CHAPTER III

DETERMINATION OF $pK_a$ OF HYDROXAMIC ACIDS BY NUCLEOPHILIC SUBSTITUTION REACTION

Part of this work has been published in Indian J. Chem., 2007, 46A, 1630-1631
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BY NUCLEOPHILIC SUBSTITUTION REACTION

3.0 INTRODUCTION

Hydroxamate ions (I) are $\alpha$-effect nucleophiles, whose reactivities are higher than that predicted by relationship between nucleophilicity and basicity$^{1-11}$. These are effective agents$^{10-11}$ of detoxification. The enhanced reactivity compared with normal nucleophiles of similar basicity is due to the presence of an unshared electron pair adjacent to the nucleophilic centre, i.e. the $\alpha$-effect.$^{12-13}$ Despite their interesting properties and various applications, hydroxamic acids still remain one of the less characterized classes of organic compounds. Assignments of the correct structure have been a major difficulty because these compounds may adopt either the $Z$ (cis) or $E$ (trans) conformation. Studies on the analytical and biomedical applications of hydroxamic acids require knowledge of their $pK_a$ values.

\[
\begin{array}{c}
\text{O} \\
\text{R} - \text{C} - \text{N} - \text{R}' \\
\text{O}^- \\
\end{array}
\]

(I)

However, no attention has been devoted for the study of $pK_a$ of N-methyl substituted hydroxamic acids and $p$-substituted benzohydroxamic acids by nucleophilic substitution reaction. In the present investigation N- methyl substituted hydroxamic acids and $p$-substituted benzohydroxamic acids have been undertaken for nucleophilic substitution reaction of $p$-nitrophenyl acetate.

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3.1 REVIEW OF THE EARLIER WORK

Hydroxamic acid, is a family of weak organic acid containing the \(-\text{C(O)}\text{NHOH}\) functionality. The advent of recent technological advancements and the exciting developments in the sophisticated physico-chemical methods have paved the way for a clear understanding of the structures and chemistry of hydroxamic acids.\(^6\)\(^-\)\(^8\) In recent years, constant attention has been devoted to synthesize various hydroxamic acid derivatives as well as their metal complexes.\(^9\)\(^-\)\(^{15}\)

The various applications of hydroxamic acids such as acid-base catalyzed hydrolysis of hydroxamic acids,\(^15\)\(^-\)\(^{19}\) protonation equilibria,\(^20\)\(^-\)\(^{23}\) micellar catalysis,\(^24\)\(^-\)\(^{26}\) micellar-polymer effect\(^27\)\(^,\)\(^28\) and DNA cleavage\(^29\) have been studied extensively. The ionization dissociation constant \((pK_a)\) of nucleophiles play a important role in the hydrolysis of carboxylic and phosphate esters.\(^30\)\(^-\)\(^{37}\)

Acid dissociation constants and their temperature dependence for a series of carbon and nitrogen substituted hydroxamic acids in aqueous solution by pH metric method has been extensively studied by Crumbliss et al.\(^37\)\(^-\)\(^{39}\). Similarly Agrawal et al.\(^38\)\(^-\)\(^{42}\) determined \(pK_a\) of several hydroxamic acids in aqueous and organic solvent media. Garcia et al.\(^26\) measured acid dissociation constant of some hydroxamic acids by spectrophotometric and potentiometric methods. Surprisingly no attempts seem to have been made to determine \(pK_a\) of hydroxamic acid using nucleophilic substitution reactions.

3.2 PRESENT INVESTIGATION

In the present investigation, acid dissociation constant \((pK_a)\) of the \(p\)-substituted benzohydroxamic (II) and N-methyl \(p\)-substituted benzohydroxamic acids (III) have been studied by the \(\alpha\)-effect nucleophilicity of
the hydroxamate ions towards the hydrolysis of p-nitrophenyl acetate (PNPA) at different pH (Scheme 3.1).

\[
\text{H}_3\text{C} - \text{C} - \text{O} - \text{C}_6\text{H}_4\text{NO}_2 + \text{Nu}^- \rightarrow \text{H}_3\text{C} - \text{C} - \text{Nu}^- + \text{O} - \text{C}_6\text{H}_4\text{NO}_2
\]

PNPA

\[
\begin{align*}
&\text{H-N-OH} \\
&\text{X} = \text{H, CH}_3, \text{CH}_2\text{O}, \text{Cl, NO}_2
\end{align*}
\]

\[
\begin{align*}
&\text{H}_3\text{C}-\text{N-OH} \\
&\text{X} = \text{H, CH}_3, \text{CH}_2\text{O}, \text{Cl}
\end{align*}
\]

\(\text{Nu}^- = \text{Hydroxamate ions (II & III)}\)

Scheme 3.1

The acid dissociation constants \(pK_a\) (\(pK_a = -\log K_a\)) can be represented as equation 1

\[
4 - \text{XC}_6\text{H}_4\text{CON} (\text{OH}) \text{R'} \xleftrightarrow{\text{H}_2\text{O}} \ [4 - \text{XC}_6\text{H}_4\text{C} (\text{O}) \text{N} (\text{O}) \text{R'}]^- + \text{H}^+_{\text{aq}} \quad (1)
\]

\(\text{R'} = \text{H; CH}_3\)
3.3 EXPERIMENTAL

Material:

*p-Nitrophenyl acetate* was obtained from S D fine chemicals and *p*-substituted benzohydroxamic acid (II) was prepared by the reaction of corresponding *p*-substituted benzoyl chloride and hydroxylamine hydrochloride in the presence of sodium carbonate according to the scheme 3.2. Benzohydroxamic acid M.P. 124°C (lit 26 M.P. 123-127). *p*-chloro benzohydroxamic acid M.P. 159°C (lit27 M.P 158-159°C). *p*-Methyl benzoxydroxamic acid M.P. 131°C. *p*-methoxy benzohydroxamic acid M.P. 178°C (with decomposed). The synthesis of N-methyl substituted benzohydroxamic acids were discussed in Chapter II.

\[
\begin{align*}
\text{Scheme 3.2}
\end{align*}
\]
Determination of pKₐ of Hydroxamic Acids

The rate of nucleophilic reaction with PNPA was determined by following the increase in absorption of p-nitrophenoxide anion (400nm) using Varian Carry-50, Systronics (Type 104) spectrophotometer. All of the kinetic experiments were performed at an ionic strength of 0.1M (with KCl). Phosphate buffer was employed. All reactions were conducted under pseudo-first-order conditions. For all of the kinetic runs, the absorbance/time results fit very well to the first order rate equation.

\[
\ln (A_\infty - A) = \ln (A_\infty - A_0) - kt
\]  

(2)

The pseudo-first-order rate constant can be determined by least squares fits. 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. The spectrum exhibits an increase in absorbance at 400 nm with the formation of p-nitrophenoxide ion during the course of reaction (Fig. 3.1).

3.4 RESULTS AND DISCUSSION

The pseudo-first order rate constants (k_{obs}) for the reaction of PNPA (1.0 x 10⁻⁴ M) with hydroxamate ions have been determined spectrophotometrically at 27°C under excess hydroxamic acid concentration (1.0 x 10⁻³ M). All the reactions obey Eq. (3), where k₀ and kₐ⁻ are the rate constants for hydrolytic reactions in buffer alone and nucleophilic hydrolysis of PNPA.

\[
k_{obs} = k_0 + k_{A^-} [A^-]  
\]  

(3)

\[
k_0 = k_{H_2O} + k_{OH^-} [OH^-]
\]  

(4)
Fig. 3.1 Repeat scans graph showing increasing absorbance at 400 nm. [PNPA] = 1.0 x 10^{-4} M, [HA] = 1.0 x 10^{-3} M, [KCl] = 1.0 M Temp. 27 °C.
Hydroxamate ions are α-nucleophiles, i.e., very strong nucleophiles, so that competition with other nucleophiles and, in particular, \( \text{OH}^- \) and \( \text{H}_2\text{O} \) is not expected and \( k_{\text{obs}} \) is simply given by \( k_{\text{obs}} = k_{A^-} [A^-] \). The plots of \( k_{\text{obs}} \) vs. nucleophilic concentrations were linear and passed through origin.

The values of \( k_{\text{obs}} \) depends upon the ionization state of the hydroxamic acid, eq. (5) may be written to describe \( k_{\text{obs}} \) where \( k_{A^-} \) is second order rate constant, \([HA]_r\) is the analytical concentration of hydroxamic acid, and \( \alpha_{A^-} \) is the fraction of \([HA]_r\) ionized.

\[
k_{\text{obs}} = k_0 + k_{A^-} [HA]_r \alpha_{A^-} \quad (5)
\]

In this equation \( \alpha_{A^-} \) is equal to \( K_a / K_a+[H^+] \) and \( K_a \) is the kinetically apparent dissociation constant of hydroxamic acids. Eq. (6) may be expressed the observed rate constants for the hydrolysis of PNPA.

\[
k_{\text{obs}} = k_0 + k_{A^-} [HA]_r \frac{K_a}{K_a+[H^+]} \quad (6)
\]

Utilizing eq. (6), the \( pK_a \) values of the hydroxamic acid have been determined by the pH dependent rate constant of the hydrolysis of PNPA. The \( pK_a \) values of the hydroxamic acid have been determined by measuring the pseudo first order rate constants for PNPA cleavage at 27°C at different pH values between 6.6 to 11.0. Table: 3.1 summarize the pH-dependent pseudo-first order rate constants \( (k_{\text{obs}}) \) for the reaction of PNPA with hydroxamate ions. The \( k_{\text{obs}} \) values increase with increasing pH values of the reaction medium. The \( k_{\text{obs}} \) values for the reaction depends on the active concentration of the hydroxamate ions, obtained upon the deprotonation of the hydroxamic acids with increasing pH.
Table 3.1. pH dependent observed first order rate constants for the reaction of PNPA and hydroxamate ions.

<table>
<thead>
<tr>
<th>pH</th>
<th>p-X C₆H₄(C=O)NHOH</th>
<th>p-X C₆H₄(C=O)N(OH)CH₃</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kₐobs x 10¹³ s⁻¹</td>
<td></td>
</tr>
<tr>
<td>6.6</td>
<td>0.46</td>
<td>0.34</td>
</tr>
<tr>
<td>7.1</td>
<td>0.86</td>
<td>0.70</td>
</tr>
<tr>
<td>7.9</td>
<td>8.87</td>
<td>2.46</td>
</tr>
<tr>
<td>8.5</td>
<td>21.0</td>
<td>3.05</td>
</tr>
<tr>
<td>9.1</td>
<td>27.5</td>
<td>3.55</td>
</tr>
<tr>
<td>10.1</td>
<td>34.0</td>
<td>8.54</td>
</tr>
<tr>
<td>11.1</td>
<td>38.1</td>
<td>21.1</td>
</tr>
</tbody>
</table>

[PNPA] = 1.0 x 10⁻⁴ M, [HA] = 1.0 x 10⁻³ M, ionic strength 0.1 M (KCl), Temp. =300K
The rate of reaction shows drastic change at the pH where the 50% hydroxamic acid deprotonated, i.e. $pK_a$ of hydroxamic acid. Hydroxamic acids have been suggested to behave either as NH or OH acids depending on solvents.\textsuperscript{15-18} It is known that the anion of hydroxamic acid acts as a reactive species in the hydrolysis of esters. Consequently, the $pK_a$ for the conversion of the N-OH to N-O$^-$ form plays an important role for the hydrolysis of PNPA. A pH-rate constant profile for the nucleophilic reaction of $1.0 \times 10^{-4}$ M PNPA with $1.0 \times 10^{-3}$ M hydroxamate ions gave the apparent $pK_a$ values for each of the hydroxamic acids. Typically, the pseudo first order rate constants for the reaction of PNPA were determined at different pH values between 6.6 and 11.0.

In Fig.3.2-3.9, pH-rate constant profile for the cleavage of PNPA by nucleophiles 3a, 3b, 3c, 3d, 1a, 1b, 1c,1d and 1e at 27° C are shown. The plots of log $k_{obs}$ vs. pH (Fig. 3.2-3.10) gave a break, which were taken as an apparent $pK_a$ for the hydroxamic acid. The $pK_a$ values obtained by kinetic studies are in close agreement with literature values. The $pK_a$ values exhibit a small variation with the substitutent $X$.

The dependence of the acid dissociation constant on the Hammett substituent constant is expressed by the equation:

$$pK_a = -\rho \sigma$$  \hspace{1cm} (7)

The plots between $pK_a$ of nucleophile (II) and nucleophile (III) and substituted constants are shown in Figure 3.11 and Figure 3.12. The $\rho$ value were obtained from the plots as follows: $\rho = (-0.73)$ for nucleophiles (II) and $\rho = (-0.91)$ nucleophiles (III).
Fig. 3.2 Plot of log $k_{obs}$ vs. pH for the reaction of PNPA with nucleophile 3a.

Fig. 3.3 Plot of log $k_{obs}$ vs. pH for the reaction of PNPA with nucleophile 3b.
Fig. 3.4 Plot of log $k_{obs}$ vs. pH for the reaction of PNPA with nucleophile 3c.

Fig. 3.5 Plot of log $k_{obs}$ vs. pH for the reaction of PNPA with nucleophile 3d.
Fig. 3.6 Plot of log $k_{obs}$ vs. pH for the reaction of PNPA with nucleophile 1a.

Fig. 3.7 Plot of log $k_{obs}$ vs. pH for the reaction of PNPA with nucleophile 1b.
Fig. 3.8 Plot of $\log k_{\text{obs}}$ vs. pH for the reaction of PNPA with nucleophile 1c.

Fig. 3.9 Plot of $\log k_{\text{obs}}$ vs. pH for the reaction of PNPA with nucleophile 1d.
Fig. 3.10 Plots of log $k_{obs}$ vs. pH for the reaction of PNPA with nucleophile 1e.
Fig. 3.11 Plot between pK_α of nucleophiles 3a – 3d and substituent constant of nucleophile (II) Series

Fig. 3.12 Plot between pKa of 1a- 1e and substituent constant of nucleophile (III) series
In discussing the influence of $R'$ on $pK_a$, it is instructive to consider the possible resonance forms for the conjugate base anion (IV-V). The electron density donating ability of $R'$ helps to stabilize resonance form V relative to IV. This allows positive charge density to build up on nitrogen and thereby stabilizes the conjugate base anion by induction. Stabilization of the negative charge on $O_2$ in the hydroxamate ion is possible only through induction. Therefore, this analysis indicates that the delocalization of the lone pair of electrons on N in anyway to provide a formal positive charge adjacent to the negative charge on $O_2$ is of importance in determining acid strength. The inductive and/or resonance effects of the carbonyl function, R and $R'$ influence this delocalization.

![Chemical Structures](image)

The basicity of nucleophile has most commonly been used as a measure of nucleophilicity. However, a certain group of nucleophiles has been found to exhibit abnormally higher nucleophilic reactivity than would be predicted from their respective basicity. A common feature of these nucleophiles is the possession of one or more nonbonding electron pair at the $\alpha$-position of the nucleophilic center. To obtain the conclusive information on the $\alpha$-effect shown by the hydroxamate ion ($k_{A^-}/k_{Normal\ Nuc.}$), the nucleophilic reactivity has been compared with nucleophilicity of $p$-chlorophenoxide. Table 3.2 summarizes the $\alpha$-effect of different hydroxamate ions. Nucleophile 3a and nucleophile 3c shows large $\alpha$-effect i.e. 50.5 and 60.1.
Table 3.2. Second Order Rate Constants for the Reaction of PNPA with Hydroxamate Ions and pKₐ's of hydroxamic acids

<table>
<thead>
<tr>
<th>Hydroxamate ion</th>
<th>pKₐ</th>
<th>Lit. Value</th>
<th>k_{HA}, M⁻¹ s⁻¹</th>
<th>b_{k_{HA}/k_{ClPhO}⁻}</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>8.5</td>
<td>8.81</td>
<td>34.6</td>
<td>50.5</td>
</tr>
<tr>
<td>3b</td>
<td>7.95</td>
<td>8.58</td>
<td>3.69</td>
<td>5.38</td>
</tr>
<tr>
<td>3d</td>
<td>7.9</td>
<td>7.92</td>
<td>3.25</td>
<td>4.74</td>
</tr>
<tr>
<td>3c</td>
<td>8.6</td>
<td>8.97</td>
<td>41.2</td>
<td>60.1</td>
</tr>
<tr>
<td>1a</td>
<td>8.35</td>
<td>8.28</td>
<td>16.9</td>
<td>24.6</td>
</tr>
<tr>
<td>1b</td>
<td>8.4</td>
<td></td>
<td>21.7</td>
<td>31.6</td>
</tr>
<tr>
<td>1c</td>
<td>8.7</td>
<td>8.50</td>
<td>30.5</td>
<td>44.5</td>
</tr>
<tr>
<td>1d</td>
<td>8.8</td>
<td>8.67</td>
<td>19.0</td>
<td>27.7</td>
</tr>
<tr>
<td>1e</td>
<td>7.9</td>
<td>7.94</td>
<td>17.7</td>
<td>25.8</td>
</tr>
</tbody>
</table>

*a* Values taken from ref. 20 and 21

*b* Second order rate constant of the reaction of p-ClPhO⁻ ($k_{ClPhO}⁻ = 0.685$ M⁻¹ s⁻¹)²⁸
Many factors have been suggested to influence the magnitude of the \( \alpha \)-effect the basicity of \( \alpha \)-nucleophiles, the types of hybridization of the electrophilic centers, and the magnitude of \( \beta_{\text{nuc}} \) among them. N-methylation of hydroxamic acids had no significant effect on \( pK_a \), since literature and observed \( pK_a \) values of nucleophile II versus nucleophile III were found to be almost similar. The Role of N-methylation on hydrolytic kinetics of PNPA was influenced by the type of substituent present on the benzene ring. Hydrolytic rate constants by nucleophile 3a and nucleophile 3d were higher than their N-methyl counter parts.

It is indicated that \( N \)-methylation had negative role on hydrolytic kinetics of PNPA. The possible explanation to this behaviour could be that the species VII formed by deprotonation of one of resonating form of hydroxamic acids could have stabilize the transition state (formed in S_N2 attack of nucleophile) better as nucleophilic oxygen has adjacent nitrogen with lone pair of electrons. While in case of nucleophile 1a, species X could not generate the corresponding species XI While with presence of electron withdrawing groups on benzene ring probably the resonating structure VII does not contribute much; and \( N \)-methylbenzohydroxamic acids exhibit more nucleophilicity due to electron donating property of methyl group.
3.5 REFERENCES


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