CHAPTER III

HYDROLYTIC CLEAVAGE OF PHOSPHODIESTERS WITH NUCLEOPHILES IN CATIONIC MICELLAR MEDIA

The cleavage of bis(p-nitrophenyl) phosphate (BNPP) over the pH range 7-12 has been studied in the presence of cationic micelles of cetyl diethylethanolammonium bromide (CDEEAB), cetyl dimethylethanolammonium bromide (CDMEAB), cetylpyridinium bromide (CPB), cetyltrimethylammonium bromide (CTAB) and cetylpyridinium chloride (CPC) using different $\alpha$-nucleophiles viz. acetohydroxamate (AHA$-$), benzohydroxamate (BHA$-$), salicylhydroxamate (SHA$-$), butane, 2, 3-dione monooximate (BDMO$-$) and $\alpha$-benzoin oximate ions. With $\alpha$-nucleophiles in cationic micellar media, hydrolytic cleavage of BNPP is approximately 10^5 fold faster than its spontaneous hydrolysis. All the reactions followed pseudo-first-order kinetics. The effect of various concentrations of cationic micelles for the reaction of BNPP and $\alpha$-nucleophiles has been studied. The variation of $k_{obs}$ values of the reactions depends on the micellar structure i.e., head groups, hydrophobic tail length and counter ion.

The catalytic hydrolysis of bis(p-nitrophenyl)phosphate (BNPP) and bis(2,4-dinitrophenyl)phosphate (BDNP) by $\alpha$-nucleophiles in gemini micellar media has been investigated at 27°C. The cationic gemini surfactants, i.e. alkanediyl-$\alpha$-$\omega$-bis(hydroxyethylmethyl-hexadecylammonium bromide (16-s-16 MEA 2Br, where s = 4 and 6) have been used. Nucleophilic reactivity of $\alpha$-nucleophiles such as hydroperoxide (HOO$-$), acetohydroxamate (AHA$-$) and butane 2, 3-dione monooximate ions (BDMO$-$) is compared. The kinetic data have been treated by applying the pseudophase model. The cationic gemini surfactants show unusual rate acceleration towards the cleavage of phosphodiesters with nucleophiles. These studies reveal that the hydroperoxide ion shows the highest catalytic activity reported so far with an unprecedented acceleration rate, 10^7 times faster than the uncatalyzed reaction. The possible mechanisms for the BNPP and BDNP cleavage promoted by $\alpha$-nucleophiles are proposed on the basis of kinetic analysis.

CHAPTER III: HYDROLYTIC CLEAVAGE OF PHOSPHODIESTERS WITH \(\alpha\)-NUCLEOPHILES IN CATIONIC MICELLAR MEDIA

3.0 INTRODUCTION

Hydrolysis and nucleophilic substitution reactions of phosphoesters are involved in many biological processes [1]. Extensive studies have been attempted to understand the mechanism of such crucial reactions [2-4]. Phosphodiesters are extremely stable compounds and are highly resistant toward hydrolytic cleavage at physiological pH. The effective catalyst for the cleavage of phosphodiester was employed to dissect the catalysis mechanism which is significant for directing the design of artificial phosphate diesterase [5]. The efficient catalysts would constitute a remarkable achievement particularly for biomedical applications as well as utility in the design of special scissors for gene therapy [6, 7]. In aqueous solution, the stability of DNA afforded by its phosphodiester linkages poses a problem in the development of sequence specific hydrolytic agents for DNA cleavage [8]. This high stability is in agreement with the fundamental role of DNA in the preservation of genetic information and underscores the importance of studies of the mechanism of phosphodiester hydrolysis [9-11]. Many \(\alpha\)-nucleophiles such as hydroxylamine [12], hydrazine [13], hydroxamic acid [4, 14] and oxime [15] are truly effective dephosphorylating agents for phosphodiesters and triesters. There are unshared electron pairs on the atom adjacent to the nucleophilic center of these \(\alpha\)-nucleophiles. It has been observed that cationic micellar media accelerate the reactivity of \(\alpha\)-nucleophiles [16-28]. Kinetic aspect of micellar catalysis [29] represents a significant contribution to the observed rate in this case of hydrolysis. Micellar rate effects have been quantitatively determined on the basis of pseudophase models [30, 31] that high local concentrations of nucleophiles are largely responsible for these rate acceleration in the interfacial micellar region [28]. It was observed that cationic micelles accelerate the spontaneous hydrolysis of bis(\(p\)-nitrophenyl) phosphate (BNPP) up to \(2\times10^5\)-fold [32] and accelerate the reaction of hydroxamates and phosphate triesters [28, 29, 31]. The reaction of \(\alpha\)-nucleophiles with phosphodiesters is expected to have a fundamental impact on the development of artificial possibly
sequence-specific nucleases for use in biotechnology as well as clean up operations involving organophosphorus toxins [33-34]. Metal complexes are well established as phosphodiester cleavage agents and in general their action is mediated by a powerful nucleophilic attack [35]. The reactivity of $\alpha$-nucleophiles is increased by incorporation with polymer and surfactants in aqueous media [36,37].

$$R = C_{16}H_{33}, C_{14}H_{29}, C_{12}H_{25}$$

Alkyldiethylethanolammonium bromide

$$X^- = Br^-, Cl^-$$

Cetylpyridinium halide (CPX)

$\text{Cetyltrimethylammonium bromide (CTAB)}$

3.1 REVIEW OF THE EARLIER WORK

In the past several years, different $\alpha$-nucleophiles i.e. hydroperoxide, hydroxamate, hydroxylamine, $\alpha$-iodosylcarboxylates and oximate ions have been used for the cleavage of phosphate esters in cationic micellar media [38-41]. It has been determined that surfactants provide better medium for cleavage of phosphate esters [42-43]. Cheng et al. [32] have studied one of the most effective metallomicellar ($Cu^{2+}$-CTAB) systems for hydrolysis of bis($\rho$-nitrophenyl) phosphate (BNPP). They found kinetic rate data $k_{obs}$, $3.23 \times 10^{-5}$ s$^{-1}$ (buffer + CTAB) at pH 7.12, are over ca. $10^5$ fold acceleration with micellar media than spontaneous. It is well known that micelles and similar colloidal assemblies generally increase the nucleophilic reactivity [44]. The structure and properties of surfactants play an important role in determining chemical reactivity.
The reaction of α-nucleophiles with bis(p-nitrophenyl)phosphate and bis(2,4-dinitrophenyl)phosphate are reported previously [45, 46]. Most significant contribution made by Nome and research groups [47] towards the cleavage of the bis(2,4-dinitrophenyl)phosphate (BDNPP) and diethyl 2,4-dinitrophenyl phosphate (DEDNPP) with different types of α-nucleophiles such as benzohydroxamic acid, laurylhydroxamic acid, hydrazine, hydrogen peroxide, polymer containing functional groups hydroxamic acid in absence and presence of micellar media. They have observed that α-nucleophiles play significant role for the hydrolysis of BDNPP, DEDNPP under the reaction conditions. For the product characterization, they have used various techniques such as NMR, GC, ESI-MS, UV spectroscopy. On the basis of these techniques they have analyzed that the nucleophilic attack by α-nucleophiles such as laurylhydroxamic acid on the aromatic carbon, giving an intermediate that decomposes to undecylamine and 2,4-dinitrophenol and at phosphorus giving an unstable intermediate that decomposes a Lossen rearrangement yielding a series of derivatives including N,N-dialkyl urea, undecyl amine, undecyl isocynate, and carbamyl hydroxamate. The kinetic acceletaion rate was $10^4$ fold faster than its spontaneous hydrolysis. Bunton and his coworkers [48] have investigated that cleavage of bis(p-nitrophenyl)phosphate (BNPP) and 4-nitrophenyl phosphorochloridate by alkaline hydrogen peroxide. They observed very high liability of the peroxo intermediate in terms of an intramolecular nucleophilic substitution by the peroxo group at phosphorus. The catalytic efficiency of two Schiff base manganese (II) complexes toward the hydrolysis of bis (p-nitrophenyl) phosphate was evaluated in 16-6-16, 2Br- at 25 °C by Jiang et al. [49]. They observed that the two Mn(III) catalysts can efficiently promote hydrolysis of BNPP with a six order of magnitude rate enhancement relative to the spontaneous rate constant. Furthermore, rate of BNPP hydrolysis in gemini 16-6-16, 2Br- micellar media are much higher than that in cetyltrimethylammonium bromide (CTAB) and n-lauroylsarcosine sodium (LSS) micelles. Meng et al. [50] have investigated that hydrolysis of bis(p-nitrophenyl) phosphate using short and long alkyl multiamine metallomicelles with Cu(II) forming complex CuL¹-CuL⁴ in buffer solution at 30 °C. They observed that the complex with 1:1 ratio of ligands (L¹-L⁴) to copper (II) ion were the kinetic active catalysts and the deprotonized Cu(II) complex formed by activated water molecule was the real active species for BNPP catalytic hydrolysis. The reaction catalyzed by active species of the
CuL₁ - CuL⁴ was 4×10⁶ s⁻¹, 7.44×10⁵ s⁻¹, 1.42×10⁵ s⁻¹ and 4.1×10⁴ s⁻¹ respectively. The order of catalytic activity was CuL⁴ > CuL³ > CuL² > CuL¹. The increasing hydrophobic hydrocarbon chain lower the polarity and dielectric constant, which lead to the change of the properties of micellar microenvironment therefore accelerate the hydrolysis of BNPP. Mao et al. [51] have studied the cleavage of bis(\(p\)-nitrophenyl) phosphate with cyclodextrin dimer ligand (2,6-bis (3-mono-amino-\(\beta\)-cycodextrinmethyl)pyridine) in neutral pH without the addition of metal. They observed that cyclodextrin dimer have higher substrate affinity than monomer cyclodextrin. Cyclodextrin dimer are linked on the primary face and they exhibit considerable promotion to bis(\(p\)-nitrophenyl) phosphate cleavage in the presence of one or more transition metal ions. Jiang et al. [52] have described the synthesis and characterization of three novel di-Ni(II) complexes, [NiL(\(\mu\)-OH)](ClO₄)₂, [NiL(DNBA)₂](CH₃CN)₂BPh₄, [NiL(BPP)₂](CH₃CN)₂BPh₄ using the novel symmetrical phenol-based ‘end off’ dinucleating ligand 2\{[(2-piperidylmethyl)amino]methyl\}-4-bromo-6-[(1-methylhomopiperazine-4-yl) methyl] phenol (HL). Moreover, they reported that the ability of the di-Ni(II) formed situ from ligand HL and nickel ions in water-ethanol (1:1 v/v), to catalytically hydrolyze the substrate bis(\(p\)-nitrophenyl) phosphate. These study reveal that the asymmetric di-Ni(II) system shows the highest catalytic activity reported so far, with the acceleration rate 1.28×10⁷ times faster than the uncatalyzed hydrolysis of the BNPP.

3.2 PRESENT INVESTIGATION

The first section of the chapter deals with the cleavage of bis (\(p\)-nitrophenyl) phosphate (BNPP) by \(\alpha\)-nucleophiles over the pH range 7-12 in cationic micellar media. Cationic surfactants i.e. cetyltrimethylammonium bromide (CTAB), cetylpyridinium bromide (CPB), cetyltrimethylammonium bromide (CTAB) and cetylpyridinium chloride (CPC) have been used. Different \(\alpha\)-nucleophiles viz. acetohydroxamate (AHA’), benzoxydroxamate (BHA’), salicylhydroxamate (SHA’), butane 2, 3-dione monoximate (BDMO’) and \(\alpha\)-benzoin oximate ions have been used (Scheme – 3.1). The variation of \(k_{obs}\) values of the reactions depends on the micellar structure i.e., head groups, tail length and counter ions. In this study, we found that the nucleophilic activities of \(\alpha\)-nucleophiles are higher with cationic micellar media toward bis (\(p\)-
Hydrolytic Cleavage of Phosphodiester with nitrophenyl phosphate cleavage. The experimental data shows that oximate ion (BDMO') has higher catalytic activity than hydroxamate ions (AHA', BHA', SHA') in cationic micellar media.

\[ R = \text{CH}_3, R' = \text{H}, \text{acetohydroxamate ion (AHA')} \]
\[ R = \text{C}_6\text{H}_5, R' = \text{H}, \text{benzohydroxamate ion (BHA')} \]
\[ R = 2-\text{HOC}_6\text{H}_4, R' = \text{H}, \text{salicylhydroxamate ion (SHA')} \]

![Scheme 3.1](attachment:image)

**Scheme 3.1**

Second section of this chapter provides hydrolysis of two phosphodiesters i.e. bis(p-nitrophenyl) phosphate (BNPP) and bis(2,4-dinitrophenyl) phosphate (BDNPP) catalyzed by hydroperoxide (HOO'), acetohydroxamate (AHA') and butane 2, 3-dione monoximate (BDMO') ions (Scheme-3.1) in the presence of cationic gemini surfactants viz. alkanediyl-\(\alpha\),\(\omega\)-bis(hydroxyethylmethylhexadecylammonium bromide) (C_{16-s-C16}, MEA 2Br, where s = 4, 6) and alkanediyl-\(\alpha\),\(\omega\)-bis(dimethylhexadecylammonium bromide) (C_{16-s-C16}, 2Br, where s = 10, 16) at 27°C (Scheme-3.2).

![Scheme 3.2](attachment:image)

**Scheme 3.2**
3.3 EXPERIMENTAL

3.3.1 MATERIALS

The phosphate diesters i.e. bis(p-nitrophenyl) phosphate was procured from Aldrich and bis(2,4-dinitrophenyl)phosphate was a gift of Prof Faruk Nome from Department of Chemistry, Federal University of Santa Catarina, Florianopolis-SC, Brazil. Acetohydroxamic acid, benzohydroxamic acid and salicylhydroxamic, butane 2, 3-dione monoxime and α-benzoin oxime were procured from Sigma, USA. Cetyldiethylethanolammonium bromide, tetradecyldiethylethanolammonium bromide, dodecyldiethylethanolammonium bromide and cetyltrimethylethanolammonium bromide were obtained from the laboratory of Prof. R. M. Palepu (Retd. Professor), St. Francis Xavier University, Antigonish, Canada. Cationic surfactants i.e. cetyltrimethylammonium bromide, cetylpyridinium bromide and cetylpyridinium chloride were obtained from Sigma (St. Louis, Missouri, USA). The gemini surfactants were synthesized as per literature procedure [39, 53]. Hydrogen peroxide was obtained from Merck chemical India. All chemicals are highly pure (98%) and used without further purification. All the solutions were prepared in triple distilled water.

3.3.2 METHOD

The reactions were studied spectrophotometrically using Systronics (Type, 104 and 118) spectrophotometer by monitoring the appearance of the leaving p-nitrophenolate ion at 400 nm and 27ºC ± 0.2ºC. All the kinetic experiments were performed at an ionic strength of 0.1 M (with KCl). Borate buffer was employed to control the pH of the reaction media. All the pH measurements were obtained using a Systronics (Type 335) pH meter. Reactions were initiated by adding 100 µL of a stock solution of the BNPP (0.005 M) in aqueous buffer solution containing other reactants. Nucleophile concentrations were taken in large excess over the substrates assuring pseudo-first-order kinetics. Observed first-order rate constants (k_{obs}) were calculated by a non-linear least squares fitting of the absorbance vs. time result fit very well to the first-order rate equation.

\[
\ln (A_{\infty} - A_t) = \ln (A_{\infty} - A_0) - kt
\]

(3.1)
3.4 RESULTS AND DISCUSSION

[I] Comparative Studies on Reaction of bis(p-nitrophenyl)phosphate and α-Nucleophiles in Cationic Micellar Media

The first-order rate constant, \( k_{\text{obs}} \) for the reaction of BNPP with AHA, BHA, SHA, BDMO and α-benzoin oxime have been measured spectrophotometrically under pseudo first-order conditions in absence and presence of cationic surfactants at 27°C. Fig. 3.1 illustrates the pH-rate profiles for the dephosphorylation of BNPP by various α-nucleophiles at pH 7.0-12.0, in the presence of CDEEAB, where \( k_{\text{obs}} \) increases with pH and reaches a maximum in the pH region where AHA, BHA, SHA, BDMO and α-benzoin oxime are fully deprotonated. The apparent dissociation constant (pKa) are 8.64, 9.71, 9.50, 9.78 and 11.5 for AHA, BHA, SHA, BDMO and α-benzoin oxime in CDEEAB (2.0×10^{-3} M), at constant [Nu] = 1.6 ×10^{-3} M, gives a rate constant in the plateau region of 0.40×10^{-5} s^{-1}, 1.95×10^{-5} s^{-1}, 1.75×10^{-5} s^{-1}, 2.00×10^{-5} s^{-1} and 1.10×10^{-5} s^{-1}. The first order rate constant for the hydrolysis of BNPP by hydroxide ion at pH 11.0 is about 3.28×10^{-9} s^{-1} [54]. Table 3.1 indicates the kinetic rate data for the reaction of BNPP with different concentrations of BDMO, α-benzoin oxime, AHA, BHA and SHA at pH 11.0. It is found that α-nucleophiles in cationic micellar media display significant catalytic activity for the cleavage of BNPP (Table 3.1). Kinetic data shows additional support for the hypothesis that hydroxamate and oximate ions are acting as a nucleophilic catalyst for reaction of BNPP. All the reactions followed rate Eq. 3.2.

\[
    k_{\text{obs}} = k_o + k_{\text{Nu}} [\text{Nu}^-]
\]

where

\[
    k_o = k_{\text{H}_2\text{O}} + k_{\text{OH}^-}[\text{OH}^-]
\]

It is assumed that catalysis by the nucleophile is dependent upon the ionization state of the hydroxamic acid and oxime. It is known that the anion of hydroxamate and oximate ions acts as a reactive species for the hydrolysis of phosphate esters. The first order rate constants and calculated second-order rates constant are shown in Table 3.2. The first-order rate constants are much greater than that of the spontaneous water reaction of BNPP \((k_0) 1.1×10^{-11} \text{s}^{-1}\); that is, there is up to \(10^5\)-fold rate enhancement, typical of α-nucleophiles. Under the physiological condition the reaction of bis(p-nitrophenyl) phosphate with oximate and hydroxamte ion is slow.
**Fig. 3.1** Plots of first-order rate constants vs. pH for the reaction of BNPP with Inset (a) Oximate ions (b) Hydroxamate ions in CDEEAB (2.0×10⁻³ M).

**Table 3.1** Effect of nucleophilic concentration of various α-nucleophiles on pseudo first-order rate constant for the reaction of BNPP in absence and presence of cationic surfactant.

<table>
<thead>
<tr>
<th>[Nu], mM</th>
<th>10⁵ k_{obs} (s⁻¹)</th>
<th>Nil</th>
<th>Surfactant</th>
<th>α-benzoin oxime</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AHA</td>
<td>BHA</td>
<td>SHA</td>
</tr>
<tr>
<td>1.6</td>
<td>0.22</td>
<td>0.55</td>
<td>0.75</td>
<td>1.20</td>
</tr>
<tr>
<td>3.2</td>
<td>0.45</td>
<td>1.60</td>
<td>2.00</td>
<td>2.20</td>
</tr>
<tr>
<td>4.8</td>
<td>0.65</td>
<td>1.80</td>
<td>2.80</td>
<td>-</td>
</tr>
<tr>
<td>6.4</td>
<td>0.88</td>
<td>-</td>
<td>-</td>
<td>5.39</td>
</tr>
<tr>
<td>8.0</td>
<td>0.99</td>
<td>2.30</td>
<td>5.00</td>
<td>7.01</td>
</tr>
</tbody>
</table>

**Reaction conditions.** Temp. = 27°C, [BNPP] = 1.6×10⁻⁴ M, pH = 11.0, μ = 0.1 M KCl, [CDEEAB] = 2.0×10⁻³ M
Table 3.2 The observed first and second-order kinetic rate data for BNPP cleavage with various α-nucleophiles in absence and presence of CDEEAB surfactant.

<table>
<thead>
<tr>
<th>Nucleophiles</th>
<th>Nil</th>
<th>Surfactant</th>
<th>k_{obs} (s^{-1})</th>
<th>k_{2} (M^{-1}s^{-1})</th>
<th>k_{obs} (s^{-1})</th>
<th>k_{2} (M^{-1}s^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>k_0</td>
<td>a1.1×10^{-11}</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>k_{OH}</td>
<td>3.9×10^{-9}</td>
<td>b3.50×10^{-5}</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AHA</td>
<td>0.22×10^{-5}</td>
<td>1.20×10^{-3}</td>
<td>1.60×10^{-5}</td>
<td>9.00×10^{-3}</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BHA</td>
<td>0.55×10^{-5}</td>
<td>2.40×10^{-3}</td>
<td>2.50×10^{-5}</td>
<td>9.80×10^{-3}</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SHA</td>
<td>0.75×10^{-5}</td>
<td>6.50×10^{-3}</td>
<td>1.90×10^{-5}</td>
<td>9.67×10^{-3}</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BDMO</td>
<td>1.20×10^{-5}</td>
<td>9.20×10^{-3}</td>
<td>2.80×10^{-5}</td>
<td>1.06×10^{-2}</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>α-benzoin oxime</td>
<td>-</td>
<td>-</td>
<td>1.10×10^{-5}</td>
<td>6.00×10^{-3}</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Reaction conditions:** Temp. = 27°C, [BNPP] = 1.6×10^{-4} M, [Nu^{-}] = 1.6×10^{-3} M, \( \mu \) = 0.1M KCl, pH = 11.0 [CDEEAB] = 2.0×10^{-3} M

3.4.1 Kinetic Studies in Cationic Micellar Media

The hydrolysis of BNPP at pH 11.0 in cationic micelles of CDEEAB, CDMEAB, CPB, CTAB and CPC with AHA, BHA, SHA, BDMO and α-benzoin oxime is much faster than α-nucleophiles alone (Tables 3.3 and 3.4). Rate–surfactant profiles for hydrolytic cleavage of BNPP with various cationic surfactants are shown in Fig. 3.2. For the observed first order rate constant, \( k_{obs} \) during BNPP cleavage catalyzed by AHA, BHA, SHA, BDMO, α-benzoin oxime in cationic surfactants, increased with increasing of the concentration of surfactants (ii) reaches a maximum value and then decreased with increasing concentration of surfactants from 0.001 to 0.020 M (Figs. 3.2, 3.3 and 3.4). At the rate maxima \( k_{obs} \) for the reaction of BNPP with CDEEAB-bound BDMO\(^{+}\), SHA\(^{-}\), BHA\(^{-}\) AHA\(^{-}\) and α-benzoin oximate ions are ca. 8.4×10^{-5} s^{-1}, 4.6×10^{-5} s^{-1}, 5.7×10^{-5} s^{-1}, 3.2×10^{-5} s^{-1}, 2.6×10^{-5} s^{-1}, at pH - 11.0 (Tables 3.3 and 3.4). It has been already proved that cationic surfactants accelerate the reaction.
Table 3.3 Kinetic data for the reaction of BNPP with hydroxamate ions in micellar media.

<table>
<thead>
<tr>
<th>[Surf.] mM</th>
<th>10^5 k_{obs} (s^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AHA</td>
</tr>
<tr>
<td></td>
<td>CPC</td>
</tr>
<tr>
<td>0.00</td>
<td>0.22</td>
</tr>
<tr>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>2.00</td>
<td>2.58</td>
</tr>
<tr>
<td>5.00</td>
<td>3.41</td>
</tr>
<tr>
<td>10.0</td>
<td>2.42</td>
</tr>
<tr>
<td>20.0</td>
<td>-</td>
</tr>
</tbody>
</table>

Reaction conditions: Temp. = 27°C, pH = 11, [BNPP] = 1.6×10^{-4} M, [Nu] = 1.6×10^{-3} M, µ = 0.1 M KCl
Table 3.4 Kinetic data for the reaction of BNPP with oximate ions in cationic micellar media.

<table>
<thead>
<tr>
<th>[Surf.] mM</th>
<th>BDMO $10^5 k_{obs}$ (s$^{-1}$)</th>
<th>α-benzoin oxime $10^5 k_{obs}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CPC</td>
<td>CPB</td>
</tr>
<tr>
<td>0.00</td>
<td>1.21</td>
<td>1.21</td>
</tr>
<tr>
<td>1.00</td>
<td>-</td>
<td>2.90</td>
</tr>
<tr>
<td>2.00</td>
<td>5.50</td>
<td>3.50</td>
</tr>
<tr>
<td>5.00</td>
<td>9.50</td>
<td>7.50</td>
</tr>
<tr>
<td>10.0</td>
<td>3.40</td>
<td>4.00</td>
</tr>
</tbody>
</table>

**Reaction condition:** Temp. = 27°C, pH 11.0, [BNPP] = 1.6×10$^{-4}$ M, [Nu] = 1.6×10$^{-3}$ M, $\mu$ = 0.1M KCl

**Fig. 3.2** Plots of the kinetic rate data for the reaction of BNPP with AHA in borate buffer at pH 11 in various cationic surfactants.
Fig. 3.3 Plots of the kinetic rate data for the reaction of BNPP with BHA in borate buffer at pH 11 in various cationic surfactants.

The electrostatic attraction of the cationic head groups of the surfactants at the micelle surface to the nucleophilic anion counterions leads to increase of the local concentration of the nucleophile, whereas incorporation of the substrate in the micelle leads to a higher local concentration of the reactants [55]. The rate of the hydrolysis should be mainly dependent on the concentration of BNPP and [Nu] in the cationic micellar media. As can be seen in Fig. 3.4, rate enhancement on addition of surfactant would be expected to be more significant for the oximate ion (BDMO⁻) compared with hydroxamate ions (AHA⁻, BHA⁻ and SHA⁻). This may be due to fact that electrostatic interaction between the cationic micelle and the anionic oximate will be much larger than that between the cationic micelle and hydroxamate ions. This acceleration is due to (i) hydrophobic effect and charge effect which concentrate reagents in the cationic micelles and (ii) the nucleophilicity of monoanionic acetohydroxamate, benzohydroxamate, salicylhydroxamate and butane 2,3-dione monoximate ions, which are effective α-effect nucleophiles with unshared electron pairs on oxygen and nitrogen. Moreover, the high nucleophilicity of oximate and hydroxamate ions are typical behavior of α-nucleophiles.
In our results, we found that BHA is slightly more hydrophobic than AHA, SHA and bind to cationic micelles hydrophobically as well as by the electrostatic attraction and more efficient reaction with the bound substrate BNPP. It is considered that nucleophilicity is governed not only by the basicity but also by the strength of interaction between the nucleophile and surfactant aggregate [56]. The marked increase in nucleophilicity in micellar media was observed in the order BDMO>SHA>BHA>AHA>α-benzoin oxime. The marked increase in micellar effects observed in following order CPC>CPB>CDEEAB>CDMEAB>CTAB. In our previous work, the comparative catalytic reactivity of acetohydroxamate (AHA\(^{-}\)), benzohydroxamate (BHA\(^{-}\)) and salicylhydroxamate (SHA\(^{-}\)) ions for cleavage of triester (Paraoxon) [16] and diester (2,4-dinitrophenyl)phosphate (BDNPP) by SHA in cationic micellar media have been studied. It has been observed that SHA showed remarkable enhanced catalytic efficiency for bis (2, 4-dinitrophenyl) phosphate in identical conditions: \([\text{BDNPP}] = 1.0 \times 10^{-4} \text{ M}, [\text{SHA}] = 1.0 \times 10^{-3} \text{ M}, [\text{KCl}] = 0.1 \text{ M}, [\text{CTAB}] = 2.0 \times 10^{-3} \text{ M}, \text{pH} 9.2 \text{ at } 27^\circ\text{C}\). Therefore, it is expected that the catalytic efficiency of the SHA\(^{-}\) is further enhanced in micellar microenvironments and compared to BHA\(^{-}\) and AHA\(^{-}\) due to the active participation of dianionic species [57].
Scheme 3.3 The sketch map of BNPP catalytic cleavage by α-nucleophiles in cationic micellar media

Scheme 3.3 is outlined for the cleavage of phosphodiester BNPP with α-nucleophiles in cationic micellar medium. The experimental results show that the cleavage rate of BNPP is much smaller in only nucleophiles than that in the cationic micellar media. As a result, there is weaker hydrophobic attraction and a stronger electrostatic repulsion between the cationic micelles and nucleophiles with substrate monoanion, and formation inorganic product. Therefore, the true cause of the higher reactivity of BNPP cleavage in cationic micelles is attributed to the formation of plentiful substrate-nucleophile system. It is in agreement with similar work presenting 2-hydroxypropyl p-nitrophenyl phosphate (HNPP) cleavage in presence of cationic micelles. They observed “sandwich absorptive mode” (micelle-HNPP-catalyst mode), in which the negative HPNP molecule is sandwiched in the interlayer between the positive micelle and the catalyst [5, 58-62].
3.4.2 Effect of Head group, Chain Length and Counter ion

The effect of different surfactant head groups like quaternary ammonium (CTAB) and pyridinium groups (CPB) on the hydrolysis of BNPP with hydroxamate and oximate ions have been studied. All the data are shown in Tables 3.3 and 3.4. The structure of surfactants head group has an important role toward the molecular packing of organized self-assemblies [63-65, 56-58]. An increase in the head group size leads to an increase in the interfacial area and the space between two head groups is also enhanced [59]. The space between two head groups allow the $\alpha$-nucleophiles to solubilize itself smoothly at the interfacial region. Because of the enhanced interfacial region, the concentration of nucleophile and substrate increased and shows higher activity. There is little information about the effect of the variation of head groups on the micellar structure [66-68]. Table 3.3 clearly indicates that the nucleophilic activity of $\alpha$-nucleophiles strongly depends on the structure and charge of the surfactant head group. In comparison, we found higher, $k_{obs}$ value with pyridinium group (CPB) ca. 10.4-fold than quaternary ammonium group (CTAB) ca. 5-fold accelerated. The results indicate that specific interactions between the cationic surfactant and the nucleophile should be effective in determining the $\alpha$-nucleophiles as the hydrophobicity together with the size of the head groups are increased. The variation of $k_{obs}$ values of the reactions depends on the micellar structure i.e., hydrophobic tail length and counter ion etc. The rate constants in a variety of association colloids are slightly higher than in water and increases modestly with increasing micellar media involves variation in the surfactant tail groups and changes in the interfacial regions [69]. Analysis of kinetic data indicates that the cetylidiethylethanolammonium bromide shows higher reactivity than cetylpyridinium bromide, cetyltrimethylammonium bromide. The increase of $k_{obs}$ values with increasing alkyl chain lengths ($R = 16$) of the surfactants, i.e., with increasing aggregation number of micelle is mainly due to the increase in the electrical surface potential of the micelle and partially due to an increase of hydrophobicity of the palisade layer of the micelle.
Table 3.5 Kinetic rate data for the reaction of BNPP with BHA and BDMO in borate buffer at pH 11.0 in chain length variation of cationic surfactants.

<table>
<thead>
<tr>
<th>[Surf.] mM</th>
<th>10^5 k_{obs} (s^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BHA</td>
</tr>
<tr>
<td></td>
<td>DDEEAB</td>
</tr>
<tr>
<td>0.00</td>
<td>0.55</td>
</tr>
<tr>
<td>1.00</td>
<td>2.00</td>
</tr>
<tr>
<td>2.00</td>
<td>3.00</td>
</tr>
<tr>
<td>5.00</td>
<td>3.20</td>
</tr>
<tr>
<td>10.0</td>
<td>2.90</td>
</tr>
</tbody>
</table>

**Reaction conditions:** Temp. = 27°C, [BNPP] = 1.6×10^{-4} M, [Nu] = 1.6×10^{-3} M, pH = 11, \( \mu = 0.1 \) M KCl

**Fig. 3.5** Plots of the kinetic rate data for the reaction of BNPP with BHA in borate buffer at pH 11 in various chain lengths of cationic surfactants.
In all cases, the [CDEEAB] rate profile was maximum as compared to [TDEEAB] and [DDEAB] surfactant because [CDEEAB] corresponds to complete solubilization of the substrate in the micellar pseudo phase and alkanol group can exert a protective effect on the positive charge, thus lowering the attractive electrostatic interactions between the quaternary ammonium center of the micelle and corresponding negative charge of the dissociated acid group in Table 3.5. The reactivity order follows the trend CDEEAB>TDEEAB>DDEEAB (Fig. 3.5).

[HII Hydrolysis of Phosphate Diesters by α-Nucleophiles in Presence of Cationic Gemini Surfactants]

Kinetics of nucleophilic reaction of bis(\(p\)-nitrophenyl)phosphate (BNPP) and bis(2,4-dinitrophenyl)phosphate (BDNPP) with hydrogen peroxide (\(H_2O_2\)), butane 2,3-dione monoxime (BDMO) and acetohydroxamic acid (AHA) in novel and conventional cationic gemini micellar media have been investigated at 27°C. The reactivity of different \(\alpha\)-nucleophiles (\(H_2O_2\), AHA and BDMO) for the hydrolysis of BNPP and BDNPP is shown in Table 3.6. Nucleophilic activity was measured at various pH values ranging 7.1 to 12.0 using the activated substrates, BNPP and BDNPP, with gemini and nonmicellar media.

**Table 3.6** Catalytic reactivity of various nucleophiles for the cleavage of phosphodiesters at reaction conditions.

<table>
<thead>
<tr>
<th>Nucleophiles</th>
<th>(pK_a)</th>
<th>pH 8.0</th>
<th>pH 9.2</th>
<th>pH 11.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>-</td>
<td>3.2\times10^{-11}</td>
<td>4.1\times10^{-7}</td>
<td>5.4\times10^{-11}</td>
</tr>
<tr>
<td>OH</td>
<td>15.5</td>
<td>-</td>
<td>-</td>
<td>0.4\times10^{-5}</td>
</tr>
<tr>
<td>(H_2O_2)</td>
<td>11.6</td>
<td>0.08\times10^{-5}</td>
<td>1.3\times10^{-3}</td>
<td>0.10\times10^{-5}</td>
</tr>
<tr>
<td>AHA</td>
<td>9.71</td>
<td>0.01\times10^{-5}</td>
<td>0.09\times10^{-4}</td>
<td>0.02\times10^{-5}</td>
</tr>
<tr>
<td>BHA</td>
<td>9.16</td>
<td>0.04\times10^{-5}</td>
<td>0.6\times10^{-5}</td>
<td>-</td>
</tr>
<tr>
<td>SHA</td>
<td>7.2</td>
<td>0.06\times10^{-5}</td>
<td>-</td>
<td>1.4\times10^{-5}</td>
</tr>
<tr>
<td>BDMO</td>
<td>9.78</td>
<td>0.069\times10^{-5}</td>
<td>0.12\times10^{-4}</td>
<td>0.19\times10^{-5}</td>
</tr>
</tbody>
</table>

\(k_0 = 1.1\times10^{-11} \text{ (s}^{-1}\text{) (BNPP)} \) and \(1.9\times10^{-7} \text{ (s}^{-1}\text{) (BDNPP)} \) Ref. [7, 8]
Fig. 3.6 Changes in UV-Vis spectra of (a) absence and (b) presence of 16-6-16 MEA, 2Br⁻ with hydroperoxide ion upon addition of BNPP observed at 5 minute intervals in aqueous media at 27°C.

Fig. 3.7 Plots of log $k_{obs}$ vs. pH for the reaction of BNPP with hydroperoxide in different cationic gemini surfactants: (■) Nil (●) 16-12-16, 2Br⁻, (▲) 16-10-16, 2Br⁻, (▼) 16-4-16 MEA, 2Br⁻ (♦) 16-6-16 MEA, 2Br⁻. Temp. – 27°C, NaOH – 0.1 M, [BNPP] – $1.0 \times 10^{-4}$ M, [H₂O₂] - 0.3 M, [Surfactant] - $1.0 \times 10^{-3}$ M.
The catalytic activity of relevant nucleophiles is determined on the basis of basicity and strength of nucleophiles. It has been reported the dissociation constant of various α-nucleophiles such as hydrogen peroxide (H₂O₂), acetohydroxamic acid (AHA), benzohydroxamic acid (BHA), salicylhydroxamic acid (SHA) and butane 2, 3-dione monoxime (BDMO) respectively [70].

Kinetic experiments were carried out to assess the ability of gemini surfactants with nucleophile system to hydrolyze the BNPP. Fig. 3.6 (a) shows the UV absorption spectra in the absence of micelle and (b) in the presence of micelle (16-10-16 MEA, 2Br⁻). The reaction was monitored by following the increase of absorbance at 400 nm, an indication of the release of 4-nitrophenoxide (NP) from BNPP. The reactivity patterns of cationic gemini surfactants are shown in Fig. 3.7. Fig. 3.8 shows the effect of nucleophilic concentration on the rate data, $k_{obs}$ for the cleavage of BNPP and BDNPP. The rate of BNPP cleavage initially increased linearly with the increase of nucleophilic concentration but gradually deviated from linearity.

![Kinetic plots of $k_{obs}$ vs concentration of nucleophile for the reaction of BNPP and BDNPP.](image)

**Fig. 3.8** Kinetic plots of $k_{obs}$ vs concentration of nucleophile for the reaction of BNPP (A) (■) H₂O₂, (●) NaOH and for BDNPP Inset (B) (■) H₂O₂, (●)NaOH.
The kinetic rate data for the reaction of BNPP and BDNPP with different concentration of hydrogen peroxide and NaOH are collected. The reaction rate increases with increasing concentration of hydrogen peroxide. Kinetic data show extra support for the hypothesis that hydroperoxide anion (HOO\(^-\)) is acting as a nucleophilic catalyst for the reaction of both diesters, BDNPP and BNPP. Mixing of H\(_2\)O\(_2\) with NaOH leads to a nearly quantitative deprotonation of H\(_2\)O\(_2\). Therefore, an increase in \(k_{obs}\) on addition of H\(_2\)O\(_2\) means that HOO\(^-\) is a stronger nucleophile than OH\(^-\).

Hydrolysis of BNPP in the presence of hydroperoxide ion (\(k_{obs} = 1.0\times10^{-5}\) s\(^{-1}\)) was faster than OH\(^-\) (\(k_{obs} = 0.1\times10^{-5}\) s\(^{-1}\)). But, hydroperoxide ion with BDNPP (\(k_{obs} = 2.01\times10^{-3}\) s\(^{-1}\) in 0.1 M) caused a 10\(^2\) fold faster rate acceleration than with BNPP. The high nucleophilicity of HOO\(^-\) is typical behavior of \(\alpha\)-effect nucleophiles. This behavior is supporting the fact that the nucleophilicity of hydroperoxide anion is very high in this form. Additions of higher concentrations of H\(_2\)O\(_2\) when it is in excess over NaOH does not produce more HOO\(^-\) anions, but the reaction rate is obviously going higher, thus indicating that the reaction with HOO\(^-\) is catalyzed by neutral H\(_2\)O\(_2\). The second order rate constant for the hydroperoxide ion system was calculated from the slope of the straight line of \(k_{obs}\) vs. [Nu] and is reported in Table 3.7. It agrees with literature data for the hydrolysis of phosphodiesters [45,70].

**Table 3.7** The second order rate constant for the cleavage of diesters with nucleophiles in absence and presence of gemini surfactant.

<table>
<thead>
<tr>
<th>Nucleophiles</th>
<th>Nil</th>
<th>16-12-16, 2Br(^-)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BNPP</td>
<td>BDNPP</td>
</tr>
<tr>
<td>(k_{OH}) [M(^{-1})s(^{-1})]</td>
<td>1.45\times10^{-7}</td>
<td>0.74\times10^{-3}</td>
</tr>
<tr>
<td>(k_{HOO^-}) [M(^{-1})s(^{-1})]</td>
<td>1.29\times10^{-6}</td>
<td>0.52\times10^{-2}</td>
</tr>
<tr>
<td>(k_{AHA}) [M(^{-1})s(^{-1})]</td>
<td>0.56\times10^{-3}</td>
<td>1.5\times10^{-2}</td>
</tr>
<tr>
<td>(k_{BDMO^-}) [M(^{-1})s(^{-1})]</td>
<td>0.72\times10^{-3}</td>
<td>2.0\times10^{-2}</td>
</tr>
</tbody>
</table>

**Reaction conditions:** Temp. = 27°C, pH 11.0, [NaOH] = 0.1M, [Subs]=1.0\times10^{-8}\) M
3.4.3 Micellar Reaction Media

The influence of surfactant concentration on a micellar modified reaction has been investigated. In our previous study [53], we have investigated the physicochemical characterization of cationic gemini surfactants (16-4-16 MEA, 2Br⁻, 16-6-16 MEA, 2Br⁻) and their effect on reaction kinetics in aqueous-solvent medium.

3.4.4 Reaction of bis(2,4-dinitrophenyl)phosphate in Presence of Gemini Surfactants

The cleavage of bis(2,4-dinitrophenyl)phosphate (BDNPP) catalyzed with hydroperoxide (HOO⁻), acetohydroxamate (AHA⁻), butane 2,3-dione mono oximate (BDMO⁻) in presence and absence of cationic gemini surfactants (16-4-16 MEA, 2Br⁻, 16-6-16 MEA, 2Br⁻, 16-10-16, 2Br⁻ and 16-12-16, 2Br⁻) have been investigated. The phosphodiester hydrolysis was quite slow in the absence of surfactants. It is reported that gemini surfactants are more effective than monomeric surfactants for ester hydrolysis [71] and for nucleophilic substitution reactions. We observed that the cationic gemini surfactants, 16-s-16 MEA, 2Br⁻, (s = 4, 6) are more reactive than 16-s-16, 2Br⁻ (s = 10, 12) in the cleavage of BNPP and BDNPP. Figs. 3.9 and 3.10 show the rate-surfactant profile for the cleavage of BDNPP using various nucleophiles.

![Rate surfactant profiles for the reaction of bis(2, 4-dinitrophenyl)phosphate with acetohydroxamate ion in cationic gemini surfactants](image)

**Fig. 3.9** Rate surfactant profiles for the reaction of bis(2, 4-dinitrophenyl)phosphate with acetohydroxamate ion in cationic gemini surfactants: (▲) 16-6-16 MEA, 2Br⁻, (●) 16-4-16 MEA, 2Br⁻ and (■) 16-10-16 2Br⁻ (lines are predicted values with model).
Fig. 3.10 Rate surfactant profiles for the reaction of bis(2,4-dinitrophenyl)phosphate with butane, 2 3-dione monoximate ion in cationic gemini surfactants: (○) 16-6-16 MEA 2Br⁻, (Δ) 16-4-16 MEA 2Br⁻ and (□) 16-10-16 2Br⁻ (V) 16-12-16 2Br⁻ (lines are predicted values with model).

We can see that in all cases $k_{obs}$ increases upon augmenting the surfactant concentration. The reason is that cationic micelle catalyzes the reaction and $k_{obs}$ passed through maxima with increasing surfactant concentration. Further addition of surfactant decreases the reaction rate. It means that the reaction is taking place simultaneously in the bulk and micellar phases. The rate data shows that the variation of rate constants below the critical micelle concentration (cmc) is difficult to quantify due to reactant induced micellization and interaction with non-micellized surfactants [55]. The $k_{obs}$ values for the hydrolysis of BDNPP are accelerated at above the cmc of the surfactants in the aqueous media. This is due to the micellar accelerated nucleophilic reaction. However, it is likely that the extent of attack of oximate ion at the phosphorus (P=O) is influenced by a number of factors apart from lipophilicity, including the nucleophilicity of α-effect nucleophile, the aggregate size of the micelle and polarity of the interfacial region. From these experimental evidences, pseudophase model has been applied for the determination of binding affinity of substrate in micellized and non micellized media.
The effect of micellar rate on bimolecular reactions can generally be treated quantitatively in terms of distribution of reactants between aqueous and micellar pseudophases. The pseudophase kinetic model [72] considers the micelle as a different phase from the aqueous phase, the reaction occurring in both phases according to Scheme 3.4.

\[
\text{Substrate}_W + D_n + \text{Nu}_W \quad \overset{k_2^W}{\rightleftharpoons} \quad \text{Products}
\]

\[
\text{Substrate}_M + \text{Nu}_M \quad \overset{k_2^M}{\rightleftharpoons} \quad \text{Products}
\]

Scheme 3.4

where the subscripts W and M denote the aqueous and micellar phases respectively, \([D_n]\) is the concentration of micellized surfactant ([D1]-cmc). The cmc values are taken from literature [73]. Since dimeric surfactants show lower cmc that monomeric surfactants [74], the dimeric surfactants should behave better in accelerating the reaction since they can incorporate the reactants into the micelles at lower surfactant concentration and for any surfactant concentration they produce a larger number of micelles. In fact, the distribution of nucleophiles and substrate between bulk and micellar phases is considered through the nucleophilic distribution constant \(K_{M \text{Nu}}\) and substrate equilibrium constant \(K_{M \text{Substrate}}\). The \(k_2^W\) and \(k_2^M\) are second order rate constant in aqueous and micellar phases respectively. The nucleophilic concentration in the micellar pseudophase has been defined as the local, molar concentration within the micellar pseudophase: \(k_2^M = k_M \times V_M\) where \(V_M\) is the molar volume of the reaction region. We have used a \(V_M\) value of 0.63 dm\(^3\)mol\(^{-1}\) for gemini micelles [74]. The influences of surfactant on the cleavage of BDNPP with \(\alpha\)-nucleophiles are considered according to pseudophase assumptions, which are given by the following Eq (3.3).

\[
K_{obs} = \frac{k_2^W + \frac{k_2^W}{V_M} K_{m \text{Substrate}} K_{\text{Nu}} [D_n]}{1 + K_{W \text{Substrate}} [D_n] (1 + K_{M \text{Substrate}} [D_n]) [\text{Nu}]} (3.3)
\]
The rate data are fitted by using Eq 3.3, the adjustable parameters, $K_{M_{\text{substrate}}}$ and $k_2^M$ would be obtained. The fitting data are represented by solid lines shown in Figs 3.9 and 3.10. The kinetic data obtained for the all gemini surfactants concentration up to 0.02 M. The $K_{M_{\text{substrate}}}$ and $k_2^M$ adjustable parameter values obtained from these fitting are summarized in Tables 3.8 and 3.9.

Table 3.8 Kinetic parameters obtained by applying pseudophase model for the nucleophilic reaction of BDNPP with acetohydroxamate ion (AHA$^-$) in the presence of cationic surfactants.

<table>
<thead>
<tr>
<th>Gemini Surfactants</th>
<th>$k_2^W$ (M$^{-1}$s$^{-1}$)</th>
<th>$K_M^{BDNPP}$ (M$^{-1}$)</th>
<th>$K_M^{Nu}$ (M$^{-1}$)</th>
<th>$k_2^M$ (M$^{-1}$s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-6-16, MEA, 2Br$^-$</td>
<td>1.50×10$^{-2}$</td>
<td>4200</td>
<td>133±13</td>
<td>(4.0±0.17)×10$^{-2}$</td>
</tr>
<tr>
<td>16-4-16, MEA, 2Br$^-$</td>
<td>1.50×10$^{-2}$</td>
<td>4000</td>
<td>99±19</td>
<td>(7.0±0.99)×10$^{-2}$</td>
</tr>
<tr>
<td>16-12-16, 2Br$^-$</td>
<td>1.50×10$^{-2}$</td>
<td>3800</td>
<td>50±15</td>
<td>(9.5±0.22)×10$^{-3}$</td>
</tr>
<tr>
<td>16-10-16, 2Br$^-$</td>
<td>1.50×10$^{-2}$</td>
<td>3800</td>
<td>48±10</td>
<td>(9.0±0.12)×10$^{-3}$</td>
</tr>
</tbody>
</table>

**Reaction conditions:** Temp. = 27°C, [substrate] = 1×10$^{-4}$ M, [AHA] = 1×10$^{-3}$ M, [KCl]-0.1 M

Table 3.9 Kinetic parameters obtained by applying pseudophase model for the nucleophilic reaction of BDNPP with oximate ion (BDMO$^-$) in the presence of cationic surfactants.

<table>
<thead>
<tr>
<th>Gemini Surf.</th>
<th>$k_2^W$ (M$^{-1}$s$^{-1}$)</th>
<th>$K_M^{BDNPP}$ (M$^{-1}$)</th>
<th>$K_M^{Nu}$ (M$^{-1}$)</th>
<th>$k_2^M$ (M$^{-1}$s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-6-16 MEA, 2Br$^-$</td>
<td>2×10$^{-2}$</td>
<td>4200</td>
<td>192±10</td>
<td>(5.8±0.4)×10$^{-2}$</td>
</tr>
<tr>
<td>16-4-16 MEA, 2Br$^-$</td>
<td>2×10$^{-2}$</td>
<td>4000</td>
<td>86±01</td>
<td>(8.2±0.6)×10$^{-2}$</td>
</tr>
<tr>
<td>16-12-16, 2Br$^-$</td>
<td>2×10$^{-2}$</td>
<td>3000</td>
<td>32±04</td>
<td>(1.12±0.9)×10$^{-2}$</td>
</tr>
<tr>
<td>16-10-16, 2Br$^-$</td>
<td>2×10$^{-2}$</td>
<td>3800</td>
<td>91±05</td>
<td>(7.1±0.2)×10$^{-3}$</td>
</tr>
</tbody>
</table>

**Reaction conditions:** Temp. = 27°C, [substrate] = 1×10$^{-4}$ M, [BDMO] = 1×10$^{-3}$ M, [KCl]-0.1 M
The nucleophilic association constant of the nucleophiles BDMO, AHA to the micelles, $K_{M^{\text{Nu}}}^N$ are higher for gemini surfactants than for the conventional surfactants (Tables 3.8 and 3.9). Cationic gemini micelles induce large rate enhancements in reactions of organic substrates with nucleophiles that are anions of weak acids such as oximes. Weakly acidic functional groups such as hydroxamic acid have also been attached directly to surfactant head groups or comicellized with inert surfactant. The large rate enhancement are produced by high local anionic nucleophile concentrations generated by strong binding of the organic acids and their enhanced deprotonation by high local OH- concentration at cationic interface [75-76]. It has also been investigated that spacer chain maintain the equilibrium distance between two cationic head groups, then spacer tends to loop inside micellar core to minimize its contact with water. Increased looping of the spacer also separates substrate and reagent at the Stern layer region and thereby mitigates the efficiency of the reaction [42].

For 16-s-16 MEA, 2Br$^-$ (s = 4, 6) hydrogen bonding can take place with water and among the head groups through oxygen atom of –C$_2$H$_4$OH groups. This is likely to provide additional hydration at the head group level resulting in screening of Coulombic forces of repulsion between charged heads and also enforcing a connection among head groups, helping 16-s-16 MEA 2Br$^-$ surfactants to form aggregates at a lower concentration than those of conventional surfactants. The ethanol moiety (–C$_2$H$_4$OH) of 16-s-16 MEA 2Br$^-$, (s = 4, 6) can help to stabilize the micelles due to hydrogen bonding affinity to anions [77]. This implies that the gemini surfactants possibly provide a more favorable microenvironment for the BDNPP cleavage than monomeric surfactant CTAB [4]. Therefore, the rate of BDNPP cleavage in gemini 16-4-16 MEA, Br$^-$ and 16-6-16 MEA, Br$^-$ micellar solution are faster than 16-10-16, Br$^-$ and 16-12-16, Br$^-$ micellar solution. Furthermore, the presence of the ethanol moiety that can help in the modulation of properties of surfactants can be exploited in the near future to prepare more versatile structures, to be conceived as tailor made surfactants to fit proper applications [78].

The second order rate constant for hydrolytic cleavage of phosphodiester with $\alpha$-nucleophiles in the aqueous and micellar phase can be estimated by fitting of data.
From the fitting of Eq. 3.3, we obtained $k_2^W = 1.5 \times 10^{-2} \text{ M}^{-1} \text{s}^{-1}$ (AHA-aqueous), $2.0 \times 10^{-2} \text{ M}^{-1} \text{s}^{-1}$ (BDMO-aqueous) and $k_2^M = 4.0 \times 10^{-2} \text{ M}^{-1} \text{s}^{-1}$ (AHA-16-6-16, MEA, 2Br$^-$), $k_2^M = 5.8 \times 10^{-2} \text{ M}^{-1} \text{s}^{-1}$ (BDMO-16-6-16 MEA, 2Br$^-$), in micellar media. The results show that the second-order rate constant is higher in micellar media compared to aqueous media. Thus, the reaction is confirmed to be faster in the cationic micellar solutions than in water. For hydroperoxide ion, it is noted that the fit of Eq. 3.3 is not sensitive to the adjustable parameters due to an insignificant contribution from the micellar reaction relative to the aqueous reaction in surfactant solutions. The oximate ion (BDMO) shows 27.4 fold rate acceleration with addition of 16-6-16 MEA, 2Br$^-$ in reaction solution for the cleavage of BDNPP. As stated earlier, oximate associate with 16-6-16 MEA, 2Br$^-$ micelle through hydrophobic interactions ($K_M^\text{BDMO} = 192 \text{ M}^{-1}$). The N-OH groups are considerably ionized as N-O$^-$ at pH 9.2 and therefore they also bind to dicationic head group through electrostatic attractions. BDMO show higher micellar catalytic effect ($k_2^M/k_2^W$) towards reaction of BDNPP (2.9 fold), whereas AHA shows around 3.2 fold catalysis respectively. The satisfactory fit obtained for these experiments supports the validity of the model employed. In previous study [57], we have investigated the hydrolysis of bis(2,4-dinitrophenyl)phosphate with salicylhydroxamate ion in CTAB micellar media. The salicylhydroxamate ion has shown an important role towards cleavage of BDNPP. In this study, we observed that BDMO shows higher catalytic activity compared to other nucleophiles for the cleavage of BDNPP in gemini micellar media. Gemini micellar media accelerate $10^2$ fold the micellar catalysis than CPC, CDEEAB, CDMEAB, CPB and CTAB [33].

### 3.4.5 Reaction of bis($p$-nitrophenyl)phosphate in presence gemini surfactants

The effect of gemini surfactants (16-4-16 MEA 2Br$^-$, 16-6-16 MEA, 2Br$^-$, 16-10-16, 2Br$^-$ and 16-12-16, 2Br$^-$) for the nucleophilic promoted reactions of BNPP and BDNPP with HOO$^-$ ion has been studied at fixed nucleophile and surfactants concentrations which were varied between 0.0005 and 0.010 M. The reaction rates initially increase as the surfactant concentration is increased. The effect is very significant in 16-s-16 MEA, 2Br$^-$ (where $s = 4, 6$). The activity of the nucleophilic catalyst is explained as due to an electrostatic effect, the cationic nature of the surfactant favors the presence of hydroperoxide (HOO$^-$) at the micellar surface,
accelerating the cleavage of the substrate associated to micelles. The $k_{obs}$ values in the presence of cationic micelles gradually accelerated at low surfactant concentration and at high surfactant concentration show the inhibition effects for hydrolysis of BNPP. A similar dependence of $k_{obs}$ on surfactant concentration was previously observed for conventional monomeric and dimeric surfactants [79]. The hydroperoxide ion catalytic activity is 61 fold ($6.1 \times 10^{-5}$ s$^{-1}$/0.10×$10^{-5}$ s$^{-1}$) higher for BNPP cleavage than that of the hydroxide ion. At the rate maxima, $k_{obs}$ for the reaction of BNPP with 16-6-16, 2Br$^-$ - HOO$^-$ ($26.1 \times 10^{-5}$ s$^{-1}$) that is up to 4.27 fold catalysis over the reaction of HOO$^-$ alone ($6.10 \times 10^{-5}$ s$^{-1}$) and $2 \times 10^7$ fold are enhancement over the spontaneous aqueous reaction. For BDNPP reaction with 16-6-16 MEA 2Br$^-$ - HOO$^-$ ($32.3 \times 10^{-3}$ s$^{-1}$) the increase is 10 fold over the reaction of HOO$^-$ alone ($3.23 \times 10^{-3}$ s$^{-1}$) and a $1.7 \times 10^4$ fold rate enhancement over the spontaneous water reaction occurs.

The pseudophase model explains the variation of the rate constant with different cationic gemini surfactants. The binding constant is calculated using Eq. 3.4.

$$k_{obs} = \frac{k_W \left( k^M/M \right) K_M [D_n]}{1 + K_s [D_n]}$$

The calculated data are shown in Table 3.10. Analysis of kinetic data indicates that the 16-s-16 MEA, 2Br$^-$ (where $s = 4, 6$) series show higher reactivity compared to the 16-s-16, 2Br$^-$ ($s = 10, 12$) one. The micellar bound nucleophiles show large catalytic activity for the hydrolytic reactions [49]. The result of fitting the kinetic data by using Eq. 3.4 is shown in Fig. 3.11 by solid lines. We observed that the maximum binding constant for MEA gemini surfactants occurring for 16-4-16 MEA, 2Br$^-$ ($7681 \pm 420$ M$^{-1}$) while the maximum for the DMA series is 16-10-16, 2Br$^-$ ($1793 \pm 609$ M$^{-1}$). While not completely being comparable, since the different spacer, those values could indicate that the DMA series find it much difficult to accommodate the reactants. Since the MEA series should form hydrogen bonding among the headgroups, due to ethanol moieties, this series of surfactants has a higher micellar surface compactness. We recently demonstrated that the DMA series show low micellar surface compactness, since those surfactants cannot promptly accommodate the pyrene, normally used as a fluorescence probe to find the cmc [80]. Once more, the gemini system exhibits better catalytic activity toward the cleavage of triesters compared to CTAB system, as already shown in the literature [49].
Fig. 3.11 Influence of surfactant concentration upon pseudo-first-order rate constant for hydrolysis of BNPP in different cationic gemini surfactants. The solid line is calculated by the pseudophase model.

A comparative study of the different hydrolytic parameters associated with nucleophiles for the hydrolysis of BNPP show that by comparison with the uncatalyzed hydrolysis of BNPP ($k_{\text{uncat}} = 2.0 \times 10^{-13} \text{s}^{-1}$ and $1.1 \times 10^{-11} \text{s}^{-1}$) at pH 6.0 and 7.0 [81], the nucleophilic catalyzed reaction rates are $5.5 \times 10^6$ (for HOO⁻) and $2.3 \times 10^7$ (for 16-6-16 MEA, 2Br⁻ + HOO⁻ system) times faster. Besides, the uncatalyzed rate of BDNPP is $k_{\text{uncat}} = 1.7 \times 10^{-7} \text{s}^{-1}$ and nucleophilic catalyzed reactions are $1.9 \times 10^4$ (for HOO⁻) and $1.9 \times 10^5$ (with 16-6-16 MEA, 2Br⁻) and $1.3 \times 10^6$ (for 16-6-16 MEA, 2Br⁻ + AHA⁻) and $8.3 \times 10^6$ (for 16-6-16 MEA, 2Br⁻ + BDMO) times faster. On the basis of calculated data, we obtained the binding constant $K_M$ and the second order rate constant for BNPP and BDNPP, here reported in Table 3.10. It can be clearly noted that the binding constants are higher for bis(2,4-dinitrophenyl)phosphate compared to bis(p-nitrophenyl)phosphate. The reason may be that influence of electron withdrawing groups in the para-position to a phenolic oxygen donor on the magnitude of the rate data ($k_{\text{obs}}$) has shown that, generally, the more electron withdrawing the substituent the augment the $k_{\text{obs}}$ value [82] compared to bis(p-nitrophenyl)phosphate. Therefore, the binding constants could be higher in BDNPP than BNPP.
Table 3.10 Kinetic parameters obtained by applying pseudophase model for the nucleophilic reaction of diesters with hydroperoxide ion (HOO\(^-\)) in the presence of cationic surfactants.

<table>
<thead>
<tr>
<th></th>
<th>16-6-16, MEA 2Br(^-)</th>
<th>16-4-16 MEA, 2Br(^-)</th>
<th>16-12-16, 2Br(^-)</th>
<th>16-10-16, 2Br(^-)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>K(_M) (M(^{-1}))</strong></td>
<td>7681±420</td>
<td>6572±101</td>
<td>1962±485</td>
<td>1793±609</td>
</tr>
<tr>
<td><strong>k(_2)(_m) (M(^{-1})s(^{-1}))</strong></td>
<td>0.1625±0.007</td>
<td>0.1562±0.008</td>
<td>0.1187±0.005</td>
<td>0.0998±0.006</td>
</tr>
</tbody>
</table>

**Reaction conditions:** Temp. = 27 °C, [substrate] = 1×10\(^{-4}\) M, [Nu] = 0.3 M, [KCl] = 0.1 M (CMC = 16-6-16 MEA = 3.63×10\(^{-6}\) M, 16-4-16 MEA = 1.81×10\(^{-6}\) M, 16-12-16 = 0.02×10\(^{-3}\) M and 16-10-16 = 0.027×10\(^{-3}\)) Ref [32, 36]

We observed that hydrogen peroxide accelerate 10\(^7\) fold with gemini surfactant with respect to the reaction performed without surfactants. Nome and coworkers have reported [45] that the bis(2,4-dinitrophenyl)phosphate hydrolysis is catalyzed by hydroperoxide ions in aqueous media. They observed that \(\alpha\)-nucleophile (hydroperoxide) 10\(^5\) fold accelerate the rate of reaction for the cleavage of BDNPP than normal nucleophiles. Mao et al. [81] have studied that the hydrolysis of BNPP with metal ions (Ce(IV)–L1, Ce(IV)–L2). They observed that metal ion exhibits 1.3×10\(^8\) fold acceleration with respect to the spontaneous hydrolysis of BNPP [41] which means the half-life time is reduced from hundreds of years to about 2 minutes. The value of in that case is ca. 10\(^7\), indicating still better catalytic activity of the reaction system in micellar media. In order to obtain insight for the \(\alpha\)-nucleophile catalyzed hydrolysis of BNPP and other related phosphate esters further studies are in progress.

### 3.4.6 Reaction Mechanism

On the basis of the above studies, nucleophilic catalyzed mechanism is proposed for the catalytic cleavage of BNPP and BDNPP by hydroperoxide and butane 2, 3-dione
monoximate ions, which are similar to other reported data [40, 46]. As shown in Scheme 3.5 the hydroperoxide (HOO⁻) is proposed to be the active ion in the hydrolysis of BNPP under the reaction conditions employed in these kinetics studies. In this reaction the HOO⁻ mediated scission of the peroxy intermediate provides a way for completion of the nucleophilic reaction. We believe that the reaction of HOO⁻ with BNPP, simultaneously release almost 1 equivalent of p-nitrophenolate ion (NP) and mono(p-nitrophenyl)peroxyphenolate (1), which could readily form peroxyphosphate (2) which then give inorganic products (3) under reaction conditions [40, 46]. This could be supported by the literature reporting fast intramolecular substitution by a peroxide ion that was postulated in the peroxydolysis of bisaryl oxalates, e.g. bis(2,4-dinitrophenyl)oxalate, leading to formation of a cyclic peroxoxyxalate [48].

Takasaki [43], Bunton [48], Nome [40] and their research group have reported detailed mechanism for the cleavage of BNPP and BDNPP with hydroperoxide, benzohydroxamate, lauryl hydroxamte, ions. The reaction of BDNPP with hydroperoxide ion form peroxy intermediate that gives organic products indentified by ESI-MS and NMR spectra data. In these studies the catalytic rate constants $k_{cat}$ were all in order of magnitude of about $1 \times 10^{-6}$ s$^{-1}$ or less under the reaction conditions. Herein we present to the best of our knowledge the most highly effective HOO⁻ system reported, speeding up the hydrolysis reaction rate by a factor of $10^7$ compared to
uncatalyzed hydrolysis of BNPP. Over the past recent years, the potent α-nucleophiles such as benzohydroxamic acid, lauryl hydroxamic acids have been used for the cleavage of bis(2,4-dinitrophenylphosphate). Their products are characterized by NMR spectroscopy and ESI-MS, the study allows monitoring of the most important species with time for the reaction of BNPP and BDNPP [82]. In our previous study [57], we have proposed mechanism for the nucleophilic attack of hydroxamate ion at the \( P=O \) center of phosphate esters. Herein, we found that butane 2,3-dione monoximate ion (BDMO\(^-\)) show higher catalytic reactivity towards the cleavage of BDNPP compared to acetohydroxamate ion (AHA\(^-\)). The reason may reside in the fact that oximes are α-nucleophiles, and the charge on the oxido atom (\(-N-O^-\)) of the conjugate bases is responsible for the hydrolytic cleavage of carboxylate and phosphate esters (Scheme 3.6). Many research groups have reported the reactivity of α-nucleophiles towards the cleavage of carboxylate and phosphate esters in micellar media. Furthermore, the computational calculations show nucleophilic reactivity for the substrate hydrolysis [83]. Thus, the highly efficient catalytic activity may be induced by the unshared lone pair of electron on adjacent center of BDMO and AHA. It has also been observed that nucleophilic reactivity of α-nucleophiles is much higher than that predicted by Bronsted relationship between nucleophilicity and basicity [84].

\[
\begin{align*}
\text{Ka oxime} & \quad + \\
\text{BDNPP} & \quad \rightarrow \\
\text{Products}
\end{align*}
\]

\textbf{Scheme 3.6}
3.5 CONCLUSION

In this study, we found that the nucleophilic activities of α-nucleophiles are higher with the cationic micellar media toward bis(p-nitrophenyl) phosphate cleavage. The experimental data shows that oximate ion (BDMO⁻) has higher catalytic activity compared with hydroxamate ions (AHA⁻, BHA⁻, SHA⁻) in cationic micellar media. The CPC shows higher catalytic activity toward hydrolytic cleavage of diester compared with CPB, CDEEAB, CDMEAB and CTAB. The hydrolytic cleavage of diester is very slow therefore, kinetic rate data are limited, but α-nucleophiles with cationic surfactants are better for BNPP cleavage. This study will help to elucidate the cleavage mechanisms of phosphodiester bonds and design of efficient artificial nucleases.

A few α-nucleophiles, hydroperoxide, AHA, BDMO were employed as effective catalyst for the hydrolysis of phosphate esters bis (p-nitrophenyl) phosphate (BNPP) and bis(2,4-dinitrophenyl)phosphate (BDNPP) in micellar media, using two series of gemini surfactants, 16-4-16 MEA, Br⁻ and 16-4-16 DMA, Br⁻. Gemini surfactants showed to be very effective for the catalytic hydrolysis of phosphoric diesters with anionic nucleophiles, in particular α-nucleophiles, and this will be significant for directing the design of artificial phosphate diesters. The results from the kinetic study showed that the general order of reactivity for micellar media is: 16-6-16 MEA, Br⁻ >16-4-16 MEA, Br⁻ >16-12-16, Br⁻ >16-10-16, Br⁻ while among the α-nucleophiles, the hydroperoxide ion (HOO⁻) was evidenced as the most reactive one for the cleavage of BNPP and BDNPP, more reactive than AHA and BDMO. In particular, cationic gemini surfactants turned out to be very effective to increase the nucleophilic reactivity of HOO⁻. The reactivity of HOO⁻ in presence of 16-6-16 MEA, Br⁻ was about 4.27 time faster for BNPP and 10 times faster for BDNPP over the reaction of HOO⁻ alone, and 2×10⁷ (for BNPP) and 1.7×10⁴ (for BDNPP) faster over the spontaneous reaction in water. The increase of 2×10⁷ times is the better result ever obtained in the acceleration of BNPP ester hydrolysis with nucleophiles or α-nucleophiles. These results showed that cationic gemini micelles are excellent reaction media in combination with α-nucleophiles for the phosphate esters hydrolysis, cleaving the P=O bond. The improvement for phosphate esters hydrolysis catalysis could be pursued by searching for both better performing gemini surfactants and α-nucleophiles, for which studies are actively in progress.
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