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# Rate of Oxidation of Thiamine by Hg(II) in Alkaline Medium

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A differential kinetic spectrophotometric method is proposed for studying the rate of oxidation of thiamine, TM (vitamin $B_1$) by mercury(II) in basic medium (pH = 12.5). The reaction has been studied in the range of 277.7 K to 320.5 K. The concentration of thiamine used: $0.35 \times 10^{-5}$ M, $0.70 \times 10^{-5}$ M and $1.40 \times 10^{-5}$ M. The concentrations of $OH^-$ and Hg(II) were kept constant by using 2 M NaOH and $2.0 \times 10^{-3}$ M Hg(II). Under these conditions, the only reaction product detected was thiochrome (TC). The initial rate was followed by recording the rate of formation of TC at $\lambda = 350$ nm for the first 20 seconds. Different kinetic parameters have been measured under reaction conditions.

## INTRODUCTION

The oxidation of thiamine (TM) by various oxidizing agents, and its conversion to thiochrome (TC) was the subject of several studies[1–5]. These studies demonstrated the formation of side products which greatly affected the amount of TC produced. The study of Ryan[2], which was based on the kinetic fluorometric method at 296 K, showed that the amount of TC depends on the kind of the oxidizing agent used and the value of pH of the medium. This was confirmed by other workers[6,7]. A study[8] used a photochemical spectrofluorimetric flow-injection method for the determination of thiamine, based on conversion of TM in alkaline medium into an intensively fluorescent compound (TC). Previous studies[9,10] indicated that the oxidation of TM by Hg(II) is appropriate for the study of the rate of oxidation of TM. Nicos[1] has used the TM-TC reaction successfully for the determination of thiamine by continuous flow chemiluminescence measurement. The oxidation of TM to TC is considered as a standard method recommended by U.S.P.[11] for the assay of TM. The study of Ryan[2] showed that when TM was oxidized by Hg(II) in basic medium, the only product was TC and the reaction remained pseudo-first order until the half life time of the reaction, then it followed a different path. Previous studies[9,12] confirmed the use of TM-Hg(II) reaction successfully in the quantitative analysis of TM. A differential kinetic fluorometric method has also been proposed[4] for the determination of a mixture of TM and pyrophosphate, based on the oxidation of these analytes by Hg(II) to TC. Another study[13] used a microminiaturized tubular heating system, with continuous heating and cooling, for kinetic study of thiamine degradation at high temperatures.

Nobody studied in detail the oxidation of TM in order to have all kinetic parameters in a wide range of temperatures. The aim of this work is to determine the initial rate of the reaction of TM-Hg(II) spectrophotometrically in a wide temperature range and to investigate the oxidation of TM by Hg(II) kinetically.

## EXPERIMENTAL

Standard solutions of thiamine hydrochloride (Sigma, purity 98%) and $HgCl_2$ (BDH) were prepared at $1 \times 10^{-3}$ M and $2 \times 10^{-3}$ M respectively at pH = 4.0 adjusted with hydrochloric acid. Working solutions of thiamine of lower concentrations were prepared daily by diluting to the appropriate volume with distilled water. Thiochrome (TC) was obtained from sigma (reagent grade). A computerized Jassco double beam UV/Vis spectrophotometer with CRT was used to follow the rate of production of TC and to record all the absorption spectra.

To a 10 mL thermostated standard flask containing 3 mL of $2 \times 10^{-3}$ M mercuric chloride solution were added an appropriate volume of diluted solution of TM to give the required concentration, and about 0.50 mL of 2 M NaOH to adjust the pH at 12.5 in this order. The stopwatch was started and the solution was directly diluted to the mark with distilled water. An aliquot of the reaction mixture was transferred to a thermostated cell at the required temperature. The cell was cleaned thoroughly with soap and distilled water. The reaction temperature was controlled by using a special water jacket which was attached to a water bath (Teche instrument, with heating and refrigeration bath and a digital temperature display); the water jacket surrounded the flask and the cells of the reaction. The temperature of the reaction mixture was recorded directly by a digital thermometer. The TM and TC concentrations were determined using calibration curve of TC which had been plotted between absorbance (A) and concentration. The slope of the straight line produced equals $8.42 \times 10^{-5}$ M/A. In order to calculate the maximum amount of TC at equilibrium the relationship between (A) and time (t) was followed by the sepectrophotometer, then the absorbance ($A_t$) at time (t) was drawn against $e^{-kt}$ (Figure 3) according to the Arrhenius equation:

$$A_t = A_\infty - (A_\infty - A_0)\, e^{-kt}$$

## RESULTS AND DISCUSSION

The initial concentrations after mixing are indicated as $[TM]_i$ in mol/litre. The reactant Hg(II) was introduced to the reaction mixture before adding NaOH solution, to ensure that no side reactions are taking place, and that the only formed product is TC. A suitable concentration of Hg(II) equal to $2.0 \times 10^{-3}$ M with pH = 12.5 was chosen to prevent the formation of any precipitate during measurements, like $Hg(OH)_2$ and hydroxo complexes. The reaction rate was measured for a wide range of temperatures (from 277.7 to 320.5 K). The absorption spectra of each of the solutions, TM, Hg(II) and TC, were recorded in the range of 200 to 400 nm, to ensure that the only compound which has an absorption at $\lambda_{max} = 350$ nm is TC.

The rate of formation of TC was followed for the first twenty seconds; the extent of reaction during this period of time does not exceed 5% of the original concentration. The initial rate for the reaction was calculated from the slope of the straight line drawn between absorbance and the time for the first 25 seconds of reaction time (average of three trials) according to the first order rate equation:

$$\text{Rate} = -d[TM]/dt = k[TM]_i$$

The value of the rate constant (k), at a given TM concentration, for the pseudo-first order reaction has been calculated (Table-1), which also includes the average value ($k_{av}$) at each temperature. Upon the application of Arrhenius equation (Fig. 1) using $k_{av}$ at various temperatures, the least mean square Arrhenius equation becomes

$$\log k/s^{-1} = 5.23 \pm 0.18 + 44.30 \pm 0.99 \text{ kJ mol}^{-1}/RT \ln 10$$

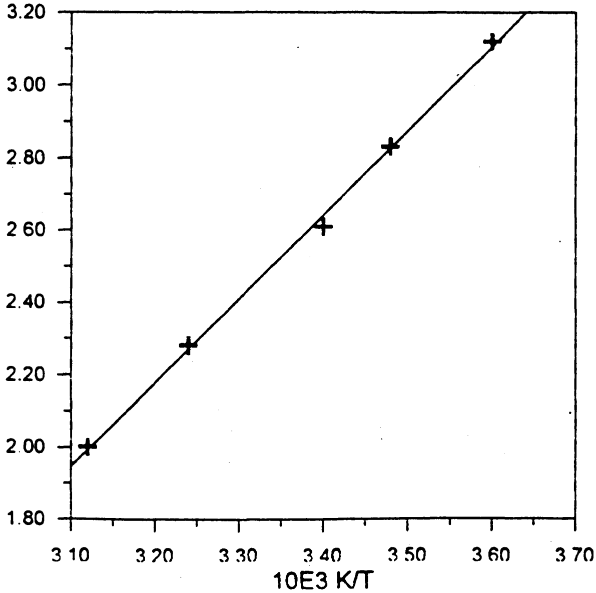

Fig. 1. Arrhenius Plot.

The values of Arrhenius parameters for the oxidation of thiamine under these conditions are determined for the first time. Table-1 clearly demonstrates the continuous increase in the values of the rate constant at 296 K, which as reported by Ryan[2] is $3.64 \times 10^{-2}$ $s^{-1}$, while in other studies[14, 15] the value of the rate constant calculated from the half-life measurements at the same temperature is $2.00 \times 10^{-3}$ $s^{-1}$. In this study the experimental value of the rate constant (k) at 294.8 K equals to $2.46 \times 10^{-3}$ $s^{-1}$, which shows very good agreement with the average of the two calculated rate constants.

The chosen concentrations and the measured corresponding initial rates are listed in Table-1 and Table-2. The slopes of the striaght lines in Fig. 2 represent the order of the reaction with respect to thiamine at each temperature. The average of these orders is equal to 1.02 ± 0.04, which is in excellent agreement with the value obtained by Ryan[2]. From Fig. 3 and the values of $[TM]_i$ ($0.7 \times 10^{-5}$ M and $1.4 \times 10^{-5}$ M), the maximum calculated values of TC at equilibrium recorded with respect to $[TM]_i$ equal 81.7% and 76.4% at 297.0 K, and equal to 79.4% and 68.0% at 308.5 K. These values and the effect of increasing temperatures on the percentage of TC produced at equilibrium are consistent with the values obtained from the study of Rogers *et al.*[16]

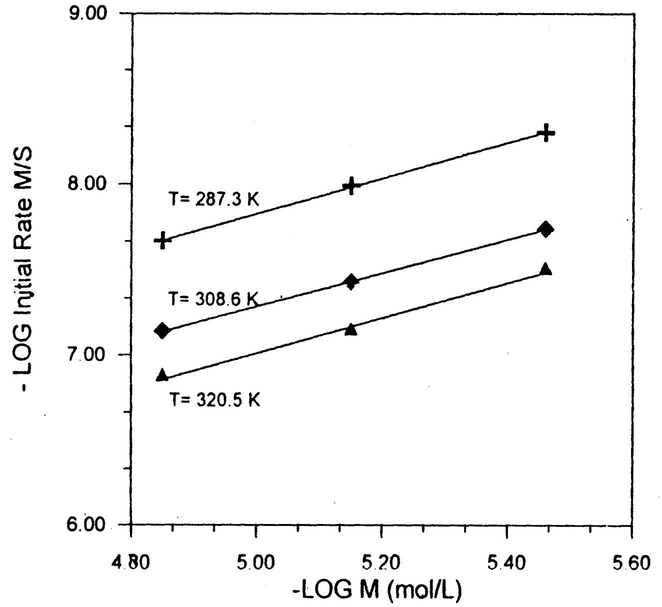

Fig. 2. Log-Log plot of initial rates vs. intial concentration.

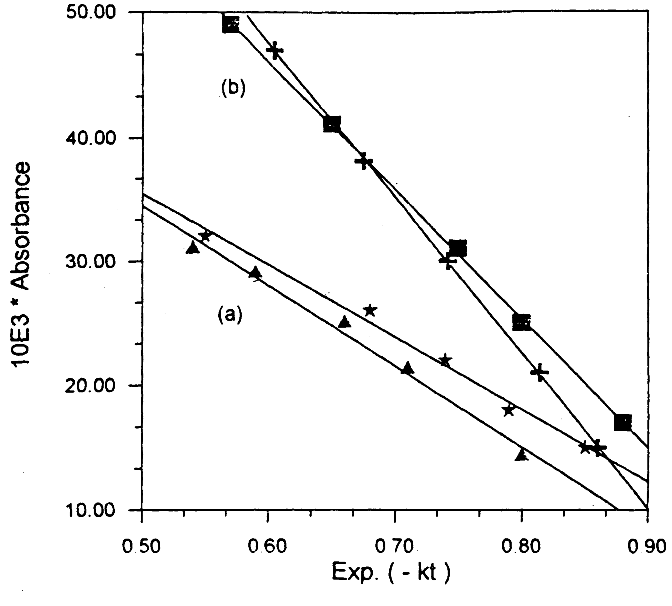

Fig. 3. Plot of Exp. (–kt) versus absorbance (A) from arrhenius equation, TM initial conc. (a) = 0.70 × 0E-5 M, (b) = 1.4 × 10E-5 M. cross and triangle lines at 297 K, square and star lines at 308.6 K

TABLE-1
PSEUDO-FIRST-ORDER RATE CONSTANTS FOR OXIDATION OF THIAMINE

| $[TM]_i$ mol/L Rate const. ($s^{-1}$) Temp./K | $0.35 \times 10^{-5}$ $k \times 10^3$ | $0.70 \times 10^{-5}$ $k \times 10^3$ | $1.40 \times 10^{-5}$ $k \times 10^3$ | $k_{av.} \times 10^3$ |
|---|---|---|---|---|
| 277.7 | – | 0.73 | 0.79 | 0.76 ± 0.03 |
| 287.3 | 1.49 | 1.46 | 1.52 | 1.49 ± 0.03 |
| 294.8 | 2.45 | 2.42 | 2.52 | 2.46 ± 0.05 |
| 308.6 | 5.26 | 5.31 | 5.17 | 5.25 ± 0.07 |
| 320.5 | 9.31 | 10.70 | 10.12 | 10.04 ± 0.30 |

TABLE-2
INITIAL RATES AT TEMPERATURES AND CONCENTRATIONS USED

| $[TM]_i$ mol/L Initial rate ($MS^{-1}$) Temp. K | $0.35 \times 10^{-5}$ Rate $\times 10^9$ | $0.70 \times 10^{-5}$ Rate $\times 10^9$ | $1.40 \times 10^{-5}$ Rate $\times 10^9$ |
|---|---|---|---|
| 287.3 | 5.21 | 10.22 | 21.3 |
| 308.6 | 18.4 | 37.2 | 72.4 |
| 320.5 | 32.6 | 75.0 | 141.7 |

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