CHAPTER IV: S\textsubscript{N}2 REACTIONS OF ORGANOPHOSPHATE AND THIOPHOSPHATE WITH N-HYDROXYAMIDES IN CATIONIC MICELLAR MEDIA\#.

4.0 INTRODUCTION

Synthetic organophosphates (OPs) are among the most toxic substances known. These neurotoxic chemicals are used not only as pesticides and insecticides but also as chemical warfare agents. \textit{p-Nitrophenyl diphenyl substituted} OPs, such as methyl parathion, parathion and paraoxon are some of the most widely used pesticides in agriculture. These esters are potent acetylcholinesterase (AChE) and butyrylcholinesterase inhibitors. Due to their biological and environmental significance, their degradation has been extensively investigated using different hydrolysing \textit{α}-nucleophiles\textsuperscript{1-21}. The active OPs block AChE at the synapses. The extensive usage of these pesticides generates large volumes of excess aqueous pesticide-containing waste.

Although some very effective methods are available for the detoxification of organophosphates, none of them is well suited to all classes of these compounds. Due to their extreme toxicity, there are immediate needs for innovative analytical and environmental friendly methods to decompose neurotoxic phosphate esters. Nucleophilic hydrolysis and oxidation\textsuperscript{22-26} are the most preferred reactions to detoxify them. However, the high toxicity and licensing problems associated with such compounds mandates that most university research laboratories employ simulants instead of the actual target compounds.

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4.1 REVIEW OF THE EARLIER WORK

Organized assemblies and biomimetic models containing nucleophiles have been extensively employed as potent esterolytic agents. Micelles and other association colloids act as self-assembled microreactors, compartmentalizing, concentrating or separating and diluting reactants thereby altering rate equilibrium constants of chemical reactions. Most of these catalytic processes proceed with the attack of nucleophiles on the acyl carbonyl and phosphate groups. Consequently such processes are potentiated by performing the reactions in aggregates such as cationic micelles or vesicles which assist in bringing together nucleophilic reagents and hydrophobic substrates. These aqueous surfactant dispersions such as micelles, microemulsions, vesicles etc. along with their ability to support the rate enhancement of esterolytic reactions, also provide the best means for solubilization of hydrophobic compounds in aqueous media. Enhanced nucleophilic reactivity is generally governed by micellar effects and nature of the particular nucleophile. The nucleophiles exhibiting α-effect are oximate (R₂C=NO⁻)¹,², hydroxamate (RC(O)NHO⁻)³⁻⁵, peroxides (ROO⁻)⁶⁻⁹, hydroxylamine (NH₂OH)¹⁰⁻¹², hydrazine (RNH-NH₂)¹³, hypochlorite¹⁴, o-iodosylcarboxylates¹⁵⁻¹⁸ hydroxybenzotriazoles¹⁹,²⁰ and tetrazoles²¹ etc. N-hydroxyamides are structural analogue of hydroxamic acids with N-OH functional group.

4.2 PRESENT INVESTIGATION

It is the quest for a more complete understanding of biologically important phosphoryl transfer reactions that drives the continuing mechanistic studies of nucleophilic substitution at P=O ans P=S centres.²⁷⁻²⁹

In the preceding chapters, catalytic hydrolysis of carboxylic and phosphoric acid esters were studied in various cationic surfactants.
The present chapter focuses on the nucleophilic decomposition of PNPDPD, paraoxon and parathion (Scheme I) using N-hydroxyamides in cationic micellar media (Scheme II). Phosphate and phosphorothioate esters were employed in order to see effect of changing the electrophilic centre from P=O to P=S on the rates of reaction. The esterolytic cleavage of substrate were governed both by the strength of micellar binding and basicity of anionic nucleophiles used.

\[
\begin{align*}
\text{R}_2\text{O-} &\text{P-} &\text{O-} &\text{nitro} &\text{NO}_2 &\text{+ Nu}^- &\xrightarrow{\text{CTAX}} &\text{R}_2\text{O-} &\text{P-} &\text{Nu} &\text{+ O-} &\text{nitro} &\text{NO}_2 \\
\text{H}_5\text{C}_2\text{O-} &\text{P-} &\text{O-} &\text{nitro} &\text{NO}_2 &\text{OC}_2\text{H}_5 & &\text{H}_5\text{C}_2\text{O-} &\text{P-} &\text{S-O-} &\text{nitro} &\text{NO}_2 &\text{OC}_2\text{H}_5 \\
\text{R}_1 = \text{R}_2 = \text{C}_2\text{H}_5, Z = \text{O}, \text{PARAOXON} & &\text{R}_1 = \text{R}_2 = \text{C}_2\text{H}_5, Z = \text{S}, \text{PARATHION} \\
\text{C}_6\text{H}_5\text{O-} &\text{P-} &\text{O-} &\text{nitro} &\text{NO}_2 &\text{OC}_6\text{H}_5 \\
\text{R}_1 = \text{R}_2 = \text{C}_6\text{H}_5, Z = \text{O}, \text{PNPDPP} \\
\text{Nu}^- & &
\end{align*}
\]

\[\text{N-hydroxysuccinimide} \quad \text{N-hydroxyphthalimide}\]

\textbf{Scheme- I}
CTAX = Cetyltrimethylammonium halide

\[
\text{CTA}^+ = n-C_{16}H_{33}N^+(CH_3)_3, \ X = Br^- \quad \text{CTAB}
\]

\[
\text{CTA}^+ = n-C_{16}H_{33}N^+(CH_3)_3, \ X = Cl^- \quad \text{CTACL}
\]

Cetyltrimethylammonium bromide  Cetyltrimethylammonium chloride

Scheme II

4.3 EXPERIMENTAL

Paraoxon and parathion were prepared by literature method at the Vertox laboratory of Defence Research Development Establishment, Gwalior (Scheme III). All the esters were characterized by spectroscopic techniques. All other reactants (nucleophiles) and surfactants used were obtained from Sigma / Aldrich and are of the highest purity available commercially and were used as such without further purification. The concentrations of nucleophiles used were in the range of 0.5 – 1.83 mM. Due to the low solubility of PNPDP in water, its solution was prepared in 50% (v/v) acetonitrile.

\[
\text{Diethyl chloro phosphate} + \text{p-nitrophenol} \xrightarrow{\text{Triethylamine}} \text{p-nitrophenyl diethyl phosphate (Paraoxon)}
\]

Scheme III

Kinetic experiments were monitored by observing the rate of formation
of p-nitrophenoxide ion at 400 nm using Unicam UV-2 300 Spectrophotometer. All the experiments were performed at an ionic strength (μ) of 0.1M KCl. The values of rate constants were reproducible within 3%. The buffer solutions employed were phosphate and borate.

4.4 RESULTS AND DISCUSSION

The rate of hydrolyses of PNPDPP, paraoxon and parathion were studied spectrophotometrically with varying concentrations of N-hydroxyamides in micellar and non-micellar media at 27 °C under pseudo-first-order conditions. The first order rate constants, $k_{obs}$ were obtained from linear plots of $\log (A_x - A_o)/(A_x - A_t)$ versus time. The pH-dependent pseudo-first-order rate constants for PNPDPP, paraoxon and parathion cleavages at 27 °C were determined at different pH values between 8.0-12.4 by following the release of p-nitrophenoxide ion at 400 nm spectrophotometrically. The pH – rate constant profiles data for all the three esters under CTAB micellar condition are shown in Table 5.1. The observed first order rate constant increases with increasing pH values. At higher pH, the reaction was very fast and the rate of decomposition can not be measured.

The decomposition reaction is nucleophile concentration dependent. The reaction rate increases with increasing concentration of N-hydroxyamide. The linear nature of plot (rate-nucleophile concentration) with small intercept value shows true catalysis by the N-hydroxyamides against the hydrolysis of phosphate esters. The rate of hydrolytic reactions in aqueous media can be shown as;

$$k_{obs} = k_o + k_{Nu} [Nu^-]$$  \hspace{1cm} (1)

$$k_o = k_{H_2O} + k_{OH^-}[OH^-]$$  \hspace{1cm} (2)

N-hydroxyamides are α-nucleophiles, so that competition with other nucleophiles in particular H$_2$O and OH$^-$ is not expected.
Table 4.1

pH-dependent pseudo-first-order rate constants for the reactions of $N$-hydroxyamides with PNPDPP, paraoxon and parathion.

<table>
<thead>
<tr>
<th>pH</th>
<th>PARAOXON</th>
<th>PNPDP</th>
<th>PARATHION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NHS</td>
<td>NHP</td>
<td>NHS</td>
</tr>
<tr>
<td>8.00</td>
<td>a</td>
<td>a</td>
<td>2.30</td>
</tr>
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<td>9.00</td>
<td>a</td>
<td>a</td>
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<td>10.0</td>
<td>0.34</td>
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<td>5.47</td>
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<td>10.5</td>
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<td>–</td>
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<tr>
<td>11.0</td>
<td>1.22</td>
<td>1.56</td>
<td>14.3</td>
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<tr>
<td>11.5</td>
<td>2.11</td>
<td>2.35</td>
<td>b</td>
</tr>
<tr>
<td>12.0</td>
<td>7.51</td>
<td>3.60</td>
<td>b</td>
</tr>
<tr>
<td>12.4</td>
<td>13.3</td>
<td>15.6</td>
<td>b</td>
</tr>
</tbody>
</table>

Temp. 27°C [Substrate] = 1.0 x 10^{-4} M a = reaction is very slow
[Nu] = 1.0 x 10^{-3} M [Surfactant] = 1.0 x 10^{-3} M b = reaction is very fast
μ = 0.1 M KCl

4.4.1 Effect of Cationic Surfactant

Micellar effects represent the most prominent and extensively studied examples of surfactant influenced on the kinetics of organic reactions in water and aqueous-organic mixtures. These systems open wide prospects for controlling the rate of chemical transformations and the state of equilibria existing^{30-32}. The ability of
micellized surfactants to control rates of moderately slower reactions is well established. Acceleration of organic reactions in micelle solution is determined mainly by two factors i.e. concentration of the reactants in the micelle pseudophase and considerable increase in the rate of reaction.

Aqueous cationic surfactant solutions are known to accelerate the spontaneous hydrolysis of carboxylic and phosphate esters. The reactivity depends upon nature of the substrate, pH and type of nucleophile. The kinetic results of all the three substrates in different concentration of CTAB and CTACl are shown in Table 5.2. Reactivities in surfactant microorganized media is illustrated by results on micellar catalysis in micelles. Degradation of paraoxon and parathion by N-hydroxyamides, in presence of cationic surfactants i.e., cetyltrimethylammonium salts, CTAX is given in Table 5.2. As is apparent in Table 5.2, the degradation of substrates is accelerated by the presence of CTA\(^+\) surfactant in case of both nucleophiles, N-hydroxysuccinimide and N-hydroxyphthalimide. The rate constants increase with increase in surfactant concentration culminating in a maximum above the cmc value (Figure 4.1).

It has been amply demonstrated in a number of studies that at concentrations below the cmc, hydrophobic substrates induce the formation of submicellar aggregates where the reaction takes place. Above the cmc, the rate of decomposition of Paraoxon becomes double in case of N-hydroxysuccinimide and three times in case of N-hydroxyphthalimide at the rate maxima. The degradation of PNPDP in the presence of NHS and NHP is not very significant whereas in the case of paraoxon and parathion the observed first order rate constant increases with surfactant concentration.
Table 4.2

Kinetic rate data for the reaction of PNPDPD, Paraoxon and Parathion with N-hydroxyamides in cationic micellar media at 27^\textdegree C.

<table>
<thead>
<tr>
<th>[Surfactant] [mM]</th>
<th></th>
<th>k\textsubscript{obs.} 10\textsuperscript{4} / s\textsuperscript{-1}</th>
<th></th>
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<tr>
<td></td>
<td>NPNPDP</td>
<td>PARAOXON</td>
<td>PARATHION</td>
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<tr>
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<td>2.64</td>
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<td>1.92</td>
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<td>1.76</td>
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<td>12.6</td>
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<td>18.2</td>
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<td>1.68</td>
<td>1.87</td>
<td>10.0</td>
<td>14.2</td>
<td>12.1</td>
<td>–</td>
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\( a = \text{pH} \ 9.1 \quad b = \text{pH} \ 12.0 \quad \mu = 0.1 \text{ M KCl} \)
**Figure 4.1 (a)** Rate surfactant plots of $k_{\text{obs.}}$ (rate constant) vs. [surfactant] for the reaction of PNPDPP with NHS and NHP.

**Figure 4.1 (b)** Rate surfactant plots of $k_{\text{obs.}}$ (rate constant) vs. [surfactant] for the reaction of Paraoxon and Parathion with NHS and NHP.
The rate of reaction of paraoxon with N-hydroxyamide increases with increasing surfactant concentration, thus no rate maxima is observed. The rate of reaction for PNPDPP increases with increasing surfactant concentration up to maximum and then decreases. Paraoxon is much more hydrophilic in nature as compared to p-nitrophenyl diphenyl phosphate (PNPDPP). Rate data shows that the nucleophilic reaction of paraoxon is more significant with N-hydroxysuccinimide in aqueous media. But in micellar solution the reaction is not as significant as compared to N-hydroxyphthalimide. The nucleophilic reactivity in micelle depends upon the binding of substrate and interaction with anionic nucleophiles\(^41\). These reactions have an advantage in micellar media for solubility reasons since, the substrate, PNPDPP is highly hydrophobic.

The kinetic counterion effect is quite significant since the overall micellar effect, \(k_{\text{max}} / k_{\text{Nu}}\) is higher on changing from bromide to chloride for the reaction of paraoxon and PNPDPP. High concentration of anionic nucleophiles in the Stern layer of a cationic micelle implies that the surfactant counterion is readily transferred to the aqueous phase since the reaction must occur at this interface and the micellar surface cannot be oversaturated by anions\(^42\). The reactivity of N-hydroxyamides with Paraoxon and PNPDPP is higher in CTACl micelle because Cl\(^-\) counterion is readily exchangeable as compared to Br\(^-\) ion.
References


37. L. S. Romsted, C. A. Bunton and J. Yao, *Curr. Opin. Colloid Interface Sci.*, 128
1997, 2, 622.


