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EQUILIBRIUM AND KINETIC STUDIES OF METAL ION PROMOTED HYDRATION AND ENOLIZATION REACTIONS OF OXALOACETATE IN VARIOUS BUFFERS: THE COOPERATIVITY WITH BASE CATALYSTS

The Ohio State University

Ph.D. 1984

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by

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HYDRATION AND ENOLIZATION REACTIONS OF OXALOACETATE IN
VARIOUS BUFFERS: THE COOPERATIVITY WITH BASE CATALYSTS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of the Ohio State University

By
Suh-Jen Jane Tsai, B.S.

The Ohio State University
1984

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ACKNOWLEDGEMENTS

I wish to thank Dr. Daniel L. Leussing for his guidance throughout the course of the research project and in the preparation of this manuscript. I am indebted to the members in this group with whom I have spent unforgettable years.

Special thanks go to Dr. Hsi-Pai Hsu for sharing his experience in computer programming.

I also wish to thank my parents and other members in my family, especially my mother, for their taking care of my beloved son Philip. I could not have finished this work in an appropriate time without their help.

Finally, I wish to thank my husband Tsun-Chung for his encouragement and his confidence in me. His continuous support makes the successful completion of the Ph.D requirements possible.
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34. A Bronsted Plot of Metal Promoted Enolization
of Oxaloacetate ................................. 276
Oxaloacetic acid plays an important role in the biological system. The investigations of Britten (1) and Villafranca (2) showed that oxaloacetic acid was a competitive inhibitor of aconitase which catalyzes the dehydration of citrate and isocitrate to cis-aconitate. In aqueous solution, oxaloacetic acid exists as a mixture of enol (I), keto (II) and hydrate (III) species (3,4). In addition, oxaloacetic acid also decomposes spontaneously into pyruvic acid (IV) and carbon dioxide (5,6,7,8).
Although intensive investigations on the role of polyvalent cations and enzymes in the decarboxylation of oxaloacetic acid have been performed (9-24) only relatively few studies on metal ion promoted hydration-dehydration of oxaloacetic acid have been done.

Many carbonyl compounds are known to exist in equilibrium with their enol forms and hydrate forms in aqueous solution. From spectrophotometric measurements, Herold, Wolf (26,27) and Schou (28) found 60% to 26% hydrate acetaldehyde (V) was present in aqueous solution. The kinetics of the hydration of acetaldehydes (VI) have been extensively studied by Lauder (26) and by Bell et al. (29,30). In addition, Bell and Darwent (31) claimed that the hydration reaction of acetaldehyde at low temperature was a first order reaction with respect to acetaldehyde. The result also indicated that a general relationship between the catalytic ability of various catalysts and their dissociation constants in aqueous solution existed. Based on those results a reaction mechanism was proposed (31). Further studies were performed by Bell and Clunie (32,88).

\[
\begin{align*}
\text{CH}_3\text{CH(OH)}_2 & \quad \text{CH}_3\text{CHO} \\
V & \quad \text{VI}
\end{align*}
\]
Bell, Rand and Wynne-Jones (33) investigated the catalytic effects of carboxylic acids and a series of substituted pyridines on the hydration reaction of acetaldehyde. Their work showed that the hydration reaction was general acid and general base catalyzed. These authors concluded that the following reaction steps, which were earlier proposed by Bell and Darwent (31), represent the mechanism of the hydration of acetaldehyde.

**Acid catalysis:**

\[
\begin{align*}
\text{CH}_3\text{CHO} + \text{H}_2\text{O} + \text{HA} & \rightleftharpoons \text{CH}_3\text{CH(OH)}^+\text{O}_2\text{H} + \text{A} \\
\text{CH}_3\text{CH(OH)}^+\text{O}_2\text{H} + \text{A} & \rightleftharpoons \text{CH}_3\text{CH(OH)}_2 + \text{HA}
\end{align*}
\]  

**Base catalysis:**

\[
\begin{align*}
\text{CH}_3\text{CHO} + \text{H}_2\text{O} + \text{B} & \rightleftharpoons \text{CH}_3\text{CH(OH)}^\text{O}_2\text{H} + \text{HB} \\
\text{CH}_3\text{CH(OH)}^\text{O}_2\text{H} + \text{HB} & \rightleftharpoons \text{CH}_3\text{CH(OH)}_2 + \text{B}
\end{align*}
\]

in which HA and B stand for general acid and general base. Reactions (1) and (3) are the rate-determining steps.

Gruen and McTigue (34,35) studied the kinetics of the hydration of aliphatic aldehydes which included acetaldehyde, propionaldehyde, n-butylaldehyde, and iso-butylaldehyde. In acidic buffer solutions, the rate constants for hydrogen ion catalysis of these aldehydes...
were almost constant. These authors claimed that the serious deviations from the usual Bronsted relation in few base-catalyzed reaction were due to the formation of appreciable amounts of nucleophilic addition compounds between the aldehydes and the catalyzing bases. They also found that the equilibrium ratio of (hydrate)/(aldehyde) was susceptible to the salt concentration. Kinetic and spectrophotometric evidence supported this finding. Concurrently, a detailed report on the kinetics of dehydration of methylene glycol in aqueous solution was contributed by Bell et al (25).

The dehydration-hydration reactions of aldehydes and their derivatives are important processes in biological systems. The enzymatic dehydration of aldehydes was investigated by Trentham, McMurry and Pogson (36) over a wide range of enzyme concentrations. As shown in equation (5), D-Glyceraldehyde 3-phosphate exists as the hydrate form, also called geminal diol (VIII) and free aldehyde (IX) in aqueous solution. The kinetic studies of the interconversion between hydrate and aldehyde by a rapid temperature perturbation of the hydrate-aldehyde equilibrium indicated the dehydration of the diol form is the rate-determining step in the catalytic oxidation of D-
gyceraldehyde 3-phosphate by NAD\(^+\). The values of the thermodynamic parameters, \(\Delta H\) and \(\Delta S\) for the reversible hydration of various aldehydes were reported by Pocker and Dickerson in 1969 (37). The observed \(\Delta H\) and \(\Delta S\) of propionaldehyde, isobutyraldehyde and pivalaldehyde were \(-5.4\) kcal mol\(^{-1}\), \(-18.3\) cal mol\(^{-1}\) deg\(^{-1}\); \(-5.8\) kcal mol\(^{-1}\), \(-20.4\) cal mol\(^{-1}\) deg\(^{-1}\); \(-4.4\) kcal mol\(^{-1}\), and \(-17.6\) cal mol\(^{-1}\) deg\(^{-1}\), respectively. Their results showed that all of the hydration reactions of these three aldehydes in acetate, diethylmalonate and phosphate buffered solutions were general acid and general base catalyzed. In order to explain their observed results, a concerted mechanism which involved a change in hybridization of carbon instead of transferring a proton from or to oxygen was postulated.

Pyruvic acid (X) is one of the most important intermediate involved in the metabolism of carbohydrates and proteins. Due to its biological significance, many investigations of the hydration reaction of pyruvic acid
have been done (38-49). By integrating the NMR spectra, Pocker et al. (47) evaluated the equilibrium concentration of pyruvic acid (X) and its hydrate (XI), 2,2-dihydroxypropionic acid, as a function of temperature and pH. From these equilibrium data, the thermodynamic parameters of the hydration of pyruvic acid, \( \Delta G = -0.24 \) kcal mol\(^{-1} \), \( \Delta H = -7.8 \) kcal mol\(^{-1} \) and \( \Delta S = -26.8 \) eu.

\[
\begin{align*}
\text{CH}_3\text{CO}_2\text{H} & \quad \text{OH} \\
\text{CH}_3\text{CO}_2\text{H} & \quad \text{OH} \\
\text{CH}_3\text{CO}_2\text{H} & \quad \text{OH} \\
\text{CH}_3\text{CO}_2\text{H} & \quad \text{OH}
\end{align*}
\]

were calculated. In order to elucidate the high negative entropy of hydrate in aqueous solution, a highly structured model in which 2,2-dihydroxypropionic acid bridged with two water molecules through hydrogen bonding was proposed (VII).
Pyruvic acid undergoes a relatively fast spontaneous hydration reaction in aqueous solution, \( k = 2.0 \, \text{min}^{-1} \) at 0.0 °C(42,43,48). Eigen, Kustin and Strehlow claimed (42,43) this interesting observation was due to intramolecular acid catalysis. That is, an intramolecular hydrogen bond is formed between the oxygen of the carbonyl group and the hydrogen of the neighboring carboxyl group as shown in (XII). Further thermodynamic and kinetic studies were performed by Pocker et al. (49). These authors examined the reversible hydration of methyl pyruvate and ethyl pyruvate. At 0.0 °C, the corresponding spontaneous hydration rates of pyruvic acid, methyl pyruvate and ethyl pyruvate were equal to 2 min\(^{-1}\), 1.98 min\(^{-1}\) and 1.55 min\(^{-1}\), respectively. The slightly lower spontaneous hydration rate of ethyl pyruvate was attributed to the steric hindrance of ethyl group. Obviously, the hypothesis of intramolecular acid catalysis does not give a satisfactory explanation of the relatively rapid hydration rates of pyruvic acid and pyruvate esters.

Reynolds, Yates and Pogson (50) have examined the equilibria of dihydroxyacetone phosphate in neutral aqueous solution. These authors found that dihydroxyacetone phosphate existed as a mixture of enol(XIII), gem-diol(XIV)
and keto(XV) forms in the ratio 1:44:55. In fact, the distribution of these species is temperature dependent. Consequently, at 37 °C, 83% of dihydroxyacetone phosphate was presented as keto form. The interconversion rates of these three species were also determined.

The reaction rates and equilibrium constants for hydration and enolization of acetopyruvate (2,4-dioxovalerate, XVI) have been determined by J.P. Guthrie (51). In CDCl₃ solution, the NMR spectrum of acetopyruvate only showed signals which were contributed by the enol acetopyruvate (XVI). However in aqueous solution where a molecule of water is added to the α-carbonyl group of acetopyruvate, the methylene signal of the hydrate (XVII) was observed at higher field and the keto form(XVIII) at lower field. The distribution of keto, enol and hydrate species was dependent on the concentration of proton ions in aqueous solution. By integrating the NMR spectra of acetopyruvate, the relative amounts of these
three forms of acetopyruvate were determined. In addition, rate constants of 0.1 sec\(^{-1}\) and 0.012 sec\(^{-1}\) were reported for the enolization and dehydration of acetopyruvate at pH 5.

Soon after Pocker et al. published their result on pyruvate, an investigation of the kinetics and equilibria for the reversible hydration of glyoxylic acid (XIX) in aqueous solution was reported (52). The scavenger technique was used to evaluate the spontaneous and catalytic dehydration rates of hydrated glyoxylic acid and glyoxylate. The observation showed the dehydration was catalyzed by general acids and general bases.
The hydrate of oxaloacetate, also called 2,2-dihydroxy succinate, was first detected in tris buffer solution by Pogson and Wolfe using a stopped-flow spectrophotometer (3). A triphasic reaction was observed in the reduction of oxaloacetate by NADH catalyzed by mitochondrial L-malate dehydrogenase. The first, fastest, phase of the reaction was assigned to the reaction between keto-oxaloacetate and the enzyme. The slowest phase represented the ketonization of the enol and the intermediate rate was a result of the dehydration of hydrated oxaloacetate. Although keto-oxaloacetate was the predominant species (74.3%) in pH 7.4 tris buffer solution, the equilibrium concentrations of enol and hydrate oxaloacetate were not negligible.

Kokesh(4) utilized NMR spectroscopy to prove the existence of the hydrate oxaloacetic acid and its dianion in aqueous solution at 38 °C. The distribution of enol, keto and hydrate forms was also determined by integrating the peak intensities of hydrate species. A detailed discussion of the significance of hydrate species in the kinetics of enolization and decarboxylation of oxaloacetic acid and its anion was reported.

A pH jump technique was employed in the rate determination of the buffer catalyzed dehydration-hydration
reaction of diethyloxaloacetate (54). In aqueous solution, diethyloxaloacetate undergoes both ketonization-enolization and dehydration-hydration equilibra as shown in equations 6 and 7. Since \(-\text{COO}^-\) is an electron-donating group and \(\text{CH}_2\text{COO}^-\) is a much weaker electron withdrawing group than \(-\text{COOC}_2\text{H}_5^-\) and \(-\text{CH}_2\text{COOC}_2\text{H}_5^-\) groups, diethyloxaloacetate has a greater tendency to hydrate than oxaloacetate dianion. The results showed that the dehydration reaction was catalyzed by general acids.

The significance of metal ions in the hydration reactions of carbonyl compounds has also been a popular subject for many years. The reversible hydrations of 2- and 4- pyridinecarboxaldehydes are strongly promoted by the presence of zinc metalloenzyme and erythrocyte carbonic anhydrase (55,56). In order to understand the role of metal ions in the biological system, an intensive study of the divalent cation effects on the hydration of 2- and 4-
pyridinecarboxaldehydes was done by Pocker and Meany (46). In the absence of metal ions, the hydration of these compounds were both general acid and base catalyzed. The catalytic power of various divalent cations were related to their ability to form complexes with certain bidentate ligands. The rate of the hydrate reaction was highly enhanced by the coordination between the divalent cations and the conjugate hydrate 2-pyridinecarboxaldehyde. On the contrary, the corresponding divalent cations only caused little enhancement on the hydration reaction rates of 4-pyridinecarboxaldehydes.

The metal ions effects on the enzyme catalyzed hydration of pyruvic acid and dehydration of 2,2-dihydroxypropionate anion were examined by Pocker and Meany (57). In diethyl malonate buffer solutions, the catalytic power of various divalent ions on the dehydration of 2,2-dihydroxypropionate follow the order: Cu(II) > Zn(II) > Ni(II) > Co(II) > Cd(II) > Mn(II). This is similar to the order which is predicted by the coordination tendency of these divalent metal ions with the hydrate pyruvate anion. The promotion of the dehydration rates of hydrated pyruvate anions by these divalent metal ions is due either to very effective polarization of the carbonyl
group of pyruvate or to participation of these metal ions in the transfer of water molecules. Detailed studies of the coordination between metal ions and bases in the hydration of acetaldehyde which involved nucleophilic attack upon the carbonyl group were performed by Paul Woolley (58-60). According to his results, the catalytic effect of zinc ion on the hydration of acetylateddehyde is accelerated by hydroxide, acetate and pyridine ions.

Although most of the enolization reactions of carbonyl compounds are susceptible to general acid and general base catalysis, the enolization of keto-acetylacetone (XX) has been shown not to be sensitive to any acid catalysis

\[
\begin{align*}
\text{CH}_3\text{CCH}_2\text{CCH}_3 & \rightleftharpoons \text{CH}_3\text{C}^\equiv\text{HCCH}_3 \\
\text{XX} & \\
\text{CH}_3\text{CCH}_2\text{CCOOH} & \text{XXI}
\end{align*}
\]

including hydronium ions (74). An observation of the metal ion catalyzed tautomerization of acetylacetone was performed by Meany (75). The kinetics were studied in a dilute acetate buffer solution by a spectrophotometric
technique. The observations showed that the tautomerization of acetylacetone is catalyzed by various divalent cations as general acids.

Acetopyruvic acid (XXI) also undergoes hydration and enolization in aqueous solutions. Due to slow interconversion rates (about $5 \sec^{-1}$), the keto, enol and hydrate species gave distinct and sharp peaks in the NMR spectrum (51). The iodination reaction was employed to measure the reaction rate of the enolization process. Guthrie's report (51) indicated that the enolization of acetopyruvic acid was faster than hydration at pH 5.

Oxaloacetic acid is a component of the Krebs cycle which occupies a center position in the biological metabolism systems. Enol oxaloacetate is responsible for reducing the activity of succinate dehydrogenase (61) and citrate lyase (62). Keto oxaloacetic acid has been found to be the active substrate of malate dehydrogenase (63), phosphoenol pyruvate carboxykinase (64) and citrate synthase (65). Due to its significance, the mechanism of enolization and ketonization has been the subject of many investigations for over 70 years. Meyer (66) determined the enol content of oxaloacetic acid in water solution by bromination titrations.
In the evaluation of the extinction coefficients of oxaloacetic acid in ether and petroleum ether solutions, Hantzsch found that the observed extinction coefficients of oxaloacetic acid in these two solutions were essentially the same (91). By using the extinction coefficient of diethyl α,α-dieethyl oxaloacetate as $\epsilon^{\text{keto}}$ and that of oxaloacetic acid in ether as $\epsilon^{\text{enol}}$, Hantzsch was able to evaluate the concentration of enol-keto acid present in aqueous solution. Bell et al. (67) measured the enolization rate of oxaloacetic acid by bromination. A similar measurement was also done by Long and Watson (68). Both results showed that in the presence of either acid or base catalysts, the halogenation rate was only dependent on the concentrations of keto species and catalysts, but not on the concentration of halogens. This indicated the addition of halogen to enol oxaloacetate was not the rate-determining step. In order to further understand the mechanism of the enolization of oxaloacetic acid, G.W.Kosicki (69) examined the isotope effects on the enolization reaction rates. By comparing the enolization rates in H$_2$O and D$_2$O, Kosicki drew a conclusion that the bond cleavage between proton and carbon was the rate-determining step of enolization reaction. A value of 5600
M⁻¹ cm⁻¹ was reported for the extinction coefficient of enol oxaloacetic acid in ether.

An investigation of the nature of oxaloacetic acid in the solid state and in neutral, aqueous solution has been done by Banks (70). Oxaloacetic acid exists as enol form in the solid state. Banks noticed a large UV absorption decrease when the solid enol oxaloacetic acid was dissolved in aqueous solution. The decrease was attributed to the ketonization of enol species. Thus, the ketonization was easily followed by a direct spectrophotometric method. Based on the experimental results, two different possible mechanisms, the consecutive and concerted mechanisms, were proposed for the tautomerization reaction of carbonyl compounds as shown in the reaction equations 9-11. The extinction coefficient for dianion at 280 nm, was measured by extrapolating to the time when an appropriate amount of oxaloacetic acid in ethanol was mixed with pH 7.38 buffer at 1.5 °C, and a value of 3385 M⁻¹ cm⁻¹ was reported. The apparent extinction coefficient of α-ketoglutarate at pH 7.38, ε²⁸⁰ = 24 M⁻¹ cm⁻¹, was adopted as the extinction coefficient of keto dianion. The distribution of enol and keto species was calculated from the extinction coefficients of these two species. Banks also examined the
Consecutive Mechanism:

Acid Catalysis

\[ 
\overset{\text{\footnotesize H}}{\text{\footnotesize C-C- + HA}} \rightleftharpoons \overset{\text{\footnotesize O}}{\text{\footnotesize C-C- + A}} \rightleftharpoons \overset{\text{\footnotesize O}}{\text{\footnotesize C=C- + HA}} \ldots (9) 
\]

Base Catalysis

\[ 
\overset{\text{\footnotesize O}}{\text{\footnotesize C-C- + B}} \rightleftharpoons \overset{\text{\footnotesize O}}{\text{\footnotesize C=C- + HB}} \rightleftharpoons \overset{\text{\footnotesize O}}{\text{\footnotesize C=C- + A}} \ldots (10) 
\]

Concerted Mechanism:

\[ 
\overset{\text{\footnotesize O}}{\text{\footnotesize HA + C-C- + B}} \rightleftharpoons \overset{\text{\footnotesize O}}{\text{\footnotesize A + C=C- + B}} \ldots (11) 
\]

where \( \text{HA} \) and \( \text{B} \) stand for acid and base.

buffer effects on the enolization of oxaloacetic acid (71). The report showed the tautomerization mechanism of oxaloacetic acid was buffer dependent. Among those buffers examined by Banks, acetate, N-methyldiethanolamine, and carbonate-bicarbonate buffers were consistent with the consecutive mechanism while phosphate, succinate, triethanolamine, imidazole and tri(hydroxymethylamino)methane were not. The ionization constants of oxaloacetic acid in aqueous solution at 25 °C and \( \mu = 0.1 \) have been potentiometrically determined by Tate.
et al. (72). For the enol species, $pK_{a1} = 1.89$ and $pK_{a2} = 3.72$, whereas for keto species, $pK_{a1} = 2.24$ and $pK_{a2} = 3.90$. In addition, the equilibrium distribution of enol oxaloacetate was determined spectrophotometrically. About 14% of enol oxaloacetic acid was present in pH 5.2 to 8.4 aqueous solution. Although many spectrophotometric studies of oxaloacetic acid had been done in a variety of buffers, solvents and temperature, the extinction coefficients of keto and enol species were evaluated without recognizing the existence of hydrate species until its existence was demonstrated by Pogson and Wolfe (3).

Since Shoolery and his coworkers (95) contributed the first NMR study of oxaloacetic acid and diethyl ester in 1962, special attention has been devoted to this application (81,92-94). NMR data for the enol and keto oxaloacetic acid in deuterated dioxane, acetone and $d_6$-dimethyl sulfoxide were published by Hess and Reed (92). In $d_6$-dimethyl sulfoxide ($d_6$-DMSO) solution, 53% of oxaloacetic acid existed as enol form. By combining the spectroscopic measurements including NMR, IR and UV, these authors were able to evaluate the molar absorptivity of enol species at 260 nm and a value of $1.1 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ was reported. Detailed investigations employed proton magnetic
resonance methods were also reported by Kokesh(4). By reexamining the NMR study of oxaloacetic acid performed by Shoolery et al., Kokesh found that one of the peak assignments made in their report was incorrect. Kokesh claimed(4) that the peak which was located about 0.6 ppm further upfield than that for the pure ester actually represented the methyl group of a hemiketal derivative(XXII) not the keto form of diacid or diester in methanol as assigned by Shoolery et al.. Thus the enol/keto ratio reported by Shoolery and his coworkers is

\[
\begin{align*}
\text{OCH}_3 \\
\text{R-O-R'} \\
\text{OH}
\end{align*}
\]

XXII

actually a ratio between enol and hemiketal. The hydrate of oxaloacetic acid is the predominant species at low pH. The equilibrium ratio of enol/keto decreases as the pH increases. In very basic solutions, the enol/keto ratio increases again due to the formation of enolate ions. Table 1 gives a summary of the distribution of each species in aqueous solution at various pH values.
### TABLE 1

**Summary of the Equilibrium Values of Enol, Keto and Hydrate Oxaloacetic Acid**

<table>
<thead>
<tr>
<th>pH</th>
<th>%hydrate</th>
<th>%keto</th>
<th>%enol</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>54.9</td>
<td>11.1</td>
<td>34.0</td>
</tr>
<tr>
<td>5.0</td>
<td>19.0</td>
<td>60.3</td>
<td>20.7</td>
</tr>
<tr>
<td>7.4(phosphate)</td>
<td>7.1</td>
<td>76.2</td>
<td>16.7</td>
</tr>
<tr>
<td>7.4(tris)</td>
<td>7.8</td>
<td>74.3</td>
<td>17.8</td>
</tr>
<tr>
<td>8.0</td>
<td>7.7</td>
<td>65.5</td>
<td>27.7</td>
</tr>
</tbody>
</table>

---

**Pogson and Wolfe (3) 20 °C Malate Dehydrogenase-NADH assay method**

<table>
<thead>
<tr>
<th>pH</th>
<th>%hydrate</th>
<th>%keto</th>
<th>%enol</th>
</tr>
</thead>
<tbody>
<tr>
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<td>95</td>
<td>small</td>
<td>5</td>
</tr>
<tr>
<td>1.0</td>
<td>85</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>3.1</td>
<td>35</td>
<td>55</td>
<td>10</td>
</tr>
<tr>
<td>3.7</td>
<td>25</td>
<td>65</td>
<td>10</td>
</tr>
<tr>
<td>4.7</td>
<td>13</td>
<td>76</td>
<td>11</td>
</tr>
<tr>
<td>6.9</td>
<td>7</td>
<td>81</td>
<td>12</td>
</tr>
<tr>
<td>12.7</td>
<td>6</td>
<td>73</td>
<td>21</td>
</tr>
</tbody>
</table>

---

**Emly and Leussing(86) 25 °C NMR(4) UV(92)**

<table>
<thead>
<tr>
<th>pH</th>
<th>%hydrate</th>
<th>%keto</th>
<th>%enol</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>95</td>
<td>small</td>
<td>5</td>
</tr>
<tr>
<td>1.0</td>
<td>85</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>3.1</td>
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<td>10</td>
</tr>
<tr>
<td>3.7</td>
<td>25</td>
<td>65</td>
<td>10</td>
</tr>
<tr>
<td>4.7</td>
<td>13</td>
<td>76</td>
<td>11</td>
</tr>
<tr>
<td>6.9</td>
<td>7</td>
<td>81</td>
<td>12</td>
</tr>
<tr>
<td>12.7</td>
<td>6</td>
<td>73</td>
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</tr>
</tbody>
</table>
TABLE 1
(continue)

Kokesh (4) 38 °C
Proton Nuclear Magnetic Resonance

<table>
<thead>
<tr>
<th>pH</th>
<th>%hydrate</th>
<th>%keto</th>
<th>%enol</th>
</tr>
</thead>
<tbody>
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<td>6.7</td>
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<tr>
<td>6.70</td>
<td>5.5</td>
<td>87.3</td>
<td>7.2</td>
</tr>
<tr>
<td>6.89</td>
<td>5.2</td>
<td>87.4</td>
<td>7.4</td>
</tr>
</tbody>
</table>

Tate et al. (69) 25 °C
Absorbance Analysis $\varepsilon^{280} = 3700 \text{ M}^{-1} \text{ cm}^{-1}$

<table>
<thead>
<tr>
<th>pH</th>
<th>%keto</th>
<th>%enol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>93.7</td>
<td>6.3</td>
</tr>
<tr>
<td>1.8</td>
<td>91.6</td>
<td>8.4</td>
</tr>
<tr>
<td>2.2</td>
<td>90.8</td>
<td>9.2</td>
</tr>
<tr>
<td>2.6</td>
<td>89.6</td>
<td>10.4</td>
</tr>
<tr>
<td>3.0</td>
<td>89.5</td>
<td>10.5</td>
</tr>
<tr>
<td>4.0</td>
<td>87.9</td>
<td>12.2</td>
</tr>
<tr>
<td>4.4</td>
<td>87.3</td>
<td>12.7</td>
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<tr>
<td>4.8</td>
<td>86.6</td>
<td>13.4</td>
</tr>
<tr>
<td>5.2</td>
<td>87.7</td>
<td>12.3</td>
</tr>
</tbody>
</table>
The solid enol oxaloacetic acid, also called hydroxymaleic acid, ketonizes when dissolved in pH 5 to 9.9 aqueous solution. The equilibrium mixture of enol and keto species shows a broad peak or plateau in the wavelength range of 242 μm to 262 μm (63). With the addition of Mg(II) ions to the equilibrium solution, the optical density at 267 μm increases rapidly due to the fast formation of a complexed enol oxaloacetate. Pedersen (76) also observed a similar effects of Cu(II) ions on the enolization of ethylacetoacetate (XXIII).

Diverse metal ion effects on the decarboxylation of acetoacetic acid (XXIV) and oxaloacetic acid were reported by Krebs (5). Based on the observation that oxaloacetic acid was activated by metal ions whereas acetoacetic acid was not, the α-enol moiety (XXV) was believed to be the major coordination structure of metal complexes. However, the formation of 6-membered chelate (XXVI) was still possible based on the reason that the decarboxylation of acetonedicarboxylic acid (XXVII) was also promoted by metal
ions. Because acetonedicarboxylic acid formed 6-membered chelate with divalent metal ion as shown in structure

XXV

XXVI

XXVII

XXVIII

During the spectrophotometric study of Al(III) and Mn(II) ions effects on the UV absorption of oxalosuccinic acid (XXVIII) and oxaloacetic acid, Kornberg and his coworkers(10) found that the initial UV absorption of oxalosuccinic acid (252 nm) and that of oxaloacetic acid (274 nm) were increased dramatically. These authors
believed the enhancement of UV absorption was caused by the formation of enol metal complexes before those dicarboxylic acids decarboxylated.

Steinberger and Westheimer (77) found that when Cu(II) ions were added into $\alpha,\alpha$-dimethyloxaloacetate solution, the absorbance increased rapidly and reached a maximum value in a few seconds. The absorbance change was followed by a slower absorbance decrease. A similar result was obtained when Fe(II) ions was added. Since $\alpha,\alpha$-dimethyloxaloacetate could not enolize, these author claimed the increase in absorbance was due to the formation of dimethyl derivative of pyruvate enolate produced by the decarboxylation of $\alpha,\alpha$-dimethyloxaloacetate. The protonation and ketonization of pyruvate enolate were responsible for the slower absorbance decrease. These reactions are shown in equation 12. Detailed studies of the protonation equilibria and metal ion promoted decarboxylation of oxaloacetic acid were performed by Pedersen (99,13). His conclusion about the decarboxylation order with: Cu(II) $>$ Zn(II) $>$ monoanion $>$ dianion $>$ neutral oxaloacetic acid was consistent with the result obtained by Steinberger and Westheimer (77). Two plausible complexing
where M stands for the metal ion.

structures(XXIX, XXX) were postulated. Monoprotonated anion of oxaloacetic acid also formed M(HOX) complexes with Cu(II) and Zn(II) ions. A 7-membered chelated(XXXI) where metal ion was coordinated with both carboxylate groups was proposed by Williams(16) to explain the inhibition of decarboxylation occurred in high metal concentrations. The
overall interactions between oxaloacetate and metal ions

were described in equation 13.

Further studies of oxaloacetate reaction in the presence of various divalent metal ions including Ca(II), Co(II), Cu(II), Mn(II), Ni(II) and Zn(II) were performed by Gelles et al. (79,19). The nature of those chelate compounds formed between metal ions and oxaloacetate was thoroughly studied potentiometrically and photometrically. Two identical absorption spectra of this α-keto acid in ether and light petroleum ether were also obtained by Gelles and Hay(79). The apparent extinction coefficients of enol oxaloacetic acid, α,α-dimethyloxaloacetic acid and
its dianion were reported as $8800 \text{M}^{-1}\text{cm}^{-1}$, $22 \text{M}^{-1}\text{cm}^{-1}$ and $92 \text{M}^{-1}\text{cm}^{-1}$, respectively. Based on the assumption that the reaction rate of tautomeration was faster than that of decarboxylation, the enol contents of metal bonded oxaloacetate were measured by the zero time absorbancies immediately after mixing solutions of metal ions and oxaloacetate. It was claimed that the enol percentages of MnOx, ZnOx and CuOx were 6%, 15% and 40%, respectively.

The formation of dinuclear complex was proposed to account for the inhibition of the decarboxylation rate which occurs at high metal ion concentrations. The formation constants $K_1$ and $K_2$, of 1:1 metal ion-ox complex, MOX and dinuclear complex $M_2OX^{2+}$, respectively, were determined potentiometrically. However, the rate-concentration profiles defined by $K_1$ and $K_2$ were proved to be inconsistent with those measured spectrophotometrically (90).

Tate et al. have determined the proton-dissociation constants and the stability constants of magnesium(II)-oxaloacetate complexes by pH and optical-density measurements (72,78). According to their result, the log value of the overall stability constant for the complex formed between Mg(II) ion and oxaloacetate dianion was
1.96 at 25 °C and I=0.1. There was no evidence which indicated the formation of 1:2 Mg(II)-oxaloacetate complex. They also found Mg(II)-enol oxaloacetate complex had greater stability than Mg(II)-keto oxaloacetate complex. Consequently, the equilibrium distribution of enol form was increased by the addition of Mg(II) ions. The equilibrium constant of MgOX(keto) ⇌ MgOX(enol), K=0.30 was reported.

Based on the observation that the intensity of the absorption maximum varied with the nature of the divalent cations, Bamann and Sethi (80) insisted that the fast absorbance changes which occur when metal ion solutions were mixed with oxaloacetate solution is caused by the metal ion catalyzed enolization of oxaloacetate not by the formation of pyruvate enolate complexes. Tsai et al. (81) have examined the NMR spectra of europium-oxaloacetate complexes. The NMR isotropic shift of the CH₂ group of these complexes indicated the formation of the α-oxocarboxylate complex between oxaloacetic acid and metal ions in aqueous solution.

Quantitative studies of the catalytic effect of Zn(II) on oxaloacetate reactions were done by Covey and Leussing (23). The formation of binuclear enolate Zn₂H⁻₁OX⁺(XXXII) and deprotonated hydrate Zn₂H⁻₁OX⁺(XXXIII) was kinetically
proved. Two relaxations were observed when metal ion solutions and OX solutions were mixed. Based on the assumption that hydration-dehydration interaction was not significant in this isopH condition, these authors claimed that the UV absorbance increase which completed in about 30 seconds was attributable to the enol-keto tautomeration of oxaloacetate. Whereas the slower absorbance decrease which was completed in 15 to 30 minutes was a result of decarboxylation. The resolution of observed relaxation time into microscopic rate constants and definition of numerous formation constant for Zn(II)-oxaloacetate complexes were made possible by using high speed computation techniques. The computer programs RLXFT and CORNEK (101) were used.

Raghavan and Leussing also studied the Copper (II) ions influence on the tautomeration and decarboxylation
of oxaloacetate (89). A summary of hydration-dehydration

\[ HOX^- (enol) \rightleftharpoons HOX^- (keto) \rightarrow pyr^- (enol) \rightarrow pyr^- \]

\[
\begin{array}{c}
\text{fast} \\
\text{fast}
\end{array}
\]

\[ OX^2^- (enol) \rightleftharpoons OX^2^- (keto) \rightarrow pyr^- (enolate) \rightarrow pyr^- \]

\[
\begin{array}{c}
\text{fast} \\
\text{fast}
\end{array}
\]

\[ CuOX(enol) \rightleftharpoons CuOX(keto) \rightarrow Cupyr(enolate) \rightarrow Cupyr \]

\[ Cu_2H_{-1}OX^+ \]

\[ Cu_2H_{-2}OX^2_- \]

\[ \ldots (14) \]

of these reactions is given in equation 14. Again, the isopH method was employed. A two-relaxation decay was initiated by rapidly mixing metal ion solution with oxaloacetate solution. These two solutions were previously buffered with the same concentration of buffer and were adjusted to the same pH level, the isopH level. To
simplify the investigation an assumption, which stated that the contribution of hydration-dehydration is negligible in isoph experiments, was made. In the presence of Cu (II) ions the enolization and decarboxylation rates were competitive. According to their report most of oxaloacetate is initially present as keto form. However on adding Cu(II) only 80% of keto-oxaloacetate was converted to enol and enolate while in the same time interval (ca. 30 seconds) the remaining 20% decarboxylated. The equilibrium constant of Cu-oxac(keto) ⇌ Cu-oxac(enol) reaction was calculated with appropriate correction of the interference of decarboxylation.

After Munakata and his coworkers (24) reported their studies of the influence of various ligands on the activity of Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) metal ions toward oxaloacetate, detailed investigations of the mixed ligand effects on the enolization and decarboxylation of Cu(II)-oxaloacetate were performed by Raghavan and Leussing (83). Although the enolization of oxaloacetate has been identified for a long time(91) the real enolization mechanism especially which involves the catalysis by metal ions and buffer components are still the subject of many arguments. In addition to the consecutive and concerted
general acid and general base mechanisms, a third possible mechanism was proposed by Bruice and Bruice in their short communication (84). Their reinvestigation of phosphate and imidazole buffer effects on enolization showed no evidence for concerted mechanism which was previously reported by Bank (70,71).

In a detailed report, Bruice and Bruice (85) stated that the tautomeration of oxaloacetate in acetate and pyridine buffer solutions exhibited general-acid catalysis. The tautomeration proceeded via general-base catalysis in carbonate and phosphate($\text{HPO}_4^{2-}/\text{PO}_4^{3-}$) solutions. However, the tautomeration exhibited both general-acid and general-base catalyzed pathways in the presence of imidazole and phosphate($\text{H}_2\text{PO}_4^-/\text{HPO}_4^{2-}$) buffers. The Bronsted constants $-\alpha$ and $\beta$ were 0.43 and 0.35, respectively. These buffers showed no evidence of the concerted general-acid and general-base catalyzed mechanism. These authors claimed that in the presence of a tertiary amine with $pK_a > 8$, the enolization of oxaloacetate occurs via nucleophilic catalysis which involves the formation of a zwitterionic carbinolamine intermediate followed by amine-promoted elimination. The reaction process is described in equations 15 and 16.
A similar result was obtained by Bruice and Bruice (54) in their kinetic studies of dimethyloxaloacetate. Further investigations of tautomerization and reversible hydration rates of oxaloacetate were performed by Emly and Leussing (86). Both reaction rates were susceptible to general acid and general base catalysis. Oxaloacetate underwent fast dehydration and slow enolization over a wide range of buffer concentrations of oxyanion bases and unsaturated amines. Diverse catalytic effects of tertiary amines on tautomerization and hydration reactions of oxaloacetate were observed. Enolization rates were highly susceptible to the total tertiary amine concentration.

Although, the dehydration rate is faster than that of enolization in low tertiary amine concentration, after passing a certain crossover point the enolization rate is
enhanced dramatically and becomes the faster reaction. In fact, the nonlinear relationship between reaction rates and the tertiary amine buffer concentration which lead Bruice and Bruice (84,85) to postulate the mechanism involved the carbinolamine intermediate is attributed to the incorrect identification of these reactions in higher buffer concentration. With appropriate identification of these reactions, a linear relationship between reaction rates and buffer concentration was observed. Both base catalyzed mechanisms of hydration and enolization thus proposed give

Hydration

\[
\begin{align*}
\text{H-OH} & \quad \text{O} \\
\text{B} & \quad \text{H-} \\
\text{O}_2\text{CCH}_2\text{CO}_2^- & \rightleftharpoons \text{BH}^+ + \overset{\text{OH}}{\text{O}_2\text{CCH}_2\text{CO}_2^-} \quad \text{(17)}
\end{align*}
\]

Enolization

\[
\begin{align*}
\text{O} \\
\text{B} & \quad \overset{\text{O}}{\text{O}_2\text{CCH}_2\text{CO}_2^-} \\
\text{O}_2\text{CCH}_2\text{CO}_2^- & \rightleftharpoons \text{BH}^+ + \overset{\text{O}}{\text{O}_2\text{C-C=CHCO}_2^-} \quad \text{(18)}
\end{align*}
\]

oxyanion intermediates as shown in equations 17 and 18. Further studies showed that the catalytic effects of general bases were greatly promoted by metal ions(97). To elucidate this observation, the coordination of metal ion with oxaloacetate was pictured as in equation 19. Due to
the complexing with metal ion, the oxyanion intermediate is stabilized in the reaction coordination.

In a recent report published by Mao and Leussing(98), the enol/keto ratio of MnOX complex, K=0.74 was obtained kinetically. Plausible reaction mechanisms of coordinated oxaloacetate involved in the presence of either general acid or general base were proposed as shown in equation 20.
All of the experiments were performed by isopH method. That is, a pair of reaction solutions were prepared for each kinetic run. One of these solutions contained divalent metal ions, the other contained oxaloacetate. Both solutions were made up with the same buffer concentration and were maintained at the same pHs. The kinetic data resolution and interpretation were done under the assumption that only tautomerization and decarboxylation occurred in isopH condition. They have proved that the enolization rate-concentration profiles are highly susceptible to K. Therefore, the equilibrium constant, K can be kinetically resolved. The stability of enol MnOX complex is attributable to the formation of dicarboxylate(XXXIV) and dinuclear complexes(XXXV and XXXVI). The stabilization of enol dinuclear complexes may be due to the charge transfer from the carboxyl group to the 2-oxygen atom through the carbon-carbon bonding.
The enolization reaction of oxaloacetic acid in tertiary amine buffer solution has been studied once again by Bruice (87). According to this result, the reaction rates showed a linear dependence on tertiary amine concentration only at amine concentration > 0.02 M in the pH increase experiments. However this relationship between the reaction rates and amine concentration only existed when the buffer concentration was larger than 0.05 M in the pH decrease experiments. They also found the intersection between the buffer dilution plots for enolization and hydration which was first observed by Emly and Leussing (86). The second-order rate constants of tertiary amines are from 800 to 2200 times greater than the oxygen base second-order rate constants. The nonlinear dependence of the enolization rates on amine concentration at low tertiary amine concentrations and the extremely large second-order rate constants of tertiary amines were claimed to indicate the formation of carbinolamine intermediate in the enolization reaction. Among the nine tertiary amines studied, only 2-(diisopropylamino)ethanol undergoes general base catalysis, like oxygen bases, owing to its great steric hinderance. A most recent report states that the specific and general base catalytic pathways of the
enolization of oxaloacetate in N,N,N',N'-tetramethylethylene diamine (tetrameen) buffer solution are greatly activated by the presence of Mg(II) ions (100).

The computer programs, R LXFT and CORNEK (101) have been extensively used in these laboratories to compute the microscopic rate constants of each catalytic pathway involved in the reactions of oxaloacetate. R LXFT calculates the pseudo first order rates from the digitized stopped-flow traces. CORNEK then resolves the microscopic rate constants from the pseudo first order rate-concentration profiles. This method gives satisfactory results for one-relaxation system. However, inconsistent results are obtained in analyzing the rate constants of two-relaxation decays when the hydration-dehydration and tautomerization occur at comparable rates.

In Emly's study of the tetrameen promoted dehydration and enolization of oxaloacetate, pH decrease experiments were performed to measure the enolization rates whereas the pH increase experiments were employed to determine both dehydration rates and enolization rates. Therefore, the pseudo first order rates obtained in pH decrease experiments should be identical to those obtained in pH
increase experiments however, a great discrepancy was observed as shown in Figure 1 (86). This discrepancy can be explained by two reasons. One, the assumption that only enolization occurs in pH decrease experiment is not completely true. Two, the pseudo first order rates of pH jump experiments resolved by RLXFT do not accurately describe the corresponding two-relaxation decays. Similar discrepancies were also observed in Emly's study of the magnesium activated reaction of oxaloacetate (97). The isopH method was employed to determine the enolization rate of oxaloacetate, however, the resultant pseudo first order reaction rates are not identical to any reaction rate defined by the corresponding two-relaxation decay observed in pH jump experiments. This inconsistency which is clearly shown in the rate-concentration profiles (Figure 2) indicates the necessity of developing a new method to resolve the reaction rate constants from the biphasic decay.
Figure 1: Enolization Rate Constants of Oxaloacetate as a Function of Tetramethen Concentration

0.200 mM oxaloacetate

pH decrease experiments:

\[ \text{pH(initial)} = 12.7 \]

\[ \triangle \quad \text{pH(final)} = 7.2 \]

\[ \square \quad \text{pH(final)} = 8.9 \]

\[ \circ \quad \text{pH(final)} = 9.6 \]

pH increase experiments

\[ \text{pH(initial)} = 1.0 \]

\[ \triangle \quad \text{pH(final)} = 7.2 \]

\[ \blacksquare \quad \text{pH(final)} = 8.9 \]

\[ \bullet \quad \text{pH(final)} = 9.8 \]
Figure 1
Figure 2: Mg(II) Promoted Enolization Rate Constants of Oxaloacetate as a Function of Tetrameen Concentration

pH(final) = 9.0
15.0 mM Magnesium Chloride
0.200 mM oxaloacetate

○ --- data obtained in isopH experiments

▽,● --- data obtained in pH increase experiments, pH(initial) = 1.0
$PH \ 9.0$

$[MgCl_2] = 0.015 \text{ M}$

$[OX] = 0.200 \text{ mM}$

$PH \ triangle$

$PH \ circle$

$ISOPH$

Figure 2
RESEARCH OBJECTIVES

Due to the significance of metal ion in biological and nonbiological systems, the influence of metal ion on the reaction of oxaloacetate have been extensively studied with the hope of shedding light on the mysteries of metal ion - substrate interactions. Hydration and enolization of oxaloacetate behave differently toward general acid and base catalysis (100). The interaction between oxaloacetate and catalysts are even more complicated in the presence of metal ions. Although a large number of investigations on the reactions of oxaloacetate had been done, only relatively few quantitative studies on how metal ions alter the mechanisms of general acid and general base catalyzed hydration enolization had been performed.

Therefore, the present research was undertaken with the following goals.

1. To develop a computer program to calculate the microscopic rate constants of hydration and enolization directly from the digitized stopped-flow traces and compare the result with those obtained by RLXFT-CORNEK.

2. To perform quantitative studies of hydration and enolization reactions of oxaloacetate in various buffers including acetate, MES, Bicine and 3,3,-dimethylglutarate to define the Bronsted constants for these reactions.
3. To test the assumption that only enolization takes place in isopH experiments and hydration can be neglected.

4. To obtain quantitative kinetic data of hydration and enolization in various buffer solutions in the presence of different divalent metal ions including magnesium, manganese and zinc ions.

5. To determine the equilibrium and rate constants for the interconversions of $\text{MgOX(keto)} \rightleftharpoons \text{MgOX(hydrate)}$, $\text{MnOX(keto)}' \rightleftharpoons \text{MnOX(hydrate)}$ and $\text{ZnOX(keto)} \rightleftharpoons \text{ZnOX(hydrate)}$ and to determine how these metal ions change the buffer catalytic abilities toward enolization and hydration of oxaloacetate.

6. To determine the effect on the Bronsted constants for buffer catalyzed enolization of Mg(II), Mn(II) and Zn(II) as the activating metal ions.

7. To correlate the change in the values of Bronsted constants with the complexing ability of divalent metal ions toward oxaloacetate dianion.
Chapter II
EXPERIMENTAL PROCEDURES

Reagents

Solvent

All of the equilibrium and kinetic studies were done in aqueous solution. Demineralized doubly distilled water which was made by the Ohio State University laboratory reagent stores was thoroughly boiled to remove all dissolved carbon dioxide before using for solution preparation.

Oxaloacetic Acid, $\text{HOOCCH}_2\text{COOH}$:

Oxaloacetic acid in its solid, cis-enol form was purchased from Sigma Chemical Company. The recrystallization method used by Bamann and Sethi (105) was employed here to purify the material. The purity of the oxaloacetic acid was determined by titration with standard sodium hydroxide solution. The purities found for oxaloacetic acid before and after recrystallization were ca. 95% and 97%,
respectively. This reagent was stored in the refrigerator. The stock solution was freshly prepared by weight prior to use.

**Acetic Acid, HOAC:**

Mallinckrodt analytical grade glacial acetic acid was used to prepare stock solutions which were analyzed by titration with standard sodium hydroxide solution using 1% phenolphthalein in alcohol as an indicator.

**Hydrochloric Acid, HCl:**

Analytical reagent grade concentrated hydrochloric acid made by Mallinckrodt, INC. was used.

**Sodium Acetate, NaOAC:**

The crystalline hydrate salt, NaOAC·3H₂O, was made by J.T. Baker Chemical Corporation.

**Sodium Chloride, NaCl:**

Analytic reagent grade sodium chloride made by Mallinckrodt, INC.
**Sodium Hydroxide, NaOH:**

Saturated solution of analytical reagent grade sodium hydroxide (Mallinckrodt) was skimmed to remove Na$_2$CO$_3$ precipitate. Stock NaOH solutions were volumetrically prepared from the saturated solution and the concentration was determined by using potassium hydrogen phthalate (analytical reagent grade, Mallinckrodt, INC.) as a primary standard reagent. 1% phenolphthalein in alcohol was used as an indicator.

**Ethylenediaminetetraacetic Acid, EDTA:**

The standard EDTA solutions were obtained from the reagent store in the chemistry department.

**Ammonia-Ammonia Chloride Buffer, NH$_4$OH/NH$_4$Cl:**

Ammonia chloride was made by Allied Chemical Corporation. 70 grams of NH$_4$Cl was dissolved in 570 ml concentrated ammonia (Mallinckrodt) and diluted to the one liter mark to make one liter pH 10.0 buffer solution.

**Magnesium(II) Chloride, MgCl$_2$:**

Reagent grade MgCl$_2$·6H$_2$O (98%, Baker Analyzed) was used to prepare stock solutions by weight. The concentration of
Mg(II) ions in the stock solution was then determined by EDTA titration (102).

**Zinc(II) Chloride, ZnCl₂:**

Reagent grade zinc metal (99.9%, Baker Analyzed) was made by J.T. Baker Chemical Corporation. In order to remove trace amounts of zinc oxide, the 20 mesh granular zinc metal was first washed with dilute hydrochloric acid and then rinsed with demineralized doubly distilled water followed by reagent grade acetone. The washed zinc metal was oven-dried at 115 °C for one hour. The slightly acidic zinc chloride solution was prepared by dissolving the clean zinc metal in appropriate amount of concentrated hydrochloric acid. The solution was filtered and diluted with boiled demineralized doubly distilled water. The resultant concentration was determined by EDTA titration using Eriochrome Black T as an indicator (102).

**Manganese(II) Chloride, MnCl₂:**

Analytical reagent grade MnCl₂·4H₂O crystals made by Mallinckrodt, INC. was purchased from the storeroom in the chemistry department. The stock solution was directly prepared from MnCl₂·4H₂O crystals and the concentration was determined by EDTA titration (102).
2-(N-Morpholino)ethanesulfonic Acid, MES:

Monohydrate 2-(N-Morpholine)ethanesulfonic acid with molecular weight of 213.3 and pKa = 6.15 (20 °C) was purchased from Calbiochem-Behring Corp.

N,N-bis[2-Hydroxyethyl]glycine, Bicine:

Anhydrous Bicine with molecular weight 163.2 was made by Sigma Chemical Company. The pKa equals to 8.3 at 25 °C and the useful range is pH 7.6 to 9.0.

3,3-Dimethylglutaric Acid, DMG:

3,3-Dimethylglutaric Acid, pKa1 = 3.70 and pKa2 = 6.34 (25 °C), in its solid form was purchased from Sigma Chemical Company. The molecular weight of anhydrous compound is 160.2.

Standard Buffers:

Four different buffers routinely used in pH meter calibrations at 25 °C were prepared according to National Bureau of Standard specifications. They are:

A. pH 9.183 buffer
   0.01 molar Sodium Tetraborate Decahydrate (Borax)

B. pH 6.863 buffer
   0.025 molar Potassium Dihydrogen Phosphate and
0.025 molar Disodium Hydrogen Phosphate
C. pH 7.00 buffer
0.0275 molar Disodium Hydrogen Phosphate and
0.0200 molar Potassium Dihydrogen Phosphate
D. pH 4.004 buffer
0.0500 molar Potassium Hydrogen Phthalate

Preparation of Metal Free Glassware:

Glassware used in all of the experiments was cleaned carefully according to the method reported in Biochemical Analysis (106). Glassware was first cleaned by MICRO liquid detergent (International Products Corporation, Trenton, NJ) followed by soaking in dilute nitric acid solution for ca. 20 minutes to ensure that the glassware was completely metal free. The acid-cleaned glassware was then thoroughly rinsed with demineralized doubly distilled water and air dried.

Equilibrium Investigations

pH Measurements:

All of the pH measurements were done by using a Radiometer (Copenhagen) model 26 pH meter which was equipped with a Corning glass 476050 combination electrode and a
thermostated glass container. The thermostated glass container was constantly circulated with distilled water to keep the temperature at 25.0 °C. The pH meter was calibrated with two NBS buffers to cover the desired pH region.

**Potentiometric Titrations:**

Appropriate amounts of the solution to be titrated were placed in the thermostated container. The titrant was added by a Radiometer ABU 12 autoburette. The corresponding pH was recorded manually. Batch pH titration was used for unstable solution.

**Kinetic Investigations**

All kinetic studies were performed at 25.0 ± 0.2 °C. For each kinetic run two sample solutions were freshly prepared. These two solutions were either oxaloacetic acid solution and buffer solution as in the pH decrease experiments or oxaloacetic acid-buffer solution and metal-buffer solution as in the isopH experiments. Each kinetic run was initiated by mixing those two sample solutions and
the absorbance at 260-320 nm was monitored. The Durrum-Gibson stopped-flow spectrophotometer interfaced with either Nova minicomputer or Apple II microcomputer was used for the spectrophotometric data collection. The pre-amplified photomultiplier current was output to a log amplifier and an A-D converter. The digitized data were stored for data analysis. For each reaction mixture, 100 to 248 absorbance points were logged and three to ten replicate runs were performed. The pH values of the resultant mixtures were measured.

Data Resolution

The equilibrium constants and the first order reaction rates were calculated using PHFIT and RLXFT programs run by a Nova minicomputer equipped with 24 K memory, a 252 K fixed-head disk, Centronics line printer, multichannel A-D converter, Hewlett-Packard Model 7225A plotter and a Hazeltine video terminal. The second order reaction rates were calculated by an Amdahl 470 V/8 macrocomputer which was located at the Ohio State University Instruction and Research Computer Center. The least squares data fitting
programs used include PHFIT, RLXFT, LINFIT, GENDIS, CORNEK and MINABS written in FORTRAN. A brief description of each program except MINABS is given below. A more detailed discussion about MINABS is given in Chapter III.

**GENDIS**:

The subroutine GENDIS calculates the general distribution of species concentrations from the total species concentration and complex formation constants. The iterative procedures is based on the Newton-Raphson method.

**PHFIT**:

The PHFIT program includes the subroutine GENDIS calculates the optimum values for formation constants from pH titration data by curve fitting technique.

**RLXFT**:

The RLXFT program calculates the first-order reaction rates (k) from digitized stopped-flow readings, A(t). For a single relaxation, \( A(t) = A^{\infty} + A \cdot e^{-kt} \), where \( A(t) \) is the measured absorbance at time \( t \), \( A^{\infty} \) is the absorbance value at the completion of the reaction, and \( A \) is the amplitude of the relaxation phase. For a biphasic
relaxation, $A(t) = A_{\text{inf}} + A_1 \cdot e^{-k_1 t} + A_2 \cdot e^{-k_2 t}$, where $k_1$ and $k_2$ stand for the two pseudo first order reaction rates of these two relaxations. The optimum values of $A_{\text{inf}}$, $A$, $A_1$, $A_2$, $k$, $k_1$, and $k_2$ were calculated by the least-squares refinement.

LINFIT:

The LINFIT program calculates the slope and intercept from experimental values by the least-squares refinement (103).

CORNEK:

The CORNEK program calculates the second-order catalytic constants from rate-concentration profiles (101). Some modification of CORNEK had been done in order to improve the data fitting. The new version of CORNEK employs a group of software packages, MINUIT (104), to do the curve fitting. The subroutine SEEK, SIMPLX (107) and MIGRAD (108) are employed to obtain the absolute minima of the data. Four subroutines are included in the new version of CORNEK program. The subroutine GENDIS calculates the equilibrium concentration of each species and complex from the total concentration. The subroutine FITFC gives a rate law definition and the correlation between the catalytic
constant and the concentration of each species. The subroutine FCN was called by MINUIT to calculate the SUMSQ value from the calculated values, CALC(I) and observed values, OBS(I). SUMSQ value controls the curve fitting and is defined as \[ \text{SUMSQ} = \left[ \text{weight}(I) \cdot (\text{OBS}(I) - \text{CALC}(I))^2 \right], \]

where \( \text{weight}(I) \) is the weighting function.
Chapter III  
A GENERAL COMPUTER PROGRAM : MINABS

This chapter describes the derivation of rate equations involved in reversible hydration-dehydration and tautomerization. In addition, a recently developed general computer program, MINABS, in which the microscopic rate constants are obtained by fitting theoretical data points directly to digitized experimental absorbance-time curves is presented. A brief discussion of other procedures which have been used to treat the data is also presented.

Derivation of Rate Equations

The two coupled reversible processes, hydration-dehydration and tautomerization, yield biphasic rates when the equilibrium is suddenly disturbed by changing the concentration of a certain species, e.g. the proton. In aqueous solution, the equilibria and kinetics between hydrate, keto and enol are described by the following reactions. (The charge on each species has been omitted.)
\[
\begin{align*}
\text{R}_2 & \quad \text{F}_1 \\
\text{OX(}\text{enol}) & \quad \text{OX(}\text{keto}) \quad \text{OX(}\text{hyd}) \\
\text{F}_2 & \quad \text{R}_1
\end{align*}
\]

where \( \text{F}_1, \text{R}_1, \text{F}_2 \) and \( \text{R}_2 \) are the reaction rate constants of hydration, dehydration, enolization and ketonization, respectively. Equation (21) implies two independent rate equations.

\[
\begin{align*}
-\frac{d\text{OX(}\text{hyd})}{dt} &= \text{R}_1[\text{OX(}\text{hyd})] - \text{F}_1[\text{OX(}\text{keto})] \quad \text{...(22)} \\
-\frac{d\text{OX(}\text{keto})}{dt} &= -\text{R}_1[\text{OX(}\text{hyd})] + (\text{F}_1 + \text{F}_2)[\text{OX(}\text{keto})] - \text{R}_2[\text{OX(}\text{enol})] \quad \text{...(23)}
\end{align*}
\]

In addition,

\[
[\text{OX(}\text{total})] = [\text{OX(}\text{hyd})] + [\text{OX(}\text{keto})] + [\text{OX(}\text{enol})] \quad \text{...(24)}
\]

The equilibrium of this system is governed by the rate process defined in equation 21. At any non-equilibrium stage, the concentration of oxaloacetate can be represented by a sum of its concentration at equilibrium, \( \text{OX}^{\text{eq}} \), and a small component, \( \delta \text{OX} \).

\[
[\text{OX}] = [\text{OX}]^{\text{eq}} + \delta [\text{OX}] \quad \text{...(25)}
\]

As the system approaches to the steady state, the small component, \( \text{OX} \) decreases and eventually vanishes. Therefore, the concentration of oxaloacetate in equations
22-24 can be represented by equation 25. Equations 22-24 are rewritten as

\[-d([\text{OX(hyd)}]_{eq} + \delta [\text{OX(hyd)}])/dt =
\]
\[R_1([\text{OX(hyd)}]_{eq} + \delta [\text{OX(hyd)}])
- F_1([\text{OX(keto)}]_{eq} + \delta [\text{OX(keto)}]) \quad ...(26)\]

\[-d([\text{OX(keto)}]_{eq} + \delta [\text{OX(keto)}])/dt =
\]
\[-R_1([\text{OX(hyd)}]_{eq} + \delta [\text{OX(hyd)}])
+ (F_1 + F_2)([\text{OX(keto)}]_{eq} + \delta [\text{OX(keto)}]) -
R_2([\text{OX(enol)}]_{eq} + \delta [\text{OX(enol)}]) \quad ...(27)\]

\[[\text{OX(total)}]_{eq} + \delta [\text{OX(total)}] =
[\text{OX(hyd)}]_{eq} + \delta [\text{OX(hyd)}] +
[\text{OX(keto)}]_{eq} + \delta [\text{OX(keto)}] +
[\text{OX(enol)}]_{eq} + \delta [\text{OX(enol)}] \quad ...(28)\]

At steady state, rate equations 22 and 23 are identically equal to zero. By subtracting the equilibrium form of equation 22 from equation 26 and equation 23 from equation 27, the following equations are obtained.

\[-d\delta[\text{OX(hyd)}]/dt = R_1 \delta [\text{OX(hyd)}] - F_1 \delta [\text{OX(keto)}]\]
\[\quad ...(29)\]

\[-d \delta [\text{OX(keto)}]/dt =
-R_1 \delta [\text{OX(hyd)}] + (F_1 + F_2) \delta [\text{OX(keto)}]
- R_2 \delta [\text{OX(enol)}] \quad ...(30)\]
Equation 31 is obtained by subtracting equation 24 from equation 28.

\[ [\text{OX(total)}] = \delta [\text{OX(hyd)}] + \delta [\text{OX(keto)}] + \delta [\text{OX(enol)}] = 0 \]

\[ \ldots (31) \]

The above near-equilibrium mass balance expression allows the near-equilibrium concentration of one species to be represented by other two species.

\[ [\text{OX(enol)}] = -\delta [\text{OX(keto)}] - \delta [\text{OX(hyd)}] \]

\[ \ldots (32) \]

Expressions describing the relaxation times for the two processes are obtained by substituting equation 32 into equations 29 and 30.

\[ -d\delta [\text{OX(hyd)}]/dt = R_1 \delta [\text{OX(hyd)}] - F_1 \delta [\text{OX(keto)}] \]

\[ = 1/T \delta [\text{OX(hyd)}] \]

\[ \ldots (33) \]

\[ -d \delta [\text{OX(keto)}]/dt = -R_1 \delta [\text{OX(hyd)}] + (F_1 + F_2) \delta [\text{OX(keto)}] - R_2 \delta [\text{OX(enol)}] \]

\[ = -R_1 \delta [\text{OX(hyd)}] + (F_1 + F_2) \delta [\text{OX(keto)}] + R_2 \delta [\text{OX(keto)}] \]

\[ = -R_1 + R_2) \delta [\text{OX(hyd)}] + (F_1 + F_2 + R_2) \delta [\text{OX(keto)}] \]

\[ = 1/T \delta [\text{OX(keto)}] \]

\[ \ldots (34) \]

Equations (33) and (34) are two simultaneous equations.

\[ R_1 \delta [\text{OX(hyd)}] - F_1 \delta [\text{OX(keto)}] - 1/T \delta [\text{OX(hyd)}] = 0 \]

\[ (-R_1 + R_2) \delta [\text{OX(hyd)}] + (F_1 + F_2 + R_2) \delta [\text{OX(keto)}] - \]
\[
\frac{1}{\tau} \delta [OX(keto)] = 0
\]

The above simultaneous equations can be represented in a 2X2 matrix notation as the product of a 2X2 coefficient matrix and a 2X1 matrix.

\[
\begin{bmatrix}
R_1 - 1/\tau & -F_1 \\
-R_1 + R_2 & F_1 + F_2 + R_2 -1/\tau
\end{bmatrix}
\begin{bmatrix}
\delta [OX(hyd)] \\
\delta [OX(keto)]
\end{bmatrix} = 0
\]

Equation 35 implies that

\[
(R_1 - 1/\tau)(F_1 + F_2 + R_2 -1/\tau) - F_1(R_1-R_2) = 0
\]

Therefore,

\[
(1/\tau)^2 - (R_1 + F_1 + F_2 + R_2)1/\tau + R_1(R_2 + F_2) + F_1R_2 = 0
\]

\[
1/\tau = 1/2[(R_1+F_1+R_2+F_2) \pm [(R_1+F_1+R_2+F_2)^2 - 4(R_1F_2+R_1R_2+F_1R_2)]^{1/2}]
\]

or \[1/\tau \pm = 1/2[(R_1+F_1+F_2+R_2) \pm [(R_1+F_1-F_2-R_2)^2 + 4F_1F_2]^{1/2}]

Equation 36 can be simplified as:

\[
1/\tau \pm \text{or } k_1 = 1/2 [Y \pm (B^2 + 4C)^{1/2}] \quad \ldots(37)
\]

where \( \tau \pm \) stand for the relaxation times of two relaxations,

\[
Y = F_2 + R_2 + F_1 + R_1 \\
B = F_2 + R_2 -(F_1 + R_1) \\
C = F_2F_1 \\
i = 1 \text{ or } 2
\]
Since reversible hydration-dehydration and tautomerization are susceptible to general acid and base catalysis, these reactions can be represented by a function of microscopic rate constants and the equilibrium concentration of all species present in the solution.

\[ F_i \text{ or } R_i = k[H_2O] + k^{OH}[OH] + k^H[H] + k^{HA}[HA] + k^A[A] + \ldots \]  

...(38)

where \( i = 1 \) or \( 2 \), \( HA \) and \( A \) stand for acidic buffer component and basic buffer component, respectively. In aqueous solution, the equilibrium concentration of solvent is extremely high, ca. 55.5 M at 25 °C. Therefore, \( k[H_2O] \) is regarded as a constant and is represented by \( k^\ast \). Consequently, equation 38 is rewritten as

\[ F_i \text{ or } R_i = k^\ast + k^{OH}[OH] + k^H[H] + k^{HA}[HA] + k^A[A] + \ldots \]  

...(39)

The coupling term \( 4C \) in equation 37 becomes negligible small when these two relaxation rates are greatly different. In this circumstance,

\[ k_i = F_i + R_i \quad i=1 \text{ or } 2 \]

That is, one of the pseudo first order rate constants, \( k_i \), determines the relaxation rate of the reversible enolization-ketonization process and the other defines the relaxation rate of the reversible hydration-dehydration.
Only some reaction rate constants of those reactions which fulfill the above conditions can be satisfactorily resolved by RLXFT-CORNEK. When the reaction rates of two processes are only slightly different, then the coupling term $4C$ is not negligible. Under this condition, each relaxation is a function of both hydration-dehydration and tautomerization. Therefore, a more reliable non-linear least-squares fitting routine is required to resolve the microscopic rate constants directly from the time dependent absorbance curves.

**Computer Program: MINABS**

This section describes the procedures developed to calculate the the microscopic rate constants and fit theoretical time dependent absorbance curves to those observed. A general expression for a two-relaxation exponential decay is written:

$$\text{OBS}(I) = A_1 \cdot \exp(-k_1 \cdot t(I)) + A_2 \cdot \exp(-k_2 \cdot t(I)) + A^{\text{inf}}$$

...(40)

where $\text{OBS}(I)$ is the observed absorbance at time $t(I)$, $A^{\text{inf}}$ is the absorbance at the completion of the reaction, $A_1$ and $A_2$ are the amplitudes of these two relaxations, and $k_1$ and $k_2$ are the reciprocals of the relaxation times which
determine the shape of the absorbance-time curves. I indexes a data point. Since $A^\text{inf}$, $A_1$ and $A_2$ are constants in one experiment, those constants can be calculated by the following manipulation.

Let $X(I)$ and $Y(I)$ stand for the exponential components, the equation 40 can be simplified as:

$$OBS(I) = A_1 X(I) + A_2 Y(I) + A^\text{inf} \quad \ldots(41)$$

For $N$ data points, there are $N$ simultaneous equations with five unknowns, namely $A_1$, $X(I)$, $A_2$, $Y(I)$, and $A^\text{inf}$. However, $A_1$, $A_2$ and $A^\text{inf}$ are constants under a given set of experimental conditions. Therefore, these $N$ simultaneous equations can be represented by a product of a $N \times 3$ coefficient matrix times a $3 \times 1$ matrix.

$$\begin{align*}
OBS(1) & = X(1) \quad Y(1) \quad 1 \\
OBS(2) & = X(2) \quad Y(2) \quad 1 \\
OBS(3) & = X(3) \quad Y(3) \quad 1 \\
. & = . \quad . \quad . \\
. & = . \quad . \quad . \\
OBS(I) & = X(I) \quad Y(I) \quad 1 \\
\end{align*}$$

$$\begin{bmatrix}
A_1 \\
A_2 \\
A^\text{inf}
\end{bmatrix}$$
\[
\begin{align*}
X(1) & \quad X(2) \quad X(3) \quad \ldots\quad X(I) \\
Y(1) & \quad Y(2) \quad Y(3) \quad \ldots\quad Y(I) \\
1 & \quad 1 \quad 1 \quad \ldots\quad 1
\end{align*}
\]

\[
OBS(1) \\
OBS(2) \\
OBS(3) \\
\vdots \\
OBS(I)
\]

\[
\begin{align*}
X(1) & \quad X(2) \quad X(3) \quad \ldots\quad X(I) \\
Y(1) & \quad Y(2) \quad Y(3) \quad \ldots\quad Y(I) \\
1 & \quad 1 \quad 1 \quad \ldots\quad 1
\end{align*}
\]

\[
= 
\begin{align*}
X(1) & \quad Y(1) \quad 1 \\
X(2) & \quad Y(2) \quad 1 \\
X(3) & \quad Y(3) \quad 1 \\
\vdots & \quad \vdots \quad \vdots \\
X(I) & \quad Y(I) \quad 1
\end{align*}
\]

\[
A_1 \\
A_2 \\
A_{\inf}
\]

\[
X(1)OBS(1) + X(2)OBS(2) + \ldots + X(I)OBS(I) \\
Y(1)OBS(1) + Y(2)OBS(2) + \ldots + Y(I)OBS(I) \\
OBS(1) + OBS(2) + OBS(3) + \ldots + OBS(I)
\]
\[
\begin{align*}
X(1)^2 & \quad X(1)Y(1) & \quad X(1)Y(I) & \quad X(1) + \ldots + X(I) \\
X(I)Y(1) & \quad \ldots & \quad X(I)Y(I) & \quad Y(1)^2 + \ldots + Y^2(I) \\
X(1) + X(2) + \ldots + X(I) & \quad Y(1) + Y(2) + \ldots + Y(I) & \quad I
\end{align*}
\]

<table>
<thead>
<tr>
<th>(\sum_{I=1}^{N} X(I)OBS(I))</th>
<th>(\sum_{I=1}^{N} X(I)^2)</th>
<th>(\sum_{I=1}^{N} X(I)Y(I))</th>
<th>(\sum_{I=1}^{N} X(I))</th>
<th>(A_1)</th>
<th>(A_2)</th>
<th>(A_{\text{inf}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\sum_{I=1}^{N} Y(I)OBS(I))</td>
<td>(\sum_{I=1}^{N} X(I)Y(I))</td>
<td>(\sum_{I=1}^{N} Y(I)^2)</td>
<td>(\sum_{I=1}^{N} Y(I))</td>
<td>(A_1)</td>
<td>(A_2)</td>
<td>(A_{\text{inf}})</td>
</tr>
<tr>
<td>(\sum_{I=1}^{N} OBS(I))</td>
<td>(\sum_{I=1}^{N} X(I))</td>
<td>(\sum_{I=1}^{N} Y(I))</td>
<td>(\sum_{I=1}^{N} I)</td>
<td>(A_{\text{inf}})</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The above equation is simplified as:

\[
\begin{pmatrix}
A_{11} & A_{12} & A_{13} \\
A_{14} & A_{15} & A_{16} \\
A_{17} & A_{18} & A_{19}
\end{pmatrix}
= \begin{pmatrix}
A_{21} & A_{22} & A_{23} \\
A_{31} & A_{32} & A_{33} \\
A_{41} & A_{42} & A_{43}
\end{pmatrix}
= \begin{pmatrix}
A_{51} & A_{52} & A_{53} \\
A_{54} & A_{55} & A_{56} \\
A_{57} & A_{58} & A_{59}
\end{pmatrix}
\]  

\[\ldots (42)\]
\[
\begin{bmatrix}
A^{11} & A^{12} & A^{13} & -1 \\
A^{21} & A^{22} & A^{23} & . \\
A^{31} & A^{32} & A^{33} & .
\end{bmatrix}
\begin{bmatrix}
A^{14} \\
A^{15} \\
A^{16}
\end{bmatrix}
= \begin{bmatrix}
1 & 0 & 0 \\
0 & 1 & 0 \\
0 & 0 & 1
\end{bmatrix}
\begin{bmatrix}
A^1 \\
A^2 \\
A^{inf}
\end{bmatrix}
\]

\[
(A^{11})^{-1}A^{14} + (A^{12})^{-1}A^{15} + (A^{13})^{-1}A^{16} = A^1 \\
(A^{21})^{-1}A^{14} + (A^{22})^{-1}A^{15} + (A^{23})^{-1}A^{16} = A^2 \\
(A^{31})^{-1}A^{14} + (A^{32})^{-1}A^{15} + (A^{33})^{-1}A^{16} = A^{inf}
\]

...(43)

where the superscript \(-1\) stands for matrix inversion, for example, \((A^{11})^{-1}\) represents the matrix inversion of \(A^{11}\) matrix. \(A^1, A^2\) and \(A^{inf}\) are functions of OBS(I),X(I) and Y(I). Therefore, these three parameters can be calculated according to equation 43 once OBS(I),X(I) and Y(I) are defined.

Based on the above computation, the FORTRAN computer program, MINABS which fits the microscopic rate constants directly from the digitized stopped-flow traces is developed. MINABS is composed of a main program and five subroutines including subroutine GENDIS, MINUIT, FITFC, MATIV and FCN. A copy of MINABS program is given in Appendix A. Definitions of all symbols used in MINABS are also included in this appendix. The manipulation performed by MINABS is clearly shown in a flow chart (Figure 3).
Subroutine GENDIS calculates the general distribution of species concentrations from the total species concentrations and complex formation constants by Newton-Raphson approximation. Subroutine MINUIT (104) is a data fitting routine which computes the absolute minimum of the sum squares of the residuals of observed and calculated data points. MINUIT has three minimizing subroutines, namely, SEEK, SIMPLX and MIGRAD. SEEK which is a Monte Carlo searching subroutine is usually called initially when several minima are anticipated or when no reasonable estimates are available.

SIMPLX performs a simplex minimization (107). This is useful when the initial estimate is far from the minimum. MIGRAD is also a minimization method based on a procedure developed by Fletcher (108). MIGRAD is used when the estimated value is close to the minimum. Subroutine FITFC gives a rate law definition which is identical to equation 37. The correlation between catalytic constant and the equilibrium concentration of each species as shown in equation 39 is also given. Based on this information, the reciprocals of the relaxation times, $k_1$ and $k_2$ are calculated.
Subroutine MATIV calculates the matrix inversion by Gauss-Jordan elimination (103). Subroutine FCN is called by MINUIT. Subroutine FCN then calls FITFC and MATIV. Here, $A_1, A_2$ and $A_{\infty}$ are calculated according to equations 41-43. Consequently, the theoretical absorbance at time, $t(I)$, i.e. $A(I)$ is defined by:

$$A(I) = A_{\infty} + A_1 \cdot \exp(-k_1 \cdot t(I)) + A_2 \cdot \exp(-k_2 \cdot t(I))$$

...(44)

The SUMSQ value is then calculated according to the following equation:

$$\text{SUMSQ} = \left[ \text{weight}(I) \cdot \left[ \text{OBS}(I) - A(I) \right] \right]^2 \quad ...(45)$$

where the weight$(I)$ is usually defined as the following:

$$\text{weight}(I) = 1/\left[ \text{OBS}(I) \right]^2$$

OBS$(I)$ is the observed absorbance at time, $t(I)$. The magnitude of the microscopic rate constants are adjusted by SIMPLEX and MIGRAD to minimize the SUMSQ. The curve fitting process is repeated until the best values of the microscopic rate constants are obtained.
Figure 3: A Flow Chart of MINABS
START

Read and Write Parameters

Call GENDIS

Call MINUIT

STOP

Subroutine FCN

START

Call FITFC:
calculate k1 and k2

Call MATIV:
calculate $A^\text{inf}, A_1$ and $A_2$

Calculate $A(I)$

calculate SUMSQ

write Parameters

Return
The goodness of the nonlinear curve fitting result is not only decided by SUMSQ, but in addition, the standard deviations, the internal and global correlation coefficients of all the variables, i.e. the microscopic rate constants also indicate the accuracy of the estimated rate constants. The internal correlation coefficient which relates one variable to another is measured on a zero to one scale. It indicates strong correlation between two variables when the internal correlation of the corresponding variables closes to or equal to one. This indicates that the two variables may actually represent one parameter. The global correlation coefficient which implies the relation of one variable to all the other variables is also measured on a zero to one scale.

The acetate buffer catalysis of hydration and enolization of oxaloacetate is employed as an example to illustrate the data fitting process performed by the MINABS program. The experimental conditions, namely pH, the total concentrations of buffer and oxaloacetate and the observed absorbances, OBS(I) and the time, t(I) of these data were input into the computer. The equilibrium constants which defined the equilibria of acetate buffer and oxaloacetic acid as described in equation 46 were also
included. The initial estimates of the microscopic rate constants of all possible catalytic pathways, e.g. $k^\text{OH}$, and $k^\text{H}$ were also included as a part of the input data.

\[
\begin{align*}
H^+ + \text{OAC}^- & \rightleftharpoons \text{HOAC} \quad \log \beta = 4.53 \\
H^+ + \text{OX}^2- & \rightleftharpoons \text{HOX}^- \quad \log \beta = 3.821 \\
2H^+ + \text{OX}^2- & \rightleftharpoons \text{H}_2\text{OX} \quad \log \beta = 6.041 \\
\end{align*}
\]

\[\ldots(46)\]

The subroutine GENDIS then calculated the equilibrium distribution of proton, acetate, acetic acid, oxaloacetate dianion, mono oxaloacetate and oxaloacetic acid from their equilibrium constants and the initial total concentrations of these species.

The initial estimates of the microscopic rate constants were employed in the subroutine FITFC. In this subroutine the enolization and hydration rate functions were calculated from estimated microscopic rate constants according to equation 39. From the hydration and enolization rate functions, $F_1$ and $F_2$, the reciprocals of the relaxation times, $k_1$ and $k_2$ were then calculated according to equation 36.
Matrix inversion was performed by subroutine MATIV. \( A_1, A_2 \) and \( A_{\text{inf}} \) of each absorbance vs. time curve were calculated according to equations 40-43. After the \( A_1, A_2 \) and \( A_{\text{inf}} \) were obtained, the theoretical absorbance, \( A(I) \) at each time, \( t(I) \) was calculated according to equation 44.

The differences between the theoretical absorbances and the experimental values were then obtained and the SUMSQ value was calculated according to equation 45. The magnitudes of the microscopic rate constants were then adjusted by subroutine SIMPLX so as to minimize the value of SUMSQ. This computational sequence was repeated until further improvements in SUMSQ became insignificant.

As mentioned before, instead of resolving the microscopic rate constants from the buffer dependency of pseudo first order rate constants, MINABS determines the microscopic rate constants from the digitized absorbance-time curves. This general computer approach which handles two-relaxation exponential decays allows the rate constants to be completely resolved, even in cases where either hydration rates are comparable with the enolization rates or one amplitude is relatively small compared with the other. Furthermore, the equilibrium constants of metal oxaloacetate complexes may also be determined by MINABS.
The application of MINABS in the kinetic studies will be found in the following chapters.

Computer Program: PPFIT

This section presents another computer program, PPFIT, which is also developed in this work. In exploring the Zn(II) ion promoted reactions of oxaloacetate (see Chapter VIII), some time dependent absorbance profiles with undefined $A^{\text{inf}}$ were obtained. In order to meaningfully evaluate the relaxation time from those digitized stopped-flow traces, a FORTRAN computer program which includes the phase-plane method and the MINUIT data fitting process has been written.

The phase-plane method was first introduced by Huen (109) and Bernalte et al. (110) to evaluate a simple exponential decay. Following their paper, a modified phase-plane method was published by Bacon et al. (111) and was proved to be an efficient method for evaluation of the kinetic data in cases where base lines were not known. The base line may be undefined due to 1) instrument base line drift or 2) insufficient time allowed for the signal to decay to the base level or 3) the occurrence of another very slow relaxation which causes a variation in the optical
density of the reaction solution. Based on the work contributed by Bacon et al., a data fitting program, PPFIT is developed.

One-relaxation decay is represented as:

\[ A(t) = A \cdot \exp(-t \cdot k) + A_{\text{inf}} \]  

...(47)

where \( A(t) \) is the absorbance at time, \( t \), measured in second,

\( A \) is the amplitude of the relaxation phase which is a proportionality constant.

\( A_{\text{inf}} \) is the baseline of the decay, i.e. the absorbance at the completion of the reaction.

\( k \) is the first order reaction rate constant, i.e. the reciprocal of the relaxation time. Integrating equation 47 over the time limits, from 0 to \( t \), yields the phase-plane solution.

\[
\int_0^t A(t) \, dt = \int_0^t [A \cdot \exp(-t \cdot k) + A_{\text{inf}}] \, dt
\]

\[
\int_0^t A(t) \, dt = \int_0^t A \cdot \exp(-t \cdot k) \, dt + \int_0^t A_{\text{inf}} \, dt
\]

\[
\int_0^t A(t) \, dt = (-A/k) \cdot \exp(-t \cdot k) \bigg|_0^t + A_{\text{inf}} t
\]

\[
\int_0^t A(t) \, dt = (-A/k) \cdot \exp(-t \cdot k) + A/k + A_{\text{inf}} t - 1/(k \cdot A_{\text{inf}}) + 1/(k \cdot A_{\text{inf}})
\]

\[
\int_0^t A(t) \, dt = (-1/k)[A \exp(-t \cdot k) + A_{\text{inf}}] + (1/k)[A + A_{\text{inf}}] + A_{\text{inf}} \cdot t
\]
\[ \int_0^t A(t)dt = (-1/k)A(t) + (1/k)[A + A_{\text{inf}}] + A_{\text{inf}} t \]

\[ A(t) = [A + A_{\text{inf}}] - k \int_0^t A(t)dt + A_{\text{inf}} t \cdot k \quad \ldots (48) \]

Equation 48 shows \( A(t) \) is a linear function of variables \( t \) and \( \int_0^t A(t)dt \).

\( \int_0^t A(t)dt \) is numerically integrated. According to trapezoidal rule,

\[ \int_0^t A(t)dt \approx (\Delta t/2) C_1 \quad \ldots (49) \]

\[ t = t_{i+1} - t_i = \text{time between each point} \]

\[ C_1 = A(t_i) + A(t_{i-1}) + C_{i-1} \quad i > 1 \quad \ldots (50) \]

\( i \) indicates the number of each point.

let \( C_0 = 0 \quad \ldots (51) \)

By substituting equations 49-51 into equation 48

\[ A(t_1) = [A + A_{\text{inf}}] - k(\Delta t/2)C_1 + A_{\text{inf}} t \cdot k \]

\[ A(t_1) = [A+A_{\text{inf}}] - k(\Delta t/2)[A(t_1)+A(t_{1-1})+C_{1-1}]+A_{\text{inf}} t \cdot k \]

let \( P_1 = k \)

\[ P_2 = A_{\text{inf}} k \]

\[ P_3 = A + A_{\text{inf}} \]

The above equation is rewritten as

\[ A(t_1) = P_3 - P_1(\Delta t/2) [A(t_1) + A(t_{1-1}) + C_{1-1}] \]

\[ + P_2 t \]

The optimum values of these three parameters, \( P_1, P_2 \) and \( P_3 \) are evaluated by the curve fitting routine, MINUIT and the first order reaction rate constant is precisely obtained.
A copy of the PPFIT program which includes the phase-plane method and MINUIT to perform the data fitting is given in Appendix A. In order to evaluate the accuracy and precision of the results obtained by PPFIT program a short computer program was written in FORTRAN language to generate some data of an one-relaxation decay with the relaxation rate constant $= 0.1 \text{ sec}^{-1}$. Those simulated data were analysed by both PPFIT and RLXFT. The overall results are summarized in Table 2. As clearly shown in this table, by analysing the first ten data points, RLXFT gives 116% deviation while PPFIT only gives 25% deviation. Furthermore, by analysing the first fifteen data points, RLXFT gives 35% deviation while PPFIT only gives 8.2% deviation. Apparently, the rate constant evaluated by PPFIT is more reliable than that evaluated by RLXFT in the circumstance when a valid absorbance at $t = \infty$ is not obtained.
TABLE 2

A Comparison of the First Order Rate Constants Obtained by RLXFT and PPFIT

A. The first ten points were analysed.

<table>
<thead>
<tr>
<th>true value</th>
<th>PPFIT</th>
<th>RLXFT</th>
<th>% deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>the rate constant, $k$</td>
<td>$0.100 \text{ sec}^{-1}$</td>
<td>$0.216 \pm 0.010 \text{ sec}^{-1}$</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>$0.0751 \pm 0.0607 \text{ sec}^{-1}$</td>
<td>$0.0918 \pm 0.0515 \text{ sec}^{-1}$</td>
<td>25%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$0.135 \pm 0.010 \text{ sec}^{-1}$</td>
<td>116%</td>
</tr>
</tbody>
</table>

B. The first 15 data points were analysed.

<table>
<thead>
<tr>
<th>true value</th>
<th>PPFIT</th>
<th>RLXFT</th>
<th>% deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>the rate constant, $k$</td>
<td>$0.100 \text{ sec}^{-1}$</td>
<td>$0.135 \pm 0.010 \text{ sec}^{-1}$</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>$0.0918 \pm 0.0515 \text{ sec}^{-1}$</td>
<td>$0.135 \pm 0.010 \text{ sec}^{-1}$</td>
<td>35%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8.2%</td>
</tr>
</tbody>
</table>
Chapter IV

BUFFER CATALYSIS OF HYDRATION AND ENOLIZATION OF OXALOACETATE

A thorough understanding of the general acid and base catalysis of the hydration and enolization of oxaloacetate is essential before exploring the influence of metal ions on the Bronsted and values. This chapter describes investigations on the catalytic effects of various buffers including acetic acid, 3,3-dimethylglutaric acid (DMG, XXXVIII), N,N-Bis (2-hydroxyethyl)glycine (Bicine, XXXX) and 2-(N-Morpholino)ethanesulphonic acid (MES, XXXIX) on the hydration and enolization rates of oxaloacetate at 25.0 ± 0.2 °C, ionic strength, u=0.275

\[
\begin{align*}
\text{HOCH}_2\text{C(CH}_3\text{)}_2\text{CH}_2\text{COH} & \quad \text{XXXVIII} \\
\text{O} & \quad \text{O} \\
\text{HOCCH}_2\text{C(CH}_3\text{)}_2\text{CH}_2\text{COH} & \quad \text{O}^{+}\text{HCH}_2\text{CH}_2\text{SO}_3^- \\
\text{XXXIX} & \\
\text{(HOCH}_2\text{CH}_2\text{)}_2\text{N}^{+}\text{HCH}_2\text{COO}^- \\
\text{XXXX} &
\end{align*}
\]
M(NaCl). These buffers allow hydration and enolization to be studied in a wide pH range of 3.5 to 9.5.

Hydration and enolization are susceptible to general acid and base catalysis (100) as shown below.

**Hydration:**

\[
\text{hydration:} \quad \text{enolization:} \\
\begin{align*}
\ce{\text{O}} & \quad \ce{\text{II}} \\
\ce{-O_2C-C-CH_2-CH_2-CO_2-} + \text{H} + \text{H}_2\text{O} & \quad \text{slow} \\
\ce{\text{O}} & \quad \ce{\text{II}} \\
\ce{-O_2C-C-C-C-C-C-C-CH_2-CH_2-CO_2-} + \text{B} & \quad \text{fast} \\
\ce{\text{O}} & \quad \ce{\text{II}} \\
\ce{\text{O}} & \quad \ce{\text{II}}
\end{align*}
\]

Both general acid and general base promoted enolization undergo two consecutive steps: the protonation of a keto-oxygen atom and the removal of a proton from the carbon which is next to the keto group. The deprotonation is the rate-determining step. In highly alkaline condition, enol oxaloacetate can be deprotonated and gives enolate as shown in equation 53. The deprotonation of enol species is a
preequilibrium step relative to hydration and enolization reactions. In addition to the kinetic investigations, potentiometric and spectrophotometric equilibrium studies are also performed to facilitate the elucidation of those kinetic data.

**Equilibrium Investigation**

**Potentiometric Studies**

A. The Determination of Proton Activity Coefficient

The proton activity coefficient in $u=0.275 \text{ M(NaCl)}$ aqueous solution was determined potentiometrically at 25.0 °C. The correlation between the proton concentration and the proton activity measured by a pH meter is described by the following equation:

$$-\log[H^+] = \text{slope} \cdot \text{pH(obs)} + \log(f)$$
where $[H^+]$ is the proton concentration, $pH(\text{obs})$ is the pH value observed potentiometrically. $f$ is the proton activity coefficient.

A 0.1061 M, $u=0.275$ M HCl solution was prepared and used as the stock solution. Eight different solutions with HCl concentration ranging from $10^{-1}$ M to $10^{-5}$ M were prepared volumetrically by diluting the stock solution with appropriate amounts of boiled demineralized doubly distilled water. Suitable amounts of NaCl were added to keep the ionic strength at 0.275 M and the values of pH was measured. Table 3 gives the values of $-\log[H^+]$ and the corresponding pH(\text{obs}). As is shown in Figure 4, a well defined straight line is obtained by plotting $-\log[H^+]$ vs. pH(\text{obs}). The intercept which is a log function of the proton activity coefficient, $f$ was evaluated by a linear, least-squares routine. The intercept was found to correspond to $\log f = -0.02340$. Therefore, the proton activity coefficient, $f$, is 0.9475.

B. The Proton Dissociation Constants of Oxaloacetic Acid

Oxaloacetic acid, an important substance in biological systems, has three acid sites. Oxaloacetic acid is a fairly strong acid. However, the removal of the third
TABLE 3

The Determination of Proton Activity Coefficient

<table>
<thead>
<tr>
<th>solution</th>
<th>pH(obs)</th>
<th>-log[H+]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.051</td>
<td>0.9743</td>
</tr>
<tr>
<td>2</td>
<td>2.014</td>
<td>1.974</td>
</tr>
<tr>
<td>3</td>
<td>2.323</td>
<td>2.275</td>
</tr>
<tr>
<td>4</td>
<td>3.005</td>
<td>2.974</td>
</tr>
<tr>
<td>5</td>
<td>3.320</td>
<td>3.275</td>
</tr>
<tr>
<td>6</td>
<td>4.026</td>
<td>3.974</td>
</tr>
<tr>
<td>7</td>
<td>4.366</td>
<td>4.275</td>
</tr>
<tr>
<td>8</td>
<td>5.110</td>
<td>4.975</td>
</tr>
</tbody>
</table>
proton to give enolate occurs at extremely alkaline solution. The equilibrium among oxaloacetic acid ($H_2OX$), its monoanion ($HOX$) and dianion ($OX$) is shown as in equation 54. According to Tate and his coworkers (78), $pK_{a3} = 13.06$ ($u=0.1$, at 25 °C). Oxaloacetic acid is quite unstable in aqueous solution due to its spontaneous decarboxylation which gives pyruvic acid and carbon dioxide as the products. Therefore, batch titrations were performed to determine $pK_a$s in 0.275 M ionic strength at 25.0 °C.

The overall proton dissociation constants were determined potentiometrically. Duplicate titrations were performed with 20.10 ml 30.00 mM oxaloacetic acid. Suitable amount of NaCl was added to keep the ionic strength, $u=0.275$. During the titration, precise amounts of alkaline titrant, 0.2785 M sodium hydroxide solution were added from an ABU12 autoburette and the corresponding pH value was measured by a Radiometer Model 26 pH meter. A
least-squares computer program, PHFIT was employed to compute the proton dissociation constant from the titration data. The proton dissociation constants of oxaloacetic acid thus determined give a good agreement with the literature values (Table 4).

C. Proton Dissociation Constants of Various Buffers

The acid-base titration and data analysis procedures performed to determine the proton dissociation constants of 3,3-dimethylglutaric acid and acetic acid were essentially the same as those described above. Except batch titrations were not necessary owing to the relatively high stability of those buffers. The pKas determined in this work along with those reported in literature are presented in Table 4. The equilibrium constants determined in this work are in excellent agreement with those reported in the literature. The potentiometric titration data are given in Appendix C.
Figure 4: A Plot of $-\log[H^+]$ vs. pH(obs) --- Determination of Proton Activity Coefficient
Figure 4
Spectrophotometric Studies

Hydration, enolization and decarboxylation of oxaloacetate complicate the spectrophotometric characteristic of oxaloacetate. The conjugation between an olefinic double bond and the doubly bonded oxygen of enol oxaloacetic acid give stronger absorption in UV region than does keto oxaloacetic acid. The molar absorptivity of enol oxaloacetic acid is ca. $1.1 \times 10^4 \text{M}^{-1}\text{cm}^{-1}(192)$. Figure 5 shows the UV spectra of $5.00 \times 10^{-4}$ M oxaloacetic acid at pH 1.30. The absorption rapidly decreases as the result of the decarboxylation of oxaloacetic acid.

Since the enolization and hydration mechanisms are usually studied by monitoring the UV absorption of oxaloacetate solution at 265 nm-290 nm, the buffer employed should be transparent in the desired wavelength region. In order to decide the applicabilities of buffers in the kinetic studies of hydration and enolization, the UV absorption spectra were also taken. Figures 6-8 show the UV spectra of 3,3-dimethylglutaric acid (DMG), N,N-Bis(2-hydroxyethyl)glycine (Bicine) and 2-(N-Morpholino)ethanesulphonic acid (MES). The results show that the optical density of pH 3.51, 10.00 mM DMG is relatively low at wavelength down to 240 nm. Identical UV
### TABLE 4

Equilibrium Constants of Oxaloacetic Acid, 3,3,-Dimethylglutaric Acid and Acetic Acid

\[
\begin{align*}
\text{H}_2\text{OX} & \rightleftharpoons \text{HOX}^- + \text{H}^+ & \text{Ka}_2 & \text{OX}^- + 2 \text{H}^+ \\
\text{pKa}_1 & \text{pKa}_2 & \chi^2 & \text{reference} \\
2.220 \pm 0.006 & 3.821 \pm 0.005 & 2.17 \times 10^{-5} & \text{this work(a)} \\
2.242 \pm 0.006 & 3.883 \pm 0.009 & 8.30 \times 10^{-5} & \text{this work(a)} \\
2.208 & 3.864 & --- & \text{Pedersen(b)} \\
2.31 & 3.89 & --- & \text{Gelles(c)} \\
2.22 & 3.89 & --- & \text{Tate, et al.}(d) \\
2.18 & 3.94 & --- & \text{Duc, et al.}(e) \\
2.35 & 4.03 & --- & \text{Mao(f)} \\
\end{align*}
\]

\[
\begin{align*}
\text{H}_2\text{DMG} & \rightleftharpoons \text{HDMG}^- + \text{H}^+ & \text{Ka}_2 & \text{DMG}^- + 2 \text{H}^+ \\
\text{pKa}_1 & \text{pKa}_2 & \chi^2 & \text{reference} \\
3.421 \pm 0.022 & 6.676 \pm 0.021 & 2.51 \times 10^{-3} & \text{this work(a)} \\
3.70 & 6.34 & --- & \text{Stafford et al.}(g) \\
\end{align*}
\]
<table>
<thead>
<tr>
<th>pKa&lt;sup&gt;1&lt;/sup&gt;</th>
<th>X&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.530 ± 0.007</td>
<td>1.96x10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>this work (a)</td>
</tr>
<tr>
<td>4.526 ± 0.008</td>
<td>1.95x10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>this work (a)</td>
</tr>
<tr>
<td>4.53</td>
<td>---</td>
<td>Mao (f)</td>
</tr>
<tr>
<td>4.612</td>
<td>---</td>
<td>Emly (h)</td>
</tr>
</tbody>
</table>

---

a. u=0.275M(NaCl), 25.0 °C.
b. u=0.10M(NaCl), 37 °C (13).
c. u=0.10M(KCl), 25 °C (79).
d. u=0.10M(KCl), 25 °C (72).
e. u=0.20M(KClO<sub>4</sub>), 25 °C (113).
f. u=0.10M(KCl), 25 °C (98).
g. 25 °C (116).
h. u=0.10M(KCl), 25 °C (97).
spectrophotometric feature was also observed by Rand and his coworkers (54). MES solution is also almost transparent down to wavelength 240 nm. With regard to the UV spectrum of 10.00 mM, pH 8.95 Bicine solution, significant absorption is only observed in wavelength range of 260 nm and shorter. Based on this observation, the kinetic investigations are performed by monitoring the UV absorption change of 270 nm to 290 nm.

**Kinetic Investigations**

Concentration-jump chemical relaxation methods including pH increase and pH decrease methods have been employed in this study. In order to determine the influence of general acid and general base catalysts on these reactions kinetic studies have been done in various buffers covering a wide range of buffer and proton concentrations. In all of the kinetic studies the equilibrium concentration of buffer is two to three order of magnitude greater than oxaloacetate. Kinetic studies of hydration and enolization of OX in either acetate, DMG, Bicine and MES were performed with 0.275 (NaCl) ionic strength at 25.0 ± 0.2 °C.
Figure 5: Time Dependent UV Absorption Spectrum of Oxaloacetic Acid

0.500 mM oxaloacetic acid   pH = 1.30
light path = 1 cm

1 --- collected at 5 min.
2 --- collected at 1 min.
3 --- collected at 10 min.
Figure 5
Figure 6: A UV Absorption Spectrum of pH 3.61 3,3-Dimethylglutaric Acid

10.0 mM DMG

light path = 1 cm
Figure 6

WAVELENGTH nm
Figure 7: A UV Absorption Spectrum of pH 8.95 Bicine

10.0 mM Bicine

1 cm light path
Figure 7
Figure 8: A UV Absorption Spectrum of pH 6.20 MES

10.0 mM MES

1 cm light path
Figure 8

WAVELENGTH nm
The Hydration and Enolization of Oxaloacetate in Acetate Buffer

The reversible hydration-dehydration and tautomeration of OX in acetate (OAC) buffer solutions were studied in 30.0 mM to 140 mM acetate. NaCl was added to maintain the ionic strength at 0.275 M. Standard HCl and NaOH solutions were used to adjust the pH to the desired values. In pH increase experiments, pH 1.30 oxaloacetate solutions were rapidly mixed with equal volumes of alkaline buffer solution to give mixed solutions in the pH range of 4.0 to 5.6. In pH decrease experiments, pH 12.70 OX solutions were rapidly mixed with equal volumes of acidic buffer solution to give mixed solutions in the same pH range as that of the pH increase experiments. Mixing was performed by using a Durrum Model D-132 multi-mixing system. The pH value of each mixed solution was determined immediately after mixing by a Radiometer Model 26 pH meter. The UV absorption changes of the mixed solutions were monitored at 275 nm.

Based on the observation that Bromocresol Green indicator solution was yellow at pH 3.8 and blue at pH 5.4, some supporting experiments which had been done by Emly
(97) were repeated to measure the mixing time of stopped-flow instrument. A yellow, pH 1.6 acetic acid and indicator solution was mixed with a blue pH 12.7 oxaloacetate and indicator solution to give a mixed solution with an equilibrium pH 4.7. The results of several identical experiments showed that the absorbance change in either 420 nm, yellow region or 615 nm, blue region was completed within 1 millisecond.

During the curve fitting process, the literature values of the equilibrium constants of oxaloacetate species as summarized in Table 5 were employed to define the equilibrium distribution of oxaloacetate. A general definition of all of the microscopic rate constants determined in this research are given in Table 6. The experimental and theoretical absorbance vs. time curves as a function of acetate buffer concentration are given in Figure 9. These spectra were taken with 0.200 mM oxaloacetate. Triangles, diamonds and squares give the results for 30.0 mM, 90.0 mM and 120 mM acetate, respectively. The solid curves give the best theoretical values which were obtained by MINABS.

Oxaloacetic acid is highly hydrated in the extremely acidic solution. According to Table 7, there are 83 %
# TABLE 5

**Equilibrium Constants of Oxaloacetate Species**

<table>
<thead>
<tr>
<th>Reaction Equation</th>
<th>Equilibrium Constant, $K_E$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{OX}(\text{keto}) \rightleftharpoons \text{OX}(\text{enol})$</td>
<td>0.142</td>
</tr>
<tr>
<td>$\text{HOX}(\text{keto}) \rightleftharpoons \text{HOX}(\text{enol})$</td>
<td>0.127</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction Equation</th>
<th>Equilibrium Constant, $K_H$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{OX}(\text{keto}) \rightleftharpoons \text{OX}(\text{hyd})$</td>
<td>0.084</td>
</tr>
<tr>
<td>$\text{HOX}(\text{keto}) \rightleftharpoons \text{HOX}(\text{hyd})$</td>
<td>0.77</td>
</tr>
</tbody>
</table>

a. $K_E = [\text{OX}(\text{enol})]/[\text{OX}(\text{keto})]$  
or $K_E = [\text{HOX}(\text{enol})]/[\text{HOX}(\text{keto})]$  

b. $K_H = [\text{OX}(\text{hyd})]/[\text{OX}(\text{keto})]$  
or $K_H = [\text{HOX}(\text{hyd})]/[\text{HOX}(\text{keto})]$
hydrate, 8% enol and 9% keto at pH 1.30. After mixing with alkaline acetate buffer solution, the equilibrium distribution of hydrate species reduced dramatically to only ca. 9%. Consequently, the equilibrium distribution of enol and keto species were enhanced and gave final distribution of ca. 12% and 79%, respectively. The redistribution of hydrate, keto and enol species give biphasic absorption curves which shift upward as the buffer concentration increase as shown in Figure 9. As clearly shown in these spectra, each buffer concentration gives a unique biphasic absorbance-time curves as the result of the redistribution of oxaloacetate species. The microscopic rate constants thus determined by MINABS are summarized in Table 8.

Under the experimental conditions employed, the hydroxide term is negligible. Both hydration and enolization are accelerated by acetic acid and proton catalytic pathways. Only trivial acetate catalysis is observed, \( k^{2,A} = 0.009 \text{ M}^{-1}\text{sec}^{-1} \). The solvent catalytic pathway is only observable in hydration. Since acetate buffer has been employed by Leussing and Mao (98) in their kinetic studies on the influence of solvent on the metal ion-catalyzed decarboxylation of oxaloacetate and by
### TABLE 6

**Definition of the Microscopic Rate Constants**

<table>
<thead>
<tr>
<th>Equilibrium Reaction</th>
<th>Definition ( (a, b, c) )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Hydration</strong></td>
<td></td>
</tr>
<tr>
<td>( \text{HOX(keto)} \xrightarrow{k^4} \text{HOX(hyd)} )</td>
<td>( k^4 = k^4, [H] + k^4, [HA] )</td>
</tr>
<tr>
<td>( \text{OX(keto)} \xrightarrow{k^5} \text{OX(hyd)} )</td>
<td>( k^5 = k^5, [H] + k^5, [HA] + k^5, [OH] + k^5, [A] + k^5, H_2O )</td>
</tr>
<tr>
<td><strong>B. Enolization</strong></td>
<td></td>
</tr>
<tr>
<td>( \text{HOX(keto)} \xrightarrow{k^1} \text{HOX(enol)} )</td>
<td>( k^1 = k^1, [H] + k^1, [HA] )</td>
</tr>
<tr>
<td>( \text{OX(keto)} \xrightarrow{k^2} \text{OX(enol)} )</td>
<td>( k^2 = k^2, [H] + k^2, [HA] + k^2, [OH] + k^2, [A] + k^2, H_2O )</td>
</tr>
</tbody>
</table>

a. \( HA \) stands for the acidic buffer component.
b. \( A \) stands for the basic buffer component.
c. \( k^x, y \) stands for the microscopic rate constants of \( y \) species involved in \( x \) reaction.

For example, \( k^4, [H] \) stands for the microscopic rate constant of proton involved in the enolization of \( \text{HOX} \) species.
### TABLE 7

Equilibrium Distribution of Oxaloacetate in Acetate Buffer

<table>
<thead>
<tr>
<th>[OAC] mM</th>
<th>PH</th>
<th>% keto</th>
<th>% enol</th>
<th>% hydrate (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>1.30</td>
<td>9.0</td>
<td>8.0</td>
<td>83</td>
</tr>
<tr>
<td>0.00</td>
<td>12.70</td>
<td>73.0</td>
<td>21</td>
<td>6.0</td>
</tr>
<tr>
<td>30.0</td>
<td>5.01</td>
<td>79.3</td>
<td>11.7</td>
<td>8.99</td>
</tr>
<tr>
<td>60.0</td>
<td>5.04</td>
<td>79.4</td>
<td>11.8</td>
<td>8.86</td>
</tr>
<tr>
<td>90.0</td>
<td>5.06</td>
<td>79.5</td>
<td>11.8</td>
<td>8.78</td>
</tr>
<tr>
<td>120</td>
<td>5.06</td>
<td>79.5</td>
<td>11.8</td>
<td>8.78</td>
</tr>
<tr>
<td>30.0</td>
<td>4.62</td>
<td>77.0</td>
<td>11.4</td>
<td>11.6</td>
</tr>
<tr>
<td>60.0</td>
<td>4.57</td>
<td>76.6</td>
<td>11.4</td>
<td>12.0</td>
</tr>
<tr>
<td>140</td>
<td>4.64</td>
<td>77.2</td>
<td>11.4</td>
<td>11.4</td>
</tr>
<tr>
<td>30.0</td>
<td>5.00</td>
<td>79.2</td>
<td>11.7</td>
<td>9.04</td>
</tr>
<tr>
<td>45.0</td>
<td>4.97</td>
<td>79.1</td>
<td>11.7</td>
<td>9.18</td>
</tr>
<tr>
<td>60.0</td>
<td>5.00</td>
<td>79.2</td>
<td>11.7</td>
<td>9.04</td>
</tr>
<tr>
<td>75.0</td>
<td>5.02</td>
<td>79.3</td>
<td>11.7</td>
<td>8.95</td>
</tr>
<tr>
<td>20.0</td>
<td>5.41</td>
<td>80.3</td>
<td>11.9</td>
<td>7.80</td>
</tr>
<tr>
<td>30.0</td>
<td>5.45</td>
<td>80.4</td>
<td>11.9</td>
<td>7.73</td>
</tr>
<tr>
<td>45.0</td>
<td>5.55</td>
<td>80.5</td>
<td>11.9</td>
<td>7.58</td>
</tr>
<tr>
<td>60.0</td>
<td>5.57</td>
<td>80.5</td>
<td>11.9</td>
<td>7.55</td>
</tr>
<tr>
<td>75.0</td>
<td>5.63</td>
<td>80.6</td>
<td>11.9</td>
<td>7.48</td>
</tr>
</tbody>
</table>

a. These data were collected with 0.200 mM oxaloacetic acid.
Leussing and Emly (97) in their kinetic studies of metal ion activated tautomerization, their results are represented here for comparison purpose (Table 9). With regard to enolization, the rate constant of specific acid, i.e. proton, is two to three orders of magnitude greater than that of the acetic acid. Identical relationships were reported by both Mao and Emly.

The acid buffer dependency of enolization and hydration are also implied in the correlation among the pseudo first order rate constants, buffer concentration and pH values as indicated in Table 10. The pseudo first order hydration rate constants for 30.0 mM acetate at pH 5.0 and 4.6 are 0.0342 M⁻¹sec⁻¹ and 0.0361 M⁻¹sec⁻¹, respectively. As the acetate concentration increases to 120 mM, the pseudo first order hydration rate constants obtained at pH 5.0 and pH 4.6 are 0.0432 M⁻¹sec⁻¹ and 0.0543 M⁻¹sec⁻¹, respectively. Quantitatively, by increase the acetate concentration by a factor of four causes 50% increase in hydration rate at pH 4.6 while this only causes less than 30% increase in the hydration rate at pH 5.0. The pseudo first order enolization rate constants for 30.0 mM and 120 mM acetate at pH 5.0 are 0.0175 sec⁻¹ and 0.0333 sec⁻¹, respectively. By increasing the acetate concentration by a
TABLE 8

Forward Microscopic Rate Constants of Acetate Buffer

\[ T = 25.0 \pm 0.2 \, ^\circ\text{C} \quad u = 0.275\text{M(NaCl)} \]

---

A. Hydration

\[ OX(\text{keto}) \xrightleftharpoons{k^5} OX(\text{hyd}) \]

\[ k^5 = k^5,H[H] + k^5,HA[HA] + k^5,H_2O \]

\[ k^5,H = 541.5 \pm 60.1 \, \text{M}^{-1}\text{sec}^{-1} \]

\[ k^5,HA = 0.146 \pm 0.026 \, \text{M}^{-1}\text{sec}^{-1} \]

\[ k^5,H_2O = 0.026 \pm 0.007 \, \text{M}^{-1}\text{sec}^{-1} \]

B. Enolization

\[ OX(\text{keto}) \xrightleftharpoons{k^2} OX(\text{enol}) \]

\[ k^2 = k^2,H[H] + k^2,HA[HA] + k^2,A[A] \]

\[ k^2,H = 1073.4 \pm 71.1 \, \text{M}^{-1}\text{sec}^{-1} \]

\[ k^2,HA = 1.71 \pm 0.30 \, \text{M}^{-1}\text{sec}^{-1} \]

\[ k^2,A = 0.009 \pm 0.002 \, \text{M}^{-1}\text{sec}^{-1} \]
TABLE 9

Comparison of Microscopic Rate Constants for Enolization of Oxaloacetate in Acetate Buffer

<table>
<thead>
<tr>
<th>catalyst</th>
<th>this work</th>
<th>Mao(98)</th>
<th>Emly(97)</th>
</tr>
</thead>
<tbody>
<tr>
<td>solvent</td>
<td>--(a)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>proton</td>
<td>1073.4</td>
<td>1450</td>
<td>900</td>
</tr>
<tr>
<td>acetic acid</td>
<td>1.71</td>
<td>0.7</td>
<td>1.2</td>
</tr>
<tr>
<td>acetate</td>
<td>0.009</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

a. The corresponding catalytic constant was not observable.
b. The unit of each microscopic rate constant is M$^{-1}$sec$^{-1}$.  

---
factor of four causes almost 100% increase in the enolization rate constant.

**The Hydration and Enolization of Oxaloacetate in 3,3-Dimethylglutarate Buffer**

In this section, kinetic studies on the hydration and enolization of oxaloacetate in 3,3-dimethylglutaric acid (DMG, \( pK_{a1} = 3.42 \), \( pK_{a2} = 6.67 \)) is presented. The kinetic data were collected by both pH increase and pH decrease techniques in 0.275 ionic strength at 25.0 °C. In pH increase experiments OX solutions with initial pH of 1.30 were rapidly mixed with alkaline DMG solutions to give solutions in the pH range of 3.5 to 7.2. In pH decrease experiments, the highly alkaline oxaloacetate solutions at pH = 12.7 were rapidly mixed with acidic buffer solutions. The biphasic UV absorbance changes were monitored at 275 nm.

The kinetic investigation of hydration and enolization of OX species were performed with 0.200 mM oxaloacetate and 5.00 mM to 90.0 mM DMG at different buffer ratios. Results of representative experiments are pictured in Figure 10. Solid line shows the theoretical absorbance vs. time curves whereas triangles, diamonds and squares stand for the
**TABLE 10**

Hydration and Enolization Rate Constants of Oxaloacetate in Acetate Buffer System

---

**A. pH increase experiments (a)**

<table>
<thead>
<tr>
<th>[OAC] mM</th>
<th>pH</th>
<th>$k^5$, sec$^{-1}$</th>
<th>$k^2$, sec$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.0</td>
<td>5.01</td>
<td>0.0342</td>
<td>0.0175</td>
</tr>
<tr>
<td>60.0</td>
<td>5.04</td>
<td>0.0372</td>
<td>0.0225</td>
</tr>
<tr>
<td>90.0</td>
<td>5.06</td>
<td>0.0401</td>
<td>0.0275</td>
</tr>
<tr>
<td>120</td>
<td>5.06</td>
<td>0.0432</td>
<td>0.0333</td>
</tr>
<tr>
<td>30.0</td>
<td>4.62</td>
<td>0.0361</td>
<td>0.0365</td>
</tr>
<tr>
<td>60.0</td>
<td>4.57</td>
<td>0.0432</td>
<td>0.0509</td>
</tr>
<tr>
<td>90.0</td>
<td>4.57</td>
<td>0.0483</td>
<td>0.0620</td>
</tr>
<tr>
<td>120</td>
<td>4.57</td>
<td>0.0543</td>
<td>0.0730</td>
</tr>
<tr>
<td>140</td>
<td>4.64</td>
<td>0.0565</td>
<td>0.0729</td>
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</table>

**B. pH decrease experiments (b)**

<table>
<thead>
<tr>
<th>[OAC] mM</th>
<th>pH</th>
<th>$k^5$, sec$^{-1}$</th>
<th>$k^2$, sec$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.0</td>
<td>5.00</td>
<td>0.0342</td>
<td>0.0179</td>
</tr>
<tr>
<td>45.0</td>
<td>4.97</td>
<td>0.0361</td>
<td>0.0223</td>
</tr>
<tr>
<td>60.0</td>
<td>5.00</td>
<td>0.0376</td>
<td>0.0243</td>
</tr>
<tr>
<td>75.0</td>
<td>5.02</td>
<td>0.0390</td>
<td>0.0265</td>
</tr>
<tr>
<td>20.0</td>
<td>5.41</td>
<td>0.0321</td>
<td>0.00669</td>
</tr>
<tr>
<td>30.0</td>
<td>5.45</td>
<td>0.0325</td>
<td>0.00713</td>
</tr>
<tr>
<td>45.0</td>
<td>5.55</td>
<td>0.0328</td>
<td>0.00793</td>
</tr>
<tr>
<td>60.0</td>
<td>5.57</td>
<td>0.0333</td>
<td>0.00793</td>
</tr>
<tr>
<td>75.0</td>
<td>5.63</td>
<td>0.0336</td>
<td>0.00810</td>
</tr>
</tbody>
</table>

---

*a. 0.200 mM oxaloacetate.*

*b. 1.00 mM oxaloacetate.*
experimental values obtained with 20.0 mM, 30.0 mM and 60.0 mM DMG, respectively. The microscopic rate constants resolved by non-linear curve fitting routine, MINABS are given in Table 11.

Quantitatively, the second order rate constant of monoacid (HDMG) involved in the hydration has about the same order of magnitude of the microscopic rate constant of solvent \( k^{5,H^A} = 0.01 \text{ M}^{-1} \text{sec}^{-1}, k^{5,H_2O} = 0.026 \text{ sec}^{-1} \). As shown in Table 11, the effect of buffer for catalysis of enolization is greater than its effect on the hydration rates. For enolization, the second order rate constant of \( H_2DMG \) diacid, \( 3.16 \text{ M}^{-1} \text{sec}^{-1} \), is about 5 fold greater than that of HDMG, \( k^{2,H^A} = 0.63 \text{ M}^{-1} \text{sec}^{-1} \). The rate constant of DMG dianion, \( k^{2,A} = 0.11 \text{ M}^{-1} \text{sec}^{-1} \) is only few hundredth of the rate constant of \( H_2DMG \) diacid. Those microscopic rate constants give satisfactory description of those absorbance vs. time curves. This is revealed in the good agreement between the absorbances obtained experimentally and those calculated by MINABS as shown in Figure 10. Table 12 gives the hydration and enolization rate constants determined in this work.
Figure 9: Absorbance vs. Time Curves of Oxaloacetate in Acetate Buffer

0.200 mM oxaloacetate

pH(initial) = 1.30

pH(final) = 5.05

wavelength = 275 nm

Δ = 30.0 mM acetate

◊ = 90.0 mM acetate

□ = 120 mM acetate
Figure 9
Figure 10: Absorbance vs. Time Curves of Oxaloacetate in 3,3-Dimethylglutarate Buffer

- 0.200 mM oxaloacetate
- pH(initial) = 1.30
- pH(final) = 3.65
- wavelength = 275 nm
- △ = 20.0 mM DMG
- ◊ = 30.0 mM DMG
- □ = 60.0 mM DMG
Figure 10

ABSORBANCE (275NM)

TIME X 10E-01 (SECONDS)

0.00 0.16 0.32 0.48 0.64 0.80 0.96 1.12 1.28 1.44 1.60

0.00 0.08 0.16 0.24 0.32 0.40 0.48 0.56 0.64 0.72 0.80

-2 -8 -20 -80 -220 -820 -2820 -8820 -28820 -88820 -288820 -888820

P < 3 □ P < 3 □ P < 3 □
The Hydration and Enolization of Oxaloacetate in Bicine Buffer

The kinetic studies of hydration-dehydration and tautomerization of oxaloacetate in tertiary amine buffer systems, namely, N,N-Bis (2-hydroxyethyl)glycine (Bicine, XXXX) and 2-(N-Morpholino)ethanesulphonic acid (MES, XXXIX) will be presented in this and subsequent sections. Detailed investigations have been performed to correlate the catalytic effect of Bicine and MES with their pKas. Attempts to verify these tertiary amine buffers as either acting like an oxygen base catalysis or undergoing the nucleophilic addition-elimination mechanism are also performed.

Identical procedures performed in the kinetic study of the hydration and enolization of oxaloacetate in the presence of either acetate or DMC buffer were also employed to study Bicine buffer (pKa = 8.35) catalysis on these reactions. The reactions were performed with 0.500 mM OX and a wide range of buffer concentrations, namely 10.0 mM to 120 mM. The final pH of the mixed solutions were measured immediately after mixing and were in the range of pH 8.1 to pH 8.9. The absorbance vs. time curves were
monitored at 285 nm. Triplicate runs have been performed for one experimental condition. For each run, 248 data points were collected.

The kinetic data were analyzed simultaneously with a non-linear least-squares refinement, MINABS programs. Since the hydroxide terms of hydration and enolization have been determined in separate experiments (86), $k^{5,\text{OH}} = 545.0 \text{ M}^{-1}\text{sec}^{-1}$ and $k^{2,\text{OH}} = 80.0 \text{ M}^{-1}\text{sec}^{-1}$, these values were used during the curve fitting process. The microscopic rate constants thus found iteratively by MINABS are summarized in Table 13. General base, i.e. Bicine, catalysis on both hydration and enolization is observed. Quantitatively, $k^{5,A} = 0.34 \pm 0.12 \text{ M}^{-1}\text{sec}^{-1}$, $k^{2,A} = 0.102 \pm 0.007 \text{ M}^{-1}\text{sec}^{-1}$ for hydration and enolization, respectively.

Furthermore, the satisfactory description of biphasic absorption-time curves by this model is shown in Figure 11. In these curves, the solid lines represent the theoretical values whereas triangles, diamonds, and squares, represent the observed absorption data obtained with 60.0 mM, 120 mM and 150 mM Bicine, respectively. The dramatic absorbance change, from -4 to 8, was due to the rapid enolization of oxaloacetate when the pH value of the reaction solution rapidly increased from pH 1.30 to ca. pH 9. Because the
### TABLE 11

Microscopic Rate Constants of Oxaloacetate in 3,3-Dimethylglutarate Buffer

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Microscopic Rate Constant</th>
<th>T = 25.0 ± 0.2 °C</th>
<th>u = 0.275M(NaCl)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Hydration</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k^5$</td>
<td>$k^5 = k^5, H[H] + k^5, HA[HA] + k^5, H_2O$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k^5, H = 541.5 \text{ M}^{-1}\text{sec}^{-1}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k^5, HA = 0.010 \pm 0.002 \text{ M}^{-1}\text{sec}^{-1}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k^5, H_2O = 0.026 \text{ M}^{-1}\text{sec}^{-1}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k^5, H_2A = 0.50 \pm 0.12 \text{ M}^{-1}\text{sec}^{-1}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>B. Enolization</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k^2$</td>
<td>$k^2 = k^2, H[H] + k^2, HA[HA] + k^2, A[A]$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k^2, H = 1073.4 \text{ M}^{-1}\text{sec}^{-1}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k^2, HA = 0.63 \pm 0.17 \text{ M}^{-1}\text{sec}^{-1}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k^2, A = 0.11 \pm 0.03 \text{ M}^{-1}\text{sec}^{-1}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k^2, H_2A = 3.16 \pm 0.90 \text{ M}^{-1}\text{sec}^{-1}$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
# TABLE 12

The Hydration and Enolization Rate Constants of Oxaloacetate in 3,3-Dimethylglutarate Buffer System

<table>
<thead>
<tr>
<th>[DMG] mM</th>
<th>pH</th>
<th>k₁, sec⁻¹</th>
<th>k₂, sec⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.0</td>
<td>4.27</td>
<td>0.0460</td>
<td>0.0622</td>
</tr>
<tr>
<td>30.0</td>
<td>3.68</td>
<td>0.0766</td>
<td>0.143</td>
</tr>
<tr>
<td>60.0</td>
<td>3.61</td>
<td>0.0837</td>
<td>0.177</td>
</tr>
<tr>
<td>90.0</td>
<td>3.58</td>
<td>0.0884</td>
<td>0.204</td>
</tr>
<tr>
<td>5.00</td>
<td>5.91</td>
<td>0.0266</td>
<td>0.00410</td>
</tr>
<tr>
<td>10.0</td>
<td>6.06</td>
<td>0.0265</td>
<td>0.00624</td>
</tr>
<tr>
<td>20.0</td>
<td>6.05</td>
<td>0.0266</td>
<td>0.0116</td>
</tr>
<tr>
<td>30.0</td>
<td>6.04</td>
<td>0.0267</td>
<td>0.0170</td>
</tr>
<tr>
<td>60.0</td>
<td>5.10</td>
<td>0.0304</td>
<td>0.0470</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>[DMG] mM</th>
<th>pH</th>
<th>k₁, sec⁻¹</th>
<th>k₂, sec⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.0</td>
<td>7.12</td>
<td>0.0261</td>
<td>0.00501</td>
</tr>
<tr>
<td>15.0</td>
<td>6.84</td>
<td>0.0261</td>
<td>0.00496</td>
</tr>
<tr>
<td>10.0</td>
<td>6.83</td>
<td>0.0261</td>
<td>0.00339</td>
</tr>
<tr>
<td>15.0</td>
<td>6.84</td>
<td>0.0261</td>
<td>0.00496</td>
</tr>
<tr>
<td>1.00</td>
<td>5.70</td>
<td>0.0269</td>
<td>0.00271</td>
</tr>
<tr>
<td>20.0</td>
<td>6.34</td>
<td>0.0263</td>
<td>0.00980</td>
</tr>
<tr>
<td>30.0</td>
<td>6.76</td>
<td>0.0262</td>
<td>0.0105</td>
</tr>
<tr>
<td>60.0</td>
<td>6.65</td>
<td>0.0264</td>
<td>0.0228</td>
</tr>
</tbody>
</table>

a. 0.200 mM oxaloacetate.
b. 0.400 mM oxaloacetate.
reaction rates are highly susceptible to the buffer concentration the microscopic rate constants can be easily resolved from the absorbance vs. time data.

The pseudo first order rate constants are summarized in Table 14. In addition, the linear buffer dependency of these pseudo first order rate constants is shown in Figure 12. Under these experimental conditions, the hydration rates are about one order of magnitude faster than enolization rates.

The Hydration and Enolization of Oxaloacetate in MES Buffer

PH increase and pH decrease methods described previously were employed to study the buffer catalysis of hydration and enolization of oxaloacetate in 2-(N-Morpholino)ethanesulphonic acid, MES, buffer solution. Kinetic data were collected with 0.200 mM and 0.400 mM oxaloacetate over a wide range of buffer concentration, namely from 22.9 mM to 150 mM.
Figure 11: Absorbance vs. Time Curves of Oxaloacetate in Bicine Buffer

0.500 mM oxaloacetate
pH(initial) = 1.30
pH(final) = 8.23
wavelength = 285 nm

△ --- 60.0 mM Bicine
◊ --- 120 mM Bicine
□ --- 150 mM Bicine
Figure 11

TIME X 10E-01 (SECONDS)

ABSORBANCE (285NM)
Figure 12: Hydration and Enolization Rate Constants as Functions of Bicine Concentration

0.500 oxaloacetate

\[ \text{pH(initial)} = 1.30 \]

Enolization rate constants:

- O --- pH(final) = 8.80
- O --- pH(final) = 8.20

Hydration rate constants:

- \( \triangle \) --- pH(final) = 8.80
- \( \Box \) --- pH(final) = 8.20
Figure 12

Hydration

Enolization

$k \times 10^2 \text{ sec}^{-1}$ vs. Bicine mM
Figure 13: Absorbance vs. Time Curves of Oxaloacetate in MES Buffer

0.200 mM oxaloacetate

pH(initial) = 1.30

pH(final) = 5.65

wavelength = 285 nm

\( \triangle = 25.0 \text{ mM MES} \)

\( \Diamond = 90.0 \text{ mM MES} \)

\( \bigcirc = 150 \text{ mM MES} \)
Figure 13
**TABLE 13**

**Microscopic Rate Constants of Oxaloacetate in Bicine Buffer**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>microscopic rate constant(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Hydration</strong></td>
<td></td>
</tr>
<tr>
<td>$k^5$</td>
<td>$k^5, H_2^0 = 0.026 M^{-1} sec^{-1}$</td>
</tr>
<tr>
<td>$k^5, A = 0.34 \pm 0.12 M^{-1} sec^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$k^5, OH = 545.0 M^{-1} sec^{-1}$ (b)</td>
<td></td>
</tr>
<tr>
<td>$k^5 = k^5, H_2^0 + k^5, A + k^5, OH$</td>
<td></td>
</tr>
<tr>
<td>$k^5 = k^5, H + k^5, A + k^5, OH$</td>
<td></td>
</tr>
<tr>
<td>$k^5, H = 541.5 M^{-1} sec^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$k^5, A = 0.34 \pm 0.12 M^{-1} sec^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$k^5, OH = 545.0 M^{-1} sec^{-1}$ (b)</td>
<td></td>
</tr>
</tbody>
</table>

| **B. Enolization** | |
| $k^2$ | $k^2, A = 0.102 \pm 0.007 M^{-1} sec^{-1}$ |
| $k^2 = k^2, H + k^2, A + k^2, OH$ |
| $k^2, H = 1073.4 M^{-1} sec^{-1}$ |
| $k^2, OH = 80.0 M^{-1} sec^{-1}$ (b) |
| $k^2, A = 0.102 \pm 0.007 M^{-1} sec^{-1}$ |

---

*a. A represents (HOCH₂CH₂)₂NCH₂COO⁻.*

*b. reference (86).*
Since \( pK_a = 6.15 \) at \( 20 ^\circ C \) has been reported by Good et al. (115), the buffer solutions were carefully adjusted with standard \( \text{HCl} \) or standard \( \text{NaOH} \) to give a final pH range of 5.4 to 6.2. The biphasic stopped-flow traces were monitored at 285 nm.

The buffer dependence of absorbance vs. time curves is shown in figure 13. Solid lines give the theoretical values while triangles, diamonds and squares give the experimental values obtained with 25.0 mM, 90.0 mM, 150 mM buffer. The biphasic absorption curves are characterized by a small absorption decreases followed by a large absorption increases. The slope of the absorption increase becomes steeper as the buffer concentration increases. The excellent agreement between the theoretical absorbances and experimental values demonstrates the reliability of the microscopic rate constants thus determined.

The pseudo first order hydration rate constants and enolization rate constants obtained in these experiments are summarized in Table 15. The linear buffer dependence of pseudo first order rate constants observed in Bicine buffer system is also observed in the presence of MES (Figure 14). As described in Table 16, general acid, i.e. \( \text{HMES} \), catalysis is observed in the enolization reaction with a rate constant of \( k^{2,HA} = 0.618 \pm 0.034 \, \text{M}^{-1}\text{sec}^{-1} \).
### Table 14

Hydration and Enolization Rate Constants of Oxaloacetate in Bicine Buffer

<table>
<thead>
<tr>
<th>[BIC] mM</th>
<th>pH</th>
<th>( k^5 ), sec(^{-1} )</th>
<th>( k^2 ), sec(^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.0</td>
<td>8.10</td>
<td>0.0272</td>
<td>0.0005</td>
</tr>
<tr>
<td>30.0</td>
<td>8.19</td>
<td>0.0283</td>
<td>0.00142</td>
</tr>
<tr>
<td>60.0</td>
<td>8.21</td>
<td>0.0297</td>
<td>0.00274</td>
</tr>
<tr>
<td>90.0</td>
<td>8.23</td>
<td>0.0311</td>
<td>0.00414</td>
</tr>
<tr>
<td>120</td>
<td>8.24</td>
<td>0.0325</td>
<td>0.00553</td>
</tr>
<tr>
<td>150</td>
<td>8.24</td>
<td>0.0338</td>
<td>0.00687</td>
</tr>
<tr>
<td>10.0</td>
<td>8.61</td>
<td>0.0295</td>
<td>0.00108</td>
</tr>
<tr>
<td>30.0</td>
<td>8.74</td>
<td>0.0320</td>
<td>0.00274</td>
</tr>
<tr>
<td>60.0</td>
<td>8.81</td>
<td>0.0350</td>
<td>0.00520</td>
</tr>
<tr>
<td>90.0</td>
<td>8.78</td>
<td>0.0368</td>
<td>0.00731</td>
</tr>
<tr>
<td>120</td>
<td>8.81</td>
<td>0.0394</td>
<td>0.00975</td>
</tr>
</tbody>
</table>

---

a. \( k^5 \) = the hydration rate constant.
b. \( k^2 \) = the enolization rate constant.
contrast, the hydration reaction is not promoted by either HMES or MES catalysts.

Discussion

Interconversion between hydrate, keto and enol oxaloacetate have been studied in acetate, DMG, Bicine and MES buffers. These buffers are chosen due to their small complexing tendency toward divalent metal ions. Hydration and enolization are promoted via different catalytic pathways. According to this work, the hydration rates are highly susceptible to hydroxide and solvent catalysis whereas enolization rates are not dependent on solvent catalysis term. This is consistent with the Leussing and Emly work (86) and the Bruce work (87) on dehydration and enolization of oxaloacetate in the presence of tertiary amines. In addition, proton-catalyzed pathways contribute to both hydration and enolization rates.

The hydration reaction of oxaloacetate involves the addition of a relatively heavy group, namely hydroxide ion, to the carbonyl carbon and the addition of a proton to the oxygen as shown in equation 55.
TABLE 15

Microscopic Rate Constants of Oxaloacetate in MES Buffer

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Rate Constants</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Hydration</strong></td>
<td></td>
</tr>
<tr>
<td>$OX(keto) \rightarrow OX(hyd)$</td>
<td>$k^5 = k^5_H[H]+k^5_OH[OH]+k^5_H_2O$</td>
</tr>
<tr>
<td>$k^5_H = 541.5 \text{ M}^{-1}\text{sec}^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$k^5_OH = 545.0 \text{ M}^{-1}\text{sec}^{-1}$</td>
<td></td>
</tr>
<tr>
<td><strong>B. Enolization</strong></td>
<td></td>
</tr>
<tr>
<td>$OX(keto) \rightarrow OX(enol)$</td>
<td>$k^2 = k^2_H[H]+k^2_A[A]+k^2_OH[OH]$</td>
</tr>
<tr>
<td>+ $k^2_HA[HA]$</td>
<td></td>
</tr>
<tr>
<td>$k^2_H = 1073.4 \text{ M}^{-1}\text{sec}^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$k^2_OH = 80.0 \text{ M}^{-1}\text{sec}^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$k^2_A = 0.050 \pm 0.017 \text{ M}^{-1}\text{sec}^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$k^2_HA = 0.618 \pm 0.034 \text{ M}^{-1}\text{sec}^{-1}$</td>
<td></td>
</tr>
</tbody>
</table>

a. A represents $\text{NCH}_2\text{CH}_2\text{SO}_3^-$. 
TABLE 16

The Hydration and Enolization Rate Constants of Oxaloacetate in MES Buffer

<table>
<thead>
<tr>
<th>[MES] mM</th>
<th>pH</th>
<th>$k_5, \text{sec}^{-1}$</th>
<th>$k_2, \text{sec}^{-1}$ (a,b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22.9</td>
<td>6.16</td>
<td>0.0299</td>
<td>0.00771</td>
</tr>
<tr>
<td>41.2</td>
<td>6.16</td>
<td>0.0299</td>
<td>0.0133</td>
</tr>
<tr>
<td>54.9</td>
<td>6.19</td>
<td>0.0299</td>
<td>0.0168</td>
</tr>
<tr>
<td>82.4</td>
<td>6.17</td>
<td>0.0299</td>
<td>0.0255</td>
</tr>
<tr>
<td>110</td>
<td>6.16</td>
<td>0.0299</td>
<td>0.0342</td>
</tr>
<tr>
<td>137</td>
<td>6.17</td>
<td>0.0299</td>
<td>0.0419</td>
</tr>
<tr>
<td>25.0</td>
<td>5.61</td>
<td>0.0306</td>
<td>0.0145</td>
</tr>
<tr>
<td>45.0</td>
<td>5.59</td>
<td>0.0307</td>
<td>0.0243</td>
</tr>
<tr>
<td>60.0</td>
<td>5.59</td>
<td>0.0307</td>
<td>0.0315</td>
</tr>
<tr>
<td>90.0</td>
<td>5.62</td>
<td>0.0306</td>
<td>0.0450</td>
</tr>
<tr>
<td>120</td>
<td>5.67</td>
<td>0.0305</td>
<td>0.0574</td>
</tr>
<tr>
<td>150</td>
<td>5.68</td>
<td>0.0305</td>
<td>0.0708</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>[MES] mM</th>
<th>pH</th>
<th>$k_5, \text{sec}^{-1}$</th>
<th>$k_2, \text{sec}^{-1}$ (c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22.9</td>
<td>5.94</td>
<td>0.0301</td>
<td>0.00993</td>
</tr>
<tr>
<td>41.2</td>
<td>6.07</td>
<td>0.0300</td>
<td>0.0148</td>
</tr>
<tr>
<td>82.4</td>
<td>6.13</td>
<td>0.0299</td>
<td>0.0267</td>
</tr>
<tr>
<td>110</td>
<td>6.14</td>
<td>0.0299</td>
<td>0.0350</td>
</tr>
<tr>
<td>137</td>
<td>6.15</td>
<td>0.0299</td>
<td>0.0429</td>
</tr>
<tr>
<td>25.0</td>
<td>5.49</td>
<td>0.0309</td>
<td>0.0159</td>
</tr>
<tr>
<td>45.0</td>
<td>5.51</td>
<td>0.0308</td>
<td>0.0256</td>
</tr>
<tr>
<td>60.0</td>
<td>5.53</td>
<td>0.0308</td>
<td>0.0326</td>
</tr>
<tr>
<td>90.0</td>
<td>5.56</td>
<td>0.0307</td>
<td>0.0466</td>
</tr>
<tr>
<td>120</td>
<td>5.57</td>
<td>0.0307</td>
<td>0.0609</td>
</tr>
<tr>
<td>150</td>
<td>5.58</td>
<td>0.0307</td>
<td>0.0750</td>
</tr>
</tbody>
</table>

a. $k_5$ and $k_2$ are hydration and enolization rate constants, respectively.
b. pH increase experiments.
c. pH decrease experiments.
Figure 14: Hydration and Enolization Rate Constants as Functions of Total MES Concentration

0.200 oxaloacetate

\[ \text{pH}(\text{initial}) = 1.30 \]

Enolization rate constants:

- \( \square \) --- \( \text{pH}(\text{final}) = 5.65 \)
- \( \triangle \) --- \( \text{pH}(\text{final}) = 6.16 \)

Hydration rate constants:

- \( \bigcirc \) --- \( \text{pH}(\text{final}) = 5.65 \)
- \( \bigcirc \) --- \( \text{pH}(\text{final}) = 6.16 \)
Figure 14

$\text{k} \times 10^2 \text{ sec}^{-1}$

$\text{MES mM}$

$\text{pH 5.65}$

$\text{pH 6.16}$

$\text{Enolization}$

$\text{pH 6.16}$
In contrast, enolization only involves the transfer of a proton as shown in equation 56. An OH\(^-\) ion could directly attack the carbonyl carbon in hydration reaction. Consequently, the microscopic rate constant of hydroxide ion involved in the hydration is almost sixfold greater than which is involved in the enolization as shown in Table 17.

The reversible hydration-dehydration of several \(\alpha\)-keto carboxylic acids have been studied and some of the literature values are presented in Table 18. According to Bruice's result (87), the overall microscopic rate constants of the solvent and hydroxide pathways are 0.567 sec\(^{-1}\) and 6190 M\(^{-1}\)sec\(^{-1}\), respectively. By calculating with
TABLE 17
Microscopic Rate Constants of Catalysts Involved in Hydration and Enolization

\[ T = 25.0 \, ^\circ \text{C} \quad u = 0.275 \, \text{M} (\text{NaCl}) \]

A. Hydration

<table>
<thead>
<tr>
<th>buffer, A</th>
<th>pKₐ</th>
<th>H</th>
<th>H₂O</th>
<th>H₂A</th>
<th>HA</th>
<th>A</th>
<th>OH</th>
</tr>
</thead>
<tbody>
<tr>
<td>acetate</td>
<td>4.53</td>
<td>541.5</td>
<td>0.026</td>
<td>--</td>
<td>0.146</td>
<td>--</td>
<td>545.0</td>
</tr>
<tr>
<td>DNG</td>
<td>3.42</td>
<td>541.5</td>
<td>0.026</td>
<td>0.50</td>
<td>0.01</td>
<td>--</td>
<td>545.0</td>
</tr>
<tr>
<td>MES</td>
<td>6.15</td>
<td>541.5</td>
<td>0.026</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>545.0</td>
</tr>
<tr>
<td>Bicine</td>
<td>8.35</td>
<td>541.5</td>
<td>0.026</td>
<td>--</td>
<td>--</td>
<td>0.34</td>
<td>545.0</td>
</tr>
</tbody>
</table>

B. Enolization

<table>
<thead>
<tr>
<th>buffer, A</th>
<th>pKₐ</th>
<th>H</th>
<th>H₂O</th>
<th>H₂A</th>
<th>HA</th>
<th>A</th>
<th>OH</th>
</tr>
</thead>
<tbody>
<tr>
<td>acetate</td>
<td>4.53</td>
<td>1073.4</td>
<td>--</td>
<td>--</td>
<td>1.71</td>
<td>0.009</td>
<td>80.0</td>
</tr>
<tr>
<td>DNG</td>
<td>3.42</td>
<td>1073.4</td>
<td>--</td>
<td>3.16</td>
<td>0.63</td>
<td>0.11</td>
<td>80.0</td>
</tr>
<tr>
<td>MES</td>
<td>6.15</td>
<td>1073.4</td>
<td>--</td>
<td>--</td>
<td>0.618</td>
<td>0.050</td>
<td>80.0</td>
</tr>
<tr>
<td>Bicine</td>
<td>8.35</td>
<td>1073.4</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.102</td>
<td>80.0</td>
</tr>
</tbody>
</table>
TABLE 18
Microscopic Rate Constants and Equilibrium Constants Involved in Hydration-Dehydration of Some \(\alpha\)-keto Carboxylic Acids

<table>
<thead>
<tr>
<th>Acid</th>
<th>Equilibrium Constant</th>
<th>Rate Constants</th>
</tr>
</thead>
<tbody>
<tr>
<td>oxaloacetate (87, 30 °C)</td>
<td>(\text{solvent term: } k^h + k^d = 0.567 \text{ sec}^{-1})</td>
<td>(k^h = 1.70 \times 10^5 \text{ M}^{-1} \text{ sec}^{-1})</td>
</tr>
<tr>
<td></td>
<td>(\text{OH term: } k^h + k^d = 6190 \text{ M}^{-1} \text{ sec}^{-1})</td>
<td></td>
</tr>
<tr>
<td>diethyloxyaloacetate (54, 30 °C)</td>
<td>(\text{proton term: } k^h + k^d = 1.70 \times 10^6 \text{ M}^{-1} \text{ sec}^{-1})</td>
<td>(k^h = 0.17 \text{ M}^{-1} \text{ sec}^{-1})</td>
</tr>
<tr>
<td>(\alpha)-ketoglutaric acid (53, 0 °C)</td>
<td>(K = k^h / k^d = 1.35)</td>
<td>(k^h = 0.133 \text{ sec}^{-1}, k^d = 0.099 \text{ sec}^{-1})</td>
</tr>
<tr>
<td>pyruvic acid (47, 48, 0 °C)</td>
<td>(K = k^h / k^d = 5.24)</td>
<td>(k^h = 0.033 \text{ sec}^{-1}, k^d = 0.0063 \text{ sec}^{-1})</td>
</tr>
</tbody>
</table>
TABLE 18
(Continue)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Equilibrium Constant</th>
<th>Reaction Rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate (47, 48, 0 °C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH₃COCOOC⁻ ⇌ CH₃C(OH)₂COO⁻</td>
<td>K = kₜ / kₐ = 0.17</td>
<td></td>
</tr>
<tr>
<td>Solvent term: kₜ = 0.00016 sec⁻¹, kₐ = 0.00092 sec⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proton term: kₜ = 38.5 M⁻¹sec⁻¹, kₐ = 700 M⁻¹sec⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylpyruvate (49, 0 °C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH₃COCOCH₃ ⇌ CH₃C(OH)₂COOCH₃</td>
<td>K = kₜ / kₐ = 6.46</td>
<td></td>
</tr>
<tr>
<td>Solvent term: kₜ = 0.033 sec⁻¹, kₐ = 0.0051 sec⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proton term: kₜ = 0.700 M⁻¹sec⁻¹, kₐ = 0.108 M⁻¹sec⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethylpyruvate (49, 0 °C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH₃COCOCH₂H₅ ⇌ CH₃C(OH)₂COCH₂H₅</td>
<td>K = kₜ / kₐ = 5.11</td>
<td></td>
</tr>
<tr>
<td>Solvent term: kₜ = 0.026 sec⁻¹, kₐ = 0.0051 sec⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proton term: kₜ = 0.680 M⁻¹sec⁻¹, kₐ = 0.133 sec⁻¹</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
K = [OX(hyd)] / [OX(keto)] = 0.084 (Table 5), the microscopic rate constants of hydration reaction, $k^h,3 = 0.044 \text{ sec}^{-1}$ and $k^h,2 = 480 \text{ M}^{-1}\text{sec}^{-1}$ which are practically identical with this work are obtained. An interesting correlation between the microscopic rate constants of oxaloacetate, $\overset{\text{O}}{\overset{\text{O}}{\text{O}}}\text{OCCH}_2\text{COO}^-$, and diethyloxaloacetate, $\overset{\text{C}_2\text{H}_5\text{OOCCH}_2\text{COOC}_2\text{H}_5}$, is observed. Although substituting an electron-donating group, $-\text{COO}^-$ ($\sigma_1 = -0.17$) and a weakly electron-withdrawing group, $-\text{CH}_2\text{COO}^-$ ($\sigma_1 = 0.01$) with electron-withdrawing groups, $-\text{COOC}_2\text{H}_5$ ($\sigma_1 = 0.34$) and $-\text{CH}_2\text{COOC}_2\text{H}_5$ ($\sigma_1 = 0.13$), highly increases the tendency of diethyloxaloacetate to hydrate (54). However, this enhancement in the hydration rate does not increase the microscopic rate constant of either solvent or hydroxide pathway. In contrast, the solvent and hydroxide catalyzed terms of oxaloacetate are few order of magnitude greater than diethyloxaloacetate. Significant contribution of solvent and proton catalyzed pathways to the reversible hydration-dehydration of pyruvate and its derivatives are also obtained.

Regarding to the buffer catalysis on the heavy group addition, e.g. the hydration reaction, general acid catalysis is observed in both acetic acid and DMG buffers.
as summarized in Table 17 whereas general base catalysis is only observed in Bicine buffers. In Bruice's attempts to correlate the structure with the reactivity (87), a Bronsted constant, $\beta = 0.67$ was reported for the dehydration of oxaloacetate in a variety of tertiary amines. However, the correlation between the logarithms of reaction constants and $pK_a$s of the two tertiary amines employed in this work, namely MES and Bicine, are not defined by $\beta = 0.67$.

General acids, $H_2DMG$ and $HDMG^-$, and specific acid, $H^+$, are the predominant catalytic species with regard to enolization reaction (Table 17). $H_2DMG$ has the largest microscopic rate constant, $k_{H_2A}^2 = 3.16 \text{ M}^{-1}\text{sec}^{-1}$. Qualitatively, the effect of those acid buffer components follows this order: $H_2DMG$ diacid $> \text{acetic acid} > H\text{MES} > HDMG$ which is parallel to their $K_a$s. Under these experimental conditions, the general base and specific base catalysts are less effective than either general acid or specific acid catalysts. The basic Bicine component, $(\text{HOCH}_2\text{CH}_2)_2\text{NCH}_2\text{COO}^-$ gives the strongest general base catalysis. Actually, the general base catalysis follows this order: $\text{Bicine}^- > \text{DMG}^{2-} > \text{MES}^- > \text{acetate}^-$. 
As mentioned earlier, the tertiary amine promoted enolization might proceed through the formation of carbinolamine intermediate formation instead of general acid and general base catalysis. Evidence for the formation of carbinolamine intermediate was claimed by Bruice from results of a kinetic investigation of the enolization of oxaloacetate (87) in eight out of nine tertiary amines studied, namely, quinuclidine (XXXIII), triethylamine, trimethylamine, 3-quinuclidinol (XXXIV), N,N,N',N'-tetramethylethylene diamine (XXXV), 1,4-diazabicyclo[2,2,2]octane (XXXVI), 3-chloroquinuclidine (XXXVII) and monoprotonated N,N,N',N'-tetramethylethylene diamine (XXXVIII).
A low Bronsted value, $\beta = 0.24$ which was identical to that had been reported by Leussing and Emly (86) was obtained. However, this low Bronsted value may be explained by the fact that the intrinsic basicities of tertiary amines are not truly measured by their solution pKas. A possible reason proposed by Leussing and Emly is that solvational energies in the polar solvents cause the proton basicities of tertiary amines to be significantly lower than their intrinsic gas-phase basicities. If only a small amount of charge transfer occurs in the transition state then the gas-phase basicities would be a better measure of the catalytic effect. Furthermore, an electrostatic interaction could account for at least part of the difference between tertiary amines and oxyanion bases. In the transition state the positively charged protonated tertiary amine will be stabilized by electrostatic interactions with the negative charges on the substrate. There is no such electrostatic stabilization for the transition state for oxyanion catalysis (86).

According to this work, the enolization of oxaloacetate in the presence of either MES, or Bicine proceeds via general acid and/or general base catalysis. Since the formation of carbinolamine intermediate requires
the strong nucleophilic attack of nitrogen atom on $\alpha$-carbon, the nucleophilic addition-elimination reaction only occurs when less sterically bulky tertiary amines are involved. HMES gives a higher catalytic effect than MES as shown in Table 17. The negative charge on either $\text{-SO}_3^-$ group of MES or the $\text{-COO}^-$ group of Bicine highly prevents the formation of zwitterionic intermediates.

\[
\begin{align*}
\text{XXXXXIX} & \quad \text{XXXXX} \\
\begin{array}{c}
\text{O}^- \\
\text{OOC-C-CH}_2\text{COO}^- \\
\text{N-CH}_2\text{CH}_2\text{SO}_3 \\
\end{array} & \quad \begin{array}{c}
\text{O}^- \\
\text{OOC-C-CH}_2\text{COO}^- \\
\text{HOCH}_2\text{CH}_2\text{N-CH}_2\text{COO}^- \\
\text{H-C-H} \\
\text{H-C-H} \\
\text{OH} \\
\end{array}
\end{align*}
\]

(XXXXIX) and (XXXXX). Under these experimental conditions, the pseudo first order rate constants and the buffer concentrations are linearly related as shown in Figure 12 and Figure 14. Furthermore, the correlation between reactivities and structures of MES and Bicine are well defined by Bronsted equations:

- general acid catalysis
  \[
  \log(k_{\text{catalyst}}) = a \log(K_{\text{acid}}) + \text{constant}
  \]
- general base catalysis
\[ \log(k_{\text{catalyst}}) = -\beta \log(K_{\text{acid}}) + \text{constant} \]

with \(-a = 0.40\) and \(\beta = 0.34\). These Bronsted values are essentially identical to those reported by Bruice (87) for oxyanion buffers. The above results indicate that both MES and Bicine act like oxygen base buffers. The microscopic rate constants of general acid catalysis and those reported by Bruice (87) are summarized in Table 19. The Bronsted plots of enolization of oxaloacetate is given in Figure 15.
### TABLE 19

**Microscopic Rate Constants for the Enolization of Oxaloacetate at 25.0 °C, u = 0.275**

<table>
<thead>
<tr>
<th>catalyst(A)</th>
<th>pKa of HA</th>
<th>k²</th>
<th>log k²</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. HO⁻</td>
<td>15.75</td>
<td>73.5</td>
<td>1.87</td>
<td>(87)</td>
</tr>
<tr>
<td>2. PO₃⁻</td>
<td>11.35</td>
<td>8.00</td>
<td>0.90</td>
<td>(87)</td>
</tr>
<tr>
<td>3. 2-(diisopropylamino)ethanol</td>
<td>10.25</td>
<td>3.06</td>
<td>0.49</td>
<td>(87)</td>
</tr>
<tr>
<td>4. CO₂⁻</td>
<td>9.77</td>
<td>0.682</td>
<td>-0.17</td>
<td>(87)</td>
</tr>
<tr>
<td>5. HPO₂⁻</td>
<td>6.70</td>
<td>0.11</td>
<td>-0.96</td>
<td>(87)</td>
</tr>
<tr>
<td>6. Bicine</td>
<td>8.35</td>
<td>0.102</td>
<td>-0.99</td>
<td>this work</td>
</tr>
<tr>
<td>7. DMG</td>
<td>6.67</td>
<td>0.11</td>
<td>-0.96</td>
<td>this work</td>
</tr>
<tr>
<td>8. MES</td>
<td>6.15</td>
<td>0.050</td>
<td>-1.30</td>
<td>this work</td>
</tr>
<tr>
<td>9. OAC</td>
<td>4.53</td>
<td>0.009</td>
<td>-2.05</td>
<td>this work</td>
</tr>
</tbody>
</table>

### B. General Acid Catalysis

<table>
<thead>
<tr>
<th>catalyst(A)</th>
<th>pKa of HA</th>
<th>k²</th>
<th>log k²</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. HBIC</td>
<td>8.35</td>
<td>--</td>
<td>--</td>
<td>this work</td>
</tr>
<tr>
<td>2. HDMG</td>
<td>6.67</td>
<td>0.63</td>
<td>-0.20</td>
<td>this work</td>
</tr>
<tr>
<td>3. HMES</td>
<td>6.15</td>
<td>0.618</td>
<td>-0.21</td>
<td>this work</td>
</tr>
<tr>
<td>4. HOAC</td>
<td>4.53</td>
<td>1.71</td>
<td>0.23</td>
<td>this work</td>
</tr>
<tr>
<td>5. H₂DMG</td>
<td>3.42</td>
<td>3.16</td>
<td>0.5</td>
<td>this work</td>
</tr>
<tr>
<td>6. H₃O⁺</td>
<td>-1.7</td>
<td>1073.4</td>
<td>3.03</td>
<td>this work</td>
</tr>
</tbody>
</table>
Figure 15: A Bronsted Plot of Enolization of Oxaloacetate
Figure 15
Chapter V

EQUILIBRIUM INVESTIGATIONS OF SELECTED METAL COMPLEXES

In order to interpret how metal ions influence the rates it is necessary to know their equilibrium interactions. The complexing abilities of Mg(II), Mn(II) and Zn(II) with either oxaloacetate or base buffer components have been determined and the results are described in this chapter. The equilibrium studies have been performed with potentiometric titrations and spectrophotometric measurements. All of the equilibrium studies are performed in \( u = 0.275 \text{ M(NaCl)} \) aqueous solution at 25 °C. Some equilibrium constants of metal-oxaloacetate complexes reported in literature are also summarized in this chapter.

Potentiometric Studies

A. The Equilibrium Constants of the Mg(II)-Oxaloacetate Complexes

The overall formation constant of MgOX complex was determined by potentiometric titration. The titration was
performed with a 50.00 ml sample solution which contained 1.771 mM oxaloacetic acid and 91.67 mM magnesium chloride at constant temperature, T=25.0 °C. During the titration precise volumes of 0.05012 M NaOH (u=0.275 M) were added using an ABU12 autoburette. The corresponding pH value was measured by a Radiometer Model 26 pH meter. Because the oxaloacetate aqueous solutions are quite unstable due to spontaneous decarboxylation, batch titrations were performed.

The least-squares data fitting computer program, PHFIT was employed to compute the metal complex formation constant from those titration data. The logarithm of the formation constant of Mg(II)OX complex thus determined is 

\[ \log K = 1.156 \pm 0.073 \]

The formation constant of MgOX determined in this work along with those literature values are given in Table 20. Apparently, the equilibrium value reported by Tate et al. is higher than expected. Table 20 also shows the Mg(II) complexes of several dicarboxylate dianions. The formation constants of Mg(II) complexes of the dicarboxylate dianions,

- oxaloacetate \(-\text{OOCC(O)CH}_2\text{COO}^-\)
- succinate \(-\text{OOCCCH}_2\text{CH}_2\text{COO}^-\)
- malate \(-\text{OOCCCH}_2\text{CH(OH)COO}^-\)
- tartarate \(-\text{OOCC(OH)CH(OH)COO}^-\)
have approximately same order of magnitude as that of oxaloacetate.

Complexing to Mg(II) ion had been shown by Tate to increase the equilibrium concentration of enol species. In addition, the coordination compounds including MGOX, MgH$_{-1}$OX$^-$ and Mg$_2$H$_{-1}$OX$^+$ were observed in the kinetic studies of decarboxylation of oxaloacetate. A great increase in the absorbance of the OX solution with pH > 8 indicating that the mononuclear complex, MgH$_{-1}$OX$^-$ and the binuclear complex, Mg$_2$H$_{-1}$OX$^+$ are enolate complexes. The pKa 8.3 of mononuclear enolate complex was much lower than free OX(enol), pKa = 12.8 (97). Those constants as summarized in Table 21 are adopted to define the equilibrium distribution of each reactant species in the interpretation of kinetic data.

B. The Equilibrium Constants of Mn(II)-Oxaloacetate Complexes

The equilibrium constants of Mn-oxaloacetate complexes were determined by Mao and Leussing (98). In the kinetic studies which will be discussed in the following chapters, those constants (Table 22) are employed to define the equilibrium concentrations of all oxaloacetate species presented in the reaction solutions.
TABLE 20

The Formation Constants of Mg(II) Dicarboxylate Complexes

<table>
<thead>
<tr>
<th>Metal Complex</th>
<th>Log β</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg-oxaloacetate</td>
<td>1.156</td>
<td>this work, u=0.275, 25.0 °C</td>
</tr>
<tr>
<td>Mg-oxaloacetate</td>
<td>1.02</td>
<td>Mao(98), u=0.10, 25 °C</td>
</tr>
<tr>
<td>Mg-oxaloacetate</td>
<td>1.015</td>
<td>Emly(97), u=0.10, 25 °C</td>
</tr>
<tr>
<td>Mg-oxaloacetate</td>
<td>1.96</td>
<td>Tate et al. (78), u=0.1, 25 °C</td>
</tr>
<tr>
<td>Mg-succinate</td>
<td>1.2</td>
<td>Cannan and Kibrick(128), u=0.2, 25 °C</td>
</tr>
<tr>
<td>Mg-malate</td>
<td>1.55</td>
<td>ibid.</td>
</tr>
<tr>
<td>Mg-tartarate</td>
<td>1.36</td>
<td>ibid.</td>
</tr>
</tbody>
</table>
TABLE 21

Equilibrium Constants of Mg(II)-Oxaloacetate Complexes

<table>
<thead>
<tr>
<th>Reaction</th>
<th>log $\beta$ (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Mg}^{2+} + \text{OX}^2-(\text{keto}) \rightleftharpoons \text{MgOX(} \text{keto})$</td>
<td>0.96</td>
</tr>
<tr>
<td>$\text{Mg}^{2+} + \text{OX}^2-(\text{enol}) \rightleftharpoons \text{MgOX(} \text{enol})$</td>
<td>1.29</td>
</tr>
<tr>
<td>$\text{Mg}^{2+} + \text{OX}^2-(\text{enol}) \rightleftharpoons \text{MgH}_1\text{OX}^-(\text{enol}) + \text{H}^+$</td>
<td>-7.01</td>
</tr>
<tr>
<td>$2\text{Mg}^{2+} + \text{OX}^2-(\text{enol}) \rightleftharpoons \text{Mg}_2\text{H}_1\text{OX}^+(\text{enol}) + \text{H}^+$</td>
<td>-5.32</td>
</tr>
<tr>
<td>$\text{Mg}^+ + \text{OX}^2- \rightleftharpoons \text{MgH}_1\text{OX}^- + \text{H}^+$</td>
<td>-7.91</td>
</tr>
<tr>
<td>$2\text{Mg}^{+2} + \text{OX}^2- \rightleftharpoons \text{Mg}_2\text{H}_1\text{OX}^+ + \text{H}^+$</td>
<td>-6.22</td>
</tr>
</tbody>
</table>

* a. reference (97).
### TABLE 22

**Equilibrium Constants of Acetate, Oxaloacetate and Their Mn(II) Complexes**

<table>
<thead>
<tr>
<th>reaction</th>
<th>log $\beta$</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Mn}^{2+} + \text{OX}^{2-} \rightleftharpoons \text{MnOX}$</td>
<td>1.58</td>
<td>Mao(98) (a)</td>
</tr>
<tr>
<td>$2\text{Mn}^{2+} + \text{OX}^{2-} \rightleftharpoons \text{Mn}_2(\text{H}_2\text{OX})^+ + \text{H}^+$</td>
<td>-3.67</td>
<td>Mao(98) (a)</td>
</tr>
<tr>
<td>$\text{Mn}^{2+} + \text{OX}^{2-}($keto$) \rightleftharpoons \text{MnOX}($keto$)$</td>
<td>1.41</td>
<td>Mao(98) (a)</td>
</tr>
<tr>
<td>$\text{Mn}^{2+} + \text{OX}^{2-}($enol$) \rightleftharpoons \text{MnOX}($enol$)$</td>
<td>2.11</td>
<td>Mao(98) (a)</td>
</tr>
<tr>
<td>$\text{MnOX}($keto$) \rightleftharpoons \text{MnOX}($enol$)$</td>
<td>-0.130</td>
<td>Mao(98) (a)</td>
</tr>
</tbody>
</table>

*a. u=0.10 M(NaCl), 25 °C.*
C. Equilibrium Constants of Zn(II)-Oxaloacetate complexes

Zn(II) has been proved to form relatively strong complexes with oxaloacetate and promote decarboxylation and enolization (97,98). Zn(II) stabilizes the enol oxaloacetate by forming various complexes with this dicarboxylate. A value of 4.7 was reported for the equilibrium constant of \( \text{ZnOX(keto)} \rightleftharpoons \text{ZnOX(enol)} \) (97). In Table 23 the literature values of the equilibrium constants of Zn(II)-oxaloacetate complexes are summarized. These constants were employed in the following chapters to calculate the equilibrium distribution of OX species.

D. Equilibrium Constants of Acetate and Its Metal Complexes

Numerous equilibrium constants of acetate and its metal complexes had been reported in the literature. Since the equilibrium constants are susceptible to ionic strength and the temperature, equilibrium constants have been redetermined under identical experimental conditions to the kinetic studies and have been used to calculate the equilibrium distribution between acetate and metal ions. Those constants are summarized in Table 24.
TABLE 23

Equilibrium Constants of Zn(II)-Oxaloacetate Complexes

<table>
<thead>
<tr>
<th>reaction</th>
<th>$\log \beta$</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Zn}^{2+} + \text{O}_x^2^- \rightleftharpoons \text{ZnOX}$</td>
<td>2.34</td>
<td>(98)</td>
</tr>
<tr>
<td>$\text{Zn}^{2+} + \text{O}_x^2^-(\text{keto}) \rightleftharpoons \text{ZnOX(keto)}$</td>
<td>1.643</td>
<td>(98)</td>
</tr>
<tr>
<td>$\text{Zn}^{2+} + \text{O}_x^2^-\text{(enol)} \rightleftharpoons \text{ZnOX(enol)}$</td>
<td>3.161</td>
<td>(98)</td>
</tr>
<tr>
<td>$\text{ZnOX(keto)} \rightleftharpoons \text{ZnOX(enol)}$</td>
<td>0.67</td>
<td>(97)</td>
</tr>
<tr>
<td>$2\text{Zn}^{2+} + \text{O}_x^2^-(\text{enol}) \rightleftharpoons \text{Zn}<em>2\text{H}</em>{-1}\text{O}_x^+(\text{enol}) + \text{H}^+$</td>
<td>-.621</td>
<td>(97)</td>
</tr>
<tr>
<td>$2\text{Zn}^{2+} + \text{O}_x^2^-\text{(hyd)} \rightleftharpoons \text{Zn}<em>2\text{H}</em>{-1}\text{O}_x^+(\text{hyd}) + \text{H}^+$</td>
<td>-.249</td>
<td>(97)</td>
</tr>
<tr>
<td>$2\text{Zn}^{2+} + \text{O}_x^2^- \rightleftharpoons \text{Zn}<em>2\text{H}</em>{-1}\text{O}_x^+ + \text{H}^+$</td>
<td>-1.16</td>
<td>(98)</td>
</tr>
</tbody>
</table>
TABLE 24

Equilibrium Constants of Acetate and Selected Metal Complexes

<table>
<thead>
<tr>
<th>reaction</th>
<th>log $\beta$</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{H}^+ + \text{OAC}^- \rightleftharpoons \text{HOAC}$</td>
<td>4.53</td>
<td>this work(a)</td>
</tr>
<tr>
<td>$\text{Mg}^{2+} + \text{OAC}^- \rightleftharpoons \text{MgOAC}^+$</td>
<td>0.50</td>
<td>(127)</td>
</tr>
<tr>
<td>$\text{Mn}^{2+} + \text{OAC}^- \rightleftharpoons \text{MnOAC}^+$</td>
<td>1.00</td>
<td>Mao(98)(b)</td>
</tr>
<tr>
<td>$\text{Zn}^{2+} + \text{OAC}^- \rightleftharpoons \text{ZnOAC}^+$</td>
<td>1.00</td>
<td>Mao(98)(b)</td>
</tr>
<tr>
<td>$\text{Zn}^{2+} + 2\text{OAC}^- \rightleftharpoons \text{Zn(OAC)}_2$</td>
<td>0.81</td>
<td>20 °C(129)</td>
</tr>
</tbody>
</table>

---

a. 25.0 °C, $u = 0.275$ M (NaCl)
b. 25 °C, $u = 0.10$ M (KCl)
E. The Formation Constants of 3,3-Dimethylglutarate Complexes

3,3-Dimethylglutaric acid (DMG), which is one of the most popular buffers used in the biological studies with pKa1=3.70 and pKa2=6.34 (116), allows the catalytic process of hydration and enolization be studied at relative low pH. The dianion of this alkyl substituted dicarboxylic acid tends to form stable complexes with divalent metal ions due to the presence of oxygen anions of the carboxylate groups.

The formation constants of these complexes have been determined potentiometrically at 25.0 ± 0.2 °C and u=0.275 M (NaCl). To determine the formation constant of the Mg(II)-DMG complex, a 60.00 ml sample solution which contained 1.000 mM DMG and 10.00 mM magnesium chloride was titrated with a 0.2785 M NaOH solution. For the Mn(II)-DMG complex, a 50.00 ml sample solution with 1.000 mM DMG and 0.500 mM manganese(II) chloride was titrated with 0.2785 M NaOH. For Zn(II)-DMG complex, a 60.00 ml sample solution which contained 1.00 mM DMG and 1.000 mM zinc (II) chloride was also titrated with 0.2785 M NaOH. Relatively low concentrations of Mn(II) and Zn(II) ions were employed in the titrations to prevent the precipitation of the hydroxide salts of metal ions. The formation constants
thus resolved by PHFIT program from the titration data are summarized in Table 25. Among these three metal ions, Zn(II) has the strongest affinity toward the dicarboxylate groups. The formation constants of these coordinated complexes follow this order:

\[
\text{Zn(II)} > \text{Mn(II)} > \text{Mg(II)}.
\]

This is essentially identical to the order of the stabilities of the metal-OX complexes. Representative titration curves are shown in Figure 16. A summary of the potentiometric titration data is given in Appendix C.

### F. Equilibrium Constants of Selected Metal Complexes of Bicine and MES

The literature values of the equilibrium constants of Bicine and MES complexes are summarized in Table 26. These constants are employed in the subsequent chapters to define the equilibrium concentration of the corresponding species.

### Spectrophotometric Investigations

Because the influence of metal ions on buffer catalyzed hydration and enolization were studied using acetate, DMG, Bicine and MES, it is important to know...
### TABLE 25

**Equilibrium Constants of 3,3-Dimethylglutaric Acids and Its Metal Complexes**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Equilibrium Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H_2DMG \leftrightarrow HDMG^- + H^+$</td>
<td>$pK_{al} = 3.70$ (116)</td>
</tr>
<tr>
<td>$HDMG^- \leftrightarrow DMG^{2-} + H^+$</td>
<td>$pK_{a2} = 6.34$ (116)</td>
</tr>
<tr>
<td>$H_2DMG \leftrightarrow HDMG^- + H^+$</td>
<td>$pK_{al} = 3.42 \pm 0.23$</td>
</tr>
<tr>
<td>$HDMG^- \leftrightarrow DMG^{2-} + H^+$</td>
<td>$pK_{a2} = 6.67 \pm 0.22$</td>
</tr>
<tr>
<td>$Mg^{2+} + DMG^{2-} \leftrightarrow MgDMG$</td>
<td>$\log = 3.10 \pm 0.21$</td>
</tr>
<tr>
<td>$Mn^{2+} + DMG^{2-} \leftrightarrow MnDMG$</td>
<td>$\log = 3.48 \pm 0.18$</td>
</tr>
<tr>
<td>$Zn^{2+} + DMG^{2-} \leftrightarrow ZnDMG$</td>
<td>$\log = 4.05 \pm 0.53$</td>
</tr>
</tbody>
</table>
TABLE 26

Equilibrium Constants of Bicine, MES and Their Metal Complexes

<table>
<thead>
<tr>
<th>Reaction</th>
<th>$\log \beta$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{BIC}^- + H^+ \rightleftharpoons \text{HBIC}$</td>
<td>8.35</td>
<td>(a)</td>
</tr>
<tr>
<td>$\text{Mg}^{2+} + \text{BIC}^- \rightleftharpoons \text{MgBIC}^+$</td>
<td>1.5</td>
<td>(a)</td>
</tr>
<tr>
<td>$\text{Mn}^{2+} + \text{BIC}^- \rightleftharpoons \text{MnBIC}^+$</td>
<td>3.1</td>
<td>(a)</td>
</tr>
<tr>
<td>$\text{Zn}^{2+} + \text{BIC}^- \rightleftharpoons \text{ZnBIC}^+$</td>
<td>5.37</td>
<td>(b)</td>
</tr>
<tr>
<td>$\text{Zn}^{2+} + 2\text{BIC}^- \rightleftharpoons \text{Zn(BIC)}_2$</td>
<td>2.67</td>
<td>(b)</td>
</tr>
<tr>
<td>$\text{MES}^- + H^+ \rightleftharpoons \text{HMES}$</td>
<td>6.15</td>
<td>(a)</td>
</tr>
<tr>
<td>$\text{Mg}^{2+} + \text{MES}^- \rightleftharpoons \text{MgMES}^+$</td>
<td>0.8</td>
<td>(a)</td>
</tr>
<tr>
<td>$\text{Mn}^{2+} + \text{MES}^- \rightleftharpoons \text{MnMES}^+$</td>
<td>0.7</td>
<td>(a)</td>
</tr>
</tbody>
</table>

a. 20 °C, $u = 0.1$ M (115)
b. 25.0 °C, $u = 0.1$ M (KNO$_3$) (132)
whether the addition of metal ions causes any variation in the UV absorption of these buffers. The UV spectra of these buffers were taken in the presence of divalent metal ions to assure the applicability of these systems. All of the equilibrium UV spectra were taken with a UVIKON 810 spectrophotometer (double-beam, Kontron Electronics, INC.). This spectrophotometer is equipped with a constant temperature sample cell. All of the spectra were taken in 0.275 M ionic strength aqueous solution with a 1 cm path length quartz cuvette at 25.0 ± 0.2 °C.

The UV spectra of DMG were taken at two proton concentrations, namely pH 3.51 and pH 6.62. Figures 17-18 show the UV spectra of DMG solution with or without divalent metal ions. At pH 3.51, the absorption curves of DMG and its metal complexes are superimposable. That is, the addition of divalent metal ions do not cause any measurable variation in the UV absorbility of DMG. At pH 6.62, the UV absorption curve of DMG in the presence of Mn(II) is shifted ca. 6 nm to higher wavelengths whereas in the presence of Mg(II) or Zn(II) the spectra are practically superimposable.

The UV spectra of Bicine solutions in the presence of the metal ions are shown in Figure 19. Bicine solutions only give relatively small absorption at 270 nm to 340 nm.
MES solutions are almost transparent down to 245 nm (Figure 20), even though the divalent metal ions are present in the aqueous solution. Therefore, the kinetic studies of the interconversions among hydrate, keto and enol species in these buffer solutions can be performed by monitoring the UV absorbance change at 280 nm or even higher wavelengths.
Figure 16: Titration curves of 3,3-Dimethylglutarate and Its Metal Complexes at 25.0 °C, $u = 0.275 \text{ M}$

- $\square$ --- 1.000 mM DMG
- $\triangle$ --- 1.000 mM DMG + 10.00 mM Mg(II)
- $\circ$ --- 1.000 mM DMG + 0.5000 mM Mn(II)
- $\Diamond$ --- 1.000 mM DMG + 1.000 mM Zn(II)
Figure 16
Figure 17: UV Spectra of 3,3-Dimethylglutarate and Metal Complexes at pH 3.51

1. 10.0 mM DMG
2. 10.0 mM DMG + 127 mM Mg(II)
3. 10.0 mM DMG + 88.5 mM Mn(II)
4. 10.0 mM DMG + 9.30 mM Zn(II)
Figure 17
Figure 18: UV Spectra of 3,3-Dimethylglutarate and Metal Complexes at pH 6.62

1. 10.0 mM DMG
2. 10.0 mM DMG + 127 mM Mg(II)
3. 10.0 mM DMG + 85.5 mM Mn(II)
4. 10.0 mM DMG + 9.30 mM Zn(II)
Figure 19: UV Spectra of Bicine and Metal Complexes at pH 8.95

1. 10.0 mM Bicine
2. 10.0 mM Bicine + 120 mM Mg(II)
3. 10.0 mM Bicine + 85.0 mM Mn(II)
4. 10.0 mM Bicine + 9.20 mM Zn(II)
Figure 19

Absorbance vs. Wavelength (nm)

- BICINE + Mg
- BICINE
- BICINE + Mn
- BICINE + Zn
Figure 20: UV Spectra of MES and Metal Complexes at pH 6.20

1. 10.0 mM MES + 85.0 mM Mn(II)

2. 10.0 mM MES
   10.0 mM MES + 120 mM Mg(II)
   10.0 mM MES + 9.20 mM Zn(II)
Figure 20
Chapter VI

MG(II) ION PROMOTED HYDRATION AND ENOLIZATION OF OXALOACETAE DIANION

Equation 57 describes the interconversions of OX and metal-oxaloacetate complex (MOX) in dehydration-hydration and tautomerization. The protonation of OX and MOX species, indicated by perpendicular arrows, are much faster and are treated as preequilibrium steps. A completed, detailed derivation of the relaxation rate equations is given in Appendix B. The data fitting processes are essentially the same as those discussed in previous chapters. However, the FITFC subroutine was modified to include metal ion activated reaction pathways. The microscopic rate constants involved in hydration and enolization of metal ion activated oxaloacetate are defined in Table 27.

The kinetic studies of the influence of Mg(II) on hydration and enolization of OX in various buffer systems, namely acetate, DMG, Bicine and MES have been done by pH increase and/or isopH techniques. Since the decarboxylation rate is relatively slow under these conditions, the hydration and enolization of OX can be studied without the interference from decarboxylation. All
where $k^1$, $k^2$, $k^3$ are the pseudo first order enolization rate constants of HOX, OX and MOX, respectively and $k^4$, $k^5$ and $k^6$ are the pseudo first order hydration rate constants of HOX, OX and MOX, respectively.

...\((57)\)

of the experiments are performed in 0.275 M (NaCl) ionic strength aqueous solution at 25.0 ± 0.2 °C.
TABLE 27

Definition of the Microscopic Rate Constants Involved in Hydration and Enolization of Metal Oxaloacetate

<table>
<thead>
<tr>
<th>reaction</th>
<th>microscopic rate constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Hydration</td>
<td></td>
</tr>
<tr>
<td>$\text{MnOX(keto) } \rightleftharpoons \text{MnOX(hyd)}$</td>
<td>$k^6 = k^6, [H^+] + k^6, [OH^-] + k^6, [H_2O]^2$</td>
</tr>
<tr>
<td></td>
<td>$+ k^6, [H_2A]^2 + k^6, [HA]^2 + k^6, [A]^2$</td>
</tr>
<tr>
<td>B. Enolization</td>
<td></td>
</tr>
<tr>
<td>$\text{MnOX(keto) } \rightleftharpoons \text{MnOX(enol)}$</td>
<td>$k^3 = k^3, [H^+] + k^3, [OH^-] + k^3, [H_2O]^2$</td>
</tr>
<tr>
<td></td>
<td>$+ k^3, [H_2A]^2 + k^3, [HA]^2 + k^3, [A]^2$</td>
</tr>
</tbody>
</table>
Chloride salt of magnesium is used as the source of Mg(II) ions. Two reaction solutions, one containing oxaloacetic acid, the other containing Mg(II) ions are prepared separately for each experiment. Stock solutions of NaCl, buffer and magnesium chloride are prepared with 500 ml volumetric flasks. Appropriate volumes of those stock solutions are measured into 100 ml volumetric flasks. 10 ml and 25 ml burettes are used for the volume measurement. The proton concentration of these two solutions are adjusted to the desired level by either standard HCl or NaOH solution. Each oxaloacetate solution is freshly prepared before use because keto oxaloacetate decarboxylates spontaneously.

In order to determine the effect of Mg(II) on the hydration and enolization of OX, a number of experiments have been performed over a wide range of Mg(II), proton and buffer concentrations. Triplicate runs have been performed for each experiment. Each reaction was initiated by mixing the oxaloacetate solution with the metal ion solution using a Durrum-Gibson stopped-flow spectrophotometer. An adequate collection time was allowed in order to define the amplitudes and the relaxation times of the two relaxations. The absorbance vs. time curves
were digitized and 248 data points were collected for each spectrum.

**Acetate Buffer**

**IsopH Experiments**

In isopH experiments, two solutions equilibrated and buffered with acetate to the same pH were prepared. Equal amounts of the two solutions, one containing oxaloacetate, the other containing Mg(II) ions were mixed and the resultant relaxation profiles were monitored spectrophotometrically. The isopH technique had been employed by Covey and Leussing (23), Raghavan and Leussing (89,125) and Mao and Leussing (98) to study metal ion effects on the tautomerization reactions with the assumption that the redistribution between hydrate and keto species was negligible. Here, the validity of this assumption is tested.

Since both tautomerization and hydration are involved in the isopH experiments, the absorbance at time, \( t \), \( A(t) \) is defined by

\[
A(t) = A_{\text{inf}} + A_1 \cdot \exp(-k_1 t) + A_2 \cdot \exp(-k_2 t) \quad \ldots\ldots (58)
\]
where the pseudo first order rate constants $k_1$ and $k_2$ rooted in the above equation do not stand for either tautomerization rate or hydration-dehydration rate. Instead, $k_1$ and $k_2$ are functions of both of the reaction rates. To prove this point, the absorbance vs. time data collected were analysed by both RLXFT and MINABS programs. IsopH experiments were performed with 1.00 mM oxaloacetate, 25.0 mM and 50.0 mM Mg(II) and a wide range of acetate buffer concentrations, namely from 30.0 mM to 150 mM acetate.

Although the two reaction solutions were buffered at the same total acetate concentration and were equilibrated at the same pH value, after mixing the equilibrium distribution of hydrate, keto and enol species were perturbed due to the formation of metal-oxaloacetate complexes. This indicates that the equilibrium distribution of OX species is a function of proton and metal ion concentrations. The RLXFT program which employed least-squares refinement to optimize the amplitudes and relaxation times only gave a satisfactory definition of one of the two relaxations (primarily enolization) owing to the relatively small amplitude of the other relaxation (primarily hydration). With the manipulation performed by
MINABS program (Chapter III) the $A_{\text{inf}}$, amplitudes and the reciprocals of the relaxation times were obtained without difficulty.

The amplitudes and the first order rate constants obtained in various experiments are summarized in Table 28. The results obtained by RLXFT and MINABS are given in part A and B, respectively. As mentioned previously, RLXFT can only define one relaxation under these conditions whereas MINABS gives satisfactory results for both relaxations. As shown by the values of A1 and A2 the amplitude of the faster reaction is only a few percent of that of the slower reaction.

The agreement between the results obtained by RLXFT and MINABS is also displayed in Figure 21. As shown in the plots of the pseudo first order rate constant vs. concentration, in pH 5.50 and 25.0 mM Mg(II) solutions, a relatively good agreement exists between the rate constant obtained assuming one-relaxation, and the slower rate constant obtained by assuming two relaxations. Under these conditions, the assumption is reasonably valid that only tautomerization is involved in isopH experiments. However, when the isopH experiments were performed at lower pH (pH 4.20) and at a higher metal ion concentration (50.0 mM),
TABLE 28

The Amplitudes and the Reciprocals of Relaxation times, k1 and k2, Obtained in Various IsoP experiments

<table>
<thead>
<tr>
<th>[OAC] mM</th>
<th>[MgCl2] mM</th>
<th>pH</th>
<th>A1</th>
<th>k1, sec⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.0</td>
<td>25.0</td>
<td>5.45</td>
<td>1.83</td>
<td>0.0918</td>
</tr>
<tr>
<td>60.0</td>
<td>25.0</td>
<td>5.50</td>
<td>1.84</td>
<td>0.129</td>
</tr>
<tr>
<td>90.0</td>
<td>25.0</td>
<td>5.57</td>
<td>1.80</td>
<td>0.163</td>
</tr>
<tr>
<td>120</td>
<td>25.0</td>
<td>5.61</td>
<td>1.77</td>
<td>0.200</td>
</tr>
<tr>
<td>150</td>
<td>25.0</td>
<td>5.64</td>
<td>1.74</td>
<td>0.250</td>
</tr>
<tr>
<td>30.0</td>
<td>50.0</td>
<td>4.15</td>
<td>4.61</td>
<td>0.403</td>
</tr>
<tr>
<td>60.0</td>
<td>50.0</td>
<td>4.19</td>
<td>4.63</td>
<td>0.546</td>
</tr>
<tr>
<td>90.0</td>
<td>50.0</td>
<td>4.21</td>
<td>4.47</td>
<td>0.717</td>
</tr>
<tr>
<td>150</td>
<td>50.0</td>
<td>4.23</td>
<td>4.56</td>
<td>0.957</td>
</tr>
</tbody>
</table>

B. analysed by MINARS

<table>
<thead>
<tr>
<th>[OAC] mM</th>
<th>[MgCl2] mM</th>
<th>pH</th>
<th>A1</th>
<th>k1, sec⁻¹</th>
<th>A2</th>
<th>k2, sec⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.0</td>
<td>25.0</td>
<td>5.45</td>
<td>1.80</td>
<td>0.0872</td>
<td>-0.0678</td>
<td>0.557</td>
</tr>
<tr>
<td>60.0</td>
<td>25.0</td>
<td>5.50</td>
<td>2.01</td>
<td>0.143</td>
<td>-0.345</td>
<td>0.541</td>
</tr>
<tr>
<td>90.0</td>
<td>25.0</td>
<td>5.57</td>
<td>2.04</td>
<td>0.187</td>
<td>-0.378</td>
<td>0.524</td>
</tr>
<tr>
<td>120</td>
<td>25.0</td>
<td>5.61</td>
<td>2.03</td>
<td>0.230</td>
<td>-0.350</td>
<td>0.511</td>
</tr>
<tr>
<td>150</td>
<td>25.0</td>
<td>5.64</td>
<td>2.00</td>
<td>0.270</td>
<td>-0.340</td>
<td>0.500</td>
</tr>
<tr>
<td>30.0</td>
<td>50.0</td>
<td>4.15</td>
<td>5.20</td>
<td>0.448</td>
<td>-0.680</td>
<td>0.939</td>
</tr>
<tr>
<td>60.0</td>
<td>50.0</td>
<td>4.19</td>
<td>5.09</td>
<td>0.576</td>
<td>-0.506</td>
<td>0.947</td>
</tr>
<tr>
<td>90.0</td>
<td>50.0</td>
<td>4.21</td>
<td>4.20</td>
<td>0.709</td>
<td>-0.281</td>
<td>0.969</td>
</tr>
<tr>
<td>150</td>
<td>50.0</td>
<td>4.23</td>
<td>6.62</td>
<td>0.893</td>
<td>-0.210</td>
<td>1.12</td>
</tr>
</tbody>
</table>

a. A2 and k2 were not obtained.
the discrepancy between one-relaxation and two-relaxations is significant. This is clearly shown in Figure 21.

**PH Increase Experiments**

In the pH increase experiments, pH 1.30, 0.400 mM oxaloacetic acid solutions were rapidly mixed with equal volumes of acetate buffered Mg(II) ion solutions. The resultant biphasic two-relaxation decay profiles were monitored at 275 nm. The influence of the concentration of Mg(II) on the reversible hydration-dehydration and tautomerization of oxaloacetate is clearly shown in the biphasic absorbance vs. time curves (Figure 22). The absorption curves shift upward when the metal concentrations increase. This is attributed to the complex formation between metal ion and oxaloacetate. When the pH of the reaction solution increases rapidly from 1.30 to 4.12 or even higher, the concentration of hydrate species decreases. Accompanying this decrease, the concentration of keto species increases due to the increasing of monoacid and dianion. The increase in keto is also accompanied by an increase in the enol form which is the species monitored spectrophotometrically.
Figure 21: Comparison of the Pseudo First Order Rate Constants of Mg(II)-Oxaloacetate Determined by MINABS and RLXFT

all of the data were collected with 1.00 mM OX

○ -- pH 4.20, 50.0 mM Mg(II) analysed by RLXFT
△ -- pH 5.50, 25.0 mM Mg(II) analysed by RLXFT
□ -- pH 4.20, 50.0 mM Mg(II) analysed by MINABS
○ -- pH 5.50, 25.0 mM Mg(II) analysed by MINABS
Figure 21
In order to completely define the microscopic rate constants of all possible catalytic pathways, the kinetic data were collected over a wide range of reactant concentrations. The determination of the general acid/base and specific acid/base catalytic pathways of free OX in acetate buffer system has been discussed in Chapter IV. The microscopic hydration rate constants of proton, acid buffer component and solvent are $541.5 \text{ M}^{-1}\text{sec}^{-1}$, $0.146 \text{ M}^{-1}\text{sec}^{-1}$ and $0.026 \text{ sec}^{-1}$, respectively. While those involved in enolization reaction are $1073.4 \text{ M}^{-1}\text{sec}^{-1}$, $1.71 \text{ M}^{-1}\text{sec}^{-1}$ and $0.009 \text{ M}^{-1}\text{sec}^{-1}$ for proton, acidic buffer and base buffer catalyzed pathways, respectively.

In the presence of Mg(II), the overall hydration rate constant is a sum of the hydration constants of MgOX and free OX. Similarly, the overall enolization rate constant is also a sum of the enolization rate constants of MgOX and free OX. The equilibrium constants which define the equilibria of MgOX and OX except $\text{MgOX(keto)} \rightleftharpoons \text{MgOX(hyd)}$ are given in Chapter V. During the data fitting process, those equilibrium constants and the microscopic rate constants of the reaction of free OX were used.

The microscopic rate constants for hydration and enolization of MgOX and the equilibrium constant for $\text{MgOX(keto)} \rightleftharpoons \text{MgOX(hyd)}$ were optimized iteratively by
fitting the theoretical curves to experimental absorbance vs. time curves. The MgOX(hyd) / MgOX(keto) ratio, $K_{\text{hyd}} = 0.475 \pm 0.290$ and the microscopic rate constants summarized below give excellent agreement between the theoretical absorbances and the observed values.

For hydration
$$k^6,H_2O = 0.18 \pm 0.03 \text{ sec}^{-1}$$

For enolization
$$k^3,H = 118 \pm 11 \text{ M}^{-1}\text{sec}^{-1}$$
$$k^3,H_2O = 0.0272 \pm 0.0022 \text{ sec}^{-1}$$
$$k^3,HA = 1.08 \pm 0.12 \text{ M}^{-1}\text{sec}^{-1}$$
$$k^3,A = 0.784 \pm 0.351 \text{ M}^{-1}\text{sec}^{-1}$$

These constants are defined in Table 27.

The results show that the solvent, i.e., $H_2O$, and acetate catalyzed pathways are highly promoted by the presence of Mg(II) ions. Complexing with Mg(II) causes ca. 7 fold increase in the solvent catalytic pathway for hydration. In contrast, proton and acetic acid catalysis are inhibited and are not observed in hydration.

With regard to the enolization, the specific acid and general acid catalytic pathways which was observed in the tautomeration of CuOX complex (89) are also observed in the enolization of MgOX complex. For enolization, proton catalysis of MgOX is only one tenth of free OX whereas
acetic acid catalysis has approximately the same order of magnitude as free OX. Complexing with Mg(II) highly activates solvent catalysis toward the enolization. The value $k_{3, H_2 O} = 0.0272 \text{ sec}^{-1}$ is obtained for the Mg(II) complex while solvent catalysis is not observed in the absence of Mg(II). Cooperativity between acetate and Mg(II) causes a substantial increase in the reaction rate. The value of acetate catalysis rate constant in the presence of Mg(II), 0.784 M$^{-1}$sec$^{-1}$ is ca. 87 times greater than the value of acetate catalysis rate constant in the absence of Mg(II), 0.009 M$^{-1}$sec$^{-1}$.

Figure 22 shows the biphasic absorbance vs. time curves of various representative experiments in which the solid line represents the theoretical absorbances and squares, diamonds and triangles stand for the experimental values obtained with 50.0 mM, 35.0 mM and 10.0 mM Mg(II), respectively. There is excellent agreement between the experimental and theoretical values.

The equilibrium constant for MgOX(keto) $\rightleftharpoons$ MgOX(hyd) obtained here ($K_{hyd} = 0.475$) is slightly larger than that reported by Emly ($K_{hyd} = 0.16$) (97). The difference between Emly's value and that determined in this work might be attributed to the fact that in Emly's isopH experiments,
RLXFT data analysis process tends to overlook the formation of MgOX(hyd) species.

From the equilibrium constant of OX and metal complexes, the equilibrium distribution of hydrate, keto and enol species were calculated. The equilibrium distribution of keto, enol and hydrate OX species in 30.0 mM to 150 mM acetate, 10.0 mM to 50.0 mM Mg(II) and various pH values are summarized in Table 29. This table indicates that the equilibrium distribution of hydrate, keto and enol species is not only dependent on proton, acetate concentrations but also dependent on Mg(II) concentration.

3,3-Dimethylglutarate Buffer

Kinetic data were obtained by pH increase method. The freshly prepared acidic OX solution (pH 1.30) was rapidly mixed with alkaline DMG buffered Mg(II) solution. Since 3,3-dimethylglutaric acid is a diprotic acid with pKal = 3.42 and pKa2 = 6.67 as determined in Chapter IV, the pH increase experiments were performed with final pH range of 3.4 to 6.7. Consequently, the buffer catalysis of diacid, monoacid and base component on the hydration and enolization of MgOX complex can be determined. In order to completely characterize the interactions among reactants and catalysts, numerous experiments were performed with
TABLE 29

Equilibrium Distribution of Oxaloacetate in Mg(II) Promoted Acetate Buffer

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<th>[Mg(II)] mM</th>
<th>pH</th>
<th>% keto</th>
<th>% enol</th>
<th>% hydrate(a)</th>
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a. These data were collected with 0.200 mM oxaloacetic acid.
0.200 mM oxaloacetate, 10.0 mM to 150 mM buffer and 50.0 mM to 2.00 mM Mg(II). The biphasic absorbance vs. time curves which corresponding to the redistribution of OX species were monitored at 285 nm.

The Mg(II) ion concentration dependence of the biphasic absorbance vs. time curves is shown in Figure 23. The digitized absorbance vs. time data were analysed by the non-linear least-squares refinement, MINABS program. In the data fitting process, the equilibrium constant of

$\text{MgOX(keto)} \rightleftharpoons \text{MgOX(hyd)}$, $k_{\text{hyd}} = 0.475$ determined previously and the second order rate constants of general acid/base and specific acid/base determined in Chapter IV were used.

Figure 23 shows the experimental and theoretical values of several representative experiments. Solid lines stand for the theoretical values whereas squares, diamonds and triangles represent the experimental values. There is excellent agreement between the theoretical absorbances and the experimental values. According to this work the hydration of MgOX complex is only promoted by solvent pathway, $k_{6, H^2O} = 0.18 \text{ sec}^{-1}$, which is the same value as that was determined in acetate buffers. Furthermore, the general base catalysis of enolization is greatly enhanced by the presence of Mg(II) ion. As shown below:

for enolization
\[ k^3,_{H_2O} = 0.0272 \text{ sec}^{-1} \]
\[ k^3,_{A} = 3.85 \pm 2.31 \text{ M}^{-1}\text{sec}^{-1} \]

where \( k^3,_{H_2O} \) and \( k^3,_{A} \) stand for the rate constants of solvent and the base buffer, \( \text{DMG}^{2-} \) respectively. It appears that \( \text{DMG}^{2-} \) dianion is the predominant catalytic pathway for the enolization of \( \text{MgOX} \). As determined in Chapter IV, the microscopic rate constant of the \( \text{DMG}^{2-} \) dianion for free \( \text{OX} \) is 0.11 M\(^{-1}\)sec\(^{-1}\). Quantitatively, the addition of \( \text{Mg(II)} \) ion causes 35 fold enhancement in the rate constant. A summary of the equilibrium distribution of keto, enol, hydrate \( \text{OX} \) of various experimental conditions employed is given in Table 30.

**Bicine Buffer**

\( \text{pH} \) increase procedures were employed to study the hydration and enolization of oxaloacetate in \( \text{N}-\text{substituted glycine, namely } \text{N,N-Bis(2-hydroxyethyl)glycine, Bicine, solution. Kinetic investigations of the buffer catalysis on the hydration and enolization of } \text{MgOX complexes have been carried out with } 10.0 \text{ mM to } 150 \text{ mM Bicine, } 5.00 \text{ mM to } 25.0 \text{ mM Mg(II) at various } \text{pH. The relative high pK}_a \text{ of Bicine (pK}_a = 8.35), allowed hydroxide catalysis to be} \)
### TABLE 30

Equilibrium Distribution of Oxaloacetate in Mg(II) Promoted 3,3-Dimethylglutarate Buffer

<table>
<thead>
<tr>
<th>[DMG] mM</th>
<th>[Mg(II)] mM</th>
<th>pH</th>
<th>%keto</th>
<th>%enol</th>
<th>%hydrate(a)</th>
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</table>

a. These data were collected with 0.200 mM oxaloacetic acid.
**TABLE 31**

Equilibrium Distribution of Oxaloacetate in Mg(II) Promoted Bicine

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<th>[BIC]mM</th>
<th>[Mg(II)]mM</th>
<th>pH</th>
<th>%keto</th>
<th>%enol</th>
<th>%hydrate(a)</th>
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a. These data were collected with 0.200 mM oxaloacetic acid.
studied. As clearly shown in Table 31, each experiment gives different distribution of keto, enol and hydrate species.

In these experiments, 83% hydrate, 9% keto and 8% enol were initially present in pH 1.30 oxaloacetic acid solution. After mixing oxaloacetate solutions with Bicine buffered Mg(II) solutions, the interconversions of OX(keto) \rightleftarrows OX(hyd) and OX(keto) \rightleftarrows OX(enol) rapidly occurred and the biphasic absorbance decays were monitored spectrophotometrically at 285 nm. The non-linear least-squares curve fitting process was performed by MINABS program.

The rate of the hydration reaction is highly susceptible to an oxygen donar base. Hydroxide is a very strong catalyst and a value of \( k_{6,OH} = 4.1 \times 10^5 \text{ M}^{-1}\text{sec}^{-1} \) was obtained. This hydroxide catalyzed pathway for the hydration of MgOX is ca. two order of magnitude greater than that for uncomplexed OX. This constant has approximately the same order of magnitude as which reported by Leussing and Emly, \( k = 1.0 \times 10^6 \text{ M}^{-1}\text{sec}^{-1}(100) \). The activation by Mg(II) is also observed in general base catalysis. The rate constant for the Bicine, \((\text{HOCH}_2\text{CH}_2)_2\text{NCH}_2\text{COO}^-\), catalyzed hydration of MgOX, \( k_{6,A} = \)

### TABLE 32

**Microscopic Rate Constants of Mg(II) Promoted Bicine system**

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<tr>
<th>Reaction</th>
<th>Microscopic Rate Constant (a)</th>
</tr>
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<td></td>
</tr>
<tr>
<td>A. Hydration</td>
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<tr>
<td>k^5</td>
<td>OX(keto) ⇌ OX(hyd)</td>
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<tr>
<td>k^6</td>
<td>MgOX(keto) ⇌ MgOX(hyd)</td>
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<td>B. Enolization</td>
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<td>OX(keto) ⇌ OX(enol)</td>
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<td>k^3</td>
<td>MgOX(keto) ⇌ MgOX(enol)</td>
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59.8 M\(^{-1}\)sec\(^{-1}\) is ca. 170 times greater than that for uncomplexed OX, \(k^5,A = 0.34\) M\(^{-1}\)sec\(^{-1}\).

Mg(II) complex formation also has relatively strong effect on base catalyzed enolization. The \(\text{OH}^-\) catalyzed rate constant for the enolization for MgOX, \(k^3,\text{OH}^+ = 3.0\times10^4\) M\(^{-1}\)sec\(^{-1}\) is also ca. three order of magnitude greater than that for OX, \(k^2,\text{OH}^- = 80.0\) M\(^{-1}\)sec\(^{-1}\). Cooperativity between Mg(II) and Bicine catalyst causes ca. 147 times increase in this term (\(k^3,A/k^2,A = 14.70/0.102 = 147\)).

As shown in Figure 24, there is excellent agreement between the observed absorbances and the theoretical values which are represented by triangles, diamonds, squares and solid curves respectively. Again, these experiments prove that the general base and specific base catalyzed pathways are promoted by the presence of Mg(II) ion. The results are as summarized in Table 32.
Figure 22: Absorbance vs. Time Curves of 
Mg(II)-Oxaloacetate Mixtures in Acetate Buffers

0.200 mM oxaloacetate
60.0 mM acetate
pH(initial) = 1.30
pH(final) = 4.55

□ --- 50.0 mM Mg(II)
◊ --- 35.0 mM Mg(II)
△ --- 10.0 mM Mg(II)
Figure 23: Time Dependent Absorbance Curves of Mg(II)-Oxaloacetate Mixtures in 3,3-Dimethylglutarate Buffers

0.200 mM oxaloacetate

60.0 mM DMG

pH(initial) = 1.30

pH(final) = 3.60

△ --- 2.00 mM Mg(II)

◇ --- 6.00 mM Mg(II)

□ --- 10.00 mM Mg(II)
Figure 23
Figure 24: Absorbance vs. Time Curves of Mg(II)-Oxaloacetate Mixtures in Bicine Buffers

0.200 mM oxaloacetate
60.0 mM Bicine
pH(initial) = 1.30
pH(final) = 8.55

Δ --- 10.0 mM Mg(II)
◊ --- 20.0 mM Mg(II)
□ --- 35.0 mM Mg(II)
Figure 24
Similar experiments have been performed to examine the hydration and enolization of MgOX complexes in 2-(N-Morpholino)ethanesulphonic Acid, MES, buffer solution. Interconversion among keto, enol and hydrate species was investigated with 5.00 mM to 75.0 mM Mg(II) and 10.0 mM to 120 mM buffer at pH 5.5 to 6.9. PH 1.30 oxaloacetate solutions were mixed with various MES buffered Mg(II) solutions, the resultant relaxations were monitored by following the UV absorbance change of the OX species at 285 nm. When the OX solution was mixed with a MES buffered metal ion solution, a biphasic UV absorbance change was observed.

According to this work neither general acid nor general base catalysis is observed for the hydration of MgOX in MES buffer. This may be attributed to the relative low acidity and basicity of this buffer. The uncomplexed OX species undergoes general acid catalyzed enolization. However, the MgOX species only proceed via the MES anion catalyzed pathway and \( k^3, A = 2.27 \pm 0.38 \, M^{-1} \text{sec}^{-1} \) is determined iteratively.
As found previously, enolization is also promoted by proton and hydroxide ions. However, under these experimental conditions, $6.0 < \text{pH} < 7.0$, these two species are insignificant as compared to the metal ions and buffer components. Figure 25 shows the Mg(II) concentration dependence of the biphasic two-relaxation profiles. The solid lines stand for the theoretical values defined by those calculated microscopic rate constants. Triangles, diamonds and squares stand for the experimental absorbances collected in 35.0 mM, 65.0 mM and 10.0 mM Mg(II) solution, respectively. Again, an excellent agreement between the theoretical and observed values is obtained. The overall equilibrium distribution of keto, enol and hydrate OX as well as the experimental conditions employed in this work are summarized in Table 33.
**TABLE 33**

Equilibrium Distribution of Oxaloacetate in Mg(II) Promoted MES Buffer

<table>
<thead>
<tr>
<th>[MES] mM</th>
<th>[Mg(II)] mM</th>
<th>pH</th>
<th>% keto</th>
<th>% enol</th>
<th>% hydrate(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>41.2</td>
<td>35.0</td>
<td>6.84</td>
<td>73.8</td>
<td>13.4</td>
<td>12.8</td>
</tr>
<tr>
<td>54.9</td>
<td>35.0</td>
<td>6.84</td>
<td>74.0</td>
<td>13.4</td>
<td>12.6</td>
</tr>
<tr>
<td>82.4</td>
<td>35.0</td>
<td>6.88</td>
<td>74.5</td>
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<td>25.0</td>
<td>35.0</td>
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<td>73.1</td>
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</tr>
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<tr>
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<td>100</td>
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<td>67.1</td>
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<td>45.0</td>
<td>75.0</td>
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</tr>
<tr>
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<td>6.06</td>
<td>69.8</td>
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<td>16.0</td>
</tr>
<tr>
<td>45.0</td>
<td>55.0</td>
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<tr>
<td>45.0</td>
<td>45.0</td>
<td>6.08</td>
<td>72.0</td>
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<td>14.2</td>
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<td>73.4</td>
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<td>13.1</td>
<td>11.8</td>
</tr>
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<td>76.0</td>
<td>13.0</td>
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<tr>
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<td>6.14</td>
<td>76.8</td>
<td>12.8</td>
<td>10.4</td>
</tr>
<tr>
<td>41.2</td>
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<td>6.15</td>
<td>78.2</td>
<td>12.5</td>
<td>9.30</td>
</tr>
<tr>
<td>41.2</td>
<td>5.00</td>
<td>6.16</td>
<td>79.5</td>
<td>12.3</td>
<td>8.27</td>
</tr>
</tbody>
</table>

a. These data were collected with 0.200 mM oxaloacetic acid.
Figure 25: Absorbance vs. Time Curves of Mg(II)-Oxaloacetate Mixtures in MES Buffers

0.200 mM oxaloacetate
45.0 mM MES
pH(initial) = 1.30
pH(final) = 6.08

\( \triangle --- 35.0 \text{ mM Mg(II)} \)
\( \diamond --- 65.0 \text{ mM Mg(II)} \)
\( \square --- 100 \text{ mM Mg(II)} \)
Chapter VII

MN(II) ION PROMOTED HYDRATION AND ENOLIZATION OF OXALOACETATE DIANION

Extensive investigations of the influence of Mn(II) and buffers on the hydration and enolization of oxaloacetate dianion are presented in this chapter. The reaction rates are determined in acetate, DMG, Bicine and MES buffers. In this chapter, the kinetic determination of the equilibrium constant of \( \text{MnOX(keto)} \rightleftharpoons \text{MnOX(hyd)} \) is also presented.

Again, the pH increase technique was employed. All of the investigations were performed with 0.275 M ionic strength aqueous solution at 25.0 °C. The experimental procedures were identical to those described in Chapter VI. However, some of the procedures will be repeated whenever the repeating is necessary to clear up the subject. The equilibrium constants required to define the equilibria of OX, MnOX and buffers are summarized in Chapter V.
Mn(II) ion which exists as pale pink hexaquo ion, \([\text{Mn(H}_2\text{O})_6]^{2+}\) in neutral or acidic aqueous solution is the most stable oxidation state for manganese. The hexaquo ion is quite stable with regard to oxidation. However, the manganese hydroxide, Mn(OH)_2 which found in alkaline solution is highly susceptible to oxidation and gives Mn_2O_3 and MnO_2. Since the divalent metal ion effect on the interconversion of keto ⇌ hydrate and keto ⇌ enol was the major interest of this research, Mn(II) solutions were prepared at relatively low pH. The chloride salt was used as the source of Mn(II) ions.

pH 1.30 oxaloacetic acid solutions were prepared over a wide range of Mn(II) ion concentrations in order to estimate the equilibrium constant of 
\[\text{MnOX(keto)} \rightleftharpoons \text{MnOX(hyd)}\]. Reversible hydration-dehydration and tautomerization were followed with 7.00 mM to 50.0 mM Mn(II), 30.0 mM to 150 mM acetate buffer at pH 4.2 to 5.8. The resultant biphasic absorbance vs. time curves were monitored at 285 nm. The microscopic rate constants of catalysts and the equilibrium constant, \(k_{\text{hyd}}\) of 
\[\text{MnOX(keto)} \rightleftharpoons \text{MnOX(hyd)}\] were estimated by fitting the
experimental absorbance vs. time curves. This was performed by MINABS program.

Since Mg(II) has been proved to complex with hydrate oxaloacetate (97), identical complex formation between Mn(II) and hydrate oxaloacetate was expected. If MnOX(hyd) existed in aqueous solution, the overall hydration constants would be a sum of the hydration constants of OX and MOX. Detailed derivation of the rate laws is given in Appendix B. The equilibrium constant which defines the interconversion between MnOX(keto) and MnOX(hyd) and the microscopic rate constants were optimized by the non-linear curve fitting process, MINABS. According to the result of curve fitting the equilibrium constant of \( \text{MnOX}(\text{keto}) \rightleftharpoons \text{MnOX}(\text{hyd}) \), \( k_{\text{hyd}} = 0.790 \pm 0.063 \) was obtained. The theoretical absorbance vs. time curves defined by the equilibrium constant of \( \text{MnOX}(\text{keto}) \rightleftharpoons \text{MnOX}(\text{hyd}) \) and the microscopic rate constants agree very well with the experimental absorbance vs. time curves. This is demonstrated in Figure 26. In these curves, solid lines stand for the theoretical absorbance vs. time curves whereas diamonds, squares and triangles show the experimental absorbance vs. time curves obtained with 37.9 mM, 50.0 mM and 7.00 mM Mn(II), respectively.
Figure 26: Absorbance vs. Time Curves of Mn(II)-Oxaloacetate Mixtures in Acetate Buffers

- 0.100 mM oxaloacetate
- 60.0 mM acetate
- pH\(_{\text{initial}}\) = 1.30
- pH\(_{\text{final}}\) = 4.25
- \(\Delta = 7.00\) mM Mn(II)
- \(\Diamond = 37.9\) mM Mn(II)
- \(\Box = 50.0\) mM Mn(II)
Figure 26
The microscopic rate constants thus resolved are summarized in Table 34. The hydration of MnOX is predominantly promoted by the solvent catalyzed pathway. Mn(II)-oxaloacetate complex reacts 17 times faster than uncomplexed oxaloacetate.

Regarding to enolization rate, the reactions of both OX and MnOX are promoted by acetic acid and acetate catalysis. Cooperativity between catalysts and Mn(II) ions greatly improves the catalytic abilities of these catalysts. The acetic acid catalysis of MnOX, \( k^3,HA = 3.31 \text{ M}^{-1}\text{sec}^{-1} \), is twice as much as free OX, \( k^2,HA = 1.71 \text{ M}^{-1}\text{sec}^{-1} \). The general base catalysis, i.e. acetate catalysis, is enhanced with an even larger factor as shown in the following:

\[
\frac{k^3,A}{k^2,A} = 1.07 \text{ M}^{-1}\text{sec}^{-1} / 0.009 \text{ M}^{-1}\text{sec}^{-1} = 119
\]

Furthermore, the solvent term which is not observable in free OX makes significant contribution toward the enolization rate of the Mn(II) complex. The microscopic rate constant for solvent catalyst is equal to 0.0519 sec\(^{-1}\).

The promotion of general acid and base catalysis by Mn(II) toward enolization was also observed by Leussing and Mao (98). For comparison, the microscopic rate constants
### TABLE 34

Microscopic Rate Constants of Oxaloacetate in Mn(II) Promoted Acetate Buffer

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Microscopic Rate Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Hydration</strong></td>
<td></td>
</tr>
<tr>
<td>( k_5 )</td>
<td>( k_5 = k_5, [H] + k_5, [HA] + k_5, H_2O )</td>
</tr>
<tr>
<td>( k_6 )</td>
<td>( k_6 = k_6, H_2O )</td>
</tr>
<tr>
<td><strong>B. Enolization</strong></td>
<td></td>
</tr>
<tr>
<td>( k_2 )</td>
<td>( k_2 = k_2, [H] + k_2, [HA] + k_2, [A] )</td>
</tr>
<tr>
<td>( k_3 )</td>
<td>( k_3, H_2O = 0.0519 \pm 0.0032 \text{ sec}^{-1} )</td>
</tr>
</tbody>
</table>

\( T = 25.0 \pm 0.2 \degree C \)  
\( u = 0.275 \text{ M} (\text{NaCl}) \)
determined in this work and those reported by Leussing and Mao are summarized in Table 35. It seems that there is reasonable agreement between the results obtained in this work and the literature values. The microscopic rate constants for solvent catalyst reported by Mao and this work are 0.040 sec\(^{-1}\) and 0.0519 sec\(^{-1}\), respectively. Proton catalysis was not observed either in Mao's work or in this work. For buffer catalysis, the microscopic rate constants of acetic acid determined in Mao's work and this work are 3.7 M\(^{-1}\)sec\(^{-1}\) and 3.31 M\(^{-1}\)sec\(^{-1}\), respectively. Both works give ca. 1.0 M\(^{-1}\)sec\(^{-1}\) for the rate constant for acetate catalyst. In Table 36 the equilibrium distribution of hydrate, keto and enol species are summarized for all of the experiments performed.

3,3-Dimethylglutarate Buffer

Similar pH jump experiments have been performed to investigate the Mn(II) ion influence on the hydration and enolization of OX in 3,3-dimethylglutaric acid buffer. In order to determine the effects of Mn(II) ions on these reactions, kinetic data were collected with constant buffer concentrations and a wide range of Mn(II) concentrations, i.e. 1.70 mM to .200 mM, at various pH values. In the data fitting process which was performed by MINABS, the
TABLE 35

Comparison of Enolization Rate Constants of Oxaloacetate in Mn(II) Promoted Acetate Buffer

<table>
<thead>
<tr>
<th>complex</th>
<th>catalyst</th>
<th>Mao(a)</th>
<th>this work</th>
</tr>
</thead>
<tbody>
<tr>
<td>OX</td>
<td>H₂O</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>MnOX</td>
<td>H₂O</td>
<td>0.04 sec⁻¹</td>
<td>0.0519 sec⁻¹</td>
</tr>
<tr>
<td>OX</td>
<td>H</td>
<td>900 M⁻¹sec⁻¹</td>
<td>1073 M⁻¹sec⁻¹</td>
</tr>
<tr>
<td>MnOX</td>
<td>H</td>
<td>--(b)</td>
<td>--</td>
</tr>
<tr>
<td>OX</td>
<td>HOAC</td>
<td>0.7 M⁻¹sec⁻¹</td>
<td>1.71 M⁻¹sec⁻¹</td>
</tr>
<tr>
<td>MnOX</td>
<td>HOAC</td>
<td>3.7 M⁻¹sec⁻¹</td>
<td>3.31 M⁻¹sec⁻¹</td>
</tr>
<tr>
<td>OX</td>
<td>OAC</td>
<td>--</td>
<td>0.009 M⁻¹sec⁻¹</td>
</tr>
<tr>
<td>MnOX</td>
<td>OAC</td>
<td>1.1 M⁻¹sec⁻¹</td>
<td>1.07 M⁻¹sec⁻¹</td>
</tr>
</tbody>
</table>

a. reference (98).
b. The corresponding constant was not observable.
### TABLE 36

Equilibrium Distribution of Oxaloacetate in Mn(II) Promoted Acetate Buffer

<table>
<thead>
<tr>
<th>[OAC] mM</th>
<th>[Mn(II)] mM</th>
<th>pH</th>
<th>%keto</th>
<th>%enol</th>
<th>%hydrate(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.0</td>
<td>7.00</td>
<td>5.02</td>
<td>72.5</td>
<td>14.8</td>
<td>12.7</td>
</tr>
<tr>
<td>90.0</td>
<td>7.00</td>
<td>5.03</td>
<td>74.1</td>
<td>14.1</td>
<td>11.8</td>
</tr>
<tr>
<td>120</td>
<td>7.00</td>
<td>5.03</td>
<td>74.7</td>
<td>13.8</td>
<td>11.5</td>
</tr>
<tr>
<td>150</td>
<td>7.00</td>
<td>5.03</td>
<td>75.1</td>
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<td>11.3</td>
</tr>
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<td>60.0</td>
<td>7.00</td>
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<td>5.14</td>
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<tr>
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<td>50.0</td>
<td>4.20</td>
<td>56.4</td>
<td>19.8</td>
<td>23.8</td>
</tr>
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</table>

a. These data were collected with 0.200 mM oxaloacetic acid.
equilibrium constant of MnOX(hyd) determined previously was employed to define the correlation between MnOX(keto) and MnOX(hyd). Also, the microscopic rate constants of proton and solvent involved in hydration and enolization were used.

Table 37 gives the overall results. For the hydration of free OX the microscopic rate constants of H$_2$DMG diacid and HDMG mono acid are 0.50 M$^{-1}$sec$^{-1}$ and 0.010 M$^{-1}$sec$^{-1}$, respectively, these catalysts are inhibited by the presence of Mn(II) so that general acid catalysis was not observed. Hydration of MnOX is predominantly promoted by solvent catalysis, $k_6^{H_2O} = 0.436$ sec$^{-1}$. This rate constant of solvent catalyst of MnOX is 17 fold greater than that for uncomplexed OX.

Enolization of MnOX does not proceed through either proton or general acid catalysis. In contrast, the complexing with Mn(II) causes pronounced activation of general base catalysis on enolization. The general base catalyzed pathway, i.e. DMG$^{2-}$, on the enolization of MnOX, $k_3^{A}=11.75$ M$^{-1}$sec$^{-1}$ is 100 fold greater than that of free OX, $k_2^{A}=0.11$ M$^{-1}$sec$^{-1}$.

The microscopic rate constants of solvent catalyzed pathway on both hydration and enolization, $k_6^{H_2O} = 0.436$.
sec\(^{-1}\) and \(k^3, H_2O = 0.0519\) sec\(^{-1}\), determined in acetate buffer along with those microscopic rate constants determined in this section give relatively good data fitting result of the biphasic two-relaxation absorbance curves. This is shown in Figure 27. Again, the solid curves show the theoretical values whereas triangles, diamonds and squares show the experimental values. The redistribution of hydrate, enol and keto species as functions of proton, buffer and Mn(II) concentrations can be calculated by the equilibrium constants which define the equilibria of OX and MnOX. A summary of the equilibrium distribution of keto, enol and hydrate species is given in Table 38.

**Bicine Buffer**

The divalent metal ion influence on the hydration and enolization of oxaloacetate dianion has also been investigated in N,N-Bis(2-Hydroxyethyl)glycine, i.e. Bicine buffer. Since OX species is extensively hydrated in acidic solution, both keto ⇄ hydrate and keto ⇄ enol transformations can be studied by rapidly changing the pH of the reactant solution. Relative low metal concentrations which ranged from 0.200 mM to 1.20 mM were
TABLE 37

Microscopic Rate Constants of Oxaloacetate in Mn(II) Promoted 3,3-Dimethylglutarate Buffer

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Microscopic Rate Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T = 25.0 \pm 0.2 ^\circ C$</td>
<td>$u = 0.275M(NaCl)$</td>
</tr>
</tbody>
</table>

**A. Hydration**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Rate Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k^5$</td>
<td>$k^5 = k^5[H]+k^5[HA]+k^5[H_2O]$</td>
</tr>
<tr>
<td>$k^6$</td>
<td>$k^6 = k^6[H_2O]$</td>
</tr>
</tbody>
</table>

**B. Enolization**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Rate Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k^3$</td>
<td>$k^3 = k^3[H_2O] \pm k^3[A]$</td>
</tr>
<tr>
<td>$k^3[H_2O]$</td>
<td>$k^3[H_2O] = 0.0519 \text{ sec}^{-1}$</td>
</tr>
<tr>
<td>$k^3[A]$</td>
<td>$k^3[A] = 11.75 \pm 2.05 \text{ M}^{-1} \text{sec}^{-1}$</td>
</tr>
<tr>
<td>[DMG]mM</td>
<td>[Mn(II)]mM</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>60.0</td>
<td>0.200</td>
</tr>
<tr>
<td>60.0</td>
<td>0.600</td>
</tr>
<tr>
<td>60.0</td>
<td>0.800</td>
</tr>
<tr>
<td>60.0</td>
<td>1.200</td>
</tr>
<tr>
<td>60.0</td>
<td>1.700</td>
</tr>
<tr>
<td>60.0</td>
<td>0.400</td>
</tr>
<tr>
<td>60.0</td>
<td>0.600</td>
</tr>
<tr>
<td>60.0</td>
<td>0.200</td>
</tr>
<tr>
<td>60.0</td>
<td>1.200</td>
</tr>
<tr>
<td>60.0</td>
<td>1.700</td>
</tr>
</tbody>
</table>

*a. These data were collected with 0.200 mM oxaloacetic acid.*
employed owing to the high catalytic ability of metal ions in these alkaline buffer solutions (pKa of Bicine = 8.35, at 25 °C).

For each experiment, the acidic Mn(II) ion and OX solution which was freshly prepared was rapidly mixed with equal amount of alkaline buffer solution. The biphasic absorbance changes monitored at 285 nm for all of the experiments were analysed by MINABS program. The Mn(II) ion dependence of the absorbance vs. time curves is clearly shown in Figure 28.

Owing to cooperativity of Mn(II), rate constants of the hydroxide and solvent catalyzed pathways for both hydration and enolization are tremendously enhanced by Mn(II) (Table 39). In the absence of Mn(II) hydration of the OX species is promoted by proton, hydroxide ion and solvent catalytic pathways. However, only the base and solvent terms are observable in the hydration of MnOX. Quantitatively, the microscopic rate constants of hydroxide, Bicine and solvent pathways of MnOX are 8.8X10^5 M^-1 sec^-1, 70.9 M^-1 sec^-1 and 0.436 sec^-1, respectively. The microscopic rate constant for hydration of MnOX by hydroxide is 1600 fold greater than that for uncomplexed OX. The cooperation of general base catalyst, i.e. Bicine,
with Mn(II) results in 208 fold increment in the hydration rate constant.

Under these circumstances, substantial promotion of base catalytic pathways is observed in the enolization of MnOX. The hydroxide component of MnOX is $9.0 \times 10^4 \text{ M}^{-1} \text{sec}^{-1}$ which is ca. 1000 fold greater than uncomplexed OX. The base component, $k^3, A = 16.20 \text{ M}^{-1} \text{sec}^{-1}$ of MnOX is 162 fold enhanced. Table 39 gives a summary of those microscopic reaction rate constants.

Figure 28 gives the time dependent absorbance curves of some representative experiments. The excellent agreement between experimental absorbances and those defined by the microscopic rate constants given in Table 39 is clearly shown in these curves.

**MES Buffer**

Similar pH jump experiments as described previously were performed with one of N-substituted taurine buffers, namely, 2- (N-morpholino)ethanesulphonic acid. The relatively high pKa (pKa = 6.15 at 20°C and $u=0.1$) of this weak acid permits the Mn(II) ions influence on the transformation among keto, enol and hydrate species to be studied in approximately neutral solutions. The two-
TABLE 39

Microscopic Rate Constants of Oxaloacetate in Mn(II) Promoted Bicine Buffer

<table>
<thead>
<tr>
<th>reaction</th>
<th>microscopic rate constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T = 25.0 \pm 0.2^\circ C$</td>
<td>$u = 0.275 M (NaCl)$</td>
</tr>
<tr>
<td>A. Hydration</td>
<td></td>
</tr>
<tr>
<td>$k^5$</td>
<td></td>
</tr>
<tr>
<td>$\text{OX(keto)} \rightleftharpoons \text{OX(hyd)}$</td>
<td>$k^5 = k^5, H^[-][H] + k^5, A^[-][A] + k^5, H_2O^{(a)}$</td>
</tr>
<tr>
<td></td>
<td>$+ k^5, O^[-][OH]$</td>
</tr>
<tr>
<td></td>
<td>$k^5 = 541.5[H] + 0.34[A] + 0.026$</td>
</tr>
<tr>
<td></td>
<td>$+ 545[OH]$</td>
</tr>
<tr>
<td>$k^6$</td>
<td></td>
</tr>
<tr>
<td>$\text{MnOX(keto)} \rightleftharpoons \text{MnOX(hyd)}$</td>
<td>$k^6 = k^6, H_2O + k^6, O^[-][OH] + k^6, A^[-][A]$</td>
</tr>
<tr>
<td></td>
<td>$k^6, H_2O^0 = 0.436 \text{ sec}^{-1}$</td>
</tr>
<tr>
<td></td>
<td>$k^6, O^[-][OH] = (8.8 \pm 0.8) \times 10^5 \text{ M}^{-1} \text{sec}^{-1}$</td>
</tr>
<tr>
<td></td>
<td>$k^6, A = 70.9 \pm 11.2 \text{ M}^{-1} \text{sec}^{-1}$</td>
</tr>
<tr>
<td>B. Enolization</td>
<td></td>
</tr>
<tr>
<td>$k^2$</td>
<td></td>
</tr>
<tr>
<td>$\text{OX(keto)} \rightleftharpoons \text{OX(enol)}$</td>
<td>$k^2 = k^2, H^[-][H] + k^2, O^[-][OH] + k^2, A^[-][A]$</td>
</tr>
<tr>
<td></td>
<td>$k^2 = 1073.4[H] + 80.0[OH] + 0.102[A]$</td>
</tr>
<tr>
<td>$k^3$</td>
<td></td>
</tr>
<tr>
<td>$\text{MnOX(keto)} \rightleftharpoons \text{MnOX(enol)}$</td>
<td>$k^3 = k^3, H_2O + k^3, A^[-][A]$</td>
</tr>
<tr>
<td></td>
<td>$+ k^3, O^[-][OH]$</td>
</tr>
<tr>
<td></td>
<td>$k^3, H_2O^0 = 0.0519 \text{ sec}^{-1}$</td>
</tr>
<tr>
<td></td>
<td>$k^3, O^[-][OH] = (9.0 \pm 5.0) \times 10^4 \text{ M}^{-1} \text{sec}^{-1}$</td>
</tr>
<tr>
<td></td>
<td>$k^3, A = 16.20 \pm 5.40 \text{ M}^{-1} \text{sec}^{-1}$</td>
</tr>
</tbody>
</table>
relaxation decay, which was a result of the interconversions of keto ⇔ enol and keto ⇔ hydrate, was monitored at 285 nm.

The influence of Mn(II) ions on the hydration and enolization has been investigated in various metal concentrations range from 10.1 mM to 1.00 mM. All of the pH jump experiments were performed with a final pH of 6.22 to pH 6.81. Table 40 which was calculated by the equilibrium constants of OX and MnOX gives a summary of the equilibrium distribution of hydrate, keto and enol species of all of the experiments performed. The digitized stopped-flow traces thus obtained were analysed by MINABS program. The equilibrium constant of MnOX(keto) ⇌ MnOX(hyd), $K_{hyd} = 0.790$ which was determined in acetate buffer was also employed here to describe the equilibrium correction between hydrate and keto species of Mn(II)OX.

The contribution of either proton or hydroxide ion to the rates of these reactions was negligible due to the relative low proton and hydroxide concentration (ca. $10^{-7}$ M). Under these experimental conditions, only the solvent catalyzed pathway for the hydration of the MnOX complex was observed, $k^6_{H_2O} = 0.436 \text{ sec}^{-1}$. Neither general acid nor general base catalysis was observed.
<table>
<thead>
<tr>
<th>[MES] mM</th>
<th>[Mn(II)] mM</th>
<th>pH</th>
<th>% keto</th>
<th>% enol</th>
<th>% hyd. (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45.0</td>
<td>5.00</td>
<td>6.22</td>
<td>74.5</td>
<td>15.0</td>
<td>10.5</td>
</tr>
<tr>
<td>45.0</td>
<td>6.00</td>
<td>6.22</td>
<td>73.4</td>
<td>15.6</td>
<td>11.1</td>
</tr>
<tr>
<td>45.0</td>
<td>7.00</td>
<td>6.22</td>
<td>72.2</td>
<td>16.2</td>
<td>11.6</td>
</tr>
<tr>
<td>45.0</td>
<td>8.00</td>
<td>6.23</td>
<td>71.1</td>
<td>16.8</td>
<td>12.0</td>
</tr>
<tr>
<td>45.0</td>
<td>10.0</td>
<td>6.13</td>
<td>69.2</td>
<td>17.8</td>
<td>13.0</td>
</tr>
<tr>
<td>45.0</td>
<td>1.00</td>
<td>6.81</td>
<td>79.7</td>
<td>12.6</td>
<td>7.74</td>
</tr>
<tr>
<td>45.0</td>
<td>5.00</td>
<td>6.61</td>
<td>74.2</td>
<td>15.5</td>
<td>10.3</td>
</tr>
<tr>
<td>45.0</td>
<td>10.0</td>
<td>6.66</td>
<td>67.3</td>
<td>20.5</td>
<td>12.3</td>
</tr>
</tbody>
</table>

(a) These data were collected with 0.200 mM oxaloacetic acid.
The enolization of uncomplexed \( \text{OX} \) undergoes general acid catalysis in MES buffer and \( k_{2,HA} = 0.618 \ \text{M}^{-1} \text{sec}^{-1} \) as determined in Chapter IV. According to the curve fitting result, the enolization of \( \text{MnOX} \) was only promoted by the base buffer, MES\(^-\), \( k_{3,A} = 3.56 \pm 0.60 \ \text{M}^{-1} \text{sec}^{-1} \).

The Mn(II) ion dependence of the absorbance vs. time curves as well as the excellent agreement between the observed absorbances and calculated values are visualized in Figure 29. Again, the solid curves stand for calculated values whereas triangles, diamonds and squares stand for those absorbances obtained with 5.00 mM, 6.00 mM and 7.00 mM Mn(II), respectively.
Figure 27: Absorbance vs. Time Curves of Mn(II)-Oxaloacetate Mixtures in 3,3-Dimethylglutarate Buffers

- 0.200 mM oxaloacetate
- 60.0 mM DMG
- pH(initial) = 1.30
- pH(final) = 3.95
- △ = 0.800 mM Mn(II)
- ◊ = 1.20 mM Mn(II)
- □ = 1.70 mM Mn(II)
Figure 28: Absorbance vs. Time Curves of Mn(II)-Oxaloacetate Mixtures in Bicine Buffers

0.200 mM oxaloacetate
60.0 mM Bicine
\( \text{pH(initial)} = 1.30 \)
\( \text{pH(final)} = 8.55 \)
\( \triangle = 0.400 \text{ mM Mn(II)} \)
\( \diamond = 0.600 \text{ mM Mn(II)} \)
\( \square = 0.800 \text{ mM Mn(II)} \)
Figure 28
Figure 29: Absorbance vs. Time Curves of Mn(II)-Oxaloacetate Mixtures in MES Buffers

0.100 mM oxaloacetate
45.0 mM MES
pH(initial) = 1.30
pH(final) = 6.22

□ = 7.00 mM Mn(II)
◊ = 6.00 mM Mn(II)
△ = 5.00 mM Mn(II)
Chapter VIII

ZN(II) ION PROMOTED HYDRATION AND ENOLIZATION OF OXALOACETATE DIANION

In this chapter the effectiveness of Zn(II) on hydration and enolization rates of oxaloacetate is discussed. The promotion of hydration and enolization of oxaloacetate by the hard metal ions, Mg(II) and Mn(II), has been discussed in earlier chapters. It is of interest to extend this topic with a softer metal, i.e. Zn(II) ion. The investigations have allowed the equilibrium constant of ZnOX(keto) $\rightleftharpoons$ ZnOX(hyd) to be estimated. Consequently, the correlation between the thermodynamic stability of metal-oxaloacetate complex and the catalytic ability of corresponding metal ion can be ascertained. Also, reaction models for hydration and enolization of metal-OX can be proposed. Detailed investigations have been done with acetic acid, 3,3-dimethylglutaric acid, Bicine and MES buffers.

Besides being a biologically important metal, Zn(II) has been proved to activate several chemical reactions including the decarboxylation of oxaloacetate (130,131). Furthermore, the hydration of acetaldehyde was greatly
enhanced by the cooperation of Zn(II) ion with hydroxide ion as proved by Woolley (59,60). Therefore, similar enhancement is expected in the hydration and enolization of oxaloacetate. A series of pH increase experiments with various zinc chloride, proton and buffer concentration have been performed at 25.0 °C and 0.275 M (NaCl) ionic strength aqueous solution to verify this prediction. The equilibrium constants required to define the equilibria of buffers, OX and Zn(II)OX except the equilibrium constant of ZnOX(hyd) are summarized in Chapter V.

**Acetate Buffer**

Detailed experimental procedures for studies using Zn(II) are similar to those which have been described in Chapter VI. The kinetic study was initially performed with 35.0 mM Zn(II), pH 4.0 solution in various acetate buffer concentrations, namely from 60.0 mM to 120 mM acetate. The resultant time dependent absorption curves showed relatively fast initial UV absorption increase followed by rapid UV absorption decrease. The UV absorption increase, which was complete in ca. 30 seconds, was due to the enolization and hydration of oxaloacetate whereas the absorption decrease which was complete in about 15 minutes,
was due to the decarboxylation of oxaloacetate as reported by Leussing and Covey (23).

In these experiments, 83% hydrate species was initially present in the oxaloacetic acid solution. As the pH rapidly increase from 1.30 to 4.10, the equilibrium distribution of hydrated form rapidly dropped to ca. 25%. Consequently, the equilibrium distribution of enol form increase concurrently. However, due to the strong catalysis by Zn(II) the reversible hydration-dehydration was too fast to be measured on the time scale of stopped-flow spectrophotometer under these experimental conditions. Similar catalytic effects were also observed by Pocker et al. in their study of pyruvate hydration-dehydration in the presence of metal ions (48).

These experiments did not supply quantitative information about how Zn(II) promotes the interconversion between hydrate and keto species. Further investigations were performed using relatively low metal concentrations of ca. 10^{-4} M Zn(II). The resultant relaxation curves were analysed by MINABS program which had been discussed in Chapter III.

The equilibrium constant of ZnOX(keto) $\rightleftharpoons$ ZnOX(hyd) along with the microscopic rate constants for catalysts
were kinetically determined by optimizing these variables to fit the experimental time dependent absorbance curves. A value of 2.00 ± 0.67 for the equilibrium constant, $k_{\text{hyd}}$, was obtained. The microscopic rate constants thus resolved are summarized in Table 41. The goodness of the non-linear curve fitting result is shown in Figure 30. In Figure 30, the solid curves represent the theoretical absorbances whereas squares, diamonds and triangles stand for the experimental values collected with 0.800 mM, 0.560 mM and 0.200 mM Zn(II). It can be seen that the absorption curves are highly susceptible to the concentration of divalent metal ions. A summary of the equilibrium distribution of oxaloacetate species involved in these experiments is given in Table 42.

The hydration rate of uncomplexed oxaloacetate undergoes acetic acid, proton and solvent catalysis as determined in Chapter IV. However, ZnOX shows different catalytic pathways. In the presence of Zn(II) ions, hydration of the Zn(II) oxaloacetate complex is promoted only by solvent pathway with $k_{6,H_2O}^6 = 0.94 \pm 0.06$ sec$^{-1}$. This microscopic rate constant for solvent catalyzed hydration of ZnOX is ca. 36 fold greater than that for uncomplexed OX.
TABLE 41

Microscopic Rate Constants of Oxaloacetate in Zn(II)
Activated Acetate Buffer

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Microscopic Rate Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>T = 25.0 ± 0.2 °C</td>
<td>u = 0.275M(NaCl)</td>
</tr>
</tbody>
</table>

A. Hydration

\[
\begin{align*}
OX(keto) & \rightleftharpoons OX(hyd) \\
ZnOX(keto) & \rightleftharpoons ZnOX(hyd)
\end{align*}
\]

\[
\begin{align*}
k^5 & = k^5_H[H] + k^5_H[A][A] + k^5_H_2O \\
k^6 & = k^6_H_2O \\
k^6_H_2O & = 0.94 ± 0.06 \text{ sec}^{-1}
\end{align*}
\]

B. Enolization

\[
\begin{align*}
OX(keto) & \rightleftharpoons OX(enol) \\
ZnOX(keto) & \rightleftharpoons ZnOX(enol)
\end{align*}
\]

\[
\begin{align*}
k^2 & = k^2_H[H] + k^2_H[A][A] + k^2_A[A] \\
k^3 & = k^3_H[H] + k^3_H_2O + k^3_A[A] + k^3_H[A][A] \\
k^3_H & = 814 ± 390 \text{ M}^{-1}\text{sec}^{-1} \\
k^3_H_2O & = 0.37 ± 0.11 \text{ sec}^{-1} \\
k^3_H[A] & = 8.02 ± 2.13 \text{ M}^{-1}\text{sec}^{-1} \\
k^3_A & = 7.48 ± 0.16 \text{ M}^{-1}\text{sec}^{-1}
\end{align*}
\]
TABLE 42

Equilibrium Distribution of Oxaloacetate in Zn(II)
Activated Acetate Buffer

<table>
<thead>
<tr>
<th>[HOAc] mM</th>
<th>[Zn(II)] mM</th>
<th>pH</th>
<th>%keto</th>
<th>%enol</th>
<th>%hydrate(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>90.0</td>
<td>0.560</td>
<td>4.99</td>
<td>75.9</td>
<td>14.2</td>
<td>9.91</td>
</tr>
<tr>
<td>120</td>
<td>0.560</td>
<td>5.04</td>
<td>76.6</td>
<td>13.8</td>
<td>9.57</td>
</tr>
<tr>
<td>150</td>
<td>0.560</td>
<td>5.03</td>
<td>76.9</td>
<td>13.6</td>
<td>9.52</td>
</tr>
<tr>
<td>30.0</td>
<td>0.560</td>
<td>4.54</td>
<td>71.7</td>
<td>15.0</td>
<td>13.3</td>
</tr>
<tr>
<td>60.0</td>
<td>0.560</td>
<td>4.56</td>
<td>72.6</td>
<td>14.4</td>
<td>13.0</td>
</tr>
<tr>
<td>90.0</td>
<td>0.560</td>
<td>4.55</td>
<td>73.1</td>
<td>14.0</td>
<td>13.0</td>
</tr>
<tr>
<td>150</td>
<td>0.560</td>
<td>4.57</td>
<td>74.0</td>
<td>13.4</td>
<td>12.6</td>
</tr>
<tr>
<td>30.0</td>
<td>0.404</td>
<td>5.05</td>
<td>75.7</td>
<td>14.5</td>
<td>9.75</td>
</tr>
<tr>
<td>60.0</td>
<td>0.404</td>
<td>5.00</td>
<td>76.3</td>
<td>13.9</td>
<td>9.76</td>
</tr>
<tr>
<td>90.0</td>
<td>0.404</td>
<td>5.04</td>
<td>77.0</td>
<td>13.5</td>
<td>9.47</td>
</tr>
<tr>
<td>120</td>
<td>0.404</td>
<td>5.05</td>
<td>77.4</td>
<td>13.3</td>
<td>9.34</td>
</tr>
<tr>
<td>60.0</td>
<td>0.0200</td>
<td>5.03</td>
<td>79.2</td>
<td>11.9</td>
<td>8.94</td>
</tr>
<tr>
<td>60.0</td>
<td>0.0960</td>
<td>5.01</td>
<td>78.6</td>
<td>12.3</td>
<td>9.17</td>
</tr>
<tr>
<td>60.0</td>
<td>0.200</td>
<td>5.01</td>
<td>77.8</td>
<td>12.8</td>
<td>9.36</td>
</tr>
<tr>
<td>60.0</td>
<td>0.0200</td>
<td>4.54</td>
<td>76.2</td>
<td>11.4</td>
<td>12.4</td>
</tr>
<tr>
<td>60.0</td>
<td>0.0670</td>
<td>4.55</td>
<td>75.9</td>
<td>11.7</td>
<td>12.3</td>
</tr>
<tr>
<td>60.0</td>
<td>0.0800</td>
<td>4.56</td>
<td>75.9</td>
<td>11.8</td>
<td>12.3</td>
</tr>
<tr>
<td>60.0</td>
<td>0.200</td>
<td>4.50</td>
<td>74.5</td>
<td>12.4</td>
<td>13.1</td>
</tr>
<tr>
<td>60.0</td>
<td>0.800</td>
<td>4.54</td>
<td>70.9</td>
<td>15.6</td>
<td>13.5</td>
</tr>
<tr>
<td>60.0</td>
<td>0.200</td>
<td>3.82</td>
<td>64.8</td>
<td>10.7</td>
<td>24.5</td>
</tr>
<tr>
<td>60.0</td>
<td>0.800</td>
<td>3.91</td>
<td>63.5</td>
<td>13.7</td>
<td>22.8</td>
</tr>
</tbody>
</table>

a. These data were collected with 0.200 mM oxaloacetic acid.
General acid and base catalysis toward enolization rates are also greatly enhanced. As shown in Table 41, the microscopic rate constants for the proton, acetic acid and acetate catalyzed pathways are 814 ± 390 M⁻¹sec⁻¹, 8.02 ± 2.13 M⁻¹sec⁻¹ and 7.48 ± 0.16 M⁻¹sec⁻¹, respectively. Complexing to Zn(II) causes approximately 20% decrease in the microscopic rate constant for proton catalysis. In contrast, acetic acid catalysis on the enolization reaction is increased by a factor of 5. Tremendous enhancement of acetate catalysis by Zn(II) is also observed. Quantitatively, the microscopic rate constants of acetate in the enolization of ZnOX is 83 fold greater than that of uncomplexed OX (k³,A /k²,A = 7.48 M⁻¹sec⁻¹ / 0.009 M⁻¹sec⁻¹ = 83). In addition to these catalytic constants, the solvent catalyzed pathway is also promoted by Zn(II), k³,H₂O = 0.37 sec⁻¹, which is about the same order of magnitude as the solvent catalyzed pathway for the hydration of ZnOX. The solvent term appears not to involve in the enolization of free OX. Cooperativity between solvent and Zn(II) greatly enhances the solvent catalysis on enolization.

Identical activation on the enolization of ZnOX were also reported by Leussing and Emly (97) and Leussing and
Mao (98). The microscopic rate constants determined in this work and these literature values are summarized in Table 43. For solvent catalysis, my result, 0.37 sec\(^{-1}\) is slightly higher than either Mao's, 0.11 sec\(^{-1}\), or Emly's result, 0.149 sec\(^{-1}\). In the earlier works this may be attributed to the inaccurate resolution of pseudo first order enolization rates by RLXFT. However, inhibition of proton catalysis has been observed in all of these studies. The microscopic rate constants for acetate catalysis obtained in Emly's work and this work, 7.3 M\(^{-1}\)sec\(^{-1}\) and 7.48 M\(^{-1}\)sec\(^{-1}\), respectively, are in a good agreement.

*3,3-Dimethylglutarate Buffer*

Detailed investigations of Zn(II) ion effect on the buffer catalysis on hydration and enolization have been performed with a series of pH increase experiments. All of the experiments were initiated at relatively low pH, namely pH 1.30. Under this condition oxaloacetic acid was highly hydrated. Because the precipitation of Zn(OH)\(_2\) was inevitable in alkaline buffer solutions, Zn(II) was added to the oxaloacetic acid solution before mixing with the buffer solution. The freshly prepared acidic Zn(II) and OX solution (pH 1.30) was rapidly mixed with alkaline DMG buffer solution. Since 3,3-dimethylglutaric acid is a
### TABLE 43

Comparison of the Enolization Rate Constants of Zn(II) Promoted Oxaloacetate in Acetate Buffer

<table>
<thead>
<tr>
<th>complex catalyst</th>
<th>Mao(a)</th>
<th>Emly(b)</th>
<th>this work</th>
</tr>
</thead>
<tbody>
<tr>
<td>OX H₂O</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>ZnOX H₂O</td>
<td>0.11 sec⁻¹</td>
<td>0.149 sec⁻¹</td>
<td>0.37 sec⁻¹</td>
</tr>
<tr>
<td>OX H</td>
<td>900 M⁻¹sec⁻¹</td>
<td>1450 M⁻¹sec⁻¹</td>
<td>1073 M⁻¹sec⁻¹</td>
</tr>
<tr>
<td>ZnOX H</td>
<td>620 M⁻¹sec⁻¹</td>
<td>1210 M⁻¹sec⁻¹</td>
<td>814 M⁻¹sec⁻¹</td>
</tr>
<tr>
<td>OX HOAC</td>
<td>0.7 M⁻¹sec⁻¹</td>
<td>1.2 M⁻¹sec⁻¹</td>
<td>1.71 M⁻¹sec⁻¹</td>
</tr>
<tr>
<td>ZnOX HOAC</td>
<td>4.1 M⁻¹sec⁻¹</td>
<td>7.3 M⁻¹sec⁻¹</td>
<td>8.02 M⁻¹sec⁻¹</td>
</tr>
<tr>
<td>OX OAC</td>
<td>--</td>
<td>--</td>
<td>0.009 M⁻¹sec⁻¹</td>
</tr>
<tr>
<td>ZnOX OAC</td>
<td>(c)</td>
<td>7.3 M⁻¹sec⁻¹</td>
<td>7.48 M⁻¹sec⁻¹</td>
</tr>
</tbody>
</table>

a. reference (98).
b. reference (97).
c. The corresponding rate constant was not observable.
diprotic acid with pKa1 = 3.42 and pKa2 = 6.67 as determined in Chapter IV, the pH increase experiments were performed with final pH range of 3.4 to 6.7. Consequently, the buffer catalysis of diacid, monoacid and base component on the hydration and enolization of ZnOX complex could be determined.

In order to completely characterize the interactions among reactants and catalysts, numerous experiments were performed with 0.200 mM oxaloacetate, 20.0 mM to 130 mM buffer and 1.70 mM to 0.0500 mM Zn(II). The biphasic absorbance vs. time curves which corresponding to the redistribution of OX species were monitored at 285 nm. The Zn(II) ion concentration dependence of the biphasic absorbance vs. time curves is clearly demonstrated in Figure 31 by some representative experiments. The resultant digitized stopped-flow traces were analysed as described earlier by MINABS.
Figure 30: Absorbance vs. Time Curves of Zn(II)-Oxaloacetate Mixtures in Acetate Buffers

0.200 mM oxaloacetate
60.0 mM acetate
pH(initial) = 1.30
pH(final) = 4.56

△ --- 0.200 mM Zn(II)
◊ --- 0.560 mM Zn(II)
□ --- 0.800 mM Zn(II)
Figure 30

Absorbance (285nm)

Absorbance vs. Time (seconds)
Figure 31: Absorbance vs. Time Curves of Zn(II)-Oxaloacetate Mixtures in 3,3-Dimethylglutarate Buffers

0.200 mM oxaloacetate
60.0 mM DMG
pH(initial) = 1.30
pH(final) = 3.55

Δ --- 0.400 mM Zn(II)
◊ --- 0.600 mM Zn(II)
□ --- 0.800 mM Zn(II)
In the data fitting process, the equilibrium constant of $\text{ZnOX(keto)} \rightleftharpoons \text{ZnOX(hyd)}$, $K_{\text{hyd}} = 2.00$ determined previously and the second order rate constants for general acid, general base, specific acid and specific base determined in Chapter IV were used. In addition, the microscopic rate constants for solvent and proton catalytic pathways involved in the hydration and enolization were also used. By comparing the experimental absorbances with the calculated absorbances, the optimum values of microscopic rate constants were resolved. Figure 31 shows the experimental and theoretical values of several representative experiments. Solid lines stand for the theoretical values whereas squares, diamonds and triangles represent the experimental values.

According to this work, the hydration of ZnOX complex is only promoted by solvent pathway as determined in acetate buffer. Tremendous enhancement of $\text{DMG}^{2-}$ catalytic ability toward the enolization reaction was uncovered. As summarized in Table 44, the $\text{DMG}$ dianion term thus determined is $42.98 \pm 30.71 \ M^{-1}\text{sec}^{-1}$. Again, the activation of general base catalyst by cooperativity with a divalent metal ion is observed. As determined in Chapter IV, the microscopic rate constant of the $\text{DMG}^{2-}$ catalyst of free OX is $0.11 \ M^{-1}\text{sec}^{-1}$. Quantitatively, the addition of
Zn(II) ion causes ca. 400 fold enhancement in the general base catalyst term.

A summary of the equilibrium distribution of keto, enol, hydrate species for the various experimental conditions employed is given in Table 45. This table shows the Zn(II) concentration dependency of the equilibrium distribution of hydrate, keto and enol species. The equilibrium constants employed to define the equilibria except ZnOX(keto) ⇌ ZnOX(hyd) are summarized in Chapter V. The equilibrium constant of ZnOX(hyd) had been earlier determined in acetate buffer.

**Bicine Buffer**

A series of pH increase experiments have been performed to study Zn(II) influence on the hydration and enolization of oxaloacetate in N-substituted glycine, N,N-Bis(2-hydroxyethyl)glycine, i.e. Bicine, solution. The relative high pKa (pKa = 8.35, at 25 °C) of Bicine allowed the kinetic studies to be performed in more alkaline solutions. Since the equilibrium constant of ZnOX(keto) ZnOX(hyd) had been determined in acetate buffer this constant, $K_{\text{hyd}} = 2.00$, was used in the non-linear data fitting process.
### TABLE 44

**Microscopic Rate Constants of Oxaloacetate in Zn(II) Activated 3,3-Dimethylglutarate Buffer**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Microscopic Rate Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Hydration</strong></td>
<td></td>
</tr>
<tr>
<td>$k^5$</td>
<td>$k^5 = k^5, [H] + k^5, [HA][HA] + k^5, H_2^0$</td>
</tr>
<tr>
<td>$O_X(keto) \rightleftharpoons O_X(hyd)$</td>
<td>+ $k^5, H_2A[H_2A]$</td>
</tr>
<tr>
<td>$ZnO_X(keto) \rightleftharpoons ZnO_X(hyd)$</td>
<td>$k^5 = 541.5[H] + 0.010[HA] + 0.026$</td>
</tr>
<tr>
<td></td>
<td>$+ 0.50[H_2A]$</td>
</tr>
<tr>
<td><strong>B. Enolization</strong></td>
<td></td>
</tr>
<tr>
<td>$k^6$</td>
<td>$k^6 = k^6, H_2^0$</td>
</tr>
<tr>
<td>$O_X(keto) \rightleftharpoons O_X(enol)$</td>
<td>$k^6, H_2^0 = 0.94 \text{ sec}^{-1}$</td>
</tr>
<tr>
<td>$ZnO_X(keto) \rightleftharpoons ZnO_X(enol)$</td>
<td>$k^3 = k_3, H_2^0 + k_3, [A]$</td>
</tr>
<tr>
<td></td>
<td>$k^3, H_2^0 = 0.37 \text{ sec}^{-1}$</td>
</tr>
<tr>
<td></td>
<td>$k^3, [A] = 42.98 \pm 30.71 \text{ M}^{-1}\text{sec}^{-1}$</td>
</tr>
<tr>
<td>[DMG] mM</td>
<td>[Zn(II)] mM</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>60.0</td>
<td>0.0500</td>
</tr>
<tr>
<td>60.0</td>
<td>0.100</td>
</tr>
<tr>
<td>60.0</td>
<td>0.200</td>
</tr>
<tr>
<td>60.0</td>
<td>0.400</td>
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<tr>
<td>60.0</td>
<td>0.600</td>
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<tr>
<td>60.0</td>
<td>0.800</td>
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<td>1.000</td>
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<tr>
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<td>30.0</td>
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<tr>
<td>60.0</td>
<td>1.70</td>
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<tr>
<td>90.0</td>
<td>1.70</td>
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<tr>
<td>120</td>
<td>1.70</td>
</tr>
<tr>
<td>130</td>
<td>1.70</td>
</tr>
</tbody>
</table>

*a. These data were collected with 0.200 mM oxaloacetic acid.*
The interconversions of keto ⇌ hydrate and keto ⇌ enol were studied with 0.100 mM to 5.00 mM Zn(II), 10.0 mM to 120.0 mM buffer at pH 7.0 to 8.9. As is shown in Table 46, each experiment gives different distribution of keto, enol and hydrate species. The biphasic absorbance curves were monitored at 285 nm. The non-linear least-squares curve fitting process performed by MINABS has been discussed in Chapter III.

As shown in Figure 32, there is excellent agreement between the observed absorbances and the theoretical values which are represented by triangles, diamonds, squares and solid curves respectively. Again, these experiments prove that the general base and specific base catalytic pathways are highly promoted by the presence of Zn(II) ions as summarized in Table 47.

Bicine$^-\text{ }$ catalyzed hydration rate constant of uncomplexed OX is only 0.34 $M^{-1}\text{ }\sec^{-1}$. By complexing with Zn(II), Bicine catalysis is dramatically increased with $k_b^A = 250.3 \pm 87.6 \text{ } M^{-1}\text{ }\sec^{-1}$. There is ca. 740 fold increase in the Bicine catalysis on the enolization. An even larger enhancement is observed in the rate constant for catalysis by hydroxide.
Figure 32: Absorbance vs. Time Curves of Zn(II)-Oxaloacetate Mixtures in Bicine Buffers

0.200 mM oxaloacetate

60.0 mM Bicine

pH(initial) = 1.30

pH(final) = 8.35

\( \triangle \) --- 5.00 mM Zn(II)

\( \diamond \) --- 1.40 mM Zn(II)

\( \square \) --- 1.00 mM Zn(II)
Figure 32
TABLE 46

Equilibrium Distribution of Oxaloacetate in Zn(II)
Activated Bicine Buffer

<table>
<thead>
<tr>
<th>[BIC] mM</th>
<th>[Zn(II)] mM</th>
<th>pH</th>
<th>%keto</th>
<th>%enol</th>
<th>%hydrate(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.0</td>
<td>5.00</td>
<td>8.58</td>
<td>80.8</td>
<td>12.2</td>
<td>6.99</td>
</tr>
<tr>
<td>30.0</td>
<td>5.00</td>
<td>8.62</td>
<td>81.0</td>
<td>12.0</td>
<td>6.97</td>
</tr>
<tr>
<td>60.0</td>
<td>5.00</td>
<td>8.70</td>
<td>81.0</td>
<td>12.0</td>
<td>6.98</td>
</tr>
<tr>
<td>90.0</td>
<td>5.00</td>
<td>8.72</td>
<td>81.1</td>
<td>12.0</td>
<td>6.99</td>
</tr>
<tr>
<td>120</td>
<td>5.00</td>
<td>8.73</td>
<td>80.9</td>
<td>12.1</td>
<td>7.00</td>
</tr>
<tr>
<td>150</td>
<td>5.00</td>
<td>8.19</td>
<td>80.8</td>
<td>12.0</td>
<td>7.20</td>
</tr>
<tr>
<td>10.0</td>
<td>5.00</td>
<td>6.94</td>
<td>78.9</td>
<td>13.7</td>
<td>7.38</td>
</tr>
<tr>
<td>30.0</td>
<td>5.00</td>
<td>7.83</td>
<td>81.0</td>
<td>12.0</td>
<td>6.99</td>
</tr>
<tr>
<td>60.0</td>
<td>5.00</td>
<td>8.05</td>
<td>81.0</td>
<td>12.0</td>
<td>6.98</td>
</tr>
<tr>
<td>90.0</td>
<td>5.00</td>
<td>8.21</td>
<td>81.0</td>
<td>12.0</td>
<td>6.97</td>
</tr>
<tr>
<td>60.0</td>
<td>0.350</td>
<td>6.79</td>
<td>76.4</td>
<td>15.9</td>
<td>7.72</td>
</tr>
<tr>
<td>60.0</td>
<td>0.200</td>
<td>7.77</td>
<td>80.8</td>
<td>12.1</td>
<td>7.01</td>
</tr>
<tr>
<td>60.0</td>
<td>0.160</td>
<td>8.05</td>
<td>81.0</td>
<td>12.1</td>
<td>6.99</td>
</tr>
<tr>
<td>60.0</td>
<td>0.140</td>
<td>8.16</td>
<td>81.0</td>
<td>12.0</td>
<td>6.98</td>
</tr>
<tr>
<td>60.0</td>
<td>0.100</td>
<td>8.29</td>
<td>80.9</td>
<td>12.0</td>
<td>7.00</td>
</tr>
</tbody>
</table>

a. These data were collected with 0.200 mM oxaloacetic acid.
### TABLE 47

**Microscopic Rate Constants of Oxaloacetate in Zn(II) Activated Bicine Buffer**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Microscopic Rate Constant(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T = 25.0 \pm 0.2^\circ C$</td>
<td>$u = 0.275M(\text{NaCl})$</td>
</tr>
</tbody>
</table>

#### A. Hydration

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Rate Constant(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k^5$</td>
<td>$k^5 = k^5,<em>{\text{H}^+} + k^5,</em>{\text{A}^-} + k^5,_{\text{H}<em>2\text{O}} + k^5,</em>{\text{OH}^-}$</td>
</tr>
<tr>
<td>$k^6$</td>
<td>$k^6 = 541.5[H] + 0.34[A] + 0.026 + 545[\text{OH}]$</td>
</tr>
</tbody>
</table>

#### B. Enolization

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Rate Constant(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k^2$</td>
<td>$k^2 = k^2,<em>{\text{H}^+} + k^2,</em>{\text{OH}^-} + k^2,_{\text{A}^-}$</td>
</tr>
<tr>
<td>$k^3$</td>
<td>$k^3 = k^3,<em>{\text{OH}^-} + k^3,</em>{\text{H}<em>2\text{O}} + k^3,</em>{\text{A}^-}$</td>
</tr>
</tbody>
</table>

- $k^2,_{\text{H}^+} = (1.1 \pm 0.5 \times 10^{-7})M^{-1}\text{sec}^{-1}$
- $k^2,_{\text{A}^-} = 250.3 \pm 87.6M^{-1}\text{sec}^{-1}$
- $k^3,_{\text{OH}^-} = (1.6 \pm 1.0 \times 10^{-6})M^{-1}\text{sec}^{-1}$
- $k^3,_{\text{H}_2\text{O}} = 0.37\text{ sec}^{-1}$
- $k^3,_{\text{A}^-} = 447 \pm 249M^{-1}\text{sec}^{-1}$
The rate constant for the hydroxide pathway of ZnOX is \((1.1 \pm 0.5) \times 10^7 \text{ M}^{-1}\text{sec}^{-1}\) whereas the microscopic rate constant of hydroxide catalysis of uncomplexed OX is only 545 \(\text{M}^{-1}\text{sec}^{-1}\). Complexing with Zn(II) causes four order of magnitude increase in hydroxide catalysis.

A similar large increase in catalysis by Bicine\(^{-1}\) and proton is also observed in the enolization reaction as shown in Table 47. The rate constant of Bicine catalyst of the enolization of free OX is only 0.102 \(\text{M}^{-1}\text{sec}^{-1}\) whereas that of ZnOX is 447 \(\text{M}^{-1}\text{sec}^{-1}\). The Bicine catalysis of ZnOX is ca. three order of magnitude higher than that of uncomplexed OX. Pronounced promotion is also observed in hydroxide catalysis. The hydroxide catalysis of enolization is also highly susceptible to Zn(II). The microscopic rate constant of hydroxide is only 80.0 \(\text{M}^{-1}\text{sec}^{-1}\). The complexing of OX with Zn(II) causes approximately \(10^4\) times increase in hydroxide catalysis.

**MES Buffer**

PH increase experiments were performed to examine the hydration and enolization of ZnOX complexes in 2-\((N\text{-Morpholino})\text{ethanesulphonic Acid, MES buffer solution. PH 1.30 oxaloacetic acid and Zn(II) solutions were mixed with various MES buffer solutions, and the resultant biphasic**
two-relaxation profiles were monitored by tracing the UV absorbance change of OX species at 285 nm. The pH of the corresponding solution for each experiment was taken immediately after each reaction. Similar data analysis process was employed to analyse the UV absorbance-time curves by optimize the second order reaction rates of buffer components.

Since pKa of HMES, $\overset{+}{\text{NHCH}_2\text{CH}_2\text{SO}_3}$ reported is 6.15, at 20 °C, $u=0.10$ M (115), the applicable buffer range of this buffer falls between 5.15 and 7.15. Those experiments were done with 35.0 mM to 0.500 mM Zn(II), 10.0 mM to 120 mM buffer at pH 5.20 to 7.00. Within this pH range, the acidic buffer and basic buffer concentration was about 4 order of magnitude greater than either proton or hydroxide ion concentrations. Based on this reason, the proton and hydroxide ions catalytic pathways were assumed to be negligible in those experiments.

In the data fitting process, the pKa of HMES and equilibrium constants of diacid OX, mono OX, buffer and the formation constants of ZnOX complexes were employed to define the equilibrium distribution of all of the species involved. The proton, hydroxide and solvent catalytic pathways of hydration and enolization of OX species defined
previously were also employed as constants in data fitting process.

According to this work, neither general acid nor general base catalysis was observed in Zn(II) activated hydration reaction of OX in MES system. Only solvent catalysis, $k^6,H_2O=0.94 \text{ sec}^{-1}$ appeared to be the predominant catalytic pathway. This might be attributed to the relative low acidity and basicity of the buffer. Although enolization of uncomplexed OX showed HMES catalysis, enolization of ZnOX complex only proceeded via MES-catalytic pathway with $k^3,A=30.70 \pm 1.50 \text{ M}^{-1}\text{sec}^{-1}$.

Again, base catalysis is greatly increased through cooperativity with Zn(II). Figure 33 shows the Zn(II) concentration dependence of the biphasic two-relaxation profiles as well as the excellent agreement between the theoretical and observed values. The solid lines stand for the theoretical values defined by those calculated microscopic rate constants. Triangles, diamonds and squares stand for the experimental absorbances collected in 0.500 mM, 1.00 mM and 5.00 mM Zn(II) solution, respectively. The overall equilibrium distribution of keto, enol and hydrate OX as well as the experimental conditions employed in this work are summarized in Table 48.
### TABLE 48

**Equilibrium Distribution of Oxaloacetate in Zn(II) Activated MES Buffer**

<table>
<thead>
<tr>
<th>[MES] mM</th>
<th>[Zn(II)] mM</th>
<th>pH</th>
<th>%keto</th>
<th>%enol</th>
<th>%hydrate(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45.0</td>
<td>35.0</td>
<td>6.01</td>
<td>1.91</td>
<td>95.9</td>
<td>2.18</td>
</tr>
<tr>
<td>45.0</td>
<td>25.0</td>
<td>6.01</td>
<td>3.04</td>
<td>94.0</td>
<td>2.97</td>
</tr>
<tr>
<td>45.0</td>
<td>10.0</td>
<td>6.02</td>
<td>10.7</td>
<td>83.1</td>
<td>6.22</td>
</tr>
<tr>
<td>10.0</td>
<td>5.00</td>
<td>6.21</td>
<td>20.1</td>
<td>72.5</td>
<td>7.37</td>
</tr>
<tr>
<td>50.0</td>
<td>5.00</td>
<td>6.09</td>
<td>23.2</td>
<td>68.2</td>
<td>8.53</td>
</tr>
<tr>
<td>90.0</td>
<td>5.00</td>
<td>6.08</td>
<td>23.5</td>
<td>67.9</td>
<td>8.63</td>
</tr>
<tr>
<td>120</td>
<td>5.00</td>
<td>6.08</td>
<td>23.5</td>
<td>67.9</td>
<td>8.63</td>
</tr>
<tr>
<td>10.0</td>
<td>25.0</td>
<td>6.21</td>
<td>2.01</td>
<td>96.0</td>
<td>1.97</td>
</tr>
<tr>
<td>25.0</td>
<td>25.0</td>
<td>6.08</td>
<td>2.64</td>
<td>94.8</td>
<td>2.58</td>
</tr>
<tr>
<td>25.0</td>
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<td>6.12</td>
<td>9.12</td>
<td>85.6</td>
<td>5.27</td>
</tr>
<tr>
<td>50.0</td>
<td>10.0</td>
<td>6.08</td>
<td>9.75</td>
<td>84.6</td>
<td>5.64</td>
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<tr>
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<td>10.0</td>
<td>6.08</td>
<td>9.76</td>
<td>84.6</td>
<td>5.64</td>
</tr>
<tr>
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<td>6.91</td>
<td>1.90</td>
<td>97.0</td>
<td>1.10</td>
</tr>
<tr>
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<td>10.0</td>
<td>6.49</td>
<td>4.59</td>
<td>92.8</td>
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<td>8.23</td>
<td>87.0</td>
<td>4.75</td>
</tr>
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<td>45.0</td>
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<td>5.89</td>
<td>13.1</td>
<td>79.4</td>
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</tr>
<tr>
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<td>19.5</td>
<td>69.1</td>
<td>11.4</td>
</tr>
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<td>10.0</td>
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<td>25.2</td>
<td>60.1</td>
<td>14.7</td>
</tr>
<tr>
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<td>1.00</td>
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<td>48.9</td>
<td>43.9</td>
<td>7.18</td>
</tr>
<tr>
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<td>1.00</td>
<td>6.50</td>
<td>59.4</td>
<td>31.8</td>
<td>8.85</td>
</tr>
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<td>1.00</td>
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<td>63.8</td>
<td>26.6</td>
<td>9.61</td>
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<td>1.00</td>
<td>5.59</td>
<td>67.4</td>
<td>22.1</td>
<td>10.5</td>
</tr>
<tr>
<td>45.0</td>
<td>1.00</td>
<td>5.23</td>
<td>67.8</td>
<td>21.0</td>
<td>11.2</td>
</tr>
<tr>
<td>45.0</td>
<td>0.500</td>
<td>6.92</td>
<td>66.9</td>
<td>25.2</td>
<td>7.86</td>
</tr>
<tr>
<td>45.0</td>
<td>0.500</td>
<td>6.49</td>
<td>71.3</td>
<td>20.2</td>
<td>8.47</td>
</tr>
<tr>
<td>45.0</td>
<td>0.500</td>
<td>6.18</td>
<td>72.8</td>
<td>18.5</td>
<td>8.73</td>
</tr>
<tr>
<td>30.0</td>
<td>0.500</td>
<td>6.56</td>
<td>70.8</td>
<td>20.8</td>
<td>8.39</td>
</tr>
<tr>
<td>45.0</td>
<td>0.500</td>
<td>6.67</td>
<td>69.9</td>
<td>21.9</td>
<td>8.26</td>
</tr>
<tr>
<td>66.0</td>
<td>0.500</td>
<td>6.66</td>
<td>70.0</td>
<td>21.8</td>
<td>8.27</td>
</tr>
<tr>
<td>75.0</td>
<td>0.500</td>
<td>6.66</td>
<td>70.0</td>
<td>21.8</td>
<td>8.27</td>
</tr>
</tbody>
</table>

a. These data were collected with 0.100 mM oxaloacetic acid.
Figure 33: Absorbance vs. Time Curves of Zn(II)-Oxaloacetate Mixtures in MES Buffers

0.100 mM oxaloacetate
45.0 mM MES
pH(initial) = 1.30
pH(final) = 6.15

- Δ --- 0.500 mM Zn(II)
- ◊ --- 1.00 mM Zn(II)
- □ --- 5.00 mM Zn(II)
Chapter IX

SUMMARY

In earlier chapters, a series of systematic investigations on the metal ion accelerated hydration and enolization of oxaloacetate in acetate, 3,3-dimethylglutarate, Bicine and MES buffers have been described. Before this work, limited quantitative information on how metal ion effects hydration and enolization rates had been reported due to the lack of a proper way of resolving the reaction rate constants for these two comparable reactions. This formed a substantial gap in characterizing the participation of metal ions in biological reactions. The MINABS computer program which is developed in this work gives satisfactory results by fitting functions of the microscopic rate constants directly to the absorbance vs. time curves.

Mg(II), Mn(II) and Zn(II) have been employed to probe divalent metal ion effects on general acid and general base catalysis and to correlate the variation of the Bronsted coefficients with the thermodynamic stability of the corresponding metal-oxaloacetate complexes. The
equilibrium constants of some of the metal complexes have been determined kinetically. Based on the equilibrium constants of enol complexes reported in the literatures (see Chapter V), one may predict that the equilibrium constants of hydrate complexes will also follow the Irving-Williams natural order of complex stability. The experimental results reported here have been found to be in accord with the prediction.

The equilibrium constants for the 1:1 complexes of Mg(II), Mn(II) and Zn(II) with OX(hyd) and OX(enol) are summarized in Table 49. Among these metal ions, Zn(II), being the softest metal, forms the most stable oxaloacetate complex. The equilibrium constants for the hydrate complexes decrease in the order:

$$Zn(II) > Mn(II) > Mg(II)$$

An identical trend is followed by the stabilities of enol complexes. Also, the equilibrium constant of hydrate complex has approximately the same order of magnitude as that of corresponding enol complexes.

The level of metal enhancement in these catalytic terms is directly proportional to the pKas of buffers. Among these catalysts, hydroxide is the most alkaline species, therefore, it shows the greatest enhancement.
### TABLE 49
Equilibrium Constants of Oxaloacetate and Its Metal Complexes at 25.0 °C

#### A. Hydration

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Equilibrium Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{OX(keto)} \rightleftharpoons \text{OX(hyd)}$</td>
<td>0.086</td>
</tr>
<tr>
<td>$\text{MgOX(keto)} \rightleftharpoons \text{MgOX(hyd)}$</td>
<td>0.475 ± 0.290</td>
</tr>
<tr>
<td>$\text{MnOX(keto)} \rightleftharpoons \text{MnOX(hyd)}$</td>
<td>0.790 ± 0.063</td>
</tr>
<tr>
<td>$\text{ZnOX(keto)} \rightleftharpoons \text{ZnOX(hyd)}$</td>
<td>2.00 ± 0.67</td>
</tr>
</tbody>
</table>

#### B. Enolization

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Equilibrium Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{OX(keto)} \rightleftharpoons \text{OX(enol)}$</td>
<td>0.148</td>
</tr>
<tr>
<td>$\text{MgOX(keto)} \rightleftharpoons \text{MgOX(enol)}$</td>
<td>0.30</td>
</tr>
<tr>
<td>$\text{MnOX(keto)} \rightleftharpoons \text{MnOX(enol)}$</td>
<td>0.74</td>
</tr>
<tr>
<td>$\text{ZnOX(keto)} \rightleftharpoons \text{ZnOX(enol)}$</td>
<td>4.70</td>
</tr>
</tbody>
</table>
Furthermore, the increment of catalytic ability is also
dependent on the nature of metal ion. Zn(II) forms the
most stable complex with hydrate species. Consequently, Zn(II) induces the highest promotion in
catalytic ability.

Interestingly, besides solvent, Bicine and hydroxide
catalytic pathways, other catalysts appear not to be
involved in metal ion promoted hydration as summarized in
Table 50. At neutral pH, or even lower, a hydrolyzed metal
ion may be a major source of hydroxide ion. Therefore, the
hydroxide catalysis is greatly activated by metal ion. A
plausible reaction mechanism which explains the
extraordinary activation of hydroxide pathway is presented
in equation 59. The participation of divalent metal ion
not only facilitates the generation of hydroxide ions, but
also electrostatically stabilizes the hydrate intermediate.
The activation energy for the hydration reaction is lower
due to the formation of a chelate ring between a metal ion
and oxyanion as shown in equations 59 and 60.
\[
\begin{align*}
2^+\text{M(OH}_2\text{)}^\dagger & \rightleftharpoons \text{M(OH)}^\dagger \text{BH} \\
\text{C-CH}_2\text{CO}_2^- & \rightleftharpoons \text{C-CH}_2\text{CO}_2^- \\
\text{M}^+ & \rightleftharpoons \text{M}^+ \rightleftharpoons \text{C-CH}_2\text{CO}_2^- \text{OH} \\
\text{C-CH}_2\text{CO}_2^- & \rightleftharpoons \text{C-CH}_2\text{CO}_2^- \\
\text{(59)}
\end{align*}
\]
TABLE 50

Microscopic Rate Constants Involved in the Hydration of Oxaloacetate and Its Metal Complexes

\[ T = 25.0 \pm 0.2 \, ^\circ C \quad u = 0.275M(NaCl) \]

<table>
<thead>
<tr>
<th>catalyst</th>
<th>pKa</th>
<th>OX</th>
<th>MgOX</th>
<th>MnOX</th>
<th>ZnOX</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{H}_3\text{O}^+ )</td>
<td>-1.70</td>
<td>541.5</td>
<td>--(b)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>( \text{H}_2\text{DMG} )</td>
<td>3.42</td>
<td>0.50</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>( \text{HOAC} )</td>
<td>4.53</td>
<td>0.146</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>( \text{MES} )</td>
<td>6.15</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>( \text{HDMG} )</td>
<td>6.67</td>
<td>0.01</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>( \text{H}_2\text{O} )</td>
<td>-1.7</td>
<td>0.026</td>
<td>0.18</td>
<td>0.436</td>
<td>0.94</td>
</tr>
<tr>
<td>OAC</td>
<td>4.53</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>MES</td>
<td>6.15</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>DMG</td>
<td>6.67</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Bicine</td>
<td>8.35</td>
<td>0.34</td>
<td>59.8</td>
<td>70.9</td>
<td>250.3</td>
</tr>
<tr>
<td>OH</td>
<td>15.75</td>
<td>545.0</td>
<td>4.1x10^5</td>
<td>8.8x10^5</td>
<td>1.1x10^7</td>
</tr>
</tbody>
</table>

a. The unit of the microscopic rate constant of each catalyst is \( \text{M}^{-1}\text{sec}^{-1} \) except solvent. Solvent catalyst is measured in sec\(^{-1}\).
b. The corresponding constant was not observed.
This accounts for the facilitation of solvent, hydroxide and general base catalytic pathways by metal ion. According to this work, the general base catalysis is only observed in Bicine buffer. Acetate, DMG and MES do not show any general base catalysis on the hydration reaction. This proves that the Guthrie's theory (134) is applicable in the hydration reaction. That is, if the general base is too weak to permit the transfer of a proton from a solvent molecule, then hydration undergoes solvent catalysis and base catalysis is not observable. If it is too strong the reaction proceeds via specific base catalysis, i.e. hydroxide pathway. Only base having strength in the intermediate range gives general base catalysis.

General acid and general base catalyzed rates of enolization are also greatly susceptible to metal ions. The overall microscopic rate constants involved in the enolization reaction of oxaloacetate and metal-oxaloacetate are summarized in Table 51. While base catalyzed enolization rates are increased by complexing, the rate constants for general acid and proton catalysis are somewhat smaller than those for uncomplexed OX. This gives important information to visualize the reaction mechanism involved in the metal ion promoted enolization of oxaloacetate.
TABLE 51

Microscopic Rate Constants involved in the Enolization of Oxaloacetate and Its Metal Complexes

\[ T = 25.0 \pm 0.2 \, ^\circ C \quad u = 0.275M(NaCl) \]

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>pKa</th>
<th>Ox</th>
<th>MgOX</th>
<th>MnOX</th>
<th>ZnOX</th>
</tr>
</thead>
<tbody>
<tr>
<td>(H_3O^+)</td>
<td>-1.7</td>
<td>1073.4</td>
<td>118</td>
<td>--(b)</td>
<td>814</td>
</tr>
<tr>
<td>H₂DMG</td>
<td>3.42</td>
<td>3.16</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>HOAC</td>
<td>4.53</td>
<td>1.71</td>
<td>1.08</td>
<td>3.31</td>
<td>8.02</td>
</tr>
<tr>
<td>HMES</td>
<td>6.15</td>
<td>0.618</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>HDMS</td>
<td>6.67</td>
<td>0.63</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>H₂O</td>
<td>-1.7</td>
<td>--</td>
<td>0.0272</td>
<td>0.0519</td>
<td>0.37</td>
</tr>
<tr>
<td>OAC</td>
<td>4.53</td>
<td>0.009</td>
<td>0.784</td>
<td>1.07</td>
<td>7.48</td>
</tr>
<tr>
<td>MES</td>
<td>6.15</td>
<td>0.05</td>
<td>2.27</td>
<td>3.56</td>
<td>30.70</td>
</tr>
<tr>
<td>DMG</td>
<td>6.67</td>
<td>0.11</td>
<td>3.85</td>
<td>11.75</td>
<td>42.98</td>
</tr>
<tr>
<td>Bicine</td>
<td>8.35</td>
<td>0.102</td>
<td>14.70</td>
<td>16.2</td>
<td>447</td>
</tr>
<tr>
<td>OH</td>
<td>15.75</td>
<td>80.0</td>
<td>3.0×10⁴</td>
<td>9.0×10⁴</td>
<td>1.6×10⁶</td>
</tr>
</tbody>
</table>

\(a\). The unit of the microscopic rate constant of each catalyst is \text{M}^{-1}\text{sec}^{-1} except solvent.
Solvent catalyst is measured in \text{sec}^{-1}.
\(b\). The corresponding constant was not observed.
The activation of solvent, general base and specific base catalysis on enolization follows the same order as predicted by the coordination tendency of these divalent metal ion toward oxaloacetate dianion. The stabilities of metal complexes follow this order:

\[ \text{ZnOX(enol)} > \text{MnOX(enol)} > \text{MgOX(enol)}. \]

For a given catalyst, e.g. MES\(^-\), the microscopic rate constants also follow the same trend. The values of MgOX, MnOX and ZnOX are \(2.27 \text{ M}^{-1}\text{sec}^{-1}\), \(3.56 \text{ M}^{-1}\text{sec}^{-1}\) and \(30.70 \text{ M}^{-1}\text{sec}^{-1}\), respectively. Among the buffers studied, the catalytic abilities of base components are directly related to their pKas. This relationship is generally applicable to all of the three metal complexes. For example, the microscopic rate constants of Mg(II) promoted general base catalysis follow this order:

\[ \text{Bicine}^\text{-} > \text{DMG}^2^- > \text{MES}^- > \text{OAC}^- \]

which is the order of their pKas. Identical trend is also observed in Mn(II) and Zn(II) accelerated enolization.

The enolization of oxaloacetate dianion provides a relatively good model system for studying the variation of Bronsted coefficients caused by metal ions. The Bronsted coefficients are common measures of the sensitivity of a reactant to changes in the acidity or basicity of a catalyst. As shown in Table 52, the Bronsted \(\beta\)s which
indicate the pKa dependency of general base catalysis of uncomplexed OX, MgOX, MnOX and ZnOX are 0.34, 0.27, 0.26 and 0.25, respectively. It is seen that complexing to a metal ion causes a small decrease in the β value. Although the differences between the Bronsted values are relative small, the trends follow the coordinating ability of the metal ion. Table 52 gives the overall formation constants of metal-oxaloacetate complexes and the corresponding Bronsted values.

A metal ion may be regarded as an electron withdrawing substituent. As shown in equation 61 the electrostatic interaction between metal ion and keto oxygen atom stabilizes the intermediate and eases the deprotonation of the carbon which is next to keto group. Consequently, the removal of proton by general base catalysis is less sensitive to the basicity of this component. This is reflected in the smaller Bronsted coefficient for enolization reaction. A Bronsted plot showing the pKa dependency of the catalytic constants of free oxaloacetate and metal-oxaloacetate complexes is give in Figure 34.

The metal activation of general acid catalysis is somewhat more complicated than general base catalysis. The energy barrier height is reduced due to the presence of metal ion. However, the stabilization of oxyanion
TABLE 52

The Bronsted Coefficients of Oxaloacetate and Its Metal Complexes Involved in the Enolization Reactions

<table>
<thead>
<tr>
<th>reaction</th>
<th>log K (a)</th>
<th>Bronsted β</th>
</tr>
</thead>
<tbody>
<tr>
<td>uncomplexed OX²⁻</td>
<td>---</td>
<td>0.34</td>
</tr>
<tr>
<td>Mg²⁺ + OX²⁻ ⇌ MgOX</td>
<td>1.15</td>
<td>0.27</td>
</tr>
<tr>
<td>Mn²⁺ + OX²⁻ ⇌ MnOX</td>
<td>1.58</td>
<td>0.26</td>
</tr>
<tr>
<td>Zn²⁺ + OX²⁻ ⇌ ZnOX</td>
<td>2.34</td>
<td>0.25</td>
</tr>
</tbody>
</table>

a. K is the formation constant of a metal complex (MOX).
intermediate by metal ion is not as much as in the base catalysis because proton also stabilizes the negative charge on oxanion. In general, the overall enhancement of general acid catalysis by metal ion is the result of the competition between metal ion and proton. Therefore, the activation of acid catalysis is less substantial than that of base catalysis. The reaction mechanism involved in general acid catalyzed enolization is shown in equation 62.
\[
\begin{align*}
\text{FAST} & \quad \longrightarrow \\
\text{SLOW} & \\
\text{SLOW} & \\
\text{FAST} & \\
\end{align*}
\]
Figure 34: A Bronsted Plot of Metal Promoted Enolization of Oxaloacetate

- O --- OX
- O --- MgOX
- △ --- MnOX
- □ --- ZnOX
Figure 34
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Appendix A

THE FORTRAN PROGRAMS OF MINABS, RLXFT, CORNEK AND PPFIT
MINABS Program

// JOB
// TIME=(2,30), MSGCLASS=A, REGION=2048K
/* JOBPARM DISKIO=1000, LINES=5000, SERVICE=REGULAR
// PROCLIB DD DSN=HEP.PROCLIB, DISP=SHR
// EXEC FTXCLG
// A EXEC FTXCLG, GOREGN=2048K
// FORT.SYSIN DD *
C     MAIN DRIVER FOR MINUIT-CORNEK -RLXFT
C     BY SJT
C     NDAS=# OF DATA SETS
C     NPTS = # OF DATA POINTS
C     SEC = TIME BETWEEN EACH POINT
C     AINF = A INFINITE
C     TM = TIME OF EACH CORRESPONDING POINT
C     H1=K1  H2= K2
IMPLICIT REAL*8(A-H,O-Z)
COMMON/DAPTS/ OBS(4000), CALC(4000), WEIGHT(4000), NPTS, NDAS
COMMON/A/ TSP(4000, 5), SP(4000, 5), COMP(4000, 15), BETA(15), M(5, 15)
COMMON/H/ NBV
COMMON/E/ HY(35), EN(35), SEC(35), NPTS1
COMMON/C/TLIM1, NIND, NASS, NKN, NUNK, NC, NCNTL
COMMON/D/ TH(4000), H1(4000), H2(4000), X(4000), Y(4000)
COMMON/CONSTS/RK(25), FR(4000)
CALL UNDFRL
READ(5,*) NIND, NASS, NKN, NCNTL, TLIM1
WRITE(6,001) NIND, NASS, NKN, NCNTL, TLIM1
DO 20 I=1,NASS
READ(5,*)(M(J, I), J=1, NIND), BETA(I)
WRITE(6,002)(M(J, I), J=1, NIND)
WRITE(6,003) BETA(I)
BETA(I)=10**BETA(I)
20 CONTINUE
READ(5,*) NBV
READ(5,*) NDAS
READ(5,*) NPTS1
NPTS=0
NPTS=NPTS1*NDAS
DO 95 N=1,NDAS
READ(5,*) SEC(N)
READ(5,*)(TSP(N, J), J=1, NIND)
CONTINUE
READ(5,*) (OBS(I),I=1,NPTS)
DO 25 I=1,NPTS
   N=0
   N=(I-1)/NPTS1 + 1
   TM(I)=SEC(N)*(I-NPTS1*(N-1))
25 CONTINUE
DO 40 I=1,NPTS
   WEIGHT(I)=OBS(I)
   WEIGHT(I)=1.0/WEIGHT(I)**2
   TSP(I,NIND)=10**(-TSP(I,NIND))
40 CONTINUE
NUNK=NIND-NKN
NC=NUNK+1
DO 50 I=1,NDAS
   DO 45 J=1,NIND
      SP(I,J)=TSP(I,J)
   45 CONTINUE
IF(NASS.GT.0) CALL GENDIS(I)
50 CONTINUE
001 FORMAT(5X,4I5,1D12.3)
002 FORMAT(5X,5I5)
003 FORMAT(10X,1PD12.3)
004 FORMAT(5X,1D12.3)
006 FORMAT(5X,I3,1P6D12.3)
CALL MINUIT
STOP
END

SUBROUTINE GENDIS(I)
IMPLICIT REAL*8(A-H,O-Z)
COMMON/A/TSP(4000,5),SP(4000,5),COMP(4000,15),
   BETA(15),M(5,15)
COMMON/H/NBV
COMMON/E/HY(35),EN(35),SEC(35),NPTS1
COMMON/C/TLIM1,NIND,NASS,NKN,NUNK,NC,NCNTL
COMMON/DUM/AR(5,6)
TLIM=TLIM1*SP(I,1)
NCNT=0
UL=9.0
PL=0.9
20 DO 30 J=1,NUNK
   AR(J,NC)=TSP(I,J)-SP(I,J)
   DO 30 K=1,NASS
      COMP(I,K)=BETA(K)
DO 29 L=1,NIND
29 IF(M(L,K).NE.0) COMP(I,K)=COMP(I,K)*SP(I,L)**M(L,K)
30 AR(J,NC)=AR(J,NC)-M(J,K)*COMP(I,K)
    DO 40 J=1,NUNK
        IF(DABS(AR(J,NC)).GT.TLIM) GO TO 42
    CONTINUE
    RETURN
40 DO 50 J=1,NUNK
    DO 49 K=1,NUNK
        AR(J,K)=0.0
    CONTINUE
49 IF((M(K,L).NE.0).AND.(M(J,L).NE.0)) AR(J,K)=AR(J,K) +M(J,L)*M(K,L)*
    COMP(I,L)
50 AR(J,J)=AR(J,J)+1.0000
    K1=NUNK-1
    DO 70 J=1,K1
        K2=J+1
        DO 70 K=K2,NUNK
            IF(AR(K,J).EQ.0.0) GO TO 70
            R=AR(K,J)/AR(J,J)
            IF(DABS(R).LE.1.0) GO TO 61
        CONTINUE
        DO 69 N=J,NC
            T=AR(K,N)
            AR(K,N)=AR(J,N)
        CONTINUE
        DO 69 L=J,NC
            AR(K,L)=AR(K,L)-R*AR(J,L)
        CONTINUE
42 DO 50 J=1,NUNK
    DO 49 K=1,NUNK
        AR(J,K)=0.0
    CONTINUE
48 IF((M(K,L).NE.0).AND.(M(J,L).NE.0)) AR(J,K)=AR(J,K) +M(J,L)*M(K,L)*
    COMP(I,L)
49 AR(J,K)=AR(J,K)/SP(I,K)
50 AR(J,J)=AR(J,J)+1.0000
    K1=NUNK-1
    DO 70 J=1,K1
        K2=J+1
        DO 70 K=K2,NUNK
            IF(AR(K,J).EQ.0.0) GO TO 70
            R=AR(K,J)/AR(J,J)
            IF(DABS(R).LE.1.0) GO TO 61
        CONTINUE
        DO 69 N=J,NC
            T=AR(K,N)
            AR(K,N)=AR(J,N)
        CONTINUE
        DO 69 L=J,NC
            AR(K,L)=AR(K,L)-R*AR(J,L)
        CONTINUE
70 CONTINUE
    DO 80 J=1,NUNK
        NU=NC-J
        L=J-1
        IF(L.LE.0) GO TO 80
    CONTINUE
    RETURN
79  AR(NU,NC)=AR(NU,NC)-AR(NU,N)*AR(N,NC)
80  AR(NU,NC)=AR(NU,NC)/AR(NU,NU)
    DO 90 J=1,NUNK
    IF(AR(J,NC).GT.UL*SP(I,J)) AR(J,NC)=UL*SP(I,J)
    IF(-AR(J,NC).GT.PL*SP(I,J)) AR(J,NC)=-PL*SP(I,J)
90  SP(I,J)=SP(I,J)+AR(J,NC)
    NCNT=NCNT+1
    IF(NCNT.GT.NCNTL) GO TO 100
    IF(NCNT.NE.NCNTL/2) GO TO 20
    UL=1.0
    PL=0.5
    GO TO 20
100 WRITE(6,101) I
    WRITE(6,102) NCNT
102 FORMAT(’NCNT = ’,I5)
101 FORMAT(5X,’NONCONVERGENCE FOR EXP. 0’,I5)
    WRITE(6,1) (SP(I,II),II=1,NIND)
    WRITE(6,1) (COMP(I,II), II=1,NASS)
    WRITE(6,1) (AR(J,NC),J=1,NUNK)
    STOP
1 FORMAT(1P6D12.2)
END
SUBROUTINE MATIV(N)
IMPLICIT REAL*8(A-H,O-Z)
COMMON/F/ A1(35),A2(35),AINF(35),ARRAY(3,3)
DIMENSION IK(3), JK(3)
DET=1.
DO 100 K=1,3
AMAX=0.
21 DO 30 I=K,3
   DO 30 J=K,3
      IF(DABS(AMAX)-DABS(ARRAY(I,J))) 24,24,30
24 AMAX=ARRAY(I,J)
   IK(K)=I
   JK(K)=J
30 CONTINUE
   IF(AMAX) 41,32,41
32 DET=0.
   GO TO 140
41 I=IK(K)
   IF(I-K) 21,51,43
43 DO 50 J=1,3
      SAVE=ARRAY(K,J)
      ARRAY(K,J)=ARRAY(I,J)
50 ARRAY(I,J)=-SAVE
51 J=JK(K)
   IF(J-K) 21,61,53
53 DO 60 I=1,3
      SAVE=ARRAY(I,K)
      ARRAY(I,K)=ARRAY(I,J)
60 ARRAY(I,J)=-SAVE
61 DO 70 I=1,3
      IF(I-K) 63,70,63
63 ARRAY(I,K)=ARRAY(I,K)/AMAX
70 CONTINUE
   DO 80 I=1,3
   DO 80 J=1,3
      IF(I-K) 74,80,74
74 IF(J-K) 75,80,75

75 ARRAY(I,J)=ARRAY(I,J)+ARRAY(I,K)*ARRAY(K,J)
80 CONTINUE
   DO 90 J=1,3
5 IF(J-K) 83,90,83
83 ARRAY(K,J)=ARRAY(K,J)/AMAX
90 CONTINUE
   ARRAY(K,K)=1./AMAX
100 DET=DET*AMAX
   DO 130 L=1,3
      K=3-L+1
      J=IK(K)
   IF(J-K) 111,111,105
105 DO 110 I=1,3
      SAVE=ARRAY(I,K)
ARRAY(I,K)=-ARRAY(I,J)
110 ARRAY(I,J)=SAVE
111 I=JK(K)
113 DO 120 J=1,3
115 SAVE=ARRAY(K,J)
116 ARRAY(K,J)=-ARRAY(I,J)
117 CONTINUE
120 ARRAY(I,J)=SAVE
130 CONTINUE
140 RETURN
END

SUBROUTINE FCN(NPAR,GV,FVAL,XV,IFLAG)
IMPLICIT REAL*8(A-H,O-Z)
COMMON/A/TSP(4000,5),SP(4000,5),COMP(4000,15),BETA(15),M(5,15)
COMMON/H/NBV
COMMON/E/HY(35),EN(35),SEC(35),NPTS1
COMMON/C/TLIM1,NIND,NASS,NKN,NUNK,NC,NCNTL
COMMON/DAPTS/OBS(4000),CALC(4000),WEIGHT(4000),NPTS,NDAS
COMMON/D/TM(4000),H1(4000),H2(4000),X(4000),Y(4000)
COMMON/CONSTS/RK(25),FR(4000)
COMMON/F/A1(35),A2(35),AINF(35),ARRAY(3,3)
DIMENSION XV(23),GV(23)
DIMENSION A14(35),A15(35),A16(35),SQ(35)
DIMENSION A11(35),A12(35),A13(35),A22(35),A23(39)
DIMENSION A21(35),A32(35),A31(35),A33(35)
IF(IFLAG .LT. 3 ) RETURN
C ASSIGN THE XV TO THE APPROPRIATE CONSTANTS
DO 30 I=1,21
30 RK(I)=XV(I)
C SUMSQ=0.0
DO 100 I=1,NPTS
IF(NBV .EQ. 0) GO TO 50
CALL GENDIS(I)
FR(I)=0.0
100 CONTINUE
C DO 60 N=1,NDAS
CALL FITFC(N)
A11(N)=0.
A12(N)=0.
A13(N)=0.
293
A14(N)=0.
A15(N)=0.
A16(N)=0.
A22(N)=0.
A23(N)=0.
A1(N)=0.0
A2(N)=0.0
AINF(N)=0.0
SQ(N)=0.
60 CONTINUE
DO 70 I=1,NPTS
   N=0
   N=(I-1)/NPTS1 + 1
   X(I)=DEXP(-H1(N)*TM(I))
   Y(I)=DEXP(-H2(N)*TM(I))
   A11(N)=X(I)*X(I)+A11(N)
   A12(N)=X(I)*Y(I)+A12(N)
   A13(N)=X(I)+A13(N)
   A22(N)=Y(I)*Y(I)+A22(N)
   A23(N)=Y(I)+A23(N)
   A14(N)=X(I)*OBS(I)+A14(N)
   A15(N)=Y(I)*OBS(I)+A15(N)
   A16(N)=OBS(I)+A16(N)
   A21(N)=A12(N)
   A32(N)=A23(N)
   A31(N)=A13(N)
   A33(N)=NPTS1
70 CONTINUE
DO 80 N=1,NDAS
   ARRAY(1,1)=A11(N)
   ARRAY(2,1)=A21(N)
   ARRAY(3,1)=A31(N)
   ARRAY(1,2)=A12(N)
   ARRAY(2,2)=A22(N)
   ARRAY(3,2)=A32(N)
   ARRAY(1,3)=A13(N)
   ARRAY(2,3)=A23(N)
   ARRAY(3,3)=A33(N)
   CALL MATIV(N)
   A1(N)=ARRAY(1,1)*A14(N)+ARRAY(1,2)*
1A15(N)+ARRAY(1,3)*A16(N)
   A2(N)=ARRAY(2,1)*A14(N)+ARRAY(2,2)*A15(N)+
1ARRAY(2,3)*A16(N)
   AINF(N)=ARRAY(3,1)*A14(N)+ARRAY(3,2)*A15(N)+
1ARRAY(3,3)*A16(N)
80 CONTINUE
DO 90 I=1,NPTS
  N=0
  N=(I-1)/NPTS1 + 1
  FR(I)=A1(N)*X(I)+A2(N)*Y(I)+AINF(N)
  YCALC=FR(I)
  SUMSQ=SUMSQ+WEIGHT(I)*(OBS(I)-YCALC)**2
  CALC(I)=YCALC
90 CONTINUE
  SUMSQ=SUMSQ/(NPTS)
  FVAL=SUMSQ
  IF (IFLAG .NE. 3 ) RETURN
  WRITE(6,25)NIND,NASS,NKN,NCNTL,NBV,TLIM1
  DO 150 I=1,NASS
    WRITE(6,26)(M(J,I),J=1,NIND)
    BETA(I)= DLOG10(BETA(I))
    WRITE(6,27) BETA(I)
150 CONTINUE
  DO 85 I=1,NPTS
    N=0
    N=(I-1)/NPTS1 +1
    SQ(N)=SQ(N)+WEIGHT(I)*(OBS(I)-FR(I))**2
85 CONTINUE
  DO 95 N=1,NDAS
    SQ(N)=SQ(N)/NPTS1
95 CONTINUE
25 FORMAT(///,5X,5I5,1D12.3,///)
26 FORMAT(5X,10I5)
27 FORMAT(20X,'LOG10 BETA = ',1P1D12.3)
  WRITE(6,3)
  WRITE(6,4)
  WRITE(6,5)
  WRITE(6,6)
  WRITE(6,7)
  WRITE(6,8)
  WRITE(6,9)
  WRITE(6,10)
  WRITE(6,11)
  WRITE(6,12)
  WRITE(6,13)
  WRITE(6,14)
  WRITE(6,15)
  WRITE(6,16)
  WRITE(6,17)
WRITE(6,18)
WRITE(6,19)
WRITE(6,20)
WRITE(6,21)
WRITE(6,22)
WRITE (6,001) SUMSQ
DO 300 N=1,NDAS
TSP(N,NIND)=-DLOG10(TSP(N,NIND))
WRITE(6,37) N, (TSP(N,J),J=1,NIND)
300 CONTINUE
DO 330 N=1,NDAS
WRITE(6,39) N,HY(N),EN(N)
330 CONTINUE
WRITE(6,36)
36 FORMAT(//,10X,'N',8X,'A1',7X,'H1',9X,'A2',8X,
1'H2',7X,'AINF',7X,'SUMSQ',//)
DO 110 N=1,NDAS
WRITE(6,38) N, A1(N), H1(N), A2(N), H2(N), AINF(N), SQ(N)
110 CONTINUE
34 FORMAT(5X,1P2D10.2)
DO 200 I=1,NPTS
WRITE(6,002) I, OBS(I), FR(I)
200 CONTINUE
RETURN
001 FORMAT(//,5X,'SUMSQ = ',1PD12.3,//)
002 FORMAT(2X,I5,1P6D10.2)
37 FORMAT(10X,I3,1P6D10.2,//)
38 FORMAT(10X,I3,1P6D10.2,//)
39 FORMAT(8X,I3,'HY = ',1PD12.3,5X,'EN = ',1PD12.3)
3 FORMAT(//,10X,'RK1: AC+OXK = OXHY'
4 FORMAT(10X,'RK2: HAC + OXK = OXHY'
5 FORMAT(10X,'RK3: H + OXK = = OXHY')
6 FORMAT(10X,'RK4: OH + OXK = OXHY')
7 FORMAT(10X,'RK5: OXK = OXHY')
8 FORMAT(10X,'RK6: H + M + OXK = M + OXHY')
9 FORMAT(10X,'RK7: HAC + M+OXK = M+OXHY')
10 FORMAT(10X,'RK8: AC + M+OXK = M+OXHY')
11 FORMAT(10X,'RK9: M+OXK = M+OXHY')
12 FORMAT(10X,'RK10: OXK + AC = OXE')
13 FORMAT(10X,'RK11: OXK + HAC = OXE')
14 FORMAT(10X,'RK12: OXK + M+OH = OXH')
SUBROUTINE FITFC(I)
IMPLICIT REAL*8(A-H,0-Z)
COMMON/A/TSP(4000,5),SP(4000,5),COMP(4000,15),
BETA(15),M(5,15)
COMMON/H/NBV
COMMON/E/HY(35),EN(35),SEC(35),NPTS1
COMMON/D/TM(4000),H1(4000),H2(4000),X(4000),Y(4000)
COMMON/CONSTS/RK(25),FR(4000)

C
C   FORWARD RATES: KETO -> HYD; KETO -> ENOL
C   ENTER FITTING FUNCTION HERE
C
OXAC HYD AND ENOL RATES IN HOAC BUFFERS
H=SP(I,4)
OXK=SP(I,2)/(1.0+0.148+0.086)
HOXK=COMP(I,2)/(1.0+0.150+0.77)
OXE=.148*OXK
OXY=.086*OXK
HOXY=0.77*HOXK
HOXE=0.15*HOXK
AC=SP(I,1)
HAC=COMP(I,1)
OH=1.27D-14/H
AM=SP(I,3)
AMOXK=COMP(I,3)/(1.0+RK(21)+0.3)
AMOXE=0.3*AMOXK
AMOXH=AMOXK*RK(21)
F1=(RK(1)*AC+RK(2)*HAC+RK(4)*OH+RK(5)+RK(3)*H)
F1=F1+(RK(6)*H+RK(7)*HAC+RK(8)*AC+RK(9)+RK(12)*OH)
F1=F1*AMOXK/OXK
F3=RK(10)*AC+RK(11)*HAC+RK(13)*OH+RK(17)*H
F3=F3+(RK(14)*AC+RK(15)+RK(16)*HAC+RK(19)*H+RK(20)*OH)
F3=F3+AMOXK/OXK
F1=F1*OXK/(OXK+HOXK+AMOXK)
R1=F1/0.086
F3=F3*0XK/(0XK+HOXK+AMOXK)
R3=F3/0.148
B=-(F1+F3+R1+R3)
C=R1*R3+F1*R3+R1*F3
SQ=DSQRT(B*B-4.0*C)
H1(1)=(-B-SQ)/2.0
H2(1)=(-B+SQ)/2.0
HY(1)=F1
EN(1)=F3
RETURN
END

/GO.FT07FO01 DD DSN=TS1403.SJT,
// UNIT=USERDA,DCB=(RECFM=FB,LRECL=80,BLKSIZE=3120),
// SPACE=(TRK,(1,1)),DISP=(OLD,KEEP)
//GO.SYSIN DD *
SJT AC-OXAC EXP3, 06/28/84

   1.  RK1  .000  .0000  0.0  0.00
   2.  RK2  .146  .00  .0  1.0
   3.  RK3  541.5  0.0  000  2000.
   4.  RK4  000.  00  0.00  0.0  1.0
   5.  RK5  014  0000  0.00  0.0  1.0
   6.  RK6  0.00  0000  0.0  0.0  1.0
   7.  RK7  0.0  0000  0.0  5.0
   8.  RK8  0.0  0.00  0.0  5.0
   9.  RK9  0.18  0000  0.0  1.0
  10.  RK10  0.00  0.000  0.0  1.0
  11.  RK11  1.71  0.0  80  10.0
  12.  RK12  0.0  0.0  0.0  1.0
  13.  RK13  0.0  0.0  0.0  1.0
  14.  RK14  1.0  10  0.0  3.0
  15.  RK15  0.272  0.0000  0.0  1.0
  16.  RK16  2.0  0.2  0.0  14.0
  17.  RK17  1073.4  0.00  000  2000.
  18.  RK18  0.0  0.0  0.0  1000.
  19.  RK19  300  30.0  0.0  1500.
  20.  RK20  0.0  0.0  0.0  1000.
  21.  RK21  0.475  0.000  0.0  50

SEEK
SIMPLEX
MICRAD
EXIT
/**
// EXEC WNOTIFY
//
PLXFT PROGRAM

RELAXATION FITTING PROGRAM WITH MULTIPLE MODES
INCLUDES SUMMATION MODE FOR BINARY DATA
ANY OR ALL VARIABLES MAY BE ITERATED

NOVEMBER 30, 1978, EMLV

INTEGER CV
DOUBLE PRECISION ALABELS(7)
COMMON/LABEL/ALABELS
DIMENSION NM1(4), NM2(4), NM3(4), INV(251), SAVE(7,9), SAV2(7), VF(248)
COMMON ZF(256), VF(256), R(7), SIGA(7), CV(7), VS(256)
14 FORMAT(5X,13,3F10.3)
4 FORMAT(3X,B9.3)
5 FORMAT(10X,13," POINTS AT ",F5.3," SECS/POINT")
6 FORMAT(3X,14I5)
10 FORMAT(7X,2B10.1)
6 FORMAT(" ESTS ARE ",/ " AINF ",E13.6,
2 FORMAT(6)
9 FORMAT(7X,12,2X,S8)
3 FORMAT(15X,6."(016)")
12 FORMAT(7X,S8,F10.4,(3X,F10.4),/ ,15X,4(F10.4,3X),/)
13 FORMAT(7X,S8,F10.3,(3X,F10.3),/ ,15X,4(F10.3,3X)/)
16 FORMAT(3X,"---------------------")

**************

TYPE " OUTPUT FILE NAME? "
READ(11,2) NM3(1)
CALL FOPEN(11,HMS(1))
ACCEPT "STOPPED-FLOW" (1) OR "AUTO" (2) DATA " TYPE
ACCEPT " AUTO INCREMENTING VERSION? " NO(0) OR YES(1) ", ISV
IF(ISV EQ 1) ACCEPT " NUMBER OF REPLICATE EXPERIMENTS", NREP
DO 30 I=1,7
SAVE(1)=0
30 A(I)=0

AUTO VERSION RETURNS TO 31

C
**SECTION 31: INPUT FILE NAME**

```
31 ICVV=0
  TYPE * "INPUT FILE NAME?"
  READ(11,2) NM2(1)
  IESP=1
  ICC=0
32 CALL FOPEN(2, NM2(1))
  WRITE(10,1) NM2(1)
  WRITE(4,1) NM2(1)
  IF (ITYPE.EQ.1) GO TO 34
```

**SECTION 32: CARRY INPUT**

```
  THIS SECTION IS FOR CARRY INPUT
  ACCEPT "NUMBER OF POINTS ??", NPTS
  ACCEPT "EQUALLY SPACED POINTS ??", IESP
  IF (IESP.EQ.0) GO TO 33
  ACCEPT "SECONDS/POINT ??", ZFI
  READ(2) (Y(I), I=1, NPTS)
  WRITE(10,4) (Y(I), I=1, NPTS)
  WRITE(4,4) (Y(I), I=1, NPTS)
  GO TO 40
33 IF (IESP.EQ.0) GO TO 33
34 IF (IESP.EQ.1) GO TO 35
35 END CARRY INPUT; TRANSFER CONTROL TO 40
```

**SECTION 34: STOPPED-FLOW INPUT SECTION**

```
  34 READ(2,2) NM2(1)
  READ BINARY(2) IOF, NMS, NPTS, (INVY(I), I=1, NPTS)
  ZFI=FLOAT(NMS)/10.**(IOF)
  WRITE(10,5) NPTS, ZFI
  NNPT=NPTS/14
  DO 35 J=1, NNPT
       K5=J+14
       K3=J+13
       WRITE(10,6) (INVY(I), I=K3, K5)
       WRITE(4,6) (INVY(I), I=K3, K5)
  CONTINUE
  K4=14+NNPT+1
  IF (NPTS.LE. K4) GO TO 36
  WRITE(10,6) (INVY(I), I=K4, NPTS)
  WRITE(4,6) (INVY(I), I=K4, NPTS)
  CONTINUE
  IF (ICVV.GE.1) GO TO 37
  ACCEPT "NO. OF POINTS TO BE SUMMED ??", NSUM
  IF (NSUM.EQ.1) GO TO 40
  ZFI=ZFI/NSUM
  NPTS=NPTS/NSUM
  NPTSR=NPTSR/NSUM
  TYPE * "TOTAL NO. OF POINTS AFTER SUMMING = ", NPTSR
  37 IF (NSUM.EQ.1) GO TO 40
  DO 39 K=1, NPTSR
       IVS=INVY((K-1)*NSUM+1)
  CONTINUE
  DO 39 J=2, NSUM
       IVS=IVS+INVY((K-1)*NSUM+J)
  39 CONTINUE
  IVS=INVY(K)=IVS
  40 END STOPPED-FLOW INPUT SECTION
```

**SECTION 40: INITIAL AND FINAL POINT NUMBERS**

```
  40 IF (ICVV.GE.0) ACCEPT "INITIAL AND FINAL POINT NUMBERS ??", NSF, NF11
  IF (IESP.GE.0) GO TO 42
  NPT=0
  IVS=1=NSF, NF11
  NPT=NPT+1
  VF(1)=VF=1
```
IF(ITYPE.NE.1) VF(JPT)=Y(JPT)
IF(ITYPE.NE.1) V(JPT)=Y(I)
41 IF(ITYPE.EQ.1) V(JPT)=INVS(I)

42 TYPE "NUMBER OF POINTS FIT ", JPT
WRITE(4,7) NSF, NFITN, JPT, NPTS, ZFI
7 FORMAT(7X, "ANALYSES POINTS FROM", I3, " TO", I4, " (*, I3, " OF *, I4,
* POINTS) AT ", F7.1, " SECS")
IF(CITY, GE. 1) GO TO 47
ACCEPT "ONE(1), TWO(2), OR THREE(3) RELAXATIONS ??", IRL
TYPE " 
IF(IRL.EQ.2) TYPE " A0BS=AINF+A1*EXP(-K1*T)+A2*EXP(-K2*T)"
IF(IRL.EQ.3) TYPE " A0BS=AINF+A1*EXP(-K1*T)+A2*EXP(-K2*T)"
IF(IRL.NE.1) GO TO 43

SINGLE RELAXATION SECTION

PROVIDES FOR ITS OWN ESTIMATES AND ITERATES ALL
THREE PARAMETERS AUTOMATICALLY

TYPE " A0BS=AINF + A1*EXP(-K1*T)"
R(1)=(Y(JPT-2)+Y(JPT-1)+Y(JPT))/3.0
R(2)=Y(JPT)-R(1)
DO 43 I=1, JPT
IF(ABS(Y(I)-R(I)).LT.ABS(A(2)/2.7)) GO TO 44
43 CONTINUE

44 R(I)=1./ZFI(I)
WRITE(10,8) R(1), R(2), R(3)
NTE=3
ACCEPT " DO YOU WISH TO CONTROL PARAMETERS ??", ICIT
IF(ICIT.EQ.1) GO TO 60
NTERM=1
NCVC=10
DO 59 I=1, 3
59 CV(I)=1
GO TO 47

FOR ONE RELAXATION TRANSFER CONTROL TO 47

CONTROL RESUMES HERE FOR MULTIPLE RELAXATIONS

45 TYPE " CODE NUMBERS ARE: ENTER ESTIMATES:"
60 NTE=2*IRL+1
DO 46 I=1, NTE
WRITE(10,9) I, ALABELS(I)
46 READ(11) R(I)
ACCEPT "NO. OF CONSTANTS TO BE VARIED ?? ", NTERM
TYPE " ENTER CODE NUMBERS FOR VARIABLES 1 THROUGH ", NTERM
READ(11) (CV(I), I=1, NTERM)
ACCEPT "NUMBER OF CYCLES TO BE REFINED ??", NCVC

CURFT STARTS HERE

47 FLAMO=0.001
CHISS=0.0
NDONE=0
DO 48 II=1, NCVC
NDONE=NDONE+1
CALL CURFT(JPT, NTERM, FLAMO, CHISS, IRL)
WRITE(10,10) (ALABELS(I), R(I), I=1, NTERM)
IF(ABS(CHISS-CHISSQ)/CHISS LT 0.001) GO TO 49
48 CHISS=CHISSQ

CURFT ENDS

OUTPUT OCCURS WHEN CURFT HAS SUCCESSFULLY EXITED TO 49
49 WRITE(4,11) CHISO, NDONE
11 FORMAT(7X, "CHISO ".,F8.3, 5X, "(REFINED", I3, " CYCLES")/ & 17X, "VARIABLE", 5X, "STD. DEV.")
   NCCT=1
   DO 51 I=1, NTE
      J=CV(NCCT)
      IF(ABS(A(I)), LE, (1)) GO TO 50
      IF(J.EQ.1) WRITE(4,12) ALABELS(I), A(I), SIGA(I)
      IF(J.NE.1) WRITE(4,12) ALABELS(I), A(I)
      GO TO 51
50 IF(J.EQ.1) WRITE(4,13) ALABELS(I), A(I), SIGA(I)
      IF(J.NE.1) WRITE(4,13) ALABELS(I), A(I)
   51 IF(I.EQ.J AND NCCT.NE.LT.NTERM) NCCT=NCCT+1
      IF(ISV.EQ.1) GO TO 53
   C CONTROL IS PASSED TO 53 FOR AUTO INCREMENTING VERSION
   C OTHERWISE, THIS IS THE OUTPUT SECTION
      I.E., ISV=0
      ACCEPT "OUTPUT POINTS INITIAL, FINAL ??", NSF, NFIN
      IF(NFIN.GT.LT.NSF) GO TO 52
      IF(ITYPE.EQ.1) GO TO 62
      DO 61 K=1, JPY
         61 Y(K)=VF(K)
      62 IF(ITYPE.EQ.1) NPTS=NPTSR
      DO 57 J=1, NPTS
         VS(J)=A(1)+*A(2)*EXP(-2*J*A(3))
         IF(IRL.GE.2) VS(J)=VS(J)+A(4)*EXP(-2*J*A(5))
         IF(IRL.LE.3) VS(J)=VS(J)+A(6)*EXP(-2*J*A(7))
         57 VF(J)=VS(J)-YF(J)
      WRITE(10,18)
         18 FORMAT(2X, 12X, "A(CALC)", 4X, "OBS-CALC")
      WRITE(10,14) (J, Y(J), VS(J), YF(J), J=NSF, NFIN)
      ACCEPT "STORE THIS PART OF OUTPUT ??", IST
      IF(IST.EQ.1) WRITE(4,18)
      IF(ISET.EQ.1) WRITE(4,14) (J, Y(J), VS(J), YF(J), J=NSF, NFIN)
   52 CALL FCLOSE(2)
      IF(NPTS.NE.0) GO TO 31
      ICC=ICC+1
      IF(ICC.NE.2) GO TO 31
      ICC=0
      WRITE(4,15)
   15 FORMAT(1H1)
      GO TO 31
   C CONTROL IS PASSED TO 31 FOR NON-AUTO INCREMENTING VERSION
   C FOR AUTO VERSION THE CONTROL RESUMES HERE AND ENDS WITH
   C A TRANSFER TO 31 ALSO (ISV=1)
   C
   53 CALL FCLOSE(2)
      ICC=ICC+1
      IF(ICC.NE.2) GO TO 54
      ICC=0
      WRITE(4,15)
   54 IF(ICC.NE.0) WRITE(4,16)
      ICYY=ICYY+1
      DO 55 JJ=1, NTE
         SAVE(JJ, ICYY)=A(JJ)
      55 SAVE(JJ, ICYY)=A(JJ)
      NM2(J)=NM2(J)+1
      IF(NV.LT.NREP) GO TO 32
17 FORMAT(2X, /, 10X, "AVERAGES ARE: ")
   DO 56 JJ=1, NTE
      A(JJ)=SAV2(JJ)/FLOAT(NREP)
      SAV2(JJ)=0.
      IF(ABS(A(JJ)).LE. (1.)) WRITE(4,13) ALABELS(JJ), A(JJ)
      56 IF(ABS(A(JJ)).GT. (0.1)) WRITE(4,12) ALABELS(JJ), A(JJ)
         WRITE(4,16)
   DO 58 JJ=1, NTE
      IF(ABS(SAVE(JJ, 1)).LE. (0.1)) WRITE(4,13) ALABELS(JJ), (SAVE(JJ, KK), &KK=1, NREP)
      IF(ABS(SAVE(JJ, 1)).GT. (0.1)) WRITE(4,12) ALABELS(JJ), (SAVE(JJ, KK), &KK=1, NREP)
   58 CONTINUE
      WRITE(4,15)
      ACCEPT "NEW DATA (1) ?? OR HALT(0) ??", INDC
      IF(INDC.NE.1) STOP
      GO TO 31
   END
CORNK PROGRAM

1. // JOB
2. // TIME=(1,38), MCCLASS-A, REGION=512K
3. //NOPARM DISK=1=1000, LINES=50000, SERVICE=REGULAR
4. //PROC18 DO DS=H,F,P,PROC18,DISP=D4
5. //EXEC F1DLG
6. //A EXEC F1DLG,REGION=512K
7. //FORT.51,6 DO #
8. C MAIN DRIVER FOR AIMIT-CORNK
9. IMPLICIT REAL*8(A-H,O-Z)
10. COMMON/DAFTS/ ORS(150), CALC(150), WEIGHT(150), NPS
11. COMMON/A/ TSP(150.5), SP(150.5), CMP(150.15), BETA(15), M(5.15), MBV
12. COMMON/C/TLIN1, HINO, MINO, MNUM, MHCNTL
13. COMMON/COMSTS/RX(25), RX(150), SP(150)
14. CALL UNIT
15. READ(S,H) HINO, MINO, MHCNTL, TLI1
16. WRITE(6,001) HINO, MINO, MHCNTL, TLI1
17. DO 20 I=1, MINO
18. READ(S,H) (I,J), J=1, HINO), BETA(J)
19. WRITE(6,002) (I,J), J=1, HINO
20. WRITE(6,003) BETA(J)
21. BETA(J)=BETA(J)+BETA(J)
22. CONTINUE
23. DO 50 I=1, MINO
24. READ(S,H) MBV
25. READ(S,H) NPS
26. DO 40 J=1, MBV
27. READ(S,H) TSP(I,J), J=1, HINO), ORS(I)
28. WEIGHT(I)+W(I, I)
29. WEIGHT(I)+W(I, I)+I
30. TSP(I,J)=TSP(I,J)+TSP(I,J)
31. CONTINUE
32. MNUM=HINO-MINO
33. MHCNTL=MHCNTL+1
34. DO 50 I=1, MINO
35. DO 45 J=1, HINO
36. SP(I,J)=TSP(I,J)
37. CONTINUE
38. IF(MINO .GE. 0) CALL GENOS(I)
39. CONTINUE
40. 001 FORMAT(5X,45.10D2.3)
41. 002 FORMAT(5X,5.5)
42. 003 FORMAT(1X,1PD0.2.3)
43. 004 FORMAT(5X,1PD0.2.3)
44. CALL AIMIT
45. STOP
46. END
47. C SUBROUTINE GENOS(I)
48. IMPLICIT REAL*8(A-H,O-Z)
49. COMMON/A/ TSP(150.5), SP(150.5), CMP(150.15), BETA(15), M(5.15), MBV
50. COMMON/C/TLIN1, HINO, MINO, MNUM, MHCNTL
51. COMMON/DUM/A(5,4)
52. TLIN1=TLIN1+SP(I,1)
53. MHCNTL=MHCNTL+1
54. NPS=FL-0.9
55. FL=0.9
56. FL=0.9
57. CONTINUE
58. 20 DO 50 J=1, MNUM
59. NPS(I,J)=TSP(I,J)+SP(I,J)
DO 30 K=1,MASS
30   CONTINUE
   RETURN
STOP
118. IMPLICIT REAL*4, A-H, Q-P,Z
119. COMMON/A/ ISP(150,5), SP(150,5), COM(150,15), ETA(15), R15, 15, MUV
120. COMMON/C/TLRI, MIND, mass, MIND, HC, MXTL
121. COMMON/DMPS/ OBS(150), CALC(150), HEIGHT(150), WPTS
122. COMMON/CONS/ RX(25), FR(150), SR(150)
123. DIMENSION IV(25), GX(25)
124. IF(IFLAG .LT. 3 ) RETURN
125. C ASSIGN THE IV TO THE APPROPRIATE CONSTANTS
126. DO 30 I=1,22
127. RX(I)=IV(I)
128. 30 CONTINUE
129. C
130. SUMS=0.0
131. DO 101 I=1,NPTS
132. IF (MUV .EQ. 0) GO TO 50
133. CALL GENOS(I)
134. SUM CONTINUE
135. CALL FITFCC(I)
136. TCALC=SR(I)
137. DO 100 I=1,NOS(I)
138. DO 5 I=1,NOS(I)
139. CALC(I)=TCALC
140. CONTINUE
141. SUMS=SUMS+WEIGHT(I)* (OS(I)-TCAICP)**2
142. CONTINUE
143. IF (IFLAG .NE. 3 ) RETURN
144. WRITE(4,25) MIND, mass, MIND, HC, MXTL, MUV, TLRI
145. DO 150 I=1, MASS
146. WRITE(6,26)(A(I,J), J=1,MIND)
147. BETAl(1)= DEGT1BETA(I)
148. WRITE(6,27) BETA(I)
149. CONTINUE
150. WRITE(6,28) BETAl(1)
151. WRITE(6,29) BETAl(1)
152. WRITE(6,30) BETAl(1)
153. WRITE(6,31) BETAl(1)
154. WRITE(6,32) BETAl(1)
155. WRITE(6,33) BETAl(1)
156. WRITE(6,34) BETAl(1)
157. WRITE(6,35) BETAl(1)
158. WRITE(6,36) BETAl(1)
159. WRITE(6,37) BETAl(1)
160. WRITE(6,38) BETAl(1)
161. WRITE(6,39) BETAl(1)
162. WRITE(6,40) BETAl(1)
163. WRITE(6,41) BETAl(1)
164. WRITE(6,42) BETAl(1)
165. WRITE(6,43) BETAl(1)
166. WRITE(6,44) BETAl(1)
167. WRITE(6,45) BETAl(1)
168. WRITE(6,46) BETAl(1)
169. WRITE(6,47) BETAl(1)
170. WRITE(6,48) BETAl(1)
171. WRITE(6,49) BETAl(1)
172. WRITE(6,50) BETAl(1)
173. WRITE(6,51) BETAl(1)
174. WRITE(6,52) BETAl(1)
175. WRITE(6,53) BETAl(1)
176. WRITE(6,54) BETAl(1)
177. WRITE(6,55) BETAl(1)
178. WRITE(6,56) BETAl(1)
179. WRITE(6,57) BETAl(1)
180. WRITE(6,58) BETAl(1)
181. WRITE(6,59) BETAl(1)
182. WRITE(6,60) BETAl(1)
183. WRITE(6,61) BETAl(1)
184. WRITE(6,62) BETAl(1)
185. WRITE(6,63) BETAl(1)
186. WRITE(6,64) BETAl(1)
187. WRITE(6,65) BETAl(1)
188. WRITE(6,66) BETAl(1)
189. WRITE(6,67) BETAl(1)
190. WRITE(6,68) BETAl(1)
191. WRITE(6,69) BETAl(1)
192. WRITE(6,70) BETAl(1)
193. WRITE(6,71) BETAl(1)
194. WRITE(6,72) BETAl(1)
195. WRITE(6,73) BETAl(1)
196. WRITE(6,74) BETAl(1)
197. WRITE(6,75) BETAl(1)
198. WRITE(6,76) BETAl(1)
199. WRITE(6,77) BETAl(1)
200. WRITE(6,78) BETAl(1)
201. WRITE(6,79) BETAl(1)
306

180. 200 CONTINUE

181. RETURN

182. 001 FORMAT(IX,'SUMSO = ',1P012.3//)

183. 002 FORMAT(IX,15,1P010.2)

184. 3 FORMAT(IX,'R(K): AC+OIX = OHTD')

185. 4 FORMAT(IX,'R(K): MAC + OIX = OHTD')

186. 5 FORMAT(IX,'R(K): H + OIX = OHTD')

187. 6 FORMAT(IX,'ON + OIX = OHTD')

188. 7 FORMAT(IX,'OEX : OIX = OHTD')

189. 8 FORMAT(IX,'R(K): H + R + OIX = R + OHTD')

190. 9 FORMAT(IX,'R(K): MAC + R + OIX = R + OHTD')

191. 10 FORMAT(IX,'R(K): AC + R + OIX = R + OHTD')

192. 11 FORMAT(IX,'R(K): OIX = R + OHTD')

193. 12 FORMAT(IX,'R(K): OIX = R + OHTD')

194. 13 FORMAT(IX,'R(K): OIX = R + OHTD')

195. 14 FORMAT(IX,'R(K): OIX = R + OHTD')

196. 15 FORMAT(IX,'R(K): OIX = R + OHTD')

197. 16 FORMAT(IX,'R(K): OIX = R + OHTD')

198. 17 FORMAT(IX,'R(K): OIX = R + OHTD')

199. 18 FORMAT(IX,'R(K): OIX = R + OHTD')

200. END

201. SUBROUTINE FITFC(I)

202. IMPLICIT REAL(A-H,0-Z)

203. COMMON/A,TS(150.5),SP(150.5),COM(150.15),RETA(15),R(5,15),NIV

204. COMMON/CONSTS/EX(25),FR(150.),SR(150.)

205. C

206. C FORWARD RATES: KETO -> HTD; KETO -> ENOL

207. C ENTER FITTING FUNCTION HERE

208. C OXAC DENTOD AND ENOL RATES IN HOAC BUFFERS

209. N=SP(1.4)

210. OEX=SP(1.2)/(1.0*10.148+0.86)

211. OIX=SP(1.2)/(1.0*10.154+0.77)

212. OEX=1.148*OIX

213. OIX=1.148*OIX

214. OHTD=SP(1.4)

215. OHTD=SP(1.4)

216. OHTD=SP(1.4)

217. OHTD=SP(1.4)

218. OHTD=SP(1.4)

219. OHTD=SP(1.4)

220. AA=SP(1.3)

221. AA=SP(1.3)

222. AA=SP(1.3)

223. F1=TR(1)*AC+TR(2)+MAC+TR(4)+OH+TR(5)*TR(1)*H

224. F1=TR(1)*AC+TR(2)+MAC+TR(4)+OH+TR(5)*TR(1)*H

225. 4 = ANOX/AX

226. 4 = ANOX/AX

227. 4 = ANOX/AX

228. 4 = ANOX/AX

229. 4 = ANOX/AX

230. 4 = ANOX/AX

231. 4 = ANOX/AX

232. 4 = ANOX/AX

233. 4 = ANOX/AX

234. 4 = ANOX/AX

235. 4 = ANOX/AX

236. 4 = ANOX/AX

237. 4 = ANOX/AX

238. 4 = ANOX/AX

239. 4 = ANOX/AX

240. 4 = ANOX/AX

241. 4 = ANOX/AX

242. 4 = ANOX/AX

243. 4 = ANOX/AX

244. 4 = ANOX/AX

245. 4 = ANOX/AX

246. 4 = ANOX/AX
RJ=*R4X(I00+R4X+R0X)+R0X)
B=(F1+F3+R3)
C=R43+R43+R1+F3
SO=0
FR(I)=-(S0)/2.0
SR(I)=(S0)/2.0
RETURN
END
//GO.F1017 DO 05H=1S1443.SJT.
// UNIT=USERDA.DCB=(REC=7F,UEQ=00,AKS=SIZE=1120).
// SPACE=1RK:(1,11),DISP=(BLD,KEEP)
//GO.SY1N DO
480. 4.5.1.100.1.183.84
481. 1.0.1.11.4.330
482. 1.1.11.3.821
483. 1.1.1.1.1.115
484. 1.1.0.2.1.4.330
485. 1.0.1.0.0.58
486. 1
487. 11
488. 1.0E-02, 1.0E-03, 7.58E-02, 4.57E+00, 0.015
489. 9.0E-02, 1.0E-03, 7.58E-02, 4.57E+00, 0.035
490. 1.58E-01, 1.0E-03, 7.58E-02, 4.57E+00, 0.063
491. 1.58E-01, 1.0E-03, 7.58E-02, 4.21E+00, 0.074
492. 4.0E-02, 1.0E-03, 7.58E-02, 4.57E+00, 0.073
493. 8.0E-02, 1.0E-03, 7.58E-02, 4.58E+00, 0.022
494. 1.0E-01, 1.0E-03, 7.58E-02, 4.88E+00, 0.019
495. 2.0E-02, 1.0E-03, 7.58E-02, 5.88E+00, 0.07E+02
496. 3.0E-02, 1.0E-03, 7.58E-02, 5.38E+00, 0.117
497. 4.0E-02, 1.0E-03, 7.58E-02, 5.48E+00, 0.135
498. 5.0E-02, 1.0E-03, 7.58E-02, 5.48E+00, 0.157
500. SJT AC-0XAC EXP X 1/1983 0
501. 1. RX1 0.0
502. 2. RX2 0.
503. 3. RX3 0.000
504. 4. RX4 0. 0.
505. 5. RX5 0.0 0.000 0.0 10.
506. 6. RX6 0.
507. 7. RX7 0.
508. 8. RX8 0. 0.8 0.10 0000.
509. 9. RX9 0.
510. 10. RX10 0.0 0.0 0.0110.
511. 11. RX11 1.0 0.0 0.0 20.
512. 12. RX12 0.
513. 13. RX13 0.0
514. 14. RX14 1.0 5.5 0.00 0.0 106.
515. 15. RX15 0.0 0.000 0.0 1800.
516. 16. RX16 0.0 0.
517. 17. RX17 1.13 0.0 0.0 1800.
518. 18. RX18 0.0 0.0 0.0 1800.
519. 19. RX19 1200.0 0.0 0.0 100808.
520. 20. RX20 0.0 0.0 0.0 10080.
521. 21. RX21 1.0 0.000 0.0 1800.
522. 22. RX22 0.0
523. 23. 0
524. 24.
525. 25.
527. 27.
528. 28.
529. 29.
530. 30.
531. 31.
532. 32.
533. 33.
534. 34.
535. 35.
536. 36.
537. 37.
538. 38.
539. 39.
540. 40.
541. 41.
542. 42.
543. 43.
1. // JOB ,
2. // TIME=(2,38),RGCLASS=4,REGION=512
3. //NJCPMK DISK=1=1988, LINES=5888,5=UNCE=REGULAR
4. //PROCLIB DO DSH=REP,PROCLIB,DISP=SFR
5. //EXEC FIXCLE
6. //A EXEC FIXCLE,GOREGION=512
7. //FORST IN DO *#"C
8. C MAIN DRIVER FOR AIMUII -DEFT
9. C BT S1J
10. C ORG-AINF+ABS(1)*RE,TIME
11. C RX(1)=REA
12. C RX(2)=AINF,REA
13. C RX(3)=A-INF+ABS
15. C COMMON/DAPS/ ORS(158),CALC(158),WEIGHT(158),MPTS
16. C COMMON/A/ RS(158),EK(158),SEC
17. C COMMON/CONS/RS(25),FR(158)
18. CALL UNDEF
19. READ(5,4) MPTS
20. READ(5,4) SEC
21. WRITE(6,404) SEC
22. READ(5,4) (ORS(I),I=1,NPTS)
23. DO 48 I=1,MPTS
24. WEIGHT(I)=0.1+0.05(I)
25. CONTINUE
26. CONTINUE 48 FORMAT(53,145,10,12,3)
27. CONTINUE 49 FORMAT(53,155)
28. CONTINUE 50 FORMAT(53,145,10,12,3)
29. CONTINUE 51 FORMAT(53,155)
30. CONTINUE 52 FORMAT(53,145,10,12,3)
31. CONTINUE 53 FORMAT(53,145,10,12,3)
32. CALL AIMUII
33. STOP
34. END
35. C
36. C
37. C SUBROUTINE FCO(NPAP,GY,FW,H,Vxu,FV,IFLAG)
38. C IMPLICIT REAL(A-H,0-2)
39. C COMMON/DAPS/ ORS(158),CALC(158),WEIGHT(158),MPTS
40. C COMMON/A/ RS(158),EK(158),SEC
41. C COMMON/CONS/RS(25),FR(158)
42. C DIMENSION XVI(3),GVX(3)
43. C IFCVLAG (1, 3) RETURN
44. C ASSIGN THE RV TO THE APPROPRIATE CONSTANTS
45. DO 38 I=1,3
46. RX(I)=VW(I)
47. CONTINUE 38
48. C SUMS=0.0
49. RS(I)=MS(I)
50. DO 100 I=1,3
51. RX(I)=MS(I)+RS(I)+RS(I-1)*RS(I-1)
52. EK(I)=SEC*RS(I)/2.0
53. EK(I)=E(K-1)-RX(I)+EK(I)+EK(I)*SEC*1
54. YCALC=FR(I)
55. SUMS=SUMS+YCALC
56. SUMS=SUMS+WEIGHT(I)*MS(I)
57. SUMS=SUMS+WEIGHT(I)*YCALC
58. SUMS=SUMS+YCALC
59. CONTINUE 100
60. C
FVM=SUMSQ
IF (IFLAG .NE. 3) RETURN
REAL RX(1)
ANF=RX(1)
ABS=RX(3)-ANF
WRITE(4,7) ANF
WRITE(4,8) ABS
WRITE(4,9) RX
WRITE(6,101) SUMSQ
DO 200 I=2,NPTS
WRITE(6,102) I,OBS(I),FR(I)
200 CONTINUE
RETURN
001 FORMAT(5X,'SUMSQ = ',F10.3,/) 002 FORMAT(23.13,F8.21)
7 FORMAT(5X,'ANF = ',F10.3,/) 8 FORMAT(5X,'ABS = ',F10.3,/) 170 FORMAT(5X,'REA = ',F10.3,/) END
//GO.FTBW1 DO DSN=151483.JT,
// UNIT=USERA.DCH=(RECIF=FB,RECIF=8B,BLKSIZE=3120),
// SPACE=(1IX(1,1),DISP=(OLD,KEEP)
//GO.STSN DO #
387. 119
388. 148
310. -201,-241,-231,-225,-219,-212,-211.
311. -212,-206,-282,-198,-198,-195,-192.
312. -184,-186,-188,-176,-175,-173,-166.
313. -167,-163,-162,-159,-157,-154,-150.
314. -147,-147,-146,-146,-143,-138,-136.
315. -136,-133,-128,-130,-125,-127,-126.
316. -118,-119,-116,-113,-114,-111,-111.
318. -100,-97,-98,-95,-95,-91,-92.
319. -92,-87,-86,-86,-83,-82,-82.
320. -79,-75,-72,-71,-66,-68,-65.
322. -48,-49,-44,-39,-39,-48,-35.
323. -34,-31,-33,-20,-25,-24,-21.
325. -9,-7,-5,-4,-7,-3,-1.
326. -1,-2,-1,-4,-4,-4,-9.
566. 511 ONG-DGAC EXP. 3/12/84
581. 1. RK1 .81 .881 .0 .8 1.0
582. 2. RK2 17. 1.7 .8 .8 200.
583. 3. RK3 150. 15. 0.0 2000.
524.
688. SEEK
681. START
681.5 AGRID
682. EXIT
683. /*
684. // EXEC WMNOTIFY
685. //
Appendix B

The Derivation of the Relaxation Rate Equations for the Dehydration-Hydration and Tautomerization Interconversions of Oxaloacetate in the Presence of Metal Ions.
By controlling the experimental condition, the decarboxylation of oxalacetate can be neglected. That is, only the dehydration-hydration equilibrium and tautomerization equilibrium are concerned. In aqueous solutions, the overall equilibrium and kinetic relations between hydrate, keto and enol species are fully described by the following equations (the charge on each species has been omitted).

\[ \begin{align*}
    \text{HOX} & \xrightleftharpoons[k_{1b}]{k_{1f}} \text{HOX}_{\text{enol}} & \quad \text{HOX} & \xrightleftharpoons[k_{4f}]{k_{4b}} \text{HOX}_{\text{hyd}} \\
    \text{OX} & \xrightleftharpoons[k_{2b}]{k_{2f}} \text{OX}_{\text{enol}} & \quad \text{OX} & \xrightleftharpoons[k_{5f}]{k_{5b}} \text{OX}_{\text{hyd}} \\
    \text{MOX} & \xrightleftharpoons[k_{3b}]{k_{3f}} \text{MOX}_{\text{enol}} & \quad \text{MOX} & \xrightleftharpoons[k_{6f}]{k_{6b}} \text{MOX}_{\text{hyd}} 
\end{align*} \]

Those \( k_{1f}, k_{2f} \) and \( k_{3f} \) stand for the forward enolization rates of monoprotonated oxaloacetate, HOX, oxaloacetate dianion, OX, and metal ion coordinated oxaloacetate, MOX, respectively. In addition, \( k_{4f}, k_{5f} \) and \( k_{6f} \) represent the forward hydration rates of HOX, OX and MOX, respectively. To simplify this system, the deprotonated hydrate species \( M_2H^{-1}OX^{+} \text{hyd} \) and the enolate species including \( H^{-1}OX^{3-}, MH^{-1}OX^{-} \) and \( M_2H^{-1}OX^{+} \) are not considered in the present derivation. However, the contribution of those species to the total concentration of \( \text{OX}_{\text{hyd}} \) and \( \text{OX}_{\text{enol}} \) has been taken into account in the data fitting process.

The mass balance equations can be obtained.

\[
    \text{OX}_{\text{total}} = \text{OX}_{\text{enol}} + \text{OX}_{\text{keto}} + \text{OX}_{\text{hyd}}
\]
\( OX_{\Sigma,\text{enol}} = [OX_{\text{enol}}] + [HOX_{\text{enol}}] + [MOX_{\text{enol}}] \)

\( OX_{\Sigma,\text{keto}} = [OX_{\text{keto}}] + [HOX_{\text{keto}}] + [MOX_{\text{keto}}] \)

\( OX_{\Sigma,\text{hyd}} = [OX_{\text{hyd}}] + [HOX_{\text{hyd}}] + [MOX_{\text{hyd}}] \)

Since the coordination of \( OX \) with either proton or metal ion is much faster than hydration-dehydration and tautomerization, this reaction can be regarded as "pre-equilibrium" reaction. Thus, the equilibrium relationships of the vertical equilibria stated in equation (1) are defined by the following equations.

\[
H + OX_{\text{enol}} \overset{\beta_{H,\text{enol}}}{\longrightarrow} HOX_{\text{enol}}
\]

\[
[HOX_{\text{enol}}] = \beta_{H,\text{enol}} \cdot [H] \cdot [OX_{\text{enol}}]
\]

\[
H + OX_{\text{keto}} \overset{\beta_{H,\text{keto}}}{\longrightarrow} HOX_{\text{keto}}
\]

\[
[HOX_{\text{keto}}] = \beta_{H,\text{keto}} \cdot [H] \cdot [OX_{\text{keto}}]
\]

\[
H + OX_{\text{hyd}} \overset{\beta_{H,\text{hyd}}}{\longrightarrow} HOX_{\text{hyd}}
\]

\[
[HOX_{\text{hyd}}] = \beta_{H,\text{hyd}} \cdot [H] \cdot [OX_{\text{hyd}}]
\]

\[
M + OX_{\text{enol}} \overset{\beta_{M,\text{enol}}}{\longrightarrow} MOX_{\text{enol}}
\]

\[
[MOX_{\text{enol}}] = \beta_{M,\text{enol}} \cdot [M] \cdot [OX_{\text{enol}}]
\]

\[
M + OX_{\text{keto}} \overset{\beta_{M,\text{keto}}}{\longrightarrow} MOX_{\text{keto}}
\]

\[
[MOX_{\text{keto}}] = \beta_{M,\text{keto}} \cdot [M] \cdot [OX_{\text{keto}}]
\]
$M + OX_{hyd} \xrightleftharpoons{\beta_{M,hyd}} MOX_{hyd}$

$[MOX_{hyd}] = \beta_{M,hyd} \cdot [M] \cdot [OX_{hyd}]$

$[M]_{total}$ and $[H]_{total}$ stand for the total concentration of metal ions and that of proton. The total mass balances on metal ion and proton are:

$[M]_{total} = [MOX_{enol}] + [MOX_{keto}] + [MOX_{hyd}] + [M]$

$[M]_{total} = \beta_{M,enol} \cdot [M] \cdot [OX_{enol}] + \beta_{M,keto} \cdot [M] \cdot [OX_{keto}]$

$+ \beta_{M,hyd} \cdot [M] \cdot [OX_{hyd}] + [M]$

$[M]_{total} = [M] \cdot (\beta_{M,enol} \cdot [OX_{enol}] + \beta_{M,keto} \cdot [OX_{keto}]$

$+ \beta_{M,hyd} \cdot [OX_{hyd}] + 1)$

$[H]_{total} = [HOX_{enol}] + [HOX_{keto}] + [HOX_{hyd}] + [H]$

$[H]_{total} = \beta_{H,enol} \cdot [H] \cdot [OX_{enol}] + \beta_{H,keto} \cdot [H] \cdot [OX_{keto}]$

$+ \beta_{H,hyd} \cdot [H] \cdot [OX_{hyd}] + [H]$

$[H]_{total} = [H] \cdot (\beta_{H,enol} \cdot [OX_{enol}] + \beta_{H,keto} \cdot [OX_{keto}]$

$+ \beta_{H,hyd} \cdot [OX_{hyd}] + 1)$

Under this experimental condition, two assumptions were made. Since the total concentration of metal was much greater than the total concentration of oxalacetate, pseudo first order reaction was employed and hydrogen ions concentration, $H$ remained at a constant level because buffer solutions were employed in all of the experiments.

The following rate laws describe the interconversion between
keto, hydrate and between keto and enol species.

\[
\frac{-d OX_{\text{enol}}}{dt} = k_{1b} \cdot [HOX_{\text{enol}}] - k_{1f} \cdot [HOX_{\text{keto}}] \\
+ k_{2b} \cdot [OX_{\text{enol}}] - k_{2f} \cdot [OX_{\text{keto}}] \\
+ k_{3b} \cdot [MOX_{\text{enol}}] - k_{3f} \cdot [MOX_{\text{keto}}] \tag{2}
\]

\[
\frac{-d OX_{\text{hyd}}}{dt} = k_{4b} \cdot [HOX_{\text{hyd}}] - k_{4f} \cdot [HOX_{\text{keto}}] \\
+ k_{5b} \cdot [OX_{\text{hyd}}] - k_{5f} \cdot [OX_{\text{keto}}] \\
+ k_{6b} \cdot [MOX_{\text{hyd}}] - k_{6f} \cdot [MOX_{\text{keto}}] \tag{3}
\]

\[
\frac{-d OX_{\text{keto}}}{dt} = k_{1f} \cdot [HOX_{\text{keto}}] - k_{1b} \cdot [HOX_{\text{enol}}] \\
+ k_{4f} \cdot [HOX_{\text{hyd}}] - k_{4b} \cdot [HOX_{\text{hyd}}] \\
+ k_{2f} \cdot [OX_{\text{keto}}] - k_{2b} \cdot [OX_{\text{enol}}] \\
+ k_{5f} \cdot [OX_{\text{keto}}] - k_{5b} \cdot [OX_{\text{hyd}}] \\
+ k_{6f} \cdot [MOX_{\text{keto}}] - k_{6b} \cdot [MOX_{\text{hyd}}] \tag{4}
\]

Equation (4) can be simplified as the following:

\[
\frac{-d OX_{\text{keto}}}{dt} = (k_{1f} + k_{4f}) \cdot [HOX_{\text{keto}}] - (k_{1b} \cdot [HOX_{\text{enol}}] \\
+ k_{4b} \cdot [HOX_{\text{hyd}}]) + (k_{2f} + k_{5f}) \cdot [OX_{\text{keto}}] \\
- (k_{2b} \cdot [OX_{\text{enol}}] + k_{5b} \cdot [OX_{\text{hyd}}]) \\
+ (k_{3f} + k_{6f}) \cdot [MOX_{\text{keto}}] - (k_{3b} \cdot [MOX_{\text{enol}}] \\
+ k_{6b} \cdot [MOX_{\text{hyd}}]) \tag{5}
\]
At any non-equilibrium stage, the concentration of a species is the sum of the equilibrium concentration and the residual concentration which are indicated by $\text{eq}$ and $\delta$, respectively.

\[
\frac{-d( OX_{\Sigma,\text{enol,eq}} + OX_{\Sigma,\text{enol}} )}{dt} = k_{1b} \cdot [HOX_{\text{enol,eq}} + \delta HOX_{\text{enol}}] - k_{1f} \cdot [HOX_{\text{keto,eq}} + \delta HOX_{\text{keto}}] + k_{2b} \cdot [OX_{\text{enol,eq}} + \delta OX_{\text{enol}}] - k_{2f} \cdot [OX_{\text{keto,eq}} + \delta OX_{\text{keto}}] + k_{3b} \cdot [MOX_{\text{enol,eq}} + \delta MOX_{\text{enol}}] - k_{3f} \cdot [MOX_{\text{keto,eq}} + \delta MOX_{\text{keto}}] \tag{6}
\]

\[
\frac{-d( OX_{\Sigma,\text{hyd,eq}} + OX_{\Sigma,\text{hyd}} )}{dt} = k_{4b} \cdot [HOX_{\text{hyd,eq}} + \delta HOX_{\text{hyd}}] - k_{4f} \cdot [HOX_{\text{keto,eq}} + \delta HOX_{\text{keto}}] + k_{5b} \cdot [OX_{\text{hyd,eq}} + \delta OX_{\text{hyd}}] - k_{5f} \cdot [OX_{\text{keto,eq}} + \delta OX_{\text{keto}}] + k_{6b} \cdot [MOX_{\text{hyd,eq}} + \delta MOX_{\text{hyd}}] - k_{6f} \cdot [MOX_{\text{keto,eq}} + \delta MOX_{\text{keto}}] \tag{7}
\]
\[-\frac{d}{dt} \left( \Sigma \text{keto,eq} + \Sigma \text{enol,eq} \right) = \frac{1}{dt} \left( k_{1b} \cdot [\text{HOX enol,eq} + \Sigma \text{HOX enol}] - k_{1f} \cdot [\text{HOX keto,eq} + \Sigma \text{HOX keto}] \right) + k_{4f} \cdot [\text{HOX keto,eq} + \Sigma \text{HOX keto}] - k_{4b} \cdot [\text{HOX hyd,eq} + \Sigma \text{HOX hyd}] + k_{2f} \cdot [\text{OX keto,eq} + \Sigma \text{OX keto}] - k_{2b} \cdot [\text{OX enol,eq} + \Sigma \text{OX enol}] + k_{5f} \cdot [\text{OX keto,eq} + \Sigma \text{OX keto}] - k_{5b} \cdot [\text{OX hyd,eq} + \Sigma \text{OX hyd}] + k_{3f} \cdot [\text{MOX keto,eq} + \Sigma \text{MOX keto}] - k_{3b} \cdot [\text{MOX enol,eq} + \Sigma \text{MOX enol}] + k_{6f} \cdot [\text{MOX keto,eq} + \Sigma \text{MOX keto}] - k_{6b} \cdot [\text{MOX hyd,eq} + \Sigma \text{MOX hyd}] \]

At equilibrium, 
\[-d \frac{O \Sigma \text{enol}}{dt} = 0 \]
\[-d \frac{O \Sigma \text{hyd}}{dt} = 0 \]
\[-d \frac{O \Sigma \text{keto}}{dt} = 0 \]

By subtracting the equilibrium equations (2), (3) and (5) from equations (6), (7) and (8), the near-equilibrium rate laws give the following result:

\[-d(\Sigma \text{enol}) = k_{1b} \cdot [\Sigma \text{HOX enol}] - k_{1f} \cdot [\Sigma \text{HOX keto}] + k_{2b} \cdot [\Sigma \text{OX enol}] - k_{2f} \cdot [\Sigma \text{OX keto}] + k_{3b} \cdot [\Sigma \text{MOX enol}] - k_{3f} \cdot [\Sigma \text{MOX keto}] \]
\[ -\frac{d(\delta OX_{\Sigma,\text{hyd}})}{dt} = \frac{1}{T_x} \cdot \delta OX_{\Sigma,\text{enol}} \]  

\[ = k_{4b} \cdot [\delta HOX_{\text{hyd}}] - k_{4f} \cdot [\delta HOX_{\text{keto}}] + k_{5b} \cdot [\delta OX_{\text{hyd}}] - k_{5f} \cdot [\delta OX_{\text{keto}}] + k_{6b} \cdot [\delta MOX_{\text{hyd}}] - k_{6f} \cdot [\delta MOX_{\text{keto}}] \]

\[ = \frac{1}{T_x} \cdot \delta OX_{\Sigma,\text{hyd}} \]  

\[ -\frac{d(\delta OX_{\Sigma,\text{keto}})}{dt} = k_{1f} \cdot [\delta HOX_{\text{keto}}] - k_{1b} \cdot [\delta HOX_{\text{enol}}] + k_{4f} \cdot [\delta HOX_{\text{keto}}] - k_{4b} \cdot [\delta HOX_{\text{hyd}}] + k_{2f} \cdot [\delta OX_{\text{keto}}] - k_{2b} \cdot [\delta OX_{\text{enol}}] + k_{5f} \cdot [\delta OX_{\text{keto}}] - k_{5b} \cdot [\delta OX_{\text{hyd}}] + k_{3f} \cdot [\delta MOX_{\text{keto}}] - k_{3b} \cdot [\delta MOX_{\text{enol}}] + k_{6f} \cdot [\delta MOX_{\text{keto}}] - k_{6b} \cdot [\delta MOX_{\text{hyd}}] \]

\[ = \frac{1}{T_x} \cdot \delta OX_{\Sigma,\text{keto}} \]  

\[ -\frac{d(\delta OX_{\Sigma,\text{enol}})}{dt} = k_{1b} \cdot ([\delta HOX_{\text{enol}}] / [\delta OX_{\text{enol}}]) \cdot [\delta OX_{\text{enol}}] - k_{1f} \cdot ([\delta HOX_{\text{keto}}] / [\delta OX_{\text{keto}}]) \cdot [\delta OX_{\text{keto}}] + k_{2b} \cdot ([\delta OX_{\text{enol}}] / [\delta OX_{\text{enol}}]) \cdot [\delta OX_{\text{enol}}] - k_{2f} \cdot ([\delta OX_{\text{keto}}] / [\delta OX_{\text{keto}}]) \cdot [\delta OX_{\text{keto}}] + k_{3b} \cdot ([\delta MOX_{\text{enol}}] / [\delta OX_{\text{enol}}]) \cdot [\delta OX_{\text{enol}}] - k_{3f} \cdot ([\delta MOX_{\text{keto}}] / [\delta OX_{\text{keto}}]) \cdot [\delta OX_{\text{keto}}] \]
\[ -\frac{d(S_{Ox, hyd})}{dt} = \frac{1}{\tau_z} \cdot S_{Ox, enol} \quad \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots (12) \]

\[ -\frac{d(S_{Ox, hyd})}{dt} = k_{4b} \cdot (\frac{[HOX_{hyd}]}{[Ox_{hyd}]}) \cdot [S_{Ox_{hyd}}] \]

\[ -k_{4f} \cdot (\frac{[HOX_{keto}]}{[Ox_{keto}]}) \cdot [S_{Ox_{keto}}] \]

\[ +k_{5b} \cdot (\frac{[Ox_{hyd}]}{[Ox_{hyd}]}) \cdot [S_{Ox_{hyd}}] \]

\[ -k_{5f} \cdot (\frac{[Ox_{keto}]}{[Ox_{keto}]}) \cdot [S_{Ox_{keto}}] \]

\[ +k_{6b} \cdot (\frac{[M0X_{hyd}]}{[Ox_{hyd}]}) \cdot [S_{Ox_{hyd}}] \]

\[ -k_{6f} \cdot (\frac{[M0X_{keto}]}{[Ox_{keto}]}) \cdot [S_{Ox_{keto}}] \]

\[ = \frac{1}{\tau_z} \cdot S_{Ox, hyd} \quad \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots (13) \]

\[ -\frac{d(S_{Ox, keto})}{dt} = k_{1f} \cdot (\frac{[HOX_{keto}]}{[Ox_{keto}]}) \cdot [S_{Ox_{keto}}] \]

\[ -k_{1b} \cdot (\frac{[HOX_{enol}]}{[Ox_{enol}]}) \cdot [S_{Ox_{enol}}] \]

\[ +k_{4f} \cdot (\frac{[HOX_{keto}]}{[Ox_{keto}]}) \cdot [S_{Ox_{keto}}] \]

\[ -k_{4b} \cdot (\frac{[HOX_{hyd}]}{[Ox_{hyd}]}) \cdot [S_{Ox_{hyd}}] \]

\[ +k_{2f} \cdot (\frac{[Ox_{keto}]}{[Ox_{keto}]}) \cdot [S_{Ox_{keto}}] \]

\[ -k_{2b} \cdot (\frac{[Ox_{enol}]}{[Ox_{enol}]}) \cdot [S_{Ox_{enol}}] \]

\[ +k_{5f} \cdot (\frac{[Ox_{keto}]}{[Ox_{keto}]}) \cdot [S_{Ox_{keto}}] \]

\[ -k_{5b} \cdot (\frac{[Ox_{hyd}]}{[Ox_{hyd}]}) \cdot [S_{Ox_{hyd}}] \]

\[ +k_{3f} \cdot (\frac{[M0X_{keto}]}{[Ox_{keto}]}) \cdot [S_{Ox_{keto}}] \]

\[ -k_{3b} \cdot (\frac{[M0X_{enol}]}{[Ox_{enol}]}) \cdot [S_{Ox_{enol}}] \]
\[ + k_{6f} \cdot \left( \frac{[\text{MOX}_{\text{keto}}]}{[\text{OX}_{\text{keto}}]} \right) \cdot [\text{SOX}_{\text{keto}}] \]
\[ - k_{6b} \cdot \left( \frac{[\text{MOX}_{\text{hyd}}]}{[\text{OX}_{\text{hyd}}]} \right) \cdot [\text{SOX}_{\text{hyd}}] \]
\[ = \left( k_{1f} + k_{4f} \right) \left( \frac{[\text{HOX}_{\text{keto}}]}{[\text{OX}_{\text{keto}}]} \right) \cdot [\text{SOX}_{\text{keto}}] \]
\[ - k_{1b} \left( \frac{[\text{HOX}_{\text{enol}}]}{[\text{OX}_{\text{enol}}]} \right) \cdot [\text{SOX}_{\text{enol}}] \]
\[ - k_{4b} \left( \frac{[\text{HOX}_{\text{hyd}}]}{[\text{OX}_{\text{hyd}}]} \right) \cdot [\text{SOX}_{\text{hyd}}] \]
\[ + \left( k_{2f} + k_{5f} \right) \left( \frac{[\text{OX}_{\text{keto}}]}{[\text{OX}_{\text{keto}}]} \right) \cdot [\text{SOX}_{\text{keto}}] \]
\[ - k_{2b} \left( \frac{[\text{OX}_{\text{enol}}]}{[\text{OX}_{\text{enol}}]} \right) \cdot [\text{SOX}_{\text{enol}}] \]
\[ - k_{5b} \left( \frac{[\text{OX}_{\text{hyd}}]}{[\text{OX}_{\text{hyd}}]} \right) \cdot [\text{SOX}_{\text{hyd}}] \]
\[ + \left( k_{3f} + k_{6f} \right) \left( \frac{[\text{MOX}_{\text{keto}}]}{[\text{OX}_{\text{keto}}]} \right) \cdot [\text{SOX}_{\text{keto}}] \]
\[ - k_{3b} \left( \frac{[\text{MOX}_{\text{enol}}]}{[\text{OX}_{\text{enol}}]} \right) \cdot [\text{SOX}_{\text{enol}}] \]
\[ - k_{6b} \left( \frac{[\text{MOX}_{\text{hyd}}]}{[\text{OX}_{\text{hyd}}]} \right) \cdot [\text{SOX}_{\text{hyd}}] \]
\[ = \frac{1}{k_x} \cdot [\text{SOX}_{\Sigma, \text{keto}}] \]

From equation (12)

\[ \frac{-d \left( [\text{SOX}_{\Sigma, \text{enol}}] \right)}{dt} = \frac{1}{k_x} \cdot [\text{SOX}_{\Sigma, \text{enol}}] \]

\[ = \left( k_{1b} \cdot [\text{HOX}_{\text{enol}}] + k_{2b} \cdot [\text{OX}_{\text{enol}}] + k_{3b} \cdot [\text{MOX}_{\text{enol}}] \right) \left( \frac{1}{[\text{OX}_{\text{enol}}]} \right) \cdot [\text{SOX}_{\text{enol}}] \]
\[ - \left( k_{1f} \cdot [\text{HOX}_{\text{keto}}] + k_{2f} \cdot [\text{OX}_{\text{keto}}] + k_{3f} \cdot [\text{MOX}_{\text{keto}}] \right) \left( \frac{1}{[\text{OX}_{\text{keto}}]} \right) \cdot [\text{SOX}_{\text{keto}}] \]

From equation (13)

\[ \frac{-d \left( [\text{SOX}_{\Sigma, \text{hyd}}] \right)}{dt} = \frac{1}{k_x} \cdot [\text{SOX}_{\Sigma, \text{hyd}}] \]

\[ = \left( k_{1b} \cdot [\text{HOX}_{\text{hyd}}] + k_{2b} \cdot [\text{OX}_{\text{hyd}}] + k_{3b} \cdot [\text{MOX}_{\text{hyd}}] \right) \left( \frac{1}{[\text{OX}_{\text{hyd}}]} \right) \cdot [\text{SOX}_{\text{hyd}}] \]
\[
\frac{-d(\delta_0X_{\Sigma,\text{hyd}})}{dt} = \frac{1}{\tau_2} \cdot \delta_0X_{\Sigma,\text{hyd}}
\]
\[
= (k_{4b} \cdot [\text{HOX}_{\text{hyd}}] + k_{5b} \cdot [\text{OX}_{\text{hyd}}] + k_{6b} \cdot [\text{MOX}_{\text{hyd}}])
\]
\[
(1/[\text{OX}_{\text{hyd}}] \cdot [\delta_0X_{\text{hyd}}] - (k_{4f} \cdot [\text{HOX}_{\text{keto}}] + k_{5f} \cdot [\text{OX}_{\text{keto}}] + k_{6f} \cdot [\text{MOX}_{\text{keto}}])(1/[\text{OX}_{\text{keto}}])
\]
\[
[\delta_0X_{\text{keto}}] 
\]
\[
.............. (16)
\]

From equation (14)

\[
\frac{-d(\delta_0X_{\Sigma,\text{keto}})}{dt} = \frac{1}{\tau_2} \cdot \delta_0X_{\Sigma,\text{keto}}
\]
\[
= \{ (k_{1f} + k_{4f}) [\text{HOX}_{\text{keto}}] + (k_{2f} + k_{5f}) [\text{OX}_{\text{keto}}] + (k_{3f} + k_{6f}) [\text{MOX}_{\text{keto}}] \}
\]
\[
(1/[\text{OX}_{\text{keto}}] \cdot [\delta_0X_{\text{keto}}] - \{k_{4b} \cdot [\text{HOX}_{\text{hyd}}] + k_{5b} \cdot [\text{OX}_{\text{hyd}}] + k_{6b} \cdot [\text{MOX}_{\text{hyd}}]\}
\]
\[
[\delta_0X_{\text{hyd}}] 
\]
\[
.............. (17)
\]

Since \( \delta_0X_{\Sigma,\text{enol}} = \delta_{\text{HOX}_{\text{enol}}} + \delta_{\text{OX}_{\text{enol}}} + \delta_{\text{MOX}_{\text{enol}}} \)
\[
= ([\text{HOX}_{\text{enol}}]/[\text{OX}_{\text{enol}}]).[\delta_{\text{OX}_{\text{enol}}}] + ([\text{OX}_{\text{enol}}]/[\text{OX}_{\text{enol}}]).[\delta_{\text{OX}_{\text{enol}}}] + ([\text{MOX}_{\text{enol}}]/[\text{OX}_{\text{enol}}]).[\delta_{\text{OX}_{\text{enol}}}] 
\]
\[
\delta_{0X_{\Sigma,\text{enol}}} = ([0X_{\Sigma,\text{enol}}]/[0X_{\text{enol}}]).[\delta_{0X_{\text{enol}}}] ...........(18)
\]
\[
\delta_{0X_{\Sigma,\text{keto}}} = ([0X_{\Sigma,\text{keto}}]/[0X_{\text{keto}}]).[\delta_{0X_{\text{keto}}}] ...........(19)
\]
\[
\delta_{0X_{\Sigma,\text{hyd}}} = ([0X_{\Sigma,\text{hyd}}]/[0X_{\text{hyd}}]).[\delta_{0X_{\text{hyd}}}] ...........(20)
\]

By substituting equations (18), (19) and (20) into equations (15), (16) and (17) individually, the following equations were obtained.

From equation (15)

\[
\frac{1}{T_2} \cdot \Sigma \Delta \Sigma \text{enol} = (k_{1b} \cdot [\text{HOX}_{\text{enol}}] + k_{2b} \cdot [\text{OX}_{\text{enol}}]) \\
+ k_{3b} \cdot [\text{MOX}_{\text{enol}}]) \cdot \left( \frac{\Delta \Sigma \text{enol}}{[\Sigma \Delta \Sigma \text{enol}]} \right) \\
- (k_{1f} \cdot [\text{HOX}_{\text{keto}}] + k_{2f} \cdot [\text{OX}_{\text{keto}}]) \\
+ k_{3f} \cdot [\text{MOX}_{\text{keto}}]) \cdot \left( \frac{\Delta \Sigma \text{keto}}{[\Sigma \Delta \Sigma \text{keto}]} \right)
\]

\[0 = \left( \frac{1}{[\Sigma \Delta \Sigma \text{enol}]} \right) - \frac{1}{T_2} \cdot \left( \frac{\Delta \Sigma \text{enol}}{[\Sigma \Delta \Sigma \text{enol}]} \right) \\
- (k_{1f} \cdot [\text{HOX}_{\text{keto}}] + k_{2f} \cdot [\text{OX}_{\text{keto}}]) + k_{3f} \cdot [\text{MOX}_{\text{keto}}]) \cdot \left( \frac{\Delta \Sigma \text{keto}}{[\Sigma \Delta \Sigma \text{keto}]} \right) \tag{21}
\]

From equation (16)

\[
\frac{1}{T_2} \cdot \Sigma \Delta \Sigma \text{hyd} = (k_{4b} \cdot [\text{HOX}_{\text{hyd}}] + k_{5b} \cdot [\text{OX}_{\text{hyd}}]) \\
+ k_{6b} \cdot [\text{MOX}_{\text{hyd}}]) \cdot \left( \frac{\Delta \Sigma \text{hyd}}{[\Sigma \Delta \Sigma \text{hyd}]} \right) \\
- (k_{4f} \cdot [\text{HOX}_{\text{keto}}] + k_{5f} \cdot [\text{OX}_{\text{keto}}]) + k_{6f} \cdot [\text{MOX}_{\text{keto}}]) \cdot \left( \frac{\Delta \Sigma \text{keto}}{[\Sigma \Delta \Sigma \text{keto}]} \right)
\]

\[0 = \left( \frac{1}{[\Sigma \Delta \Sigma \text{hyd}]} \right) - \frac{1}{T_2} \cdot \left( \frac{\Delta \Sigma \text{hyd}}{[\Sigma \Delta \Sigma \text{hyd}]} \right) \\
- (k_{4f} \cdot [\text{HOX}_{\text{keto}}] + k_{5f} \cdot [\text{OX}_{\text{keto}}]) + k_{6f} \cdot [\text{MOX}_{\text{keto}}]) \cdot \left( \frac{\Delta \Sigma \text{keto}}{[\Sigma \Delta \Sigma \text{keto}]} \right) \tag{22}
\]
From equation (17)

\[ 0 = \left( \frac{k_1f + k_4f}{[\text{HOX}_\text{keto}]} + \frac{k_2f + k_5f}{[\text{OX}_\text{keto}]} \right) + \left( \frac{k_3f + k_6f}{[\text{MOX}_\text{keto}]} \right) \cdot \left( \frac{1}{[\text{OX} \Sigma, \text{keto}]} \right) \]

\[ - \left( \frac{k_1b \cdot [\text{HOX}_\text{enol}]}{[\text{OX} \Sigma, \text{enol}]} + \frac{k_2b \cdot [\text{OX}_\text{enol}]}{[\text{MOX}_\text{enol}]} + \frac{k_3b \cdot [\text{MOX}_\text{enol}]}{[\text{OX} \Sigma, \text{enol}]} \right) \cdot \left( \frac{1}{[\text{OX} \Sigma, \text{enol}]} \right) \cdot [\text{OX} \Sigma, \text{enol}] \]

\[ - \left( \frac{k_4b \cdot [\text{HOX}_\text{hyd}]}{[\text{OX} \Sigma, \text{hyd}]} + \frac{k_5b \cdot [\text{OX}_\text{hyd}]}{[\text{MOX}_\text{hyd}]} + \frac{k_6b \cdot [\text{MOX}_\text{hyd}]}{[\text{OX} \Sigma, \text{hyd}]} \right) \cdot \left( \frac{1}{[\text{OX} \Sigma, \text{hyd}]} \right) \cdot [\text{OX} \Sigma, \text{hyd}] \]

Equations (21), (22), (23) may be equivalently represented by the following matrix notation as the product of a 3x3 coefficient matrix and a 3x1 residual matrix:

\[
\begin{pmatrix}
    a_{11} - \frac{1}{\tau} & a_{12} & a_{13} \\
    a_{21} & a_{22} - \frac{1}{\tau} & a_{23} \\
    a_{31} & a_{32} & a_{33} - \frac{1}{\tau}
\end{pmatrix}
\begin{pmatrix}
    [\text{SOX} \Sigma, \text{enol}] \\
    [\text{SOX} \Sigma, \text{hyd}] \\
    [\text{SOX} \Sigma, \text{keto}]
\end{pmatrix}
= \begin{pmatrix}
    0 \\
    0 \\
    0
\end{pmatrix}
\]

\[ \cdots \cdots \cdots \cdots (24) \]
Thus, equation (24) is rewritten as the following:

\[
\begin{bmatrix}
a_{11} - \frac{1}{\tau_x} & 0 & a_{13} \\
0 & a_{22} - \frac{1}{\tau_x} & a_{23} \\
- a_{11} & - a_{22} & - a_{13} - a_{23} - \frac{1}{\tau_x}
\end{bmatrix}
\begin{bmatrix}
\delta_{0X^\Sigma, \text{enol}} \\
\delta_{0X^\Sigma, \text{hyd}} \\
\delta_{0X^\Sigma, \text{keto}}
\end{bmatrix}
= \begin{bmatrix}
0 \\
0 \\
0
\end{bmatrix}
\]

\[\text{...............}(25)\]

By adding row 1 and row 2 to row 3, equation (25) can be simplified as,

\[
\begin{bmatrix}
a_{11} - \frac{1}{\tau_x} & 0 & a_{13} \\
0 & a_{22} - \frac{1}{\tau_x} & a_{23} \\
- \frac{1}{\tau_x} & - \frac{1}{\tau_x} & - \frac{1}{\tau_x}
\end{bmatrix}
\begin{bmatrix}
\delta_{0X^\Sigma, \text{enol}} \\
\delta_{0X^\Sigma, \text{hyd}} \\
\delta_{0X^\Sigma, \text{keto}}
\end{bmatrix}
= \begin{bmatrix}
0 \\
0 \\
0
\end{bmatrix}
\]

\[\text{...............}(26)\]

Thus, a new 3x3 coefficient matrix is obtained.

Since for a 3x3 matrix

\[
\begin{bmatrix}
a & d & g \\
b & e & h \\
c & f & i
\end{bmatrix}
\]

\[aei + bfg + cdh - gec - dbi - afh = 0\]

Therefore,

\[
(a_{11} - \frac{1}{\tau_x})(a_{22} - \frac{1}{\tau_x})(- \frac{1}{\tau_x}) - (a_{13})(- \frac{1}{\tau_x})(a_{22} - \frac{1}{\tau_x})
\]

\[-(a_{11} - \frac{1}{\tau_x}) (- \frac{1}{\tau_x}) a_{23} = 0\]

\[
a_{13}(- \frac{1}{\tau_x})(a_{22} - \frac{1}{\tau_x}) + a_{23}(a_{11} - \frac{1}{\tau_x})( \frac{1}{\tau_x})
\]

\[-(a_{11} - \frac{1}{\tau_x})(a_{22} - \frac{1}{\tau_x})( \frac{1}{\tau_x}) = 0\]

\[
( \frac{1}{\tau_x}) [a_{13}(a_{22} - \frac{1}{\tau_x}) + a_{23}(a_{11} - \frac{1}{\tau_x})
\]

\[-(a_{11} - \frac{1}{\tau_x})(a_{22} - \frac{1}{\tau_x})] = 0\]
\[(a_{11} - 1/\tau) \cdot \delta_{0X,\text{enol}} + a_{12} \cdot \delta_{0X,\text{hyd}} + a_{13} \cdot \delta_{0X,\text{keto}} = 0\]

\[a_{21} \cdot \delta_{0X,\text{enol}} + (a_{22} - 1/\tau) \cdot \delta_{0X,\text{hyd}} + a_{23} \cdot \delta_{0X,\text{keto}} = 0\]

\[a_{31} \cdot \delta_{0X,\text{enol}} + a_{32} \cdot \delta_{0X,\text{hyd}} + (a_{33} - 1/\tau) \cdot \delta_{0X,\text{keto}} = 0\]

where

\[a_{11} = \left\{ k_{1b} \cdot [H\text{OX}_{\text{enol}}] + k_{2b} \cdot [O\text{X}_{\text{enol}}] + k_{3b} \cdot [M\text{OX}_{\text{enol}}] \right\} \left( \frac{1}{[0X,\text{enol}]} \right)\]

\[a_{12} = 0\]

\[a_{13} = -\left( k_{1f} \cdot [H\text{OX}_{\text{keto}}] + k_{2f} \cdot [O\text{X}_{\text{keto}}] + k_{3f} \cdot [M\text{OX}_{\text{keto}}] \right) \left( \frac{1}{[0X,\text{keto}]} \right)\]

\[a_{21} = 0\]

\[a_{22} = \left( k_{4b} \cdot [H\text{OX}_{\text{hyd}}] + k_{5b} \cdot [O\text{X}_{\text{hyd}}] + k_{6b} \cdot [M\text{OX}_{\text{hyd}}] \right) \left( \frac{1}{[0X,\text{hyd}]} \right)\]

\[a_{23} = -\left( k_{4f} \cdot [H\text{OX}_{\text{keto}}] + k_{5f} \cdot [O\text{X}_{\text{keto}}] + k_{6f} \cdot [M\text{OX}_{\text{keto}}] \right) \left( \frac{1}{[0X,\text{keto}]} \right)\]

\[a_{31} = -a_{11}\]

\[a_{32} = -a_{22}\]

\[a_{33} = \left\{ (k_{1f} + k_{4f}) \cdot [H\text{OX}_{\text{keto}}] + (k_{2f} + k_{5f}) \cdot [O\text{X}_{\text{keto}}] + (k_{3f} + k_{6f}) \cdot [M\text{OX}_{\text{keto}}] \right\} \left( \frac{1}{[0X,\text{keto}]} \right)\]

\[= -a_{13} - a_{23}\]
Apparently, \( 1/\tau_k = 0 \), one of those three roots defined by equation (27), is the result of the decarboxylation relaxation of oxaloacetate. The quadratic root which is a function of the equilibrium concentration of each species and the microscopic rate constants,

\[
\frac{1}{\tau_k} = \frac{-b \pm \sqrt{b^2 - 4c}}{2}
\]

where \( b = a_{13} + a_{23} - a_{11} - a_{22} \)

\( c = a_{11} a_{22} - a_{13} a_{22} - a_{11} a_{23} \)

give the solutions to the coupled dehydration-hydration and tautomerization relaxation.
Appendix C

Equilibrium Data of Oxaloacetic Acid, Acetic Acid, 3,3-Dimethylglutaric Acid and Metal Complexes.
**pH Titration of Oxaloacetic Acid with 0.2785 M NaOH**

\( u = 0.275 \text{ (NaCl)}, 25.0 \degree C \)

---

**initial condition:** 20.10 ml of 30.00 mM H20X

**trial 1:**

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**pH Titration of Acetic Acid with 0.2785 M NaOH**

(u = 0.275 (NaCl), 25.0°C)

**Initial condition:** 20.10 ml of 60.00 mM HOAC

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**pH Titration of Mg(II)-Oxaloacetate Complex with 0.05012 M NaOH (u = 0.275 (NaCl), 25.0 oC)**

**initial condition:**

*total sample volume = 50.00 ml*

91.67 mM MgCl

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pH Titration of 3,3-Dimethylglutaric Acid in the Presence of Mg(II) with 0.2785 M NaOH (u = 0.2785 M (NaCl), 25.0°)

Initial condition:

Total sample volume = 60.00 ml
1.000 mM DMG
10.00 mM MgCl2

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**pH Titration of 3,3-Dimethylglutaric Acid in the Presence of Mn(II) with 0.2785 M NaOH (u = 0.275 (NaCl), 25.0 °C)**

**Initial condition:**

- Total sample volume = 50.00 ml
- 1.000 mM DMG
- 0.5000 mM MnCl₂

<table>
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<th>DIFF</th>
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**pH Titration of 3,3-Dimethylglutaric Acid in the Presence of Zn(II) with 0.2785 M NaOH**

**Initial condition:**

- **Total sample volume**: 60.00 ml
- **1.000 mM DMG**
- **1.000 mM ZnCl₂**

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