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CHAPTER III-A

A.1-BACKGROUND:-

It is evident from the matter presented in the preceding part of the thesis that a great deal of work has been carried out on the kinetics and mechanism of oxidation of several organic and inorganic compounds by many workers¹⁻¹⁵. It will be worth while to present here briefly the available information regarding the oxidation of amino acids and related organic compounds by different oxidants and in varieties of media giving special reference to permanganate and to the micellar media.

Amino acids make up 75% of human body and are vital to every part of human function. The kinetic investigation of the oxidation of amino acids are important because of their biological significance and a precise understanding of the mechanism of such biological redox reactions help in the synthesis of reaction products.

Kinetic study of the oxidation of glycine by permanganate ions in acid medium was investigated by Insausti and co-workers¹. Insausti² had studied about the both spectrophotometric and kinetic evidences of the production of colloidal Mn(IV) originating from the permanganate oxidation of glycine. Studies on influence of amino acid on colloidal manganese dioxide was also carried out by Insausti and co-workers³.

Perez-Benito⁴ had reported the progress of the reaction of permanganate ion with the amino acid i.e. glycine in near-neutral aqueous solutions. The reaction was monitored with a UV-vis spectrophotometer at two different wavelengths in order to observe the decay of the oxidant (MnO_4^- , at 526 nm) and the formation of one of the reaction products (colloidal MnO_2 , at 418 nm). The oxidation of α -amino acids by permanganate under neutral conditions has been studied at various temperatures by Insausti and co-worker⁵ and the existence of linear free energy relations was confirmed for the homogeneous process.

Surfactant catalyzed oxidation of glycine by acidic permanganate was studied iodometrically by Pare and his coworkers⁶ whereas oxidation of glycine by permanganate in alkaline medium was studied by Sarathi⁷. Kinetics and mechanisms of catalysed oxidation of glycine was observed by Perez-Benito et al⁸. The kinetics and mechanism of the

permanganate-induced oxidative catalytic condensation reaction of sarcosine, as a secondary amino acid, in buffered medium has been investigated spectrophotometrically by Khatti et al⁹.

Rao and his coworker¹⁰ had studied kinetics of oxidation of some amino acids like glycine, alanine, phenylalanine, serine, threonine, aspartic and glutamic acid by permanganate in a moderately concentrated sulphuric acid medium in the presence and absence of Ag^+ . The kinetics of the permanganic oxidation of glycine, L-alanine and L-leucine in strong acidic medium were investigated spectrophotometrically by Bahrami¹¹.

Kinetics and mechanisms of the oxidation of L-alanine by permanganate was also studied by Andres et al¹². Oxidation of L-Alanine in acidic solvent by permanganate was investigated by Insausti et al¹³ which is autocatalysed by intermediate Mn(IV) species. Acid-catalysed oxidation of certain amino-acids by potassium permanganate in moderately concentrated acidic media was observed by Verma et al¹⁴. Oxidation-reduction equilibrium of D-amino acid oxidase was carried out by Brucori and his coworker¹⁵.

Silver ion catalysed oxidative decarboxylation of DL-isomer of amino acids was studied by Chourey¹⁶. Surfactant catalysed oxidation of glycine by acidic permanganate was studied by Gour et al¹⁷.

Oxidation of L-isomers of basic amino acids by acidic permanganate was performed by Pandey et al¹⁸. Effect of anionic micelle on the oxidation of DL-alanine was carried out by Katre et al¹⁹. Dubey et al²⁰ studied the micellar catalysed oxidation of L-glutamine by vanadium (V).

Reaction between glycine and permanganate was investigated first by Pokrovskaya²¹ mainly from preparative view point. Cu(II) catalysed oxidation of alkaline glycine by periodate has been studied by Kovates²² while uncatalysed oxidation of acidic and neutral glycine by peroxy-disulphate was studied by Kumar and Saxena²³. Kinetics of oxidation of glycine by Co(III) has been studied by Sthapak and Ghosh²⁴.

Bose²⁵ et al studied the oxidation of glycine, alanine and valine in alkaline media by chloramine-T. Kinetics and mechanism of the silver (I) ion catalysed oxidation of α -alanine by peroxydisulphate was investigated by Chandra and coworkers²⁶.

Bilehal et al²⁷ studied the Cr(III) catalysed oxidation of L-Leucine and Isoleucine by alkaline permanganate. Surfactant catalysed oxidation of DL-Serine by NBB was studied by Katre et

al²⁸. Effect of cationic micelle on oxidation of valine by permanganate was performed by Sheikh et al²⁹. A quite different type of work has been carried out by Sharma et al³⁰. They have studied the kinetics of the oxidation of specific amino acids by ozone and observed that ozone reacts mainly with unprotonated amino group. Cr(III) catalysed oxidation of L-Valine by alkaline permanganate was performed by Kulkarni et al³¹. Ru(III) catalysed oxidation of L-arginine by alkaline permanganate was carried out by Halligudi et al³².

Iloukhani et al³³ studied the kinetics and mechanism of oxidation of L-phenyl alanine by acidic permanganate and observed that no free radicals are produced in the system. The same observation was found by Moghadasi et al³⁴ for the oxidation of L-arginine by acidic permanganate. Ahmed et al³⁵ studied the oxidation of about twenty natural amino acids by ferryl complex and observed that reactive oxygen species are responsible to damage the proteins including oxidation of amino acids side chains. Singh et al³⁶ studied the oxidation of valine by NBP in acidic medium. Recently oxidation of arginine and lysine by hexacyanoferrate III, catalysed by Ir(III) in aqueous alkaline medium was carried out by Goel and coworkers³⁷. A detailed study in the kinetic correlation for the nonautocatalytic and autocatalytic pathways of permanganate oxidation of alpha aminoacids was carried out by Joaquin et al³⁸.

Kinetics of oxidation of L-Leucine by metallic gold and silver nanoparticles in hydrogen peroxide solution was studied by Venkatesan et al³⁹. Kinetics of Silver(I) catalysed oxidation of glycine and alpha-alanine was made by Rayappan et al⁴⁰. Studies on Mn(II) catalysed oxidation of alanine, valine and leucine by peroxomonosulphate in alkaline medium was carried out by Kuttirani et al⁴¹. Oxidation of L-amino acids i.e. glycine, alanine, valine and leucine by metal ion (Mn^{3+}) in acidic medium was studied by Kumara et al⁴². The kinetics of oxidation of alpha-amino acids by CAT in aqueous alkaline medium was studied by Prasantha et al⁴³. Kinetics of oxidation of L-valine by Cu(III) periodate complex was studied spectrophotometrically by Sharana Barsamma and coworkers⁴⁴.

A.2-SCOPE, PLAN AND SALIENT POINTS

It is evident from the above discussion and a careful critical literature survey of the existing literature that oxidation studies of amino acids by different oxidants in varieties of media under catalysed or uncatalysed conditions have been carried out by number of workers. However the literature survey also reveals that the miceller catalysed oxidative

decarboxylation and deamination of amino acids by permanganate in acidic medium has received very little attention. There comparative studies also have not, yet been studied systematically. It was, therefore planned to carry out through investigation considering various view points based on the selectivity of medium, substrate, oxidant and catalyst.

The reaction medium has its own specific properties. As acidic medium has its own acidity functions, ions, ion pairs and specific nature. The proton derived from acids play an important role in the reaction. Sulphuric acid is famous for its complexing nature than other acids and known to play a role of acid catalysis. Hence sulphuric acid was chosen as a species to exhibit homogeneous acid catalysis phenomenon. Thus the applicability of various hypotheses like Zucker-Hammet, Bunnett and Bunnett-Olsen free energy relationship may be tested to know the role of water in the acid catalysed reaction.

The substrate i.e. four amino acids viz glycine, L-alanine, L-valine and L-leucine are the basic amino acids. They are simple in structure and are constructive units of proteins, enzymes, nucleic acid, RNA, DNA, hormones, chromosomes, genes etc. Among these amino acids, two of them i.e. L-valine and L-leucine are indispensable amino acids. Due to their various importance all four have been selected for current studies.

Since salts and their respective ions have their own importance and specific action for the growth, development and different activities in the living system, therefore, studies in this respect have also been included.

Surfactant or micellar catalysis is a phenomenon of great importance. It has received relatively lesser attention from the view point of kinetics and mechanism. It is true that in case of oxidative decarboxylation literature is very scanty. It was therefore decided to undertake the problem of surfactant (micellar) catalysed oxidative decarboxylation by permanganate.

A survey of literature regarding oxidation by permanganate has been given in previous chapter. It is as an oxidant was first introduced by Marguerite in 1846.

Permanganate ion as a versatile oxidising agent has an important role far over a century in varieties of analytical, preparative and degradation chemistry. The reactions of potassium permanganate are more interesting because the states, amount and the mode of oxidation brought about the permanganate ion vary with the pH of the medium. It was, therefore decided to use permanganate ion as an oxidising species for decarboxylation to understand the nature of oxidant more deeply and widely.

Therefore, the present investigation has been undertaken to elucidate the kinetics of redox system, i.e. oxidative deamination and decarboxylation of glycine, L-alanine, L-valine

and L-leucine by potassium permanganate spectrophotometrically in moderately concentrated sulphuric acid medium in presence of sodium lauryl sulphate as anionic micellar catalyst.

In order to implement and execute the ideas experimentally and practically, within the framework the present work is planned to be made to present a detailed and systematic work in the light of the following salient points:-

(1) Determination of order of reaction:

- (a) With respect to potassium permanganate
- (b) With respect to substrate i.e. glycine, L-alanine, L-valine and L-leucine

(2) Investigating acid catalysis:

- (i) To study the effect of variation of sulphuric acid concentration $[H^+]$ at constant concentration of other reactants.
- (ii) To correlate the rate of oxidation with,
 - (a) Acid concentration and
 - (b) Activity functions
- (iii) Testing of the applicability of Zucker-Hammett and Bunnett's hypotheses.
- (iv) Testing of applicability of Bunnett-Olsen's linear free energy relationship.
- (v) Role of acidity functions and activity of water.

(3) Study of effect of surfactant as catalyst in terms of micellar/premicellar catalysis.

(4) Evaluation of kinetic and activation parameters:

- (i) To find out the rate constants at different temperatures in order to determine various kinetic and activation parameters as follows.
 - (a) Temperature coefficient θ
 - (b) Energy of activation ΔE^\ddagger
 - (c) Heat of activation ΔH^\ddagger
 - (d) Entropy of activation ΔS^\ddagger
 - (e) Free energy ΔG^\ddagger and
 - (f) Frequency factor P_z .
- (ii) To see the validity of Arrhenius plot.

(5) Study of salt effects, effect of ionic strength and specific ionic effect:

- (i) To understand the effect of ionic strength on the oxidation rate in order to know the nature of the reactive species in the rate determining step.
- (ii) To understand the applicability of the relationship between ionic strength and the logarithm of the reaction rate.
- (iii) To find out the effect of various cations and anions on the reaction velocity i.e. specific ionic effect.

(6) To investigate catalytic effect of Mn(II).

(7) Mechanism:

- (i) To propose the mechanism of oxidation reaction in presence and absence of micellar catalyst on the basis of experimental results.
- (ii) To study the free radical formation.
- (iii) To identify the reaction product of oxidation reaction.

(8) Finding the Order of reactivity of amino acids:

To suggest the order of reactivity of glycine, L-alanine, L-valine and L-leucine on the basis of experimental findings.

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CHAPTER III-B

EXPERIMENTAL

(a) Chemical Used and preparation of solutions:-

The following chemicals used were of 'Analar' or GR grade, Deionised and doubly distilled water was used to prepare all the solutions.

1. Potassium Permanganate (KMnO₄) :

Potassium Permanganate was used of Rankem, analytical grade. Its solution was prepared in deionised water as given by Vogel¹ and kept in black painted glass stoppered bottles to avoid any photochemical effect. The stock solution was freshly prepared every week and standardised by standard method.

2. Amino acids

Glycine [NH₂CH₂COOH] and other L-isomers of amino acids i.e. L-Alanine [CH₃CH(NH₂)COOH], L-Valine [(CH₃)₂CHCH(NH₂)CHCOOH] and L-Leucine [(CH₃)₂CHCH₂(NH₂)CHCOOH] were used of Loba Chemie. G.R. grade. Their stock solution was prepared by direct weighing and dissolving in the deionised water, after every three to four days.

3. Mineral Acid: Sulphuric acid (H₂SO₄) :

Sulphuric acid was used of BDH 'Merck' product. Its stock solution was prepared by dilution of known volume of acid and standardized by standard procedure.

4. Sodium Lauryl Sulphate (CH₃(CH₂)₁₁OSO₃Na) :

Sodium Lauryl Sulphate was used of Loba Chemie AR grade. Its solution was prepared in deionised water and was used after keeping overnight.

8. Salt Solutions :

All the salt solutions of neutral salts i.e. Na₂SO₄, K₂SO₄, ZnSO₄, (NH₄)₂SO₄, MgSO₄, MnSO₄, NaNO₃ and CH₃COONa were prepared by dissolving their known quantities in distilled water.

9. Solvent:

Deionised and doubly distilled water was used as a solvent.

10. Miscellaneous :

All other chemicals used were of Loba Chemie & Merck GR grade.

(b) Experimental Procedure for Kinetic measurement:-

For finding out the kinetic parameters ; the Ostwald's Isolation method was applied².

Requisite amount of the solutions of amino acids, surfactant (NaLS), sulphuric acid and water (to maintain the total volume constant for all the runs) were taken in black painted glass stoppered bottles to avoid any photochemical effect, and were thermostated (of $\pm 0.1^\circ\text{C}$ accuracy) at constant required temperature.

A measured amount of permanganate solution previously thermostated at the same temperature was then rapidly mixed with the above reaction mixture. The total volume of reaction mixture was always kept 50 ml. The reaction mixture was then shaken well. The zero time of the reaction was noted when half of the permanganate solution was added. 2ml of the reaction mixture was withdrawn at known intervals of the time and the reaction was quenched by adding it into known excess of ice cold water in the optical cell. The progress of the reaction was studied by measuring absorbance of unreacted permanganate spectrophotometrically at 525nm on Systronics 106 Spectrophotometer.

The reactions were studied almost upto 60-80% completion. The initial concentrations of potassium permanganate were kept very low as compared to those of other reactants, throughout the investigation. The pseudo first order rate constants reported are the average values from two to three similar runs. The reproducibility were found to be always within $\pm 5\%$. The Ostwald's isolation method was used to determine the order of reactants. The concentration of all amino acids was taken in large excess so that it essentially remained almost constant throughout the course of reaction.

(c) Equations for calculation of different kinetic and activation parameters.

(1) For Kinetic parameters :-

(i) Rate constant

The pseudo first order rate constants (k_1 and k_1') of the oxidation reaction which is double stage process have been calculated using equation;

$$\text{Rate constant } k_1 \text{ (and } k_1') = \frac{2.303}{t} \log_{10} \frac{a}{(a-x)}$$

where $t =$ is the time in minutes

$a =$ optical density of permanganate in reaction mixture at zero (initial) time.

$(a-x) =$ optical density of unreacted permanganate in the reaction mixture at the different time interval 't'.

(for the second stage process initial time (t_G) has been determined graphically.

k_1 and k_1^1 are rate constants for the first and second stages respectively.

(ii) Equation for determination of order(n) with respect to substrate by differential method.

$$n = \frac{\log k_2 - \log k_1}{\log C_2 - \log C_1}$$

Where $n =$ order of reaction

$k_2 =$ velocity constant at concentration C_2 of Substrate.

$k_1 =$ velocity constant at concentration C_1 of Substrate.(where $k_2 > k_1$ and $C_2 > C_1$).

(iii) Equation for Second order rate constant

$$k_s \text{ or } k_s^1 = \frac{k_1 \text{ or } k_1^1}{[\text{Substrate}]}$$

- a) k_1 or k_1^1 are pseudo first order rate constants of first and second stage processes.
- b) k_s or k_s^1 are pseudo second order rate constants for first and second stage processes.

(iv) For preiceller constant from Piskiewicz³ plot:-

$$n = \log[k_{\text{obs}} - k_0] / \log[k_m - k_{\text{obs}}]$$

Where k_0 is the velocity constant without NaLS,

k_{obs} is the velocity constant at different NaLS concentration

while k_m is the maximum velocity constant in presence of NaLS used.

(2) For Activation parameters:-

Equations used for determination of different activation parameters are as follows:

(i.) Temperature Coefficient :

It have been calculated for 10°C rise in temperature from equation,

$$\text{Temperature coefficient} = \frac{\text{Rate constant } k_{T+10}}{\text{Rate constant } k_T}$$

(ii.) Energy of activation ($\Delta E^\#$):

$\Delta E^\#$ in K.Cal mol⁻¹ have been calculated by equation;

$$\Delta E^\# = \frac{2.303RT_1T_2}{T_2 - T_1} \log \frac{k_{T+10}}{k_T}$$

(iii.) The $\Delta E^\#$ have also been determined from the slope of Arrhenius plot i.e. log k against 1/T using equation

$$\Delta E_g = 2.303 \times R \times \text{slope of Arrhenius plot}$$

where constant R = 1.987 Cal mol⁻¹ K⁻¹

(iv.) Heat of Activation ($\Delta H^\#$):

$\Delta H^\#$ in K.Cal mol⁻¹ has been calculated by equation;

$$\Delta H^\# = \Delta E^\# - RT$$

(v.) Frequency factor (PZ) :

Following equation has been used for the determination of Pz in dm³

mol⁻¹min⁻¹

$$\log Pz = \log k + \frac{\Delta E^\#}{2.303RT}$$

(vi) Entropy of activation (ΔS^\ddagger):

It have been calculated in Calories mol⁻¹Kelvin⁻¹ using equation,

$$\Delta S^\ddagger = 2.303X RX \left(\log Pz - \log \frac{KT}{h} \right)$$

where K is the Boltzman's constant (1.38066 x 10⁻¹⁶ erg/degree), h is the Plank's constant (6.625x10⁻²⁷ erg second) and T is a absolute temperature.

Or

$$\frac{\Delta S^\ddagger}{19.15} = \log k_{obs} - 10.7531 - \log T + \frac{E_a}{19.15T}$$

$$\Delta S^\ddagger = \log k_{obs} - 10.7531 - \log T + \frac{E_a}{T}$$

Where Ea is activation energy, T absolute temperature and k_{obs} is observed rate constant⁴.

(vii) Free energy of activation (ΔG^\ddagger):

ΔG^\ddagger in KCal.mol⁻¹ have been calculated using relation.

$$\Delta G^\ddagger = \Delta H^\ddagger - T\Delta S^\ddagger$$

The present investigation has been undertaken to elucidate the kinetics of redox system, i.e. oxidative deamination and decarboxylation of Glycine, L-Alanine, L-Valine and L-Lucine (Amino Acids) by potassium permanganate in moderately concentrated sulphuric acid medium in presence of Sodium Lauryl Sulphate as anionic catalyst.

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