Kinetics and Mechanism of Oxidation of Glutathione reduced (GSH) and L-cysteine (L-cyst) by aqueous solution of piperidinium chloro chromate: A comparative study

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Abstract: The oxidation reaction of Glutathione reduced (GSH) and L-cysteine (L-cyst) has been studied spectrophotometrically over the range 4.6 ≤ \( [\text{substrate}] \) ≤ 13.8 (when substrate = GSH or L-cysteine), 0.03 ≤ \([H^+]\) ≤ 0.11, \( I = 0.3 \text{ mol dm}^{-3} \) (NaClO\(_4\)) and 293 K ≤ \( T \) ≤ 313 K. The rate of the reaction has been found to increase with the increase in \([\text{Substrate}]\) and \([H^+]\). The reaction follows first order kinetics in \([\text{Substrate}]\) and \([\text{H}^+]\). The reaction proceeds in two paths \( k_1 \) and \( k_2 \), where \( k_1 \) is acid independent path and \( k_2 \) is acid dependent path, both \( k_1 \) and \( k_2 \) were found to increase with the increase in temperature. For L-cysteine and GSH, the \( \Delta H^\circ \) (kJ mol\(^{-1}\)) for \( k_1 \) (sec\(^{-1}\)) paths were found to be 43.8 + 3.7, 27.05 + 1.3 and \( \Delta S^\circ \) (JK\(^{-1}\)mol\(^{-1}\)) were -161.1 + 12 and -212.5 + 4.3. For \( k_2 \)-path (mol\(^3\)dm\(^{-6}\)sec\(^{-1}\)), the \( \Delta H^\circ \) (kJ mol\(^{-1}\)) values were 53.3 + 3.4 and 67.5 + 12.3 and \( \Delta S^\circ \) (JK\(^{-1}\)mol\(^{-1}\)) values were -182.6 + 11 and 4.6 + 40.

Negative value of \( \Delta S^\circ \) indicates the reaction passes through an ordered transition state. The oxidation product of L-cysteine and GSH (reduced) is identified as L-cystine and GSSG, respectively.

Keywords: GSH, L-cysteine, spectrophotometry, kinetics, product, analysis, cystine and GSSG, activation parameters.

I. Introduction
A variety of mild and selective oxidising chromium (VI) reagents like pyridinium chloro chromate, pyridiniumbromo chromate, 2, 2-bipyridinium chloro chromate, imidazoliumchloro chromate, quinoliniumfluorochromate, quinolinium dichromate and isoquinolinium chloro chromate have been used widely in synthetic organic chemistry.1-9 In view of the increasing importance of these chromium (VI) reagents as potential and selective oxidising agents an attempt has been made to extend the study to other halochromates. This paper presents detail kinetics studies and mechanism of oxidation of GSH and L-cysteine by piperidinium chlorochromate10 in perchloric acid medium.

II. Experimental
a) Method And Material
The reactant complex, piperidinium chloro chromate (Pipcc), was prepared and characterized according to the reported method. All other chemicals used were of Analar grade. Doubly distilled water was used to prepare the solutions. The pH of the solution was adjusted by adding NaOH/HClO\(_4\) and the pH measurements were carried out with the help of a prestandardised Elico (India) digital pH meter equipped with glass electrode with an accuracy of ±0.01 pH unit. During kinetic investigation, a constant ionic strength (0.3 mol dm\(^{-3}\)NaClO\(_4\)) was maintained.

On mixing L-cysteine with Pipcc solution, there is decrease of absorbance at \( \lambda_{\text{max}} = 350 \text{ nm} \) and at \( \lambda_{\text{max}} = 424 \text{ nm} \) (Fig. 2a). Similar plot is obtained for GSH (Fig. 2b). The change in absorbance of the mixture at different time intervals is shown in (Fig. 2b). The reaction progress was monitored at \( \lambda_{\text{max}} = 424 \text{ nm} \) as there was significant decrease of absorbance.

b) Kinetic Studies
Kinetic measurements were carried out with a CECIL 7200 UV-VIS (UK) spectrophotometer equipped with a peltier system, temperature control (accuracy = ±0.1°C). The progress of the reaction was monitored by following the decrease in absorbance at \( \lambda_{\text{max}} = 424 \text{ nm} \). The conventional mixing technique was followed and...
pseudo-first order conditions were maintained throughout the course of the reaction. The reaction was followed up to not less than 90% completion. The reaction mixture was homogeneous in solvent composition and pipcc remained stable over the period of kinetic investigation. The pseudo-first order rate constants ($k_{obs}$) were calculated from the slopes of $\ln (A_t - A_\infty) = C + t \times k_{obs}$ versus time plot, following equation-1.

$$\ln (A_t - A_\infty) = C + t \times k_{obs}$$

Where, $A_t$ and $A_\infty$ are the absorbance’s of the reaction mixture at time $t$ and at equilibrium respectively.

Rate data represented as an average of duplicate runs are reproducible within $\pm$ 3%. The correlation coefficient of plots used to determine $k_{obs}$ were found to be 0.99 in most of the cases.

C) Stoichiometry And Identification Of Product

The reaction mixture containing Pipcc and Cysteine or GSH in a molar ratio 1 : 10 was warmed at 313K to complete the reaction. The unreacted Cr(VI) and the product Cr(III) were estimated form the reported experiment [Vogel, A. I (1989). Text Book of Quantitative Analysis(5th edition), ELBS, Longman group, UK]. It was observed that 2 moles of Pipcc reacted with 6 moles of cysteine or GSH to generate 2 moles of Cr(III) and 3 moles of cystine or 3 moles of GSSG respectively.

$$2\text{Cr(VI)} + 6 \text{Cystine} \rightarrow 2\text{Cr(III)} + 3 \text{Cystin} + 6\text{H}^+$$

$$2\text{Cr(VI)} + 6 \text{GSH} \rightarrow 2\text{Cr(III)} + 3 \text{GSSG} + 6\text{H}^+$$

In order to get the reaction product, 0.2 moles of Pipcc, and 0.02 moles of cysteine or GSH were mixed at $[H^+] = 0.2$ mol/dm$^3$. The volume of solution was made 50ml. The reaction mixture was warmed for quick completion of the reaction. The reaction mixture was evaporated slowly to get the product. The product was washed with diethylether and was dried in a desiccator. The FTIR of the cysteine and its oxidation product, cysteine are shown in (fig. 1(a) and 1(b)). The FTIR of GSH and its oxidation product, GSSG are shown in fig. 1(c) and 1(d).

Fig. 1(b), FTIR spectra of the oxidation product of L-cysteine shows a broad strong peak at 3565 cm$^{-1}$ in the product which is due to N-H stretching as compared to 3179 cm$^{-1}$ in L-cystine. The shifting to higher frequency is probably due to association of water molecules with the product. The carboxylate group in the product shifted from 1587 cm$^{-1}$ to 1641 cm$^{-1}$, weak band at 2552 cm$^{-1}$ in cysteine which is due to S-H stretching is absent in the product. Suggesting that the reactant aminoacid, cysteine, dimerises to disulfanylpropanoic (cystine) having S-S linkage.

Fig.1(d), shows a broad peak at 3397 cm$^{-1}$ in the product may be assigned to N-H stretching as compared to 3252 cm$^{-1}$ in GSH (fig.1.c). The shifting to higher frequency is probably due to an association of water molecules with the product. The bending bands and a strong absorption peak of carboxylate ion are overlapped forming a broad band at 1651 cm$^{-1}$ in the product due to carboxylate group compared to 1600 cm$^{-1}$, 1538 cm$^{-1}$ & 1395 cm$^{-1}$ peak in GSH. The weak band at 2526 cm$^{-1}$ in GSH due to S-H stretching is absent in the product suggesting the dimerization of GSH to GSSG having S-S linkage.

The structure of cysteine, GSH and their respective products, cystine& GSSG are shown as:

![Structure Diagrams](image-url)
III. Result And Discussion

The kinetics of oxidation of glutathione reduced (GSH) and L-cysteine (L-cyst) by aqueous solution of piperidiniumchlorochromate (Pipcc) have been studied. The data are consistent with the rate law.

\[
\frac{-d[\text{Pipcc}]}{dt} = k_1 + k_2 [H^+]^2 [\text{Substrate}] \tag{2}
\]

The linearity of the pseudo-first order plots implies that the reaction is first order in [Pipcc], values of pseudo-first order rate constant (k_{obs}) obtained at different [Substrate] at a given [H^+] and at a particular temperature are collected in Table (1 and 4). Plot of k_{obs} as a function of [Substrate] at a given [H^+] and at constant temperature is linear with a common positive intercept (Fig. 3)

**Dependence of Rate on Substrate Concentration**

At 30°C when [Pipcc] = 4.6 x 10^{-4} mol dm^{-3}, I = 0.3 mol dm^{-3}, 10^4[GSH] was varied from 4.6 to 13.8. The values of 10^4k_{obs} increased from 10.88 to 29.1, when [H^+] = 0.01mol dm^{-3}. At the same temperature and under the similar conditions, when 10^3[L-cysteine] was varied from 4.6 to 13.8, 10^4k_{obs} increased from 8.07 to 18.48 indicating the fact that GSH, a tripeptide is reacting faster than L-cysteine.

**Acid dependence for GSH and L-cysteine**

Keeping all conditions constant, [H^+] was varied. 10^4[Pipcc] = 4.6, 10^3[GSH]=2.4, I = 0.3M, temperature = 30°C, [H^+] was varied from 0.03 to 0.11mol dm^{-3}, 10^4k_{obs} increased from 11.45 to 18.95. Under the same condition, when [H^+] was varied from 0.03 to 0.11mol dm^{-3}, 10^4k_{obs} increased from 6.72 to 10.22 for L-cysteine.

With increasing [H^+], observed pseudo first order rate constant is found to increase for both L-cysteine and GSH. This indicates that protonated form of the oxidant is taking part in the electron transfer reaction. With the increasing[H^+], the concentration of the protonated form of oxidant increases. As a result, the reaction becomes faster.

**Temperature dependence for the reaction between Pipcc and the substrate**

When 10^3[Pipcc] = 4.6mol dm^{-3}, 10^3[GSH] =2.4, I = 0.3mol dm^{-3} and [H^+] = 0.3mol dm^{-3}, 10^4k_{obs} changes from 7.5 to 16.94 by increasing the temperature from 20°C to 40°C. Under the same condition for L-cysteine, 10^4k_{obs} changed from 1.97 to 11.4. Similarly increases in temperature, k_{obs} increases for both Glutathione reduced and also for L-cysteine. Increase in k_{obs} can be explained on the basis of Arrhenius equation.

Basing on stoichiometry and identification of the product, the probable mechanism may be delineated as scheme-1.

\[
\text{2Cr(VI)+Cr(IV)} \xrightarrow{\text{fast}} 2\text{Cr(V)}
\]

\[
\text{RSH + (L-cysteine)} \xrightarrow{\text{K}} \text{Cr(V)} \xrightarrow{\text{fast}} \text{R-S-S-R}
\]

Where k_1 and k_2 are acid independent and acid dependent paths of electron transfer reactions respectively. Rapid and kinetically unimportant steps of the product formation may probably be visualised as follows.

\[
\begin{align*}
2\text{Cr(V)} + 2\text{RS} & \xrightarrow{\text{fast}} 2\text{Cr(IV)} + \text{R-S-S-R} \\
\text{Cr(VI)} + \text{Cr(IV)} & \xrightarrow{\text{fast}} 2\text{Cr(V)}
\end{align*}
\]
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Cr(V) + 2RSH $\xrightarrow{\text{fast}}$ Cr(III) + R – S – S + R + 2H⁺ 
Cr(III) is the Cr(H₂O)₆³⁺ species in aqueous acidic medium. 
(RSH may be L-Cysteine or GSH reduced) 
The rate law corresponding to above mechanism is indicated below

\[
\text{Rate} = -\frac{d[L]}{dt} = \frac{K[RSH](k_1 + k_2[RSH][H^+]^2)}{1 + K[RSH]} \]  
(3)

\[-\frac{d[Pipcc]}{dt} = k_{\text{obs}}[Pipcc]_T \]  
(4)

Hence

\[k_{\text{obs}} = \frac{K[RSH](k_1 + k_2[RSH][H^+]^2)}{1 + K[RSH]} \]  
(5)

If K [RSH] >> 1, equation (5) becomes

\[k_{\text{obs}} = (k_1 + k_2 \text{[cysteine]}) [H^+]^2 \]  
(6)

Similarly for GSH

\[k_{\text{obs}} = (k_1 + k_2 \text{[GSH]}) [H^+]^2 \]  
(7)

\(k_{\text{obs}}\) vs [H⁺]² plot is linear (figure- 4 and 5) for both GSH and L-cysteine is indicating the fact that the rate law is consistent with the mechanism. The values of \(k_1\) and \(k_2\) were calculated from the intercept and slope. The values are collected in Table 3 and 6.

The oxidation products for L-cysteine and GSH are L-cystine and GSSG respectively. Activation parameters for path \(k_1\) and \(k_2\) for both the reactants are collected in Table 3 and 6. Negative value of \(\Delta S^*\) for both the reactions indicate that the reaction passes through ordered transition states.

Table – 1: Pseudo-first order rate constant for the oxidation of Pipcc with L-cysteine at different concentrations of L-cysteine.
Variation of concentration of L-cysteine

<table>
<thead>
<tr>
<th>10⁻³[L-cysteine] mol dm⁻³</th>
<th>10⁻³k_{\text{obs}} (sec⁻¹)</th>
<th>(k_2), (k_{\text{obs}}) ([\text{L-cyst}])</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.6</td>
<td>8.07</td>
<td>0.176</td>
</tr>
<tr>
<td>7.0</td>
<td>10.27</td>
<td>0.147</td>
</tr>
<tr>
<td>9.2</td>
<td>12.27</td>
<td>0.133</td>
</tr>
<tr>
<td>11.6</td>
<td>17.65</td>
<td>0.152</td>
</tr>
<tr>
<td>13.8</td>
<td>18.48</td>
<td>0.134</td>
</tr>
</tbody>
</table>

Table – 2: Variation of 10⁻³k_{\text{obs}} at different temps and at different [H⁺] for reaction of Pipcc with L-cysteine, 

\[\text{[Pipcc]} = 4.6 \times 10^{-3}\text{mol dm}^{-3}, \text{I} = 0.3\text{mol dm}^{-3} (\text{NaClO}_3), (\text{L-cysteine}) \]

\[= 2.4 \times 10^{-3}\text{mol dm}^{-3}\]

<table>
<thead>
<tr>
<th>[H⁺]mol dm⁻³</th>
<th>20°C</th>
<th>25°C</th>
<th>30°C</th>
<th>35°C</th>
<th>40°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.03</td>
<td>1.97</td>
<td>3.47</td>
<td>6.72</td>
<td>8.38</td>
<td>11.4</td>
</tr>
<tr>
<td>0.05</td>
<td>2.30</td>
<td>4.01</td>
<td>7.50</td>
<td>9.95</td>
<td>14.08</td>
</tr>
<tr>
<td>0.07</td>
<td>3.33</td>
<td>5.30</td>
<td>8.67</td>
<td>11.89</td>
<td>16.72</td>
</tr>
<tr>
<td>0.09</td>
<td>4.10</td>
<td>6.22</td>
<td>9.70</td>
<td>12.73</td>
<td>17.28</td>
</tr>
<tr>
<td>0.11</td>
<td>4.50</td>
<td>6.76</td>
<td>10.22</td>
<td>14.03</td>
<td>19.42</td>
</tr>
</tbody>
</table>

Table – 3: Electron transfer rate constants \(k_1\) and \(k_2\) for oxidation of L-cysteine by Pipcc at different temperatures.

<table>
<thead>
<tr>
<th>Temperature(°C)</th>
<th>10⁻³k₁( Sec⁻¹)</th>
<th>10⁻³k₂ (mol dm⁻³ sec⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>5.0</td>
<td>9.41</td>
</tr>
<tr>
<td>30</td>
<td>7.0</td>
<td>13.16</td>
</tr>
<tr>
<td>35</td>
<td>9.0</td>
<td>20.12</td>
</tr>
<tr>
<td>40</td>
<td>12.0</td>
<td>27.16</td>
</tr>
</tbody>
</table>

For \(k_1\), path

For \(k_2\), path

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Table – 4: Reaction of Pipcc with GSH Pseudo-first order constant with variation of concentration of GSH

<table>
<thead>
<tr>
<th>[GSH] (mol dm⁻³)</th>
<th>10^4 kobs (sec⁻¹)</th>
<th>k₂ = (kobs / [GSH])</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.6</td>
<td>10.88</td>
<td>0.236</td>
</tr>
<tr>
<td>7.0</td>
<td>15.03</td>
<td>0.215</td>
</tr>
<tr>
<td>9.2</td>
<td>21.08</td>
<td>0.230</td>
</tr>
<tr>
<td>11.6</td>
<td>25.38</td>
<td>0.219</td>
</tr>
<tr>
<td>13.8</td>
<td>29.10</td>
<td>0.211</td>
</tr>
</tbody>
</table>

Table – 5: Variation of 10^4 kobs at different temps and at different [H⁺] for reaction of Pipcc with GSH, [Pipcc] = 4.6 x 10⁻⁴ mol dm⁻³, [GSH] = 2.4 x 10⁻³ mol dm⁻³, I = 0.3 mol dm⁻³ (NaClO₄), \( \lambda_{max} = 435 \) nm

<table>
<thead>
<tr>
<th>[H⁺] (mol dm⁻³)</th>
<th>20°C</th>
<th>25°C</th>
<th>30°C</th>
<th>35°C</th>
<th>40°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.03</td>
<td>7.50</td>
<td>9.31</td>
<td>11.45</td>
<td>13.90</td>
<td>16.94</td>
</tr>
<tr>
<td>0.05</td>
<td>7.87</td>
<td>9.93</td>
<td>12.62</td>
<td>14.93</td>
<td>18.62</td>
</tr>
<tr>
<td>0.07</td>
<td>8.17</td>
<td>11.07</td>
<td>15.63</td>
<td>17.72</td>
<td>24.10</td>
</tr>
<tr>
<td>0.09</td>
<td>8.80</td>
<td>12.16</td>
<td>17.25</td>
<td>20.92</td>
<td>28.71</td>
</tr>
<tr>
<td>0.11</td>
<td>9.33</td>
<td>13.15</td>
<td>18.95</td>
<td>23.38</td>
<td>32.58</td>
</tr>
</tbody>
</table>

Table – 6: Electron transfer rate constants k₁ and k₂ for oxidation of GSH by Pipcc at different temperatures.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>10^4 k₁ (sec⁻¹)</th>
<th>k₂ (mol dm⁻³ sec⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>9.0</td>
<td>14.50</td>
</tr>
<tr>
<td>30</td>
<td>11.0</td>
<td>28.33</td>
</tr>
<tr>
<td>35</td>
<td>13.0</td>
<td>36.79</td>
</tr>
<tr>
<td>40</td>
<td>16.0</td>
<td>60.58</td>
</tr>
</tbody>
</table>

For k₁ path
\( \Delta H₁^o = 27.05 \pm 1.3 \) kJ mol⁻¹
\( \Delta S₁^o = -212.5 \pm 4.3 \) JK⁻¹ mol⁻¹

For k₂ path
\( \Delta H₂^o = 67.5 \pm 12.3 \) kJ mol⁻¹
\( \Delta S₂^o = 4.6 \pm 40 \) JK⁻¹ mol⁻¹

Figure 1(a): FTIR spectra of the substrate cysteine
Kinetics and Mechanism of Oxidation of Glutathione reduced (GSH) and L-cysteine (L-cyst) by...

Figure 1(b) FTIR spectra of the product cystine

Figure 1(c) FTIR spectra of pure GSH

Figure 1(d) FTIR spectra of oxidation product GSSG
Kinetics and Mechanism of Oxidation of Glutathione reduced (GSH) and L-cysteine (L-cyst) by...

**Figure-2(a)** UV-vis time Scan of Pipcc and L-cysteine mixture.

(1) \[ [\text{Pipcc}] = 4.6 \times 10^{-4} \text{mol dm}^{-3}, \ [\text{H}^+] = 0.11 \text{mol dm}^{-3}, \ I = 0.3 \text{mol dm}^{-3} \]
\[ [\text{cysteine}] = 2.4 \times 10^{-3} \text{mol dm}^{-3} \]
Curves (2-8), \( \Delta t = 10 \text{min}, 30^\circ C \)

**Figure-2(b)** UV-Vis time scan of Pipcc and GSH mixture.

(1) \[ [\text{Pipcc}] = 4.6 \times 10^{-4} \text{mol dm}^{-3}, \ I = 0.3 \text{mol dm}^{-3}, [\text{GSH}] = 4.6 \times 10^{-3} \text{mol dm}^{-3} \]
\[ [\text{H}^+] = 0.01 \text{mol dm}^{-3}, \ \text{temp} = 30^\circ C, \ \lambda = 300-500 \text{nm}, \ \text{at different time intervals} \]
Curve (2-6), \( \Delta t = 15 \text{min} \)
Kinetics and Mechanism of Oxidation of Glutathione reduced (GSH) and L-cysteine (L-cyst) by...

**Figure – 3** The Plot of $10^3$ [substrate] (mol dm$^-^3$) vs $10^4$ $k_{obs}$ (sec$^-^1$)

Substrate may be L-cysteine or GSH

$[\text{Pipc}]_T = 4.6 \times 10^{-4}$ mol dm$^-^3$

$[\text{H}^+] = 0.01$ mol dm$^-^3$, 30°C

**Figure – 4** The plot of $10^4$ $[\text{H}^+]^2$ / mol$^2$ dm$^6$ vs $10^4$ $k_{obs}$ / sec$^-^1$

(For Cysteine)

$[\text{Pipc}]_T = 4.6 \times 10^{-5}$ mol dm$^-^3$

$[\text{Cysteine}] = 2.4 \times 10^{-3}$ mol dm$^-^3$

$I = 0.3$ mol dm$^-^3$

At different temperature, (1) 20°C, (2) 25°C, (3) 30°C, (4) 35°C, (5) 40°C
Figure 5 The Plot of $10^4 \frac{[H^+]^2}{mol^2 dm^6}$ vs $10^4 k_{obs}$/sec

(For GSH)

$[Pipcc] = 4.6 \times 10^{-4}$ mol dm$^{-3}$

$[GSH] = 2.4 \times 10^{-3}$ mol dm$^{-3}$

$I = 0.3$ mol dm$^{-3}$

At different temperature, (1) 20°C, (2) 25°C, (3) 30°C, (4) 35°C, (5) 40°C

IV. Conclusion

The kinetics of oxidation of GSH and L-Cysteine by piperidinium chlorochromate has been studied in acid medium. The rate of redox reaction was increased with increase in concentration of [substrate] and [H$^+$] in both cases. The reaction follows first order in [substrate] and [H$^+$]. The redox reaction involves two steps. One is acid independent path and other is acid dependent path. The reaction follows free redical mechanism. The isolated products of the redox reactions are cystine due to isomerisation of substrate cysteine and GSSG due to isomerisation of substrate GSH. Moderate values of activation parameters favour the electron transfer reaction.

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Reference


