



Chemiluminescence Determination of Atropine using Luminol-Hemin-H₂O₂ System

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Received 20 October 2013|Received in revised form 2 April 2014|Accepted 15 April 2014

Abstract: A sensitive and fast chemiluminescence (CL) method has been described for the direct determination of atropine. The method is based on the quenching effect of atropine on the CL signals of the luminol-H₂O₂ reaction catalyzed by hemin in an alkaline medium. Hemin could strongly enhance the chemiluminescence of the luminol-H₂O₂ system. The luminophor of the luminol-H₂O₂-hemin CL system was identified as the excited state 3-aminophthalate anion. The CL from luminol-H₂O₂-hemin system is strongly inhibited by the presence of atropine. Under the optimum conditions, the CL intensity is proportional to the concentration of the atropine in solution over the range 0.1-29 µg/ml. Detection limit and relative standard were 0/045 µg/ml and less than 4%, respectively. The proposed method has been utilized for the determination of atropine in injection and urine samples (The urine samples were collected from different healthy volunteers) with satisfactory results. Recovery tests were also carried out by adding a known amount of atropine in samples.

Key words: Chemiluminescence; luminol; hemin; quenching effect; Atropine

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. Introduction

Drug analysis is an important branch of analytical chemistry. It plays important role in drug quality control and has wide impact on public health. Therefore, developing sensitive, simple rapid and reliable method for the determination of active ingredient is value blend necessary.

Atropine (Fig. 1) is a principal alkaloid, extracted from 'Atropa belladonna' and is often known as deadly nightshade. It may induce hallucinogenic effects and is generally noted for its anticholinergic toxicity.

It is used for medicinal purposes in the treatment of eye diseases and as antidote to opium. It has been analytically quantified using a bulk acoustic wave sensor [1, 2]. Atropine has been used in treating Parkinson's disease, peptic ulcers, diarrhea, and bronchial asthma and also to treat nerve gas poisoning [3].

Atropine has been established as a very effective remedial substance (antidote) to ward off the effects of nerve agents like tabun (GA), sarin (GB) soman (GD) [4]. Flow-injection post chemiluminescence [5], chemiluminescence micro-

total analytical system [6], capillary electrophoresis [7], electrochemical method [8] High-performance liquid chromatography [9] have been developed for the determination of atropine.

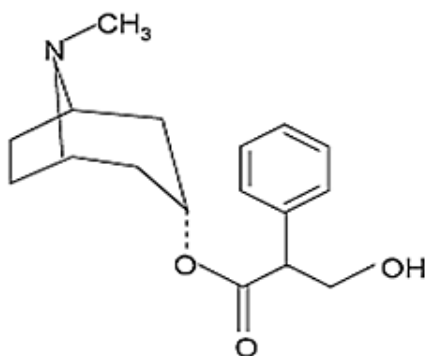


Figure 1: Molecular structure of atropine

Chemiluminescence analysis offers high sensitivity, wide linear range and simple instrument. Chemiluminescence methods provide cheap, rapid, and simple and therefore, have been successfully applied to many drugs detection [10-14]. Because of inherent functionality, metalloporphyrin complexes have been widely used in various oxidation reactions as models for cytochrome P-450. Owing to their supramolecular functions (i.e., assembly, bio mimic catalysis, and molecular recognition), metalloporphyrins have been widely used in analytical applications [15, 16].

Hemin, as natural metalloporphyrin compound, has also been applied for the determination of glucose and hydrogen peroxide [17, 18]. To the best of our knowledge, there is no report on luminol-H₂O₂-hemin CL for the determination of atropine.

In this work, we found that the emitted light from luminol-hemin- H₂O₂ CL system is quenched by atropine. Based on this fact, a new, simple and sensitive batch CL method was proposed for the analysis of atropine. The proposed method was successfully applied to the determination of

atropine in injections and in urine samples with lower detection limit and wider linear range.

2. Experimental

2.1. Apparatus

Chemiluminescence detection was performed with a home-made apparatus equipped with a Model BPY47 photocell (Leybold, Huerth, Germany). The apparatus was connected to a personal computer via a suitable interface (Micropars, Tehran, Iran).

2.2. Reagents and solutions

A 10⁻² molL⁻¹ stock solution of luminol (3-aminophthalhydrazide) was prepared by dissolving luminol (Fluka,) in 0.1mol L⁻¹ sodium hydroxide solution without purification. A stock solution of hydrogen peroxide (30%, v/v, commercially available) was prepared by appropriate dilution of 30% solution with water. Hemin (5×10⁻⁵ M in 0.1M NaOH) was obtained from Merck (Schuchardt, Germany). Stock solutions of atropine were prepared at a constant concentration of 0.5 mg/ml in 100 ml of water. Atropine (100% Analytical grade) was obtained from Sigma Aldrich (USA).

2.3. General procedure

Solution A was made by mixing 1 mL of luminol (10⁻² M), 0.1 mL of hemin (5×10⁻⁵ M) and 50 μl of atropine (various concentrations). Solution B contained various volumes of hydrogen peroxide 10⁻²M. Solution A was transferred into glass cell and then 100 μL of solution B was injected in the glass cell, chemiluminescence light intensity- time spectrum was recorded soon after mixing of the solutions.

As mentioned above, atropine was found to quench the CL emission of luminol. Therefore, the concentration of atropine was determined on the basis of changing the chemiluminescence intensity (ΔI_{CL}). ΔI_{CL} is obtained from the ratio of CL

intensity in the absence (I_0) and presence (I) of atropine i.e., $\Delta I_{CL} = I_0/I$

3. Results and discussion

3.1. Optimization of experimental conditions

The experimental conditions were optimized using 0.5 mg/mL atropine solution. An alkaline medium was introduced to improve the sensitivity of the system because of the character of luminol reaction. It was observed that higher CL signal and better repeatability could be obtained when sodium hydroxide was added into sample solution than into luminol solution.

The effect of sodium hydroxide concentration on the CL reaction was examined in the 0.04–0.4 mol/L range. Finally, 0.2 mol/L of sodium hydroxide was employed because it gave larger

signal-to-blank ratio and higher CL signal (Fig. 2A). The concentrations of hemin and luminol were important parameters in the experiments. The effect of different concentrations combination of hemin (5×10^{-6} – 5×10^{-5} mol/L) and luminol (10^{-3} – 10^{-2} mol/L) was studied. The experimental results showed that the maximum CL signal and signal-to-blank ratio was obtained when 1.6×10^{-5} mol/L of hemin and 1×10^{-2} mol/L of luminol were used (Fig. 2B and 2C). H_2O_2 is the important oxidant for determination of atropine in this CL system. The effect of hydrogen peroxide concentration on the reaction was examined in the range 0.01–0.1 mol/L. The CL signal increased by increasing of hydrogen peroxide concentration from 0.01–0.1 mol/L. Therefore, 0.1 mol/L of hydrogen peroxide concentration was employed (Fig. 2D).

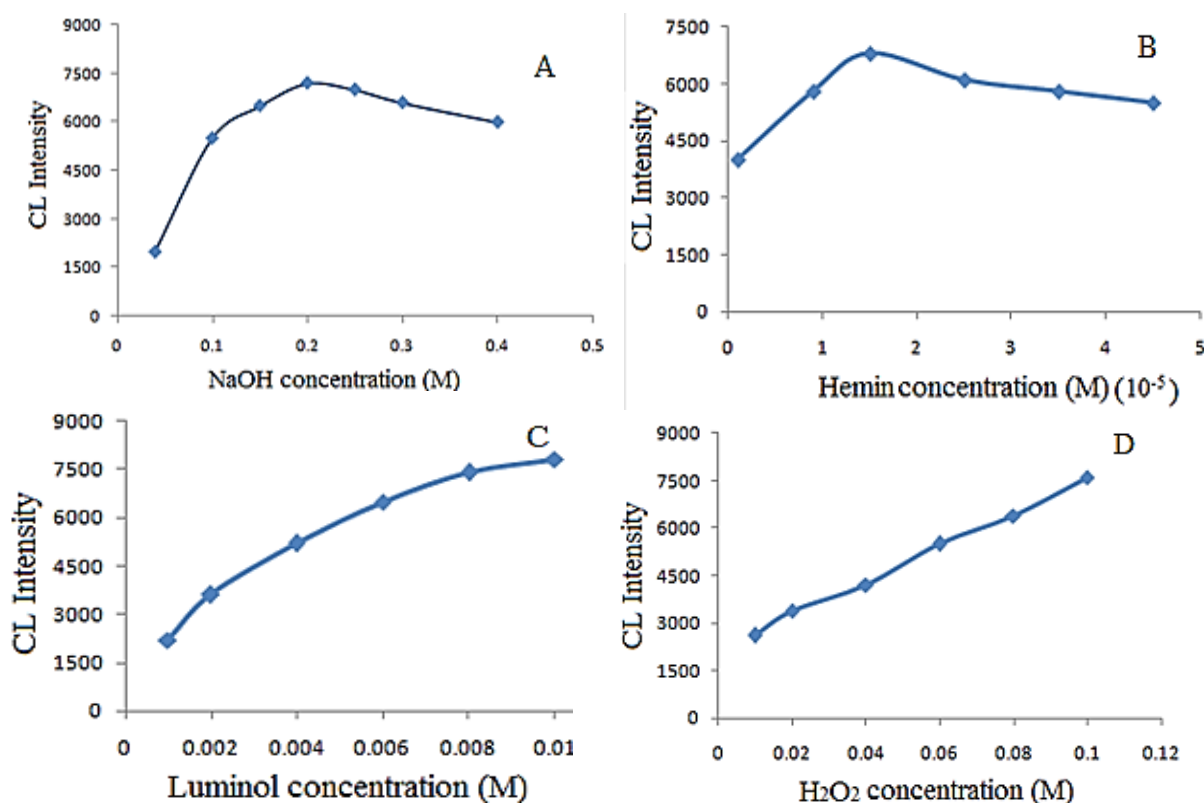


Figure 2: Effects of the reactant conditions on the luminol-hemin-NaOH- H_2O_2 CL system (A) Effects of NaOH concentration: 0.01 luminol mol/L, 0.1 mol/L H_2O_2 , 10^{-5} mol/L hemin (B) Effects of hemin concentration: 0.01 mol/L luminol, 0.2 mol/L NaOH, 0.1 mol/L H_2O_2 (C) Effects of luminol concentration: 0.2 mol/L NaOH, 0.1 mol/L H_2O_2 , 10^{-5} mol/L hemin (D) Effects of H_2O_2 concentration: 0.01 mol/L luminol, 0.2 mol/L NaOH, 10^{-5} mol/L hemin

3.2. Analytical application

3.2.1. Calibration curve and detection limit

The ratio of the initial CL intensity I_0 of luminal-hemin- H_2O_2 system to the CL intensity I at a given concentration of atropine, I_0/I , was proportional to the concentration of atropine. The atropine concentration dependence of the CL intensity was coincident to the fluorescence quenching described by a Stern- Volmer equation (Eq.2.):

$$I_0/I = 1 + K_{sv} [Q] \quad (2)$$

K_{sv} was found to be $166.53 \text{ (mol}^{-1} \text{ L)}$ and this large value provided a sensitive CL detection of atropine. The Stern–Volmer plots for atropine is shown in

insert Fig. 3. Under the optimized experimental conditions, the relative CL intensity decreased linearly in the concentration range of 0.1-29 $\mu\text{g/ml}$ for atropine ($R^2 = 0.9945$) with detection limit of $0.045(\mu\text{g/ml})$ at S/N ratio of 3. The resulting intensity–time plots are shown in Fig. 3.

3.2.2. Recovery tests and sample determination

The proposed method has been utilized for the determination of atropine in injection and urine samples with satisfactory results. Recovery tests were also carried out by adding a known amount of atropine in samples and the results were also summarized in Table 1.

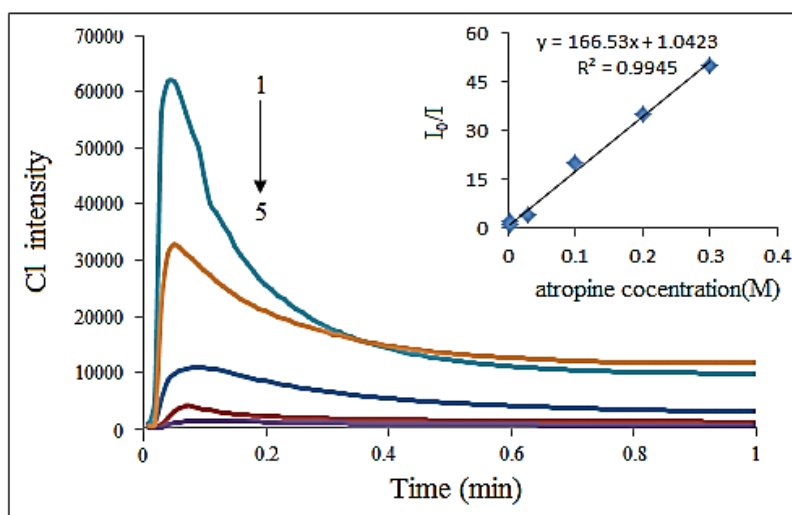


Figure 3: Chemiluminescence emission intensity as a function of time for the luminal- hemin- H_2O_2 system with constant concentration of luminal (10^{-2} M), hemin (10^{-5} M) and H_2O_2 (10^{-1} M) system, and varying concentrations of atropine: (1) 0.0 (2) 0.3 (3) 0.03 (4) 0.006 (5) 0.003

Table 1: Determination of atropine in pharmaceutical sample by the proposed method

Formulation	Claimed (mg/ml)	Found (mg/ml)	Recovery (%)	RSD(%) (n=3)
Atropine injection	0.5	0.49	98	2.37

The urine samples were collected from different healthy volunteers. To 1 mL of human urine sample, known amount of atropine standard solution was spiked and mixed. The results were

shown in Table 2. Both assays are seen to be satisfactory.

3.3. Interference study

In order to assess the possibility of applying the proposed method, the effect of foreign species was examined. Under optimal experimental conditions, the interference from selected metal ions and organic compounds was evaluated. Most of the candidate compounds had no significant influence on the determination of atropine concentration. The tolerable molar concentration ratios of foreign species to atropine were 100- fold for urea, tiourea;

uric acid; 10-fold Na^+ , K^+ , Cu^{+2} , SO_4^{-2} , Ca^{+2} , Mg^{+2} , NO_3^- , Cl^- .

3.5. Mechanism discussion

In order to explore the possible mechanism of the chemiluminescence reaction system, the CL emission spectrum of the system was studied as shown in Fig. 4.

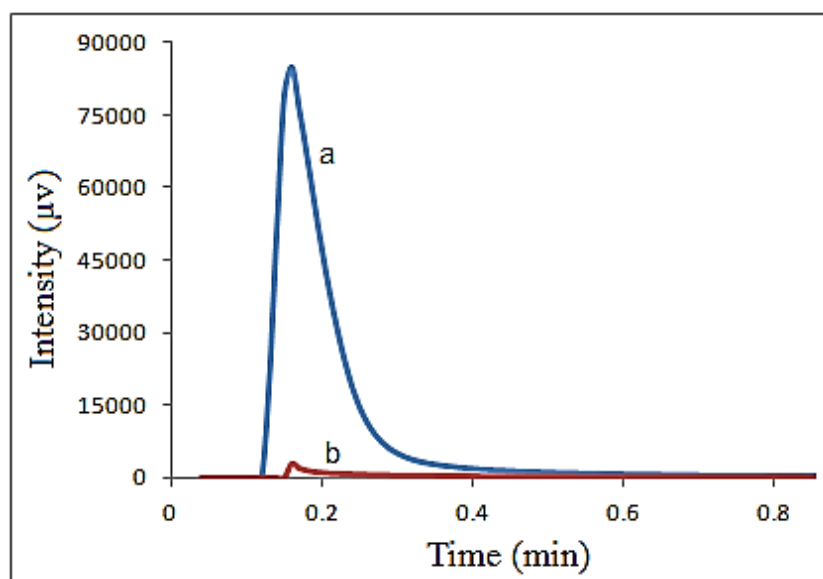


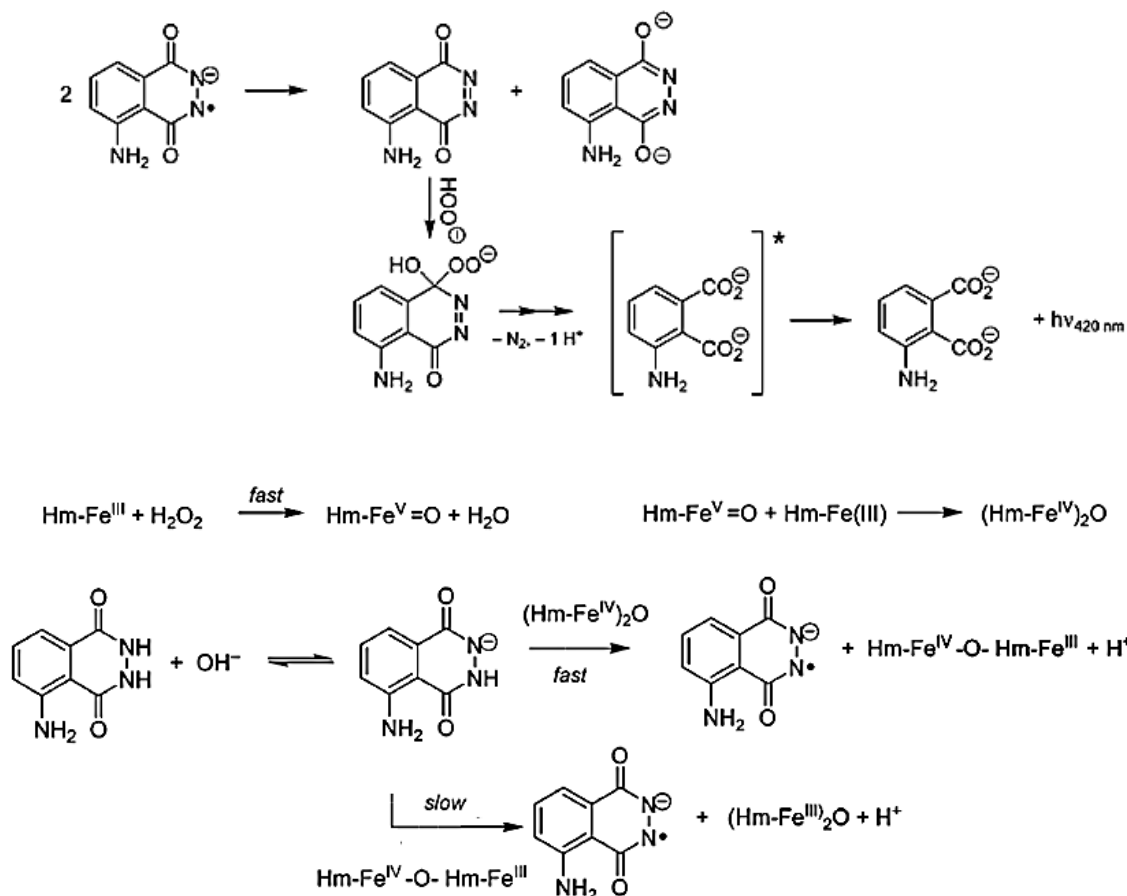
Figure 4: CL-intensity as a function of time for the Luminol- H_2O_2 system (a) and Luminol-Hemin- H_2O_2 system

Table 2: Determination of atropine in urine samples

Sample	Added (mg/ml)	Found (mg)	Recovery (%)	RSD (n=3, %)
1	0	1.02 ± 0.06	-	-
2	5	5.13 ± 0.12	102.3	3.6
3	10	8.25 ± 0.11	88.5	2.9

The results showed that the maximum wave length of CL emission intensity enhanced by hemin were 425 nm and revealing that the luminophor for the CL system was still the excited-state 3-aminophthalate anions (3-APA*) [19, 20], which is the oxidation product of luminol. Therefore, the

addition of hemin did not lead to the generation of a new luminophor for this CL system. The enhanced CL signals were ascribed to the possible catalysis from hemin consistent with the conclusion of the literature [21]. (Scheme 1)

Scheme 1: The proposed CL reaction pathway for the Luminol -Hemin -H₂O₂ system

4. Conclusion

In conclusion, hemin could strongly enhance the chemiluminescence of the luminol-H₂O₂ system. The luminophor of the luminol-H₂O₂-hemin CL system was identified as the excited state 3-aminophthalate anion.

The CL from luminol-H₂O₂-hemin system is strongly inhibited by the presence of atropine. Based on this inhibition, a novel CL method with a lower detection limit and wider linear range was developed for the determination of atropine. The detection limit is suitable for determining atropine in injections and urine samples.

5. References

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