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Determination of picomole amounts of thiamine through flow-injection analysis based on the suppression of luminol–KIO_4 chemiluminescence system

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Abstract

A continuous flow sensor for the determination of thiamine was constructed by using controlled-reagent-release technology in a FIA-CL system. The analytical reagents, luminol and KIO_4, were both immobilized on an anion-exchange column. The CL signal produced by the reaction between luminol and KIO_4, which were eluted from the column through H_2O injection, was decreased in the presence of thiamine. The decreased CL intensity was linear with thiamine concentration in the range 3.3 pmol ml^{-1}–6.7 nmol ml^{-1}; and the limit of detection was 1.0 pmol ml^{-1} ($3^{\bullet}$). The whole process, including sampling and washing, could be completed in 0.5 min with a relative standard deviation of less than 3.0%. The flow sensor showed remarkable stability and could be easily reused over 80 h. The sensor proposed was tested in determination of thiamine in pharmaceutical preparation and human urine samples.

Keywords: Flow sensor; Thiamine; Chemiluminescence; Urine

1. Introduction

Thiamine, known as vitamin B_1, is present in most plant and animal tissues. Its principal if not the sole coenzyme form is its pyrophosphate ester or cocarboxylase. The requirements for thiamine vary considerably since they are directly correlated with the amount of metabolizable carbohydrate intake. Clinically, deficiency from thiamine results in the classical beriberi disease.

Several analytical procedures were investigated for the determination of thiamine. The flourimetry methods most commonly used involve oxidation of thiamine to thiochrome through chemical [1–5] or biological way [6], then thiochrome is measured fluorimetrically. And also, HPLC [7–10] and electrophoresis [11,12] were usually developed to determine the thiamine in complicated samples. Spectrophotometry [13–17], such as UV

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adsorption, can be used for analysis of relatively pure solutions of thiamine or after HPLC separation of vitamin from other spectrally interfering compounds. Electrochemical approaches [18,19] and turbidimetric determination [20] have also been proposed for determination of thiamine. But these methods suffer the disadvantages of irreversibility, long response time or poor detection limit.

Chernysh et al. were the first to report that during oxidation of thiamine to thiochrome by potassium iron(III) cyanide, a bright chemiluminescent radiation appears [21]. A continuous flow-through chemiluminescence measurement for determination of thiamine was also developed [22,23]. However, these methods were hampered by narrow linear range and poor sensitivity.

In recent years, flow-through chemical sensors have roused the interest of analytical chemists. The type of sensor is made by filling a flow-through cell with a suitable support, usually an ion-exchange resin or controlled-pore glass beds with diameters of about 100 μm. Chemical reaction (and/or physical retention) and optical detection take place at a microzone in the sensor and the analytical signal is obtained as the sample pass through the cell and comes into direct contact with the support.

In this paper, a sensitive CL flow sensor for thiamine determination was presented. It was based on the inhibition of thiamine in the CL reaction between luminol and periodate and the CL light decrement is related to the amount of thiamine. The two CL reagents, luminol and periodate, used in this sensor, were both immobilized on Amberlyst A-27 (from Rohm and Haas Co.) anion-exchange resin. Through injection of 100 μl eluant, the reagents on the anion-exchange column were eluted from the resins and in presence of thiamine the CL reaction was inhibited, by which thiamine could be sensed. The concentration of thiamine was quantified via the peak height of the decreased CL intensity. The method was applied successfully to the determination of thiamine in pharmaceutical preparation and human urine samples without any pre-treatment.

2. Experimental

2.1. Reagents

All chemicals used were of analytical-reagent grade. Doubly distilled water was used throughout. Thiamine (Sigma Co.) and Luminol (Fluka, biochemika, Switzerland) were obtained from Xi'an Medicine Purchasing and Supply Station, China. Potassium periodate were purchased from Xi'an Chemical Reagent Plant.

A standard solution of thiamine (3.32 μmol ml^{-1}) was stored at 4 °C and protected from light. Working strength solutions were prepared daily from the above stock solution as required. Luminol was used as supplied to prepare a 0.25 mol l^{-1} stock standard solution in 0.5 mol l^{-1} NaOH in a 1000 ml calibrated flask. A 3.0×10^{-2} mol l^{-1} stock standard solution of KIO_4 was made by dissolving the solid in distilled water and diluting to 250 ml in a calibrated flask.

2.2. Preparation of resin with immobilized reagents

Amberlyst A-27 (2.0 g) was shaken with 50 ml 0.25 mol l^{-1} luminol or 0.04 mol l^{-1} potassium periodate for 96 h, then the resin was filtered, washed with doubly distilled water and dry-stored. The most convenient method to determine the amounts of luminol and potassium periodate immobilized was to measure the losses of these reagents from the immobilization solutions. The concentration was detected at 360 nm for luminol and at 225 nm for potassium periodate by UV–Vis. The amounts of luminol and periodate immobilized were 1.99 ± 0.03 mmol g^{-1} ($n = 4$) and 1.01 ± 0.02 mmol g^{-1} ($n = 3$) resin, respectively.

2.3. Apparatus

The flow injection system used in this work is shown in Fig. 1. A peristaltic pump (Shanghai meter electromotor plant, Model ND-15, 15 rpm) was used to generate the flows. PTFE tubing (1 mm i.d.) was used in the flow system. The anion-exchange resins contain immobilized luminol (0.05 g) and potassium periodate (0.10 g) were mixed

together and packed into a glass column with an internal diameter of 3 mm and total volume of about 0.5 ml, and plugged with glass wool at both ends to prevent the resins from leaking. A 100 μl of eluant was injected by a six-way valve. Before reaching the flow cell, the streams of luminol, potassium periodate, sodium hydroxide and analyte were combined in a mixing tubing (50 mm in length). The CL emission cell is a twisty glass tubing (1.0 mm i.d., 15 cm length) in order to produce a large surface area exposed to the adjacent photomultiplier tube (PMT) (HAMAMATSU, Model IP28). Extreme precautions were taken to ensure that the sample compartment and PMT were light-tight. The CL signal produced in flow was detected without wavelength discrimination, and the PMT output was amplified and quantified by a luminosity meter (Northwest nonferrous geology institute of China, Model GD-1) connected to a recorder (Shanghai Dahua Instrument and Meter Plant, Model XWT-206).

2.4. Procedures

The carrier water and the solutions (NaOH, sample and eluant) were propelled at a constant flow rate (2.0 ml min^{-1}) on each flow line. The pump was started to wash the whole flow system until a stable baseline was recorded. Then 100 μl eluant solution was injected into the carrier stream, luminol and periodate were eluted quantitatively, which was then mixed with the sample stream, the mixed solution was delivered to the CL cell, and the peak height of the CL signal was detected with the PMT and the luminometer. The concentration of sample was quantified by decreased CL intensity, $\Delta I = I_0 - I_s$, where I_0 and I_s are CL signals in the absence and in the presence of thiamine, respectively.

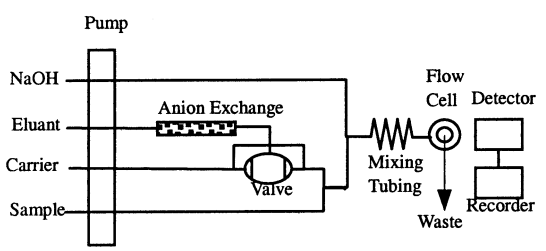

Fig. 1. Schematic diagram of the flow-injection system for thiamine determination.

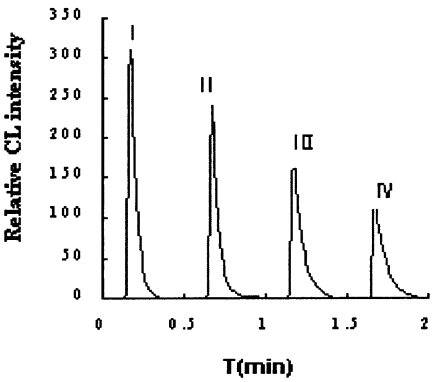

Fig. 2. CL time profile in the batch system. (I) CL intensity in the absence of thiamine. (II) CL intensity in the presence of thiamine (40 ng ml^{-1}). (III) CL intensity in the presence of thiamine (150 ng ml^{-1}). (IV) CL intensity in the presence of thiamine (500 ng ml^{-1}).

3. Results and discussion

3.1. The CL intensity–time profile

Before carrying out the flow injection method, the batch method for the CL profiles was used. Without any special eluant, the mixture of luminol and periodate rinsed by water gave out an evident CL signal. As Fig. 2 shows, the CL intensity reached a maximum 12 s after injection, and then died within 25 s. On joining of the sample into the above mixing solution, a decreased CL signal was recorded. The peak heights of the CL emission were proportional to the concentration of thiamine.

3.2. Designation for the FI-CL system

The assay could be carried out by a continuous-flow mode in two different manifolds. Through injection of 100 μl eluant (5.0×10^{-5} mol l^{-1} of Na_3PO_4), the reagents on the anion-exchange resin column were eluted and in the presence of thiamine, the CL intensity was decreased, and the decrease of CL intensity was recorded. It was

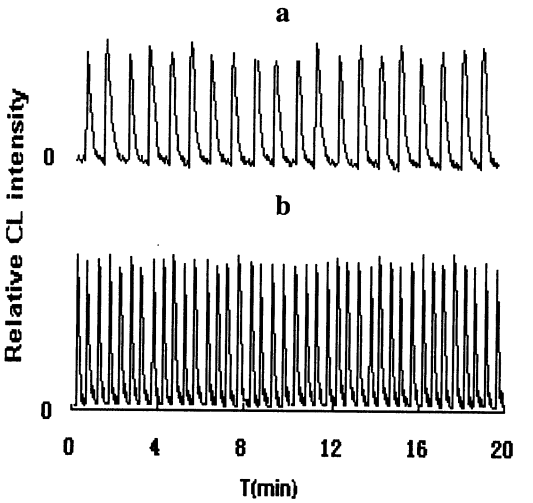

Fig. 3. CL signals in two manifolds. (A) The column set behind the injector. (B) The column set in front of the injector. The flow rate was 2.0 ml min^{-1}; the high voltage for photomultiplier was -750 V.

found that while the column with immobilized reagents was put in front of or behind the valve, two significantly different results were observed. As illustrated by results in Fig. 3, the whole analysis process, including sampling and washing, could be accomplished in 0.5 min when the column was put in front of the valve viz Fig. 1 manifold, whereas it must take more than 2.0 min when the column was put behind the valve and also manifold Fig. 1 gave the better precision. Therefore, the manifold depicted in Fig. 1 was chosen for subsequent work.

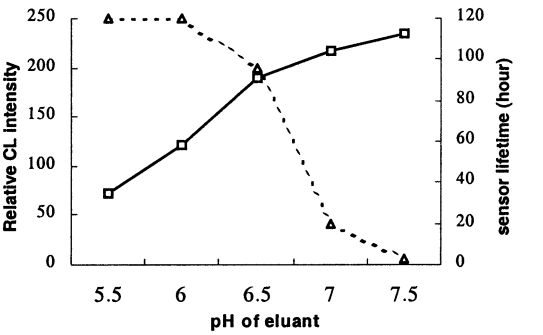

Fig. 4. (–□–) Effect of eluant pH on CL intensity. (–△–) Effect of eluant pH on sensor lifetime.

3.3. Selection of eluant

A 100 μl different eluants were injected through the resin column and releasing different amounts of luminol and periodate, thus producing the CL emission. The results are shown in Table 1. It was found that sodium sulfate gives a maximum CL emission while sodium carbonate shows some inhibitive effects on the CL reaction. Nevertheless, it was observed that a continuous flow of eluant through the column results in a rather short lifetime of sensor down to only a few hours. Considering lifetime and linear range of sensor (Discussed in detail below), water was used as eluant in subsequent work.

3.4. Effect of pH on CL and sensor lifetime

The best pH of eluant (water) on the performance of the system was evaluated. It was found that along with the increase of pH in eluant, the CL intensity decreased while the lifetime of sensor

Table 1
Character of eluants for thiamine determination

Type of CL Intensity	Relative CL intensity				
	H_2O	NaCl	Na_2CO_3	Na_2SO_4	Na_3PO_4
I	246	372	124	514	426
II	145	213	84	288	233
III	101	159	40	226	193

The concentration of each solution was 1.0×10^{-4} mol l^{-1}. (I) CL intensity in the absence of thiamine. (II) CL intensity in the presence of 40 ng ml^{-1} thiamine. (III) The decrease of CL intensity.

decreased considerably (Fig. 4). This phenomenon is probably due to the quantities of hydroxide ions in eluant were increasing. pH 6.0 was then chosen as a compromise between lifetime and a sufficient CL intensity. In this case, the column with immobilized CL reagents could be used more than 120 h with continuous injection

3.5. Effect of molar ratio of immobilized luminol and periodate

To examine the influence of the mixing ratio, resins (0.15 g) with different mixing ratios were packed into column with same internal diameter and volume. By the injection of water at a fixed volume of 100 μl, different amounts of luminol and periodate were eluted from the resins and emitted CL signals with different intensity. As Fig. 5 shows, the CL intensity dropped drastically from beginning to next day, then it went down slowly like glacis. The most stable CL signal was found with a molar ratio of 1:2 (luminol to periodate) and a middling CL intensity is in favor of measuring a catalytic effect of thiamine on CL reaction.

3.6. Effect of NaOH concentration

It was found that luminol reacts with periodate and emits CL signal only in an alkaline medium. As Fig. 6 shows, a NaOH concentration less than 0.05 M leads to an apparent decrease in ΔI. The maximum intensity was found with 0.1 M NaOH. While concentration of NaOH is higher than 0.2

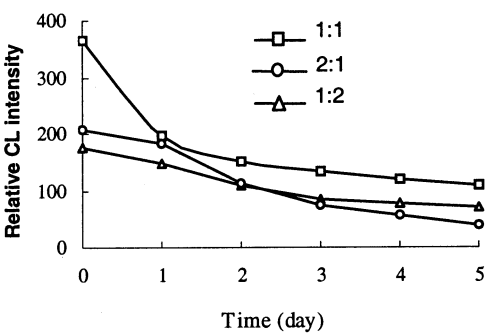

Fig. 5. Effect of molar ratio on CL intensity and sensor lifetime.

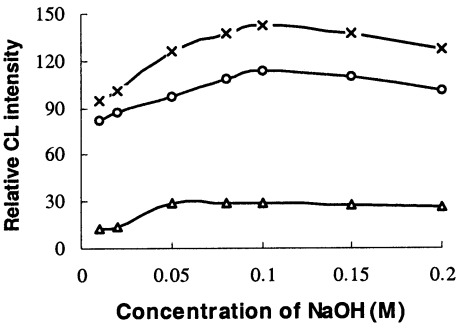

Fig. 6. Effect of concentration of NaOH on CL intensity. (–○–) CL intensity in the presence of thiamine (I_0). (– × –) CL intensity in the absence of thiamine (I_s). (–△–) The decreased of CL intensity (ΔI).

M, there is a scattering effect in flow cell due to the discrepancy between refractive index of various components. Thus 0.1 M NaOH was selected as an optimal condition.

3.7. Effect of flow rate and the length of mixing tubing

The CL signal was also dependent on the flow rate of carrier and eluant. The signal-to-noise rate decreased at a higher flow rate because the higher flow rate would impact the rate of contact of sample molecules with the ion-exchange resin. The lower flow rate caused broadening of the peak and slowing down of the sampling rates. Nevertheless, the high flow rate could leads to an unstable baseline and shortening of the sensor lifetime. A rate of 2.0 ml min^{-1} was then chosen as a compromise between good precision and lower reagent consumption.

The length of the mixing tubing was also adjusted to yield maximum light emission in the cell. It was found that a 50 mm of mixing tubing afforded the best results as regards sensitivity and reproducibility.

3.8. Performance of the sensor for thiamine measurements

A series of standard solutions were injected into the manifold depicted in Fig. 1 under the optimized conditions to test the linearity of thiamine.

At a flow rate of 2.0 ml min^{-1}, the determination of analyte could be performed in 0.5 min, including sampling and washing, giving a throughput of about 100 times per hour with a relative standard deviation of less than 3.0%.

It was found that the inhibition of CL intensity was linear with the logarithm of thiamine concentration. As Fig. 7 shows, the linear range is from 3.3 pmol ml^{-1} to 6.7 nmol ml^{-1} and the regression equation is $\Delta I = 66.603 \log C_{\text{thiamine}} - 39.174$, $\gamma = 0.9993$. The relative standard deviations of seven determinations were 2.54, 0.96 and 0.57% with thiamine concentration of 33.24, 332.44 and 3.32 nmol ml^{-1}, respectively. The limit of detection was 1.00 pmol ml^{-1}.

3.9. Interference studies

The effect of foreign ions was tested by analyzing a standard solution of thiamine (30 ng ml^{-1}) to which increasing amounts of interfering ions were added. The tolerable concentration ratios with respect to 30 ng ml^{-1} thiamine for interference at 5% level were over 800 for Cl^-, NO_3^-, Ac^-, I^-, SO_4^{2-}, PO_4^{3-}, $Cr_2O_{72}^-$, borate, oxalate, tartrate, citrate, salicylic acid, malic acid, and 600 for NH_4^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Zn^{2+}, Ni^{2+}, Mn^{2+}, Cr^{3+}, and 500 for methanol, ethanol, urea, tween-80, CTMAB, starch, polyvinyl alcohol, glucose, dextrin, sodium dodecylbenzene-

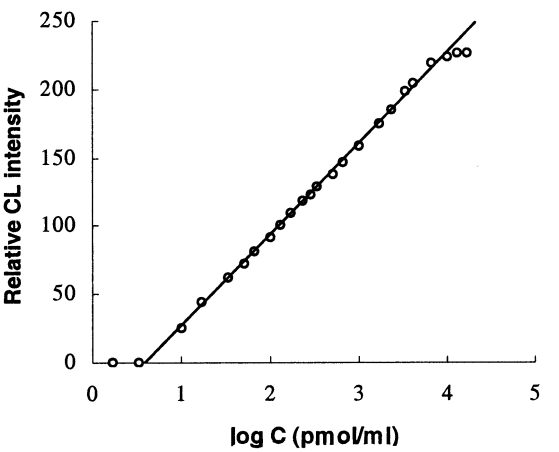

Fig. 7. Calibration graph of thiamine (3.3 pmol ml^{-1}–6.7 nmol ml^{-1}).

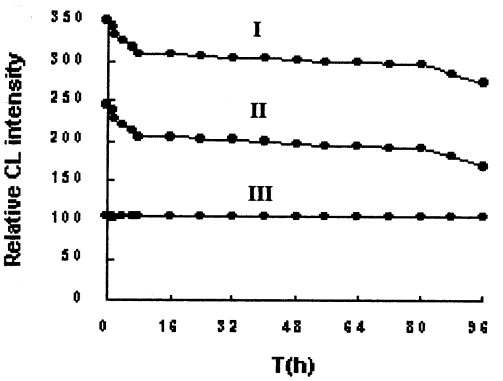

Fig. 8. Stability of the flow sensor. (I) CL intensity in absence of thiamine (I_0). (II) CL intensity in presence of 40 ng ml^{-1} thiamine (I_s). (III) The decreased of CL intensity (ΔI).

sulfonate, gelatine, sucrose, trishydroxymethlaminomethane, and 100 for urea, CO_3^{2-}, mannitol, and 10 for Cu^{2+}, and 5 for uric acid, respectively.

3.10. Operational stability of the sensor

A 100 μl eluant (water) was flow-injected through the system in presence of 30 ng ml^{-1} thiamine solution and the ΔI ($I_0 - I_s$) was recorded to test the operational stability of the sensor. The experiment lasted for 12 days and the flow system was regularly used over 8 h per day. Fig. 8 shown the stability of the flow sensor, and the average of ΔI was calculated in ten spot check determinations with RSD less than 2.0%. The flow sensor showed remarkable stability and could be easily reused over 96 h.

4. Applications

4.1. Determination of thiamine in pharmaceutical formulations

Following the procedure described in Section 2, the proposed method was applied to the determination of thiamine in pharmaceutical preparation. Eight different formulations were purchased from the local market. Not less than 20 thiamine tablets were weighed, ground to a fine powder and

Table 2
Results of thiamine in different pharmaceutical preparations

Formulations	Results by the proposed method[a]						Results by HPLC	Results by AOAC[24]
	Found (ng ml^{-1})	Added (ng ml^{-1})	Total (ng ml^{-1})	R.S.D.% $n = 5$	Recovery (%)	Content (mg tab^{-1})	Content (mg tab^{-1})	Content (mg tab^{-1})
Tablet 1	190.5	200.0	400.0	0.95	95.2	9.53	9.58	
Tablet 2	210.0	200.0	415.0	1.05	102.5	10.50	10.20	
Tablet 3	195.75	200.0	384.4	0.31	94.3	9.78	9.77	
Tablet 4	193.1	200.0	394.4	0.71	100.6	9.65	9.57	
Tablet 5	96.0	100.0	192.1	1.01	96.1	9.60	9.33	
Tablet 6	95.7	100.0	191.3	0.91	95.6	9.57	9.56	
Tablet 7	15.4	30.0	45.9	1.95	101.0	15.5		15.8
Injection	14.7	15.0	30.0	2.72	98.9	14.7		14.9

[a] The average of five determinations.

Table 3
Results of thiamine in human urine samples

Individual urine	Found (pmol ml^{-1})	Added (pmol ml^{-1})	Total (pmol ml^{-1})	Recovery (%)	$n = 5$ (R.S.D. %)	Thiamine metabolism ratio in urine (%)
1	48.90	166.33	215.57	100.2	1.43	26.40
2	110.78	332.67	451.76	102.5	0.75	54.95
3	99.80	332.67	421.49	96.7	1.82	9.60
4	9.31	166.33	184.96	105.6	1.83	2.01
5	4.66	166.33	172.65	101.0	2.37	0.04

The average of five determinations in two volunteers' urine.

mixed. A sample equivalent to approximately 100 mg of thiamine was weighed accurately, transferred into a 250 ml calibrated flask and made up to volume with water. After filtering, aliquots of the solution were diluted by a factor of 10^4 for the analysis. The measured contents were listed in Table 2. The results obtained by the proposed method were in well agreement with those obtained by HPLC or AOAC [24] with the recovery from 94.3 to 102.5% and R.S.D. less than 3.0%.

4.2. Determination of thiamine in human urine

Two apparently healthy male volunteers took thiamine tablets orally in morning with empty stomach. According to the marked content, the net dosage of thiamine they took is 60 mg per capita. From then on, first-voided urine samples were collected in dark glass bottles after 1, 1.5, 2, 3, and 4 h, respectively. Without any pre-treatment procedures, urinary thiamine could be determined and results were listed in Table 3. The metabolic profile of thiamine was shown as in Fig. 9. From the curve it could be seen that thiamine was metabolized rapidly after taking thiamine tablets. The total thiamine excreted through urine was 93.0% of that taken in a total volume of 0.73 l urine within 4 h. The thiamine concentration reached its maximum after 1.5 h and dropped sharply within a few hours.

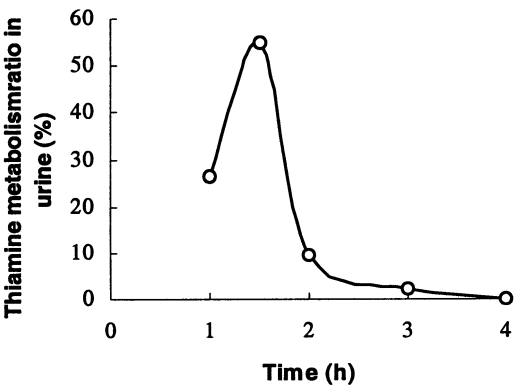

Fig. 9. Metabolic profile of thiamine in urine.

5. Conclusions

Compared with other methods for determination of thiamine, the proposed continuous flow sensor offers advantages in instrumental simplification, high sensitivity and reducing reagent consumption. The flow sensor has performed successfully in application for determination of thiamine in pharmaceutical preparation and monitoring thiamine in human urine. With the superior analytical properties performed, the flow sensor may be utilized to routinely detect thiamine in pharmaceutical formulations and study of biological process.

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