

## Research Article

# A Simple and Fast Fluorimetric Method for Thiamine (Vitamin B1) Detection by Au<sup>3+</sup>-Mediated Formation of Thiochrome

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**Abstract**

Developing an effective method for thiamine detection is of great interest in clinical analysis, food processing, and pharmaceutical industries. A hitherto unreported, simple and efficient method for thiamine detection is demonstrated in this study by using Au<sup>3+</sup> to convert the initially non-fluorescent thiamine into intensely blue-emitting thiochrome spontaneously in alkaline condition. The formation of thiochrome was characterized and confirmed by the photoluminescence and mass spectra. The linear response region for thiamine detection was observed to be  $1.0 \times 10^{-9}$ – $1.0 \times 10^{-3}$  M with a correlation coefficient of 0.994. The limit of detection was calculated to be  $2.0 \times 10^{-6}$  M. The unique homogenous-phase Au<sup>3+</sup>-assisted fluorescence detection method as developed in this study is not only highly specific to thiamine, but also simpler and faster than the conventional spectrofluorimetric methods without the need of extra separation process of thiochrome, the addition of highly toxic ions and exogenous enzymes to accelerate the formation of thiochrome.

**Keywords:** Vitamin B1; Fluorimetric detection; Thiochrome; Gold ions

**Abbreviations**

RDI: Reference Daily Intake; LC-MS: Liquid Chromatography Mass Spectrometer; IT: Ion Trap; TOF: Time-Of-Flight; LOD: Limit of Detection; GSH: L-Glutathione, Tripeptidey-Glu-Cys-Gly

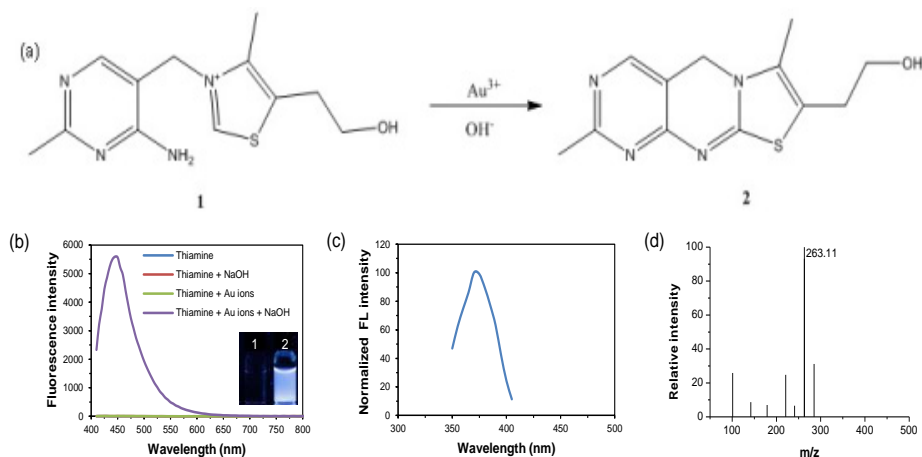
**Introduction**

Thiamine, also known as vitamin B1, is a water-soluble, S-containing vitamin consisting of an amino-pyrimidine and a thiazole ring linked by a methylene bridge [1]. Thiamine is an essential nutrient which plays a vital role in carbohydrate metabolism, maintenance of neural activity, and prevention of beriberi [2]. Human beings cannot synthesize thiamine and thus can only obtain it from their diet. The Reference Daily Intake (RDI) of thiamine for an adult human is  $1.4 \times 10^{-3}$  g/d. Medical doses of vitamin B1 pills or vitamin B1-enriched food or drinks are good means to obtain sufficient thiamine to maintain proper functions of the nervous and cardiovascular systems of the body. It is therefore of strong interest in the development of a simple (in terms of sensor construction and operation), fast, and reliable method for thiamine detection in clinical analysis, food processing, and pharmaceutical industries.

Several different analytical methods have been developed for thiamine detection [3-12]. In particular, a spectrofluorimetric method based on detection of the fluorescent substance thiochrome, which is the oxidizing product of thiamine in the presence of various oxidants, is the most frequently used one due to its simple, fast and sensitive manner [4]. The typical oxidants for thiochrome reaction include hexacyanoferrate (III) [11,12], Hg<sup>2+</sup> [5,10], Cu<sup>2+</sup> [6], and hydrogen peroxide [13,14]. However, each of these reported oxidants has their own limitations for efficient thiamine detection. When

hexacyanoferrate (III) is used as the oxidant, another separation step is needed in order to extract the fluorescent thiochrome from the aqueous phase to organic phase, or it will be quenched in the presence of hexacyanoferrate (III). Although Hg<sup>2+</sup> is an efficient oxidant for the thiochrome reaction without quenching the fluorescence, it is highly toxic and not suitable for use in the biological systems. While Cu<sup>2+</sup> is not as toxic as Hg<sup>2+</sup>, it may give false response in the presence of other biomolecules (such as cysteine) due to the formation of other fluorescent species (e.g., Cu nanoclusters [15]) under similar reaction conditions. When hydrogen peroxide is used as the oxidant, additional reagents such as enzymes are required to make the thiochrome formation more efficient, because meantime hydrogen peroxide as well as other strong oxidants such as KMnO<sub>4</sub> also tends to convert thiamine into non-fluorescent thiamine disulfide. Peroxidase extracted from horseradish [13], or Kohlrabi (*Brassica oleracea* var. *gongylodes*) [14] has been demonstrated for the detection of thiamine. However, the need of extra reagent will definitely increase the cost and add more complexity to the sensor system.

To address the above mentioned issues, herein we demonstrate a hitherto unreported method of using Au<sup>3+</sup> as oxidant for thiamine detection. In this method, non-fluorescent thiamine was facilely converted to intensely fluorescent thiochrome in the presence of Au<sup>3+</sup> under alkaline conditions, which was confirmed by the photoluminescence and mass spectra. The Au<sup>3+</sup>-mediated formation of thiochrome was further used to detect thiamine in aqueous solutions. A linear response range of  $1.0 \times 10^{-9}$ – $1.0 \times 10^{-3}$  M was achieved and the limit of detection for thiamine was as low as  $2.0 \times 10^{-6}$  M. Compared to previous studies, the current method eliminates the need of extra separation steps, involvement of highly toxic ions, and requirement of exogenous enzymes. In addition, the current method



**Figure 1:** (a) Schematic illustration showing the conversion of thiamine to thiochrome in the presence of Au<sup>3+</sup>. (b) Photoemission spectra of thiamine only (blue line), thiamine plus NaOH (red line), thiamine plus Au<sup>3+</sup>, and thiamine plus Au<sup>3+</sup> and NaOH ( $\lambda_{\text{ex}} = 370$  nm). (Inset) Digital photos of (1) thiamine and (2) thiochrome. (c) Photo excitation ( $\lambda_{\text{em}} = 445$  nm) and (d) mass (positive mode) spectra of the resultant blue emitting product.

is highly specific for thiamine detection, even in the presence of a wide range of common biomolecules and other metal ions. Presented below are the details of this investigation.

## Materials and Methods

### Reagents

Gold (III) chloride solution (HAuCl<sub>4</sub>, 30 wt.% in dilute HCl), thiamine chloride (vitamin B1, 99 wt. %), biotin (vitamin B7, 99 wt. %), L-cysteine (97 wt. %), L-glutathione (98 wt. %), D-(+)-glucose (99.5 wt. %), cellulose, copper (II) chloride (97 wt.%), cadmium chloride (99.99 wt.%), zinc chloride (97 wt. %), nickel(II) chloride (98 wt.%), lead (II) acetate trihydrate (99 wt.%), mercury (II) acetate (99.999 wt.%), cadmium nitrate tetra hydrate (99 wt.%), iron(III) nitrate nonahydrate(98 wt. %), aluminium chloride (99.0 wt. %), sodium chloride (99 wt. %), potassium chloride (99 wt. %), and lithium chloride (99 wt.%) were purchased from Sigma-Aldrich. All reagents were used as received and without further purification. Ultrapure water with a specific resistance of 18.2 M $\Omega$  was used throughout the experiment.

### Fluorescence detection of thiamine

Aqueous solutions of HAuCl<sub>4</sub> ( $2.0 \times 10^{-2}$  M,  $2.5 \times 10^{-5}$  L) and thiamine ( $2.0 \times 10^{-2}$  M,  $2.5 \times 10^{-5}$  L) were mixed in  $9.2 \times 10^{-4}$  L of ultrapure water. 2 min later,  $3.0 \times 10^{-5}$  L of NaOH (1 M) was added to adjust the solution pH as 12. The final product was collected 10 min later. A Shimadzu 2450 spectrometer and a TECAN infinite M200 plate reader were used to record the UV-vis absorption and photoluminescence spectra, respectively. A Shimadzu Liquid Chromatography Mass Spectrometer (LC-MS) equipped with an Ion Trap (IT) as well as Time-Of-Flight (TOF) technology was used to record the mass spectra of thiamine and thiochrome.

### Quantification of thiamine

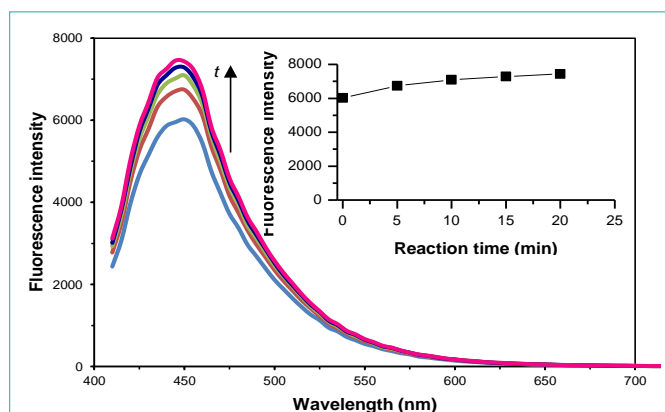
$2.5 \times 10^{-5}$  L of HAuCl<sub>4</sub> ( $2.0 \times 10^{-2}$  M) was mixed with  $2.5 \times 10^{-5}$  L of thiamine solution with varied concentrations. The mixture was topped up with ultrapure water to  $9.2 \times 10^{-4}$  L. 2 min later,  $3.0 \times 10^{-5}$  L of NaOH (1 M) was added to this mixture to adjust the solution pH as 12. The emission spectra were recorded for the resultant samples after 10 min of reaction.

### Specificity and interference measurements

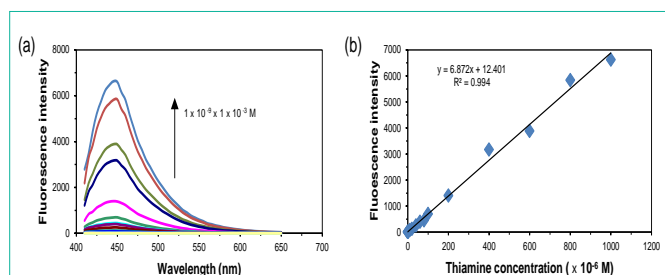
To investigate whether Au<sup>3+</sup> interact specifically with thiamine among various other biomolecules, aqueous solutions of biotin, L-cysteine, L-glutathione, D-(+)-glucose, and cellulose were used instead of thiamine to react with HAuCl<sub>4</sub>. To investigate the interference of other biomolecules, aqueous solutions of biotin, L-cysteine, L-glutathione, D-(+)-glucose, and cellulose were additionally introduced after the addition of thiamine of the same concentration. The rest reaction conditions and instrumentations were the same. To investigate the interference of other metal ions, aqueous solutions of 12 other metal ions (K<sup>+</sup>, Li<sup>+</sup>, Al<sup>3+</sup>, Zn<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup>, Pb<sup>2+</sup>, Ca<sup>2+</sup>, Hg<sup>2+</sup>, Na<sup>+</sup>, Cd<sup>2+</sup>, and Fe<sup>3+</sup>) was added in a 1:1 ratio of M-to-Au<sup>3+</sup> to the mixture of HAuCl<sub>4</sub> and thiamine.

## Results and Discussion

Figure 1a shows the conversion of thiamine (1) to thiochrome (2) in the presence of Au<sup>3+</sup> in alkaline conditions. Thiamine was initially non-fluorescent in aqueous solutions (Figure 1b, blue line, and inset item 1). Upon mixing with Au<sup>3+</sup> in alkaline conditions (pH 12), a new species exhibiting intense blue emission at 445 nm was spontaneously formed (Figure 1b, purple line, and inset item 2). It should be mentioned that the presence of both Au<sup>3+</sup> and NaOH was necessary for the formation of the blue-emitting species. No blue emission was observed in the absence of Au<sup>3+</sup> (Figure 1b, red line) or NaOH (Figure 1b, green line). Photo excitation and mass spectra were measured to further characterize the resultant blue emitting species. It showed a distinct peak at 370 nm of the excitation spectrum. Both the excitation and emission profiles were similar to those [14] reported for thiochrome previously, suggesting the formation of thiochrome. Mass spectrum further confirmed the formation of thiochrome. A base peak at 263.11 was observed on the mass spectrum, which could be assigned as thiochrome attached with a proton [C<sub>12</sub>H<sub>14</sub>N<sub>4</sub>OS + H]<sup>+</sup>. The resultant thiochrome showed very intense blue emission with quantum yield of 11% (calibrated with quinine sulfate). As shown in Figure 2, the formation of thiochrome was fast and could be completed in 10 minutes, which allowed the rapid detection of thiamine. Moreover, the as-formed thiochrome was found to be stable for several days (data not shown) in the current system



**Figure 2:** Time course photoemission spectra of the reaction mixture. (Inset) Plot of the fluorescence intensity at 445 nm against reaction time showing the completion of the reaction in ~10 min.

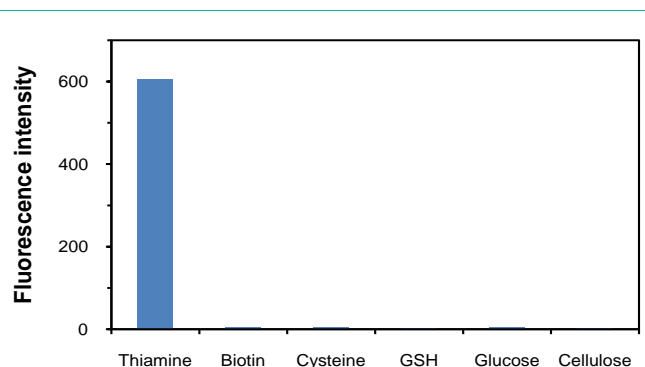


**Figure 3:** (a) Fluorescence response of 0.5 mM  $\text{Au}^{3+}$  mixed with thiamine of varied concentrations. (b) Plot of fluorescence intensity at 445 nm against the thiamine concentration.

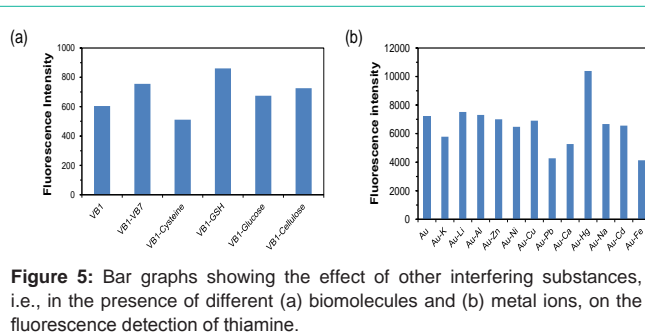
and no separation steps were needed. Therefore, the conversion of thiamine to thiochrome by  $\text{Au}^{3+}$  could be exploited for simple and fast fluorimetric detection of thiamine.

The formation of thiochrome was then used to quantify the amount of thiamine in aqueous solutions. To do so, the fluorescence spectrum of the reaction mixture was examined with thiamine of varied concentrations while the concentration of  $\text{Au}^{3+}$  was kept as constant as 0.5 mM. As shown in Figure 3a, when the thiamine concentration increased from  $1.0 \times 10^{-9}$  M to  $1.0 \times 10^{-3}$  M, the fluorescence intensity of the resultant product also increased accordingly. The fluorescence intensity at 445 nm was then plotted against the concentration of thiamine (Figure 3b), which exhibited a good linear relationship in the region of  $1.0 \times 10^{-9}$ – $1.0 \times 10^{-3}$  M ( $R^2 = 0.994$ ). The Limit Of Detection (LOD) was calculated as low as  $2.0 \times 10^{-6}$  M, where the fluorescence signal was three times higher than that of the blank.

Other than a good linearity of detection range and low detection limit, the current method is also featured by its high specificity that  $\text{Au}^{3+}$  reacted only with thiamine but not other biomolecules. As shown in Figure 4, among various common biomolecules including S-containing vitamin (e.g., biotin), biothiols (e.g., cysteine, GSH), and saccharides (e.g., glucose, cellulose), only thiamine was detected using the current method (as exhibited by a more than 100 times higher fluorescence signal). As a comparison, though other metal ions such as  $\text{Cu}^{2+}$  can also facilitate the thiochrome formation [6], it may also generate similar blue emission in the presence of other



**Figure 4:** Bar graph showing  $\text{Au}^{3+}$  reacting specifically with thiamine among various other biomolecules to generate strong emission (concentration of cellulose: 1.0g/L; the other biomolecules:  $5.0 \times 10^{-5}$  M).



**Figure 5:** Bar graphs showing the effect of other interfering substances, i.e., in the presence of different (a) biomolecules and (b) metal ions, on the fluorescence detection of thiamine.

biomolecules (e.g., cysteine) under similar reaction conditions (due to the formation of so-called Cu nanoclusters [15]).

In order to verify the performance of the current method for thiamine detection in practical applications, the fluorescence detection of thiamine was further investigated in the presence of other biomolecules or metal ions. A total of five different biomolecules including biotin, cysteine, GSH, glucose and cellulose were selected to study the interference of biomolecules in the fluorescence detection of thiamine. As shown in Figure 5a, the fluorescence of the resultant product was highly observable, though some biomolecules (e.g., cysteine) did quench the fluorescence a bit while other biomolecules (e.g., biotin, GSH, glucose, cellulose) enhanced the fluorescence for a certain degree. A total of 12 metal ions were further used to evaluate the performance of the current method. Figure 5b shows that most of the metal ions did not affect the formation of the fluorescence thiochrome. However, it was noticed that  $\text{Hg}^{2+}$  could enhance the fluorescence while  $\text{K}^+$ ,  $\text{Pb}^{2+}$ ,  $\text{Ca}^{2+}$ , and  $\text{Fe}^{3+}$  could quench the as-formed thiochrome in certain extent. The fluorescence enhancement in the presence of  $\text{Hg}^{2+}$  is not unexpected as  $\text{Hg}^{2+}$  has been demonstrated as oxidant for the thiochrome reaction in previous studies [5,10]. These results suggested the possible application of our sensor system for real samples.

## Conclusion

In summary, we have developed a simple and fast fluorimetric method for thiamine detection by using  $\text{Au}^{3+}$  to convert the non-fluorescent thiamine to intensely fluorescent thiochrome. The as-formed thiochrome exhibited an excitation peak at 370 nm and an emission peak at 445 nm with quantum yield as high as 11%. The

linear detection range for thiamine was observed to be  $1.0 \times 10^{-9}$ -  $1.0 \times 10^{-3}$  M and the correlation coefficient for the calibration curve was 0.994. The limit of detection for thiamine was calculated to be  $2.0 \times 10^{-6}$  M. In addition, this method was specific only to thiamine and was negligibly affected in the presence of other biomolecules and metal ions. The method developed in this study will find its application in a wide range of areas, such as clinical analysis, food processing, and pharmaceutical industries.

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## References

1. Kraut J, Reed H J. The crystal structure of thiamine hydrochloride (vitamin B). *Acta Crystallogr.* 1962; 15: 747-757.
2. Lonsdale D. A review of the biochemistry, metabolism and clinical benefits of thiamin(e) and its derivatives. *Evid Based Complement Alternat Med.* 2006; 3: 49-59.
3. Chen Qy, Li Dh, Yang Hh, Zhu Qz, Zheng H, Xu Jg. Novel spectrofluorimetric method for the determination of thiamine with iron(III) tetrasulfonatophthalocyanine as a catalyst. *Analyst.* 1999; 124: 771-775.
4. Fujiwara M, Matsui K. Determination of thiamine by thiochrome reaction. *Anal. Chem.* 1953; 25: 810-812.
5. Martinez-Lozano C, Perez-Ruiz T, Tomas V, Abellan C. Flow injection determination of thiamine based on its oxidation to thiochrome by mercury (II). *Analyst.* 1990; 115: 217-220.
6. Perez-Ruiz T, Martinez-Lozano C, Tomas V, Ibarra I. Flow injection fluorimetric determination of thiamine and copper based on the formation of thiochrome. *Talanta.* 1992; 39: 907-911.
7. Bohrer D, do Nascimento PC c, Ramirez AG, Mendonça JKA, de Carvalho LM, Pomblum SCG. Determination of thiamine in blood serum and urine by high-performance liquid chromatography with direct injection and post-column derivatization. *Microchem. J.* 2004; 78: 71-76.
8. Du J, Li Y, Lu J. Flow injection chemiluminescence determination of thiamine based on its enhancing effect on the luminol-hydrogen peroxide system. *Talanta.* 2002; 57: 661-665.
9. Ortega Barrales P, Fernandez de Córdova ML, Molina Diaz, A. A selective optosensor for UV spectrophotometric determination of thiamine in the presence of other vitamins B. *Anal. Chim. Acta.* 1997; 376: 227-233.
10. Ryan MA, Ingle JD. Fluorometric reaction rate method for the determination of thiamine. *Anal Chem.* 1980; 52: 2177-2184.
11. Martinez Calatayud J, Gomez Benito C, gaspar Gimenez D. FIA-fluorimetric determination of thiamine. *J Pharm Biomed Anal.* 1990; 8: 667-670.
12. Chen H, Zhu J, Cao X, Fang Q. Flow injection on-line photochemical reaction coupled to spectrofluorimetry for the determination of thiamine in pharmaceuticals and serum. *Analyst.* 1998; 123: 1017-1021.
13. Khan MA, Jin SO, Lee SH, Chung HY. Spectrofluorimetric determination of vitamin B(1) using horseradish peroxidase as catalyst in the presence of hydrogen peroxide. *Luminescence.* 2009; 24: 73-78.
14. Amjadi M, Manzoori J L, Orooji M. The use of crude extract of Kohlrabi (*Brassica oleracea gongylodes*) as a source of peroxidase in the spectrofluorimetric determination of thiamine. *Bull. Korean Chem. Soc.* 2007; 28: 246-250.
15. Yang X, Feng Y, Zhu S, Luo Y, Zhuo Y, Dou Y. One-step synthesis and applications of fluorescent Cu nanoclusters stabilized by L-cysteine in aqueous solution. *Anal Chim Acta.* 2014; 847: 49-54.