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A simple and sensitive chemiluminescence method for the determination of tiopronin for a pharmaceutical formulation

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Abstract

Here we report a rather simple and convenient chemiluminescence (CL) method for the determination of tiopronin. It was based that tiopronin could greatly enhance CL between H_2O_2 and luminol in a basic alkaline solution. Light emission is intense, and even with a simple setup a high sensitivity could be achieved. The linear range was 3 mM–500 nM with a detection limit of 200 nM. Singlet oxygen and hydroxyl radical were suggested to be produced in this reaction and was responsible for the CL of tiopronin. As a preliminary application, this simple method has been successfully applied into the determination of tiopronin in a pharmaceutical formulation.

Keywords: Tiopronin; Chemiluminescence; Luminol; Pharmaceutical formulation

1. Introduction

Tiopronin is an important thio-containing compound and is mainly used to prevent kidney stones [1,2]. Tiopronin works by removing the extra cystine from the body. Therefore, the sensitive determination of tiopronin in biological matrices and pharmaceutical preparation is highly desirable. A number of spectrometric [3–6] and fluorimetric methods [7,8] have been used for the determination of tiopronin. Several liquid chromatographic methods [9–13] have also been described. In the past few years, the sensitivity of chemiluminescence (CL) has attracted attention for the development of analytical methods for tiopronin [14–19]. Zhao et al. [14–16] reported a CL method for the determination of tiopronin, based on cerium oxidation sensitized by quinine. Perez-Ruiz et al. [17] measured tiopronin in pharmaceuticals based on cerium oxidation sensitized by rhodamine 6G and quinine. Although these methods provide a sensitivity of 10^{-7} M, but require a harsh medium, i.e. sulfuric acid. Besides, Vinas et al. [18] reported a convenient CL reaction for the determination of tiopronin, based on its inhibition of the CL generated in the copper-catalyzed oxidation of luminol. Lopez Garcia et al.

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[19] reported another CL method, based on its reduction of the CL between hypochlorite and luminol. However, the sensitivity of these methods was fairly low. Still, a simple and convenient method to measure tiopronin with a high sensitivity would be highly desirable.

Here we reported such a simple and convenient CL method for the determination of tiopronin. We found that the CL emitted after mixing H_2O_2 and luminol under alkaline conditions could be greatly and directly enhanced by tiopronin. Based on this fact, a simple and robust technique for the convenient measurement of tiopronin was developed. The linear range was 3 mM–500 nM with a detection limit of 200 nM. Besides, the mechanism of the proposed CL reaction was discussed.

2. Experimental

2.1. Chemicals

All chemicals were of analytical-reagent grade and were used as received. The water was prepared using MILLI-XQ equipment. Stock solution for 10^{-2} M tiopronin (Wako, Japan) was prepared by dissolving in 2.5 mM EDTA aqueous solution. Other standard solutions were made by gradually diluting relative stock solutions with 2.5 mM EDTA. Hydrogen peroxide, luminol and other organic compounds were also purchased from Wako, Japan. The tiopronin-containing drug, Thiola, was obtained from Shanten Pharmaceutical Company, Japan.

2.2. Apparatus

Batch CL measurements were conducted by using a BPCL chemiluminescence analyzer (Beijing, China) or a luminescence reader (BLR-201, Aloka, Japan).

2.3. CL detection procedures

Light-producing reactions were carried out in 12×75 mm disposable culture tubes containing 10 μl of 5×10^{-5} M luminol. 100 μl of 0.1 M Na_2CO_3 and 20 μl of 1.0 M H_2O_2 were added. A portion of distilled water was added to adjust the total volume and the tubes were placed in the luminescence reader. Then a portion of different concentrations of tiopronin was injected and the cover was closed to initiate CL. The signal was displayed and integrated for a 10 s interval, in arbitrary units. Kinetics of the CL was monitored on a recorder connected to the luminescence reader.

3. Results and discussion

The reaction mechanism of luminol system has been extensively studied. The excited state of 3-aminophthalic acid has been confirmed as an emitter [20–22]. The luminol reaction occurs under a wide variety of conditions. Specific analysis using luminol requires the chemistry to be controlled so that CL intensity is proportional to the concentration of the species of interest. As expected, without tiopronin, the oxidation of luminol by hydrogen peroxide is a slow reaction process and only emits a comparatively weak CL. This background can be greatly decreased by adding EDTA, since the signal is probably caused by metal impurities in CL reaction reagents [23]. Therefore, 2.5 mM EDTA was used to prepare standard and sample tiopronin solutions in the following experiments. As shown in Fig. 1,

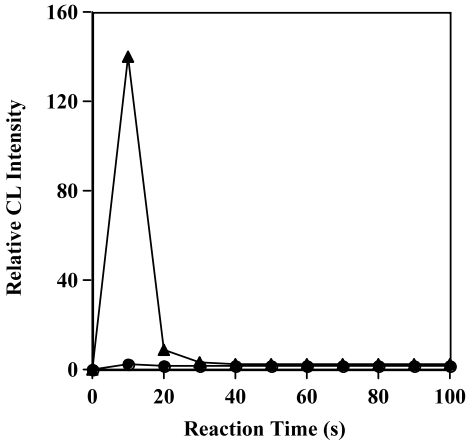


Fig. 1. Time course of the kinetic profile of the CL reaction in the absence (●) and presence (▲) of tiopronin, other components: 0.1 M H_2O_2, 0.05 M Na_2CO_3 and 2.5×10^{-6} M luminol.

CL was greatly enhanced in the presence of tiopronin.

In order to clarify the mechanism, several specific quenchers were added into the reaction solution. Firstly, as shown in Fig. 2, about 70% CL intensity was inhibited by the addition of 0.025 M dimethylfuran, 30% CL intensity was quenched by the addition of NaN_3 or 1,4-diazobicyclo[2,2,2]octane, which are generally used singlet oxygen scavengers [24–26]. Secondly, about 90% CL intensity was inhibited by the addition of 5% methanol, 80% CL intensity was quenched by the addition of 5% DMSO, which are generally used hydroxyl radical scavengers [27]. Thirdly, when the dissolved oxygen was removed from the solutions by the purge of nitrogen, the CL intensity decreased about 30%. In contrast, when the solutions purged with oxygen were used, the CL intensity increased about 70%. Fourthly, the CL spectrum of H_2O_2–tiopronin–luminol reaction was identical with that of a conventional H_2O_2–HRP–luminol reaction, this confirmed that the excited state of 3-aminophthalic acid appeared to be an emitter in this new CL reaction. Therefore, the mechanism can be summarized that singlet oxygen and hydroxyl radical were formed in this reaction, and then it reacted with luminol to emit CL. Possible mechanism was shown in Scheme 1.

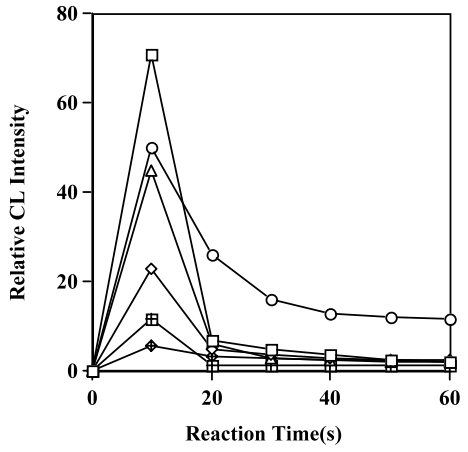


Fig. 2. Time course of the kinetic profile of the CL reaction in (□) H_2O, (○) 0.005 M 1,4-diazobicyclo[2,2,2]octane, (△) 0.025 M NaN_3, (◇) 0.025 M dimethylfuran (▩) 5% methanol and (▨) 5% DMSO; other components: 0.1 M H_2O_2, 0.05 M Na_2CO_3, 2.5×10^{-6} M luminol and 10^{-5} M tiopronin.

3.1. pH effect

The shape of the kinetic profile was greatly affected by the reaction pH. As shown in Fig. 3, at pH 9.0, the signal is slightly smaller but the signal reaches peak at 15 s and then decays slowly. At a higher pH than 10, the signal is a little bigger but it decreases quickly after reaching the maximum at 5 s. The maximum signal was the highest at a pH 10–11. However, even in an unbuffered 0.05 M Na_2CO_3 solution (pH 11.6), about 50% signal was observed. For convenience, the following experiments were done in 0.05 M Na_2CO_3 solution. A signal was also observed in other buffers, such as borate-NaOH, but the signal was comparatively smaller than that observed in $NaHCO_3$–Na_2CO_3 buffer, possibly that CO_3^{2-} participated in this CL reaction.

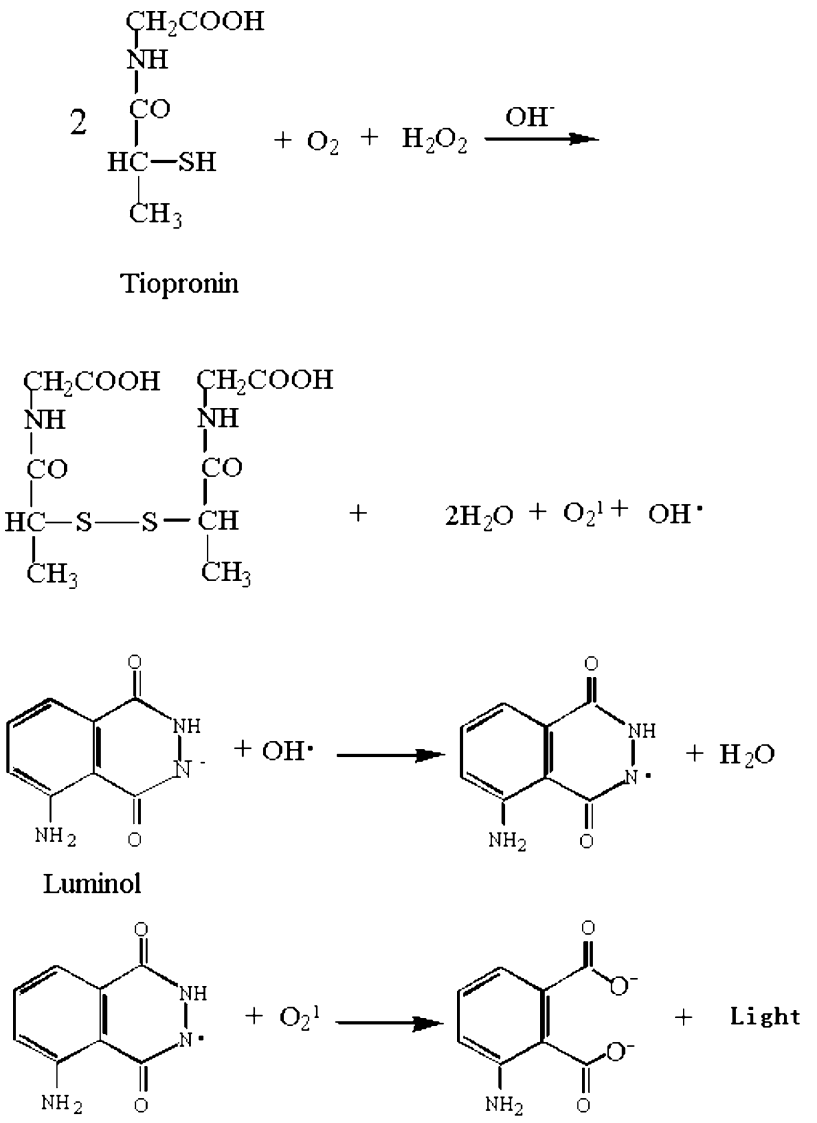
3.2. Effect of H_2O_2 and luminol the CL reaction

As shown in Fig. 4, the intensity increased when concentration of H_2O_2 was increased from 0 to 0.5 M, and then almost constant between 0.5 and 1.0 M. After that, the signal quickly decreased. Therefore, 1.0 M H_2O_2 was used as the optimum concentration.

Both of signal and background was increased with increasing luminol concentration, however, signal/noise is the highest at 5×10^{-5} M luminol (Fig. 5). Therefore, 5×10^{-5} M luminol was chosen as the optimum concentration in the following experiments.

3.3. Calibration curve

Under the proposed experimental conditions, with a batch method, a log–log calibration graph showed a linear correlation. The linear range was 3 mM–500 nM in 0.05 M Na_2CO_3 solution (the regression equation was $\log(CL) = 0.809 \log[\text{tiopronin}](uM) - 0.271$, $r^2 = 0.995$) and 0.3 mM–500 nM in pH 9.0 Na_2HCO_3–Na_2CO_3 buffer (the regression equation was $\log(CL) = 0.624 \log[\text{tiopronin}](uM) + 0.889$, $r^2 = 0.988$). The



Scheme 1. Proposed mechanism for the tiopronin-induced luminol CL.

detection limit (3σ) was 200 nM tiopronin and the quantification limit (10σ) was 600 nM tiopronin in both pH with our simple setup. The relative standard deviation was 2.7% (intra-day) and 4.0% (inter-day) at 5×10^{-6} M tiopronin. This sensitivity is higher than fluorescence and indirect CL methods [7,18,19] and comparable to that reported by using sensitized cerium oxidation CL system [14–17].

3.4. Interference studies

In order to assess the selectivity of the proposed method, the influence of common foreign species was studied by preparing solutions containing 5×10^{-6} M tiopronin and different concentrations of relative foreign species. The tolerance of each foreign species was taken as the largest concentration yielding an error of less than ±5% in the CL

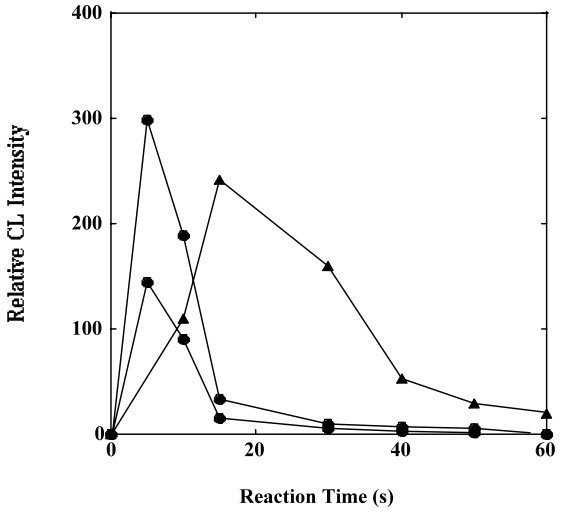


Fig. 3. pH effect on the emission intensity. (▲) pH 9; (●) pH 10; (○) pH 11.6, other components: 0.1 M H_2O_2, 0.05 M Na_2CO_3, 2.5×10^{-6} M luminol and 10^{-5} M tiopronin.

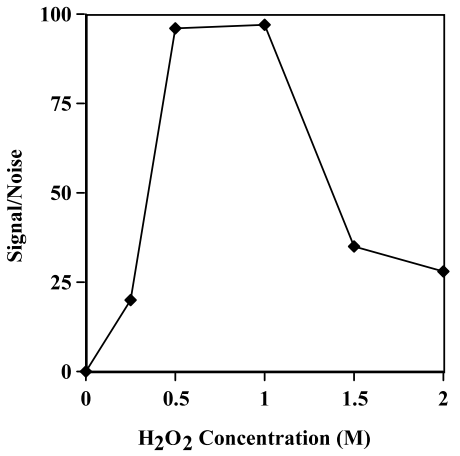


Fig. 4. Effect of hydrogen peroxide on the emission intensity, in the presence of 0.05 M Na_2CO_3, 2.5×10^{-6} M luminol and 10^{-5} M tiopronin.

signal of tiopronin. No interference was found when including up to 2000-fold Na^+, K^+, Ca^{2+}, Mg^{2+}, SO_4^{2-}, NO_3^-, Cl^-, PO_4^{3-}, $C_2O_4^{2-}$, glucose, starch, and 100-fold Zn^{2+}, Al^{3+}. However, equal amount of Fe^{3+}, Cr^{3+} and Co^{2+} increased CL signal, which could be removed by a cation exchange resin if necessary.

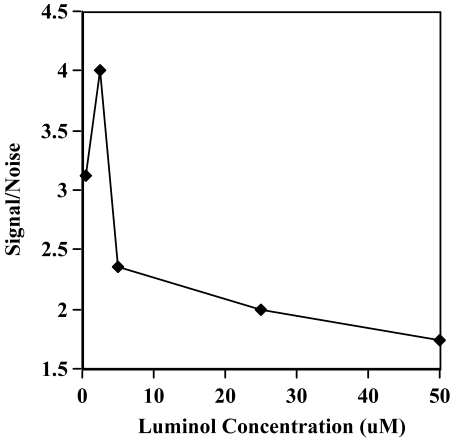


Fig. 5. Effect of luminol concentration on the signal/noise, other components: 0.1 M H_2O_2, 0.05 M Na_2CO_3 and 10^{-5} M tiopronin.

Secondly, since singlet oxygen and hydroxyl radical are intermediates, the method is susceptible to some interference. We have already found that singlet oxygen scavengers such as dimethylfuran and 1,4-diazabicyclo[2,2,2]octane and hydroxyl radical scavengers such as methanol and DMSO quenched the CL. Other compounds, like olefin dienes, terpenes, flavones and bile pigment possibly also interfere with the determination of tiopronin since they react with singlet oxygen. Although these compounds normally may not exist in most samples, special care should be taken when using this simple method in relative samples. Thirdly, we found that other thiol-containing compounds such as cysteine, homocysteine, 1-thioglycerol, 2-mercaptopropionic acid enhanced the CL whereas the disulfide-containing compounds such as cystine and homocystine did not have any effect on the CL. Thus, separation methods needed to be employed before the CL determination of each thiol compound in relative samples.

3.5. Sample analysis

The proposed CL technique can be easily used for the determination of tiopronin in the tablets. Each tablet was ground and dissolved in 100 ml water. After filtering, 10 ml of the supernatant was diluted to 100 ml with 2.5 mM EDTA solution.

The tiopronin amounts were determined to be 101 mg with a relative standard deviation of 5.0% (n = 5), which were in agreement with the labeled values (100 mg/tablet).

4. Conclusions

A simple and convenient technique for determining tiopronin was reported, based on the enhancement of tiopronin on weak CL between H_2O_2 and luminol. Tiopronin could be sensitively detected even with a simple setup. It was believed that singlet oxygen and hydroxyl radical were formed in this CL reaction and was responsible for the CL emission of tiopronin. Overall, the proposed method is not only simple and convenient, but also sensitive and user-friendly. Right now in this laboratory, further studies are being carried out in order to optimize the experimental conditions and improve the sensitivity for the post-column detection of tiopronin in biological samples.

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