

KINETICS OF HYDROLYSIS OF DI-2-METHYL-5-NITROANILINE PHOSPHATE CATALYZED BY HYDROCHLORIC ACID

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The kinetics of acid catalyzed hydrolysis of di-2-methyl-5-nitroaniline phosphate was studied in 0.1 to 7.0 mol dm⁻³ hydrochloric acid at 70 ± 0.5°C in 30/70 (v/v) dioxane-water mixture. The rate of reaction increases with increase in acid molarity up to 5.0 mol dm⁻³ which was largely contributed by the conjugate acid species. Rate data at constant ionic strength was used to identify the reactive species and to determine theoretical rates. Theoretical rates, estimated from second empirical term of Debye-Huckel equation, were found in close agreement with experimental rates. Hydrolysis of diester via conjugate acid species was assigned the bimolecular behaviour of the reaction on the basis of Arrhenius parameters, Zucker-Hammett hypothesis, Bunnett and Bunnett Olsen parameters. Solvent effect on hydrolytic reaction was found to indicate the formation of a transition state in which charge dispersion occurs.

INTRODUCTION

Phosphate esters play an important role in the physiology of cells. Most prominent is the participation of phosphate esters as a structural and functional part in DNA, RNA, and its monomeric building blocks, the occurrence as a post-translational signal in proteins and as a head group in phospholipids [1]. Phosphate is an indispensable nutrient that is usually taken up by plant cells and stored intracellularly [2] which helps in photosynthesis, energy transformations and activity of some hormones in both plants and animals [3]. These esters have found numerous industrial applications due to their overall stability, especially in the presence of oxygen [4-5]. They are artificially synthesized for their practical uses as lubricants [4], plasticizers [6], fertilizers [7], fire retardants [8] and smoke generation [9]. They are also used in human medicines and in various public hygiene products for use, both by professional operators and general public [10]. Other significant applications are as environmentally degradable pesticides due to their toxicity to a wide range of insects and their relatively short residence time in the environment [4, 11]. They are found throughout the environment and in close contact with humans. The facile hydrolysis of these esters is found everywhere on account of their great values in various chemical and biochemical reactions [12-14].

Phosphoryl and thiophosphoryl transfer reactions are of prime importance where transfer of a phosphoryl group between ATP and ADP is the fundamental mechanism for energy transfer that allows the processes of synthesis, active transport, muscle action and nerve function in biological systems [15]. There is a growing interest of investigators in the mechanism of biochemical processes, in which individual phosphate esters play important

roles. Kinetics of the reaction of simple organic phosphates provides an insight into more complicated reactions during their metabolism. The determination of mechanism of hydrolysis of organic phosphate is expected to reveal possible correlation between the reaction path of chemical and enzymatic hydrolysis of biologically important phosphate esters [16]. The kinetic study, deals all the important aspects of chemical reaction including mechanism. Various reviews regarding the kinetics and mechanisms of hydrolysis of phosphate esters in neutral, alkaline and acidic media have been reported time to time [17-21]. In present investigation attempt was made to study the kinetics of acid catalyzed hydrolysis of di-2-methyl-5-nitroaniline phosphate in dioxane-water medium.

EXPERIMENTAL

Di-2-methyl-5-nitroaniline phosphate was prepared by literature method [22] in our laboratory, which involves the reaction of 2-methyl-5-nitroaniline with phosphorylating agent phosphorus oxychloride in 2 : 1 mol ratio in solvent benzene. All chemicals used were of AR grade. All the solutions were prepared in triple distilled water.

Hydrolytic reactions of di-2-methyl-5-nitroaniline phosphate were performed at $70 \pm 0.5^\circ\text{C}$ by employing $5.0 \times 10^{-4} \text{ mol dm}^{-3}$ solution in 30/70 (v/v) dioxane-water medium. The progress of kinetics of hydrolytic reactions was studied spectrophotometrically by Allens modified method [23] at 735nm. The method involves the measurement of inorganic phosphate formed from the ester, during the course of its hydrolysis.

RESULTS AND DISCUSSION

Hydrolysis of di-2-methyl-5-nitroaniline phosphate was carried out in acid region from 0.1 to 7.0 mol dm^{-3} hydrochloric acid at $70 \pm 0.5^\circ\text{C}$. Pseudo first order rate constants were obtained at different acid molarities are summarized in Table 2 and shown in Fig. 1.

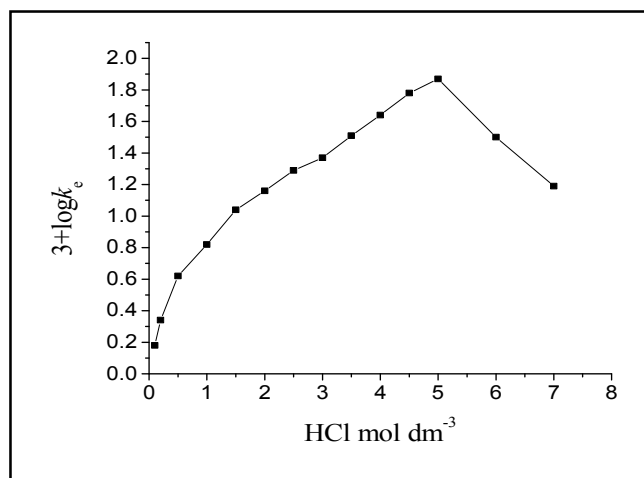


Fig. 1. Log rate constant Vs acid molarity for the acid catalyzed hydrolysis of di-2-methyl-5-nitroaniline phosphate

It is clear from the results that the rate of reaction increases with increase in acid molarity up to 5.0 mol dm^{-3} hydrochloric acid. Further rise in acid molarity decreases the rate of

reaction. The maximum rate at 5.0 mol dm^{-3} hydrochloric acid was attributed to complete conversion of substrate into its conjugate acid species as described in Scheme I. The decrease in rate after 5.0 mol dm^{-3} hydrochloric acid was attributed to lowering in concentration of attacking nucleophile (water) taking part in the reaction, *i.e.*, due to variations in water activity.

In order to determine whether or not there is effect of ionic strength or acid catalysis, a series of kinetic runs were made at six different ionic strengths from 0.5 to 3.0μ , using an appropriate mixture of sodium chloride and hydrochloric acid. The plot between rate constants and acid molarity at different ionic strength is shown in Fig. 2. Six linear curves are obtained and each represents the hydrolysis at that ionic strength. Since each linear curve makes the positive slope with the acid axis, hydrolysis is subjected to acid-catalyzed at that ionic strength. The slope of lines increases with increase in ionic strength. Hence, acid-catalyzed hydrolysis is subjected to the positive salt effect. The linear curves make intercepts on the rate axis, indicating the presence of a side reaction of the neutral species.

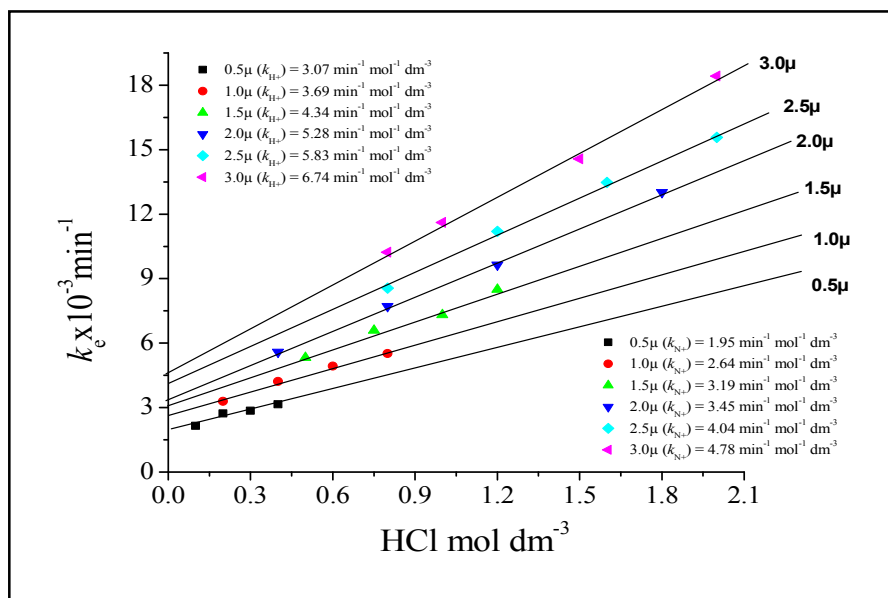


Fig. 2. Rate constant Vs acid molarity for acid catalyzed hydrolysis of di-2-methyl-5-nitroaniline phosphate at constant ionic strength

Six linear plots show the acid-catalyzed hydrolysis of di-2-methyl-5-nitroaniline phosphate at different ionic strengths by the following rate equation:

$$k_e = k_{H^+} C_{H^+} \quad \dots (1)$$

where, k_e , k_{H^+} and C_{H^+} are experimental rate constant, specific acid catalyzed rate at that ionic strength, and concentration of H^+ ion respectively. The accelerating ionic effect indicates that di-2-methyl-5-nitroaniline phosphate undergoes hydrolysis with positive effect of ionic strength. The corresponding slope (Specific acid catalyzed rate) and intercept values (neutral rate) at constant ionic strength are shown in Table 1. The specific acid catalyzed rates with their logarithmic value at that ionic strength are illustrated in Fig. 3.

The slope of lines represents a constant b'_{H^+} , where $b' = b'/2.303$ and the intercepts on the log rate axis represent the specific acid-catalyzed rates ($\log k_{H^+}$). Linearity of plot proves the validity of an empirical equation of Debye-Huckel theory [24] and can be represented as

				Estd.	Expt.		
0.1	0.28	2.40	-	2.32	1.51	0.36	0.18
0.2	0.58	2.09	-	2.67	2.21	0.43	0.34
0.5	1.58	2.29	-	3.87	4.15	0.59	0.62
1.0	3.72	2.63	-	6.35	6.68	0.80	0.82
1.5	6.61	3.02	-	9.63	10.99	0.98	1.04
2.0	10.23	3.47	-	13.70	14.31	1.14	1.16
2.5	15.14	3.98	-	19.12	19.33	1.28	1.29
3.0	21.38	4.57	-	25.95	23.72	1.41	1.38
3.5	28.84	5.25	-	34.09	32.66	1.53	1.51
4.0	38.90	6.03	-	44.09	43.46	1.65	1.64
4.5	51.29	6.92	-	58.21	60.43	1.76	1.78
5.0	67.61	7.94	-	75.55	73.58	1.88	1.87
6.0	26.30	2.45	(0.21) ³	28.75	31.37	1.46	1.50
7.0	13.80	1.05	(0.28) ⁴	14.85	15.34	1.72	1.19

Equation (7) was used to calculate the theoretically estimated rates which are then compared with the experimental rates. Table 4 summarizes both the observed and theoretical rates of hydrolysis. There is a good agreement between the theoretically estimated and experimental rates up to 5.0 mol dm⁻³ hydrochloric acid. However, there is disagreement in rate of 6 and 7 mol dm⁻³ hydrochloric acid may be attributed to lowering in the concentration and participation of water molecules. The rate beyond 5.0 mol dm⁻³ HCl was calculated employing the Bronsted-Bjerrum equation [25].

$$k_e = k_{H^+} C_{H^+} (a_{H_2O})^n + k_N (a_{H_2O})^n \quad \dots (8)$$

$$k_{H^+} C_{H^+} = k_{HO^+} C_{H^+} \exp. b'_{H^+} \mu (a_{H_2O})^n \quad \dots (9)$$

$$\log k_{H^+} C_{H^+} = \log k_{HO^+} + \log C_{H^+} = b'_{H^+} \mu + n \log (a_{H_2O}) \quad \dots (10)$$

The neutral rate at higher concentration is as follows :

$$k_N = k_{N_0} \exp b'_{N_0} \cdot \mu \cdot (a_{H_2O})^n \quad \dots (11)$$

$$\log k_N = \log k_{N_0} + b'_{N_0} \cdot \mu \cdot n \log (a_{H_2O}) \quad \dots (12)$$

where n is an integer and a_{H_2O} is water activity. The revised estimated rates agree well with the experimentally observed rates. It is clear from the results that the hydrolysis of di-2-methyl-5-nitroaniline phosphate in acid solution occurs via both conjugate acid and neutral species and their rates are subjected to positive effect of ionic strength or water activity.

In the region from 0.1 to 5.0 mol dm⁻³ the rate law is calculated and shown in Equation (13)

$$k_e = 2.69 \times 10^{-3} \cdot C_{H^+} \exp. (0.14 \times 2.303) \cdot \mu + 2.00 \times 10^{-3} \cdot \exp. (0.12 \times 2.303) \cdot \mu \dots (13)$$

The rate laws beyond 5.0 mol dm⁻³ HCl were calculated and shown in Equation (14)

$$k_e = 2.69 \times 10^{-3} \cdot C_{H^+} \cdot \exp. (0.14 \times 2.303) \cdot \mu \cdot (a_{H_2O})^n$$

$$+ (2.00 \times 2.303) \cdot \exp. (0.12 \times 2.303) \cdot \mu \cdot (a_{\text{H}_2\text{O}})^n \dots (14)$$

Molecularity of reaction

Molecularity and kinetic order of a solvolytic reaction may not be the same. Molecularity of hydrolysis of di-2-methyl-5-nitroaniline phosphate was determined by Zucker-Hammett hypothesis, Bunnett and Bunnett Olsen parameters [26-27]. Zucker-Hammett hypothesis consists of two parts, first part deals the linear plot of log rate constants against acidity function ($-H_0$). The slope value 0.67 (Fig. not shown) which is far from unity indicates the absence of unimolecular reaction, means the reaction is bimolecular. The second part of hypothesis deals with the plot between log rate constant and log acid molarity. The slope of plot is 1.22 (figure not shown) clearly shows the bimolecular nature of the reaction.

Bunnett suggested two parameters ω , ω^* . Former is the slope of plot between log rate constant + H_0 and log activity of water ($-\log a_{\text{H}_2\text{O}}$). The slope value ω is 6.52, which is greater than 3.3, so the reaction is bimolecular in nature (fig. not shown) [28]. Another is the slope of plot between log rate constant $-\log C_{\text{H}^+}$ and log activity of water ($-\log a_{\text{H}_2\text{O}}$). The slope value ω^* is 2.03 (fig not shown). The small curvature and positive slope (2.03) also supports the bimolecular reaction path of hydrolysis. New parameter ϕ suggested by Bunnett and Olsen [29] which is the slope of plot between $-\log C_{\text{H}^+} + H_0$ and $3 + \log k_c + H_0$. The slope value ϕ is 1.12 (Fig. not shown), which is in favour of the fact that water activity is playing an important role in deciding the rate at higher acidic concentration. Water is involved in slow step and behaves as a proton transfer agent. The relevant data for these plots is summarized in Table 3.

Table 3. Zucker-Hammett, Hammett, Bunnett and Bunnett-Olsen Plot data for acid catalyzed hydrolysis of di-2-methyl-5-nitroaniline phosphate

HCl (mol dm ⁻³)	$k_c \times 10^3$ (min ⁻¹)	$3 + \log k_c$	$-H_0$	$\log C_{\text{H}^+}$	b'_{H^+}	$-\log a_{\text{H}_2\text{O}}$	$3 + \log k_c + H_0$	$3 + \log k_c - \log C_{\text{H}^+}$	$-\log C_{\text{H}^+} + H_0$
1.0	6.68	0.82	0.20	0.00	0.14	–	0.62	0.82	0.20
1.5	10.99	1.04	0.47	0.18	0.21	–	0.57	0.86	0.29
2.0	14.31	1.16	0.69	0.30	0.28	–	0.47	0.86	0.39
2.5	19.33	1.29	0.87	0.40	0.35	–	0.42	0.89	0.47
3.0	23.72	1.38	1.05	0.48	0.42	–	0.33	0.90	0.57
3.5	32.66	1.51	1.23	0.54	0.49	–	0.28	0.97	0.69
4.0	43.46	1.64	1.40	0.60	0.56	–	0.24	1.04	0.80
4.5	60.43	1.78	1.58	0.65	0.63	–	0.20	1.13	0.93
5.0	73.58	1.87	1.76	0.70	0.70	–	0.11	1.17	1.06
6.0	31.37	1.50	2.12	0.78	0.84	(0.21) ³	–0.62	0.72	1.34
7.0	15.34	1.19	2.53	0.84	0.98	(0.28) ⁴	–1.34	0.35	1.69

Effect of temperature

In order to determine Arrhenius parameters [30], the hydrolysis of di-2-methyl-5-nitroaniline phosphate at 4 and 6 mol dm⁻³ hydrochloric acid was carried out at different

temperatures from 60 to 80°C with the interval of 5°C. Fig. 4 describes a plot between log rate constant and reciprocal of the absolute temperature.

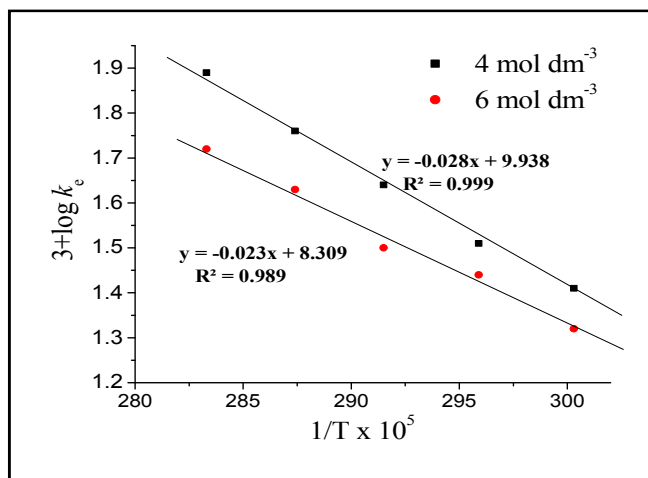


Fig. 4. Arrhenius plot for acid catalyzed hydrolysis of di-2-methyl-5-nitroaniline phosphate

Linearity of curve shows the validity of the Arrhenius equation. Slope values of the linear curve were used to determine Arrhenius parameters which are shown in Table 4. The magnitude of Arrhenius parameters [28] falls in the range of bimolecular reaction.

Table 4: Arrhenius parameters for acid catalyzed hydrolysis of di-2-methyl-5-nitroaniline phosphate

HCl (mol dm ⁻³)	Slope	E_a (kcal/mol)	A (sec ⁻¹)	$-\Delta S^\ddagger$ (e.u.)
4	-0.028	12.81	3.79×10^8	29.78
6	-0.023	10.52	9.51×10^6	43.92

Effect of solvent

Table 5 shows that the rate constant value gradually increases with the gradual addition of 1, 4 dioxane. Dioxane is regarded as a polar aprotic solvent. The effect of solvent on rate of hydrolytic reaction indicates the transition state in which charge is dispersed. This is accordance with Chanley's observation [31].

Table 5. Solvent effect rate data for acid catalyzed hydrolysis of di-2-methyl-5-nitroaniline phosphate

HCl (mol dm ⁻³)	Percentage of dioxane	$k_e \times 10^3$ (min ⁻¹)	HCl (mol dm ⁻³)	Percentage of dioxane	$k_e \times 10^3$ (min ⁻¹)
4.00	30	43.46	6.00	30	31.37
	40	46.21		40	33.12
	50	50.19		50	37.92
	60	53.24		60	40.56
	70	57.84		70	46.12

Effect of substrate Concentration

The effect of concentration of diester on rate of hydrolysis confirms the order of reaction to be one with respect to the diester by reducing half ($k_e = 72.96 \times 10^3$ min⁻¹) or double

($k_e = 73.89 \times 10^3 \text{ min}^{-1}$) of the normal concentration ($k_e = 73.58 \times 10^3 \text{ min}^{-1}$) at 5 mol dm^{-3} HCl, where hydrolysis is mainly governed by conjugate acid species.

Bond-cleavage of reaction

Bimolecular nature of hydrolysis of di-2-methyl-5-nitroaniline phosphate involving P-N bond fission is further supported by comparative kinetic rate data and isokinetic relationship plot. Comparative kinetic rate data for hydrolysis of other diesters studied kinetically is summarized in Table 6. The isokinetic relationship plot ($-\Delta S^\ddagger$ vs E_a) shows point of di-2-methyl-5-nitroaniline phosphate is collinear with other diesters which are known to undergo bimolecular hydrolysis via P-N bond fission (Fig not Shown).

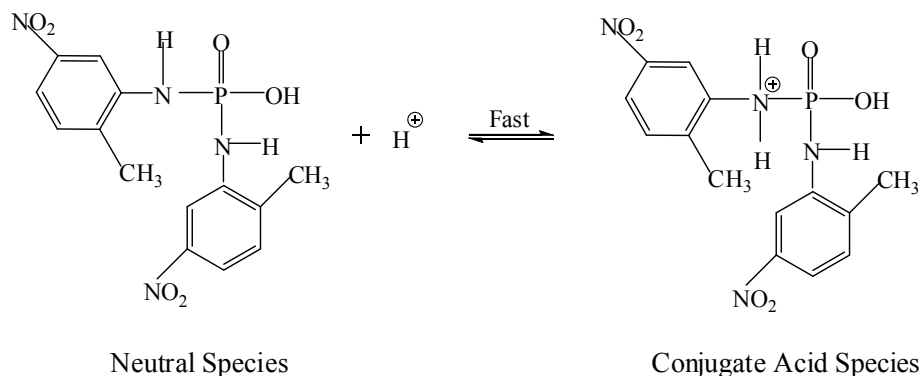
Table 6. Comparative kinetic rate data for hydrolysis of some diesters via conjugate acid species

Diesters	HCl mol dm^{-3}	E_a (kcal/mol)	$-\Delta S^\ddagger$ (e.u.)	Molecularity	Bond fission
Cyclohexyl amine phosphate	3	12.09	37.11	2	P-N
<i>O</i> -toluidine	1	11.40	38.66	2	P-N
Phenatadine phosphate	3	5.72	70.55	2	P-N
<i>m</i> -toluidine	3	15.56	21.29	2	P-N
2-Chloroaniline	3	13.70	28.90	2	P-N
2-methyl-5-nitroaniline	4	12.81	29.78	2	Present work
	6	10.52	43.92	2	

Mechanism

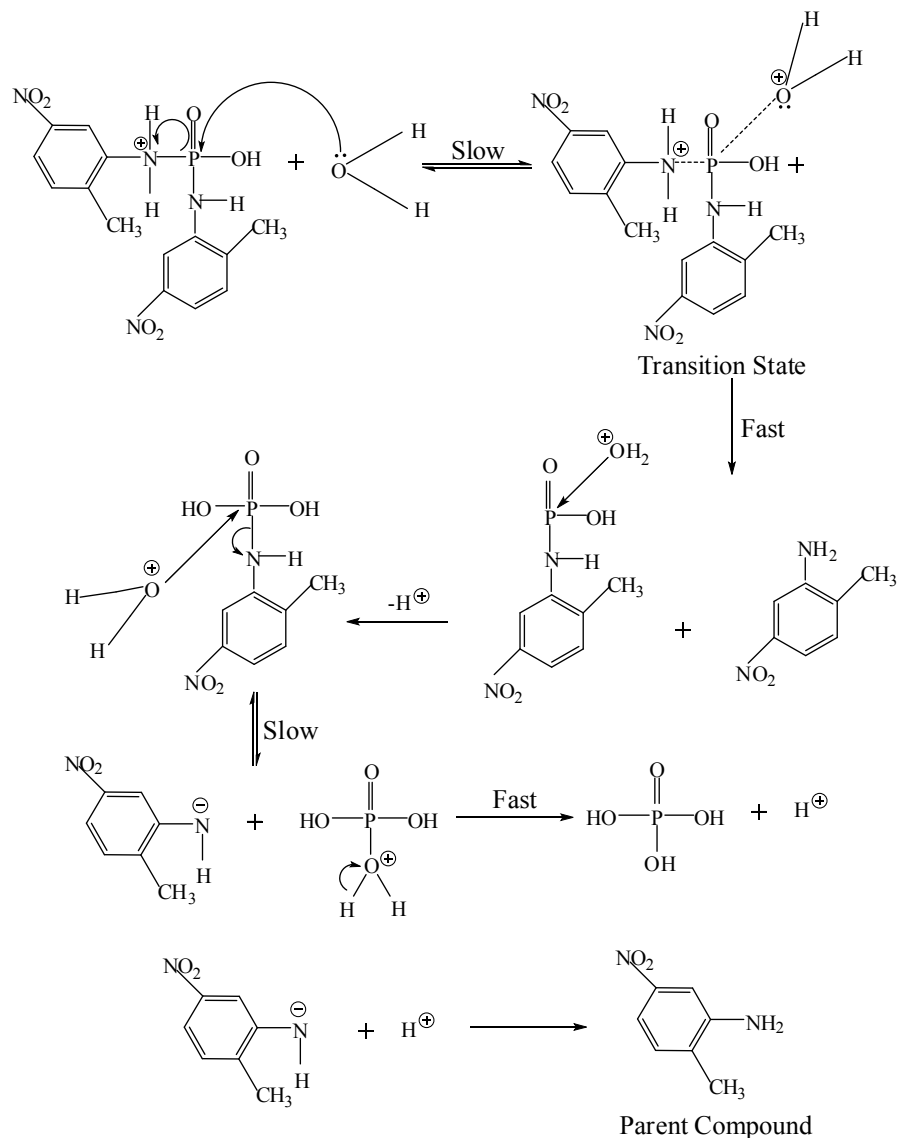
The mechanism of hydrolysis of di-2-methyl-5-nitroaniline phosphate via conjugate acid species is shown Scheme I and Scheme II.

(a) formation of conjugate acid species by fast pre-equilibrium proton transfer;



Scheme I

(b) bimolecular nucleophilic attack of water on phosphorus via conjugate acid species $S_N^2(P)$



Scheme II

CONCLUSION

Di-2-methyl-5-nitroaniline phosphate in 0.1 - 7.0 mol dm⁻³ hydrochloric acid was found to hydrolyze via neutral and conjugate acid species. The acid catalyzed hydrolysis is subjected to positive effect of ionic strength. Bimolecular nature of hydrolysis was supported by Zucker-Hammett hypothesis, Arrhenius parameters, Bunnett and Bunnett-Olsen parameters.

Bimolecular hydrolysis with P-N bond fission of di-2-methyl-5-nitroaniline phosphate was proposed. S_N^2 (p) mechanism has been suggested for the hydrolysis via conjugate acid species.

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