Kinetic Fluorescence Determination of Vitamin B_1

Abstract

This technical note describes kinetic fluorescence as an analytical technique to quantify non-fluorescent species. The technique is applied to the determination of thiamine (vitamin B_1) in solution. Fluorescence intensity is monitored as thiamine converts to fluorescent thiochrome, and a relationship is established between the rate of increase of intensity and the concentration of thiamine. The technique is shown to have good sensitivity and selectivity.

Introduction

Luminescent substances are quantified readily by sensitive fluorescence and phosphorescence techniques. Kinetic fluorescent methods extend the sensitivity advantage of fluorescence analysis to non-fluorescing samples by converting them chemically to fluorescent species. For example, the technique can be used to analyze pharmaceutical preparations to quantify non-fluorescing thiamine (vitamin B_1), which oxidizes to fluorescent thiochrome.

Kinetic fluorescence is borrowed from methods for studying reaction rates of chemical or physical transformations. In these studies, the increase or decrease in fluorescence intensity (corresponding to formation or degradation of a fluorescent species) is monitored during a reaction in order to understand the reaction mechanism. Kinetic fluorescence establishes a relationship between the change in fluorescence intensity during a reaction and the concentration of the non-fluorescing reactant. Kinetic fluorescence's precision and specificity allow quantification of individual components in a multi-component solution without complicated separations.

To illustrate the applicability of kinetic fluorescence for analytical work, we describe herein a reaction-rate method for the determination of thiamine, an experiment using commercial multiple-vitamin tablets, and a calibration procedure for thiamine determination with the Fluorolog® modular spectrofluorometer system.

Because thiamine is involved in numerous metabolic processes, proper maintenance of thiamine levels in the diet is essential. Thiamine deficiency can produce degenerative disorders such as edema, muscular atrophy, or even beriberi. In addition to foods containing thiamine (e.g., nuts, vegetables, pork, and liver), thiamine is also available in many commercial supplements, including vitamin-B complexes and anti-stress preparations.

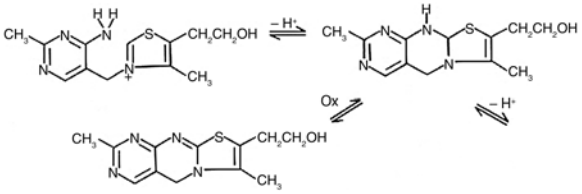

Fig. 1. *Reaction of thiamine and Hg^{2+} to form thiachrome.*

The determination of thiamine by kinetic fluorescence involves the conversion of thiamine to fluorescent thiochrome, during which changes in fluorescent are monitored. Ryan and Ingle[1], who developed the fluorometric reac-

[1] Ryan, M.A; Ingle, J.D. *Anal. Chem.* **1980**, *52*, 2177–2184.

tion-rate method for thiamine determination, found Hg^{2+} to be a suitable oxidizing agent, as shown in Fig. 1.

Ryan and Ingle applied the technique to the determination of thiamine in a synthetic vitamin-mineral preparation. Bowers modified their procedure for a student-laboratory experiment to quantify thiamine in a variety of commercial vitamin preparations.[2]

Experimental method

For the calibration procedure, four thiamine standards were prepared in concentrations of 2.5, 1.25, 0.625, and 0.313 ppm. Mercuric chloride was dissolved in concentrated HCl and diluted to make a 500-ppm Hg^{2+} solution, and a phosphate buffer (pH = 12.2) was prepared from $Na_3PO_4 \cdot 12H_2O$ and Na_2HPO_4.

To 1 mL of the thiamine standard in a cuvette was added 1 mL of the Hg^{2+} solution followed by 1 mL of buffer. The cuvette was then placed in the sample compartment of a Fluorolog® spectrofluorometer system. A variable-temperature accessory maintained the sample temperature at 22.8°C, and a magnetic stirrer provided continuous mixing during the scan.

A single-grating monochromator provided excitation at 365 nm, and a double-grating monochromator monotored fluorescence at 444 nm. Slits were set to a bandpass of 5 nm. For each calibration standard, the computer acquired a data file representing the fluorescence intensity during the reaction, measured at intervals of 100 ms over a total time of 50 s. The fastest integration time for data acquisition was 1 ms.

[2] Bower, J. *J. Chem. Ed.* **1982**, *59*, 975–977.

Results and discussion

Fig. 2 shows four time-based fluorescence scans obtained with the Fluorolog® spectrofluorometer during the oxidation of thiamine to thiochrome. The four scans trace the reaction for standard concentrations of 2.5, 1.25, 0.625, and 0.313 ppm. The reaction rates, determined from the slope of the intensity-versus-time plots in Fig. 2, are given in Table 1.

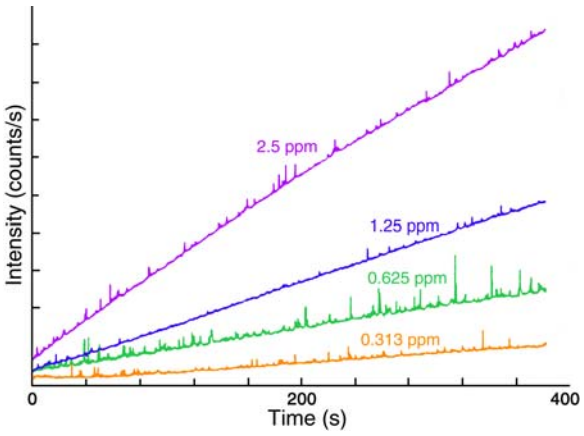

Fig. 2. *Plots of fluorescence intensity versus time for conversion of thiamine to thiachrome for four thiamine standards. Linearity indicates a constant reaction rate for each standard.*

Thiamine concentration (ppm)	Reaction rate (counts/s^2)
2.5	4.6×10^3
1.25	2.4×10^3
0.625	1.6×10^3
0.313	5.4×10^2

Table 1. *Reaction rates for thiamine standards.*

Reaction rates were plotted against concentration; the best fit was found by the linear least-squares method. The resulting calibration curve (Fig. 3) can be used to quantify trace amounts of thiamine in samples containing other vitamins and minerals.

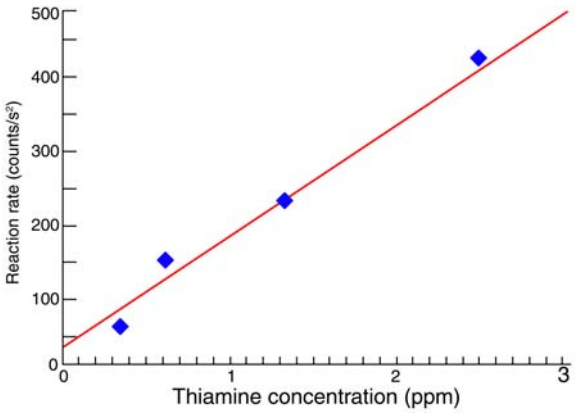

Fig. 3. *Calibration curve for reaction-rate determination of thiamine. The line is the best fit to the four thiamine standards.*

Ryan and Ingle demonstrated that their reaction-rate method for determination of thiamine gave reasonable results for commercial and synthetic vitamin-mineral preparations. Kinetic analysis of a synthetic preparation compared favorably with results obtained by the USP standard method of analysis. Moreover, the kinetic method was shown to be faster and cheaper.

Bower's students analyzed five types of commercial vitamin tables using a simplified technique: samples were run at room temperature without stirring, the reaction was followed on a recorder, and rates were determined by hand from the plots. Typical results included determinations within ±2% of the stated amount of thiamine in a "therapeutic" type, within ±5% in a "chewable" type, and within ±6% in a "one-a-day" type. Replication was within ±5% RSD for tablets from the same bottle and for preparations of the same type by different manufacturers.

Through their development of a highly sensitive and selective technique for thiamine determinations, Ryan and Ingle have demonstrated the applicability of kinetic fluorescence to the analytical laboratory. Bower's experiments emphasize the simplicity and repeatability of the technique.

Conclusion

Because it offers sensitive fluorescence measurements, full system control with our exclusive FluorEssence™ software, and modular construction, the Fluorolog® spectrofluorometer is ideal for kinetic fluorescence determinations. The calibration procedure described herein can be applied readily to reaction-rate determinations with your Fluorolog® system.

HORIBA JOBIN YVON

USA: HORIBA Jobin Yvon Inc., 3880 Park Avenue, Edison, NJ 08820-3012, Toll-Free: **+1-866-jobinyvon**
Tel: +1-732-494-8660, Fax: +1-732-549-5125, E-mail: info@jobinyvon.com, www.jobinyvon.com

France: HORIBA Jobin Yvon S.A.S., 16-18, rue du Canal, 91165 Longjumeau Cedex,
Tel: +33 (0) 1 64 54 13 00, Fax: +33 (0) 1 69 09 93 19, www.jobinyvon.fr

Japan: HORIBA Ltd., JY Optical Sales Dept, Higashi-Kanda, Daiji Building, 1-7-8 Higashi-Kanda
Chiyoda-ku, Tokyo 101-0031, Tel: +81 (0) 3 3861 8231, www.jyhoriba.jp

Germany: +49 (0) 89 462317-0 **Italy:** +39 0 2 57603050 **UK:** +44 (0) 20 8204 8142
China: +86 (0) 10 8567 9966