



Oxidative transformation of methionine by pyridinium chlorochromate in aqueous DMF medium: A kinetic study

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Abstract

The Kinetics of Oxidative transformation of Methionine by pyridinium chlorochromate in aqueous DMF medium, containing perchloric acid has been studied. The rate of reaction was found to be of first order dependence on PCC, Methionine, and H^+ ion Concentration. Michaelis–Menten type kinetics is observed respect to substrate. The increase in the rate of reaction with increase in acidity indicates the involvement of a protonated chromium(VI) species in the rate-determining step. The product of oxidation has been identified as methionine sulfoxide. The rate of reaction increased with increase in dielectric constant of the reaction medium. The plots of $\log k_{obs}$ versus $(D-1) / (2D+1)$ are non linear Indicating that there is an involvement of positive ion-dipole in the rate determining step. The activation parameters have been evaluated. On the basis of the experimental findings, a suitable mechanism and rate law has been proposed.

Keywords: Kinetics; Oxidation; DMF (N; N-dimethylformamide); Methionine; PCC (pyridinium chlorochromate)

1. Introduction

Pyridinium chlorochromate being one of the most versatile available oxidizing agent [1]. A number of reports on the oxidation of several substrates by pyridinium chlorochromate (PCC) are available in the literature like methionine [2], oximes [3], unsaturated acids [4], cysteine [5], alcohols [6]. There seems to be no report on the oxidation of Tyrosine by pyridinium chlorochromate.

Extensive studies on the mechanism of oxidation of methionine by several oxidants have been reported. Even though methionine, a naturally occurring sulfur containing amino acid, has three coordinating centers, viz., O, N and S, it has been reported by McAuley and coworkers [7-9] that the sulfur is more susceptible to attack by Cr(VI). Methionine is a methyl donor and this process in the body is activated by adenosine triphosphate (atp) and a liver enzyme such as phosphatase or dehydrogenase⁴. Active methionine can transfer its methyl group to a variety of compounds and the high energy S-methyl bond is the prime reason for this behavior. The oxidation of this biologically important amino acid is very important because it may reveal the mechanism of amino acid metabolism.

Literature survey reveals that the oxidation of methionine by different oxidants has received a limited attention so we are particularly interested to see the mechanism of the oxidation of methionine by pyridinium chlorochromate in acidic DMF-Water media.

2. Materials

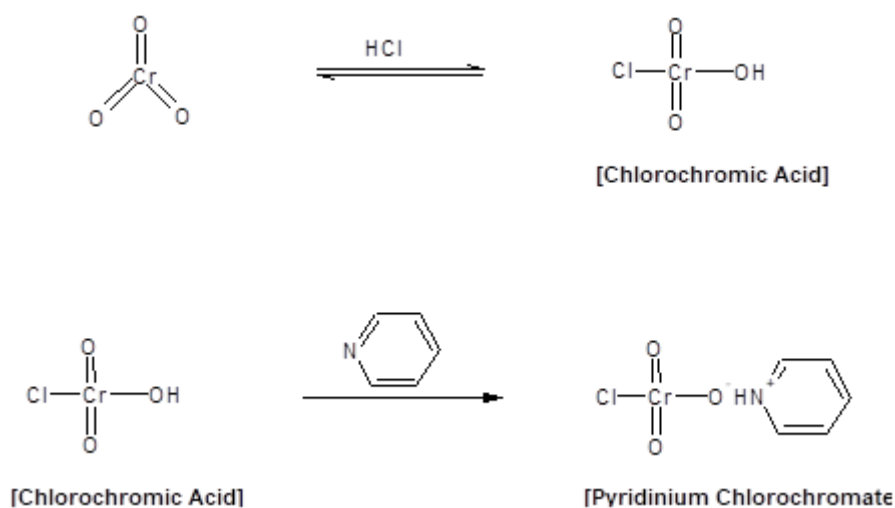
In this investigation methionine Analar grade (SRL) were used as supplied and purity was checked by its melting point. All other chemicals used in this investigation were of analytical reagent grade. Solution of $HClO_4$ was prepared by diluting known volume of acid in water. The prepared solution was standardized by titration with previously

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standardized NaOH using phenolphthalein as an indicator. Doubly distilled analytical grade DMF (SRL) was used. And all other Chemicals used were of analytical grade with 99.9% purity.

2.1. Synthesis of Pyridinium Chlorochromate

Pyridinium chlorochromate was synthesized by the method of Corey and Suggs [10] and Agrawal [11]. To 184 ml of 6 M hydrochloric acid (1.1 mol.) was added 100 g (1.0 mol) of CrO_3 rapidly with stirring. After 5 min. the homogeneous solution was cooled to 0°C and 79.1 g (1.0 mol) of pyridine was carefully added over 10 min. recooling to 0°C gave a yellow orange solid which was collected on a sintered glass funnel and dried for 1 hr in vacuum; yield 172.9 g (80 %). The solid is not appreciably hygroscopic and can be stored for extended periods at room temperature without change. Purity was checked by iodometrically, melting point, elemental analysis and by the IR and UV-VIS spectral analysis [U. V. - $\lambda_{\text{max}}=354 \text{ nm}$; I. R. - ν_{max} (KBr) = 3066, 2033, 1898, 1599, 1533, 1485, 1033, 949, 750 cm^{-1}]



2.2. Method

The measurements of rate were carried out at $40 \pm 0.1^\circ\text{C}$ in $0.3 \text{ mole dm}^{-3} \text{ HClO}_4$ under the condition [Methionine] \gg [PCC], in the solvent system of 70-30 % (v/v) DMF- H_2O . The reaction was initiated by mixing a calculated amount of thermostatted pyridinium chlorochromate in to the reaction mixture. The progress of the reaction was followed by measuring the absorbance of PCC at 354 nm in 1 cm cell placed in the thermostatted compartment of JASCO model 7800 UV/VIS spectrophotometer.

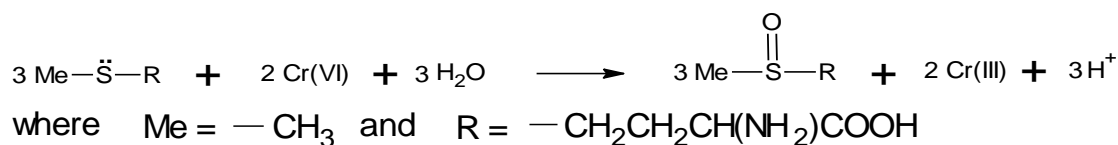
The kinetics runs were followed for more then 60-70% completion of the reaction and good first order kinetics were observed. Pseudo-first order rate constant k_{obs} were obtained from the slope of the plots of log (absorbance) versus time.

2.3. Stoichiometry and Product Analysis

A known slight excess of pyridinium chlorochromate was added to a known amount of methionine, by keeping constant all other condition of reaction, and after 12 hours the residual pyridinium chlorochromate was determined spectrophotometrically at 354 nm. Absorbance was noted before and after the completion of the reaction. The results indicated that 2 mol of PCC consumed 3 mol of Methionine for complete oxidation.

The product study has been done under kinetic conditions. For quantitative product analysis, substrate and oxidant were taken in 10:1 ratio in the reaction mixture. In the reaction mixture, amino acid and pyridinium chlorochromate concentrations were M /50 and M /500 respectively and kept in dark for a few hours. Then sodium bicarbonate was added and stirred vigorously, followed by a drop wise addition of benzyl chloride solution. The precipitate N-benzyl methionine sulfoxide [12] was confirmed by its melting point 183°C . The procedure is similar one employed in the oxidation of methionine [13] by aqueous Cr(VI). Acetone-ethanol mixture (1:1) added to the reaction mixture resulted in the precipitate of methionine sulfoxide [14], which was identified by its melting point 238°C .

The stoichiometry of the reactions was found correspond to the equation.



3. Result and discussion

3.1. Effect of Oxidant Concentration

The reaction has been investigated by varying pyridinium chlorochromate concentration in the range $0.70 \times 10^{-3}\text{M}$ to $1.8 \times 10^{-3}\text{M}$. The rate constant did not change with increase in concentration of PCC. Table -1.

Table 1 Variation of rate with PCC, Methionine, and perchloric acid concentrations

| $10^3 \times [\text{PCC}]$ mol dm^{-3} | $10^2 \times [\text{Methionine}]$ mol dm^{-3} | $10^2 \times [\text{H}^+]$ mol dm^{-3} | $k_{\text{obs}} \times 10^5$ s^{-1} |
|--|---|--|---|
| 0.7 | 1.0 | 3.0 | 34.24 |
| 0.9 | 1.0 | 3.0 | 34.15 |
| 1.0 | 1.0 | 3.0 | 34.37 |
| 1.3 | 1.0 | 3.0 | 34.80 |
| 1.6 | 1.0 | 3.0 | 35.12 |
| 1.8 | 1.0 | 3.0 | 34.40 |
| 1.0 | 0.4 | 3.0 | 16.12 |
| 1.0 | 0.6 | 3.0 | 22.90 |
| 1.0 | 1.0 | 3.0 | 34.46 |
| 1.0 | 1.8 | 3.0 | 46.82 |
| 1.0 | 2.2 | 3.0 | 49.89 |
| 1.0 | 3.0 | 3.0 | 79.64 |
| 1.0 | 4.0 | 3.0 | 95.80 |
| 1.0 | 4.4 | 3.0 | 100.44 |
| 1.0 | 1.0 | 0.75 | 12.43 |
| 1.0 | 1.0 | 1.0 | 14.30 |
| 1.0 | 1.0 | 1.5 | 23.03 |
| 1.0 | 1.0 | 3.0 | 35.40 |
| 1.0 | 1.0 | 3.375 | 42.60 |
| 1.0 | 1.0 | 4.5 | 54.50 |
| 1.0 | 1.0 | 7.5 | 116.68 |
| 1.0 | 1.0 | 10.0 | 140.20 |

T=313 K DMF: Water = 70:30 % (v/v)

3.2. Effect of Substrate Concentration

The rate of reaction increased with an increase in the concentration of substrate from 0.4×10^{-2} to 4.4×10^{-2} mole/dm³ (Table-1). The plot of $\log k_{\text{obs}}$ versus $\log[\text{substrate}]$ [Figure-1] was linear with positive slope value ≈ 1 , indicating first-order dependence of the rate on [substrate]. The plot of $1/k_{\text{obs}}$ versus $1/[\text{substrate}]$, [Figure- 2] gives straight line with positive intercept, which indicates that Michaelis–Menten type kinetics is followed with respect to substrate.

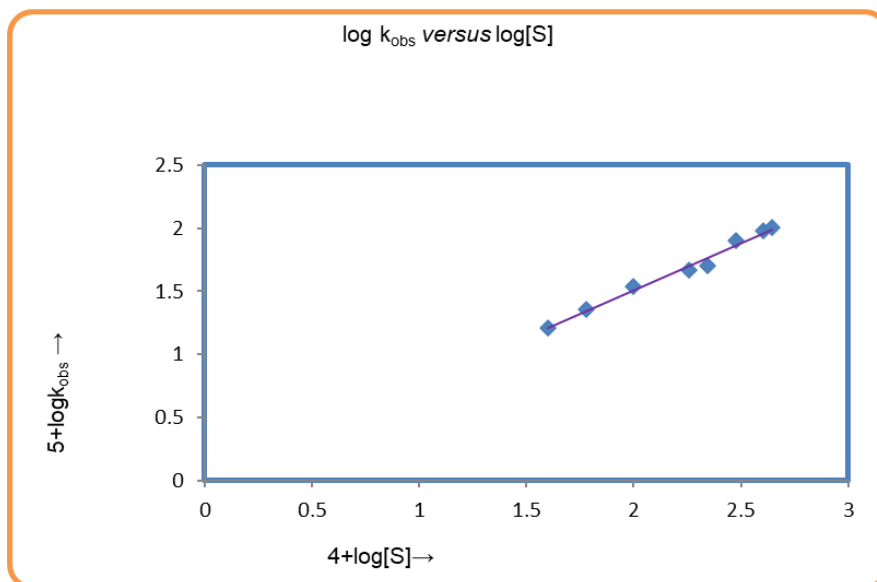


Figure 1 Variation of rate with substrate concentration

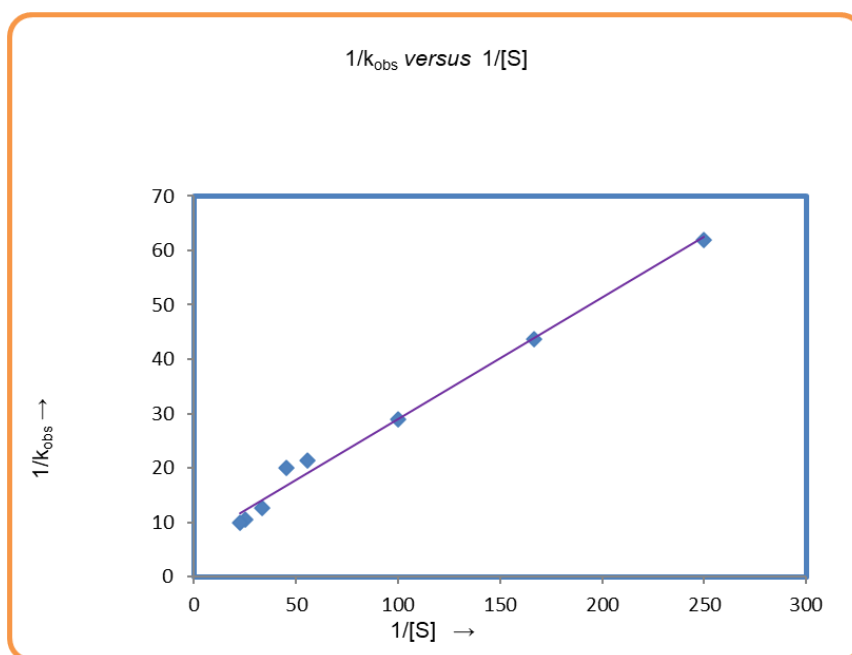


Figure 2 Variation of rate with substrate concentration

3.3. Effect of Hydrogen Ion Concentration

The rate constant increases with increase in hydrogen ion concentration (Table-1). A plot of $\log k_{\text{obs}}$ versus $\log[\text{HClO}_4]$ [Figure- 3] is a straight line with a positive slope ≈ 1 . This shows that reaction is of first order with respect to the hydrogen ion concentration.

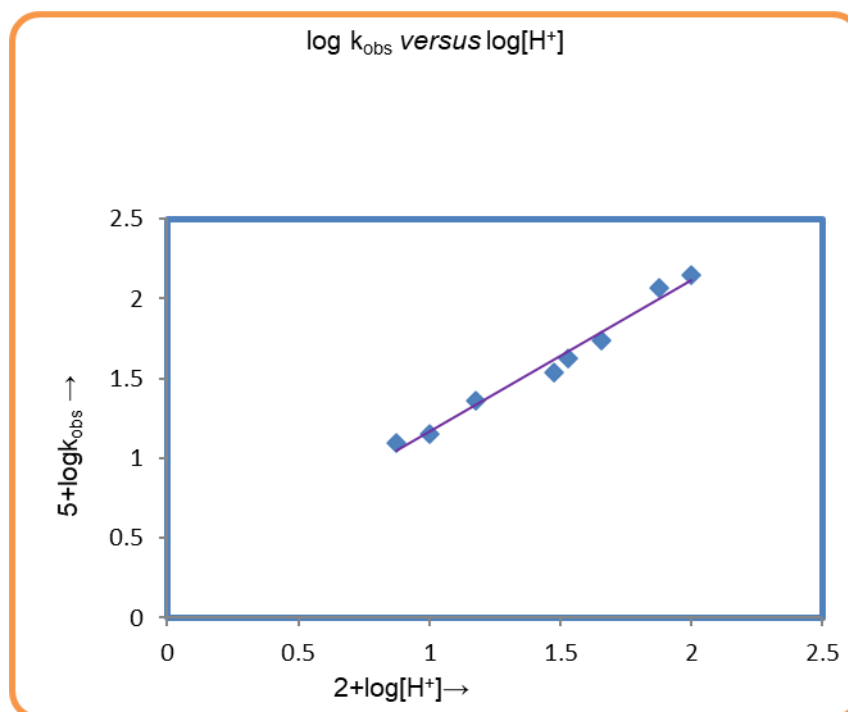
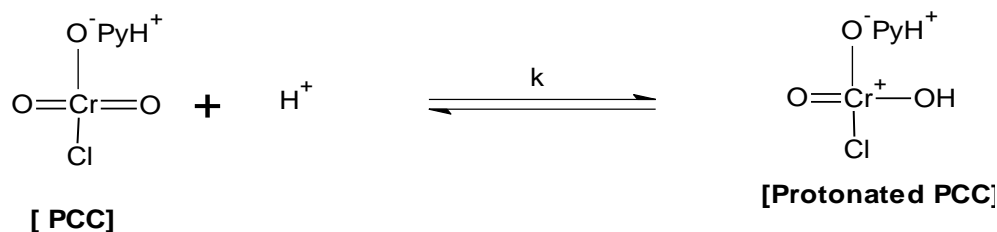


Figure 3 Variation of rate with perchloric acid concentration

The result is similar to the imidazolium fluorochromate [15] oxidation of methionine, chromic Acid [16], oxidation of serine, methionine and cysteine, quinolinium chlorochromate [17] oxidation of methionine, tripropylammonium fluorochromate [18] oxidation of methionine and pyridinium chlorochromate [19] oxidation of alcohols. Where, the rates of reaction were first order with respect to hydrogen ion concentration. The rate of oxidation increased with increase in hydrogen ion concentration indicating the reaction is acid catalyzed. The acid catalysis may well be attributed to a protonation of PCC to yield a stronger oxidant and an electrophile with both the protonated and unprotonated forms being reactive. The formation of a protonated species of PCC has been also reported [19-22].



3.4. Effect of ionic strength

The effect of ionic strength was studied in range of Debye-Huckel limiting law by varying the sodium sulfate concentration. It has been observed that there was no significant effect of ionic strength on the rate (Table-2). This indicates that the reaction may be between an ion and a neutral molecule or between neutral molecules.

Table 2 Variation of rate with sodium sulfate concentration of Methionine at 313 K

| [Na ₂ SO ₄] × 10 ³ mol dm ⁻³ | 1.0 | 3.0 | 5.0 | 7.0 | 9.0 | 11.0 |
|---|-------|-------|-------|-------|-------|-------|
| k _{obs} × 10 ⁵ sec ⁻¹ | 34.80 | 34.20 | 34.15 | 34.51 | 33.72 | 34.46 |

[Methionine] = 1.0 × 10⁻² mol dm⁻³ [HClO₄] = 3 × 10⁻² mol dm⁻³ [PCC] = 1.0 × 10⁻³ mol dm⁻³ DMF: Water = 70:30 % (v/v)

3.5. Effect of Dielectric Constant on Rate of Reaction

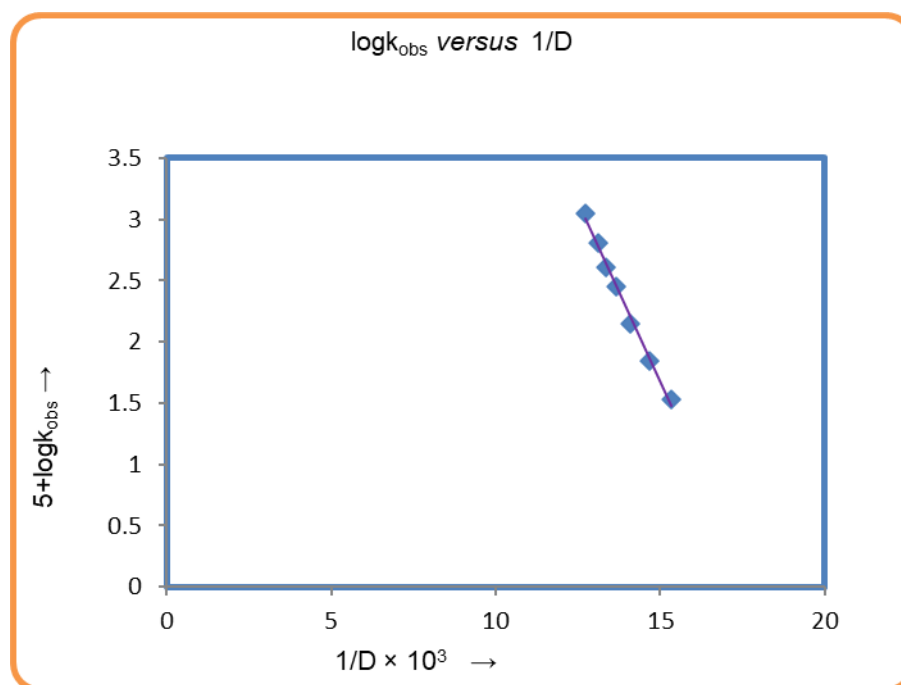
The rate of reactions decreased with an increase in volume percentage of DMF. In other word the rate of reaction increased with increase in dielectric constant of the reaction medium (Table-3).

Table 3 Variation of rate with solvent composition

| DMF: Water %(v/v) | Dielectric Constant | $k_{\text{obs}} \times 10^5 \text{ sec}^{-1}$ |
|-------------------|---------------------|---|
| 70:30 | 64.38 | 34.12 |
| 60:40 | 68.13 | 70.30 |
| 50:50 | 70.92 | 142.01 |
| 40:60 | 73.12 | 285.13 |
| 30:70 | 74.89 | 403.02 |
| 20:80 | 76.33 | 642.00 |
| 0.0:100 | 78.54 | 1122.71 |

[Methionine] = $1.0 \times 10^{-2} \text{ mol dm}^{-3}$; $[\text{HClO}_4]$ = $3 \times 10^{-2} \text{ mol dm}^{-3}$; $[\text{PCC}] = 1.0 \times 10^{-3} \text{ mol dm}^{-3}$; $T=313 \text{ K}$

The plots of $\log k_{\text{obs}}$ versus $1/D$ are straight lines (figure-4). The effect is in accordance with observation of Sherigara et al [23] and also to that observed in the oxidation of alkyl aryl sulphides and diaryl sulphides [24] in which the rate of oxidation was accelerated by an increase in water-content of the medium. It can be explained by the formation of a charge-separated (dipolar activated) complex in the rate-determining step. It means that there is dispersal of charges over a larger area on activated complex compared to the reactants. However, Pitchumani et al [25] observed a decrease in rate with increase in polarity of the medium in Cr(VI) oxidation of N-acetyl phenothiazine; the rate increased with polarity of the medium for N-methyl phenothiazine.

**Figure 4** Variation of rate with solvent composition

Amis [26] had shown that in a linear plot of $\log k_{\text{obs}}$ versus $1/D$ indicates a two dipoles or an ion-dipole in the rate determining step. The plots of $\log k_{\text{obs}}$ versus $(D-1) / (2D+1)$ are non linear (figure-5) clearly supporting that there is an involvement of positive ion-dipole in the rate determining step.

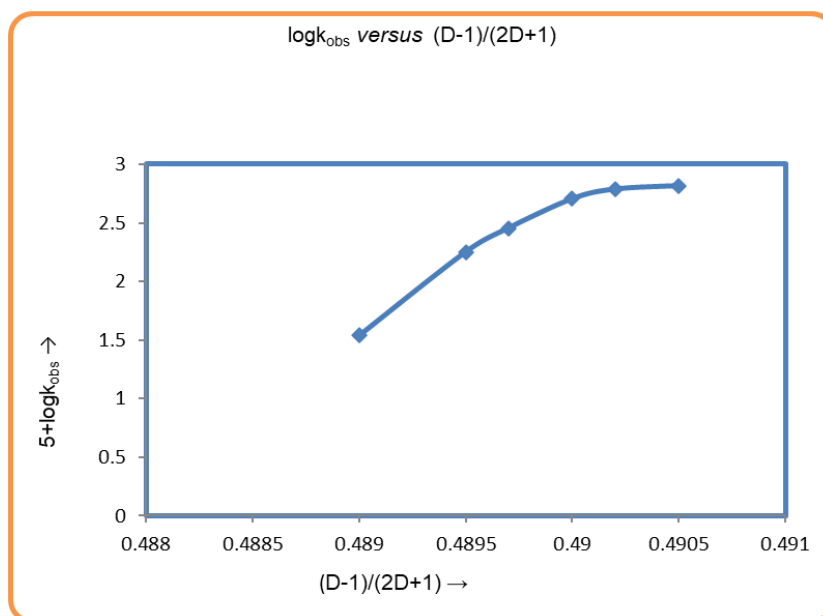


Figure 5 Variation of rate with solvent composition

3.6. Effect of MnSO₄ on Rate of Reaction

To investigate the involvement of various oxidation states of oxidant, such as Cr(VI), Cr(V), Cr(IV) and Cr(III); we studied the effect of Mn(II) ions. It was observed that an addition of Mn(II) ions retard the rate of oxidation with increase in concentration of Mn(II) ions. Table-4.

Table 4 Variation of Rate with MnSO₄ Concentration

| [MnSO ₄] × 10 ³ mole/dm ³ | 0.0 | 2.0 | 4.0 | 5.0 | 7.0 | 9.0 | 10.0 |
|---|-------|-------|-------|-------|-------|-------|-------|
| k _{obs} × 10 ⁵ sec ⁻¹ | 35.20 | 30.45 | 27.90 | 25.72 | 21.50 | 20.10 | 18.27 |

[PCC] = 1.0 × 10⁻³ mole/dm³; Temperature = 313 K; [Methionine] = 1.0 × 10⁻² mole/dm³; DMF: Water = 70:30 % (v/v); [HClO₄] = 3 × 10⁻² mole/dm³

3.7. Effect of Temperature

The rate of reaction increase with an increase in temperature Table-5. The plots of log k_{obs} versus 1/T are linear (figure -6) The Arrhenius equation is valid for the temperature range studied.

Table 5 Variation of rate with temperature

| Temperature (k) | 298 | 303 | 308 | 313 | 318 | 323 |
|--|------|-------|------|-------|-------|-------|
| k _{obs} × 10 ⁵ s ⁻¹ | 12.5 | 18.09 | 21.0 | 34.20 | 39.66 | 44.78 |

[Methionine] = 1.0 × 10⁻² mol dm⁻³; [HClO₄] = 3 × 10⁻² mol dm⁻³; [PCC] = 1.0 × 10⁻³ mol dm⁻³; DMF: Water = 70:30 % (v/v)

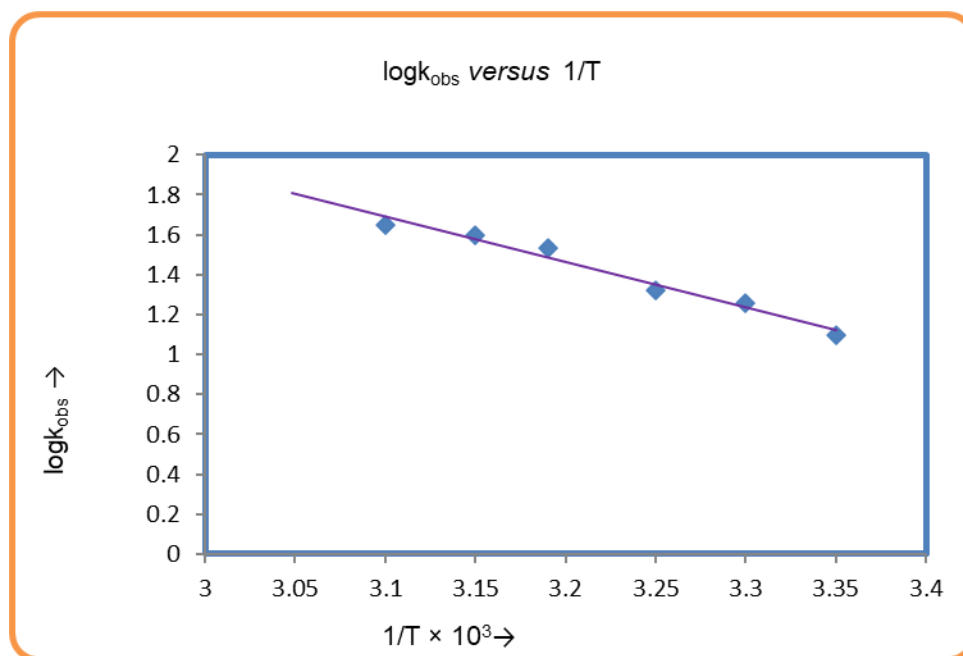


Figure 6 Variation of rate with temperature

Table 6 Activation Parameters

| Parameters | E_a (kJ mol^{-1}) | ΔH^\ddagger (kJ mol^{-1}) | ΔS^\ddagger (kJ mol^{-1}) | ΔF^\ddagger ($\text{Jk}^{-1}\text{mol}^{-1}$) | Temp. coefficient |
|------------|-----------------------------------|---|---|--|-------------------|
| values | 43.79 | 41.49 | -122.75 | 79.61 | 1.62 |

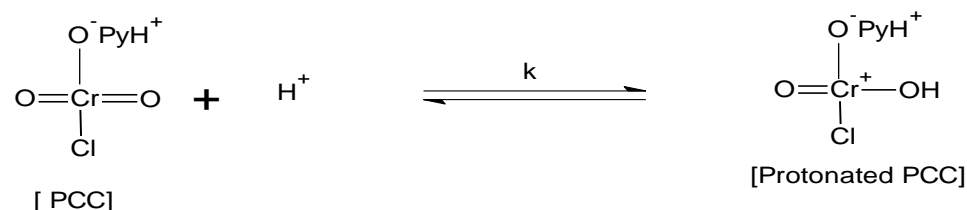
The activation parameter calculated from Arrhenius-Eyring's plot [Table-6] The value of ΔH^\ddagger and ΔS^\ddagger correspond to those expected for a bimolecular reaction. The magnitudes of activation energies are corresponding to direct attack on sulphur. The negative values of entropy suggest that the transition state was more rigid than the reactants. The charge separation takes place, in the transition state of the rate-determining step; the charged ends become highly solvated. This results in an immobilization of a large number of solvent molecules, reflected in the loss of entropy. The negative value of ΔS^\ddagger within the range of radical reactions has been ascribed to the nature of electron pairing and the loss of a degree of freedom, formerly available to the reactions on the formation of a rigid transition state. The large negative value of ΔS^\ddagger suggests that the complex was more ordered than the reactants.

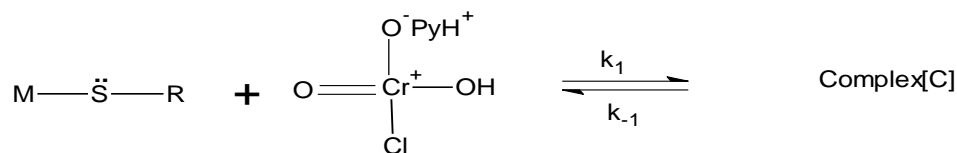
3.8. Test for Free Radical

The reaction mixture was treated with required amount of acrylonitrile (pre-washed with aqueous NaOH followed by washing with distilled water) under nitrogen atmosphere. The resulting mixture became milky indicating there by presence of free radicals during the oxidation.

3.9. Mechanism

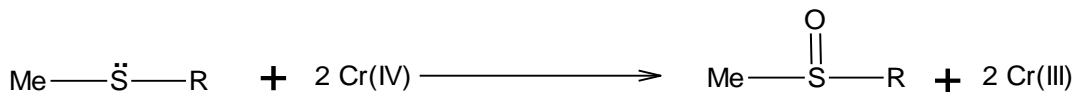
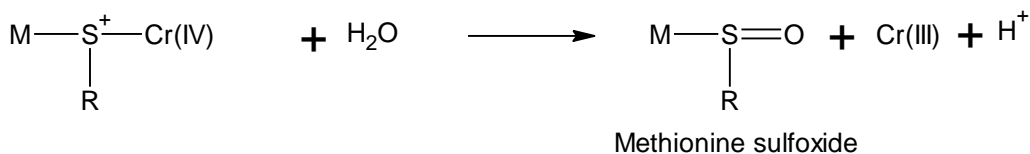
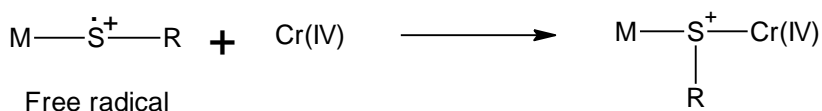
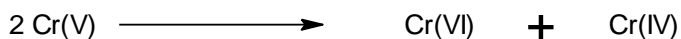
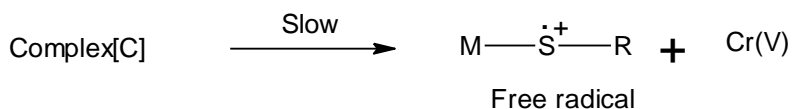
On the basis of above experimental results the following reaction mechanism has been proposed for the oxidation of methionine.



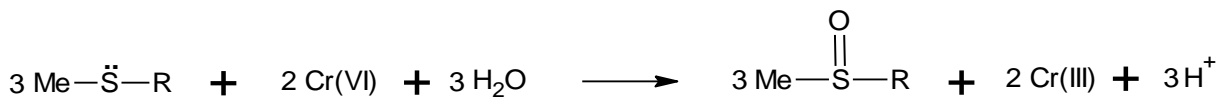


[Methionine]

[Protonated PCC]



The overall reaction reactions may be represented as:



where Me = -CH₃ and R = -CH₂CH₂CH(NH₂)COOH

Rate Law

On the basis of above mechanism the rate law can be expressed as:

$$\begin{aligned} \text{Rate of reaction} &= -\frac{d[\text{C}]}{dt} \propto [\text{C}] \\ &= k' [\text{C}] \end{aligned} \quad \dots\dots\dots 1$$

Concentration of complex, [C] can be calculated by applying steady state concept.

Rate of formation of complex = Rate of disappearance of complex

$$k_1 [\text{S}] [\text{PCC}]_{\text{prot.}} = k_{-1} [\text{C}] + k' [\text{C}] \quad \dots\dots\dots 2$$

Since $[\text{PCC}]_{\text{total}} = [\text{PCC}]_{\text{prot.}} + [\text{C}]$

Therefore $[\text{PCC}]_{\text{prot.}} = [\text{PCC}]_{\text{total}} - [\text{C}] \quad \dots\dots\dots 3$

Hence

$$k_1 [S] \{ [PCC]_{\text{total}} - [C] \} = (k_{-1} + k') [C]$$

$$k_1 [S] [PCC]_{\text{total}} - k_1 [TU] [C] = (k_{-1} + k') [C]$$

$$k_1 [S] [PCC]_{\text{total}} = k_1 [TU] [C] + (k_{-1} + k') [C]$$

$$k_1 [S] [PCC]_{\text{total}} = \{ k_{-1} + k' + k_1 [TU] \} [C]$$

$$\begin{aligned} [C] &= \frac{k_1 [S] [PCC]_{\text{total}}}{k_{-1} + k' + k_1 [S]} \\ &= \frac{[S] [PCC]_{\text{total}}}{\frac{k_{-1} + k'}{k_1} + [S]} \\ &= \frac{[S] [PCC]_{\text{total}}}{k_m + [S]} \end{aligned}$$

Hence Rate = $k' [C]$

$$= k' \frac{[S] [PCC]_{\text{total}}}{k_m + [S]}$$

$$= k [PCC]_{\text{total}} = k_{\text{obs}} [PCC]_{\text{total}} \quad (\text{when } [S] = \text{constant})$$

Where $k_{\text{obs}} = k' \frac{[S]}{k_m + [S]}$

$$\frac{1}{k_{\text{obs}}} = \frac{k_m}{k'} \frac{1}{[S]} + \frac{1}{k'}$$

There for k_m can be calculated by intercept of plot $1/k_{\text{obs}}$ versus $1/[S]$.

The rate law & mechanism suggested is supported by the observations taken.

4. Conclusion

The Study of Oxidation of Methionine by pyridinium chlorochromate in aqueous DMF medium, containing perchloric acid reveals that the reaction is first order rate dependent with respect to PCC, Methionine and H^+ ion concentration. Michaelis–Menten type kinetics is observed respect to substrate. Plots of $\log k_{\text{obs}}$ versus $(D-1) / (2D+1)$ are nonlinear which indicates that activated complex is more polar than the reactant and this also suggests the absence of dipole-dipole interaction. Activation parameter calculated from Arrhenius-Eyring's plot. The value of ΔH^\ddagger and ΔS^\ddagger correspond to those expected for a bimolecular reaction. The magnitudes of activation energies are corresponding to direct attack on sulphur. The negative values of entropy suggest that the transition state was more rigid than the reactants. The reaction involved free radicals as the active transient species. The main reaction product was identified as methionine sulfoxide.

The overall mechanistic sequence described here is consistent with the product analysis and by kinetic and mechanistic studies.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

There is no conflict of interest in this manuscript.

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