CHAPTER II

MATERIALS AND METHODS
SECTION - A

EXPERIMENTAL - SOLUTIONS AND REAGENTS

1. Oxidant Solution

Ammonium meta vanadate (Loba chemie) is used as a primary standard. The solution of oxidant was prepared by suspending weighed amount of ammonium metavanadate in distilled water in a standard flask and by adding to it a known volume of standard sulphuric acid (A.R., B.D.H.) till a clear solution was obtained. The solution was made upto the required volume. The solution so prepared remained stable for months[1] and hence such solution was used as stock solution. The concentration of stock solution was determined by titrating it ferrometrically[2]. The known volume of stock solution of oxidant and excess known volume of standard ferrous ammonium sulphate (0.01 N) was taken and titrated against 0.01 N potassium dichromate using N-phenyl anthranilic acid as a reversible redox indicator. The acidity of the solution was calculated after making allowance for hydrogen ion, used to formation as following equation:

\[ \text{VO}_3^- + 2H^+ \rightleftharpoons \text{VO}_2^+ + H_2O \]

2. **Substrate Solution**

Amino acids of Loba chemie quality were used, arginine, asparagine, glutamine and cystine were chosen as reductant in the present investigation. The stock solution of acids were prepared by weighing and dissolving the required quantity of the substrate in sulphuric acid.

3. **Sulphuric acid Solution**

Stock solution of sulphuric acid (A.R., B.D.H.) was prepared by diluting its appropriate volume with distilled water. The concentration of acid was determined by titrating it against standard alkali solution[3].

4. **Indicator Solution**

The indicator solution was prepared by dissolving 0.107 gms of N-phenyl anthranilic acid in 2ml of 5% sodium carbonate (G.R., S. Merck) solution and was then diluted it to 100 ml by distilled water [4]. Three to four drops of the indicator solution were used in each titration.

The rate of colour change of indicator was slow in the medium containing less than 1.5 M sulphuric acid. However, in the 3.0 M sulphuric acid medium, the colour change was sharp hence in each titration the overall concentration of sulphuric acid was maintained at above to 3.0 M. The colour of indicator in the oxidised form is purple, while it was pale yellow in the reduced state.

5. **Cetyltrimethyl Ammonium Bromide Solution**

The ionic surfactant (Loba chemie) cetyltrimethyl ammonium bromide \([\text{(C}_ {16}\text{H}_{33}\text{NMe}_3\text{Br})\text{CTAB}]\) was purified, by repeated recrystallization. Deionised and redistilled. CO\(_2\) free water used to prepare solution.

6. **Other Solutions**

The standard solution of potassium dichromate (B.D.H.) was prepared by dissolving the appropriate amounts in distilled water. The solution of ferrous ammonium sulphate (A.R., B.D.H.) was prepared by dissolving its calculated weight in distilled water and was standardised by titrating it against standard potassium dichromate (0.01M) solution using N-phenyl anthranilic acid as an indicator.

Glacial acetic acid (A.R., B.D.H.) was used for preparing composition of binary mixtures of acetic acid-water solution.

Mercuric chloride (B.D.H.) 2.0M aqueous solution and acrylonitrile were used for testing the free radical formation during the course of oxidation. They were also prepared by standard method.

**Determination of Critical Micelle Concentration (CMC)**

CMC of micelles was determined for each amino acid separately by adding varying concentrations of micelle in the reaction mixture and the kinetics of the reaction were followed by the procedure given below.
The pseudo first order rate constant was determined at each concentration of micelle. A plot between $k_w$ and [micelle] was drawn and from the maximum value of the rate constant, cmc is evaluated. $k_w$ represents the rate constant at different [micelles].

**Kinetic Method**

In kinetic study of the reaction, the following procedure was adopted.

The kinetic study is performed in absence and in presence of cationic surfactant i.e. cetyltrimethyl ammonium bromide.

The kinetic studies were made by taking large excess amount of amino acids and acid concentration over the oxidant concentration at constant ionic strength. Reaction mixture containing appropriate quantity of oxidant solution, acid and water were taken in 100 ml conical flask. The substrate solution was taken in another flask. To maintain the ionic strength constant, calculated volume of solium sulphate was added in the reaction mixture. These two flasks were placed in a thermostat of sensitivity ±0.1°C. When the two solutions attained the temperature of the water-bath, they were mixed to each other. The progress of the reaction was monitored by withdrawing aliquotes at definite time intervals in a conical flask containing unreacted ferrous ammonium sulphate solution.
The excess of standard ferrous ammonium sulphate solution was titrated against potassium dichromate solution using N-phenyl anthranilic acid as indicator. The same procedure is performed with surfactant. The surfactant was kept with substrate solution before final mixing in thermostat. The experimental data were fed into various integrated rate equations.
SECTION-B

KINETIC STUDIES

It was found that in each oxidation, under study, the values of 
pseudo first order rate constant obtained from the rate equation;

\[ k = \frac{2.303}{t} \log \frac{a}{(a-x)} \]

were constant within the experimental error suggesting that each reaction 
obey's first order kinetics.

I. Typical Kinetic Runs

In the oxidation studies for each substrate, some typical kinetic 
runs shall be carried out to investigate the general behaviour of the 
reaction under different experimental conditions. These studies are, 
effect of concentration of the oxidant, substrate, sulphuric acid and 
cationic surfactant (cetyltrimethyl ammonium bromide) and the variation 
of temperature. The effect of solvent was also carried out.

II. Order of the Reaction

The order of the reaction with respect to oxidant and substrate 
shall be determined by Ostwald's isolation method. Kinetic studies shall 
be carried out at varying concentrations of oxidant keeping the other 
conditions constant to obtain the order with respect to oxidant. The 
reaction order have also been found from log-log plots of initial rates 
versus concentration. The validity of plot shall be examined.
III. The Effect of Temperature

The effect of temperature was studied at four different temperatures at constant [vanadium (v)], [amino acid], [H+] and [surfactant].
SECTION-C

THERMODYNAMIC PARAMETERS

The activation parameters such as temperature co-efficient, energy of activation, frequency factor, entropy of activation, heat of reaction and free energy of activation from temperature dependent rate data, have been evaluated in this section. The following method has been applied.

A. Temperature Co-efficient

It is well established that the rate of reaction increases to an appreciable extent with rise in temperature. The value of the ratio \( \frac{k(T+10)}{k(T)} \) termed as temperature co-efficient for a 10\(^\circ\)C rise in temperature

\[
\text{Temperature co-efficient} = \frac{(k_T + 10)}{k_T} \approx 2 \text{ or } 3
\]

B. Energy of Activation \((E_a)\)

Empirically, the activation energy is the parameter which expresses the temperature dependence of the rate constant.

\[
k = A \cdot e^{-Ea/RT}
\]

The standard method for obtaining \(E_a\) is, either to evaluate from Arrhenius equation or from Arrhenius plot. Both the methods have been applied as given below;
I. Arrhenius Equation

The activation energy can be calculated by appropriately filling Arrhenius equation.

\[
\log_{10} \frac{k_1}{k_2} = \frac{\Delta E_a}{2.303 R} \left[ \frac{1}{T_2} - \frac{1}{T_1} \right]
\]

II. Graphical Method

The energy of activation was also obtained by plotting log \( k_0 \) and \( k_m \) against 1/T. Arrhenius plot was found to be linear one for the data recorded.

Gradient = \( -\frac{\Delta E_a}{2.303 R} \)

\( \Delta E_a = \text{slope} \times 2.303 \ R \ (K \ J \ Mol^{-1}) \)

C. Frequency Factor

The frequency factor (A) which represent the total frequency of encounters between the two reactant molecules, irrespective of whether they possess sufficient energy or not is given by

\[
\log A = \log k + \frac{\Delta E_a}{2.303 RT}
\]

D. Entropy of Activation (\( \Delta S^* \))

The entropy of activation is evaluated by employing equation:

\[
\frac{\Delta S^*}{R} = 2.3 \log A - 2.3 \log \frac{ekT}{h}
\]
Where,

\[ k = \text{Boltzman constant} = 1.38 \times 10^{-23} \, \text{JK}^{-1} \]
\[ T = \text{Absolute temperature} \]
\[ h = \text{Plank constant} = 6.625 \times 10^{-34} \, \text{J.Sec.} \]
\[ e = 2.718 \]
\[ R = \text{Gas constant} = 8.31 \, \text{JK}^{-1} \, \text{Mol}^{-1} \]

**E. Heat of Reaction (ΔH*)**

Theromodynamically the equation for the enthalpy of activation or heat of reaction is

\[ \Delta H^* = E_a - RT \]

**F. Free Energy of Activation (ΔG*)**

The change in free energy ΔG*, gives the extent to which a reaction goes to completion. It is determined from the following relationship.

\[ \Delta G^* = \Delta H^* - T \Delta S^* \]

where ΔH* and ΔS* are the changes in enthalpy and entropy and T is the absolute temperature. The calculated values of all the activation parameters have been recorded.

**G. Micellar Catalysis**

According to Piszkiewicz model the relationship of rate contants to detergent concentration assumes that the micelle Dₙ, forms a noncovalent complex with the substrate, S, before catalysis may takes place.
\[
D_n + S \underset{K_D}{\longleftrightarrow} D_nS \quad \quad \quad (1)
\]

\[
D_nS + V(v) \underset{k_m}{\longrightarrow} \text{Product} \quad \quad \quad (2)
\]

\[
S + V(v) \underset{k_0}{\longrightarrow} \text{Product} \quad \quad \quad (3)
\]

\(K_D\) is the dissociation constant of micelle back to its free components, \(k_m\) is the rate constant of reaction within the micelle and \(k_0\) is the rate constant of reaction in the absence of detergent, for this reaction scheme the observed rate constant \(k_\psi\) is expressed as a function of the concentration of detergent \(D\), by

\[
k_\psi = \frac{k_m [D]^n + k_0K_D}{K_D + [D]^n} \quad \quad \quad (4)
\]

On rearrangement and its log, taken to give -

\[
\log \left[ \frac{k_\psi - k_0}{k_m - k_\psi} \right] = n \log [D] - \log K_D \quad \quad \quad (5)
\]

According to the equation a plot of \(\log \left[ (k_\psi - k_0) / (k_m - k_\psi) \right] \) versus \(\log [D]\) is linear with a slope of “\(n\)” and intercept -log \(K_D\). The value of cooperativity index ‘\(n\)’ in the present study was found to be positive. It is analogous to positive co-operativity in enzymatic reaction.

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