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Kinetics and Mechanism of Oxidation of GSH by vanadium(V) in Aqueous hydrochloric acid medium

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ABSTRACT
The kinetics and oxidation of glutathione (reduced) (GSH) by vanadium(V) has been studied over the range $0.5 \leq 10^2[\mathrm{GSH}]_T \leq 2.0$, $0.5 \leq [\mathrm{H}^+] \leq 3.0$, $I = 3.1$ mol dm^{-3} and $298 \leq T \leq 313$K in aqueous hydrochloric acid medium. The reaction showed first order dependence in $[\mathrm{V(V)}]_T$, first order with respect to $[\mathrm{H}^+]$ and fractional order with respect to $[\mathrm{GSH}]_T$. The main product of the reaction is GSSG a dimer of GSH. In this reaction, one mole of vanadium(V) consumes one mole of GSH. Increase in hydrochloric acid concentration increases the rate. The activation parameters $\Delta H^\#$ (kJ mol^{-1}) and $\Delta S^\#$ (JK^{-1} mol^{-1}) for the electron transfer reaction were found to be -22.46 and -371.8. The negative values of activation parameters favor the formation of ordered transition state.

Keywords: Kinetic, oxidation, Glutathione (Reduced), Vanadium(V).

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I. INTRODUCTION

Glutathione [(2S)-2-amino-4-{[(1R)-1-[(carboxymethyl)carbamoyl]-2-sulfanylethyl]carbamoyl}butanoic acid] is a very good anti-oxidant [1]. It is a tripeptidehaving a gamma peptide linkage between the carboxyl group of the glutamate side chain and the amine group of cysteine and carboxyl group of cysteine is attached to the glycine by a general peptide linkage [2-4]. It prevents the damage to cellular compounds caused by reactive oxygen species [5]. It also controls the oxidative damage to the skin [6] and also has skin lightening ability [7]. Glutathione (GSH) is oxidized to its dimer (GSSG) during its oxidation. Healthy cells and tissues contain 90% of GSH and 10% of GSSG [8]. Elevated levels of GSH act to protect cancerous cells by conferring resistance to chemotherapeutic drugs [9]. GSH has two carboxylic groups along with one each of amino and thiol group [10], the oxidized glutathione (GSSG) has four carboxylic and two amino groups. pK_1, pK_2, pK_3, pK_4 values [11] of GSH are 2.05, 3.47, 8.63 and 9.52 respectively. Both GSH and GSSG are antioxidants and interact with metal ions [12-13].

Ammonium metavanadate is a versatile oxidizing agent and has been used to oxidize many organic compounds like hydroxylamine hydrochloride, acetanilides, hydroxyl urea, tyrosine, and piperidin-4-ol [14-18].To examine the redox nature of V(V), its reaction with GSH has been undertaken.

II. EXPERIMENTAL

All chemicals and reagents used were Merck (A.R. grade). Doubly distilled water was used throughout. Ammonium metavanadate solution was prepared by dissolving the calculated amount in hydrochloric acid and was standardized with ferrous ammoniumsulphate solution using barium diphenyl amine sulphonates indicator [19]. GSH solution was prepared by dissolving appropriate quantity of sample in double distilled water. Sodium perchlorate and hydrochloric acid were used to provide appropriate ionic strength.All the solvents used for spectroscopic studies were of spectroscopic grade.

All kinetic runs were carried out under the pseudo first order condition when $[\mathrm{GSH}]_T$ was excess over $[\mathrm{V(V)}]_T$ at 298 to 313K temperature. The thermally equilibrated solution of vanadium(V) and GSH containing the required quantities of hydrochloric acid and sodium perchlorate were used. The progress of the reaction was followed by monitoring the decrease in absorbance of V(V) as a function of time at 310 nm in one centimeter cell placed in thermostated compartment of CECIL UV-Visible spectrophotometer.

The rate constant k_{obs} were obtained from the slope of $-\ln(A_t - A_\infty)$ versus ‘t’ plots.

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

Where A_t and A_∞ are the absorbance of the reaction mixture at time 't' and at the completion of the reaction. The reported rate data represented as an average of duplicate runs were reproducible within ±3% the correction coefficient of the plots used to determine k_{obs}, were found to be 0.99 in most of the cases.

The molar absorptivity of Vanadium(V) at 310nm was found to be $\varepsilon = 575 \pm 5$ dm^3 mol^{-1} cm^{-1}. The spectral changes during the chemical reaction at 298K are shown in fig. 1.

III. RESULTS AND DISCUSSIONS

The kinetic results obtained for the above reaction may be summerised as follows.

Effect of [GSH] on reaction rate

With varying the concentration of 10^2[GSH] from 0.5 to 2.0 mol dm^{-3}, at $[H^+] = 3.0$ mol dm^{-3}, $I = 3.1$ mol dm^{-3} and $[V(V)]_T = 1.5 \times 10^{-3}$mol dm^{-3}(Table 1) 10^4k_{obs} (298K) increased from 1.23 to 9.00 s^{-1}. The plot of k_{obs} versus $[GSH]_T$was not linear at different temperatures indicating fractional order dependence of rate on $[GSH]_T$.

Effect $[H^+]$ on reaction rate

The effect of $[H^+]$ on reaction rate was studied by varying $[H^+]$ from 0.5 to 3.0 moldm^{-3}, [V(V)] = 1.5 × 10^{-3}mol dm^{-3}, [GSH] = 1.0 × 10^{-2}mol dm^{-3} and I = 3.1 mol dm^{-3}, 10^4k_{obs} (298K) increases from 1.8 to 2.35 s^{-1}. The plot of k_{obs}versus $[H^+]$ (Fig. 2) keeping other parameters constants was a straight line indicating first order dependence with respect to $[H^+]$. The composite rate constantskK_2were obtained at four different temperatures. From these data $\Delta S^{\#}(JK^{-1}\ mol^{-1})$ and $\Delta H^{\#}(kJ\ mol^{-1})$ were calculated and are collected in Table 2.

Effect of temperature

The rate of the reaction was studied varying temperature from 298 to 313K at [GSH] 0.5 × 10^{-2}, [V(V)] = 1.5 × 10^{-3}, $[H^+] = 3.0$ and $I = 3.1$ mol dm^{-3}. 10^4k_{obs}varied from 1.23 to3.34 s^{-1}.

Effect of [V(V)] on reaction rate

The rate of the reaction was studied by varying [V(V)] from 0.2 × 10^{-3} to 2.0 × 10^{-3}mol dm^{-3} keeping [GSH] = 1.0 × 10^{-2}, $[H^+] = 3.0$ at $I = 3.1$ mol dm^{-3}. 10^4k_{obs}changes from 2.34 to 2.35s^{-1}at 298 K which indicates first order dependence of rate on V(V).

Evidence in favor of free radical formation

The intervention of free radicals was examined. To the reaction mixture a known quantity of acrylonitrile has been added initially and was kept in an inert atmosphere for 2h at room temperature. Precipitate was formed when the reaction mixture was diluted with methanol, thus suggesting that a free radical is formed in the reaction.

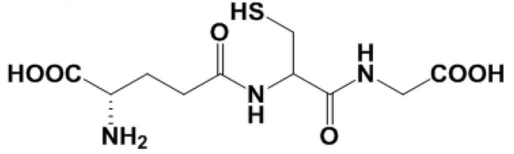
Stoichiometry and characterization of product

With V(V) in excess, in GSH, in HCl medium, [V(V)] was assayed by measuring the absorbance at 310nm. The results indicate that two moles of V(V) reacted with one mole ofGSH as shown in equation 1.

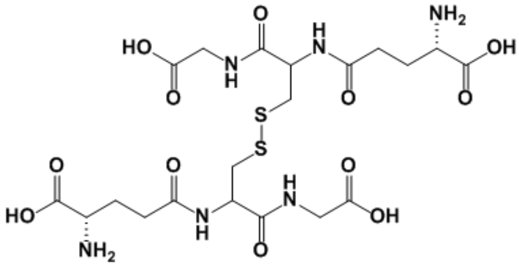
For the preparation of the product, the following solution were mixed having [V(V)] = 1.5 × 10^{-3}, [GSH] = 1.0 × 10^{-2} and [HCl] = 3.0 mol dm^{-3} and the mixture was kept for 24h at room temperature. Then the above solution was filtered. The residue was slowly dried in a desiccator. The yield of the product was nearly 70%. The FTIR spectra of the dried product was recorded (fig. 4) in a JASCO FTIR 4100 spectrophotometer using KBr pellet and was compared with the FTIR spectra of pure GSH. (Fig. 3)

Fig. 4 showed a broad peak at 3446.63 cm^{-1} in the product, may be assigned to a υ_{N-H} (NH_3^+) [20] as compared to 3026cm^{-1} in GSH. The shifting to higher frequency was probable due to an association of water molecule with the product. A weak band at 2526cm^{-1} appears in GSH due to S-H stretching which is absent in the product which suggests the dimerization of GSH to GSSG having S-S linkage. The NH_3^+ bending bands and a strong absorption peak of carboxylate ion are mixed up and a broad band is observed at 1630.53cm^{-1} in GSSG as compared to 1599.66cm^{-1}, 1538cm^{-1} and 1333cm^{-1} peak in GSH. The product was isolated as GSSG [(2S)-2-amino-5-[[(2R)-3-[(2R)-2-[[(4S)-4-amino-5-hydroxy-5-oxopentanoyl]amino]-3-[carboxymethylamino]-3-oxopropyl]disulfanyl-1-(carboxy methylamino)-1-oxopropan-2-yl]-5-oxopentanoic acid].

The structure of substrate (GSH) and product (GSSG) was shown below.



Structure of the substrate



Structure of the product (GSSG)

IV. DISCUSSION

Vanadium(V) is known to form protonated species in acid medium [21]. In higher acid concentration, VO_3^- is completely converted in to tetrahydroxovanadium(V) ion, $H_4VO_4^+$ which on protonation forms mono and deprotonated oxidant species as given below.

$$H_4VO_4^+ + H^+ \rightleftharpoons [V(OH)_3]^{2+} + H_2O$$

$$[V(OH)_3]^{2+} + H^+ \rightleftharpoons [V(OH)_2]^{3+} + H_2O$$

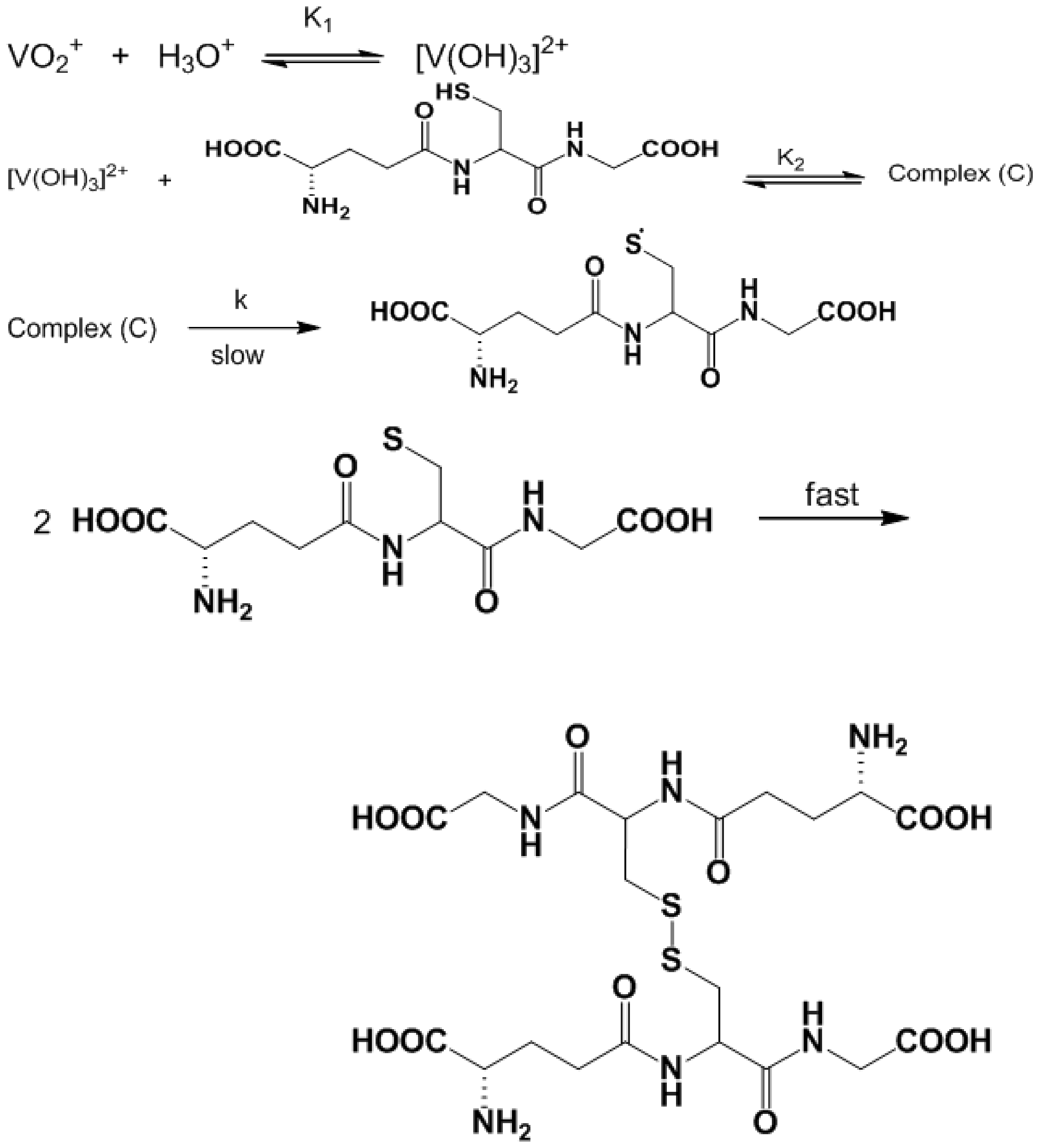
Since the order with respect to acid concentration was about unity, the monoprotonated species of vanadium(V) i.e. $V(OH)_3^{2+}$ is considered to be the kinetically active species.

The rate of reaction was measured at four different temperatures 298, 303, 308 and 313K under varying acid and GSH concentrations. The values of $kK2$ were obtained from the slope of k_{obs}versus $[H^+]$ plot at four different temperatures by knowing the values of K_1[17] at these temperatures. The values of $kK_2$are collected in the Table 2.

From the slope and intercept of the plot $\log(kK_2/T)$ versus 1/T (From Eyring equation), the values of $\Delta H^{\#}$ and $\Delta S^{\#}$ were determined. The values are collected in Table 2.

It is well known that in acid solution vanadium(V) exist [16] mainly as $[V(OH)_3]^{2+}$. The monoprotonated species of vanadium(V) combine with one mole of GSH to form complex C. In the rate determining step, the complex C is decomposed and forms the free radical and vanadium(IV). In the next fast step, two moles of the free radical react to produce GSSG [22, 23].

Scheme 1



From Scheme 1, the rate law can be derived as follows.

$$\text{Rate} = -d[V(V)] / dt$$

$$= k\,[\text{Complex (C)}] \quad (2)$$

$$[\text{Complex (C)}] = K_2\,[V(OH)_3^{2+}][GSH] \quad (3)$$

$$[V(OH)_3^{2+}] = K_1\,[VO^{2+}]\,[H_3O^+] \quad (4)$$

Therefore,

$$[\text{Complex (C)}] = K_1K_2[VO^{2+}]\,[GSH]\,[H_3O^+] \quad (5)$$

Substituting eq. 5 in eq. 2, we get,

$$\text{Rate} = k\,K_1K_2[VO^{2+}]\,[GSH]\,[H_3O^+] \quad (6)$$

$$\text{Rate} = k_{obs}[VO^{2+}] \quad (7)$$

Comparing equation (6) and (7),

$$k_{obs} = kK_1K_2\,[GSH]_e\,[H_3O^+] \quad (8)$$

V. CONCLUSION

GSH is a potential antioxidant in many cellular functions. It acts as a mediator of many physiological reactions including metabolism of xenobiotics, thiol disulfide exchange reactions and cellular signaling. To understand the oxidation of GSH by vanadium containing peroxidase, we have undertaken the electron transfer study between VO^{2+} and GSH.

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Figure 1

Overlay scan of the reaction of V(V) with GSH taken at 5 minutes difference.

$[V(V)]_T = 1.5 \times 10^{-3}$mol dm^{-3} $[GSH]_T = 2.0 \times 10^{-3}$mol dm^{-3} $[HCl]_T = 3.0$ mol dm^{-3} I = 3.01 mol dm^{-3} T = 305K

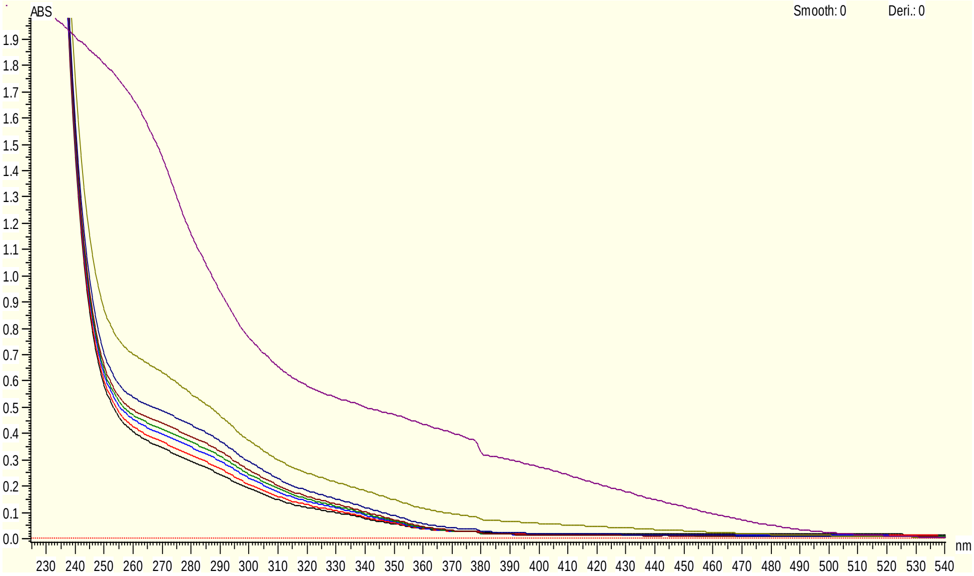

Fig. 2: Plot of k_{obs}versus $[H^+]$ at different temperatures at constant [GSH] =1.0×10^{-2}mol dm^{-3}, [V(V)] = 1.5×10^{-3}mol dm^{-3}, I = 3.1mol dm^{-3}.

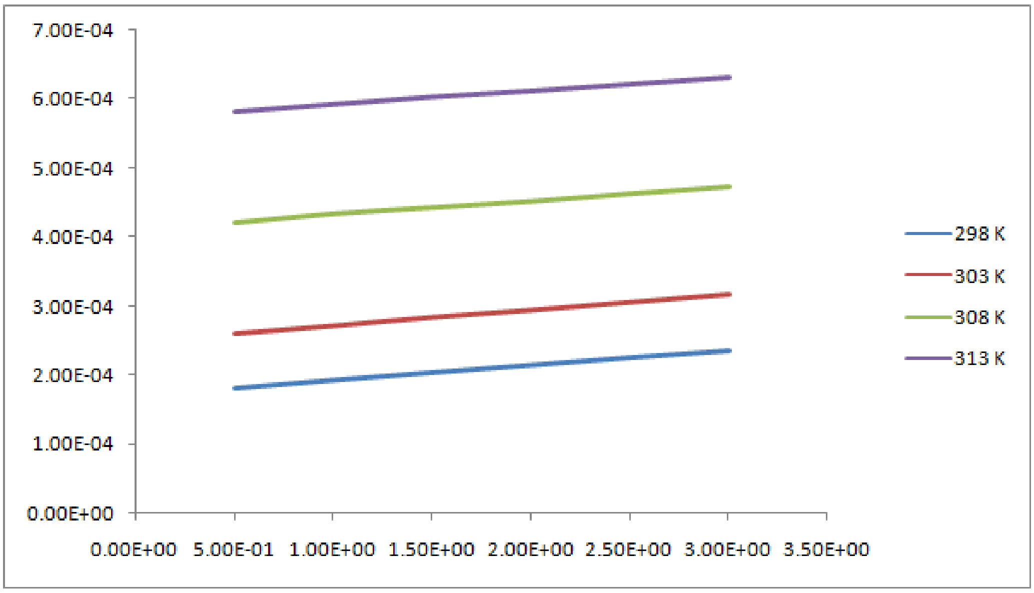

Fig. 3 FTIRspectraofpureGSH

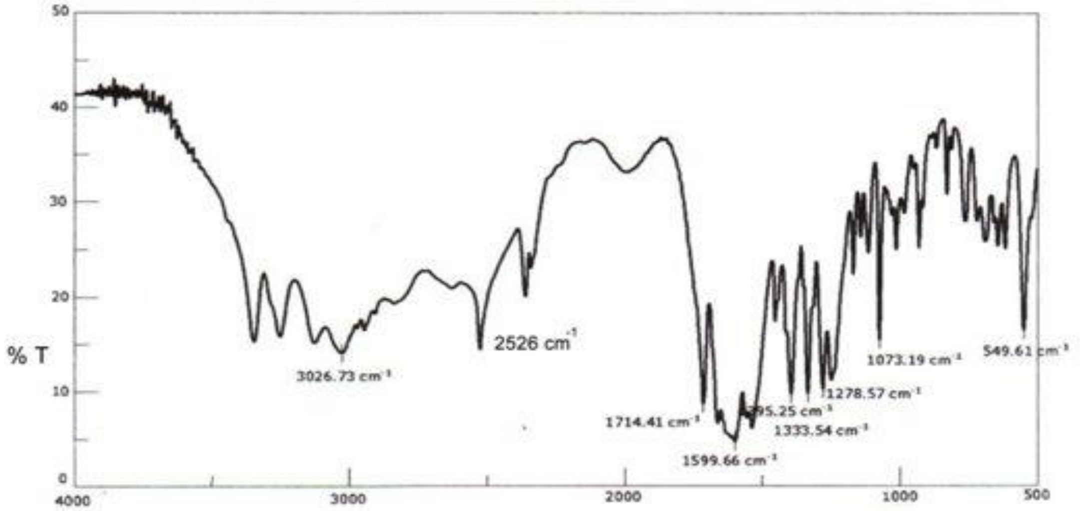

Fig. 4 FTIRspectraofoxidationproductGSSG

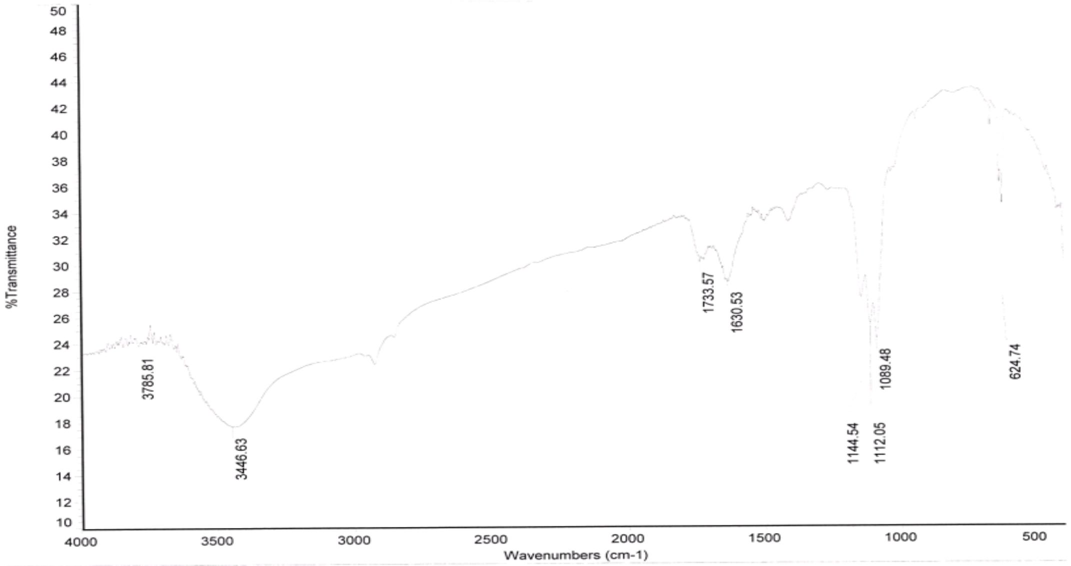

Fig. 5 Tentative structure of the oxidationproduct GSSG

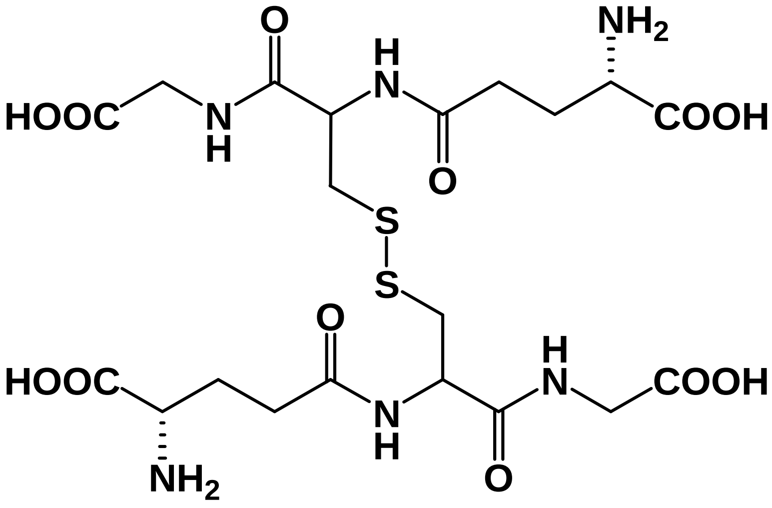

Table 1 : Effect of [V(V)], [GSH] and [HCl] on the oxidation of GSH by V(V) in aqueous hydrochloric acid medium at I = 3.1 mol dm^{-3} at different temperature

10^3[V(V)] (moldm^{-3})	10^2[GSH] (mol dm^{-3})	[HCl] (mol dm^{-3})	$10^4\,k_{obs}$(s^{-1})			
			298	303	308	313
0.2	1.0	3.0	2.34	3.16	4.72	6.31
0.5	1.0	3.0	2.34	3.16	4.71	6.32
0.8	1.0	3.0	2.35	3.15	4.71	6.32
1.0	1.0	3.0	2.34	3.16	4.71	6.31
1.5	1.0	3.0	2.35	3.16	4.72	6.32
2.0	1.0	3.0	2.35	3.16	4.72	6.33
1.5	0.5	3.0	1.23	1.67	2.50	3.34
1.5	0.8	3.0	1.60	2.16	3.21	4.32
1.5	1.0	3.0	2.35	3.16	4.72	6.32
1.5	1.2	3.0	3.49	4.67	7.00	9.31
1.5	1.5	3.0	4.63	6.17	9.25	12.32
1.5	2.0	3.0	9.00	12.00	18.01	23.98
1.5	1.0	0.5	1.80	2.61	4.20	5.81
1.5	1.0	1.0	1.92	2.72	4.33	5.92
1.5	1.0	1.5	2.03	2.84	4.42	6.03
1.5	1.0	2.0	2.14	2.94	4.51	6.12
1.5	1.0	2.5	2.25	3.05	4.62	6.22
1.5	1.0	3.0	2.35	3.16	4.72	6.32

Table 2 : Equilibrium constants and thermodynamic quantities at different temperatures for the oxidation of GSH by vanadium(V) in HCl

T / K	K_1 / dm^3 mol^{-1}	kK_2 / dm^3 mol^{-1} s^{-1}
298	1.11	1.98 x 10^{-3}
303	1.19	1.84 x 10^{-3}
308	1.31	1.55 x 10^{-3}
313	1.48	1.37 x 10^{-3}
$\Delta H^{\#}$ (kJ mol^{-1})	22.46	
$\Delta S^{\#}$ (JK^{-1} mol^{-1})	-371.88	

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