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Flow injection chemiluminescence determination of thiamine based on its enhancing effect on the luminol–hydrogen peroxide system

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Abstract

A new flow injection chemiluminescence (CL) method is proposed for the determination of thiamine, based upon its enhancing effect on the CL reaction of luminol with hydrogen peroxide in alkaline solution. The method allows the determination of thiamine within 0.05–8 μg ml^{-1} range with a detection limit (3σ) of 0.01 μg ml^{-1}. The relative standard deviation is 1.4% ($n = 11$, 0.5 μg ml^{-1} thiamine) and the sample throughput is about 90 samples h^{-1}. The method was successfully applied to the determination of thiamine in pharmaceutical preparations.

Keywords: Thiamine; Flow injection; Chemiluminescence; Luminol

1. Introduction

Thiamine (vitamin B_1), a water-soluble vitamin, is a biologically and pharmaceutically important compound. It is necessary for carbohydrate metabolism, the maintenance of normal neural activity and prevention and treatment of beriberi disease: pregnant women, infants, adolescents, and especially, elderly people are the groups at risk of hypovitaminosis of vitamin B_1 [1]. A wide variety of analytical techniques are available for the determination of thiamine in pharmaceutical preparations and biological samples including spectrophotometry [2,3], spectrofluorimetry [4,5], fluorimetry [6,7], turbidimetry [8], electrochemical methods [9–11], high performance liquid chromatography [12] and capillary electrophoresis [13].

Analytical procedures applying chemiluminescence (CL) method combines the advantages of simplicity, rapidity and sensitivity and has been frequently used for the analysis of pharmaceutical compounds. In alkaline aqueous solution, the most extensively used CL reagent is luminol, which is oxidized by hydrogen peroxide and accompanied with intensive CL in the presence of a catalyst (generally a metal ion or compounds containing metal ion [14–16]). However, to the best of our knowledge, only a few organic com-

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pounds can be determined based on its direct enhancing effect on the luminol–hydrogen peroxide reaction [17,18]. In this work, it was found that a strong CL was produced when thiamine was mixed with the combining stream of luminol with hydrogen peroxide in alkaline solution. Based on this observation, a new flow injection CL method is proposed for the determination of thiamine. The method was applied to the determination of thiamine in pharmaceutical formulations with satisfactory results. The possible CL reaction mechanism was also discussed briefly.

2. Experimental

2.1. Reagents and solutions

All chemicals used were of analytical reagent grade; and doubly de-ionized water was used throughout the experiments. Thiamine stock standard solution (1.0 mg ml^{-1}) was prepared by dissolving thiamine hydrochloride in water. The working standard solutions were prepared from this stock standard solution by the least possible number of dilution steps when used. All thiamine solutions were protected from light. A 0.01 mol l^{-1} luminol stock solution was prepared by dissolving 1.7710 g of luminol in 0.1 mol l^{-1} sodium hydroxide solution. Working solution of luminol was prepared by diluting this stock solution to 1.0×10^{-3} mol l^{-1} with 0.1 mol l^{-1} $NaHCO_3$–NaOH (pH 12) and contained 1.0×10^{-3} mol l^{-1} EDTA. Hydrogen peroxide solution (0.15 mol l^{-1}) was prepared by diluting 30% (v/v) hydrogen peroxide before use.

2.2. Apparatus

A schematic diagram of the flow injection chemiluminescence (FI-CL) system is shown in Fig. 1. A peristaltic pump was used to pump all solutions at a flow rate of 1.4 ml min^{-1} (per channel). PTFE tubing (0.8 mm i.d.) was used to connect all components in the flow system. Injection was operated using a six-way injection valve fitted with a 40-μl sample loop. Flow cell was made by coiling a 30 cm length of colorless glass tubing (1 mm i.d.) into a spiral disk shape and placed close to the photomultiplier tube. The CL signal produced in the flow cell was collected with a R_{456} photomultiplier (Hamamatsu, Japan) and recorded with an IBM-compatible computer employed a flow injection CL analysis system software (Xi'an Ruike electronic equipment Co.).

2.3. Procedure

2.3.1. Procedure for calibration

A series of working standard solutions of thiamine with different concentrations between 0.05 and 8 μg ml^{-1} were prepared by diluting a concentrated standard solution of thiamine. The CL signal was measured by injection 40 μl of thiamine standard solution into water carrier steam by a six-way injection valve, and then mixed with the combining stream of 0.15 mol l^{-1} hydrogen peroxide and 1.0×10^{-3} mol l^{-1} luminol, producing CL. The CL intensity versus the concentration of thiamine was used for the calibration.

2.3.2. Procedure for tablets

Vitamin B_1 tablets (Tianjin Quanshi Pharmaceutical Corporation), each with a nominal content of 10 mg of vitamin B_1 per tablet, were purchased from the local market. The average tablet weight was calculated from the weight of 20 tablets. They were finely powdered, homogenized and a portion of the powder, equivalent to about 50 mg of thiamine was accurately weighted and dissolved in water. The resulting mixture was filtered and the filtrate was diluted to 100 ml with water for further sample analysis.

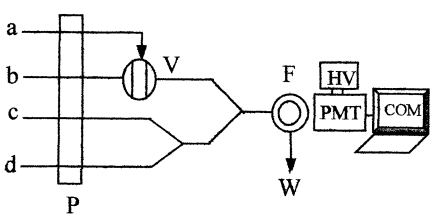

Fig. 1. Schematic diagram of the flow system for thiamine determination. (a) Sample solution; (b) water; (c) luminol solution; (d) hydrogen peroxide solution. P, peristaltic pump; V, six-way injection valve; F, flow cell; PMT, photomultiplier tube; HV, high voltage; COM, computer; W, waste.

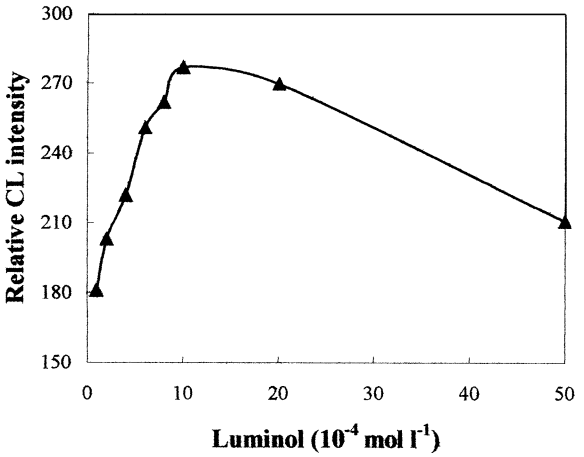

Fig. 2. Effect of luminol concentration on the CL intensity. Conditions: thiamine, 1.0 μg ml^{-1}; 0.1 mol l^{-1} $NaHCO_3$–NaOH, pH 12; H_2O_2, 0.15 mol l^{-1}; EDTA, 1.0×10^{-3} mol l^{-1}.

2.3.3. Procedure for injections

Vitamin B_1 injections (Xi'an Pharmaceutical Factory), each with a nominal content of 50 mg of vitamin B_1 in 2 ml, were also purchased from the local market. They were diluted to 100 ml with doubly de-ionized water for further sample analysis.

3. Results and discussion

3.1. Effect of pH of luminol solution

For luminol–hydrogen peroxide CL reaction, $NaHCO_3$–NaOH buffer solution was the suitable reaction medium [19]. The effect of pH of 0.1 mol l^{-1} $NaHCO_3$–NaOH buffer solution on the CL reaction was examined in the range 9–13. The CL intensity increased with the increase in pH up to 12. Further increase in pH decreased the CL intensity.

3.2. Effect of luminol concentration

The effect of 1.0×10^{-4}–5.0×10^{-3} mol l^{-1} luminol on the CL reaction is shown in Fig. 2. As can be seen, the CL intensity continues to increase with increasing luminol concentration up to 1.0×10^{-3} mol l^{-1}. Larger concentrations of luminol lowered the CL intensity.

3.3. Effect of hydrogen peroxide concentration

Fig. 3 shows the effect of hydrogen peroxide concentration on the CL reaction. As can be seen, the CL signal increased with raising hydrogen peroxide concentration up to 0.15 mol l^{-1}, whereas greater concentrations of hydrogen peroxide decreased the CL signal.

3.4. Effect of EDTA concentration

Zhang and Lu [19] have reported that EDTA can be used to decrease the effect of metal ions on the luminol–hydrogen peroxide reaction based on its chelation with metal ions. So, EDTA was added into all solutions in order to decrease the background signal. The effect of 0.0–5.0×10^{-3} mol l^{-1} EDTA on the CL reaction was examined. The experimental results show that 1.0×10^{-3} mol l^{-1} EDTA can efficiently decrease the background signal and give the highest CL signal.

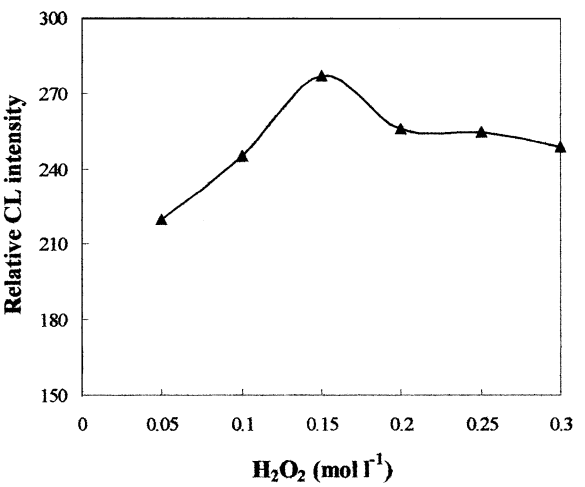

Fig. 3. Effect of hydrogen peroxide concentration on the CL intensity. Conditions: thiamine, 1.0 μg ml^{-1}; 0.1 mol l^{-1} $NaHCO_3$–NaOH, pH 12; luminol, 1.0×10^{-3} mol l^{-1}; EDTA, 1.0×10^{-3} mol l^{-1}.

Table 1
Determination of thiamine in vitamin B_1 tablets and injections

Samples	Claimed	Proposed method[a]	R.S.D. (%)	Pharmacopoeia method [20][a]
Vitamin B_1 tablets	10 mg/tablet	9.8 mg/80.5 mg	2.2	9.9 mg/80.5 mg
Vitamin B_1 injections	50 mg/injection	48.9 mg/2 ml	1.9	50.5 mg/2 ml

[a] Average of three measurements.

3.5. Performance of the system for thiamine measurement

Under the selected conditions described above, the calibration graph of CL intensity (I, relative unit) versus thiamine concentration (C, μg ml^{-1}) was linear in the range of 0.05–8 μg ml^{-1} with a detection limit (3σ) of 0.01 μg ml^{-1}. The regression equation was $I = 22.185C + 2.369$ with a correlation coefficient of 0.999 ($n = 9$). The relative standard deviation is 1.4% for 0.5 μg ml^{-1} thiamine in 11 replicate measurements. The sample measurement frequency was calculated about 90 samples h^{-1}.

3.6. Interference studies

In order to assess the possible analytical application of the proposed method to dosage forms, the effect of some common excipients and possible co-existing compounds in pharmaceutical preparations and some common vitamin was studied by analyzing a standard solution of 0.5 μg ml^{-1} thiamine. The tolerable limit of a foreign species was taken as a relative error less than 5%. No interference has been observed when including up to a 100-fold glucose, sucrose, fructose, lactose, galactose, starch, cellulose, and 10-fold magnesium stearate, riboflavin and pyridoxine hydrochloride. Ascorbic acid is the only severe interfere, but it is not very commonly found in thiamine formulations.

3.7. Application

Following the procedure detailed in Section 2, the proposed method was applied to the determination of thiamine in vitamin B_1 tablets and injections. The results are listed in Table 1 and agree well with those obtained by pharmacopoeia method [20].

3.8. Possible reaction mechanism

In order to obtain some ideas about the possible reaction mechanism, the following experiments were performed.

The CL spectrum was drawn using an RF-540 spectrofluorimeter. The spectrum obtained showed a maximum emission wavelength of 425 nm, which suggested that excited aminophthalate is the possible emission species.

The CL intensity decreased about 20% when dissolved oxygen was removed from all solutions by the flow of nitrogen. This result showed that dissolved oxygen played an important role in the CL reaction.

In order to examine if the reactive oxygen species participated in the CL reaction, the scavengers of reactive oxygen species, such as ascorbic acid, methanol, sodium benzoate and mannitol, were added into the reaction system, respectively. The CL intensity was decreased greatly in the presence of these scavengers of radical. These results showed that there has been reactive oxygen species and it participated in the CL reaction.

The reaction mechanism of thiol-containing compounds with dissolved oxygen in alkaline solution has been explored and superoxide radical is proposed as a possible reaction intermediate [21]. So, it could be supposed that thiamine may be also react with the dissolved oxygen to produce superoxide radical since it can easily convert to thiol-containing compound in alkaline solution [21]. In the presence of hydrogen peroxide, the produced superoxide radical can be converted into hydroxyl radical and singlet oxygen (Haber–Weiss reaction) [22], and hydroxyl radical could

be used as a co-oxidant for hydrogen peroxide–luminol system [23].

Based on discussion described above, the possible mechanism of the CL reaction of luminol–hydrogen peroxide–thiamine was suggested as the following reactions in its simplest form.

Thiamine → thiol-containing compound

Thiol-containing compound + dissolved oxygen → superoxide radical

Superoxide radical + hydrogen peroxide → hydroxyl radical

Luminol + hydroxyl radical + hydrogen peroxide → aminophthalate*

Aminophthalate* → aminophthalate $+ h\nu_1$ ($\lambda_{max} = 425$ nm)

4. Conclusion

In conclusion, the proposed method is simple, sensitive, and inexpensive and can be applied to the determination of thiamine in pharmaceutical preparations. The brief discussion on the reaction mechanism was also given.

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