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Post-chemiluminescence Phenomenon of NBS–Luminol Reactions and Their Applications to Flow Injection Analysis of Piroxicam

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A new post-chemiluminescence (PCL) reaction was observed when piroxicam was injected into the reaction mixture after the CL reaction of *N*-bromosuccinimide (NBS) and luminol. A possible reaction mechanism was proposed based on the studies of the CL kinetic characteristics, the CL spectra, fluorescence spectra and other experiments. A new flow injection CL method for the determination of piroxicam was established based on the PCL reaction. The relative standard deviation (RSD) for the determination of piroxicam was 1.2% (n = 11, $c = 2.0 \times 10^{-6}$ g/ml). The CL intensity responded linearly to the concentration of piroxicam in the range 1.0×10^{-7} – 1.0×10^{-5} g/ml (r = 0.9991). The detection limit was 4.0×10^{-8} g/ml. The method had been applied to the determination of piroxicam in tablets with satisfactory results.

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A peculiar chemiluminescence phenomenon, post-chemiluminescence (PCL) phenomenon, had been reported in potassium permanganate–luminol, potassium ferricyanide–luminol and Ce(IV)–sodium sulfite system.[1-6] It had been predicted that more substances could cause PCL reaction in more CL systems.

Piroxicam, chemically named 4-hydroxy-2-methyl-*N*-(2-pyridyl)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide, is a new nonsteroidal anti-inflammatory drug[7] which has been used as one of a highly effective class of drugs against various arthritic conditions and postoperative inflammation. The present methods for the determination of piroxicam are HPLC[8] and flow injection quenching CL,[9] but the quenching CL had low sensitivity.

In this work, a PCL phenomenon was observed when piroxicam was injected in the mixture after the finish of the CL reaction of alkaline *N*-bromosuccinimide (NBS) and luminol. A possible reaction mechanism was proposed based on the studies of the CL kinetic characteristics, the CL spectra, fluorescence spectra and other experiments. A new method for the determination of pixoxicam was established by combining the CL reaction with the flow injection technique. The relative standard deviation (RSD) for the determination of piroxicam was 1.2% (n = 11, $c = 2.0 \times 10^{-6}$ g/ml). The CL intensity responded linearly to the concentration of piroxicam in the range 1.0×10^{-7} – 1.0×10^{-5} g/ml (r = 0.9991). The detection limit was 4.0×10^{-8} g/ml. The method had been applied to the determination of piroxicam in tablets and the results are in good agreement with those obtained by the pharmacopoeia method.[10]

Experimental

Reagents

Piroxicam stock solution (1.00×10^{-3} g/ml) was prepared by dissolving 0.1000 g of piroxicam (National Institute for the Control of Pharmaceutical and Biological Products, China) with 15 ml ethanol and then diluting to 100 ml with water.

Luminol stock solution (1.0×10^{-2} mol/l) was prepared by dissolving 1.77 g of luminol (synthesized by the Institute of Analytical Science of Shaanxi Normal University, China) in 1000 ml of 0.01 mol/l NaOH, and luminol working solution (4.0×10^{-6} mol/l) was prepared by diluting luminol stock solution with 0.06 mol/l Na_2CO_3–0.01 mol/l $NaHCO_3$ buffer solution.

NBS stock solution (5.0×10^{-3} mol/l) was prepared by dissolving 0.0445 g of NBS (Shanghai Chemical Reagent Company, China) with water and diluting to 50 ml. NBS working solution (2.0×10^{-5} mol/l) was prepared by diluting NBS stock solution with water.

All chemicals were analytical reagent grade except luminol and doubly distilled water was used throughout the experiments.

Apparatus

The IFFM-E flow injection CL analyzer (Xi'an Remex Electronic Instrument High-Tech Ltd., China) was equipped with an automatic injection system and a detection system. PTFE tubing (0.8 mm i.d.) was used to connect all of the components in the flow system. The flow cell was a coil of glass tube that was positioned in front of the detection window of the PMT. The CL signal was treated with a personal computer. CL spectra were measured with a BPCL ultra-weak luminescence analyzer (Institute of Biophysics, Chinese Academy of Sciences). Fluorescence spectra were obtained with a 970CRT spectrophotometer (Shanghai Analysis Instrument Main Plant, China). UV absorption spectra were measured on a TU-1901 spectrophotometer (Beijing Currency Instrumental Ltd., China).

Procedure

As shown in Fig. 1, flow lines (a, b and c) were connected with piroxicam solution, luminol solution and NBS solution, respectively. The NBS stream was first merged with the luminol stream to let NBS mix and react with luminol

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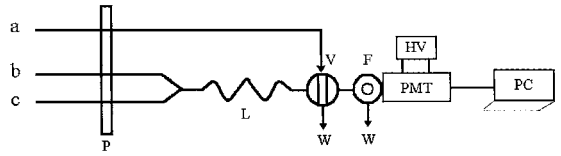

Fig. 1 Schematic diagram of CL flow system. a, Piroxicam solution; b, luminol solution; c, NBS solution; P, peristaltic pump; L, mixing tube; V, injection valve; W, waste; F, flow cell; HV, high voltage; PMT, photomultiplier tube; PC, personal computer.

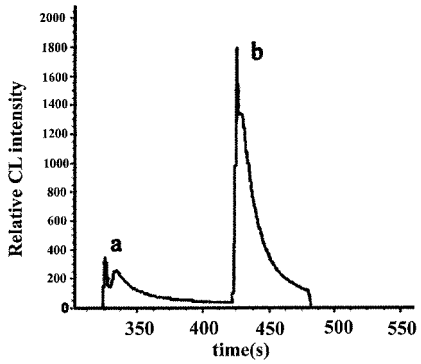

Fig. 2 Kinetic curve of the PCL reactions. a, Injecting 1.0 ml of 2.0×10^{-3} mol/l NBS solution into 1.0 ml of 1.0×10^{-5} mol/l luminol solution; b, injecting 1.0 ml of 1.0×10^{-5} g/ml piroxicam solution into the above reaction mixture.

sufficiently. When the baseline was stable, 50 μL piroxicam solution was injected into the merged steam of NBS solution and luminol solution accompanied with CL. The CL intensity (peak height) was used as the quantitative criterion.

Results and Discussion

The PCL behavior of piroxicam in NBS–luminol system

Kinetic characteristics of the CL reactions were examined using the static measuring system of the IFFM-E flow injection CL analyzer. The CL intensity–time curve is shown in Fig. 2. When 1.0 ml of 2.0×10^{-3} mol/l of NBS solution was injected into 1.0 ml of 1.0×10^{-5} mol/l of luminol solution, a CL reaction was initiated immediately (peak a). After approximately 150 s, the CL reaction terminated and the CL signal declined to baseline. Subsequently, a stronger CL reaction (peak b) was initiated when 1.0 ml of 1.0×10^{-5} g/ml of piroxicam was injected into above mixture. This CL reaction terminated and the CL signal declined to baseline again after approximately 70 s.

Under the same condition, CL signal was not detected by using the blank solution instead of the piroxicam solution.

When piroxicam was injected into NBS solution or luminol solution, there was no CL signal.

The experiments showed that the reaction initiated by piroxicam in the NBS–luminol system was a PCL reaction.

Optimization of experimental conditions

The length of the mixing tube. Since it was the PCL reaction of piroxicam in NBS–luminol system that was studied, the CL reaction between NBS and luminol must react adequately before initiating the PCL reaction. For this purpose, a mixing tube (L) (0.8 mm i.d.) was connected between the Y-piece and the injection valve (Fig. 1). If the mixing tube were too short, NBS and luminol would react deficiently and the background would be too high. If the tube were too long, the PCL signal would be too weak. The length of the mixing tube was examined in the range 15 – 100 cm when the flow rate of each solution was fixed at 1.6 ml/min. The test showed that the optimal length of mixing tube was 30 cm.

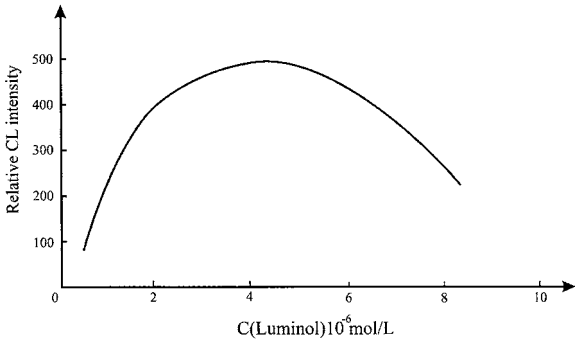

Fig. 3 Effect of luminuol concentration. NBS solution, 2.0×10^{-5} mol/l; piroxicam solution, 2.0×10^{-6} g/ml.

The pH of luminol solution. The PCL was affected by the pH of luminol solution. The pH of luminol solution was adjusted with Na_2CO_3–$NaHCO_3$ buffer solution. The result indicated that the maximal PCL signal could be observed when the pH was 9.5 (0.06 mol/l Na_2CO_3–0.01 mol/l $NaHCO_3$ solution).

The concentration of luminol solution. The effect of luminol concentration on the PCL intensity was examined from 5.0×10^{-7} to 8.0×10^{-6} mol/l. The result indicated that when the concentration of luminol was 4.0×10^{-6} mol/l, there was the strongest PCL intensity. Thus, 4.0×10^{-6} mol/l was selected as the optimum concentration of luminol solution (Fig. 3).

The concentration of NBS solution. The effect of NBS concentration on the PCL intensity was examined in the range of 5.0×10^{-6} – 5.0×10^{-5} mol/l. The maximal PCL signal was obtained when the concentration of NBS solution was 2.0×10^{-5} mol/l (Fig. 4).

NBS solution (2.0×10^{-5} mol/l) was examined to keep steady at room temperature in 2 days.

Analytical characteristics

Under the optimum conditions, the relation between the CL intensity and the concentration of piroxicam was examined. The linear range of the method was 1.0×10^{-7} – 1.0×10^{-4} g/ml with a linear regression equation of $I = 9.45c + 59.1$ ($c = 1.0 \times 10^{-7}$ g/ml, $r = 0.9991$), where I is the CL intensity (relative units) and c is the concentration of piroxicam (10^{-7} g/ml). RSD was 1.2% for 11 independent determinations of 2.0×10^{-6} g/ml piroxicam. According to the suggestions of IUPAC, the detection limit of the method was 4×10^{-8} g/ml of piroxicam.

Interference study

Under the optimum conditions, the interference tests of some excipients used in pharmaceutical preparations and some common ions to 1.0×10^{-6} g/ml piroxicam were examined. A foreign species was considered not to interfere if it caused a relative error < 5% during the determination of 1.0×10^{-6} g/ml of piroxicam solution. The tolerable concentration ratios of foreign species to 1.0×10^{-6} g/ml of piroxicam was over 1000-fold for NO_3^-, 500-fold for Ca^{2+}, Ni^{2+}, K^+, 100-fold for glucose, fecula, fructose, Fe^{2+}, Al^{3+} and Ba^{2+}, 10-fold for lactose, Zn^{2+}, Mg^{2+} and Fe^{3+}, 0.1-fold for Co^{2+}, Mn^{2+}, Cr^{3+} and Cu^{2+}.

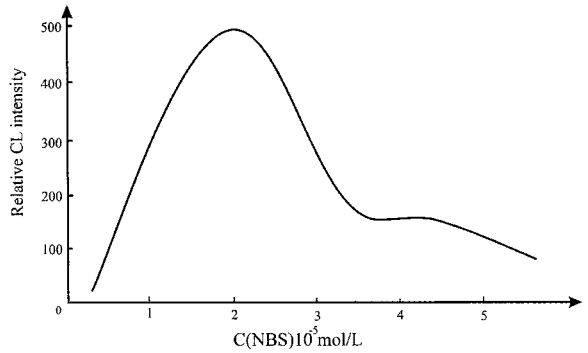

Fig. 4 Effect of NBS concentration. Luminol solution, 4.0×10^{-6} mol/l; piroxicam solution, 2.0×10^{-6} g/ml.

Table 1 Results of the determination for piroxicam tablets (mg/tablet)

Sample	This method[a]	RSD, %	Pharmacopoeia method[a]	RSD, %
1	49.6	2.6	50.1	0.5
2	47.3	2.9	47.0	0.3

a. Average of three measurements.

Sample analysis

The proposed method was applied to the determination of piroxicam in commercial piroxicam tablets. Two kinds of piroxicam tablets were purchased from a local market. The average tablet weight was calculated from the weight of 20 tablets. They were finely powered, homogenized and a portion of the powder, equivalent to one table weight, was accurately weighed and dissolved in ethanol with the aid of an ultrasonic pump. The resultant mixture was filtered and the filtrate was diluted to 100 ml with water for further sample analysis. The results are shown in Table 1. The *t*-test assumes that there was no significant difference between the proposed CL method and the pharmacopoeia method[11] at a confidence level of 95%.

Possible reaction mechanism

The CL spectra of NBS–luminol reaction and the PCL reaction were drawn using the BPCL ultra-weak luminescence analyzer. The maximum emission wavelengths were both 425 nm, which suggested that the two reactions have the same illuminant, the 3-aminophthalate ion (3-AP).[11,12]

The fluorescence spectra of luminol, the resultant solutions of NBS–luminol reaction and the PCL reaction were scanned (Fig. 5). It was observed that the maximum fluorescence wavelength of luminol was 417 nm, while the maximum fluorescence wavelengths of the resultant solution of NBS–luminol reaction and the PCL reaction were 425 nm. This gave another proof that 3-AP was the illuminant of the PCL reaction.

The absorption spectra of NBS solution and the mixtures of luminol and NBS solution were scanned (Fig. 6). It was observed that the characteristic absorption peak of piroxicam decreased gradually and disappeared finally when the quantity of NBS solution increased, which indicated that piroxicam is oxidized when NBS is added.

Under the optimum conditions, the concentration of NBS (2.0×10^{-5} mol/l) was much higher than that of luminol (4.0×10^{-6} mol/l). This result showed that excess NBS was necessary.

Based on the experimental results above, the following possible mechanism of the PCL reaction was proposed: NBS hydrolyzed to produce hypobromite with strong oxidation.[11] Hypobromite oxidized luminol producing 3-AP at an excited state (3-AP*). The 3-AP* came back to the ground state, accompanied by CL (λ_{max} = 425 nm). When piroxicam was added to the resultant solutions of NBS–luminol reaction, it was oxidized by hypobromite and released energy. 3-AP in the solution absorbed the energy and was excitated again, accompanied by CL.

The mechanism can be simply described as follows:

1 $NBS + OH^- \longrightarrow NHS + BrO^-$
2 $BrO^- + luminol \longrightarrow 3\text{-AP}^*$
3 $3\text{-AP}^* \longrightarrow 3\text{-AP} + h\nu$
4 $Piroxicam + BrO^- \longrightarrow products + energy\ (E)$
5 $3\text{-AP} + E \longrightarrow 3\text{-AP}^*$
6 $3\text{-AP}^* \longrightarrow 3\text{-AP} + h\nu \quad (\lambda_{max} = 425\ nm)$

As stated above, in conclusion the proposed method was applied to the determination of piroxicam tablets successfully.

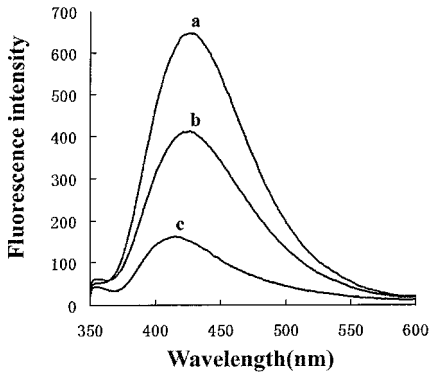

Fig. 5 Fluorescence spectra. a, NBS solution (8.0×10^{-5} mol/l) + luminol solution (1.6×10^{-5} mol/l); b, NBS solution (8.0×10^{-5} mol/l) + luminol solution (1.6×10^{-5} mol/l) + piroxicam solution (6.0×10^{-5} g/ml); c, luminol solution (1.6×10^{-5} mol/l).

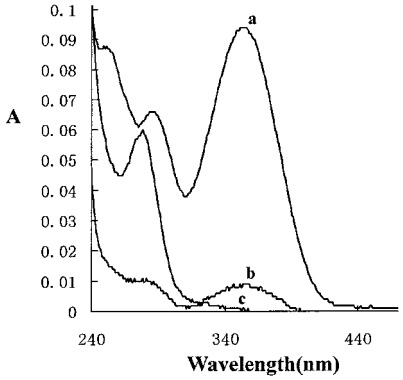

Fig. 6 UV spectra. a, Piroxicam solution (6.0×10^{-5} g/ml); b, piroxicam solution (6.0×10^{-5} g/ml) + NBS solution (8.0×10^{-5} mol/l); c, piroxicam solution (6.0×10^{-5} g/ml) + NBS solution (2.4×10^{-4} mol/l).

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