Acta Pharmaceutica Turcica
XLII (1) (23-27) 2000

A FLUOROMETRIC KINETIC METHOD FOR THE QUANTITATION OF THIAMINE IN PHARMACEUTICALS *VIA* OXIDATION WITH CERIUM IV

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The development and evaluation of a differential kinetic fluorometric method for quantifying thiamine based on oxidation with Ce(IV) is described. Conditions were developed for first order reaction in thiamine. Fluorescence ratio to a known concentration of thiochrome vs. time data were collected every 10 min for 40 min, from which the initial rate was calculated. Calibration curves were linear for thiamine concentrations between $5x10^{-4}$ and $2x10^{-6}$ M. Various concentrations of thiamine were successfully determined in different synthetic mixtures containing other vitamins.

Keywords : *Thiamine; Fluorometri; Kinetic; Thiochrome*

Introduction

There are many biological, microbiological and chemical methods which have been employed for the determination of thiamine (Vit. B_1) in pharmaceutical samples(1). The chemical methods offer accepted accuracy and speed and thus are widely accepted for routine work. The chemical methods involve the oxidation of thiamine to fluorescent thiochrome by various oxidizing agents at strongly basic mediums(2-7). Liquid chromatographic methods used the same reaction for measurement of thiamine in the presence of other vitamins (8-10).

The study of Ryan and Ingle (3) based on the kinetic fluorometric method has reported that, the amount of thiochrome depends on the kind of oxidizing agent and the pH of the medium as certified by other studies (11,12). A differential kinetic fluorometric method for the determination of a mixture of thiamine and pyrophosphate, based on the oxidation of these compounds by mercuric ions to thichrome has been also reported(5).

This paper proposes the development and evaluation of a kinetic method for the determination of thiamine *via* its oxidation to thiochrome by Ce(IV) which has not been reported previously. The method has the merit of being performed at neutral pH, free of interference of ascorbic acid and riboflavin that usually accompany thiamine in multi vitamin tablets. The kinetic behavior of the reaction was examined and appropriate conditions were selected for quantitation of thiamine.

In this method thiamine was mixed with excess of Ce(IV) and buffer to make pH=7.4, at 38±0.1°C and several fluorescence values of the produced thiochrome were measured. Initial rate was calculated from the slope of the linear least squares of data collected. The effect of temperature and pH on initial rate were studied. The method was studied for determination of thiamine in different synthetic preparations containing other vitamins.

Materials and Methods

Apparatus

All fluorometric measurements were performed by using a Kontron, SAM-25 fluorometer, Zurich, Switzerland. It was equipped with a thermostatic cell compartment to perform measurements in the range of 200-800 nm. The source was a xenon-high pressure

lamp and the excitation and emission monochromators were concave holographic gratings. Spectrophotometric measurements for the determination of Ce(IV) in the oxidizing reagent solution were performed by using a JASCO-7800 double beam spectrophotometer, Tokyo, Japan.

Reagents

Deionized-distilled water was used and all chemicals were reagent grade. One litre of cerium IV solution of 0.01 M was prepared by dissolving calculated amount of $Ce(SO_4)_2.4H_2O$ in 0.1 M H_2SO_4 solution. One litre of phosphate buffer solution (0.10 M) was prepared by dissolving calculated amount of KH_2PO_4 in deionized water and adjusting the pH to 7.92 by using 5M KOH solution. The oxidizing reagent was prepared by mixing 100 mL of the Ce(IV) solution and 900 mL buffer solution, and filtering to remove the insoluble cerium hydroxides. The pH of this solution was 7.40 and the Ce(IV) concentration determined spectrophotometrically was $2.4x10^{-4}$ M. Oxidizing reagents used for pH studies were prepared from the main oxidizing reagent having an initial pH=7.4 by adding either KOH solution or H_2SO_4 solution for pH adjustmet. Stoch solutions of $1.0x10^{-3}$ M of thiamine and $2x10^{-5}$ M thiochrome were prepared in $1.0x10^{-4}$ M sulfuric acid and these solutions were refrigerated at 4°C. Diluted solutions were prepared freshly from stock solutions.

Procedures

The initial concentration of Ce(IV) in oxidizing reagents at various pHs was determined by measuring the absorption at 280 nm of the oxidizing reagents and series of calibration standards for Ce(IV) were prepared in $1.0x10^{-4}$ M H_2SO_4 solutions. The molar absorptivity of Ce(IV) at this pH was 470 M^{-1} cm^{-1}.

All reactions except those in temperature effect studies were performed at 38±0.1°C, and all reactions except those in pH studies were performed at pH=7.4. Twenty mL of oxidizing reagent were mixed with 10 mL of thiamine standard solution or synthetic sample solution containing thiamine in 40 mL covered vials. Aliquots of 3.0 mL were pipetted every 10 min for fluorescence measurements. The excitation wavelength was 365 nm and the emission wavelength was 440 nm. These wavelengths were the most sensitive for thiochrome measurements with a detection limit of less than $1.0x10^{-8}$ M. Fluorescence intensity was measured simultaneously for the aliquot and appropriate thiochrome standard. Results were reported as equivalent thiochrome concentration.

Results and Discussion

Uncertainties for all quantitative results were quoted as one standard deviation unit. Reported concentrations were those in reaction vials.

Kinetic Dependencies

A major objective of this study was to develop a kinetic method to determine thiamine in pharmaceutical products via its oxidation with Ce(IV). To achieve that, appropriate conditions for the kinetic quantitation of thiamine have to be defined. Accordingly, effects of temperature and pH as well as Ce(IV) initial concentration were studied. Except when the parameter being varied, conditions for these studies as specified in the above procedure.

Temperature

Effects of temperatures between 30 and 60°C were studied. The slope of ln k vs. 1/T was $-6.29 \times 10^3 \pm 6.29 \times 10^2$, corresponding to an activation energy of 52 kJ mol^{-1}. Subsequent studies were done at 38°C.

Effect of pH

Initial concentration of Ce(IV) was affected by increasing pH. As pH increased cerium formed insoluble hydroxides and the initial Ce(IV) concentration became less than the prepared one. To obtain the actual Ce(IV) concentration, spectrophotometric

measurements at 280 nm were performed.

Fig.1 shows the effect of pH on both the apparent first order constant and the initial concentration of Ce(IV). The apparent rate constant increased by increasing pH and reached maxima at pH=7.9. The Ce(IV) concentration decreased as pH increased and reached almost zero at pHs above 8.5.

There were two major factors affecting the kinetics of this reaction. The formation of cyclic intermediates due to the loss of H^+, which was favored at higher pHs and the concentration of Ce(IV) to oxidize the intermediate to thiochrome which was favored at lower pHs. As a compromise between obtaining a high rate constant and keeping Ce(IV) concentration high enough to keep pseudo first order conditions, pH 7.4 was chosen for subsequent studies.

Ce(IV) concentration

Higher Ce(IV) concentrations could be used to speed the reaction, permit the use of lower temparatures and/or reduce the measurement time. However the dependence of free Ce(IV) concentration on pH disabled the use of higher concentrations, thus a value of $1.6x10^{-4}$ M (in the reaction vial) was chosen for subsequent studies.

Quantitative evaluation

The linearity of the method was evaluated by running calibration standards in the range $5x10^{-6}$ and $5x10^{-4}$ M of thiamine hydrochloride. The calibration curve was linear in this range with a slope of 0.064 ± 0.0005 h^{-1} and an intercept of $2.61x10^{-7}\pm9.5x10^{-8}$ M h^{-1}. The standard error of estimate was $2.1x10^{-7}$ M h^{-1} and the correlation

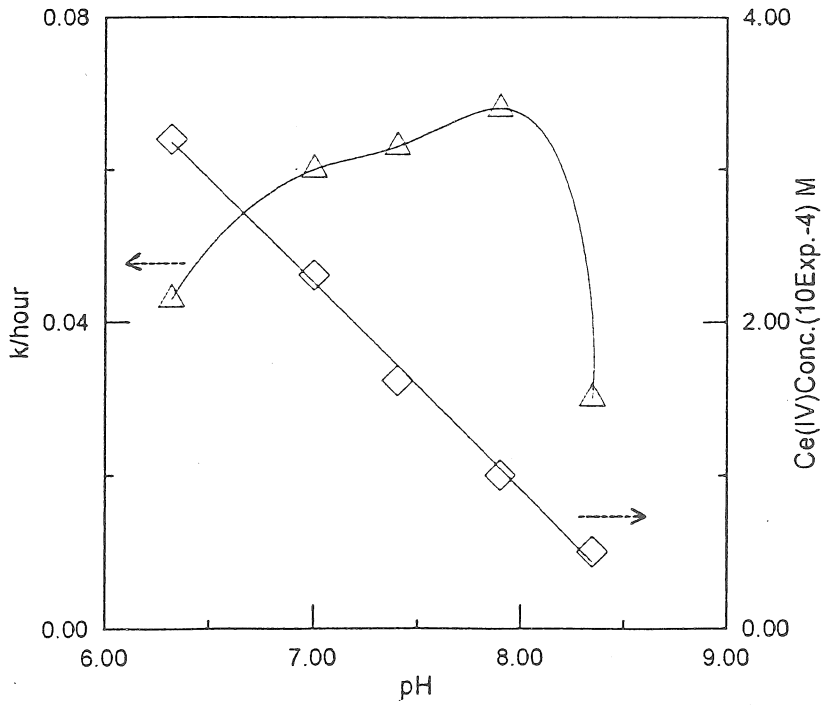

Fig.1. Effect of pH on the apparent first-order rate constant and the initial concetration o Ce(IV).

coefficient was 0.998. Uncertainty of the slope and intercept were approximately the same for calibration curves generated in five consecutive days.

The validity of the method was evaluated by performing interference studies. In one set of experiments the effect of some common excipients and coexisting compounds in pharmaceutical preparations was studied by analyzing synthetic sample solutions containing 25 and 100 μgmL^{-1} (a $\mu g\ mL^{-1}=3.33x10^{-6}$ M) of thiamine hydrochloride and excess amount of potential interferer. Results are presented in Table 1. In another set of experiments, the effect of other vitamins that may accompany thiamine in pharmaceutical preparations was studied. Resuts are present in Table 2.

Table 1. Analytical results for two levels of thiamine hydrochloride (A, 25 $\mu g\ mL^{-1}$; B, 100 $\mu g\ mL^{-1}$) in solutions containing 10 times as much additive.

Additive	Recovery of thiamine (%#)	
	A	B
Glucose	98.7	99.3
Sugar	99.2	100.8
Lactose	100.8	102.5
Starch	99.6	99.2
Sorbitol	98.8	101.6
KCl	99.4	99.8
Fructose	100.8	99.6

#Average of five measurements

To ensure proper perspectives for this study, it is emphasized that the focus has been on evaluation of Ce(IV) as potential oxidant for quantitation of thiamine. The results show that the reaction is very slow, $t_{1/2}$=10.8 hrs. and can not be accelerated significantly by using higher temperatures or pHs. Reliable equilibrium results can not be obtained since pseudo first order conditions were lost after about 10% completion of the reaction, especially for higher thiamine concentrations. Fortunately thiochrome, the product of this reaction, was highly fluorescent, concentrations of $1.0x10^{-8}$ M could be measured accurately and precisely, which makes initial rate method a proper choice for quantitation of thiamine *via* this method.

Table 2. Analytical results for two levels of thiamine hydrochloride (A, 25 $\mu g\ mL^{-1}$; B, 100 $\mu g\ mL^{-1}$) in solutions containing other vitamins.

Vitamin	Concentration (μgmL^{-1})	Recovery of thiamine (%#)	
		A	B
Ascorbic acid	1000	98.5	99.7
Riboflavin	75	96.5	99.1
Nicotiamide	150	99.2	100.8

#Average of five measurements

Linearity results showed low uncertainties for slope and intercept, the coefficient of variation of slope (CV) was about one percent. The standard error of estimate (Syx), which is a measure of the scatter about the best fit of the data, was about $\mu g\ mL^{-1}$.

Interference results showed no effects of excipients and other water soluble vitamins that may accompany thiamine in pharmaceutical formulations.

The method features the use of Ce(IV) as a potential oxidant for quantitation of thiamine in neutral media. Ascorbic acid, a potential interferant in other methods, does not interfere since it does not react with Ce(IV) under these conditions. Also the fluorescent riboflavin has little interference since the initial rate method is based on slope measurements vs. concentration.

One apparent disadvantage of the method was that it takes 40 min for each sample. Although it takes only four minutes to make four measurements for each sample, one measurement every ten minutes, enables the processing up to 10 samples at the same time.

In a series of experiments, the fixed time method was evaluated. Reliable results were obtained for measurements taken after two hours.

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Accepted: 12.07.1999