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OXIDATION OF L-CYSTEINE AND DL-METHIONINE: A COMPARATIVE KINETIC APPROACH

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ABSTRACT

Kinetics of oxidation of sulphur containing amino acids Cysteine and Methionine was investigated spectrophotometrically in alkaline medium by Potassium permanganate. The results suggest that oxidation of Cysteine is faster than Methionine, indicating of free thiol (-SH) group is more reactive than thioether(-S-) group of Methionine. Kinetic study emphasizes a significant relation between rate of the oxidation and structure of amino acids. Rate of the reaction is significantly increased by increase in concentration of amino acid and alkali whereas increase in concentration of oxidant does not have significant effect on reaction rate. pH of the medium plays a significant role in oxidation, oxidation rate increases with increase in pH of the medium.There is a good agreement between observed and calculated rate constant. The Arrhenius plot for cysteine oxidation illustrates a strong linear relationship between lnk and 1/T. similar results were obtained for Arrhenius plot of Methionine oxidation. A plausible mechanism has been proposed to account for the reaction results. cystine and Methionine sulphoxide were identified as reaction products by FTIR analysis. L-cystine is a covalently linked dimeric non essential amino acid, required for proper utilization of vitamin B_6 and healing of burns, wounds.

KEYWORDS: Kinetics, Oxidation, Amino acid, Rate of reaction.

INTRODUCTION

Kinetic investigations of amino acids have become important because of their selectivity towards oxidants. Amino acids are building block of proteins and play significant role in metabolism[1] In metabolism amino acids are subjected to many reactions and can supply precursors for many endogenous substances,[1a] for example haemoglobin in blood and various proteins and nucleotides. Oxidation of amino acids depend on whether the particular amino acid contain polar, non-polar or acidic and basic substituent. The study of oxidation of amino acids is one of the most exciting field in organic chemistry because different products are obtained by different oxidizing agents under same experimental

conditions.[2] Amino acids find a number of applications in biochemical research, pharmaceuticals, microbiology, nutrition and fortification of foods and feeds. Generally the $–NH_2$ and –COOH groups in $RCH(NH_2)COOH$ undergo chemical transformations while hydrocarbon moiety remains intact which is attributed to the higher reactivity of the former compared to R.[3]

Chemical kinetics is a branch of chemistry which deals with study of reaction rate and the factors affecting rate of reaction such as concentration, temperature, pressure, catalyst etc. Oxidation studies of peptide and pharmaceuticals have become important for clinical studies and commercialization in drug development as more biotechnology derived products .Proteins containing free cysteine such as human serum albumin and thiol containing peptides are susceptible to thiol oxidation through a variety of mechanisms. These mechanisms may involve free radical species such as superoxide or hydroxyl radical resulting in loss of thiol radical by consumption of oxygen, alternatively nucleophilic substitution reaction of thiol group with certain reactive oxygen species.[4-6]

DL- Methionine is an essential amino acid which contains sulfur. It can not be synthesized in our body and must be supplemented through the food. It supplies active methyl group and mineral sulfur to several biochemical reactions for normal brain function.[7-8] It is known that it has three coordination centers viz O,N, and S. Its oxidation path is well established and is reported that S moiety is more susceptible for oxidation.[9] Cysteine is known not only for protein synthesis and participating in electron transfer reactions. It also plays important role in cellular responses to change in redox environment which are the results of oxidative stress linked with neurological disorder pathological conditions.[9a]

The permanganate ion[MnO_4^-] is one of most important poly electron, most widely used oxidant in inorganic and organic chemistry. It is also used as an antiseptic for skin, as disinfectant in water treatment and as a fire starter in various chemicals.[9b] It has ability to interact with many active groups of organic compounds, including alcohols, aldehydes, ketones, amines and amino acids. It has ability to oxidize a great variety of substances in acidic, basic and neutral medium because the manganese ion(Mn +7) in the permanganate ion is reduced to different oxidation states in the media. [10]It also finds extensive application in organic synthesis. Kinetic studies are important source of mechanistic informations on the reaction. [11-12] It is evident that during oxidation by permanganate Mn(VII) is reduced to various oxidation states. Furthermore the mechanism of oxidation of any substrate by multivalent oxidant depends not only on the substrate but also on the medium used for the study. In present investigation oxidation is conducted in alkaline medium.Stable oxidation product in strong alkaline medium is the Permanganate ion(MnO_4^{2-}). The study of oxidation of amino acids is of interest because different oxidation products are obtained by different oxidizing agent.[13-14]

METHODOLOGY

- **L-Cysteine solution:** Fresh Solutions of different concentration of Cysteine were prepared by dissolving required quantity of substrate in double distilled water. The strength of the solutions was determined by titrating against standard iodine solution iodometrically.[15]
- **DL-Methionine solution:** Solutions of different concentrations of methionine were prepared afresh by dissolving requisite amount of D L-Methionine in double distilled water and its strength is determined by iodometric method. To an aliquot of methionine, 2.0 gms of sodium tetraborate is added and is titrated against standard iodine solution using starch indicator.

- **Potassium Permanganate Solution:** Solutions of varying concentration (0.5 -2.5 M) of oxidant were prepared by dissolving requisite amount of compound in double distilled water.
- **Potassium Chlorate Solution:** To maintain ionic strength 0.12 mol/dm^{-3} $KClO_3$ solution is prepared by dissolving requisite amount of potassium chlorate in double distilled water.
- All chemicals used were of analytical grade.
- **Kinetic Procedure:-** Kinetic measurements were carried out at constant temperatue of 30± 0.1° and at constant ionic strength of 0.12 mol/dm^{-3}, in alkaline medium. The reaction was initiated by transferring calculated amount of thermostated potassium permanganate in reaction mixture. Progress of the reaction is followed by measuring absorbance at 530 nm, at which it has maximum absorbance, using a UV-VISIBLE spectrophotometer systronic 106. The decrease in absorbance due to oxidation of cysteine is measured time to time. The graph between log absorbance vs time plots are found to be good straight lines up to 85 % completion of the reaction. Rate constants are determined from the slopes of the plot, which are represented by k, which are found to be reproducible within ± 4 %. Further the plot between k and [AA] is a good straight line, similar observations were found by varying concentration of alkali. Hence order with respect to amino acid and alkali are found to be one and concentration of oxidant does not have much effect on rate of the reaction.
- **Test of Free radicals:-** Test of free radicals was carried out by taking substrate ,oxidant and alkali in a T and humberg tube and acrylonitrile solution in a bent tube. The solutions were mixed by tilting the tube.[16] The reaction mixture was kept aside and even after 24 hrs no precipitate was observed, confirms no involvement of free radical in intermediate step.

RESULTS AND DISCUSSION

Following data was obtained under our experimental condition. Where [OH^-]>>[AA]>>[Oxidant]

- **Effect of oxidant concentration on rate**:- Effect of oxidant concentration on rate constant can be studied by varying the concentration of oxidant and keeping all other reactants concentration constant. It has been observed that there is not much change in rate constant for both of the amino acids and change is little more for cysteine than Methionine.
- **Effect of Amino acid concentration on rate**:- Effect of substrate concentration on rate constant can be studied by varying the concentration of amino acid from1 mol/dm^{-3} to 5 mol/dm^{-3} and keeping all other reactants concentration constant, linear change in rate constant k was observed. plot of logk vs log[substrate] is linear with unit slopes confirming first order dependence on substrate. First order rate constants were observed for both the amino acid.
- **Effect of Alkali concentration on rate**:- By varying alkali concentration from .01 mol/dm^{-3} to .1 mol/dm^{-3} and keeping all other reagents concentration constant, it was observed that rate of the reaction increases ,order of the reaction is found to be less than one. Change in ionic strength does not have much effect on oxidation rate.

Table 1: Effect of oxidant, substrate and alkali concentration on rate constant

S. No.	[MnO_4^-]*10^3 dm /mol^3	[AA]* 10^2 dm /mol^3	[OH^-]* 2x10^1 dm /mol^3	$k_{obs} * 10^4$	
				Cysteine	Methionine
1	2	1	0.1	24.32	1.82
2	2	2	0.1	28.32	2.42
3	2	3	0.1	32.52	2.82
4	2	4	0.1	34.54	3.41

5	2	5	0.1	35.8	3.72
6	0.5	2	0.1	34.52	4.31
7	1	2	0.1	34.58	4.33
8	1.5	2	0.1	34.62	4.35
9	2	2	0.1	34.62	4.36
10	2.5	2	0.1	34.64	4.36
11	2	2	0.01	23.82	1.62
12	2	2	0.02	23.9	1.64
13	2	2	0.04	24.1	1.72
14	2	2	0.06	24.25	1.78
15	2	2	0.08	24.3	1.82

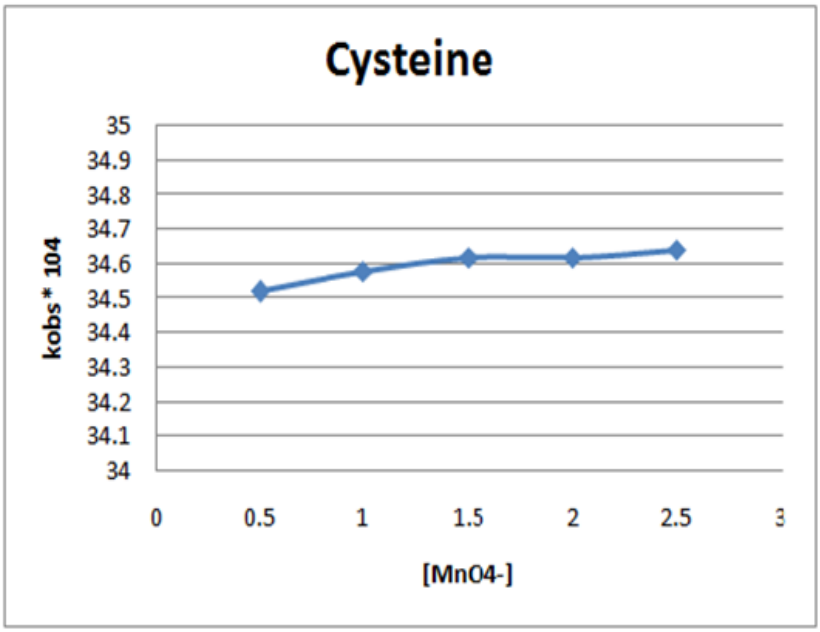

Fig. 1: Effect of Oxidant concentration on rate constant.

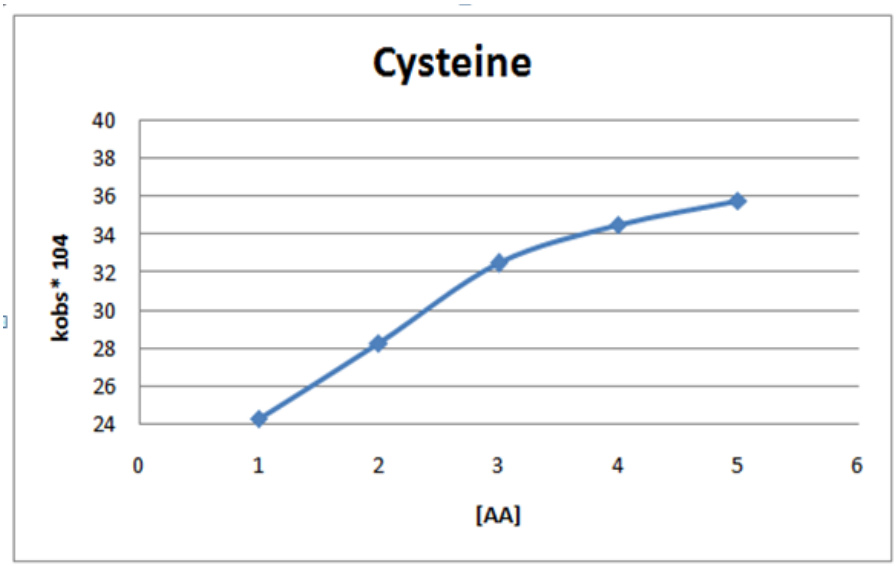

Fig. 2: Effect of Amino acid concentration on rate constant of Cyssteine.

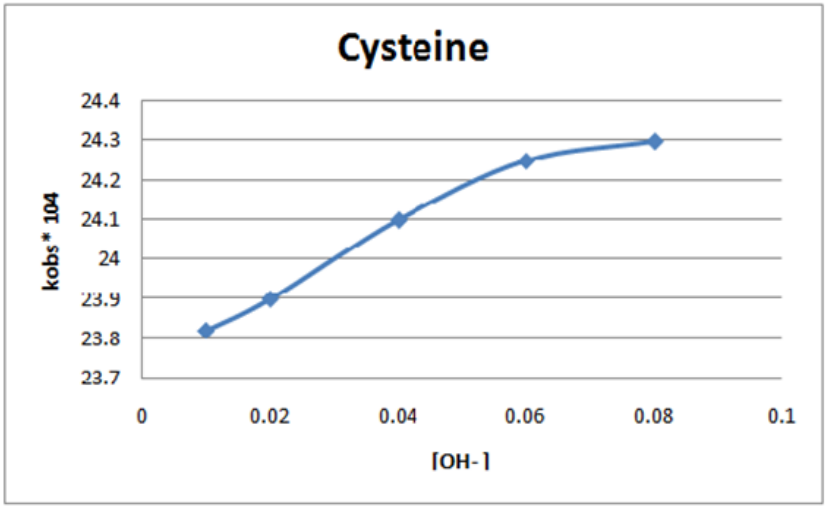

Fig. 3: Effect of Alkali concentration on rate constant of Cysteine.

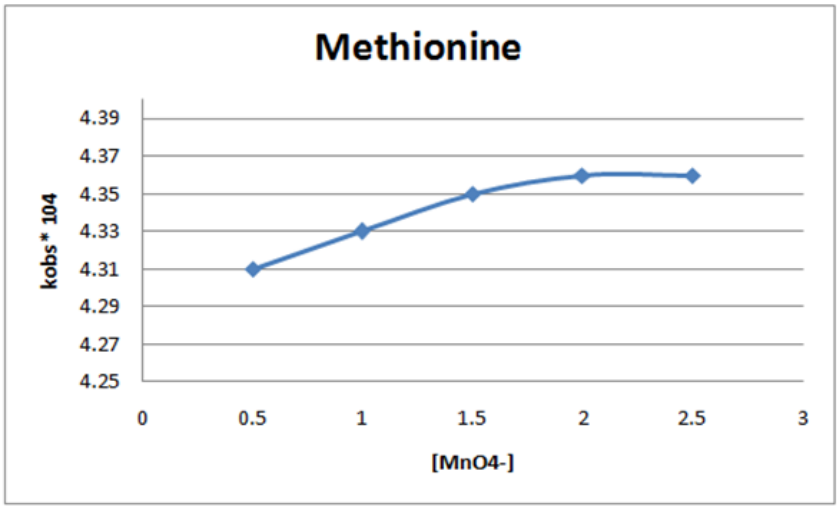

Fig. 4: Effect of Oxidant concentration on rate constant for Methionine.

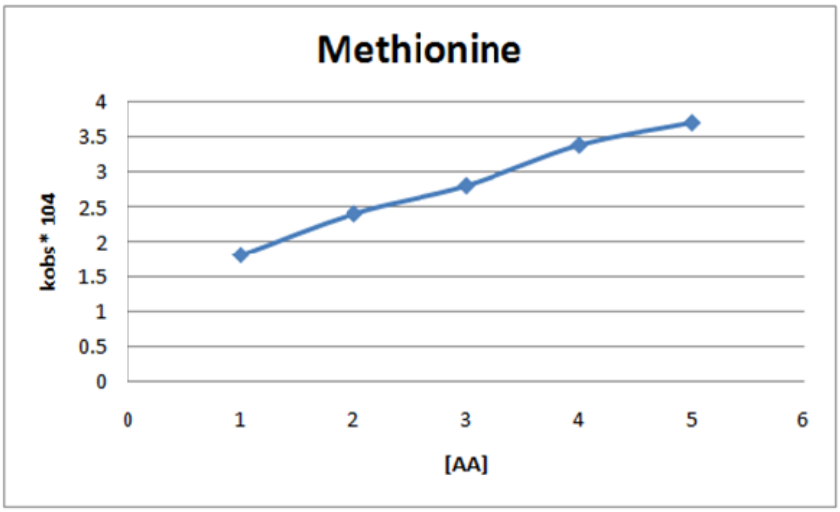

Fig. 5: Effect of Amino acid concentration on rate concentration for Methionine.

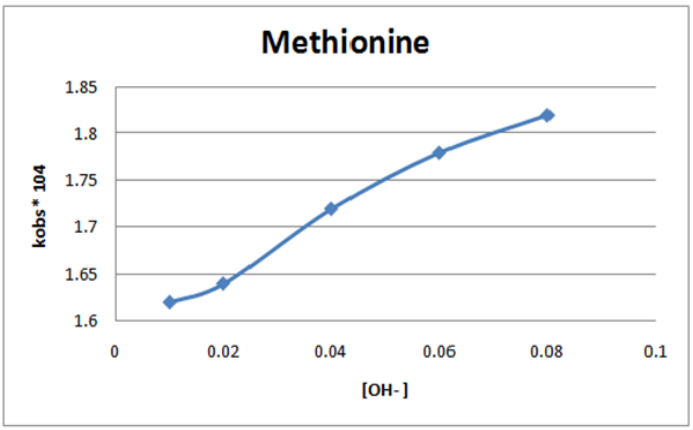

Fig. 6: Effect of Alkali concentration on Rate constant for Methionine.

- **Effect of pH on Oxidation: At** various pH levels rate costants of oxidation of Cysteine and Methionine were investigated, results indicate a distinct pH dependence on oxidation of both amino acids. The oxidation rate of both substrates increases with increase in pH of the medium. The oxidation rate of Cysteine is substantially higher than that of oxidation rate of Methionine under identical conditions, indicating thiol(-SH) group of Cysteine is more reactive than thioether group of (-S-) Methionine. The consistency in increasing k_{obs} value with hydroxide ions (OH^-) indicates that hydroxide ions are essential for oxidation, potentially by deprotonating the amino acids and increasing their susceptibility to the oxidizing agent. In general work affirms that alkalinity of the medium has significant influence on the oxidation reactions of the amino acids.
- **Effect of temperature:** Activation parameters for both the amino acids have been calculated by carrying out above reaction at two different temperatures. The output of the analysis has been given in the table below. These values are calculated by eyring and Arrhenius equation.

Table 2: Effect of temperature on rate constant.

S.No.	Temperature	Rate constant for Methionine*10^4	Rate constant for Cysteine*10^4
1	300	1.82	24.32
2	305	2.71	36.44
3	310	3.62	48.64
4	315	5.40	71.82

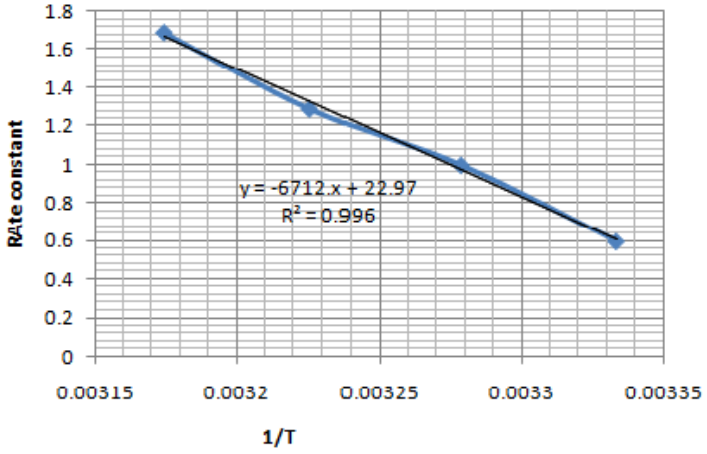

Fig. 7: Arrhenius plot for Methionine.

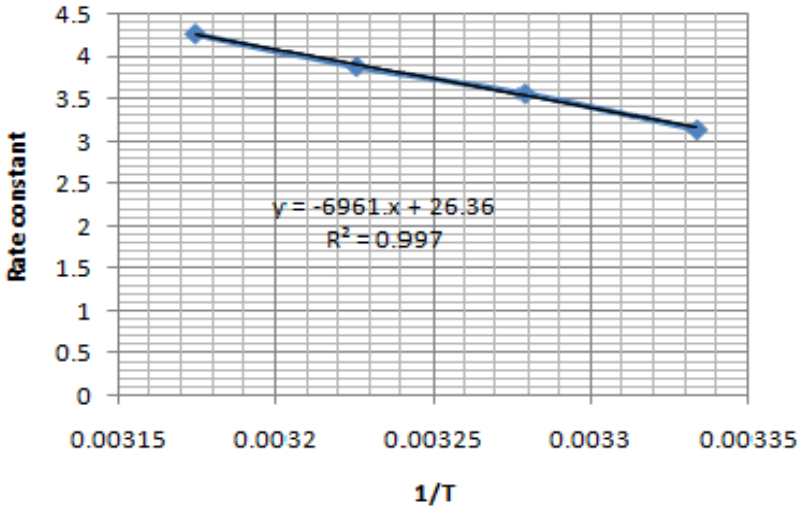

Fig. 8: Arrhenius plot for Cysteine.

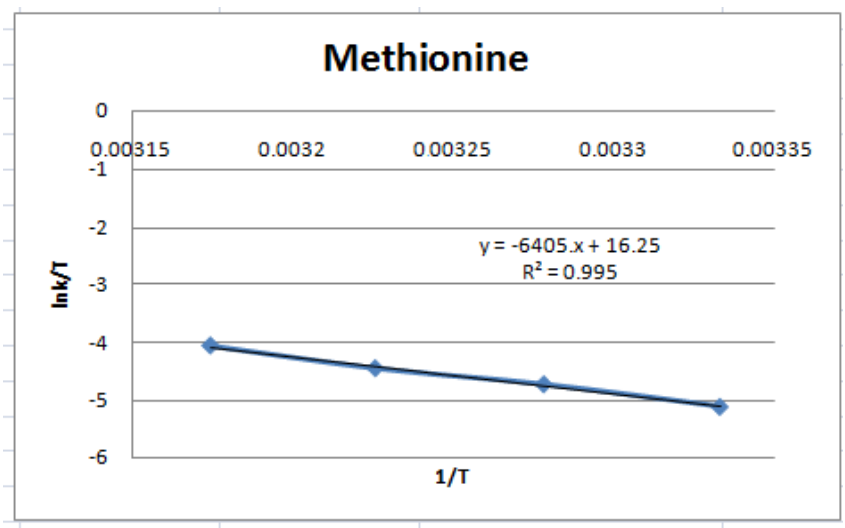

Fig. 9: Eyring plot for Methionine.

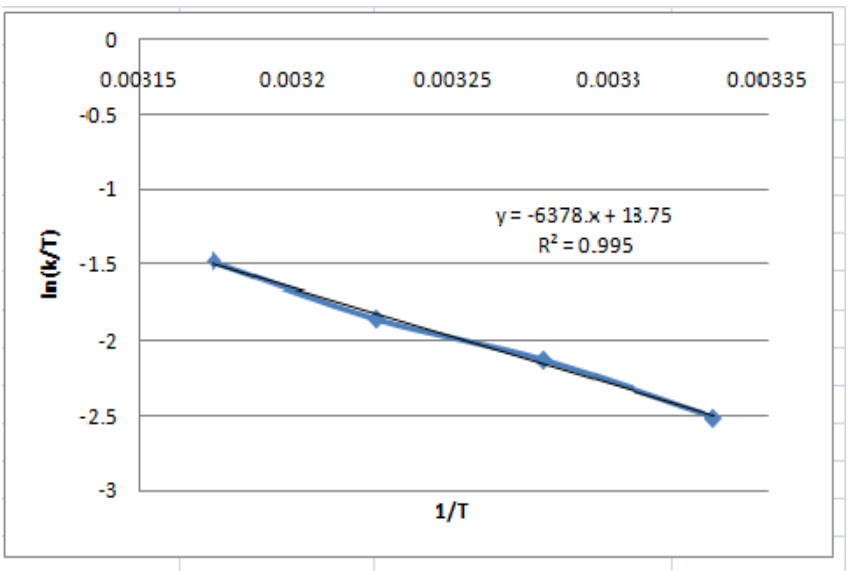

Fig. 10: Eyring plot for Cysteine.

Table: Table of Activation Parameters.

S.N.	Amino Acid	$E_a^{\neq}$ kJ/mol	$\Delta H^{\neq}$ kJ/mol	$\Delta S^{\neq}$e.u	$\Delta G^{\neq}$ kJ/mol
1	Cysteine	57.873	53.03kJ/mol	-41.636	65.34 kJ/mol
2	Methionine	55.803	53.25kJ/mol	-62.421	71.98 kJ/mol

Product Identification:-Products of the oxidation were identified by FTIR analysis.

FTIR spectra of oxidation product of **cysteine** exhibits following characteristics:-

Broad peak 3000-3200 c m^{-1} corresponds to the presence of strong hydrogen bonding between -OH and -NH_2 groups, which is common characteristic feature of all amino acids.A strong peak at 1650 cm^{-1} indicating presence of carbonyl(C=O) group, Confirming presence of –COOH group, another peak near 1450c m^{-1} corresponds to N-H bending, supporting presence of –NH_2 group .A distinct peak at 500-600 cm^{-1} corresponds to stretching vibration of disulfide (-S-S-) bond in product, which is characteristic of cystine , confirming two molecules of cysteine joined together by a disulfide bridge to form cystine. Overall spectra confirms cystine is formed by the oxidation of cysteine by alkaline potassium permanganate.

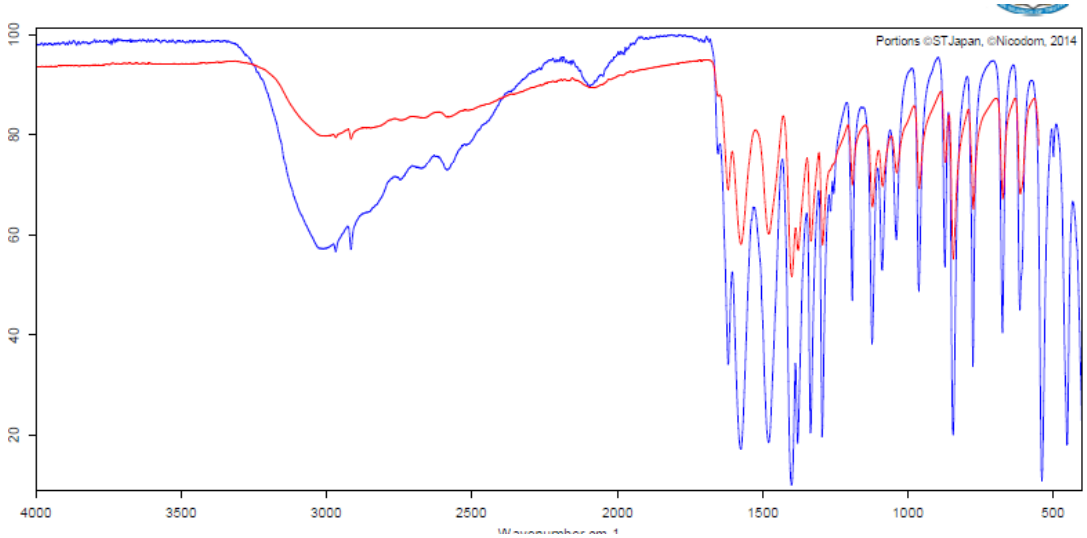

Fig. 11: IR spectra of Oxidation product of Cysteine.

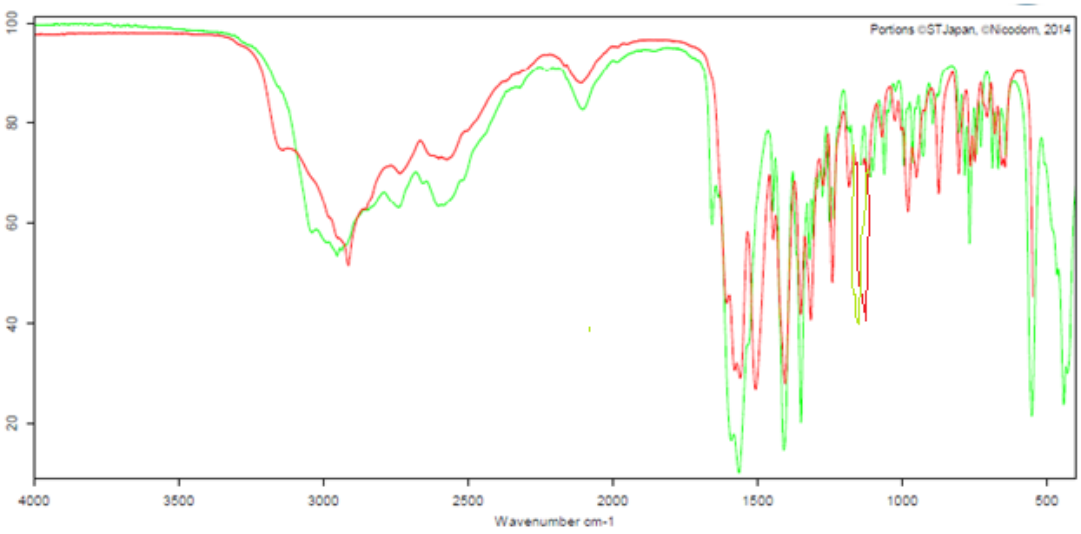

Fig. 12: IR spectra of oxidation product of Methionine.

FTIR Spectra of oxidation product of Methionine exhibits following characteristics:

The broad peak at 3000- 3200 cm^{-1} characteristics of amino acids ,indicates hydrogen bond between $–NH_2$ and –OH of carboxylic group, a peak at 2100 cm^{-1} representing the amino group of amino acids. Peaks at 1600-1700 is due to stretching of (C=O) group and a peak at 1510 cm^{-1} indicates bending vibration of the $–NH_2$ group. A strong peak at 1050-1100 cm^{-1} indicates stretching vibration of the (S=O) group. Medium to strong peaks from 1000-1300 correspond C-N stretching. The spectrum confirms methionine's structure with the presence of amine ($-NH_2$), carboxyl (-COOH), sulfoxide (-S=O), and alkyl chain peaks. Overall spectra confirms methionine on oxidation with potassium permanganate gives Methionine sulfoxide as oxidation product.

CONCLUSION

L-Cysteine and DL-Methionine ,both are sulphur containing amino acids, but from the present work it has been observed that rate constan(k) for cysteine is greater than that of Methionine which may be due to presence of free –SH group, Hence Cysteine reacts faster in comparison to Methionine. Since reaction mixture does not give ppt with acrylonitrile hence free radical is not involved in intermediate step. Rate of the reaction increases with increase in pH of the medium, indicating pH is playing a significant role in oxidation, Ionic strength does not have much influence on reaction rate. Activation energy for Cysteine is less than Methionine, indicating its higher reactivity. Enthalpy change for Cysteine and Methionine are positive mean, showing oxidation reaction is endothermic. Positive free energy for both the substrates indicate reaction is non spontaneous. FTIR analysis of the oxidation products confirms oxidation of cysteine into cystine and methionine into methionine sulfoxide. The information offers a deeper understanding of the oxidation mechanisms of amino acids.

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