Kinetic Fluorescence Determination of Vitamin B_1

Abstract

Kinetic fluorescence as an analytical technique to quantify non-fluorescent species is described, and 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 that can be converted chemically to fluorescent species. For example, the technique can be used in the analysis of pharmaceutical preparations to quantify non-fluorescing thiamine (vitamin B_1), which oxidizes to fluorescent thiochrome.

Kinetic fluorescence is borrowed from the methodology of scientists who study reaction rates of chemical, biochemical, 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. As an analytical method, kinetic fluorescence establishes a relationship between the change in fluorescence intensity during a reaction and the concentration of the non-fluorescing reactant. The precision and specificity offered by kinetic fluorescence make it possible to quantify individual components in a multi-component solution without resorting to complicated separation procedures.

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

Because thiamine is involved in numerous metabolic processes, proper maintenance of thiamine levels in the human diet is essential. A deficiency of thiamine 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 multiple-vitamin supplements, including vitamin-B complexes and anti-stress preparations.

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, who developed the fluorometric reaction-rate method for thiamine determination, found Hg^{2+} to be the most suitable oxidizing agent.[1] The reaction is shown in Figure 1.

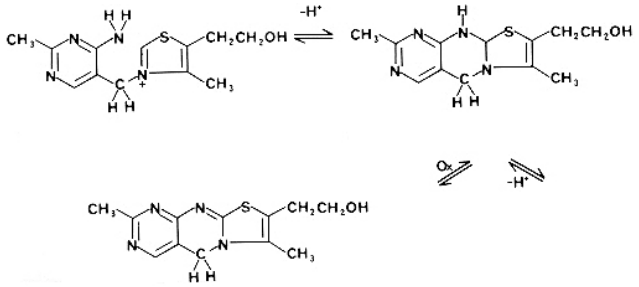

Figure 1. Reaction of thiamine and Hg^{2+} to form thiachrome.

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] The papers by Ryan and Ingle and by Bower contain detailed descriptions of their experimental methods.

[1] Ryan, M.A; Ingle, J.D. *Anal. Chem.* **1980**, *52*, 2177–2184.
[2] Bower, J. *J. Chem. Ed.* **1982**, *59*, 975–977.

Experimental Procedures

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

A single spectrometer provided excitation at 365 nm, and a double spectrometer was positioned to monitor fluorescence at 444 nm. The slits on each spectrometer were adjusted to give 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 is 1 ms.

Results and Discussion

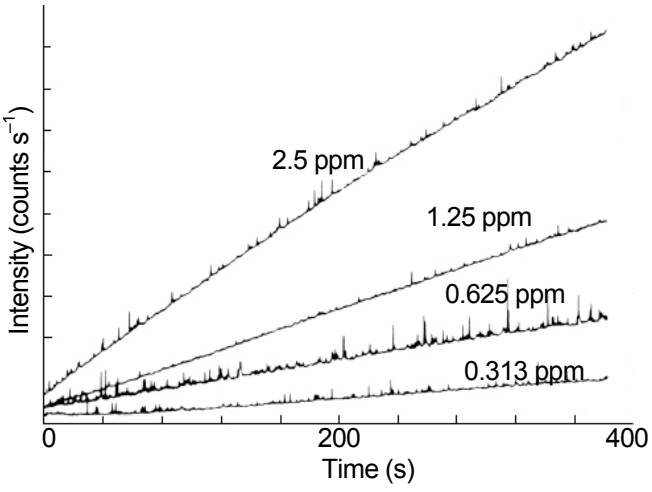

Figure 2. Plots of fluorescence intensity versus time for conversion of thiamine to thiachrome, for four thiamine standards. Linearity indicates a constant reaction rate for each standard.

Figure 2 shows four time-base 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 Figure 2, are given in Table 1.

Table 1. Reaction rates for thiamine standards

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

Using the data-processing capability of the DM3000F, reaction rates were plotted against concentration, and a linear-regression program was applied to determine the best fit. The resulting calibration curve (Figure 3) can be used selectively to quantify trace amounts of thiamine in samples containing other vitamins and minerals.

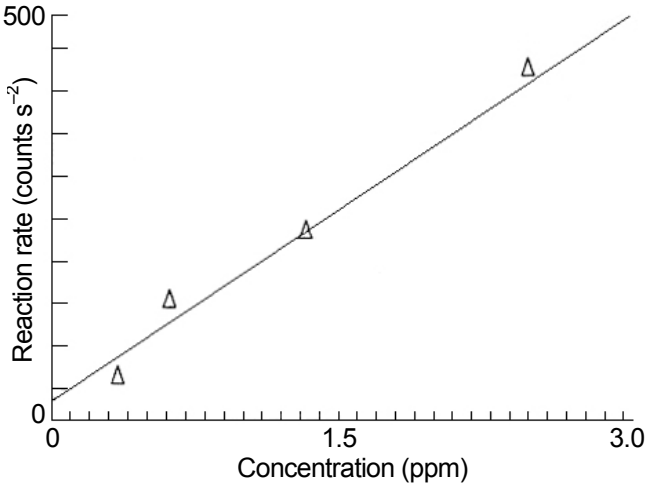

Figure 3. Calibration curve for reaction-rate determination of thiamine. The line is the best fit to the four thiamine standards.

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 less expensive.

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.

Because it offers sensitive fluorescence measurements, full system control, extensive graphics and data-processing functions and automated keystroke programming, 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.

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