Kinetics of the Oxidation of Thiamine by Hg(II) in Basic Medium

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Summary:The rate of oxidation of thiamine (Vitamin B_1) by Hg(II) ion in basic medium (pH =12.2) has been studied. The concentration of [OH⁻]and Hg(II) were kept constant by using 0.20M phosphate buffer and and 2.0×10^{-3}M Hg(II). The initial rate of formation of the only product of thiochrome was followed spectrophotometrically at λ_{max} = 350 nm for the first 2-3 minutes. The reaction has been studied in the temperature range of 279 K to 314.4 K. The reaction is pseudo-first order with respect to thiamine .Different kinetics and thermodynamics parameters such as rate costants, activation energy, enthalpy of activation, entropy of activation and Gibbs energy of activation of the reaction were reported.Various concentrations of thiamine were successfully found by measuring there initial rates in different synthetic mixtures containing other foreign species.

Introduction

The oxidation of thiamine(TA) by various oxidizing agents, and its conversion to thiochrome (TC)was the subject of several studies[1-9]. These studies demonstrated the formation of side products which greatly affects the amount of TC produced, showed that the amount of TC depends on kind of the oxidizing agent and the value of pH and described several methods for the determination of TA such as fluorometric, photochemical, spectrofluorimetric, flow-injection, continuous flow,chemiluminescence and differential kinetics fluorometric method. U.S.P [10] has considered the oxidation of TA to TC as a standared method for the assay of TA .Qiu-ying *et al* [11] were used an enzyme(FeTSPc) for the oxidation of TA by hydrogen peroxide in a spectrofluorimetric method.

Not enough kinetic and thermodynamic information is available in the literature related to TA-TC system.The aim of this work is (a) to study the reaction of TA-TC kineticaly at a wide range of temperatures in order to report its various kinetic and thermodynamic parameters and (b) to to apply the simple kinetic method for quantitative measurement of TA.

Results and Discussion

The reported TA concentrations, $[TA]_i$, were those in reaction cell after mixing. The reactant Hg(II) was introduced to the reaction before adding the buffer solution, to insure that no side reaction are taking place, and that the only formed product is TC .

A suitable concentration of [Hg(II)] equal to 2.0×10^{-3} M with pH = 12.2, has been chosen to prevent the formation of any precipitate during

measurements like $Hg(OH)_2$ and hydroxo complexes. TA aqueous solution above pH 11 react immediately to form tricyclic intermediates(CI) which can be oxidized to TC, and also converted to yellow thiol (YT) which is finally oxidized to thiamine disulfide (TDS) [1] .At pH 12.2 the concentration of YT initialy formed is inversely related to the concentration of Hg(II) [12].The ratio of TC to TDS is affected by pH [6], solvent [7], and oxidizing agent [3]. At the optimum conditions selected, pH 12.2,0.20 M phosphate buffer and 2.0×10^{-3} M of Hg(II), the only product formed is TC." Fig.(1) show just the main reactions of TA to TC through CI" . The reaction rate was measured in a range of temperatures from 279 K to 414.4 K. The absorption spectra of each solution of TA and TC has been recorded in the range of 200 to 400 nm, to insure that the only compound which has an absorbance at λ_{max} = 350 nm is TC.

Kinetics

The rate of formation of TC was followed up to 2-3 minutes after first twenty seconds needed for complete mixing of the reactants. The initial rate (A/sec.) for each experiment was obtained by measuring the slope of the tangent at zero time for the experimental absorbance - time curve, then the value of the slope of the calibration curve was used to convert the unit of the initial rate to M/sec. The following differential rate equation was used to calculate the rate constants :

$$\text{initial rate} = -d[TA]/dt = d[TC]/dt = k[TA]_i$$

At the optimum conditions mentioned above, the reaction is pseudo first order in thiamine. The
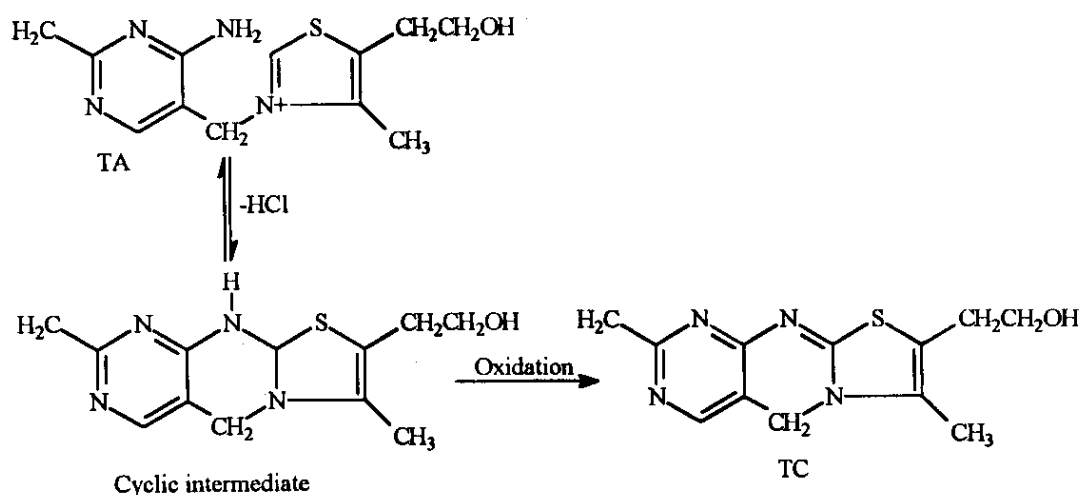
Fig. 1: Reaction pathway of thiamine hydrochloride in alkaline medium

value of the rate constant (k), at a given initial TA concentration for the pseudo-first order reaction has been calculated and presented in Table 1 which also includes the average value (k_{av}) at each temperature. From Arrhenius equation "Fig. (2)" and a suitable computer program using relation lnk/T versis $1/T$, different kinetic and thermodynamic parameters were determined for the reaction as follow: activation energy (E_a) = 44.15±3.5 kJ/mole, enthalpy of activation ($\Delta H^\#$) = 41.69±0.98 kJ/mole, entropy of activation ($\Delta S^\#$) = -151.88±3.35 J/mol K and Gibbs energy of activation ($\Delta G^\#$) = 86.41±1.96 kJ/mole. The calculated value of the pseudo-first order rate constant at 296K according to Ryan [2] was 3.64×10^{-3} s^{-1} while in other studies [2,13,14] the value of the rate constant calculated from the half-life measurements at the same temperature was 2.00×10^{-3} s^{-1}. In this study, the experimental value of the pseudo-first order rate constant (k) at 294.8 K equals to 2.47×10^{-3} S^{-1}, which shows very good agreement with the average value of the two calculated rate constants.

Table-1:Pseudo   first-order   rate   constants   for oxidation of thiamine,TA.

[TA]ᵢ mol/L
Rate Const. sec.⁻¹

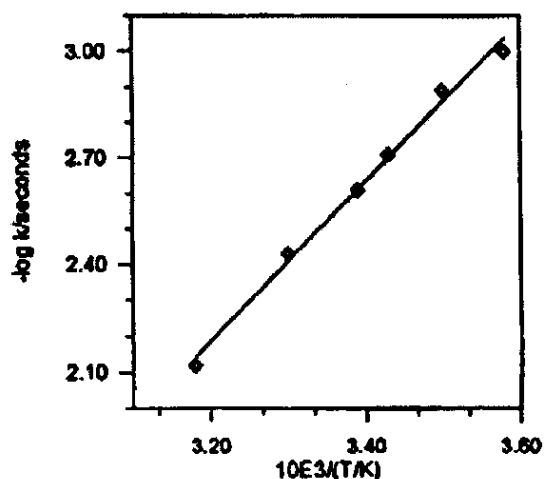| Temp./K | (3.33×10⁻⁶) | (6.66×10⁻⁶) | (1.33×10⁻⁵) | |
	k×10³	k×10³	k×10³	k_{av}×10³
279.1	—	0.86	0.9	0.88±0.04
285.5	1.34	1.31	1.25	1.30±0.04
291.1	—	1.81	—	1.81
294.8	2.47	2.40	2.53	2.47±0.06
303.1	3.59	3.69	3.78	3.69±0.10
314.4	7.42	7.54	7.52	7.49±0.06

Fig. 2:   Arrhenius plot

The values of the concentrations and the measured corresponding initial rates are listed in Table 1 and 2. The slopes of the straight lines in "Fig.(3)" represent the order of the reaction with respect to TA at each temperature,the average value calculated from the figure equal to 1.0 ± 0.04. The absorbance (A_t) at time(t) was drawn against e^{-kt} (see "Fig.(4)") according to Arrhenius equation :

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

From the intercept of this Figure and the values of initial concentration of TA (6.67×10^{-6} M and 1.33×10^{-5} M),the maximum calculated values of TC at equilibrium recorded with respect to initial

Table-2:Initial rate at temperatures and concentrations used .

[TA]; mol/L (3.33×10⁻⁶) Initial Rate×10⁹,M/sec.→ Temp./K	(3.33×10⁻⁶)	(6.66×10⁻⁶)	(1.33×10⁻⁵)
285.5	4.44	8.76	16.60
294.8	8.16	16.10	33.60
303.1	11.85	24.75	50.30
314.4	24.50	50.52	100.00

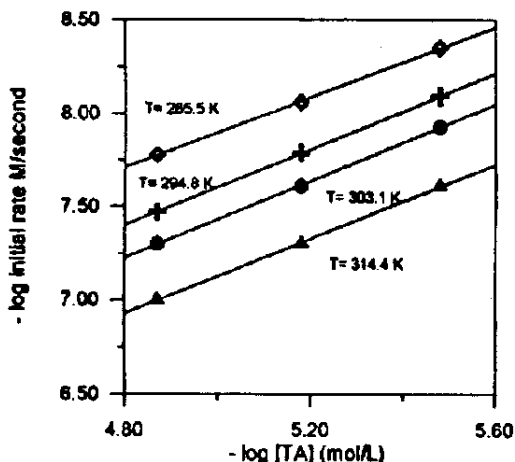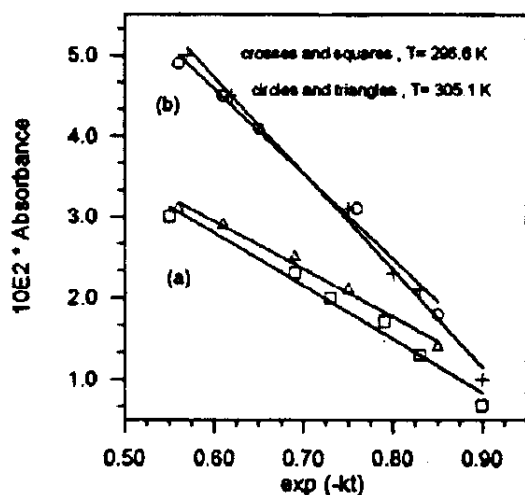
Fig. 3: log – log Plot of initial rate versis initial concentration of thiamine (TA)

Fig. 4: Plot of exp(-kt) versis absorbance, thiamine [TA] initial concentration: (a) = 6.67* 10E-6 M, (b) = 1.33*10E-5 M.

concentration of TA equal to 83.4 % and 74.7 % at 296 K , and equal to 73.4 % and 70.0% at 305 K The effect of increasing temperature on the percentage of TC produced at equilibrium are consistent with the values obtained from study [15].

Quantitative measurements

Under the reaction conditions, TA werel inear by running calibration standards from $1×10^{-6}$ to $2.0×10^{-5}$ M .The relative standard deviation was 1.9 % for $5.0×10^{-6}$ M TA (n=5)

The validity of using initial rates to determine the amount of TA, was evaluated by performing interference study . The effect of some coexisting compounds in pharmaceutical preparations was studied by analysing synthetic sample solutions containing different amounts of TA . Table 3 , shows the preparations and % recovery of TA ."Fig.(3)" was used to determine the amount TA at 294.8 K .The results showed no effect of foreign species that accompany TA in Pharmaceutical formulation, since no apreciable absorbance of any of those species at $\lambda_{max}=350$ nm, and none of them can react with Hg(II) under the reaction condition.

Table-3: Determination of thiamine (TA) in synthetic preparations from Fig.2 , at 303.1 K .

Sample	Composition	Concentration μg/ml	% recovery of thiamine#
1	Glucose	120.0	
	Riboflavin	1.1	
	Ascorbic Acid	21.0	
	Thiamine	1.0	103
2	Starch	saturated	
	Thiamine	1.1	92
3	Vitamin B₁₂	1.0	
	Ascorbic Acid	45.0	
	CuCl₂	30.0	
	MgCl₂	31.0	
	Thiamine	1.7	87
4	Vitamine B₁₂	0.9	
	Riboflavin	2.1	
	Starch	saturated	
	Thiamine	2.0	95

Average of five measurements

Experimental

Potassium monohydrogen phosphate (KHPh) and HgCl₂ , (analytical grade, BDH) .Thiamine (TA) (Sigma, purity 98%) and Hg(II) solutions were prepared at pH = 4.0 , while the buffer solution was prepared from 0.20M KHPh and the pH was adjusted to 12.2 by adding 5.0 M KOH solution .Thiochrome (TC) was obtained from Sigma (reagent grade). Stock solution of $2.0×10^{-4}$ M TC was stored indark at pH 9.0. A computerized Jasco, double beam UV/Vis is spectrophotometer with CRT, single monochromater with wavelength range of 200-1100 nm was used to follow the rate of production of TC and to record all absorption spectra .

The order and method of addition of the reagents into reaction cell were as follow: 1ml of

each TA solutions (1.0×10^{-5} M, 2.0×10^{-5} M and 4.0×10^{-5} M), 1ml of Hg(II) solution (2.0×10^{-3}M) both from auto-matic pipets,stirrer turned on,and 1ml of buffer solution from micro pipet holded over the reaction cell.The total volume of the reaction mixture was exactly 3 mL .The reaction cell was cleaned throughly with soap and distilled water then rinsed with aqueous HCl solution that has a pH of 4.0. The reaction temperature was controlled by using a suitable water jacket, which was attached to a water bath (Techne instrument ,with refrigeration and heating bath,and a digital temperature display), the water jacket surrounded the reaction and blank cells. The temperature of the reaction mixture was recorded directly by a digital thermometer, with an accuracy of $\pm$ 0.10 ^{0}C. The TA and TC concentrations were determined using a calibration curve of TC, which has been plotted between the absorbance (A) and standard concentrations of thiocrome ranged from 0.50×10^{-6} to 2.0×10^{-5}M , the relation was linear and has a slope equal to 8.42×10^{-5} M/A with correlation coefficient equal 0.979 .

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