

KINETICS OF OXIDATION OF VITAMIN D₃ 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-D₃ 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 RNH₂Cl. 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-D₃, Chloramine-B (CAB), flurometrically, Reaction kinetics, Oxidation.

INTRODUCTION:

Vitamin D is a fat soluble vitamin. UV rays trigger Vitamin D₃ synthesis in the skin by converting-hydrocholesterol to Vitamin D₃, 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 D₃ by Chloramine – B in acidic buffer fluorometrically with a view to elucidating mechanism of oxidation of the vitamin D.

MATERIAL AND METHODS: EXPERIMENTAL

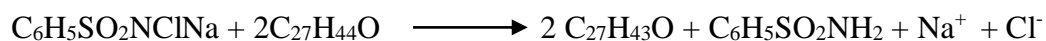
Chloramine-B was prepared by a standard procedure and its purity was checked iodometrically, Vitamin D₃ tablet was powered. This was dissolved in acidic buffer pH-S solution, ionic strength of the reaction mixture was kept at a high value using concentrated solution of NaClO₄.

KINETIC MEASUREMENTS

Kinetic runs were performed under pseudo-first order conditions with large excess of the substrate with oxidant at 303K. 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 $\pm 3\%$.

STIOCHIOMETRY

Reaction mixtures containing different compositions of Vitamin D₃ and CAB in buffer solution were equilibrated at 35°C for 24 hours. The iodometric determination of unreacted CAB in the reaction mixture showed that two molecules of vitamin D₃ consume one mole of chloramine -B.



RESULTS

The reaction performed in the acidic buffer solution under pseudo first order condition of $[\text{Vit D}_3] \gg [\text{CAB}]$ 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 $[\text{CAB}]_0$ under some experimental conditions indicate first order with respect to $[\text{CAB}]$. Under the same experimental conditions an increase in $[\text{Vit D}_3]$ increased the k^1 values. Plot of $\log k^1$ versus $\log [\text{Vit D}_3]$ was linear with slope 0.6 indicating fractional order in $[\text{Vit D}_3]$.

Table 1:- Effect of varying reactant Concentration on the reaction ratepH 5; Temperature = 308k ; $\mu=0.2\text{mol dm}^{-3}$

[CAB] $\times 10^{-3} \text{ mol dm}^{-3}$	[Vit D ₃] $\times 10^{-3} \text{ mol dm}^{-3}$	$k^1 \times 10^3 \text{ s}^{-1}$
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 $[\text{H}^+]$ increases the rate also increases i.e, the decrease of buffer pH increases the rate of reaction. A plot of $\log k^1$ versus $\log [\text{H}^+]$ gives a straight line with the slope of 2.0 indicating that the rate of oxidation vitamin D₃ is second order with respect to $[\text{H}^+]$.

Table 2; - Effect of pH on the rate of reaction[CAB] = $1.0 \times 10^{-4} \text{ mol dm}^{-3}$; [Vit D₃] = $1.25 \times 10^{-3} \text{ mol dm}^{-3}$; $\mu=0.2\text{mol dm}^{-3}$; T=308K

pH	$K^1 \times 10^3 \text{ s}^{-1}$
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

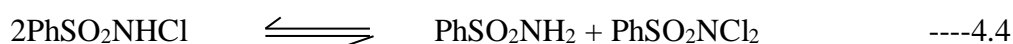
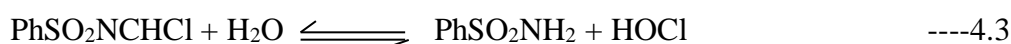
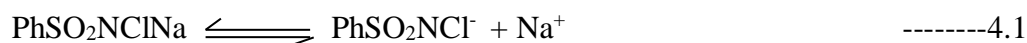
Addition of reaction mixture to aqueous acrylamide did not indicate the polymerization showing the absence of free radical species.

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

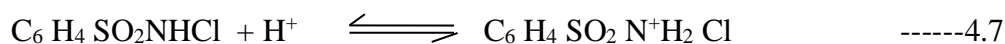
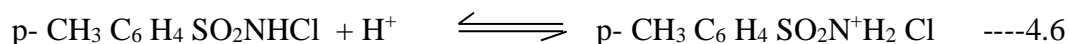
Temp. in K	$k^1 \times 10^3 \text{ s}^{-1}$	Thermodynamic Parameters
303	2.11	$\Delta H^\ddagger = 30.18 \text{ KJ mol}^{-1}$
308	2.61	$\Delta S^\ddagger = 299.46 \text{ JK}^{-1} \text{ mol}^{-1}$
313	3.15	$\Delta G^\ddagger = 93.76 \text{ KJ mol}^{-1}$
318	3.70	$E_a = 32.82 \text{ KJ mol}^{-1}$
323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

DISCUSSION AND MECHANISM

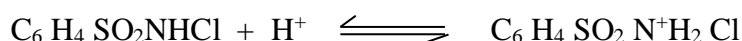
Pryde and Soper, Morris et. al. and Bishop and jening have shown the existence of similar equilibria in acid and alkaline solution of N-Metallo–N haloarylsulphanamides. Chloramine–B, being a analogous to CAT, behaves as a strong electrolyte in aqueous solutions forming different species as shown in equation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



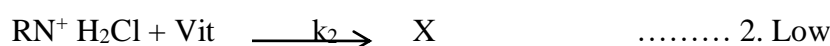
In acid solutions, the probable oxidizing species are the free acid PhSO_2NHCl , $\text{PhSO}_2\text{NCl}^-$, HOCl , H_2OCl^+ . The involvement of $\text{PhSO}_2\text{NCl}_2$ in the mechanism leads to a second order rate dependence on $[\text{CAB}]$ according to equation 4.4, which is contrary to the 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 be 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 $\text{pH} < 2$, is in the equation 4.6 and 4.7 for CAT and CAB respectively,



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



In the present case a second order in $[\text{H}^+]$ suggest that protonation of $\text{PhSO}_2\text{NCl}^-$ With two H^+ result in the generation of $\text{PhSO}_2\text{N}^+\text{H}_2\text{Cl}$, 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;



Kinetic scheme- 1

Above scheme leads to Rate law

$$k_2 k_{-1} [\text{CAB}] [\text{H}^+]^2 [\text{Vit D}_3]$$

$$\text{Rate} = \frac{\quad}{k_1 - k_2[\text{Vit D}_3]}$$

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 $[\text{H}^+]$ and fractional order w.r.t $[\text{Vit D}_3]$. The thermodynamic parameters ΔH^\ddagger , ΔS^\ddagger and ΔG^\ddagger 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 calcitriol 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 known 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 $[\text{H}^+]$ show that protonation of oxidizing species RNHCl to give RNH_2 . Further, it's observed that the fractional order w.r.t $[\text{Vit D}_3]$ 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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