

## Kinetics and Mechanism of Oxidation of Methionine by Chloramine-T in Alkaline Medium in Presence of OsO<sub>4</sub> Catalyst

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Kinetics and mechanism of oxidation of D-L methionine (Met) by sodium N-chlorotoluene-*p*-sulphonamide (CAT) in alkaline medium in presence of OsO<sub>4</sub> catalyst have been investigated at 30°C, and the results are compared to the reaction without Os(VIII) catalyst. The reaction rate shows a first order dependence each on [CAT] and [methionine] and inverse fractional order on [OH<sup>-</sup>] and fractional order on [OsO<sub>4</sub>]. Additions of halide ions and variation of ionic strength and dielectric constant of the medium do not have any significant effect on the reaction rate. Thermodynamic parameters have been evaluated.

### INTRODUCTION

There are very few reports on the mechanistic aspects of oxidation of methionine. A review of literature has shown that aromatic sulphonylhaloamines have been employed for the estimation<sup>1,2</sup> of methionine in solution and a few reactions have been kinetically investigated<sup>3</sup>.

Oxidation and kinetic study of methionine by N-haloamines has not been investigated in presence of the catalyst. It was therefore of interest to study the kinetics of oxidation of methionine by the chloramine-T in presence of catalyst OsO<sub>4</sub> in alkaline medium at 30°C.

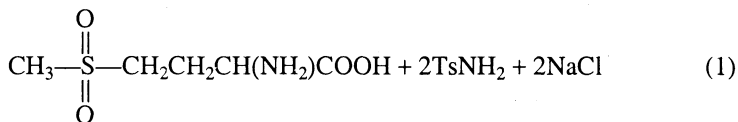
### EXPERIMENTAL

Chloramine-T (Merck) was purified by the method of Morris *et al.*<sup>4</sup> The purity of compound was checked by iodometric assay of the active halogen and by recording its Fourier transform <sup>1</sup>H and <sup>13</sup>C NMR spectra. An aqueous solution of the compound was standardized by the iodometric method and preserved in brown bottles to prevent its photochemical deterioration.

Chromatographically pure DL-methionine (Merck) was further assayed by acetous perchloric acid method<sup>5</sup> and its aqueous solution was prepared. All other reagents were of AnalaR grade. The ionic strength of the system was kept at a high value by using a concentrated NaClO<sub>4</sub> solution.

*Stoichiometry and product analysis:* Reaction mixture containing methionine and CAT in presence of  $7.86 \times 10^{-6}$  mol dm<sup>-3</sup> OsO<sub>4</sub> and 0.1 mol dm<sup>-3</sup> NaOH were

equilibrated with occasional shaking at 30°C for 24 h. The iodometric determination of unreacted oxidant in the reaction mixture showed that one mole of methionine reacted with two moles of oxidant. The observed reaction stoichiometry is given by the equation (1).



Here Ts =  $\text{CH}_3\text{C}_6\text{H}_4\text{SO}_2$ -toluene-*p*-sulphonamide ( $\text{TsNH}_2$ ) was detected by paper chromatography. Benzyl alcohol saturated with water was used as the solvent with 0.5% vanilin in 1% HCl solution in ethanol as spray reagent ( $R_f = 0.905$ ). Identification of methionine sulphone was made through paper chromatography. The solvent employed was *n*-butanol-glacial acetic acid-water (4 : 1 : 5 v/v) and a 0.2% solution of ninhydrin in butanol, water and acetic acid (95 : 4 : 0.5 v/v) was used as the spray reagent ( $R_f = 0.17$ ).

## RESULTS AND DISCUSSIONS

*Effect of varying reactant concentrations:* The kinetics of oxidation of methionine by CAT were equilibrated at several initial concentrations of the reactants in alkaline medium with  $\text{OsO}_4$  as catalyst. In the presence of excess [methionine] and fixed [NaOH] and  $[\text{OsO}_4]$ , plots of  $\log [\text{CAT}]$  versus time are linear, indicating a first order dependence of rate on [CAT]. The pseudo first-order rate constant  $k'$  are obtained (Table-1).

TABLE-1  
EFFECT OF VARYING REACTANT CONCENTRATIONS ON THE  
RATE OF REACTION

$[\text{NaOH}] = 0.10 \text{ mol dm}^{-3}$ ,  $[\text{OsO}_4] = 7.86 \times 10^{-6} \text{ mol dm}^{-3}$ ,  $\mu = 0.4 \text{ mol dm}^{-3}$

| $10^3 [\text{CAT}]$<br>$\text{mol dm}^{-3}$ | $10^2 [\text{methionine}]$<br>$\text{mol dm}^{-3}$ | $10^4 k'$<br>$\text{sec}^{-1}$ |
|---|--|--------------------------------|
| 0.5   | 1.0  | 1.64                           |
| 0.7   | 1.0  | 1.63                           |
| 0.8   | 1.0  | 1.62                           |
| 0.9   | 1.0  | 1.61                           |
| 1.0   | 1.0  | 1.60                           |
| 1.1   | 1.0  | 1.58                           |
| 1.2   | 1.0  | 1.57                           |
| 1.0   | 0.5  | 0.80                           |
| 1.0   | 1.0  | 1.60                           |
| 1.0   | 2.0  | 3.18                           |
| 1.0   | 3.0  | 4.76                           |
| 1.0   | 4.0  | 6.34                           |
| 1.0   | 5.0  | 7.94                           |

At constant  $[\text{CAT}]_0$ ,  $[\text{NaOH}]$ ,  $[\text{OsO}_4]$  and temperature the rate increased with increase of [methionine] yielding linear plot of  $\log k'$  versus  $\log [\text{methionine}]_0$  with slope equal to unity, indicating a first order dependence of the reaction rate on [methionine] (Table-1). Further, the rate increased with increase in  $[\text{OsO}_4]$  and plot of  $\log k'$  versus  $\log [\text{OsO}_4]$  was linear with slope less than unity indicating a fractional order dependence of rate on  $[\text{OsO}_4]$  (Table-2).

TABLE-2  
EFFECT OF CONCENTRATION OF CATALYST  $\text{OsO}_4$  ON THE RATE OR REACTION

$[\text{CAT}] = 0.001 \text{ mol dm}^{-3}$ ;  $[\text{NaOH}] = 0.10 \text{ mol dm}^{-3}$

$[\text{methionine}] = 0.01 \text{ mol dm}^{-3}$ ;  $\mu = 0.4 \text{ mol dm}^{-3}$

| $10^6 [\text{OsO}_4]$<br>$\text{mol dm}^{-3}$ | $10^4 k'$<br>$\text{sec}^{-1}$ |
|---|--------------------------------|
| 7.86  | 1.60                           |
| 11.78   | 2.13                           |
| 15.70   | 2.61                           |
| 19.62   | 3.05                           |
| 23.60   | 3.48                           |
| 27.50   | 3.85                           |
| 31.40   | 4.17                           |

*Effect of NaOH:* The reaction rate was studied as function of the concentration of sodium hydroxide. The rate decreased with increase in  $[\text{NaOH}]$  and a plot of  $\log k'$  versus  $\log$  of  $[\text{OH}^-]_R$  was linear with a slope of  $-0.4$ , indicating an inverse fractional order in  $[\text{NaOH}]$ . Here the reactive  $[\text{OH}^-]_R$  was determined by the relation  $[\text{OH}^-]_R = [\text{OH}^-]_T - [\text{OH}^-]_{\text{Met}}$ , where  $[\text{OH}^-]_T$  was the total concentration and  $[\text{OH}^-]_{\text{Met}}$  was the hydroxide ion concentration neutralized by the amino acid.

TABLE-3  
EFFECT OF CONCENTRATION OF  $\text{NaOH}$  ON THE RATE OF REACTION

$[\text{CAT}] = 0.001 \text{ mol dm}^{-3}$ ;  $[\text{methionine}] = 0.01 \text{ mol dm}^{-3}$

$[\text{OsO}_4] = 7.86 \times 10^{-6} \text{ mol dm}^{-3}$ ;  $\mu = 0.4 \text{ mol dm}^{-3}$

| $[\text{NaOH}]$<br>$\text{mol dm}^{-3}$ | $10^4 k'$<br>$\text{sec}^{-1}$ |
|---|--------------------------------|
| 0.05                                    | 1.94                           |
| 0.10                                    | 1.60                           |
| 0.15                                    | 1.33                           |
| 0.20                                    | 1.17                           |
| 0.25                                    | 1.03                           |

*Effect of TPS and ionic strength:* Addition of the reaction product, toluene-*p*-sulphonamide ( $0.0002 - 0.001 \text{ mol dm}^{-3}$ ) to the reaction mixture of CAT and methionine had no effect on the rate. Similarly, the reaction rate was unaffected by the variation of the ionic strength of the medium ( $0.12 - 0.4 \text{ mol dm}^{-3}$ ) by adding  $\text{NaClO}_4$ .

TABLE-4  
KINETIC AND THERMODYNAMIC PARAMETERS FOR THE OXIDATION OF  
METHIONINE BY CAT IN ALKALINE MEDIUM IN PRESENCE OF OsO<sub>4</sub>

| Temperature<br>K | 10 <sup>4</sup> k'<br>sec <sup>-1</sup> | E <sub>a</sub><br>kJ mol <sup>-1</sup> | ΔH <sup>‡</sup><br>kJ mol <sup>-1</sup> | ΔS <sup>‡</sup><br>J K mol <sup>-1</sup> | ΔG <sup>‡</sup><br>kJ mol <sup>-1</sup> |
|------------------|---|--|---|--|---|
| 298              | 1.12                                    | 54.70                                  | 52.20                                   | -145.60                                  | 96.30                                   |
| 303              | 1.60                                    | (64.70)                                | (62.20)                                 | (-142.5)                                 | (94.50)                                 |
| 308              | 2.34                                    |  |   |  |   |
| 313              | 3.23                                    |  |   |  |   |

Values in parentheses for oxidation of methionine of CAT in alkaline medium without catalyst.

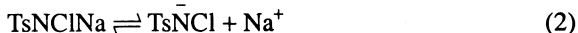
*Effect of chloride ion and bromide ion:* Addition of Cl<sup>-</sup> ion in the form of NaCl or Br<sup>-</sup> ion in the form of NaBr has no effect on the rate.

*Effect of varying dielectric constant of solvent:* The effect of dielectric constant [D] of the solvent of the reaction mixture was studied by varying the MeOH content (0–15% v/v) and indicated no significant effect on the rate.

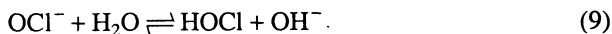
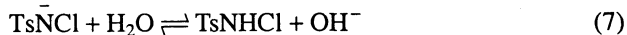
*Effect of temperature:* The reaction was carried out at different temperatures (298 to 313 K). The activation parameters were calculated from a plot of log k' versus 1/T.

Tests performed using olefinic monomers were negative indicating the absence of free radical formation in the reaction mixture of methionine and CAT.

Morris *et al.*<sup>4</sup>, Pryde and Soper<sup>6</sup>, Bishop and Jennings<sup>7</sup> have shown the existence of similar equilibria in acid and alkaline solutions of N-metallo-N-haloarylsulphonamides and chloramine-T behaves as a strong electrolyte in aqueous solutions forming different species as in equations (2)–(6).



In acidic medium, the probable oxidising species are the free acid (TsNHCl), dichloramine-T (TsNCl<sub>2</sub>), HOCl and H<sub>2</sub>OCl<sup>+</sup>. In alkaline solutions of CAT, TsNCl<sub>2</sub> and H<sub>2</sub>OCl<sup>+</sup> do not exist. The expected reactive species are TsNHCl, HOCl and TsNCl. The possible species TsNCl and OCl<sup>-</sup> which could be transformed into more reactive oxidising species TsNHCl and HOCl through reactions (7), (8) and (9).

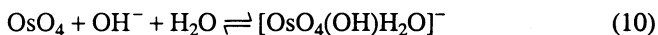


If HOCl were the primary oxidizing species as equations (8) and (9) indicate a first order retardation of the rate by the added toluene sulphonamide (TsNH<sub>2</sub>)

would be expected, which is contrary to the experimental observations. A retarding influence of  $\text{OH}^-$  ions on the reaction rate has been observed in many reactions of CAT, which has been attributed to the formation of conjugate acid  $\text{TsnHCl}$  from  $\text{TsnCl}^-$  through reaction (7). Hence an inverse fractional order dependence of rate on  $[\text{OH}^-]$  observed in presence of  $\text{OsO}_4$  indicates that  $\text{TsnHCl}$  is the most likely oxidizing species.

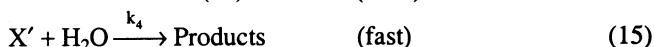
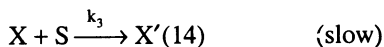
Ultraviolet spectral measurements showed that methionine solution has a sharp absorption band at 214 nm and a band around 226 nm was noticed for  $\text{OsO}_4$ , while a band at 230 nm for CAT solution was observed, in presence of  $0.1 \text{ mol dm}^{-3}$  NaOH solution. A mixture of CAT and  $\text{OsO}_4$  solution in the presence of NaOH solution showed a band around 265 nm, while for the mixture of methionine and  $\text{OsO}_4$  solution in presence of NaOH solution there was no change in  $\lambda_{\text{max}}$  at 221 nm, indicating that complex formation takes place only between the catalyst and the oxidant CAT.

Further, it has been shown that  $\text{OsO}_4^{8-10}$  is stable as  $\text{Os(VIII)}$  and exist in the following equilibria in alkaline medium.



Both  $[\text{OsO}_4(\text{OH})\text{H}_2\text{O}]^-$  and  $[\text{OsO}_4(\text{OH})_2]^{2-}$  possess octahedral geometry and they may not be able to form effective complexes with the oxidant. It is more realistic to postulate  $\text{OsO}_4$ , which possess tetrahedral geometry, as the active species that can effectively form the complex with the oxidant species.

Therefore, the first order dependence of the reaction rate on  $[\text{CAT}]$  and  $[\text{methionine}]$ , a fractional order dependence on  $[\text{OsO}_4]$ , a negative fractional order dependence on  $[\text{OH}^-]$  indicate that the formation of an intermediate species from  $\text{OsO}_4$  and CAT serves as an oxidant and the possible oxidising species in this case would be  $\text{TsnHCl}$ . The electron density around N atom in  $\text{TsnHCl}$  is decreased and N—Cl bond becomes weaker in the complex. Subsequently, the electrophilic character of  $\text{TsnHCl}$  is increased after complexation facilitating the reaction with the substrate. In view of these findings, it is likely that Scheme-1 is more probable for explaining the oxidation of methionine in CAT in alkaline medium in presence of  $\text{OsO}_4$ .



#### Scheme-I

If  $[\text{CAT}]_t$  represents total effective concentration of CAT, from Scheme-I,

$$[\text{CAT}]_t = \text{Tsn}^-\text{Cl} + \text{TsnHCl} + \text{X} \quad (16)$$

we have

$$[\text{TsNHCl}] = \frac{X}{K_2[\text{OsO}_4]}$$

$$[\text{TsNCl}^-] = \frac{[X]}{K_2[\text{OsO}_4]} \frac{[\text{OH}^-]}{K_1[\text{H}_2\text{O}]}$$

By substituting for  $[\text{TsNHCl}^-]$  and  $[\text{TsNCl}^-]$  into equation (16) one gets the following equation (17):

$$[X] = \frac{K_1 K_2 [\text{CAT}]_t [\text{OsO}_4] [\text{H}_2\text{O}]}{[\text{OH}^-] + K_1 [\text{H}_2\text{O}] + K_1 [\text{H}_2\text{O}] K_2 [\text{OsO}_4]} \quad (17)$$

Since

$$\text{Rate} = k_3 [X][\text{S}] \quad (18)$$

by substituting equation (17) into equation (18), we get

$$\text{Rate} = -\frac{d}{dt}[\text{CAT}]_t = \frac{k_3 K_1 K_2 [\text{CAT}]_t [\text{S}] [\text{OsO}_4] [\text{H}_2\text{O}]}{K_1 [\text{H}_2\text{O}] + [\text{OH}^-] + K_1 K_2 [\text{OsO}_4] [\text{H}_2\text{O}]} \quad (19)$$

which is in agreement with the experimental data including a first order each in  $[\text{CAT}]$  and  $[\text{methionine}]$ , positive fractional order in  $[\text{OsO}_4]$  and a negative fractional order in  $[\text{OH}^-]$  since  $\text{rate} = k'[\text{CAT}]_t$ , under pseudo first order condition of  $[\text{methionine}] \gg [\text{CAT}]$  the rate equation (19) can be transformed into equation (20) and (21).

$$k' = \frac{k_3 K_1 K_2 [\text{S}] [\text{OsO}_4] [\text{H}_2\text{O}]}{K_1 [\text{H}_2\text{O}] + [\text{OH}^-] + K_1 K_2 [\text{OsO}_4] [\text{H}_2\text{O}]} \quad (20)$$

$$\frac{1}{k'} = \frac{1}{k_3 K_2 [\text{S}] [\text{OsO}_4]} + \frac{[\text{OH}^-]}{k_3 K_1 K_2 [\text{S}] [\text{OsO}_4] [\text{H}_2\text{O}]} + \frac{1}{k_3 [\text{S}]} \quad (21)$$

Then,

$$\frac{1}{k'} = [\text{OH}^-] \left\{ \frac{1}{k_3 K_1 K_2 [\text{S}] [\text{OsO}_4] [\text{H}_2\text{O}]} \right\} + \left\{ \frac{1}{k_3 K_2 [\text{S}] [\text{OsO}_4]} + \frac{1}{k_3 [\text{S}]} \right\} \quad (22)$$

$$\frac{1}{k'} = \frac{1}{\text{OsO}_4} \left\{ \frac{1}{k_3 K_2 [\text{S}]} + \frac{[\text{OH}^-]}{k_3 K_1 K_2 [\text{S}] [\text{H}_2\text{O}]} \right\} + \frac{1}{k_3 [\text{S}]} \quad (23)$$

A plot of  $1/k'$  versus  $[\text{OH}^-]$  from equation (22) gives

$$\text{Slope} = \frac{1}{k_3 K_1 K_2 [\text{S}] [\text{OsO}_4] [\text{H}_2\text{O}]}$$

and

$$\text{Intercept} = \frac{1}{k_3 K_2 [\text{S}] [\text{OsO}_4]} + \frac{1}{k_3 [\text{S}]}$$

Similarly, a plot of  $1/k'$  versus  $1/[\text{OsO}_4]$  from equation (23) gives

$$\text{Slope} = \frac{1}{k_3 K_2 [\text{S}]} + \frac{[\text{OH}^-]}{k_3 K_1 K_2 [\text{S}] [\text{H}_2\text{O}]}$$

$$\text{Intercept} = \frac{1}{k_3 [\text{S}]}$$

Therefore, from the values of slopes and intercepts of equations (22) and (23) the values of  $K_1$ ,  $K_2$  and  $k_3$  are calculated for the standard run with  $[\text{CAT}]_0 = 1.00 \times 10^{-3} \text{ mol dm}^{-3}$ ,  $[\text{methionine}] = 1.0 \times 10^{-2} \text{ mol dm}^{-3}$ ,  $[\text{OsO}_4] = 7.86 \times 10^{-6} \text{ mol dm}^{-3}$  and  $[\text{NaOH}] = 0.10 \text{ mol dm}^{-3}$  at 303 K.

The values obtained are  $k_3 = 8.52 \times 10^{-2} \text{ sec}^{-1}$

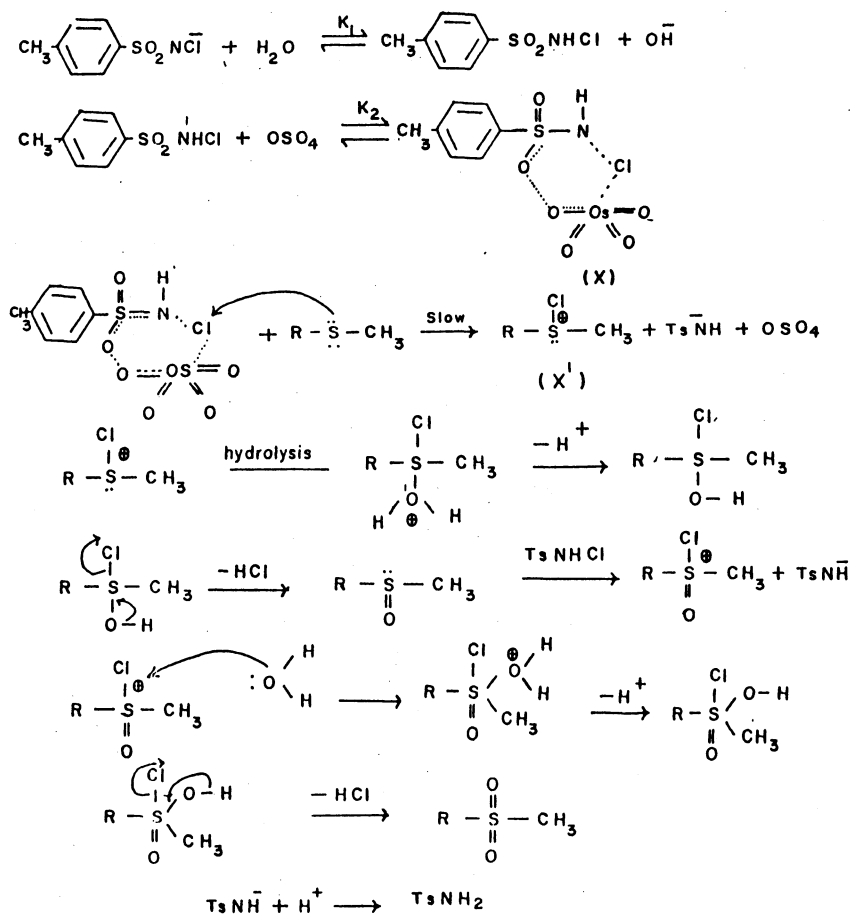
$$K_2 = 5.26 \times 10^4 \text{ lit mol}^{-1}$$

$$K_1 = 2.2 \times 10^{-3} \text{ lit mol}^{-1} \quad \text{from equation (22)}$$

and

$$K_1 = 1.8 \times 10^{-3} \text{ lit mol}^{-1} \quad \text{from equation (23)}$$

The values of  $K_1$  obtained from equations (22) and (23) are same. Further, the value of  $K_1$  is in good agreement with the value of  $K_1$  obtained in previous



Here  $R = \text{CH}_2\text{CH}_2\text{CH(NH}_2\text{)COOH}$

$Ts = \text{CH}_3\text{C}_6\text{H}_4\text{SO}_2$

Scheme-2

publications<sup>11,12</sup>. Therefore, the near constancy of  $K_1$  value forms a strong evidence for the formation of the species  $TsNHCl$  from  $TsNCl$  and  $H_2O$  (Step 12 of Scheme-1) for the oxidation of methionine by CAT in alkaline medium with  $OsO_4$  as catalyst.

A detailed mechanistic interpretation of the methionine-CAT reaction in presence of  $OsO_4$  is presented in Scheme-2. The conjugate acid  $TsNHCl$  coordinates to the catalyst  $OsO_4$  to form a cyclic complex (X), activating the CAT by stabilizing the charge on its nitrogen and polarizing the N—Cl bond. An electrophilic attack by the positive chlorine of complex species X at the sulphur atom of the substrate forms the intermediate species  $X^1$  and with regeneration of the catalyst  $OsO_4$  and  $TsNH$ .  $X^1$  is attacked by the dipolar solvent. Elimination of  $H^+$  and  $HCl$  results in the formation of methionine sulphoxide, which is oxidised by the second molecule of the oxidant, to give methionine sulphone. The four electron stoichiometry shown for the reaction clearly rules out the formation of a sulphimide as a minor product which was noticed by Ruff and Kucsman during the oxidation of sulphides by BAT.

It is of interest to compare the thermodynamic parameters obtained for the oxidation of methionine by CAT in the absence of  $OsO_4$  catalyst with the present studies. A preliminary comparison shows that the energy of activation ( $E_a$ ) is lower in the catalysed reaction. The moderate  $\Delta H^\ddagger$  and a fairly negative  $\Delta S^\ddagger$  values support the formation of a rigid transition state.

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