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Chemometric modeling of kinetic-fluorescent third-order data for thiamine determination in multivitamin complexes

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ABSTRACT

Thiamine (vitamin B1) was converted into a fluorescent thiochrome by oxidation catalyzed by Hg^{2+} in alkaline medium. The kinetic evolution for this conversion was followed by emission-excitation spectroscopy generating a third-order dataset for each sample, and a four-way data array for a group of samples. The data were analyzed by parallel factor analysis (PARAFAC), which presents the second-order advantage, allowing to estimate the analyte concentrations even in the presence of unknown fluorescent interferences. On the other hand, the second-order advantage does not prevent matrix effects or interferences affecting the reaction mechanism or kinetic properties. For this reasons, standard addition is needed in order to develop a quantitative method for thiamine determination in multivitamin complexes acquired in the local market. The thiochrome emission, excitation and time profiles are correctly isolated in all samples, and the concentration of thiamine in the latter ones was estimated with a deviation lower than 10% of their nominal values.

1. Introduction

Thiamine or vitamin B1 is a water-soluble vitamin that is naturally present in foods such as cereals, vegetables, milk and dairy, and eggs. In synthetic form, it can be found as mononitrate hydrochloride or thiamine. Its absence is associated with serious diseases like Beriberi and Wernicke–Korsakoff syndrome. Alcohol abuse can lead to thiamine deficiency, because ethanol competes with thiamine and prevents its absorption [1].

Thiamine can be deprotonated in a basic medium, and the deprotonation product can be oxidized to thiochrome, as outlined in Fig. 1. The reaction product is fluorescent, and many methods for thiamine determination are based on thiochrome emission [2–4].

A kinetic method for thiamine determination, based on thiochrome formation and fluorescence spectroscopy was proposed by Ryan and Ingle Jr. [2]. The authors applied the method in the presence of several potential interferents, such as other vitamins, anions, minerals, and excipients. Although the method is robust to many interferents, vitamins B_2, B_{12} and C, nicotinamide and folic acid, and certain metals [Mg(II), Zn(II), Cu(II), Fe(II) and Mn(II)] can cause significant interference.

Higher-order multivariate calibration methods, such as parallel factor analysis (PARAFAC) [5,6] can be used for the analyte quantification by processing complex multi-way data structures, i.e., where more than one factor is varied during the acquisition of the analytical signal. Higher-order methods have the second-order advantage, that turn possible a determination in the presence of unknown interferences. Molecular fluorescence is typically a signal of second-order, since an excitation–emission fluorescence matrix (EEFM) may be acquired per sample. In a chemical reaction, the kinetic time is an additional data way, and third-order signals can be acquired per sample, namely an excitation–emission-time array. This would be feasible when the acquisition of the data matrix can be performed in a short enough time and/or the kinetics is sufficiently slow. Nevertheless, there are few studies in the literature that explore third-order data [7–10], and only a limited number is devoted to kinetic data [11,12]. It is important to develop new third-order methodologies to be tested by the available algorithms, to probe the limits and advantages of higher-order data as regards analytical protocols for complex samples.

Even when the second-order advantage is achieved, matrix effects such as absorption of excitation or emission radiation, or light scattering due the formation of a precipitate, can hinder the use of external calibration. Many of the interferents studied by Ryan and Ingle Jr. produce such effects [2]. In fact, when matrix effects are present, standard addition is the appropriate strategy for calibration.

In this paper, we propose a new kinetic method to determine the thiamine based on third-order excitation–emission-time data, four-way PARAFAC and standard addition calibration. The method was tested in several commercial multivitamin complexes. Because each multivitamin complex is produced with a particular composition, each sample can be regarded as a specific chemical matrix. In fact, the idea is to explore the second-order advantage, proposing a robust protocol to

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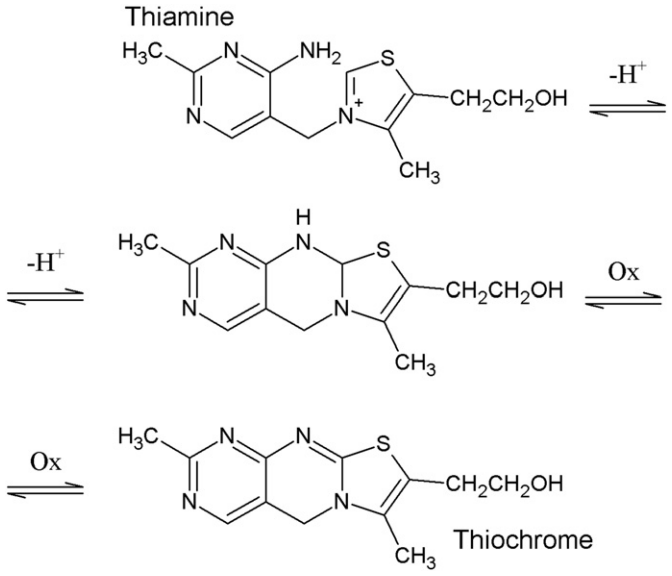

Fig. 1. Thiamine oxidation and thiochrome formation.

Table 1
Composition of commercial samples.

	S1	S2	S3	S4	S5
Vitamin					
B1 (Thiamine)	300 mg	1.5 mg	15 mg	1.4 mg	1.4 mg
A		3333 UI		2000 UI	4.8 mg
B2		1.7 mg	15 mg	1.6 mg	1.6 mg
B6		2.2 mg	10 mg	2 mg	2 mg
B12		0.003 mg	0.01 mg	0.001 mg	0.001 mg
C		60 mg	500 mg	60 mg	60 mg
D3		300 UI		200 UI	200 UI
E		15 mg		14.9 UI	10 mg
K				0.003 mg	
Nicotinamide		19 mg	50 mg	18 mg	18 mg
Calcium pantothenate		5.5 mg	20 mg	6 mg	
Biotin		0.2 mg	0.15 mg	0.15 mg	0.15 mg
Folic acid		0.1 mg		0.2 mg	0.2 mg
Minerals					
Ca		62.5 mg	100 mg		100 mg
Fe		4.5 mg			10 mg
Mg		50 mg	100 mg		40 mg
P		62.5 mg			
Mn		0.5 mg			2.5 mg
Cu		0.5 mg			2 mg
Zn		3.75 mg			1 mg

determine thiamin in different matrices, i.e., in several complex samples. To the best of our knowledge this is the first time that standard addition calibration is used with a kinetic-fluorescence third order dataset.

2. Experimental

2.1. Apparatus

Fluorescence measurements were performed on a Varian Cary Eclipse spectrophotometer. EEFM data were recorded in a 10 mm quartz cell at 25 °C, by the use of a thermostatic cell holder and a Selecta thermostatic bath. The excitation range was 320–380 nm and the emission range was 410–580 nm. The excitation and emission slits were set on 20 nm and the PMT sensitivity was set for 500 V. The scan rate was 3200 nm/min and in these conditions a complete 3D array was obtained in 40 s. The Cary Eclipse software was set to measure ten cycles of 1 min each, so that 20 s after a matrix acquisition, a new acquisition was started.

2.2. Reagents

Standards solutions of thiamine were prepared with thiamine chlorhydrate Parafarm 99.23%. Concentrated phosphate buffer was prepared by dissolution of 1.92 g of $Na_3PO_4 \cdot 12H_2O$ and 2.12 g of $NaHPO_4$ in 100 mL of water and then adjusting the pH to 12 with concentrated NaOH solution. The Hg^{2+} solution was prepared by dissolution of 0.07 g yellow mercury oxide in three drops of concentrated HCl, and dilution to 100 mL. The pH of the Hg^{2+} solution was verified an adjusted for 4 by addition of NaOH. In all these steps Mili-Q deionized water was used.

2.3. Calibration sample set

Different vitamin complexes were acquired in a local commerce of Rosario, Argentina. The pharmaceuticals presented different compositions of active principles and excipients. Table 1 presents the vitamins and minerals contained in these vitamin complexes.

In order to prepare the working samples, one pill of multivitamin was put in a 100 mL volumetric flask with sufficiently deionized water to cover the pill, and one drop of concentrated hydrochloric acid was added. The flask was then brought to a sonicator until the pill was completely dismantled. The volume was completed to the mark, and the solution was then filtered to remove the insoluble excipients. These sample solutions were diluted for a range of 120–150 ppb. The standard additions were of 50, 100, 150 and 200 ppb of thiamine.

2.4. Analytical measurement

A volume of 1.0 mL of sample solution is placed into a quartz cell, followed by 1.0 mL of Hg^{2+} solution. Then 1.0 mL of phosphate buffer is added, which indicates the start of the fluorescence measurements, because this latter addition induces the reaction. All sample solutions and reagents were kept in a 25 °C water bath prior to signal collection. After the measurements, 3.0 mL of solution was removed from the quartz cell for Hg^{2+} treatment and disposal.

2.5. Modeling and software

The four-way PARAFAC models were built with the MVC3 program for Mathwork Matlab© [13]. This software consists of a graphical user interface that provides many features for multivariate calibration. The N-way toolbox [14] is used by MVC3 to perform the specific PARAFAC calculations.

3. Results and discussion

3.1. Kinetics behavior of thiamine

Ryan and Ingle Jr. [2] established that the kinetics of thiochrome formation in the presence of Hg(II) involve a pseudo first-order process in thiamine for Hg(II)—thiamine formation, followed by a first-order in thiamine reaction to produce thiochrome. At pH 12.2 and with 2.5 mmol L^{-1} of Hg^{2+}, the increase of thiochrome fluorescence is observed up to ca. 30 min. At this pH, in the absence of Hg^{2+}, thiamine is almost completely converted into a colorless thiol in 4 min [2]. This behavior is associated with the stabilization of the intermediate compound in Fig. 1 by formation of the Hg(II)–thiamine complex, which can be then oxidized to thiochrome. Fig. 2 shows the EEFM fluorescence matrices for a standard solution of thiamine (100 ppb) in deionized water, measured at 1 (A), 4 (B), 7 (C) and 10 min (D) of reaction. It is possible to note that the fluorescence intensity of thiochrome increases three times over the acquisition time.