

## Hydrolysis of DI-2-Methoxy-4-Nitroaniline Phosphate in Buffer Media

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**Abstract:** Kinetic study of the hydrolysis of di-2-methoxy-4-nitroaniline phosphate has been investigated in the range of pH 0.00 to 7.80 in 20% (v/v) dioxane-water medium at  $70 \pm 0.5^\circ\text{C}$ . The pH log rate profile has a rate maximum at pH 4.10. Neutral and mononegative species are found to be reactive in the range of pH 0.00 to 2.20 and only mononegative species in the range of pH 2.20 to 7.80. The estimated rates agree well with those experimental rates. Bimolecular nature of hydrolytic reactions has been supported by Arrhenius parameters. Di-2-methoxy-4-nitroaniline phosphate involves P-N bond fission, which is strengthened by isokinetic relationship. Probable reaction mechanism has been proposed for the hydrolysis of di-ester *via* its neutral and mononegative species.

**Key Words:** Hydrolysis, Di-2-methoxy-4-nitroaniline phosphate, Neutral and Mononegative species, Arrhenius parameters.

### Introduction

Phosphorus is a vital element both in living matter and in the earth's crust. The human body contains about 1% by weight of phosphorus, about 0.8% of this being present as hydroxyapatite in bones and teeth and the remaining phosphorus present as organic phosphates, which are in various forms such as monoesters, diesters, and triesters according to the number of ester bonds<sup>1,2</sup>. Organic phosphate esters are important due to their broad ranges of biological properties and presence in bioactive molecules, such as natural products, amino acid analogues, pharmacological agents, and synthetic precursors<sup>3</sup>. These phosphate motifs are often used as prodrugs to enhance the water solubility or therapeutic potential of parent drugs<sup>4-6</sup>. Cyclic amine phosphate in combination with phosphoramidic acid and sulfa drugs are very important for pharmaceutical and medicinal value and as cancer chemotherapeutic agents<sup>7</sup>. Organic phosphates are important surfactants, which are widely used in many fields, such as chemical fibers, spinning and weaving, plastic, paper-making and daily chemical products<sup>8-12</sup>. Due to their surfactant properties<sup>13</sup>, they are also used as an additive in the textile and clothing dyeing industry, as well as a solvent for the extraction of uranium and plutonium from other radionuclides in nuclear fuel processing<sup>14</sup>. The hydrolysis of phosphate diester bonds, such as those found in DNA and RNA, is a reaction of fundamental importance in living systems<sup>15</sup> and it is also significant to elucidate the mechanism of such reactions in solution and in proteins<sup>16</sup>. Phosphate esters and their hydrolysis reactions underpin many of the most important reactions in biology and have therefore been the focus of continued research during the past few decades<sup>17</sup>.

### Experimental

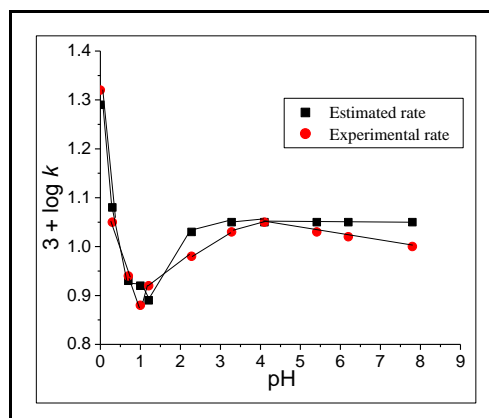
Di-2-methoxy-4-nitroaniline phosphate has been prepared from 2-methoxy-4-nitroaniline and phosphorus oxychloride by the method described earlier<sup>18</sup>. All the chemicals used were of A. R. grade and all solutions have been prepared in triply distilled water. All the reactions have been carried out at the  $70 \pm 0.5^\circ\text{C}$  employing  $5 \times 10^{-4}$  mole  $\text{dm}^{-3}$  solution of the diester in 20% (v/v) dioxane-water medium. Buffer solutions were

maintained using appropriate mixture of KCl, COOH.C<sub>6</sub>H<sub>4</sub>.COOK, NaOH and H<sub>3</sub>BO<sub>3</sub><sup>19</sup>. Inorganic phosphate produced during hydrolysis has been estimated spectrophotometrically by Allen's modified method<sup>20</sup>.

## Results and Discussion

### Hydrolysis via neutral and mononegative species

Kinetic study of hydrolysis of di-2-methoxy-4-nitroaniline phosphate has been studied in the range of pH 0.00 to 7.80 at 70°C in 20% (v/v) dioxane-water medium. Figure-1 shows the pH log rate profile in which estimated rates closely agreed with those experimental rates.



**Figure 1 – pH log rate profile for the hydrolysis of di-2-methoxy-4-nitroaniline phosphate**

In the entire pH range the hydrolysis of di-2-methoxy-4-nitroaniline phosphate has been found to be governed by two reactive species namely neutral and mononegative species. Both species are participating in hydrolysis but the reactive nature of neutral species decreases due to its conversion into mononegative species. The appearance of mononegative species starts at pH 0.00 and attains maximum value at pH 4.10. The hydrolysis after pH 4.10 has been found to be exclusively governed by mononegative species. The rise in rates in higher pH range (more than pH 1.00) is due to the incursion of more reactive mononegative species while the rise in rates in lower pH range (less than pH 1.00) is attributed to incursion of neutral and conjugated acid species. Similar nature of hydrolysis has been observed in the case of 2, 4 dinitrophenyl hydrazo phosphate<sup>21</sup>. Neutral and mononegative rates may be represented as:

$$k_N = k_{N_0} \frac{N}{N+M} \quad (1)$$

$$k_M = k_{M_0} \frac{M}{M+N} \quad (2)$$

Where  $k_{N_0}$  is specific neutral rate,  $k_{M_0}$  (specific mononegative rate) is experimental rate at pH 4.10 and,  $N/N+M$  and  $M/M+N$  are the fraction of neutral and mononegative species, respectively. The value of specific neutral rate i.e.  $k_{N_0}$  was determined from the reaction:

$$k = k_{M_0} \frac{M}{M+N} + k_{N_0} \frac{N}{N+M} + k_{H^+} \cdot C_{H^+} \quad (3)$$

Where,  $k$  is experimental rate. There is good agreement of specific neutral rate  $k_{N_0}$  values obtained by eq. (3) and ionic strength data. The value of  $k_{N_0}$  determined by eq. (3) is  $3.83 \times 10^{-3} \text{ min}^{-1}$  at different pH from 0.00 to 1.00 and the value of  $k_{N_0}$  obtained from ionic strength data is  $3.54 \times 10^{-3} \text{ min}^{-1}$ .

### Hydrolysis via mononegative species

Mono and di-esters are known to dissociate almost completely at pH 4.10, thus in range of pH 4.10 to 7.80 mononegative species of di-esters are reactive. Since they are not further converted in to dinegative species

as mononegative rates are directly proportional to the concentration of their ions, the experimental rates (pH log rate profile Figure-1) also show consistency of the rate of hydrolysis of the di-ester. A slight elevation in rates at pH 5.41 may be attributed to incursion of more nucleophilic hydroxide ion than water molecule. Similar observation has also been made for the hydrolysis of di-phenyl phosphate and di-*p*-chloro phenyl phosphate<sup>22</sup>. It is clear from Table-1 that in the range of pH 0.00 to 1.00; the hydrolysis governs by neutral, conjugate and mononegative species. In the range of pH 1.00 to 1.21, the reactions are *via* neutral and mono negative species. In the range of pH 1.21 to 7.80, only mononegative species are reactive. Kinetic rate laws for the hydrolysis of di-2-methoxy-4-nitroaniline phosphate may be represented as:

**Table-1 Estimated & experimental rates of the hydrolysis of di-2-methoxy-4-nitroaniline phosphate *via* neutral and mononegative species at different pH values**

pH	M/M+N	N/N+M	$k_M \times 10^3$ (min <sup>-1</sup> )	$k_N \times 10^3$ (min <sup>-1</sup> )	$k_{H^+} \cdot C_{H^+} \times 10^3$ (min <sup>-1</sup> )	$k \times 10^3$ (min <sup>-1</sup> ) (Estd.)	$k \times 10^3$ (min <sup>-1</sup> ) (Expt.)	3+logk (Estd.)	3+logk (Expt.)
0.00	0.06	0.94	0.68	3.60	15.14	19.42	21.06	1.29	1.32
0.30	0.12	0.88	1.36	3.37	7.41	12.14	11.23	1.08	1.05
0.70	0.25	0.75	2.83	2.87	2.86	8.56	8.80	0.93	0.94
1.00	0.40	0.60	4.54	2.30	1.45	8.29	7.67	0.92	0.88
1.21	0.54	0.45	6.12	1.72	-	7.84	8.38	0.89	0.92
2.28	0.92	0.09	10.43	0.34	-	10.77	9.56	1.03	0.98
3.28	0.99	0.01	11.23	0.04	-	11.27	10.72	1.05	1.03
4.10	1.00	0.00	11.34	0.00	-	11.34	11.34	1.05	1.05
5.41	1.00	-	11.34	-	-	11.34	10.83	1.05	1.03
6.20	1.00	-	11.34	-	-	11.34	10.47	1.05	1.02
7.80	1.00	-	11.34	-	-	11.34	10.12	1.05	1.00

In the range of pH 0.00 to 1.00

$$k = k_{H^+} C_{H^+} + 11.34 \times 10^{-3} \cdot M/(M+N) + 3.54 \times 10^{-3} \cdot N/(N+M)$$

In the range of pH 1.00 to 1.21

$$k = 11.34 \times 10^{-3} \cdot M/(M+N) + 3.54 \times 10^{-3} \cdot N/(N+M)$$

In the range of pH 1.21 to 7.80

$$k = 11.34 \times 10^{-3} \cdot M / (M+N)$$

### Solvent effect

Solvent effects play an important role in determining chemical reactivity. In particular, the rate of an elementary chemical reaction may change by order of magnitude when the solvent is changed<sup>23-27</sup>. Kinetic solvent effects on chemical reactions in different media are usually correlated in terms of "solvent polarity", which sums up all the specific and non-specific interactions of the media with initial and transition state<sup>28, 29</sup>. A change in solvent composition, or going from a protic to an aprotic solvent, can influence reactivity remarkably by affecting the transition state and ground state energy. According to Chanley's observation<sup>30</sup>, effect of solvent on the rate of hydrolysis may therefore, be taken to imply a bimolecular nucleophilic reaction with the formation of a transition state in which the charge is dispersed. Table-2 shows a significant rise in rates with increase in dioxane percentage.

**Table-2 Rate of the hydrolysis of di-2-methoxy-4-nitroaniline phosphate *via* neutral and mononegative species**

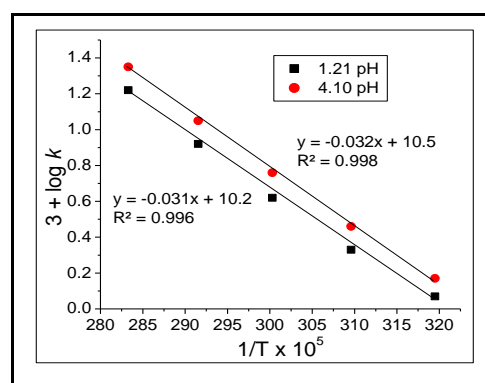
pH	Dioxane (%) V/V	$k \times 10^3$ (min <sup>-1</sup> )	pH	Dioxane (%) V/V	$k \times 10^3$ (min <sup>-1</sup> )
1.21	20.0	8.38	4.10	20.0	11.34
	30.0	9.29		30.0	12.28
	40.0	10.24		40.0	13.18
	50.0	11.17		50.0	14.10
	60.0	12.06		60.0	15.05

### Temperature effect

One of the most important relationships that provide much information to mechanism is that which connects the rate constant of a reaction with the temperature. The effect of temperature gives valuable information about the energy requirements of reaction. In order to determine the Arrhenius parameters, kinetic runs were carried out at different temperature in pH 1.21 and pH 4.10. Rate coefficients have been summarized in Table-3. Prediction of the molecularity of the reaction may be made by determining Arrhenius parameters. Figure-2 describes the Arrhenius plot between log rate coefficients and reciprocal of the absolute temperature. Linearity of the plot shows the validity of Arrhenius equation for the temperature range used. Arrhenius parameters for hydrolysis *via* neutral and mononegative species summarized in Table-4 are in favour of a bimolecular reaction<sup>31</sup>.

**Table-3 Rate of the hydrolysis of di-2-methoxy-4-nitroaniline phosphate *via* neutral and mononegative species**

pH	1/T X 10 <sup>5</sup> (K)	k x 10 <sup>-3</sup> (min <sup>-1</sup> )	pH	1/T X 10 <sup>5</sup> (K)	k X 10 <sup>-3</sup> (min <sup>-1</sup> )
1.21	319.49	2.61	4.10	319.49	4.31
	314.47	3.42		314.47	6.54
	309.60	4.61		309.60	7.90
	304.88	5.63		304.88	11.03
	300.30	7.93		300.30	13.88



**Figure 2 - Arrhenius plot for the hydrolysis of di-2-methoxy-4-nitroaniline phosphate at pH 1.21 and pH 4.10**

**Table-4 Arrhenius parameters for the hydrolysis of di-2-methoxy-4-nitroaniline phosphate *via* neutral & mononegative species**

pH	Slope	Parameters		
		E (kcal mol <sup>-1</sup> )	A (sec <sup>-1</sup> )	-ΔS <sup>‡</sup> (e.u.)
1.21	-0.031	14.18	8.91 x 10 <sup>6</sup>	29.01
4.10	-0.032	14.64	2.45 x 10 <sup>7</sup>	27.00

Di-2-methoxy-4-nitroaniline phosphate may undergo hydrolysis either by P-N or C-N bond fission. Table-5 and 6 summarizes comparative kinetic rate data<sup>32</sup> for the hydrolysis of other diesters. The point of di-2-methoxy-4-nitroaniline phosphate lies on the linear curve of those diesters which are known to undergo hydrolysis *via* P-N bond fission (Figure not shown). Thus P-N rather than C-N bond fission appears to be more probable.

**Table-5 Comparative kinetic rate data for the hydrolysis of some phosphate diester via neutral species**

S. No	Phosphate Diesters	pH	E (k cal mol <sup>-1</sup> )	-ΔS <sup>‡</sup> (e. u.)	Molecularity	Bond fission
1.	<i>p</i> -toluidine	1.24	12.81	37.29	2	P-N
2.	2,4-dinitro phenyl hydrazo phosphate	1.24	11.44	49.63	2	P-N
3.	<i>o</i> -toluidine	1.27	12.31	36.72	2	P-N
4.	<b>2-methoxy-4-nitroaniline</b>	1.21	14.18	29.01	2*	<b>Present work</b>
5.	2-chloroaniline	1.24	13.70	34.60	2	P-N
6.	<i>m</i> -toluidine	1.24	13.73	32.88	2	P-N

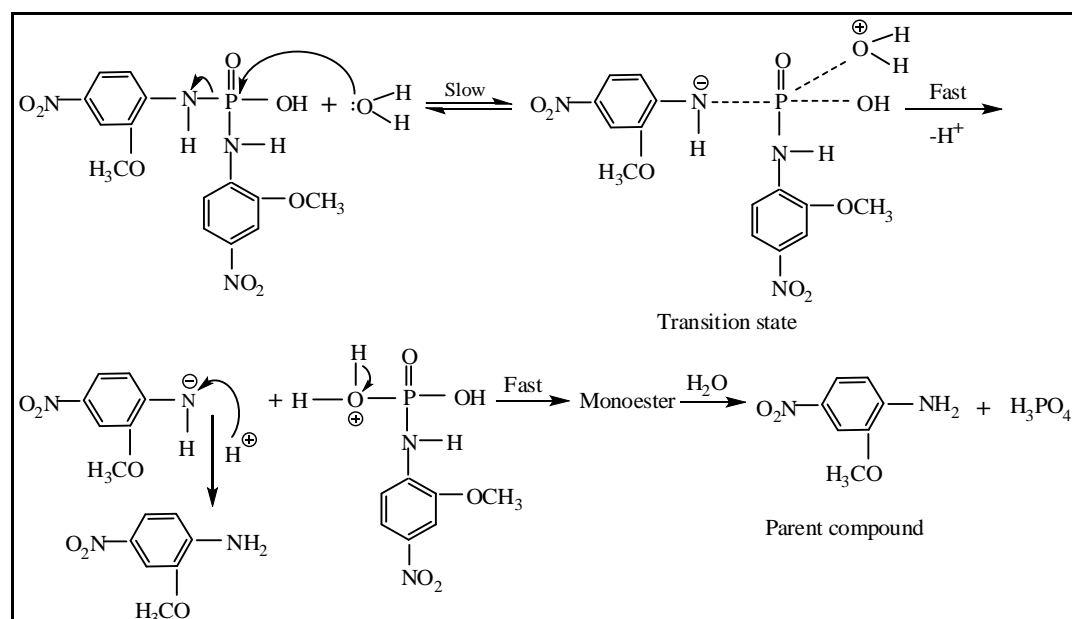
**Table-6 Comparative kinetic rate data for the hydrolysis of some phosphate diester via mononegative species**

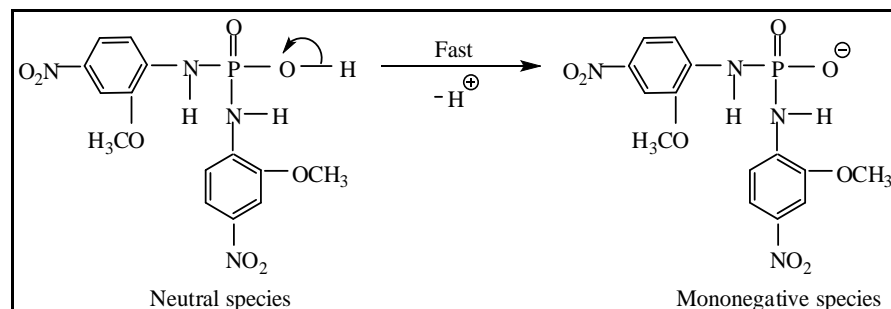
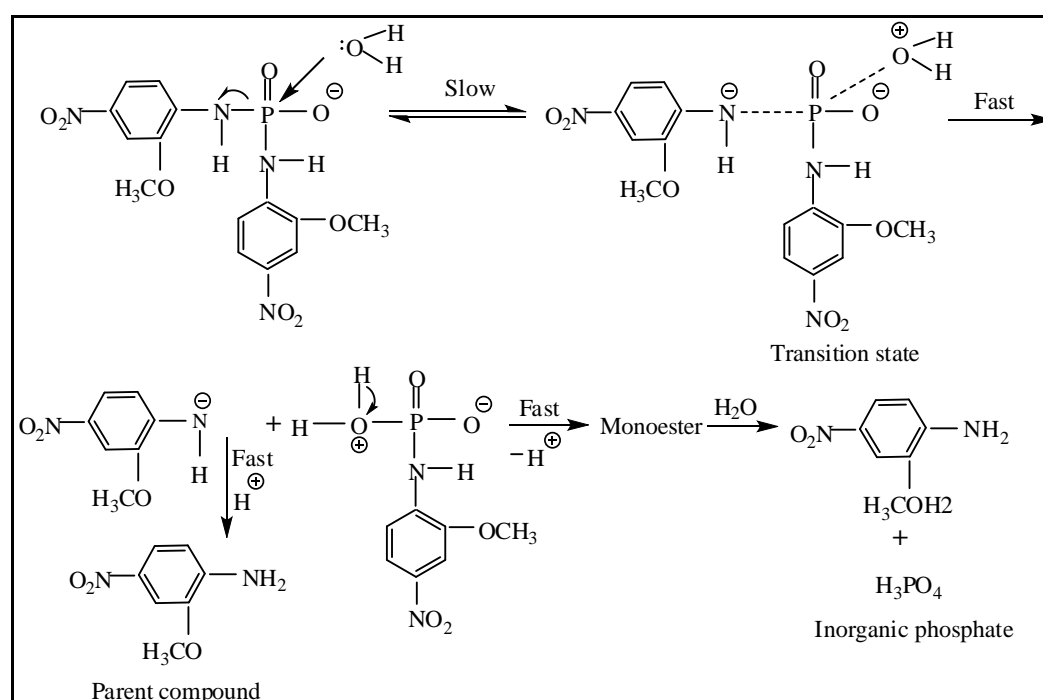
S. No.	Phosphate Diesters	pH	E (k cal mol <sup>-1</sup> )	-ΔS <sup>‡</sup> (e. u.)	Molecularity	Bond fission
1.	<i>p</i> -toluidine	4.17	9.25	62.18	2	P-N
2.	2,4-dinitro phenyl hydrazo phosphate	4.17	10.98	50.22	2	P-N
3.	<i>p</i> -chloroaniline	4.17	9.60	54.78	2	P-N
4.	<b>2-methoxy-4-nitroaniline</b>	4.10	14.64	27.00	2*	<b>Present work</b>
5.	<i>m</i> -toluidine	4.17	14.18	30.81	2	P-N

The probable reaction mechanism for the hydrolysis of mononegative and neutral species of di-2-methoxy-4-nitroaniline phosphate may be suggested as shown below:

#### Chart-1: The mechanism of hydrolysis via neutral species:

Bimolecular attack of water on phosphorus of the neutral species S<sub>N</sub><sup>2</sup> (P)



**Chart-2: The mechanism of hydrolysis via mononegative species:****(a) Formation of mononegative species****(b) Bimolecular nucleophilic attack of water on phosphorous of mononegative species  $S_N^2(P)$** **Conclusion**

Di-2-methoxy-4-nitroaniline phosphate in pH 1.00 to 7.80 was found to hydrolyze *via* neutral and mononegative species. The maximum value at pH 4.10 is due to hydrolysis *via* mononegative and neutral species. The pH log rate profile shows that the estimated rates closely agreed well with those experimental rates. Bimolecular nature of hydrolytic reactions has been supported by Arrhenius parameters. The diester involves P-N bond fission, which is strengthened by comparative kinetic rate data.

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