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KINETICS OF OXIDATION OF VITAMIN D_3 USING CHLORAMINE-B IN ACIDIC BUFFER MEDIUM BY FLUROMETRIC METHOD

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ABSTRACT: This article deals with the novel of the kinetics of oxidation of Vitamin-D_3 by N-sodio-N-chlorobenzene sulfonamide or Chloramine-B (CAB) in pH-5.0 buffer has been investigated flurometrically at 303K. Studying effects of various parameters on oxidation of vitamins help us in understanding the metabolic functions. Since vitamins are important part of our body and diet as well. Results obtained indicate second order with respect to $[H^+]$ show that protonation of oxidizing species $RNHCl^-$ to give RNH_2Cl. Further it is observed that the fractional order on [Vitamin-D] and first order dependence on [CAB]. Addition of reaction products benzenesulfonamide or bromide ions and variation of ionic strength of the medium have no influence on the reaction rate. The reaction has been studied at different temperature and activation parameters have been calculated. A general mechanism constituent with the preceding kinetic data has been proposed and the rate law derived.

KEY WORDS: Vitamin-D_3, Chloramine-B (CAB), flurometrically, Reaction kinetics, Oxidation.

INTRODUCTION:

Vitamin D is a fat soluble vitamin. UV rays trigger Vitamin D_3 synthesis in the skin by converting-hydrocholesterol to Vitamin D_3, Vitamin D exists in several forms. Calciferol is the most active form of vitamin D. The major biological function of Vitamin D is to maintain normal blood levels of calcium and phosphorous (1-2). Kinetic investigations of Vitamin D meyer in the literature. The present paper reports the kinetics of oxidation of Vitamin D_3 by Chloramine – B in acidic buffer fluorometrically with a view to elucidating mechanism of oxidation of the vitamin D.

MATERIAL AND METHODS: EXPERIMENTAL

Chloramine–B was prepared by a standard procedure and its purity was checked iodometrically, Vitamin D_3 tablet was powered. This was dissolved in acidic buffer pH-S solution, ionic strength of the reaction mixture was kept at a high value using concentrated solution of $NaClO_4$.

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KINETIC MEASUREMENTS

Kinetic runs were performed under pseudo-first order conditions with large excess of the substrate with oxidant at303K. The course of reaction was followed by measuring intensity of fluorescence of reaction mixture with time using the fluorometer. The reaction was followed for two half lives. The pseudo-first order rate constant (k^1) calculated from these plots were reproducible with in ±3%.

STIOCHIOMETRY

Reaction mixtures containing different compositions of Vitamin D_3 and CAB in buffer solution were equilibrated at 35^0C for 24hours. The iodometric determination of unreacted CAB in the reaction mixture showed dat two molecules of vitamin D_3 consume one mole of chloramine –B.

$$C_6H_5SO_2NClNa + 2C_{27}H_{44}O \longrightarrow 2\ C_{27}H_{43}O + C_6H_5SO_2NH_2 + Na^+ + Cl^-$$

RESULTS

The reaction performed in the acidic buffer solution under pseudo first order condition of [Vit D_3] >< [CAB] give linear plot of log R{R is the intensity of fluorescence}Versus time. The linearity of these plots together with constancy of k^1 of various $[CAB]_0$ under some experimental conditions indicate first order with respect to [CAB]. Under the same experimental conditions an increase in [Vit D_3] increased the k^1 values. Plot of log k^1 versus log [Vit D_3] was linear with slope 0.6 indicating fractional order in [Vit D_3].

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Table 1:- Effect of varing reactant Concentration on the reaction rate

pH 5; Temparature = 308k ; μ=0.2mol dm^{-3}

[CAB] x10^{-3} mol dm^{-3}	[Vit D_3] x 10^{-3} mol dm^{-3}	k^1 x 10^3 s^{-1}
0.25	1.25	2.41
0.5	1.25	2.41
1.0	1.25	2.44
1.5	1.25	2.42
2.0	1.25	2.43
1.0	0.75	1.85
1.0	1.00	2.15
1.0	2.00	3.6
1.0	2.5	3.6
1.0	2.5	4.26
1.0	5.0	5.65

As the $[H^+]$ increases the rate also increases i.e, the decrease of buffer pH increases the rate of reaction. A plot of log k^1 versus log $[H^+]$ gives a straight line with the slope of 2.0 indicating that the rate of oxidation vitamin D_3 is second order with respect to $[H^+]$.

Table 2; - Effect of pH on the rate of reaction

[CAB] = 1.0 x 10^{-4} mol dm^{-3} ; [Vit D_3] = 1.25 x 10^{-3} mol dm^{-3} ; μ=0.2mol dm^{-3} ;T=308K

pH	K^1 x 10^3 s^{-1}
4	3.1
4.5	2.70
5.0	2.41
5.5	2.15
6.0	2.00

Addition of reaction product benzene sulphonamide has no effect on the reaction rate. Addition of Cl ion, variation ionic strength of the medium and variation of dielectric constant has no effect on the rate of the reaction

Addition of reaction mixture to aqueous acrylamide did not indicate the polymerization showing the absence of free radical species.

The reaction was studied at varying temperature 303K to 318K from the linear plots the activation parameter were computed.

Temp. in K	k^l x 10^3 s^{-1}	Thermodynamic Parameters
303	2.11	$\Delta H^{\neq}$ = 30.18 KJ mol^{-1}
308	2.61	$\Delta S^{\neq}$ = 299.46 JK^{-1} mol^{-1}
313	3.15	$\Delta G^{\neq}$= 93.76 KJ mol^{-1}
318	3.70	E_a = 32.82 KJ mol^{-1}
323	4.29	

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DISCUSSION AND MECHANISM

Pryde and Soper, Morris et. al. and Bishop and jenning have shown the existence of similar equilibria in acid and alkaline solution of N-Metallo–N haloarylsulphanamides. Chloramine–B, being a analogous to CAT, behaves as a strong electrolyte in aqueous solutions forming different species as shown in equation 4.1 to 4.5.

$$PhSO_2NClNa \rightleftharpoons PhSO_2NCl^- + Na^+ \quad \text{--------4.1}$$

$$PhSO_2NCl^- + H^+ \rightleftharpoons PhSO_2NHCl \quad \text{------4.2}$$

$$PhSO_2NCHCl + H_2O \rightleftharpoons PhSO_2NH_2 + HOCl \quad \text{----4.3}$$

$$2PhSO_2NHCl \rightleftharpoons PhSO_2NH_2 + PhSO_2NCl_2 \quad \text{----4.4}$$

$$HOCl + H^+ \rightleftharpoons H_2OCl^+ \quad \text{------4.5}$$

In acid solutions, the probable oxidizing species are the free acid $PhSO_2NHCl$, $PhSO_2NCl^-$, HOCl, H_2OCl^+. The involvement of $PhSO_2NCl_2$ in the mechanism leads to a second order rate dependence on [CAB] according to equation 4.4, which is contrary to the experimental observation. As equation 4.3 indicates a slow hydrolysis, HOCl where the primary oxidizing species of the first order, a retardation of rate by the added $PhSO_2NH_2$ would br expected. However no such effect was noticed in the study. Narayanan and Rao and Subhasini et. al. have reported that monohaloamines can be further protonated at pH<2, is in the equation 4.6 and 4.7 for CAT and CAB respectively,

$$p\text{-}CH_3C_6H_4SO_2NHCl + H^+ \rightleftharpoons p\text{-}CH_3C_6H_4SO_2N^+H_2Cl \quad \text{----4.6}$$

$$C_6H_4SO_2NHCl + H^+ \rightleftharpoons C_6H_4SO_2N^+H_2Cl \quad \text{------4.7}$$

Therefore in higher acidic conditions, for CAB, $PhSO_2NHCl$ is expected as follows

$$C_6H_4SO_2NHCl + H^+ \rightleftharpoons C_6H_4SO_2N^+H_2Cl$$

In the present case a second order in $[H^+]$ suggest that protonation of $PhSO_2NCl^-$ With two H^+ result in the generation of $PhSO_2N^+H_2Cl$, oxidizing species involved in the mechanism of oxidation of Vit D_3 by CAB. Further the fraction order in Vit D_3 and first order with respect to CAB is observed in the experiment. In the view of these facts, the kinetic mechanism proposed for the reaction is as follows;

$$RNCl^- + 2H^+ \overset{K=k_1/k_{-1}}{\rightleftharpoons} RN^+H_2Cl \quad \text{.....1. Fast}$$

$$RN^+H_2Cl + Vit \xrightarrow{k_2} X \quad \text{..... 2. Low}$$

$$X + H_2O \xrightarrow{k_3} Product \quad \text{.....3. Fast}$$

Kinetic scheme- 1

Above scheme leads to Rate law

$$k_2\, k_{-1}[CAB][H^+]^2[Vit\ D_3]$$

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$$\text{Rate} = \frac{}{k_1 - k_2[\text{Vit } D_3]}$$

Therefore the rate law is in good agreement with the experimental results showing first order w.r.t (C.A.B), second order w.r.t $[H^+]$ and fractional order w.r.t [Vit D_3]. The thermodynamic parameters $\Delta H^{\#}$, $\Delta S^{\#}$ and $\Delta G^{\#}$ has been calculated as shown in the table 3.

CONCLUSION

In recent year Vitamin D has gained increased respect and attention. Its biological role is now known to extend behind regulation of bone mineralization and serum calcium level. Vitamin D receptors have been found in a variety of cell. It is believed that clacitriol plays an important part in the regulation of genes involved in the cell growth, differentiation and proliferation. By promoting differentiation and inhibiting proliferation, it may become an important factor in cancer prevention and therapy. Another role calcitriol involves regulation of genes which controls the production of immune factors knows as lymphokines, which effects cell mediated immunity functions.

Oxidation is an important process which goes in the biological body. Oxidation is the main process by which energy is produced. All the metabolic function which go on in the body include oxidation and reduction process. The main processes which are worthy of mentioning are electron transports chain, citric acid cycle and reactions going on it the plant.

Studying effects of various parameters on oxidation of vitamins helps us in understanding the metabolic functions. Since vitamins are important part of our body and diet as well. Results obtained indicate second order w.r.t $[H^+]$ show that protonation of oxidizing species RNHCI to give RNH_2. Further, it's observed that the fractional order w.r.t [Vit D_3] and first order w.r.t [oxidant]. The kinetics of oxidation of vitamins with CAB is not studied but we studied the kinetics by fluorometric method which is one of the easiest methods to study the effects of different parameters on the oxidation if vitamins.

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