Kinetics of Hydrolysis of Di-2,3-dichloroaniline Phosphate in Buffer Media

Nisha Chhetri and S. A. Bhoite*

School of Studies in Chemistry, Pt. RavishankarShukla University, Raipur, Chhattisgarh, 492010, India

Abstract : In present investigation, kinetic study of hydrolysis of di-2,3-dichloroaniline phosphate has been carried out in pH range from 0.00 to 7.49 in 30% dioxane-water medium at $80^{\circ}C$ (± 0.5). The pH log rate profile shows rate maximum at pH 4.11. Neutral and mononegative species have been found to be reactive in the range of pH 0.00 to 2.21 and only mononegative species in the range of pH 2.21 to 7.49. The theoretical rates determined from Debye Huckel equation have been found in close agreement with the experimental rates.Molecularity and Bond fission have been discussed in terms of isokinetic relationship. Probable reaction mechanism has been proposed for the hydrolysis of di-ester *via* its neutral and mononegative species.

Keywords: Hydrolysis, Di-2,3-dichloroaniline phosphate, Neutral and Mononegative species, P-N bond fission, Bimolecularity.

Introduction:

Phosphate esters are fundamental molecules in cellular chemistry and have a wide range of applications in the industrial, agricultural and academic field owing to their unique biological and physicochemical properties as well as their utility as synthetic intermediates[1].Other significant applications are as environmentallydegradable pesticides due to their toxicity to a wide range of insects and their relatively short residence time in the environment[2].The phosphate esters have also found application as surfactants and as brighteners in detergents[3].The cyclic and acyclic phosphate esters are normally considered as important pharmacological compounds[4-7]. Nucleoside derivatives of phosphate esters have found to be extremely important agents for anticancer and antiviral therapy[8].Prodrugs of phosphate esters have been successfully utilized to overcome a variety of drug delivery problems[9].Phosphate ester hydrolysis is a crucially important process for several biological systems, as it is involved in energy and signal transduction processes, the control of cellular metabolism, and the regulation of protein function[10, 11].Phosphate diesters play an essential role in biology, particularly as the backbone of DNA and RNA and therefore its hydrolysis represents a reaction of fundamental importance in living systems[12, 13].

Experimental:

Di-2,3-dichloroaniline phosphate was prepared from the reaction of 2,3-dichloroaniline with phosphorylating agent phosphorus oxychloride ($POCl_3$) by the procedure described earlier[14]. Characterization of the compound was done by melting point determination, elemental and IR spectral analysis techniques.Hydrolysis of di-2,3-dichloroaniline phosphate were carried out in the pH range 0.00-7.49 in 30%

dioxane-water (v/v) mixture at 80°C ($\pm$0.5) employing 5×10^{-4}mol dm^{-3} solution of diester. Buffer solutions were prepared using appropriate mixtures of KCl, COOH.C$_6$H$_4$.COOK, NaOH and H$_3$BO$_3$[15].

Inorganic phosphate produced during the hydrolysis was determined by Allen's modified method[16]spectrophotometrically. Phosphomolybdate complex[(NH$_4$)$_3$PO$_4$.12MoO$_3$] is formed by inorganic phosphate with ammonium molybdate. This complex is reduced to molybdenum blue, a soluble complex, by addition of 2,4-diaminophenol dihydrochloride solution. The blue colour so generated is fully developed within 10 minutes. The intensity of blue colour is directly proportional to the amount of the free phosphoric acid. All the chemicals used were of AR grade.

Results and Discussion:

Hydrolysis via neutral and mononegative species

Kinetics of hydrolysis of di-2,3-dichloroaniline phosphate was carried out in pH range from 0.00-7.49 at 80°C ($\pm$0.5)in 30% dioxane-water (v/v) medium. In this pH range reaction was 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 4.11. The hydrolysis at the latter pH range was found to be exclusively governed by mononegative species.
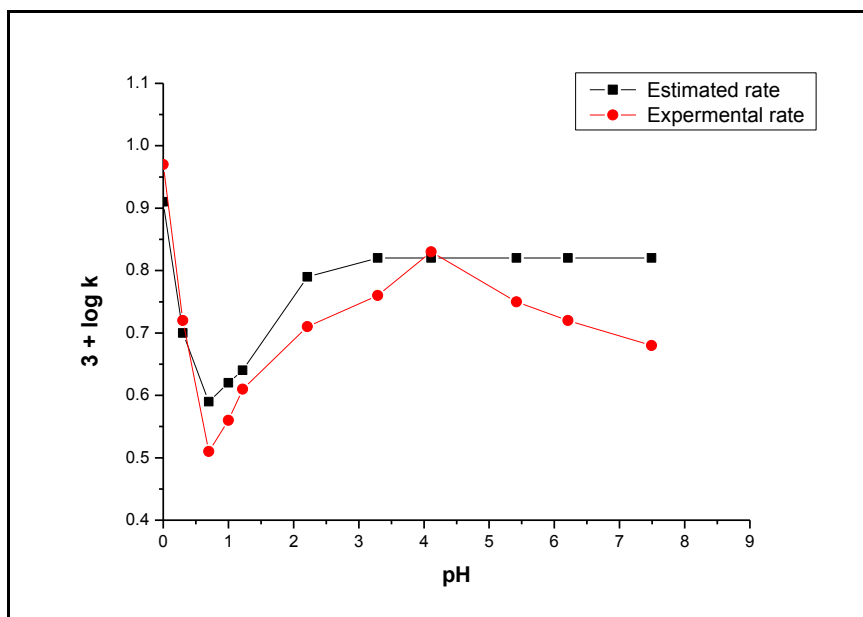

Fig.1: pH log rate profile for the hydrolysis of di-2,3-dichloroaniline phosphate

The rise in rate in higher pH range (more than pH 1.00) is due to the incursion of more reactive mononegative species while the rise in rate in lower pH range (less than pH 1.00) is attributed to the incursion of neutral and conjugate acid species. Similar nature of hydrolysis has been observed in the case of *m*-toluidine phosphate[17].Neutral and mononegative rates may be represented as:

$$k_N = k_{No} \frac{N}{N + M} \tag{1}$$

$$k_M = k_{Mo} \frac{M}{M + N} \tag{2}$$

Where k_{No} is specific neutral rate, k_{Mo} is the experimental rate (specific mononegative rate) at pH 4.11 and N/(N+M), M/(M+N) are the fraction of neutral and mononegative species, respectively. The value of specific neutral rates (k_{No}) was determined from the following equation:

$$k = k_{Mo} \frac{M}{M+N} + k_{No} \frac{N}{N+M} + k_{H^+} C_{H^+} \qquad (3)$$

Where,k is experimental rate. There is a good agreement between values of specific neutral rate k_{No} determined by equation (3) and the ionic strength data. The value of k_{No} determined byequation (3) is 1.55×10^{-3} min^{-1} at different pH from 0.00 to 1.00 and the value of k_{No} obtained from ionic strength data is 1.59×10^{-3} min^{-1}.

Hydrolysis via mononegative species:

Mono- and diesters are known to dissociate almost completely at pH 4.00, thus in pH range 4.00 to 7.00 mononegative species of diester is reactive. Since they are not further converted into dinegative species as mononegative species of monoester do, their concentration remain constant. As the mononegative rates are directly proportional to the concentration of their ions, the experimental rate of hydrolysis in this range is expected to be constant. The experimental rates (pH log rate profile fig.1) also show consistency of the rate of hydrolysis of the diester. A slight elevation in rates at 5.42 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*-chlorophenyl phosphate[18].

It is clear from the results (Table 1) that in the pH range 0.00 to 1.00, hydrolysis governs by neutral, conjugate acid and mononegative species. In the pH range 1.00 to 1.22, the reaction moves forward via neutral and mononegative species. In the pH range 1.22 to 7.49, only mononegative species are reactive.

Kinetic rate laws for the hydrolysis of di-2,3-dichloroaniline phosphate may be represented as:

I. In the region pH 0.00 to 1.00

$$k = k_{H^+} C_{H^+} + 6.68 \times 10^{-3} \frac{M}{M+N} + 1.55 \times 10^{-3} \frac{N}{N+M} \qquad (4)$$

II. In the region pH 1.00 to 1.22

$$k = 6.68 \times 10^{-3} \frac{M}{M+N} + 1.55 \times 10^{-3} \frac{N}{N+M} \qquad (5)$$

**III. In the region pH 1.22 to 7.49**

$$k = 6.68 \times 10^{-3} \frac{M}{M+N} \qquad (6)$$

Table 1. Estimated and experimental rates for the hydrolysis of di-2,3-dichloroaniline phosphate via neutral and mononegative species at different pH values

pH	N/N+M	M/M+N	$k_M \times 10^3$ (min^{-1})	$k_N \times 10^3$ (min^{-1})	$k_H^+.C_H^+ \times 10^3$ (min^{-1})	$k \times 10^3$ (min^{-1}) (Estd.)	$k \times 10^3$ (min^{-1}) (Expt.)	3+log$k$(Estd.)	3+log$k$(Expt.)
0.00	0.937	0.063	0.42	1.487	6.22	8.13	9.38	0.91	0.97
0.30	0.881	0.119	0.80	1.399	2.83	5.03	5.30	0.70	0.72
0.70	0.747	0.253	1.67	1.186	1.07	3.93	3.25	0.59	0.51
1.00	0.597	0.403	2.68	0.948	0.52	4.15	3.63	0.62	0.56
1.22	0.450	0.540	3.61	0.714	-	4.33	4.12	0.64	0.61
2.21	0.085	0.915	6.12	0.135	-	6.26	5.22	0.79	0.71
3.29	0.007	0.993	6.64	0.011	-	6.65	5.80	0.82	0.76
4.11	0.001	0.999	6.68	0.001	-	6.68	6.69	0.82	0.83
5.42		0.999	6.68			6.68	5.56	0.82	0.75
6.21		0.999	6.68			6.68	5.21	0.82	0.72
7.49		0.999	6.68			6.68	4.76	0.82	0.68

Molecularity of hydrolytic reaction

Temperature Effect:

Prediction of molecularity of the reaction may be made by determining Arrhenius parameters. The plot between log rate constant and reciprocal of absolute temperature at pH 1.22 and 4.11 is described (Fig. 2). Linearity of the plot shows the validity of Arrhenius equation for temperature range used from 70-90°C.The obtained values of Arrhenius parameters for hydrolysis via neutral and mononegative species are in favour of a bimolecular nature of reaction[19] (Table 2).
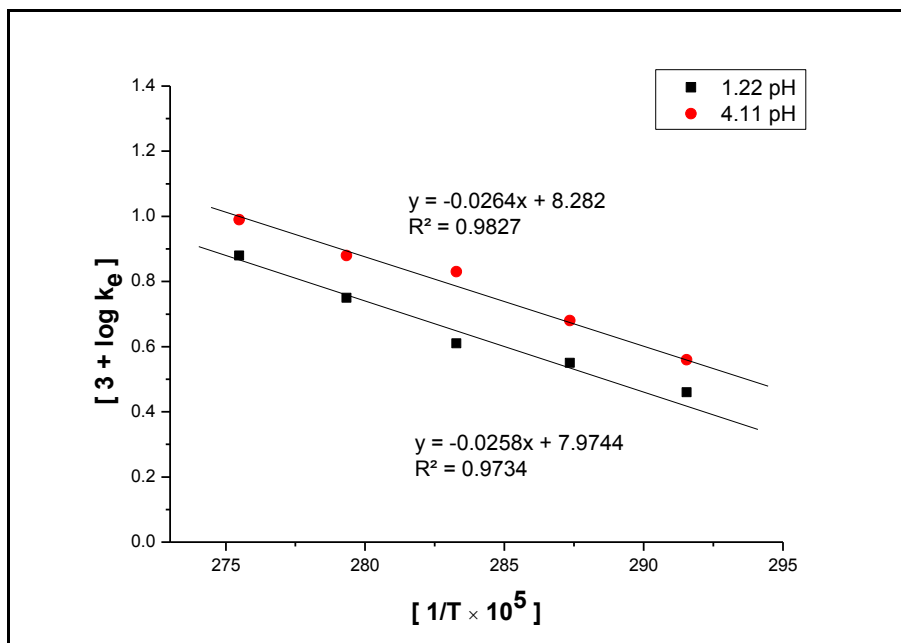
Fig. 2. Arrhenius plot for the hydrolysis of di-2,3-dichloroaniline phosphate

Table 2. Arrhenius parameters for the hydrolysis of di-2,3-dichloroaniline phosphate via neutral and mononegative species

pH	Slope	E_a (Kcal/mol)	A (Sec^{-1})	$-\Delta S^{\neq}$ (e.u.)
1.22	-0.0258	11.82	8.66×10^4	38.29
4.11	-0.0264	12.09	2.06×10^5	36.56

Effect of Solvent:

Solvent effect has been studied by taking different compositions of 1,4-dioxane-water mixture which shows that rate of reaction increase with increase in percentage of 1,4-dioxane i.e. with polarity of the medium (Table 3). Effect of solvent on rate of hydrolysis indicates the formation of a transition state in which charge is dispersed[20].

Table 3. Rate of the hydrolysis of di-2,3-dichloroaniline phosphate via neutral and mononegative species at different percentage of 1,4-dioxane

pH	1,4-dioxane (v/v)	$k_e \times 10^3$ (min^{-1})	pH	1,4-dioxane (v/v)	$k_e \times 10^3$ (min^{-1})
1.22	0.00	4.12	4.11	0.00	6.69
	5.00	4.35		5.00	6.72
	10.0	4.75		10.0	7.10
	15.0	4.98		15.0	7.43
	20.0	5.48		20.0	7.97

Bond fission of hydrolytic reaction:

Di-2,3-dichloroaniline phosphate may undergo hydrolysis either by P-N or C-N bond fission. Comparative kinetic rate data[21]for the hydrolysis of diesters studied kinetically shows isokinetic relationship. The point of di-2,3-dichloroaniline phosphate is collinear with those diesters which are known to undergo hydrolysis via P-N bond fission (Fig. 3 & 4). Thus P-N bond fission rather than C-N bond fission appears to be more probable. The probable reaction mechanism for the hydrolysis of neutral and mononegative species of di-2,3-dichloroaniline phosphate may be suggested as shown in Chart-1 & Chart-2.
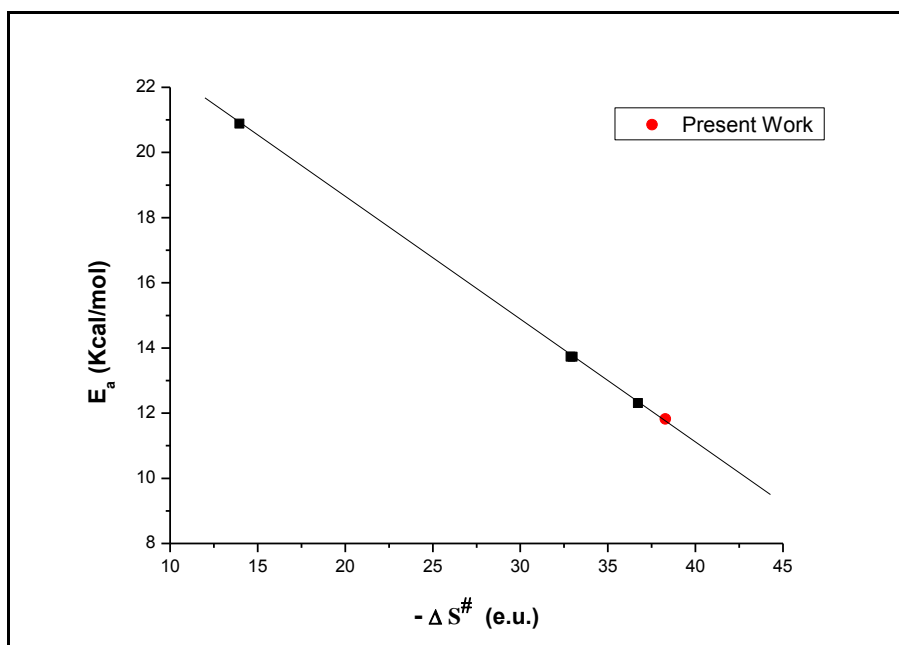

Fig. 3. Isokinetic relationship plot for the hydrolysis of some phosphate diesters via their neutral species
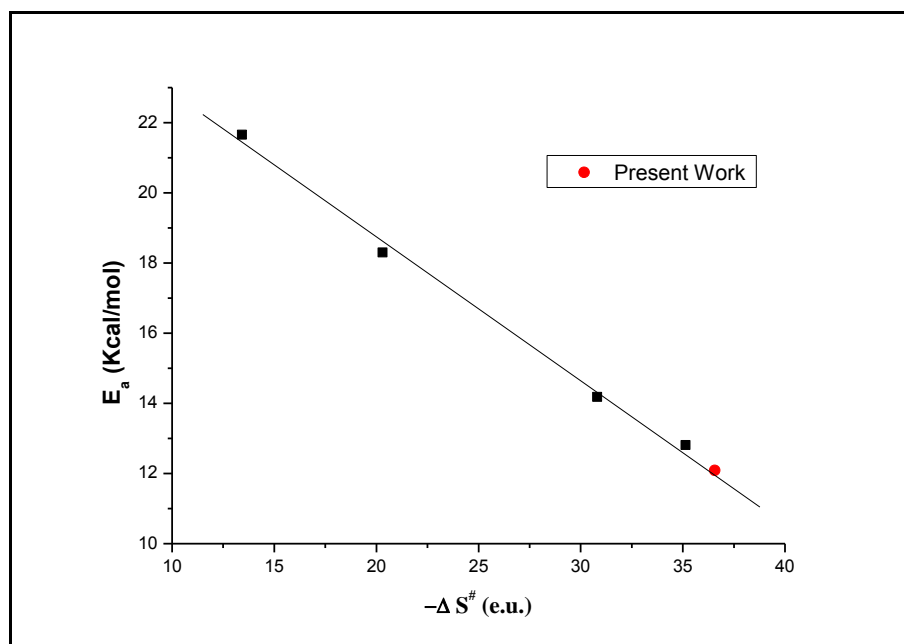
Fig. 4. Isokinetic relationship plot for the hydrolysis of some phosphate diesters via their mononegative species
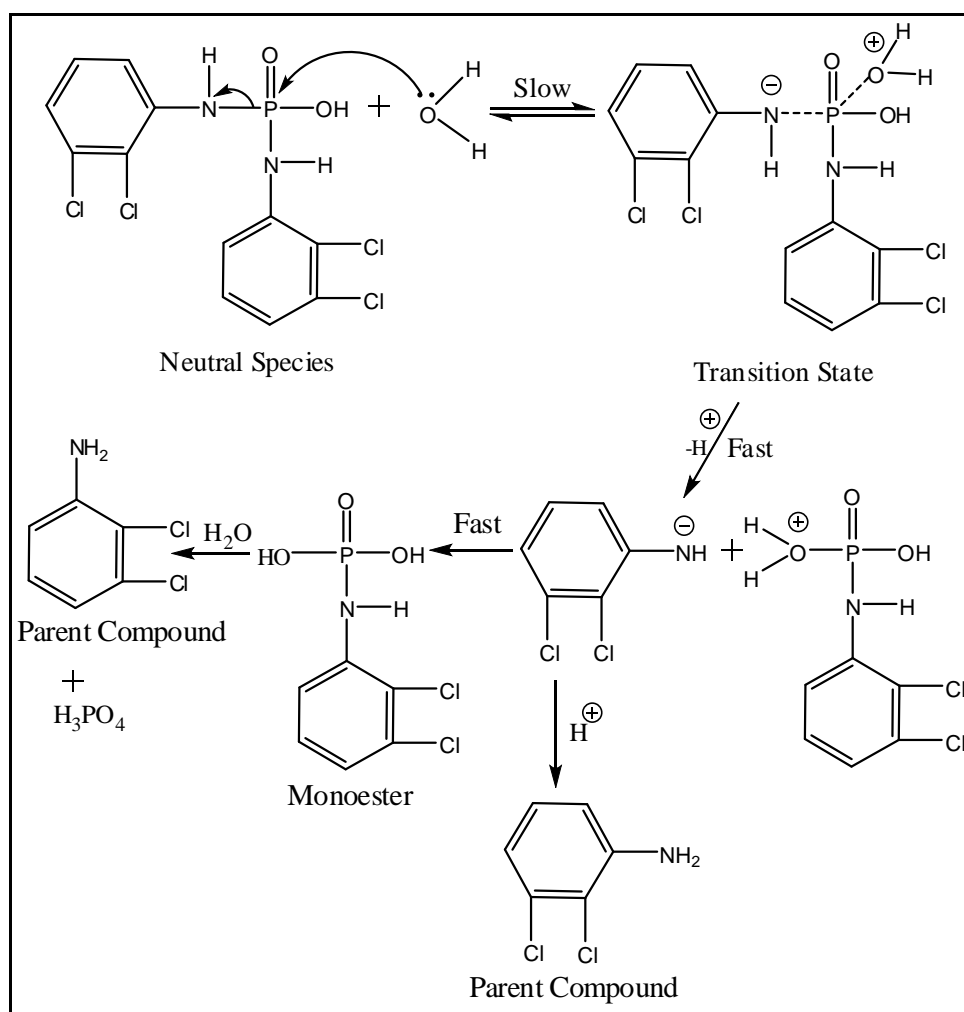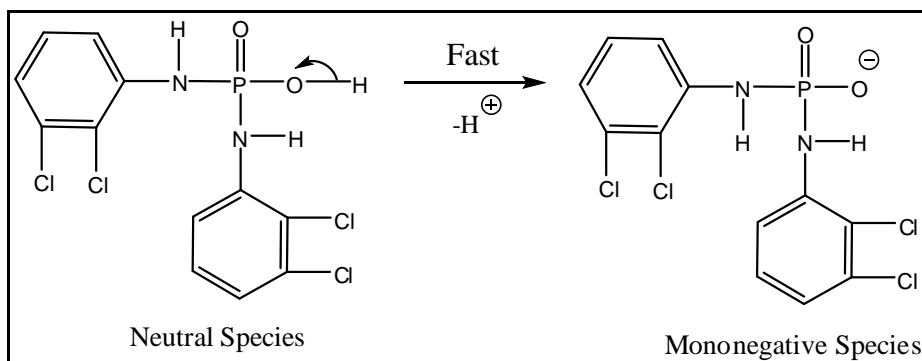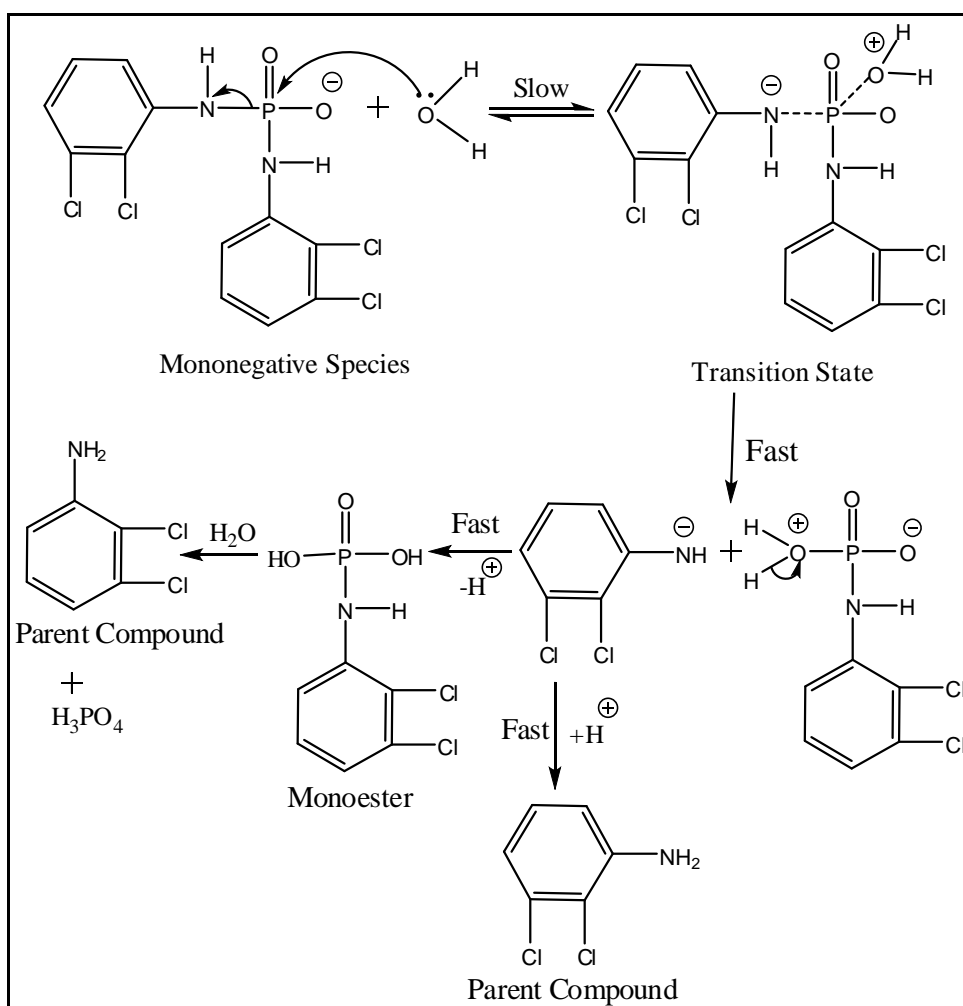

Chart-1: The Mechanism of hydrolysis of di-2,3-dichloroaniline phosphate via its neutral species: Bimolecular attack of water on phosphorus of the neutral species S_N^2 (P)

(a) Formaton of mononegative species

(b) Bimolecular nucleophilic attack of water on phosphorus of mononegative species S_N^2 (P).
Chart-2: The Mechanism of hydrolysis of di-2,3-dichloroaniline phosphate via its mononegative species:

Conclusion:

Di-2,3-dichloroaniline phosphate in pH 1.00 to 7.49 was found to hydrolyze via neutral and mononegative species. The maximum value at pH 4.11 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 strengthened by comparative kinetic rate data.

References:

1. Balakrishna A., Reddy C.S., Naik S.K., Manjunath M. and Raju C.N., "Synthesis, characterization and bio-activity of some new oe-aminophosphonates", Bull. Chem. Soc. Ethiop., 2009, 23(1), 69-75.
2. BarrD.B., Bravo R., Weerasekera G., Caltabiano L.M., Whitehead R.D., Needham L.L.,"Concentrations of dialkylphosphate metabolites of organophosphorus pesticides in the U.S. population", Environ. Health Perspect.2004, 112, 186–200.
3. Johnson D.W., Hils J.E., "Phosphate esters, thiophosphate esters and metal thiophosphates as lubricant additives", Lubricants, 2013, 1, 132-148.
4. Krishna B.S.,Rao A.J., Naidu K.R.M., Rao V.K., Raju C.N., "Synthesis and antibacterial activity studies of novel 2-substituted-1,3,2-oxazaphosphole-2-oxide derivatives of (S)-(+)-prolinol", Org.Commun., 2010, 3, 98-105.
5. Reddy K.R.K.K., Reddy C.B., Kumar K.S., Raju C.N., Reddy C.S.,"Iodine catalyzed and tertiarybutyl ammonium bromide promoted preparation of benzoxazaphosphininylphenylboronates",Org.Commun., 2009, 2, 28-33.
6. Reddy M.V.N., Annar S., Krishna A.B., Reddy G.C.S., Reddy C.S., "Tetramethyl guanidine (TMG)catalyzed synthesis of novel α-amino phosphonates by one-pot reaction", Org. Commun., 2010, 3, 39-44.
7. Kumar K.S., Reddy B., Reddy M.V.N., Reddy C.S., "Green chemical synthesis of α-hydroxyphosphonates", Org. Commun., 2012, 5 (2), 50-57.
8. Wagner C.R., Iyer V.V., McIntee E.J., "Pronucleotides: towards the in vivo delivery of antiviral and anticancer nucleotides", Med. Res. Rev., 2000, 20 (6), 417-51.
9. Gupta M.K., Jadhav S.K., Bhoite S.A., "Synthesis and in vitro antifungal activity of phosphate esters", Asian J. Chem., 2016, 28 (7), 1523-1527.
10. Cleland W.W., Henqqe A.C., "Enzymatic mechanisms of phosphate and sulfate transfer", Chem. Rev., 2006, 106 (8), 3252-78.
11. Kamerlin S.C.L., Wilkie J., "The effect of leaving group on mechanistic preference in phosphate monoester hydrolysis", Org. Biomol. Chem., 2011, 9, 5394.
12. Wentworth P., Janda K.D., "Antibody catalysis of phosphodiester hydrolysis: a survey of hapten strategies", Phosphorus, Sulfur and Silicon, 1999, 144-146, 247-250.
13. Kumar B., Tikariha D, Satnami M.L., Barbero N., Quagliotto P., Ghosh K.K., "Catalytic hydrolysis of phosphodiesters by nucleophilic ions in Gemini micellar media", J. Phys. Org. Chem., 2014, 27, 613-621.
14. Choure N., Bhoite, S.A., "Synthesis and kinetic study of hydrolysis of di-2-chloroaniline phosphate ester in acidic medium", Int. J. Chem. Kinet., 2010, 42, 126-131.
15. Sverre and Stene, Rec. Trav. Chemistry, 1930, 19, 1133.
16. Allen R.J.L., Biochem. J., 1940, 34, 858.
17. Awadhiya P., Bhoite S.A., "Kinetics of hydrolysis of di-m-toluidine phosphate in buffer media", ActaCienciaIndica, 2009, XXXV (5), 459-465.
18. Patil R., Shinde C.P., Dass K.B., Chauhan J.S., "Kinetic study and mechanism of mono phenyl hydrazo phosphate (Ba-Salt) via its conjugate acid species", Asian J. Chem., 1996, 8 (2), 197-200.
19. Long and Pritchrd, "Some buffer solutions with low nucleophilic activity" J. Am. Chem. Soc., 1957, 79,2365-2368.
20. Chanley J.D. and Feageson E., J. Am. Chem. Soc., 1955,80, 2686.
21. Gillion R.D., "Introduction to Physical Organic Chemistry", Addison-Wesley, 1970, 167.

*****