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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* $\leq 10^3$ *[substrate]* $\leq$ *13.8 (when substrate = GSH or L-cysteine), 0.03* $\leq [H^+]_T \leq$ *0.11, I=0.3 mol dm^{-3} ($NaClO_4$) and 293K* $\leq T \leq$ *313K. The rate of the reaction has been found to increase with the increase in [Substrate]$_T$ and [H$^+$]$_T$. The reaction follows first order kinetics in [Substrate]$_T$and [Pipcc]$_T$. The reaction follows second order kinetics with respect to [H$^+$]$_T$. 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 $\Delta H^{\neq}$ *(kJ mol^{-1}) for k_1 (sec^{-1}) paths were found to be 43.8 + 3.7, 27.05+1.3 and* $\Delta S^{\neq}$ *(JK^{-1}mol^{-1}) were -161.1+12 and-212.5+4.3, For $k_2$path (mol^{-3}dm^{3}sec^{-1}), the* $\Delta H^{\neq}$ *(kJ mol^{-1}) values were 53.3+3.4 and 67.5+12.3 and* $\Delta S^{\neq}$ *(JK^{-1}mol^{-1}) values were -182.6+11 and 4.6+40.*

Negative value of $\Delta S^{\neq}$ *indicates the reaction passes through a ordered transition state. The oxidation product of L-cysteine and GSH (reduced) are 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, imidazolium chloro chromate, quinoliniumfluorochromate, quinoliuium 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 oxidings 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 $\pm$ 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 λ_{max}=350 nm and at λ_{max} = 424 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 monotored at λ_{max} = 424 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 = $\pm$ 0.1^{0}C). The progress of the reaction was monitored by following the decrease in absorbance at λ_{max} = 424nm. The conventional mixing technique was followed and

pseudo-first order conditions were maintained throughout the course of the reaction. The reactionwas 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 pseuso-first order rate constants (k_{obs}) were calculated from the slopes of ln (A_t-A_∞) versus time plot, following equation-1.

$$\text{In } (A_t - A_\infty) = C + t \times k_{obs} \qquad \textbf{(1)}$$

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 ofL-cysteine or GSH to generate 2 moles of Cr(III) and 3 moles of cysteine or 3 moles of GSSGrespectively.

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

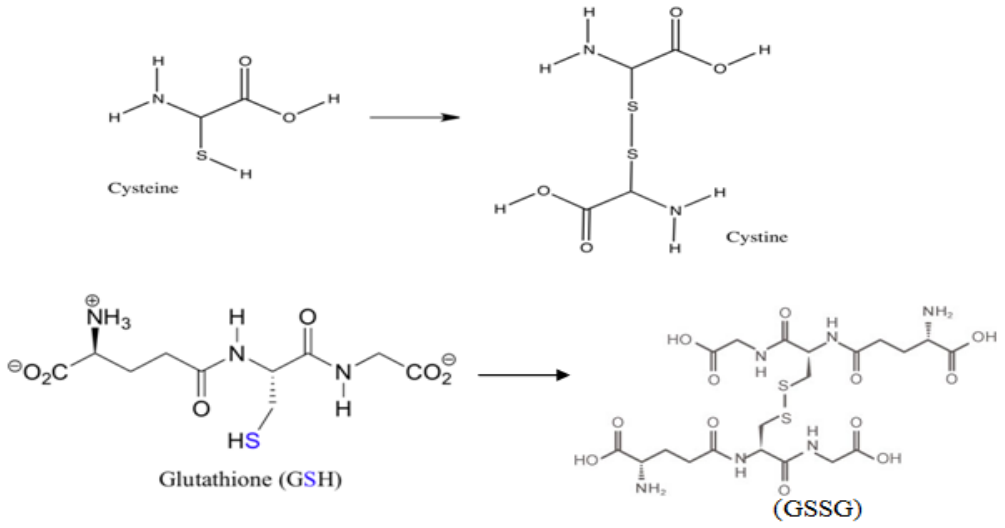
$$2Cr(VI)+6\ \text{GSH} \rightarrow\ 2Cr(III)+\ 3\ \text{GSSG}\ +6H^{+}$$

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 3565cm^{-1} in the product which is due to N-H stretching as compared to 3179cm^{-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 1587cm-1 to 1641cm^{-1}, weak band at 2552cm^{-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 3397cm^{-1} in the product may be assigned to N-H stretching as compared to 3252cm^{-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 1651cm^{-1} in the product due to carbvoxylate group compared to 1600cm^{-1}, 1538cm^{-1}& 1395cm^{-1} peak in GSH. The weak band at 2526cm^{-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 :



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.

$$\frac{-d[Pipcc]}{dt} = k_1 + k_2[H^+]^2 \text{ [Substrate]} \qquad (2)$$

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 30^0C when $[Pipcc]_T = 4.6 \times 10^{-4}$mol dm^{-3}, I = 0.3 mol dm^{-3}, 10^3[GSH] was varied from 4.6 to 13.8. The values of 10^4k_{obs} increased from 10.88 to 29.1, when $[H^+] = 0.01$moldm^{-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

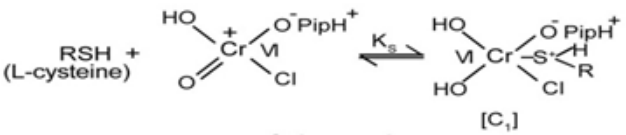
Keeping all conditions constant, $[H^+]$ was varried. $10^4[pipcc]_T$ = 4.6, $10^3[GSH]_T$=2.4, I = 0.3M, temperature = 30^0C, $[H^+]$ was varied from 0.03 to 0.11mol dm^{-3}, 10^4 k_{obs}increased from 11.45 to 18.95. Under the same condition, when $[H^+]$was varied from 0.03 to 0.11mol dm^{-3}, 10^4 k_{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 incrasing$[H^+]$, 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 $10^4[pipcc]_T$ = 4.6mol dm^{-3}, 10^3[GSH] =2.4, I = 0.3mol dm^{-3} and $[H^+]$ = 0.3mol dm^{-3}, 10^4 k_{obs} changes from 7.5 to 16.94 by increasing the temperature from 20^0C to 40^0C. 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.



Scheme - I

$$C_1 \xrightarrow[H_2O]{k_1} Cr\ (V) + R\text{-}S^{\cdot} + H^+$$

$$2H^+ + C_1 \xrightarrow[H_2O]{k_2} Cr\ (V) + R\text{-}S^{\cdot}$$

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

$$2Cr(V) + 2RS^{\cdot} \xrightarrow{\text{fast}} 2Cr(IV) + R - S - S - R$$

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

$Cr(V)+2RSH \xrightarrow{fast} Cr(III)+ R-S-S-R+ 2H^+$

Cr(III) is the $Cr(H_2O)_6^{3+}$ species in aqueous acidic medium.

(RSH may be L-Cysteine or GSH reduced)

The rate law corresponding to above mechanism is indicated below

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

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

Hence

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

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

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

Similarly for GSH

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

k_{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. Negativevalue of $\Delta S^{\neq}$ for both the reactions indicate that the reaction passes through ordered transition states.

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

Variation of concentration of L-cysteine

λ_{max}= 424nm, [Pipcc] = 4.6 x 10^{-4}mol dm^{-3}, $[H^+]$ = 0.01 mol dm^{-3}

I = 0.3mol dm^{-3}, temp = 30^0C

10^3[L-cysteine] mol dm^{-3}	10^4k_{obs} (sec^{-1})	$k_2 = \left(\frac{k_{obs}}{[L-cyst]}\right)$
4.6	8.07	0.176
7.0	10.27	0.147
9.2	12.27	0.133
11.6	17.65	0.152
13.8	18.48	0.134

Table – 2: Variation of 10^4k_{obs} at different temps and at different $[H^+]$ for reaction of Pipcc with L-cysteine, [Pipcc] = 4.6 x 10^{-4}mol dm^{-3}, I = 0.3mol dm^{-3} ($NaClO_4$), (L-cysteine) = 2.4 x 10^{-3}mol dm^{-3}

$[H^+]$mol dm^{-3}	20^0C	25^0C	30^0C	35^0C	40^0C
0.03	1.97	3.47	6.72	8.38	11.4
0.05	2.30	4.01	7.50	9.95	14.08
0.07	3.33	5.30	8.67	11.89	16.72
0.09	4.10	6.22	9.70	12.73	17.28
0.11	4.50	6.76	10.22	14.03	19.42

Table – 3:Electron transfer rate constants k_1 and k_2 for oxidation of L-cysteine by Pipcc atdifferent temperatures.

Temperature(^{0}C)	10^4k_1(Sec^{-1})	k_2($mol^{-3}dm^3sec^{-1}$)
25	5.0	9.41
30	7.0	13.16
35	9.0	20.12
40	12.0	27.16
For k_1 path		For k_2 path

$\Delta H_1^{\neq}$ = 43.8 ± . 3.7 kJ mol^{-1}	$\Delta H_2^{\neq}$ = 53.3 ± 3.4 kJ mol^{-1}
$\Delta S_1^{\neq}$ = - 161.1 ± 12 JK^{-1} mol^{-1}	$\Delta S_2^{\neq}$ = -182.6 ± 11 JK^{-1} mol^{-1}

Table – 4: Reaction of Pipcc with GSH Pseudo-first order constant with variation of concentration of GSH λ_{max} = 435nm, [Pipcc] = 4.6 x 10^{-4}mol dm^{-3}, [H$^+$] = 0.01 mol dm^{-3}
I = 0.3 mol dm^{-3} ($NaClO_4$), temp = 30^{0}C

10^3[GSH] mol dm^{-3}	10^4k_{obs} (sec^{-1})	$k_2 = \left(\frac{k_{obs}}{[GSH]}\right)$
4.6	10.88	0.236
7.0	15.03	0.215
9.2	21.08	0.230
11.6	25.38	0.219
13.8	29.10	0.211

Table – 5: Variation of 10^4k_{obs} 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 = 0.3mol dm^{-3} ($NaClO_4$), λ_{max}= 435 nm

[H$^+$]mol dm^{-3}	20^{0}C	25^{0}C	30^{0}C	35^{0}C	40^{0}C
0.03	7.50	9.31	11.45	13.90	16.94
0.05	7.87	9.93	12.62	14.93	18.62
0.07	8.17	11.07	15.63	17.72	24.10
0.09	8.80	12.16	17.25	20.92	28.71
0.11	9.35	13.15	18.95	23.38	32.58

Table – 6: Electron transfer rate constants k_1 and k_2 for oxidation of GSH by Pipcc at different temperatures.

Temperature (^{0}C)	10^4k_1(Sec^{-1})	k_2(mol^{-3}dm^{3}sec^{-1})
25	9.0	14.50
30	11.0	28.33
35	13.0	36.79
40	16.0	60.58
For k_1 path $\Delta H_1^{\neq}$ = 27.05 ± . 1.3 kJ mol^{-1} $\Delta S_1^{\neq}$ = - 212.5 ± 4.3 JK^{-1} mol^{-1}		For k_2 path $\Delta H_2^{\neq}$ = 67.5 ± 12.3kJ mol^{-1} $\Delta S_2^{\neq}$ = 4.6 ± 40 JK^{-1} mol^{-1}

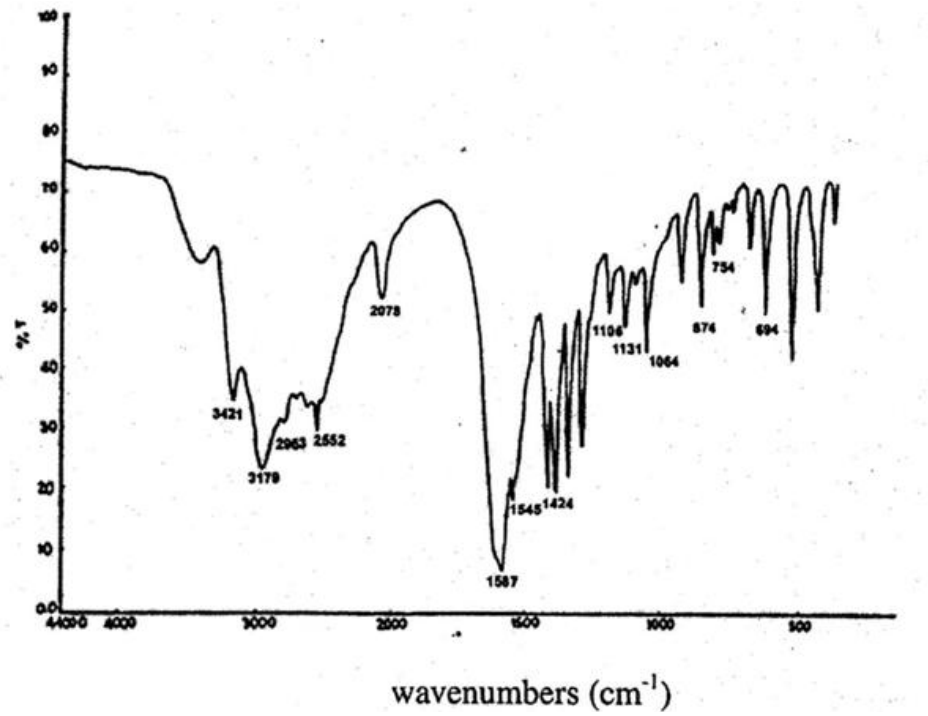


Figure- 1(a) FTIR spectra of the substrate cysteine

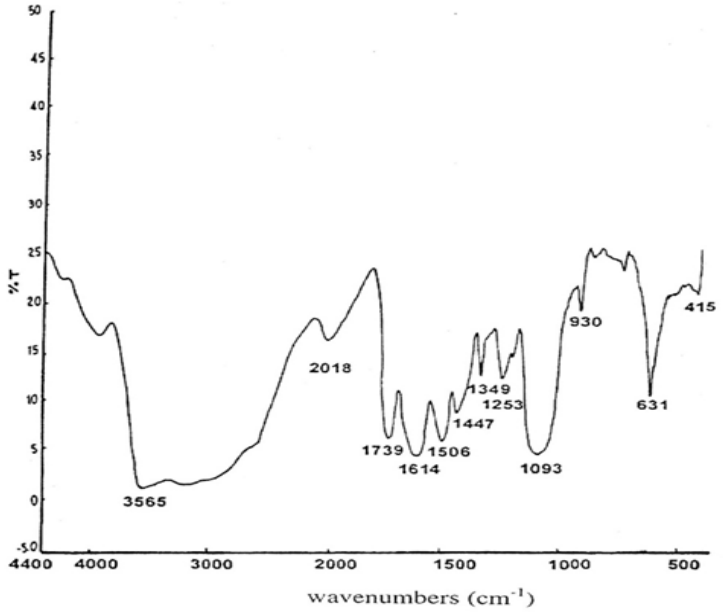


Figure 1(b)FTIR spectra of the product cystine

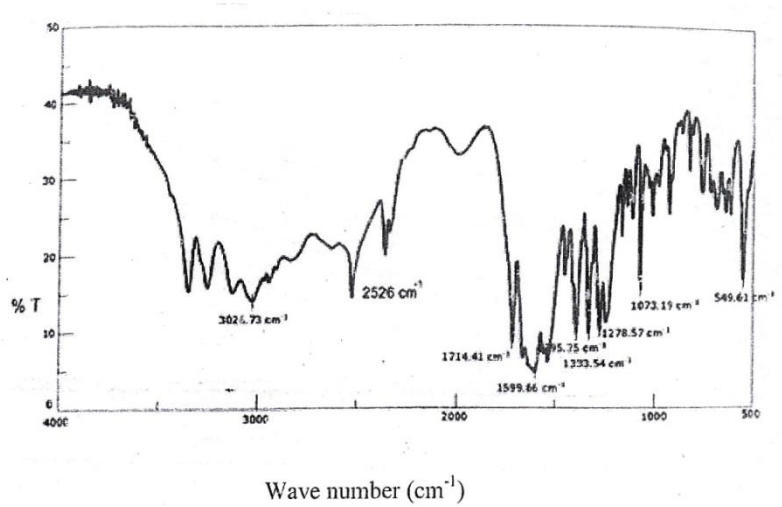


Figure 1(c) FTIR spectra of pure GSH

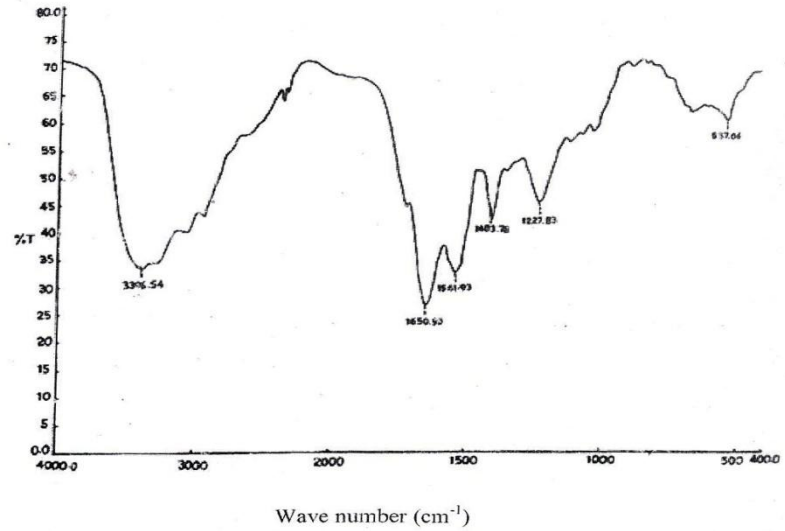


Figure 1(d) FTIR spectra of oxidation product GSSG

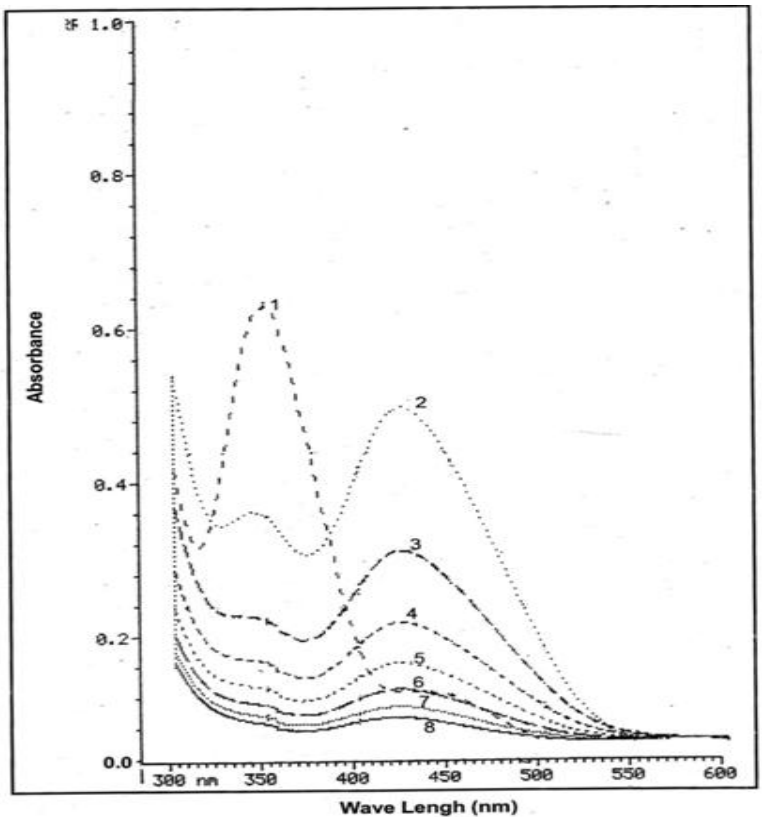


Figure-2(a)UV-vistime Scan of Pipcc and L-cysteine mixture.
(1) [Pipcc]= 4.6 x 10^{-4}mol dm^{-3}, [H^+]= 0.11 mol dm^{-3}, I= 0.3mol dm^{-3}
[cysteine]= 2.4 x 10^{-3}mol dm^{-3}
Curves (2-8), Δt = 10min, 30^0C

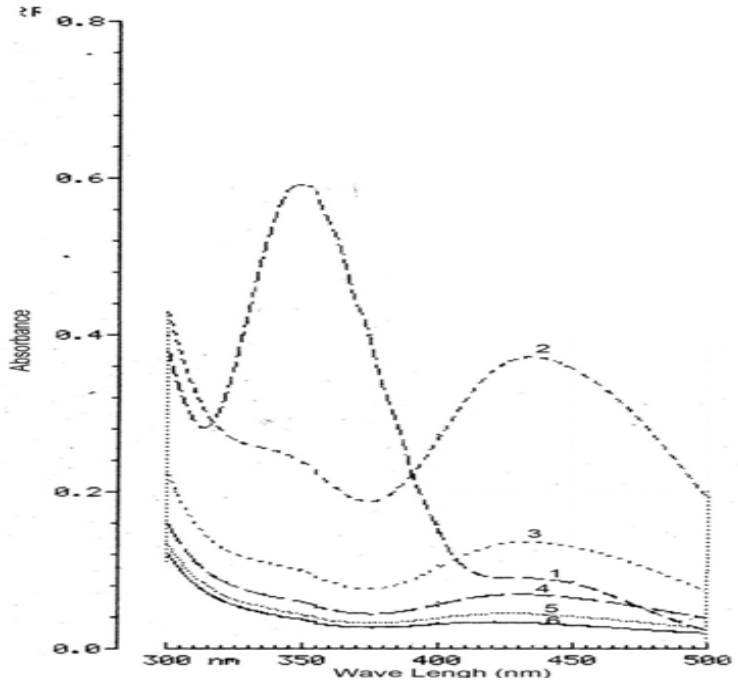


Figure-2(b)UV- Vis time scan of Pipcc and GSH mixture.
(1) $[Pipcc]_T$=4.6 x 10^{-4}mol dm^{-3}, I=0.3mol dm^{-3},[GSH]= 4.6 x 10^{-3}mol dm^{-3}
[H^+]=0.01 mol dm^{-3}, temp = 30^0C, λ=300–500nm, at different time intervals
Curve (2-6), Δt = 15min

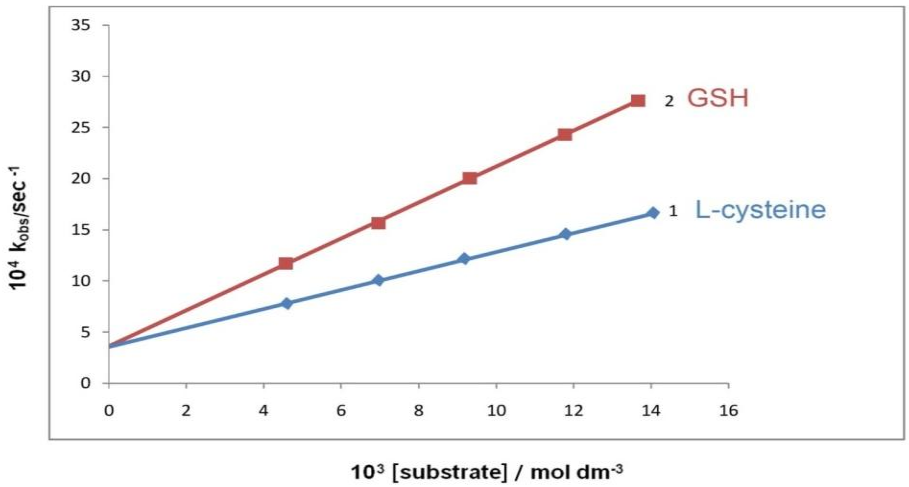
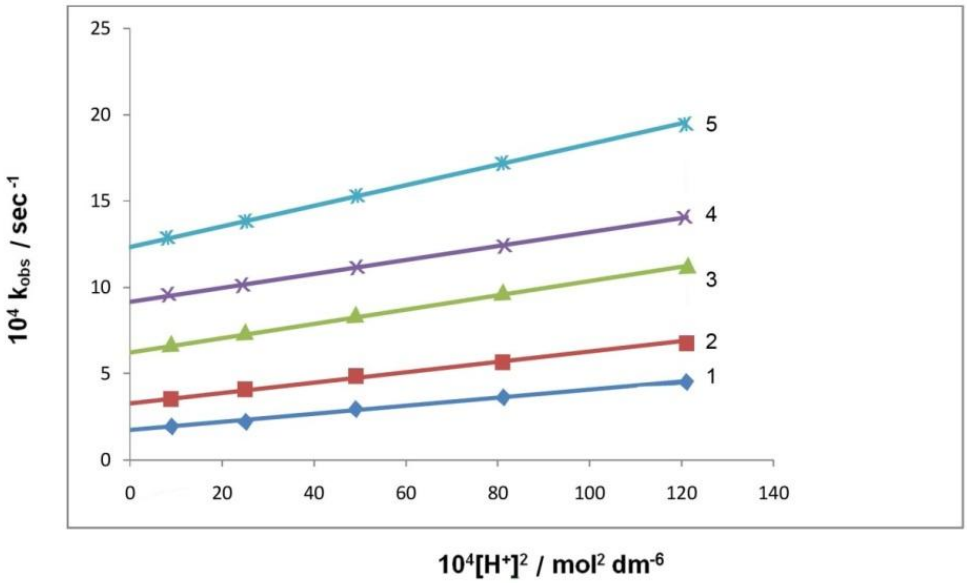


Figure – 3 The Plot of 10^3 [substrate] (mol dm^{-3}) vs 10^4 k_{obs} (sec^{-1})
Substrate may be L-cysteine or GSH
$[Pipcc]_T = 4.6 \times 10^{-4}$ mol dm^{-3}
$[H^+] = 0.01$mol dm^{-3}, 30^0C



Fugure-4 The plot of 10^4 $[H^+]^2$ / mol^2dm^{-6}/vs 10^4 k_{obs} /sec^{-1}
(For Cysteine)
$[Pipcc]_T = 4.6 \times 10^{-4}$ mol dm^{-3}
[Cysteine] = 2.4×10^{-3} mol dm^{-3}
I = 0.3 mol dm^{-3}
At different temprature, (1) 20^0C, (2) 25^0C, (3) 30^0C, (4) 35^0C, (5) 40^0C

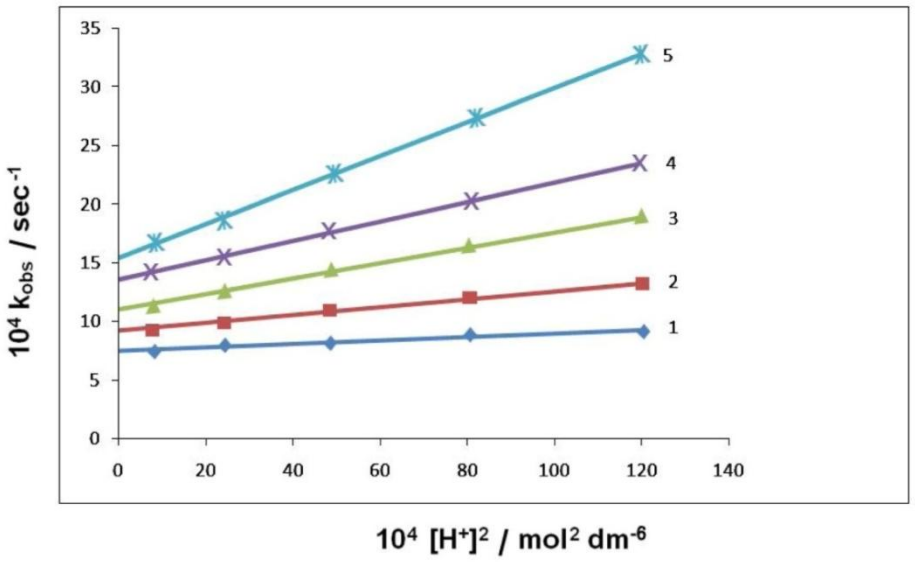


Figure -5 The Plot of $10^4\ [H^+]^2$ / $mol^2\ dm^{-6}$ vs $10^4\ k_{obs}$ / sec^{-1}
(For GSH)
$[Pipcc]_T = 4.6 \times 10^{-4}$ mol dm^{-3}
$[GSH] = 2.4 \times 10^{-3}$ mol dm^{-3}
$I = 0.3$ mol dm^{-3}
At different temprature, (1) 20^0C, (2) 25^0C, (3) 30^0C, (4) 35^0C, (5) 40^0C

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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