]
Injection 1	250 mg/2 mL	128.9 ± 0.3 mg/mL
Injection 2	250 mg/2 mL	124.6 ± 0.5 mg/mL

[a]Average of three determinations.

Table 3. Determination of chloramphenicol using this method and UV (10^{-5} mol/L)

Sample	1	2	3	4	5
This method	0.98	1.01	1.02	1.02	1.00
UV	1.00	1.01	1.00	1.00	1.01

Conclusions

A new chemiluminescence phenomenon described as the order-transform second-chemiluminescence (OTSCL) emission was observed in the alkaline luminol–sodium periodate–chloramphenicol CL system, and the mechanism of the OTSCL emission was investigated. Furthermore, the OTSCL reaction combined with a flow-injection technique was applied to the determination of chloramphenicol in pharmaceutical preparations. The discovery of the OTSCL of the alkaline luminol–sodium periodate–chloramphenicol system will enlarge the range of CL research.

Acknowledgements

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