Short communication

Kinetics and Mechanism of Oxidation of L-Cystine by Cerium (IV) in Sulphuric Acid Medium

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Abstract

The kinetics of oxidation of L-cystine by cerium (IV) was studied at 0.5–2.0 mol dm^{-3} sulphuric acid in the range 25–40 °C. The reaction exhibits first order dependence with respect to concentration of cerium (IV) and cystine. Ionic strength has negligible effect on the rate. The rate of the reaction decreases with increase in hydrogen ion concentration up to 0.5–1.0 mol dm^{-3} and remains constant thereafter. Cysteic acid was identified as the main product. A suitable mechanism was proposed and activation parameters of the slow step are computed using linear least squares method and the values of activation energy and entropy were found to be 57.4 ± 1.9 kJ mol^{-1} and –122.7 ± 5.1 JK^{-1} mol^{-1} respectively.

Keywords: Kinetics, mechanism, oxidation, L-cystine, cerium (IV).

1. Introduction

Sulphur is a soft ligand with exceptional ability to participate in coordinate with heavy metals and to bring out redox reactions. Protein structures are expected to be considerably influenced by the subsequent interactions and electron transfers. Cystine, an oxidation product and dimer of cysteine is a non essential sulphur containing amino acid that has a pivotal role to play in the inducible and endogeneous detoxication mechanisms in the body.

The paper deals with the kinetic and mechanistic study on the oxidation of L-cystine which has major biochemical and bio-technological significance since the redox properties of this amino acid play a significant role in many natural biological redox reactions.

L-cystine helps to reduce the ill effects of alcohol consumption and cigarette smoking. Also, to some extent prevents hangovers, liver and brain damage. Further, it helps to strengthen the immune system, reduce damage from free-radicals, and repair nucleic acids in the cells. The oxidation kinetics of cystine helps us in understanding the role of cystine in these beneficiary effects by acting as a scavenger for oxidants and free radicals produced in situ. The oxidation of cystine was studied earlier by very few oxidants like iodine,[1] potassium ferrate,[2,3] permanganate,[4] chlorite and chlorine dioxide,[5] and hypochlorous acid.[6] In some of its oxidations cysteic acid was identified to be the main product while with others cystine thiosulphonate, aldehyde and N,N'-dichlorocystine were reported to be the products. So, in order to have a further insight into the mechanism of oxidation of cystine, we have carried out kinetic investigations by cerium (IV), a one-electron oxidant in sulphuric acid medium.

2. Experimental

A 0.1 mol dm^{-3} solution of cystine in 0.5 mol dm^{-3} sulphuric acid was prepared from pure L-cystine (Hi media).

A 0.1mol dm^{-3} solution of cerium (IV) in 1.0 mol dm^{-3} sulphuric acid was prepared from 99.9% pure ceric sulphate(Indian Rare Earths Limited, Travancore). The strength of the solution was determined with standard iron(II) solution.

All other chemicals used in this investigation were of analytical reagent grade. Double distilled water was used throughout the investigation.

The rate measurements were carried out at 30 ± 0.1 °C in 0.5 mol dm^{-3} sulphuric acid medium under the conditions $[H^+] > [cystine] >> [cerium(IV)]$ and the progress of the reaction was followed by measuring the absorbance of cerium(IV) at 400 nm, using Milton Roy Spectronic-1201 UV-Visible spectrophotometer with 1 cm path

length silica cells. The temperature was maintained constant using a SEW (India) Lab liquid circulatory bath S–36. The rate constants were found to be reproducible within ± 5%.

The product analysis was carried out chromatographically.[7] The chromatographic plate was spotted with the reaction product and it was saturated with the vapours of phenol and then it was run in water-saturated phenol. After the development of chromatogram, the plate was removed from the tank and dried. The paper was then sprayed with 1% solution of ninhydrin in n-butanol and heated in an oven for five minutes at 100 °C. The response factor was found to be 0.13 which is in good agreement with the value of 0.1 reported by Dixit and Srivastava[7] which confirms the presence of cysteic acid. The product was further confirmed by carrying out the microscopic study by transferring the reaction product to a dish and evaporated on a water bath at 60 °C. On condensation of the solution, almost colourless mass was obtained. The mass was dissolved in 40% dilute alcohol and was recrystallised. The crystals obtained were viewed under microscope and compared with those reported earlier by Shinohara in the oxidation of cystine by iodine[1]. In Figure 1 the needle shaped crystals represents cysteic acid.

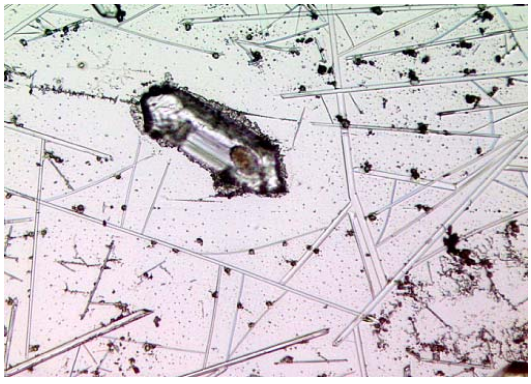

Figure 1. Cysteic acid crystals (needle shaped crystals).

The test for free radicals was carried out by taking cystine, sulphuric acid in a Thumberg tube and acrylonitrile and cerium (IV) in the bent tube. After evacuating the system the solutions were mixed by tilting the tube. The reaction mixture was kept aside and even after 24 hours no precipitate was observed which indicated the absence of free radicals.

3. Results and Discussion

To determine the stoichiometry of the reaction a known excess of cerium (IV) solution was added to a known amount of cystine at 30 °C in 0.5 mol dm^{-3} sulphuric acid medium and after 24 hours the residual cerium(IV) concentration in each case is determined spectrophotometrically. The stoichiometry of the reaction was found to correspond to the equation

$$\text{cystine} + 10\ Ce^{IV} \xrightarrow{H_2O} 2\ \text{cysteic acid} + 10\ Ce^{III}$$

Cerium (III), one of the product was found to have negligible effect on the rate of the reaction.

When the kinetic runs were made with isolation of cerium (IV) by taking cystine in excess, the plots of log(absorbance) versus time were found to be linear even beyond 80% completion of the reaction indicating that the reaction is first order with respect to cerium(IV). The pseudo first order rate constants k' were calculated from the slopes of these plots and were found to be reproducible within ±5% (Table 1).

The order with respect to cystine was determined by carrying out kinetic runs in 0.51 mol dm^{-3} sulphuric acid in the presence of 1.010^{-3} mol dm^{-3} cerium (IV) and varying the cystine concentration over 0.50–3.510^{-2} mol dm^{-3}. The pseudo first order rate constants increase with increase in cystine concentration (Table 2). Further, the plot of k' versus concentration of cystine (Figure 2) was found to be a straight line passing through origin indicating unit order dependence on concentration of cystine.

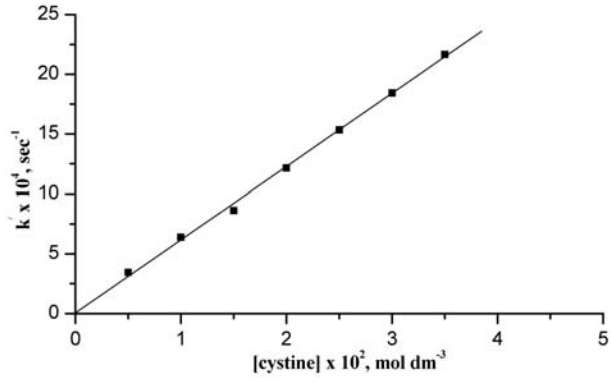

Figure 2. The dependence of pseudo first order rate constant, k' on the concentration of cystine (order with respect to cystine).

Table 1. Effect of concentration of cerium IV) on the pseudo first order rate constant, k'.

10^2[cystine] = 1.50 mol dm^{-3}; [H$^+$] = 0.51 mol dm^{-3}; [HSO$^-_4$] = 0.49 mol dm^{-3}; t = 30 ± 0.1 °C						
10^3[Ce^{IV}]/mol dm^{-3}	0.5	1.0	1.5	2.0	2.5	3.0
10^4k'/s^{-1}	7.83	8.63	8.34	7.96	8.63	7.83

Table 2. Effect of concentration of cystine on the pseudo first order rate constant, k'.

$10^3[Ce^{IV}]$ = 1.0 mol dm^{-3}; $[H^+]$ = 0.51 mol dm^{-3}; $[HSO^-_4]$ = 0.49 mol dm^{-3}; t = 30 ± 0.1 °C							
10^2[cystine]/mol dm^{-3}	0.5	1.0	1.5	2.0	2.5	3.0	3.5
10^4k'/s^{-1}	3.45	6.39	8.63	12.15	15.35	18.44	21.67

Table 3. Effect of concentration of hydrogen ion on the pseudo first order rate constant, k'.

10^2[cystine] = 1.50 mol dm^{-3}; $10^3[Ce^{IV}]$ = 1.0 mol dm^{-3}; $[HSO^-_4]$ = 0.49 mol dm^{-3}; t = 30 ± 0.1 °C							
$[H^+]$/mol dm^{-3}	0.51	0.75	1.00	1.25	1.50	1.75	2.00
10^4k'/s^{-1}	8.63	6.90	5.97	5.83	5.75	5.75	5.61

In order to study the effect of hydrogen ion concentration on the rate of the reaction, kinetic runs were carried out keeping the concentrations of all other reactants constant and varying the hydrogen ion concentration with perchloric acid at a constant bisulphate ion concentration. It was observed that the rate of the reaction decreases initially (0.5–1.00 mol dm^{-3}) and thereafter remains constant at higher hydrogen ion concentration (Table 3).

To study the effect of ionic strength on the rate of the reaction, kinetic runs were carried out keeping the concentrations of all other reactants constant and varying the ionic strength in the range 0.5–3.0 mol dm^{-3} using sodium perchlorate solution. The ionic strength was found to have negligible effect on the reaction rate (Table 4).

To study the effect of bisulphate ion on the rate of the reaction, kinetic runs were carried out at different concentrations of bisulphate varying over the range 0.49–1.97 mol dm^{-3}, keeping hydrogen ion and all other concentration of reactants constant. It was observed that increase in bisulphate concentration decreases the rate of the reaction (Table 5). Further, the plot of 1/ k' versus $[HSO_4^-]$ (Figure 3) was found to be a straight line with a small positive intercept on Y-axis.

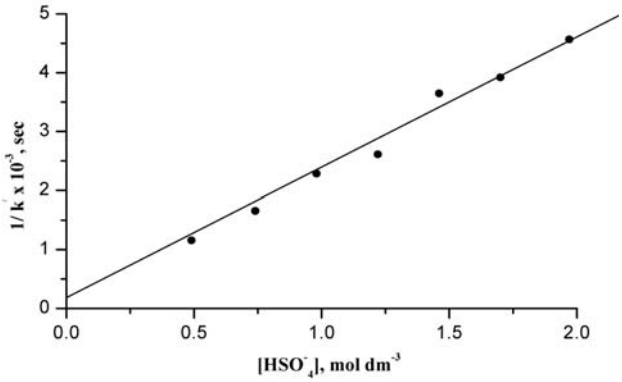

Figure 3: Plot of 1/k' as a function of concentration of HSO_4^- ions.

The effect of temperature on the rate of the reaction was studied by carrying out the reaction at four different temperatures, 35, 40, 45 and 50 °C respectively. The pseudo-first order rate constants thus obtained were tabulated in Table 6.

The energy of activation E_a and the entropy of activation $\Delta S^{\#}$ were calculated at 30 °C employing the linear

Table 4. Effect of ionic strength, μ, on the pseudo first order rate constant, k'.

10^2[cystine] = 1.50 mol dm^{-3}; $10^3[Ce^{IV}]$ = 1.0 mol dm^{-3}; $[H^+]$ = 0.51 mol dm^{-3}; $[HSO^-_4]$ = 0.49 mol dm^{-3}; t = 30 ± 0.1 °C						
μ,/mol dm^{-3}	0.5	1.0	1.5	2.0	2.5	3.0
10^4k'/s^{-1}	8.63	8.34	7.88	8.11	7.96	8.34

Table 5. Effect of concentration of bisulphate on the pseudo first order rate constant, k'.

10^2[cystine] = 1.50 mol dm^{-3}; $10^3[Ce^{IV}]$ = 1.0 mol dm^{-3}; $[H^+]$ = 0.51 mol dm^{-3}; t = 30 ± 0.1 °C							
$[HSO^-_4]$/mol dm^{-3}	0.49	0.74	0.98	1.22	1.46	1.70	1.97
10^4k'/s^{-1}	8.63	6.03	4.38	3.83	2.74	2.55	2.19

Table 6. Effect of temperature, T, on the pseudo-first order rate constant, k'.

10^2[cystine] = 1.50 mol dm^{-3}; $10^3[Ce^{IV}]$ = 1.0 mol dm^{-3}; $[H^+]$ = 0.51 mol dm^{-3}; $[HSO^-_4]$ = 0.49 mol dm^{-3}				
T/K	298	303	308	313
10^4k'/s^{-1}	6.58	8.63	14.07	19.19

least-squares method and were found to be 57.4 ± 1.9 kJ mol^{-1} and –122.7 ± 5.1 J K^{-1} mol^{-1} respectively.

3. 1. Mechanism

From the survey of literature it may be inferred that, depending upon the concentrations of H^+, HSO_4^-, H_2SO_4, cerium(IV) is reported [8,9] to be present in different forms such as Ce^{4+}, $Ce(SO_4)^{2+}$, $Ce(SO_4)_2$, $Ce(SO_4)_3^{2-}$, $HCe(SO_4)_3^-$, $HCe(SO_4)_4^{3-}$, $H_2Ce(SO_4)_4^{2-}$, $H_3Ce(SO_4)_4^-$ and $H_4Ce(SO_4)_4$ in sulphuric acid media. Basing on the effect shown by $[H^+]$ and $[HSO_4^-]$ on the rate of the reaction one or other of these species were treated to be the kinetically reactive species of cerium (IV) in the oxidation of different substrates by cerium(IV). Some of the equilibria[10,11] leading to the formation of sulphato complexes of Ce^{IV} include

$$Ce^{4+} + SO_4^{2-} \overset{K_1}{\rightleftharpoons} Ce(SO_4)^{2+} \quad (A)$$

$$Ce(SO_4)^{2+} + SO_4^{2-} \overset{K_2}{\rightleftharpoons} Ce(SO_4)_2 \quad (B)$$

$$Ce(SO_4)_2 + HSO_4^- \overset{K_3}{\rightleftharpoons} HCe(SO_4)_3^- \quad (C)$$

$$HCe(SO_4)_3^- + HSO_4^- + H^+ \rightleftharpoons H_3Ce(SO_4)_4^- \quad (D)$$

The most relevant equilibria among the above, under the conditions employed in the present investigation appear to be (C) with $Ce(SO_4)_2$ as the reactive species of cerium(IV).

L-cystine, $[-SCH_2CH(NH_2)(COOH)]_2$ is a sulphur containing amino acid and it possess four pK_a values, two corresponding to the carboxylic groups $(COOH)_1$ = 1.51; $(COOH)_2$ = 2.79 and the other two for amino groups $(NH_3^+)_1$ = 8.25; $(NH_3^+)_2$ = 8.97. Under the present experimental conditions ($[H^+]$ = 0.51 mol dm^{-3}), cystine exists in the form of $^-OOC(\overset{+}{N}H_3)CH–CH_2–S–S–CH_2–CH(\overset{+}{N}H_3)COOH$(cystine^{2+}) to the extent of 6% and as $HOOC(\overset{+}{N}H_3)CH–CH_2–S–S–CH_2–CH(\overset{+}{N}H_3)COOH$(cystine^{2+}) to the extent of 93.9%. Therefore, the protonated form of cystine, cystine$^+$ is presumed to be the reactive species and the following mechanism has been proposed.

$$\text{cystine}^+ + H^+ \overset{K}{\rightleftharpoons} \text{cystine}^{2+} \quad (1)$$

$$Ce(SO_4)_2 + HSO_4^- \overset{K_1}{\rightleftharpoons} HCe(SO_4)_3^- \quad (2)$$

$$\text{cystine}^+ + Ce(SO_4)_2 \xrightarrow[\text{slow}]{k_1} X' \quad (3)$$

$$\text{cystine}^{2+} + Ce(SO_4)_2 \xrightarrow[\text{slow}]{k_2} X'' \quad (4)$$

$$X' + Ce(SO_4)_2 \xrightarrow[\text{(in several fast steps)}]{} \text{products} \quad (5)$$

$$X'' + Ce(SO_4)_2 \xrightarrow[\text{(in several fast steps)}]{} \text{products} \quad (6)$$

where cystine$^+$ = $^-OOC(\overset{+}{N}H_3)CH–CH_2–S–S–CH_2–CH(\overset{+}{N}H_3)COOH$

cystine^{2+} = $HOOC(\overset{+}{N}H_3)CH–CH_2–S–S–CH_2–CH(\overset{+}{N}H_3)COOH$

$$\text{Rate} = \frac{-d[Ce^{IV}]}{dt} = k_1[\text{cystine}^+][Ce(SO_4)_2]_e + k_2[\text{cystine}^{2+}][Ce(SO_4)_2]_e \quad (7)$$

$$= k_1[\text{cystine}^+][Ce(SO_4)_2]_e + k_2K[\text{cystine}^+][Ce(SO_4)_2]_e[H^+]_e \quad (8)$$

$$\text{since } [Ce^{IV}]_t = [Ce(SO_4)_2]_e + [HCe(SO_4)_3^-]_e \quad (9)$$

$$\text{and } K_1 = \frac{[HCe(SO_4)_3^-]_e}{[Ce(SO_4)_2]_e[HSO_4^-]_e} \quad (10)$$

substituting $[HCe(SO_4)_3^-]_e$ from equation(10) in equation(9) leads to

$$[Ce(SO_4)_2]_e = \frac{[Ce^{IV}]_t}{1 + K_1[HSO_4^-]_e} \quad (11)$$

Also, $[\text{cystine}]_t = [\text{cystine}^+] + [\text{cystine}^{2+}]$ (12)

$$\text{and } K = \frac{[\text{cystine}^{2+}]}{[\text{cystine}^+][H^+]} \quad (13)$$

substituting $[\text{cystine}^{2+}]$ from equation(13) in equation(12) leads to

$$[\text{cystine}^+] = \frac{[\text{cystine}]_t}{1 + K[H^+]_e} \quad (14)$$

Finally, substituting for $[Ce(SO_4)_2]_e$ and $[\text{cystine}]_t$ from equation(11) and (12) in equation(8) gives

$$\text{Rate} = \frac{-d[Ce^{IV}]}{dt} = \quad (15)$$

$$= \frac{k_1[\text{cystine}]_t[Ce^{IV}] + Kk_2[\text{cystine}]_t[Ce^{IV}]_t[H^+]_e}{(1+K[H^+]_e)(1+K_1[HSO_4^-]_e)}$$

$$= \frac{[\text{cystine}]_t[Ce^{IV}]}{(1+K[H^+]_e)(1+K_1[HSO_4^-]_e)}\{k_1 + Kk_2[H^+]_e\} \quad (16)$$

Equation(16) may be written as;

$$\text{Rate} = \frac{[\text{cystine}][Ce^{IV}]}{1+K[H^+]+K_1[HSO_4^-]\{1+K[H^+]\}} \quad (17)$$

$$\{k_1 + Kk_2[H^+]\}$$

The rate equation explains the first order dependence with respect to both cystine and cerium(IV).

since $\frac{\text{Rate}}{[Ce^{IV}]_t} = k'$ and hence equation (17) may

$$k' = \frac{[\text{cystine}]\{k_1 + Kk_2[H^+]\}}{1+K[H^+]+K_1[HSO_4^-]\{1+K[H^+]\}} \quad (18)$$

Taking reciprocals on both sides for equation(18) leads to

$$\frac{1}{k'} = \frac{1+K[H^+]}{[\text{cystine}]\{k_1 + Kk_2[H^+]\}} + \quad (19)$$

$$+ \frac{K_1[HSO_4^-]\{1+K[H^+]\}}{[\text{cystine}]\{k_1 + Kk_2[H^+]\}}$$

The above equation predicts the plot of 1/k' versus HSO_4^- should be a straight line with a positive intercept on Y-axis. A similar plot(Figure 3) was obtained experimentally thus supporting the proposed mechanism. Further, the initial decrease in rate with increase in $[H^+]$ may be attributed to the progressive conversion of cystine$^+$ to cystine^{2+}.

4. Conclusion

The oxidation of L-cystine by cerium (IV) is slow compared to the oxidation of L-cysteine which was studied earlier by Srivatsava et al.[7]. These authors reported the values of E_a as 41.9 kJ mol^{-1}. However, in the present investigation the E_a value was found to be 57.4 ± 1.9 kJ mol^{-1} since the breakage of S–S bond and further oxidation requires greater activation energy while the 'SH' group of L-cysteine can easily be oxidized with lower activation barrier.

From this observation it is concluded that sulphur atom is the primary centre of attack in the oxidation of cystine or cysteine. Further, the oxidation of cystine was found to proceed slowly when compared to the oxidation of cysteine. This may be due to the disulphide (S–S) bond present in cystine molecule, whereas the thiol group is free in cysteine. Hence, the oxidation of cysteine is much faster compared to that of cystine.

5. References

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Povzetek

Raziskovali smo kinetiko oksidacije L-cistina c cerijem(IV) v prisotnosti žveplene kisline v temperaturnem območju 25–40 °C in v koncentracijskem območju kisline 0.5–2.0 mol dm^{-3}.

Ugotovili smo, da reakcija sledi hitrostnemu zakonu prvega reda glede na cerij(IV) in L-cistin. Ionska moč ima na hitrost reakcije le zanemarljiv vpliv. Hitrost reakcije pada z naraščajočo koncentracijo H^+ do nekako 0.50–1.0 mol dm^{-3} in potem ostaja konstantna. Glavni produkt reakcije, za katero smo predlagali mehanizem poteka, je cisteinska kislina. Ugotovili smo, da aktivacijska energija in E_a in entropija $\Delta S^{\#}$ najpočasnejše stopnje znašata 57.4 ± 1.9 kJ mol^{-1} in –122.7 ± 5.1 JK^{-1} mol^{-1}.