



Study of Hydrolysis of Mono-3,5-Dimethylaniline Phosphate via Conjugate Acid species

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Abstract : Hydrolysis of phosphate ester is one of the most fundamental chemical and biochemical reaction. In present investigation, kinetic study of hydrolysis of mono-3, 5-dimethylaniline phosphate has been carried out in 0.1-0.7 mol dm⁻³ hydrochloric acid at 50°C in aqueous medium. The log rate profile has rate maxima at 4.0 mol dm⁻³ hydrochloric acid. The effect of ionic strength, temperature and solvent on the rate of hydrolysis has been studied. The results show that mono-3, 5-dimethyl aniline phosphate is reactive mainly via conjugate acid species. Molecularity and order of reaction have been supported by different concepts such as Zucker Hammett hypothesis, Bunnett and Bunnett-Olsen parameters and Arrhenius parameters. The probable reaction mechanism has been suggested for the hydrolysis of monoester.

Key word : Hydrolysis, mono-3, 5-dimethylaniline phosphate, Ionic strength, bond fission.

Introduction

Phosphate ester hydrolysis is ubiquitous in biology, being involved in protein synthesis, signal, and energy transduction, as well as the replication of the genetic material¹. Phosphate mono and diesters are relatively stable and have important contribution in biochemical reaction². The importance of phosphate esters in biological processes is the basis of the continuing interest in the study of phosphoryl transfer mechanisms³. Many important biochemical processes for the maintenance of life involve the hydrolysis of phosphate esters and it is considered to be one of the most important reactions in living systems^{4,5}. Phosphate esters are ubiquitous in nature and exhibit many applications in the field of agriculture, medicine and industry^{6,7}. They are used as pesticides⁸, bactericides^{9,10}, antibiotics¹¹, and acts as HIV protease inhibitors¹². The hydrolysis of these phosphate esters is found everywhere and has attracted a great consideration due to their great values in various chemical and biochemical reaction¹³⁻¹⁵. Thus it has become the subject of intense research to the chemist and biologist.

Experimental

Mono-3,5-dimethylaniline phosphate was synthesized by Cavilier method¹⁶ in our laboratory, which

S. A. Bhoite *et al* /International Journal of ChemTech Research, 2018,11(07): 155-163.

DOI= <http://dx.doi.org/10.20902/IJCTR.2018.110719>

involves the reaction of 3,5-dimethyl aniline and phosphorus pentoxide in 1:1 mole ratio. The solid mass obtained was separated and water soluble part was precipitated out using $\text{Ba}(\text{OH})_2$ solution. Thus the Barium salt of monoester obtained as white solid was washed several times with distilled water with few drops of acetic acid to remove inorganic phosphate. All reactions were carried out at $50 \pm 0.5^\circ\text{C}$ employing $5 \times 10^{-4} \text{ mol dm}^{-3}$ solution of the monoester in aqueous medium. The inorganic phosphate produced during hydrolysis was determined spectrophotometrically using Allen's modified method¹⁷. All the solutions have been prepared in triply distilled water. All the chemicals used were of AR Grade.

Result and discussion

Hydrolysis via acid conjugate species-

The hydrolysis of mono-3,5-dimethylaniline phosphate was studied at 50°C in the range of 0.1 to 7.0 mol dm^{-3} HCl in aqueous medium. The first order rate coefficients obtained are illustrated by Figure 1. From the result it is observed that in acid region rate of hydrolytic reaction increases with increase in acid molarity up to 4.0 mol dm^{-3} HCl and after that decreases. The maximum rate at 4.0 mol dm^{-3} HCl was attributed to complete conversion of the ester molecule into their respective conjugate acid species. After complete conversion of protonated species, rate should remain constant but it further decreases with the increase in acid molarity. The decrease in the rate is attributed to the lowering of concentration of attacking nucleophile taking part in the reaction, i.e., due to variation in water activity.

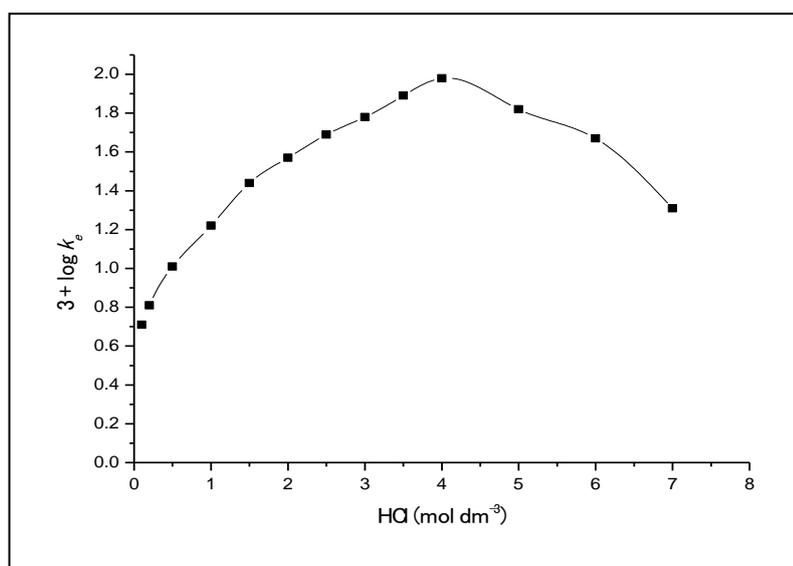


Figure 1:- Theplot of $3 + \log k_e$ versus HCl mol dm^{-3} for acid catalyzed hydrolysis of mono-3,5-dimethylanilinephosphate

Kinetic salt effect-

Kinetic salt effect was studied at different ionic strength from 0.5 to 3.0 μ , using appropriate molarities of hydrochloric acid (HCl) and sodium chloride (NaCl). The plots of rate constants of acid hydrolysis and acid molarities at different ionic strength are shown in Figure 2.

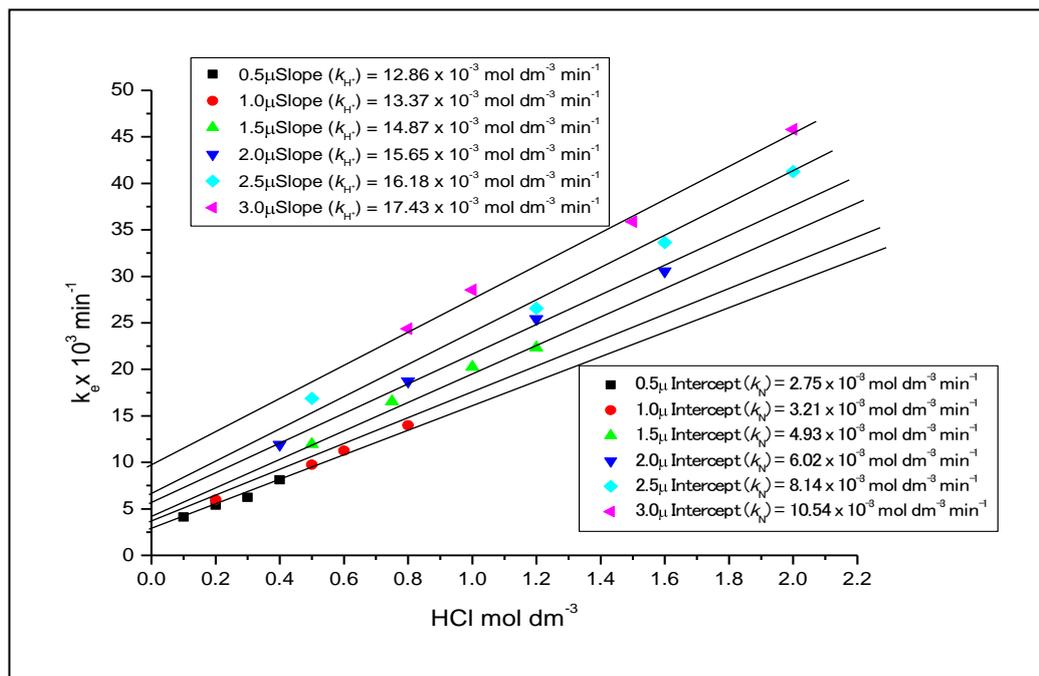


Figure 2:- Acid catalyzed hydrolysis of mono-3,5-dimethylaniline phosphate at constant ionic strength

Hydrolysis at each ionic strengths (μ) is denoted by six straight lines that make a positive slope with acid axis, hydrolysis is subjected to acid-catalysis. The slope of straight lines increases with increase in ionic strength. Thus acid catalyzed hydrolysis is attributed to positive salt effect. All the lines meet at different points on the rate axis, indicating the participation of neutral species. Different values of intercepts show that the contribution of neutral species at different acidities is varying. The accelerating ionic effect indicates that mono-3,5-dimethylaniline phosphate undergoes hydrolysis with positive effect of ionic strength. From the study of ionic strength effect, the total rates contributed by conjugate acid and neutral species may be calculated by the following 2nd empirical term of Debye-Huckle equation¹⁸.

$$k = k_{H^+} \cdot C_{H^+} + k_N \quad (1)$$

Where k , $k_{H^+} \cdot C_{H^+}$ and k_N are experimental rates, specific acid catalysed rates and neutral rates respectively. Specific acid-catalysed rate with their logarithmic values at that ionic strength are summarized in Table (1) and illustrated in Figure 3.

Table 1:-Rate data for acidic hydrolysis of mono-3,5-dimethyl aniline phosphate at constant ionic strength

Ionic strength (μ)	$k_{H^+} \times 10^3$	$3 + \log k_{H^+}$	$k_N \times 10^3$	$3 + \log k_N$
0.5	12.86	1.11	2.75	0.44
1.0	13.37	1.13	3.21	0.51
1.5	14.87	1.17	4.93	0.69
2.0	15.65	1.19	6.02	0.78
2.5	16.18	1.21	8.14	0.91
3.0	17.43	1.24	10.54	1.02

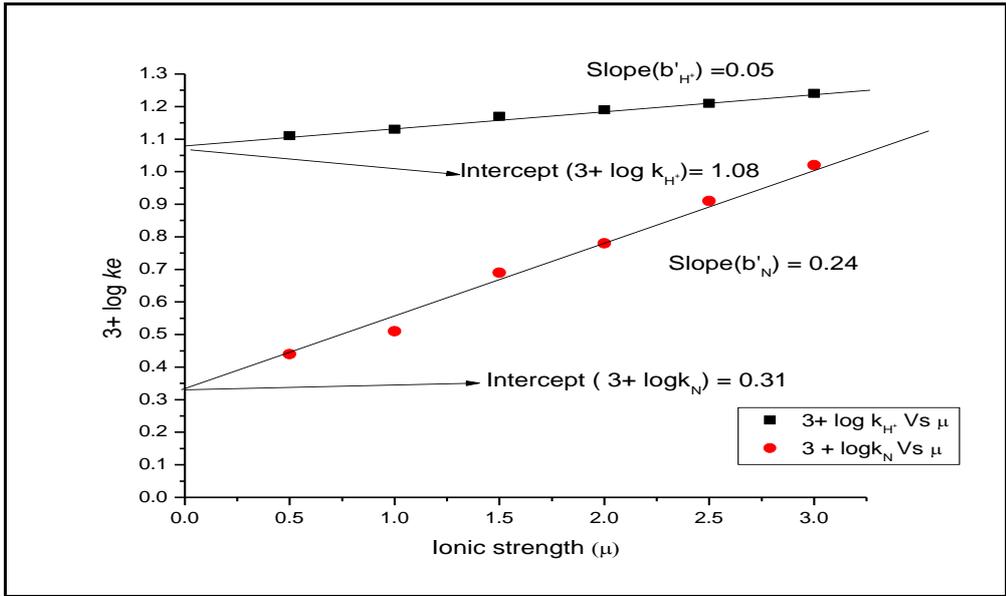


Figure 3:- Plot of $3 + \log k_e$ versus ionic strength (μ) for acidic hydrolysis of

mono-3,5-dimethylaniline phosphate

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 ($\log k_{H^+}$). The linearity of the curve shows the following relation of the rate constant with ionic strength. This presents an empirical form of Debye-Huckel equation:

$$k = k \exp b'_{H^+} \cdot \mu \tag{2}$$

The specific acid –catalysed rate may be shown as

$$k_{H^+} = k_{H_0^+} \cdot \exp \cdot b'_{H^+} \cdot \mu \tag{3}$$

Where, k_{H^+} , $k_{H_0^+}$, b'_{H^+} and μ are specific acid catalyzed rate at that ionic strength, specific acid catalyzed rate at zero ionic strength, a constant and ionic strength respectively. Specific acid catalyzed rate (k_{H^+}) can be converted into acid catalyzed rates ($k_{H^+} \cdot C_{H^+}$) as follows:

$$k_{H^+} \cdot C_{H^+} = k_{H_0^+} \cdot C_{H^+} \cdot \exp \cdot b'_{H^+} \cdot \mu \tag{4}$$

On taking logarithm

$$\log k_{H^+} \cdot C_{H^+} = \log k_{H_0^+} + \log C_{H^+} + b'_{H^+} \cdot \mu \tag{5}$$

Where, for HCl, C_{H^+} and μ are of the same value.

The neutral rate may be represented by following equation:

$$k_N = k_{N_0} \exp \cdot b'_N \cdot \mu \tag{6}$$

$$\log k_N = \log k_{N_0} + b'_N \cdot \mu \tag{7}$$

Where k_N is neutral rate and $b'_N = b'_N/2.303$. The overall hydrolysis was represented by following equation:

$$k = k_{H^+} \cdot C_{H^+} + k_N \tag{8}$$

Table 2 :- Estimated and experimental rate data for acidic hydrolysis of mono-3,5-dimethyl aniline phosphate

HCl (mol dm ⁻³)	$k_H^+ \cdot C_{H^+} \times 10^3$ (min ⁻¹)	$k_N \times 10^3$ (min ⁻¹)	$k_e \times 10^3$ (min ⁻¹)	$-\log(a_{H_2O})^n$	$k_e \times 10^3$ (min ⁻¹)Estd.	$k_e \times 10^3$ (min ⁻¹)Expt.
0.1	1.23	1.91	3.14		3.14	5.10
0.2	2.49	2.26	4.75		4.75	6.52
0.5	6.44	2.66	9.10		9.10	10.24
1.0	13.67	3.51	17.18		17.18	16.45
1.5	21.70	4.62	26.32		26.32	27.41
2.0	30.83	6.08	36.91		36.91	36.84
2.5	40.92	8.01	48.93		48.93	48.54
3.0	48.65	10.54	59.19		59.19	60.28
3.5	64.58	13.88	78.46		76.46	77.86
4.0	78.36	18.28	96.64		96.64	94.73
5.0	115.58	31.69	147.27	(0.16) ²	68.00	66.45
6.0	149.31	54.95	204.26	(0.21) ³	48.38	46.63
7.0	196.38	95.27	291.65	(0.28) ⁴	22.12	20.47

Table (2) summarizes both the observed and estimated rates of the hydrolysis in acid region. Equation (8) was used to calculate the theoretically estimated rates which are then compared with the experimental rates. The rate beyond 4.0 mol dm⁻³HCl was calculated employing the Bronsted –Bjerrum equation¹⁹.

$$k = k_H^+ C_{H^+} (a_{H_2O})^n + k_N (a_{H_2O})^n \quad (9)$$

$$k_H^+ \cdot C_{H^+} = k_{H_0^+} \cdot C_{H^+} \cdot \exp. b'_H \cdot \mu (a_{H_2O})^n \quad (10)$$

$$\log k_H^+ \cdot C_{H^+} = \log k_{H_0^+} + \log C_{H^+} + b'_H \cdot \mu + n \log (a_{H_2O}) \quad (11)$$

The neutral rate at higher concentration is as follow:

$$k_N = k_{N_0} \exp. b'_N \cdot \mu \cdot (a_{H_2O})^n \quad (12)$$

$$\log k_N = \log k_{N_0} + b'_N \cdot \mu + n \log (a_{H_2O}) \quad (13)$$

Where n is an integer and (a_{H2O}) is water activity. It is clear from the results that hydrolysis of mono-3,5-dimethylaniline phosphate in acid occurs via both conjugate acid species and neutral species, and their rates are subjected to positive ionic strength or water activity.

Molecularity of the hydrolytic reaction:-

The Zucker- Hammett hypothesis²⁰ is made up of two parts. In the first part, Hammett postulated²¹ that the reactions that give a linear plot of log rate constants against the acidity function (-H₀) did not involve water molecules in the rate determining step. The slope value 0.62 (figure not shown) of the plot is far from unity, indicating the absence of unimolecular hydrolysis. The second part of the hypothesis deals with a plot between the log rate constant and log acid molarity. A unit or approximately unit slope of this plot was used as a criterion to predict the probable mechanism to be bimolecular. The slope value 1.24 (figure not shown) clearly indicates the bimolecularity of the reaction.

Bunnett²² suggested two parameters ω, ω*. Former is the slope of plot between log rate constant + H₀ and log activity of water (-log a_{H2O}). The slope value ω = 8.37, ω* = 2.88, and φ = 1.47 for Bunnett and Bunnett –Olsen parameters²³ (figure not shown) for mono-3,5-dimethyl aniline phosphate ester also indicate a slow proton transfer with a nucleophilic attack of the water molecule.

Effect of temperature

Effect of temperature on the rate of hydrolysis of mono-3,5-dimethylaniline phosphate, a series of kinetic runs have been carried out at 2.0 mol dm⁻³HCl, using different temperatures. Arrhenius parameters²⁴ determined for the hydrolysis at 4.0 mol dm⁻³HCl which are shown in Table (3). The magnitude of the Arrhenius parameters falls in the range of bimolecular reaction.

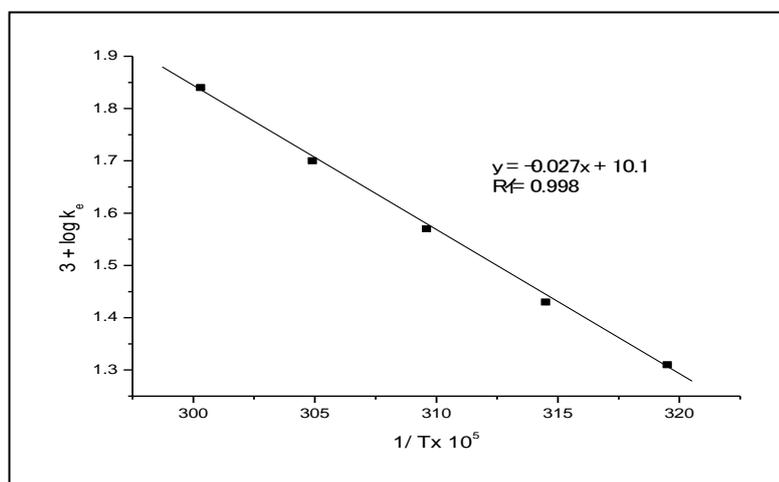


Figure 4:- Arrhenius plot for the hydrolysis of mono-3,5-dimethylaniline phosphate

Table 3:- Arrhenius parameters for acid catalyzed hydrolysis of mono-3,5-dimethylaniline phosphate

HCl (mol dm ⁻³)	Slope	E _a (kcal/mol)	A (sec ⁻¹)	-ΔS [‡] (e.u.)
2	-0.027	12.36	8.4×10 ⁶	29.01

Effect of 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 observation²⁵. Table (4) shows that the rate constant value gradually increases with the gradual addition of 1,4dioxane and DMSO. Dioxane is regarded as polar aprotic solvent while dimethylsulfoxide is regarded as dipolar aprotic solvent.

Table 4:- Solvent effect rate data for acid catalyzed hydrolysis of mono-3,5-dimethylaniline phosphate.

HCl (Mol dm ⁻³)	% solvent v/v	1,4-dioxane	DMSO
		k _e × 10 ³ (min ⁻¹)	k _e × 10 ³ (min ⁻¹)
2.0	10	40.38	46.72
2.0	20	49.73	53.44
2.0	30	54.27	60.18
2.0	40	62.54	68.33

Bond-cleavage of reaction

Bimolecular nature of hydrolysis of mono-3,5-dimethylaniline phosphate involving P-N bond fission is further supported by comparative kinetic rate data and isokinetic relationship plot. The isokinetic relationship plot (-ΔS[‡] vs E_a) shows point of mono-3,5-dimethylaniline phosphate is collinear with other monoester which are known to undergo bimolecular hydrolysis via P-N bond fission Figure 5.

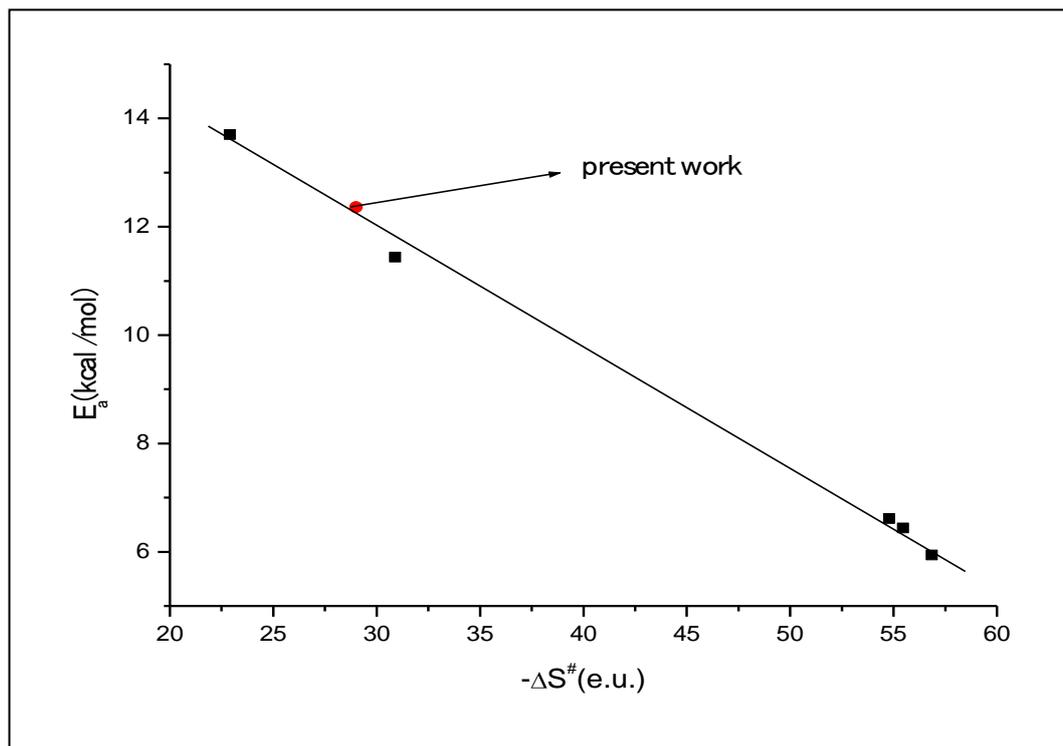
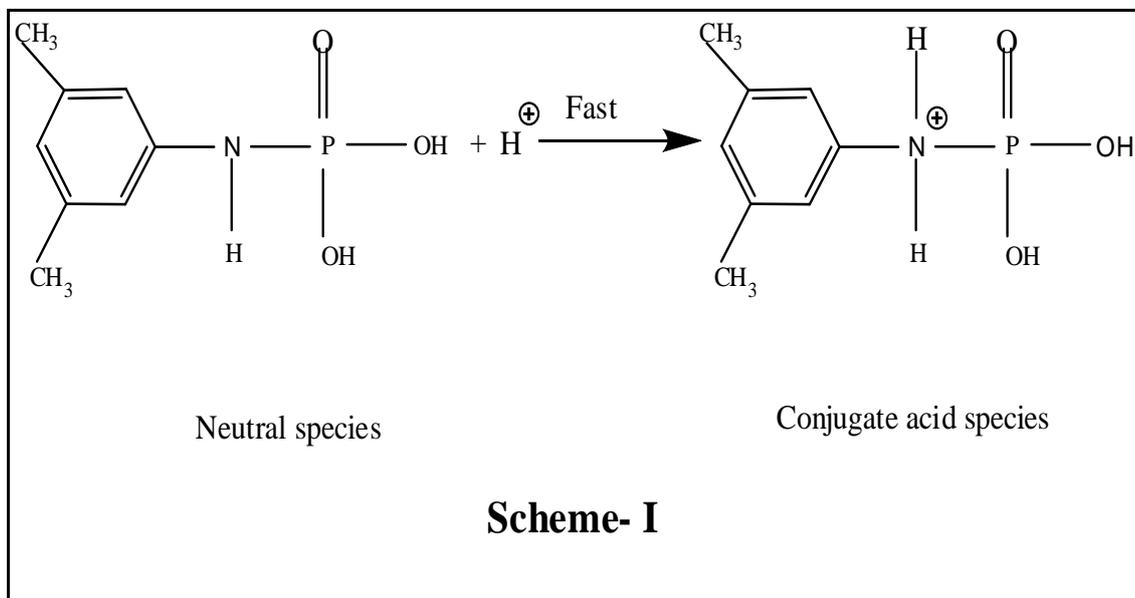


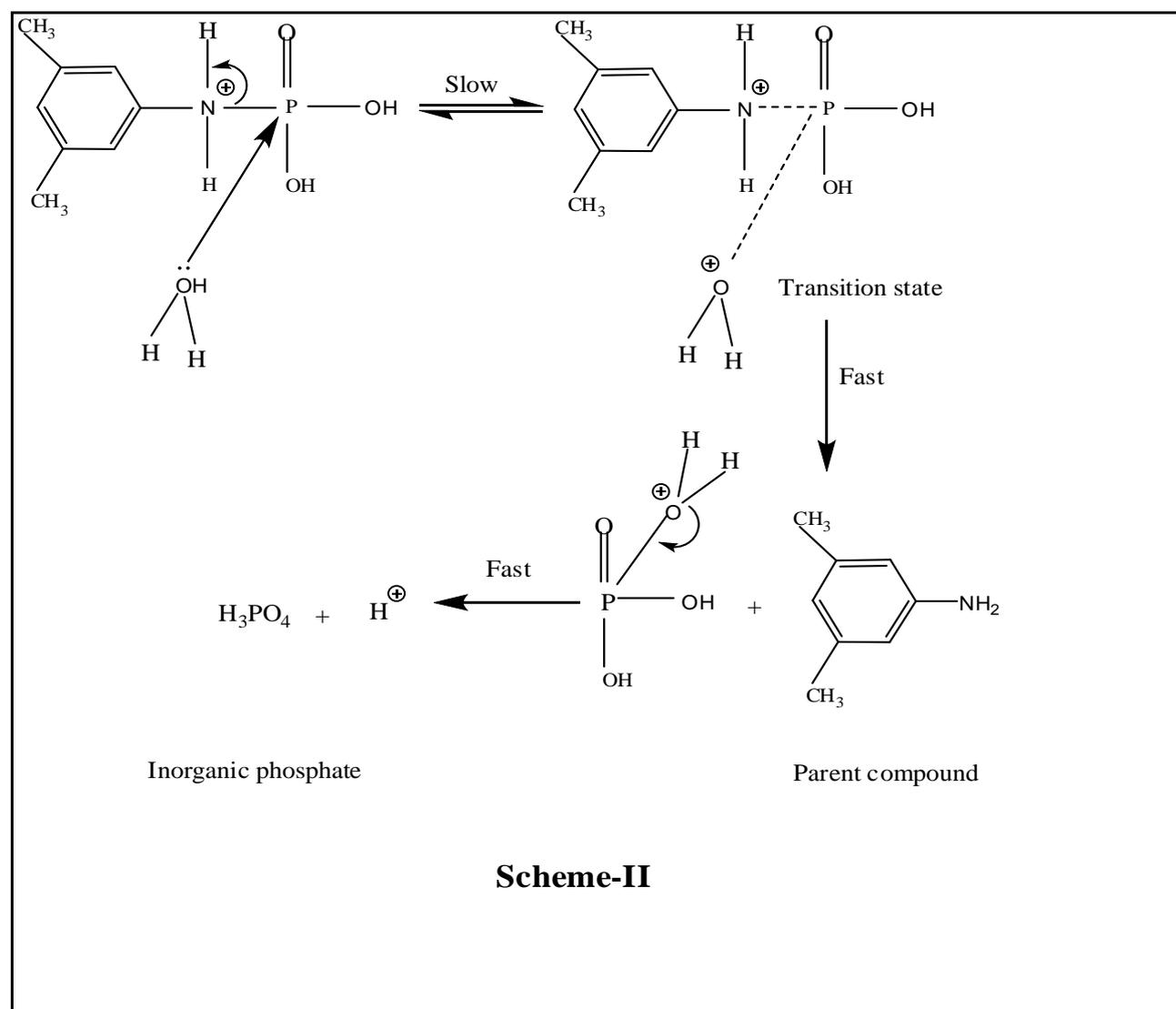
Figure 5:-Isokinetic relationship plot for the hydrolysis of some phosphate monoesters.

The probable reaction mechanism for the hydrolysis of mono-3,5-dimethylaniline phosphate may be suggested via conjugate acid species as shown in Scheme-I and Scheme-II.

(a) Formation of conjugate acid species.



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



Conclusion:-

Mono-3,5-dimethylaniline phosphate ester in 0.1 -7.0 mol dm⁻³HCl was found to hydrolyze via neutral and conjugate acid species. The acid catalysed hydrolysis is subjected to the positive effect of the ionic strength. The bimolecular nature of hydrolysis was supported by different parameters such as Hammett, Zucker – Hammett, Bunnett and Bunnett-Olsen parameters. Bimolecular hydrolysis with P-N bond fission of mono-3,5-dimethylaniline phosphate was proposed S_N²mechanism has been suggested for the hydrolysis via conjugate acid species.

Acknowledgement

The authors are thankful to the Pt. Ravishankar Shukla University, Raipur for providing University Fellowship to one of the authors (Shashibal Kindo) and also grateful to Head of School of Studies in Chemistry, Pt. Ravishankar Shukla University, Raipur (India) for providing laboratory facilities.

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