Kinetic study and Mechanistic Interpretation of Mono -4- Nitrophenyl Phosphate ester via Neutral species

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Abstract:

Kinetic study and the hydrolysis of Mono -4- nitrophenyl phosphate ester have been investigated in acid (0.1 to 6.0 mol dm^{-3} HCl) and buffer medium (pH 1.24 to 7.46) at 97 ± 0.5^0C in a 20% aqueous dioxane mixture. Ionic measurements reveal the existence of acid-catalyzed mono-ester hydrolysis. Mono ester hydrolyzes through conjugate acid, neutral, and mono negative species; however, we will address just neutral species in this study. The hydrolysis of di-ester involves monoester as an intermediate, which finally hydrolysis into organic phosphate. Since the hydrolysis of monoester is faster, the hydrolysis of the di-ester could be directly measured by the colorimetric method. Allen's modified approach determined the amount of organic phosphate recovered during total hydrolysis. The first-order rate coefficient was determined using an integrate rate equation that matches the rate measured experimentally. The influence of temperature, solvent concentration, and substrate concentration on the involvement of water, bond fission, and molecularity of a reaction have been evaluated.

Keywords: CNP Linkage, Phosphate esterHydrolysis, COP Linkage.

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Introduction

The recent interest in C-N-P-linked di esters in biochemistry reflects the present emphasis on the acid hydrolysis of a mono phosphate ester [1]. Chemical research has entered into new level in this sector resulting in organophosphate insecticides, pesticides and plant hormones [2]. As organophosphates have an essential role in the welfare of human life, they are used as fungitoxic, fertilizers, plant hormones, defoliating agents, rodenticides, miticides and aphicides, antiviral medicines, in radioactive traces technique, plasticizers, germicides, flame resistant, anti-wrinkling agents, fuel additive in industries etc [3]. Organophosphate esters have achieved tremendous academic importance also. They are used to determine the various reaction paths of biological changes [4]. Keeping in mind the importance of organophosphate for a living organism, it is essential to have a dynamic and systematic study of all the aspects of the organophosphate reactions [5].

The rate of phosphate ester hydrolysis is generally affected by factors like ionic strength, concentration, temperature, substituents, the polarity of the solvent and catalyst etc.[6]. Bunton and colleagues concluded that acid catalyzed hydrolysis [7] proceed only when electron-withdrawing substituents are present in the aryl component [8]. Hydrolysis of some substituted aryl phosphate shows that the magnitude of acid catalysis decreases with decreasing electron attracting power of the substituents [9]. In most aryl phosphate esters, there have been rate maxima, but maximum protonation is absent. Bunton and colleagues discovered that acid-catalyzed hydrolysis happens only when the aryl portion has an attractive electron substitution [10].

Experimental

The Kinetic research conducted in this manner is presented, together with a discussion of the findings. At 97 degrees Celsius, the insoluble

mono-ester was investigated in a 20% dioxane water mixture [11]. The mono-ester concentration was constant throughout the kinetic runs at "5.0 x 10^{-4} mol.dm^{-3} HCl". The appropriate influence of salt, solvent temperature, and others was used to determine the most likely hydrolysis pathways of C-O-P linkage ester [12]. The influence of ionic strength, pH log rate profile, solvent effect, and substrate concentration has been investigated [13]. The data on ionic strength and assumed pk_a values were used to create theoretical rates that were very consistent with observed rates [14].

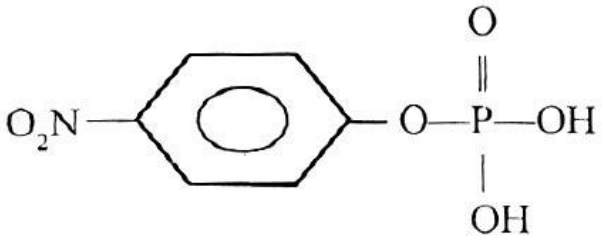
Result and Discussion

Hydrolysis via neutral species

The hydrolysis of mono-4-nitrophenyl phosphate ester demonstrates that it is reactive with neutral, mononegative, and conjugate acid species [15]. The hydrolysis of the mono-ester mentioned above was studied kinetically in acid and buffer fluids containing "0.1 to 6.0 mol.dm^{-3} HCl" and a pH range of "1.24 to 7.46 at 97 ±0.5^{0}C". Due to dioxane's solubility in water, kinetic runs were conducted in 20% aqueous dioxane. For all kinetic runs, the mono phosphate ester concentration was maintained at "5.0x10^{-4} mol.dm^{-3} HCl".

Pseudo First-order rate co-efficient of hydrolysis has been summarized in Table (1) and Figure (1) describes acid and pH log rate profile.

HYDROLYSIS VIA NEUTRAL SPECIES



Hydrolysis of mono-4-nitrophenyl phosphate via neutral species has been found to contribute the overall rate of reaction in entire acid range. The independent neutral rates have been shown the pH-log rate profile given by line (NH-----N_0----N-----NM) of (Fig.I) which can be represented by-

$$K_N = K_{N_0} \cdot \exp. \cdot b_N \cdot \mu \left(a_{H_2O}\right)^n \quad ...(12)$$

The value of 'n' is zero upto 4.0 mol.dm^3 HCI. The value of (the specific neutral rate) has been found almost of the same value obtained by two different alternate methods, one that obtained from ionic strength data (K_{N_0} = 66.07×10^{-5}mol. $dm.^{-3}$ $min.^{-1}$) while the other from pK values (K_{N_0} = 66.38×10^{-5} mol. $dm.^{-3}$ $min.^{-1}$). The former depends upon the maintenance of exact ionic strength, while the later on the accuracy of pH-values.

These values were taken to be a fair agreement in case of the hydrolysis of dimethyl phosphate for the convenience. The hydrolysis of neutral species has been found to be associated with conjugate acid species in acid region and with mononegative species in alkaline medium.

Hydrolysis of neutral species in the region of 1.0to 6.0 mol. dm^{-3} HCI explained in Part-I is represented by the equation:

$$K_e = K_{H^+} \cdot C_{H^+} + K_N \quad ... (1)$$

Hydrolysis in the region from pH 0.0 to 2.20 is mainly governed via neutral and mononegative species and can be expressed as:

$$K_e = K_M + K_N \quad ... (2)$$

where; K_M and K_N are mono negative and neutral rates.

Hydrolysis of neutral species governed by the above equation has been represented by ($NH...N_0$) and (N_0-----N----NM) in acid and alkaline medium respectively. Since, these rates are directly proportional to the concentration of their species, they may be written as:

$$K_M \propto \frac{M}{M+N} \quad ... (3)$$

$$\text{or, } K_M = K_{M_0} \cdot \frac{M}{M+N} \quad ... (4)$$

$$\text{and} \quad K_N \propto \frac{M}{M+N} \quad ... (5)$$

$$\text{or, } K_N = K_{N_0} \cdot \frac{M}{M+N} \quad .. (6)$$

where

K_{N_0} = average value of specific neutral rate at different pH values

K_{M_0} = specific mononegative rate, which is the experimental rate at pH 4.17.

$\frac{N}{N+M}$ = fraction of neutral species

$\frac{M}{M+N}$ = fraction of mono negative species

The sum of $\frac{N}{N+M}$ is always unity. The overall rate of hydrolysis in alkaline medium can be estimated at different hydrogen ion concentration provided that K_{M_0} K_{N_0} and fractions of their species are known.

Almost all the phosphate mono-esters have been found to dissociate completely at pH 4.17. (Fig.1) shows the maxima, where the hydrolysis of mono-ester may be presumed to occur exclusively via its mono-negative species at this pH, may be taken to almost unity (0.999). The observed rates at this pH are the specific mono negative rates (KM) with the above assumption. The equilibrium of the dissociation of neutral species into mono negative species and proton can be shown as:

Neutral species → Mononegative species + proton

Or

$$N \xrightarrow{K_1} M + H^+$$

where; N = Concentration of neutral species

M = concentration of mononegative species

If K_1 is the dissociation constant for the above equation then :

$$K_1 = \frac{M \times H^+}{N} \quad \ldots (7)$$

where; M, N and H^+ are the concentration of mononegative species, neutral species and hydrogen ions.

$$\frac{K_1}{H^+} = \frac{M}{N}$$

or,

$$\frac{H^+}{K_1} = \frac{N}{M}$$

or,

$$1 + \frac{H^+}{K_1} = 1 + \frac{N}{M}$$

or

$$\frac{K_1 + H^+}{K_1} = \frac{M+N}{M}$$

or,

$$\frac{K_1}{K_1 + H^+} = \frac{M}{M+N}$$

where; $\frac{M}{M+N}$ is the fraction of mononegative species (0.999) i.e. almost unity at pH 4.17.

$$\frac{K_1}{K_1 + 6.7 \times 10^{-5}} = 0.999$$

$K_1 = 0.066933$

or = 6693.3×10^{-5}

Table – 14

Hydrolysis or Mono-4-Nitrophenyl Phospate via Neutral and Mononegative Species at 97 ± 0.5°C

pH	$\frac{M}{M+N}$	$\frac{N}{N+M}$	$10^5 K_M$ (mol. dm.$^{-3}$ min.$^{-1}$)	$10^5 K_N$ (mol. dm.$^{-3}$ min.$^{-1}$)	$10^5 K_e$ (mol. dm.$^{-3}$ min.$^{-1}$) (Calcd.)	$10^5 K_e$ (mol. dm.$^{-3}$ min.$^{-1}$) (Obsd.)	5+log K_e (Calcd.)	5+log K_M (Calcd.)
0.30	0.118	0.882	17.01	58.27	75.28	80.36	1.88	1.23
0.70	0.251	0.749	36.18	49.49	85.67	84.38	1.93	1.56
1.00	0.400	0.600	57.66	39.64	97.30	95.46	1.99	1.76
1.24	0.537	0.463	77.41	30.59	108.00	106.46	2.03	1.89
2.20	0.914	0.086	131.76	5.68	137.44	133.87	2.14	2.12
3.33	0.993	0.007	143.15	0.46	143.61	142.23	2.16	2.15
4.17	0.999	0.001	144.01	0.07	144.08	144.16	2.16	2.16

Now the fractions of mononegative species can be determined at other pH values with the help of above K_1 value. We can also calculate the fractions of neutral species as the sum of fraction of neutral and mononegative species is unity [16]. The values of fraction of mononegative species are used to calculate the corresponding mononegative rates. Similarly neutral rates are obtained from their corresponding fraction Table-14 summarises the fractions of neutral species of mononegative and neutral species and their corresponding mononegative and neutral rates (Calcd.).

The above equations 1, 2 and 3 are used to calculate the specific neutral rates, which have been shown in Table-15. The value of specific neutral rate (K_{N_0}) is equal to 66.38×10^{-5} min.dm.$^{-3}$min.$^{-1}$, which is surprisingly in good agreement with K_{N_0} =66.07×10^{-5} min.dm.$^{-3}$min.$^{-1}$that obtained from ionic strength data, the difference between the two values is not much as compared to the limitations of the method used. The estimated mononegative and neutral rates by equation (4) and (6) agree well with the experimentally observed rates.

Table – 15

Specific Neutral Rates for the Hydrolysis of Mono-4-Nitrophenyl Phosphate at Different pH Values

pH	$10^5, K_e$ (mol. dm.$^{-3}$ min.$^{-1}$) (Obsd.)	$10^5.K_M$ (mol. dm.$^{-3}$ min.$^{-1}$) (Calcd.)	$10^5.(K_e-K_M) = 10^5 K_N$ (mol. dm.$^{-3}$ min.$^{-1}$)	$\frac{N}{N+M}$	$10^5.K_{N_0}$ (mol. dm.$^{-3}$ min.$^{-1}$) (Calcd.)
0.30	80.36	17.01	63.35	0.882	71.80
0.70	84.38	36.18	48.20	0.749	64.35
1.00	95.46	57.66	37.80	0.600	63.00

Average Value of K_{N_0} = 66.38×10^{-5} mol dm.$^{-3}$ min.$^{-1}$

Thus in the acid region (NH----N_0) from 1.0 to 6.0 mol dm.$^{-3}$ HCl, the rates are due to conjugate species and neutral species in alkaline medium(NH----N-----NH), from 0.1 mol. dm.$^{-3}$ HCI to 2.20 pH, the rates are due to the hydrolysis of mono-ester mainly governed by neutral species. Since the conjugate species are not reactive beyond pH 1.0.The neutral species convert into more reactive mono negative species at pH 4.17 and the contribution of neutral species towards the hydrolysis of mono-4-nitrophenyl phosphate is almost zero. The agreement between the calculated and experimentally, observed support rates the presumption.

EFFECT OF TEMPERTURE:

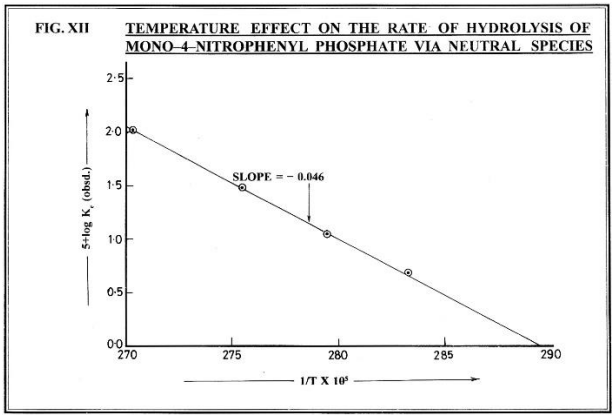
At pH 1.24, the hydrolysis of present mono-ester is most probably due to neutral species. At this pH range kinetic runs were carried out at different temperatures, in order to determine the Arrhenius parameters. The observed rate constants have been summarised in Table-16.

Table – 16

Arrhenius Plot Data for the Hydrolysis of Mono-4-Nitrophenyl Phosphate at Different Temperatures via Neutral Species

pH	t^{0}C	T K (abs.)	$10^5.\frac{1}{T}$	$10^5.K_e$	$5+\log K_e$
1.24	97	370	270.27	106.46	2.03
1.24	90	363	275.48	31.07	1.49
1.24	85	358	279.33	10.96	1.04
1.24	80	353	283.28	4.78	0.68

The linearity of plot (Fig-XII) indicates the uniformity of the reaction at that time temperature ranges used.



The Arrhenius parameters with the help of plot, suggests the bimolecular nature of the reaction. The relevant data of Arrhenius parameters have been summarised in Table-17.

TABLE-17
ARRHENIUS PARAMETERS FOR THE HYDROLYSIS OF MONO-4-NITROPHENYL PHOSPHATE VIA NEUTRAL SPECIES

pH	Parameters		Entropy
	Energy of activation (E) (K. Cal. mol.^{1})	Frequency factor (A) (Sec^{-1})	$-\Delta S^{\neq}$ (e.u.)
1.24	21.05	4.74×10^{7}	25.83

EFFECT OF CONCENTRATION:

The concentration effect is helpful to justify the order of reaction. Thus, for the recognition of order of reaction, a study is to be carried out by bringing about the change in concentration of the reactants by making the concentration of the ester half and double of the normal concentration (5.0×10^{-4} mol.dm.$^{-3}$) which is maintained through out the course of study. The kinetic runs were carried out at pH 1.24 at 97°C, the rate coefficients have been calculated, which are based upon the pseudo first order rate constants. The rates are found in decreasing order and it is concluded that the rate-coefficients are independent of the initial concentration of the ester. Hence, its a pseudo first order reaction. The relevant data is have been shown in Table-18.

Table 18

Effect of Concentration on the Rate of Hydrolysis of Mono-4-Nitrophenyl Phosphate via Neutral Species at 97°C.

pH	Mono-ester Concentration (mol.dm.$^{-3}$)	$10^{5}.K_{e}$ (mol. dm.$^{-3}$ min.$^{-1}$) (Obsd.)
1.24	0.00025	108.30
1.24	0.00050	106.46
1.24	0.00075	104.26

EFFECT OF SOLVENT:

Kinetic runs have been made in aqueous dioxan mixture in order to investigate the effect of solvent on the rate of hydrolysis at pH 1.24. According to Qualitative theory of Hughes and Ingold, the rate of hydrolysis should not be affected by a change over from more polar to less polar solvent, if there is any change, it should be very small and the rates should increase slightly. It is clear fromTable-19.

Table – 19

Effect of Solvent on the Rate of Hydrolysis of Mono-4-Nitrophenyl Phosphate Via Neutral Species at 97°C.

pH	Water Percentage (v/v)	Dioxan Percentage (v/v)	$10^{5}.K_{e}$ (mol. dm.$^{-3}$ min.$^{-1}$) (Obsd.)
1.24	100.00	00.00	106.46
1.24	60.00	40.00	95.02
1.24	50.00	50.00	84.60

The relevant data of solvent effect have been summarised in Table-19. It is clear that increase in dioxan content brings about slight increase in the rates. The increase is attributed to a better proton donating property of the acid-dioxan media. The solvent effect may therefore be taken to subject the formation of transition state in which charges are dispersed.

BOND FISSION:

The hydrolysis of mono-4-nitrophenyl phosphate via neutral species may occur unimolecularly or bimolecularly involving P–O or C–O bond fission, like many phenyl phosphate. The Arrhenius parameters* are regarded as important indications to decide the reaction path and indirectly the nature of molecularity of the reaction [17].

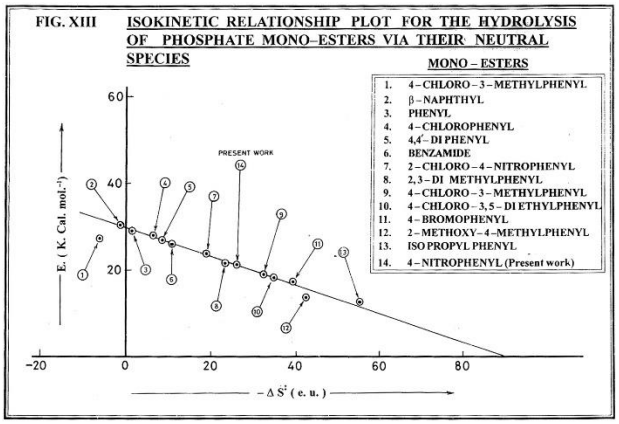
Isokinetic relationship plot and comparative kinetic rate data provide understanding of the nature of bond fission. Hence, systematic study of temperature effect and solvent effect is useful. The neutral molecule of mono-ester may undergo hydrolysis bimolecularly with P–O bond fission [18]. It has been decided by comparative isokinetic data of similarly substituted phenyl phosphate mono-esters [20]. The neutral species of these mono-esters have been found to hydrolyse bimolecularly with P-O bond fission. Since, in the isokinetic relationship plot (Fig.XIII), the point of mono-4-nitrophenyl phosphate lies on the line of other phosphate mono-esters and Table-20 indicates the range of Arrhenius parameters supporting P-O bond fission [21]. However, the activation energy and frequency factor fall in the range of bimolecular hydrolysis [22]. The value of entropy is more than sufficient to establish the nature of bond fission and molecularity of reaction.

Table -20

Comparative Isokinetic Rate Data for the Hydrolysis of Some Phosphate Mono-Esters via their Neutral Species.

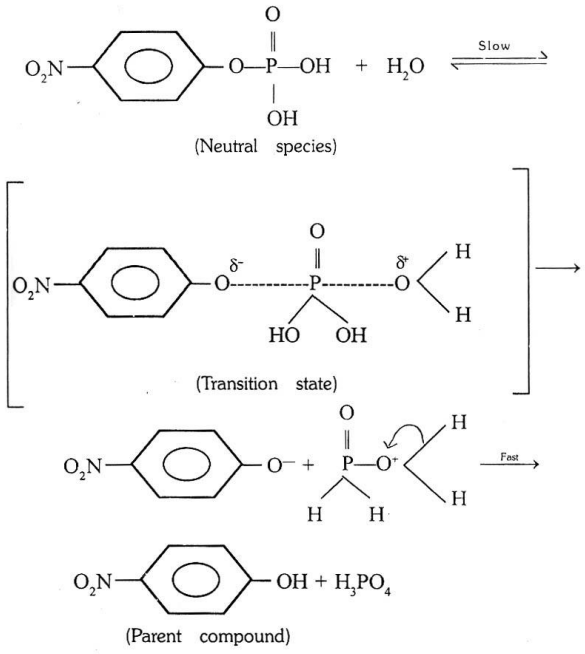
S.No.	Phosphate Mono-ester	Activation Energy (K.cal.mol.$^{-1}$)	$-\Delta S^4$ (e.u.)	Bond fission	Reference
1.	4-chloro-3-methyltollyl	27.46	-6.48	P–O	2
2.	β-naphthyl	30.48	-1.79	P–O	132
3.	Phenyl	29.00	0.90	P–O	49
4.	4-chlorophenyl	28.46	5.57	P–O	2
5.	4,4'-diphenyl	27.00	8.10	P–O	220
6.	benzamide	26.40	10.00	P–O	132
7.	2-chloro-4-nitrophenyl	24.03	18.67	P–O	231
8.	2,3-di-methoxyphenyl	22.27	23.27	P–O	232
9.	4-chloro-3-methylphenyl	18.76	31.96	P–O	93
10.	4-chloro-3,5-diethylphenyl	18.30	34.14	P–O	11
11.	4-bromophenyl	17.46	38.94	P–O	153
12.	2-methoxy-4-methylphenyl	14.14	41.90	P–O	137
13.	iso-propyl	12.93	55.20	P–O	2
14.	4-nitrophenyl	21.05	25.83	P–O*	Present Work

* Bond fission assumed for the present work.



Mechanism :

On the basis of above results and discussions, it becomes clear that hydrolysis of mono-4-nitrophenyl phosphate via neutral species is involving P-O bond fission with bimolecular attack of water on phosphorus atom. The reaction path may be formulated as-



Conclusion

The kinetic investigation of Mono-4-Nitrophanyl phosphate ester through neutral species revealed that the rate rises with increasing acid molar concentration up to 4.0-mol dm^{-3}HCl and then abruptly decreases with increasing acid concentration. The acid log rate profile bend is due to water activity, not maximum protonation. Thus, it is established that conjugate acid species developed by rapid proton transfer between conjugated and neutral species. The acid-catalyzed rate is retarded by a negative salt effect, also known as ionic retardation [23].The solvent effect results favour the formation of the transition state. Water molecules attack the neutral species at the P-O bond-fission with the biomolecular attack of water on phosphorus atom [24].This way, the reaction follows first-order kinetics and bimolecular mechanism. The order of the hydrolytic reaction is pseudo-first-order [25].

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