Effect of some pyridinium based compounds on the hydrolysis of carboxylate ester

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Nucleophilic reactivity of some pyridinium based compounds towards hydrolysis of $p$-nitrophenyl acetate (PNPA) has been studied at pH 9.0 and temperature 27ºC. The nucleophilic reactivity of newly synthesized pyridinium compounds has been compared with commercially available pyridinium based compounds such as pralidoxime and dodecylpyridinium bromide. It has been observed that with an increase in the concentration of these compounds there is an increase in the first order rate constant of the reaction. The apparent acid dissociation constant ($pK_a$) of some compounds have also been determined spectrophotometrically. Oximate ions released from 3-hydroxyiminomethyl-1-dodecylpyridinium bromide (3-HIDDPB) and pralidoxime have proved to be better nucleophilic agents for cleavage of carboxylate ester. Present investigation explores the structure-activity relationship of some pyridinium based compounds with same alkyl tail length.

Keywords: Pyridinium compounds, nucleophilic reactivity, hydrolysis, oximes, acid dissociation constant

It is well established that pyridinium based compounds are used as potential hydrolytic micellar catalysts for detoxification of organophosphorus compounds\(^1\)\(^-\)\(^3\). Pyridinium salts are categorized under cationic surfactants. Quaternary pyridinium salts are unsaturated heterocyclic compounds having different kinds of functional groups present either on pyridine ring or directly attached to the nitrogen atom. These compounds are derived from pyridine. The incorporation of different kinds of functional groups (-OH, -NH$_2$, -NOH, -OR, -COOR, etc.) in the pyridine ring makes them a versatile and functionalized compounds which can serve for various purposes\(^8\). Introduction of a nucleophilic moiety in the pyridinium based surfactant makes them more efficient and they are known as ‘functionalized surfactants’. They constitute functional micelles with a nucleophile bound covalently to the head group and help in accelerating the hydrolysis of different esters. Functionalized surfactants are the most common catalysts in the functional micellar catalysis\(^5\)\(^-\)\(^9\). Pyridinium based compounds have wide applications ranging from biological to industrial fields\(^10\). Sincere efforts have been made to study how structure and substituents on pyridinium ring influences their reactivity\(^11\)\(^-\)\(^12\). Pyridinium based functionalized surfactants are used for detoxification of organophosphorus compounds and nerve agents\(^13\)\(^-\)\(^16\). In addition, pyridinium oximes also show direct pharmacological effects\(^17\)\(^,\)\(^18\). Pyridinium based surfactants are employed in cosmetics, gene delivery, polymerization and many other industrial applications. They are also effective phase transfer agents catalysts and acylating agents\(^19\)\(^,\)\(^20\).

For the last few years we have been working on development of novel methods for detoxification of organophosphates and pesticides using some pyridinium based oximes and related compounds\(^21\)\(^-\)\(^23\). In continuation of our structure-reactivity studies of different carboxylate and phosphate esters with nucleophiles, in the present paper we focus on the synthesis and hydrolytic efficacy of three pyridinium based compounds $i.e.$ 4-((1-aminohydroxyimino)methyl)-1-dodecylpyridinium bromide (4-AHIDDPB), 3-Hydroxy-1-dodecylpyridinium bromide (3-HDDPB) and 3-Hydroxyiminomethyl-1-dodecylpyridinium bromide (3-HIDDPB) for the hydrolysis of $p$-nitrophenyl acetate (PNPA) which is
the most widely used model substrate of organophosphates. The hydrolytic efficiency of dodecylpyridinium bromide (DPB) and pralidoxime has also been studied so that a comparative study can be done (Scheme I). Among the investigated compounds 4-AHIDDPB, 3-HDDPB and 3-HIDDPB comes under the category of functionalized surfactants. Apparent $pK_a$ values of 4-AHIDDPB and 3-HDDPB have also been determined by spectrophotometric method.

## Results and Discussion

The rate of hydrolysis of $p$-nitrophenyl acetate (PNPA) was studied under pseudo first order condition at $pH$ 9.0 and 27°C with varying nucleophilic concentration of all the pyridinium compounds. The reaction rate law may be expressed as

$$\text{Rate} = k \left[ \text{Nu}^- \right] \left[ \text{Ester} \right]$$

... (1)

### Effect of concentration of nucleophile (Nu$^-$)

The present work examines the micellar effects of functionalized surfactants and the reactivity of their nucleophilic head groups in relation to cleavage of toxic esters. The rate constants for the hydrolysis of PNPA in the presence of all the pyridinium compounds are presented in Table I. The reactions were found to obey pseudo-first-order kinetics with respect to the nucleophilic concentration. It can be observed that the first-order rate constant increases sharply with increasing concentration of pyridinium compounds in the reaction medium and then decreases upon further addition of nucleophile. This is accounted on the basis that most of the nucleophilic species used in the present investigation are ‘functionalized surfactants’. Functional detergents like 4-AHIDDPB, 3HDDPB and Pralidoxime contain

### Table I — Observed first-order rate constants for the hydrolysis of PNPA in the presence of different compounds

<table>
<thead>
<tr>
<th>[Conc.] (mM)</th>
<th>4-AHIDDPB</th>
<th>3-HDDPB</th>
<th>DPB</th>
<th>Pralidoxime</th>
<th>3-HIDDPB</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>0.25</td>
<td>0.26</td>
<td>0.16</td>
<td>0.10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.50</td>
<td>0.39</td>
<td>0.26</td>
<td>0.15</td>
<td>5.03</td>
<td>8.00</td>
</tr>
<tr>
<td>1.00</td>
<td>0.51</td>
<td>0.34</td>
<td>0.27</td>
<td>11.3</td>
<td>16.5</td>
</tr>
<tr>
<td>2.00</td>
<td>0.71</td>
<td>0.53</td>
<td>0.46</td>
<td>20.8</td>
<td>24.6</td>
</tr>
<tr>
<td>3.00</td>
<td>0.68</td>
<td>0.58</td>
<td>0.43</td>
<td>22.2</td>
<td>28.4</td>
</tr>
<tr>
<td>3.50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>18.2</td>
<td>22.8</td>
</tr>
<tr>
<td>4.00</td>
<td>0.57</td>
<td>0.50</td>
<td>0.39</td>
<td>15.4</td>
<td>21.0</td>
</tr>
</tbody>
</table>

Temperature = 27 ºC, [PNPA] = 0.5 × 10$^{-4}$ M, Ionic strength (µ) = 0.1 M KCl, pH = 9.0.
a fragment of α-nucleophile in the head group to form supernucleophilic reagent for the cleavage of C=O and P=O centers of toxic esters. They are capable of forming highly reactive anionic species e.g. fragment of α-nucleophiles *i.e.* oximate anion. The nucleophilic centre is located in oximate moiety\(^{24,25}\). As the concentration of surfactant increases the reaction rate increases considerably. When the substrate is completely bound with micelles, the reaction no longer depends on the surfactant concentration and hence the rate constant decreases on subsequent addition of functionalized surfactants. Micelles bring reactants closer by hydrophobically binding of substrate and coulombically attracting negatively charged nucleophile. Initially, an increase in the surfactant concentration generates more cationic micelles and increase in the reaction rate occurs. With increasing micelles in the solution a stage appears where all the substrates are almost entrapped in the micellar phase. Further addition of surfactant increases the number of micelles to such an extent that the oximate anions are bound in the “Stern layer”, thus rate of reaction falls since the substrate in one micelle cannot react with nucleophile in another. The excess of unreactive counterions competes with oximate ions for available sites in the Stern layer.

Decrease in the observed reaction rates after maxima is due to the dilution of catalysts in the Stern layer of micelles\(^{15}\). The corresponding second order rate constants \((k_2)\) were calculated from the plots of \(k_{\text{obs}}\) versus [Nu] up to a concentration of \(2 \times 10^{-3}\) M using the equation

\[
k_{\text{obs}} = k^0_{\text{obs}} + k_{\text{Nu}} [\text{Nu}]
\]

\(k^0_{\text{obs}}\) in the equation (2) corresponds to the intercept in the \(k_{\text{obs}}\) versus [Nu] (Ref 26-28). Values of \(k_2\) obtained for 4-AHIDDPB, 3-HIDDPB, DPB, Pralidoxime and 3-HIDDPB are 0.29, 0.22, 0.20, 10.4, 12.1 \text{ M}^{-1}\text{s}^{-1}\) respectively. It is clear from the Table I that \(k_{\text{obs}}\) values are highest for the functionalized surfactant 3-HIDDPB and pralidoxime.

**Structure-activity relationship**

Oxime based functionalized surfactants are considerably reactive towards organophosphates under mild conditions. As it can be seen from Figure 1 the most reactive compound is the functionalized surfactant 3-HIDDPB which contains a hydroxyminomethyl group at position three at pyridine ring. Micelles formed by such zwitterionic species whose head part includes a covalently linked typical α-nucleophilic group can be regarded as a

![Figure 1](image-url)

*Figure 1*—Plots for the reaction of \(p\)-nitrophenyl acetate (PNPA) with different concentrations of 3-HIDDPB and pralidoxime at pH 9.0 and temperature 27 °C. Inset: Derivative plot of \(k_{\text{obs}}\) versus [Nu] (upto 2.0 mM) for the calculation of second order rate constants.
unique super-nucleophilic system ensuring high rates of substrate cleavage due to reactant concentration effect. Pralidoxime shows higher reactivity due to hydroxylimomethyl group at position two in the pyridinium ring. Structures of pyridinium detergents with oxime group as nucleophilic function able to split nerve agents were designed from the pralidoxime structure. Pralidoxime, a monoquaternary pyridinium aldoxime is known to be a good nucleophile used as an antidote in the case of nerve agent intoxication. Its antidotal effect is based on the splitting of the covalent bond between enzyme acetylcholinesterase and nerve agent. Owing to this, the design of pralidoxime like detergents seemed to be a good approach of how to improve the potency of quaternary detergents to hydrolyze nerve agents. Table I shows that the $k_{obs}$ values for 4-AHIDDPB are higher than 3-HDDPB and DPB because of amidoxime group at position four in pyridine ring. The electron withdrawing effect of quaternary nitrogen in the pyridinium oximes increases the acidity of the hydroxyimino group which is relatively low in nonsubstituted aromatic or aliphatic oximes ($pK_a \approx 12-13$). The increased acidity of pyridinium aldoximes and ketoximes ($pK_a \approx 8-10$) provides sufficient concentration of the nucleophilic oximate anion even in neutral solutions. Thus oxime based functionalized surfactants are considerably reactive when compared to normal pyridinium based surfactants. 3-HDDPB has a hydroxyl group at position three and no imino or amino functional groups in the pyridine ring hence, the rate constant values for this compound is considerably low. The surfactant DPB has no functional group attached to pyridine ring and thus the rate of hydrolysis of ester was lowest in this case. The small $k_{obs}$ values in all the cases accounts for lower alkyl chain length ($C_{12}H_{25}$).

### Table II — Melting points of some synthesized pyridinium based compounds

<table>
<thead>
<tr>
<th>Synthesized Compounds</th>
<th>Melting Points (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-AHIDDPB</td>
<td>160-161</td>
</tr>
<tr>
<td>DPB</td>
<td>104-105</td>
</tr>
<tr>
<td>3-HDDPB</td>
<td>90-92</td>
</tr>
<tr>
<td>3-HDDPB</td>
<td>138-140</td>
</tr>
</tbody>
</table>

**General synthetic procedure**

Substituted pyridines *i.e.* 4-(1-aminohydroxyliminomethyl)pyridine for (4-AHIDDPB), 3-hydroxy-pyridine (for 3-HDDPB), pure pyridine for DPB and 3-hydroxyiminomethylpyridine for 3-HDDPB was dissolved in absolute ethanol (99.5%). Further dodecyl bromide was added and the mixture was refluxed for 60 hr. The solvent was then evaporated and the crude product was dissolved in minimum amount of methanol followed by filtration with active carbon. To this, ethyl acetate was added till the crystals appeared. Product was filtered and dried. Melting points of all the synthesized compounds are presented in Table II.

### Determination of acid dissociation constant ($pK_a$)

The $pK_a$ values of 4-AHIDDPB and 3-HDDPB were determined spectrophotometrically by using Albert and Sergeant method. An aliquot (3 mL) of a stock solution (5 × 10^{-4} M) of 4-AHIDDPB in 50% (v/v) of acetonitrile and 3-HDDPB in triple distilled water was diluted with 25 mL phosphate buffer solution at pH 6.1. The pH of the solution was measured using Systronics (Type-362) pH-meter and the spectrum was recorded using buffer solution as a blank. The absorption spectrum was recorded using Varian Cary 50 UV-Vis spectrophotometer in the range of 200-400 nm and at different pH values. The average values of ten measurements were considered as the $pK_a$ of the compound. The acid dissociation constant of 4-AHIDDPB was determined spectrophotometrically at the temperature 27°C. The $pK_a$ calculations were made around half neutralization using Eqn 1.

$$pK_a = pH_{exp} + \log \frac{Abs_{Hox}}{Abs_{\psi}} = \frac{Abs_{\psi} - Abs_{ox}}{Abs_{Hox} - Abs_{\psi}}$$ (1)

Where $Abs_{Hox}$ is the absorbance of unionized form of compound, $Abs_{\psi}$ is the absorbance of partially ionized form of compound, and $Abs_{ox}$ is the absorbance of completely ionized form of compound. $pK_a$ value of 4-AHIDDPB and 3-HDDPB was found to be 7.7 and 9.0 respectively and $pK_a$ value of 3-HDDPB is 9.0.

### Kinetic measurements

The rate of nucleophilic substitution reaction was examined at 27°C by monitoring the increase in absorption of $p$-nitropenoxide ion at 400 nm using
Varian Cary 50 UV-Vis spectrophotometer. The kinetic study was performed under pseudo first order condition where the concentration of nucleophile was in large excess over the substrate concentration (10:1). Borax buffer (pH = 9.0) was used to maintain the pH of the reaction media. The liberated p-nitrophenoxide ion was identified as one of the products by comparing the UV spectrum at the end of the reaction with authentic sample under the experimental condition. Figure 2 shows a representative graph for hydrolysis of PNPA with 3-HIDDPB at pH 9.0. For all the kinetic runs, the absorbance/time result fits very well to the first order rate equation.

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

The pseudo-first-order rate constants \( k_{\text{obs}} \) were determined from the plots of log \( (A_{\text{inf}} - A/A_{\text{inf}} - A_0) \) versus time with \( A_0, A_t \) and \( A_{\text{inf}} \) being the absorbance values at zero, time and infinite time respectively (Figure 3). Each experiment was repeated at least twice, and the observed rate constant was found to be reproducible within a precision of about 3% or better.

**Conclusions**

The rate constant for cleavage of ester rises with an increase in concentration of pyridinium compounds. Among all the examined pyridinium compounds, 3-HIDDPB and pralidoxime were found to be the most effective. The presence of different substituents on the pyridinium ring increases its efficiency and it turns to be a better reagent for nucleophilic substitution reactions. They have lower \( pK_a \) values than non substituted aliphatic or aromatic oximes and hence, they prove to be efficient hydrolytic micellar catalysts for detoxification of toxic organophosphates and nerve agents. The results of the present investigation could be useful for the designing of more effective decontamination means.

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**References**