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## COMPARATIVE STUDY OF OXIDATION OF L-CYSTEINE AND DL- METHIONINE BY POTASSIUM PERMANGANATE IN ALKALINE MEDIUM

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**ABSTRACT:** Kinetics of oxidation of L-cysteine and DL-Methionine by potassium permanganate in alkaline medium was studied spectro-photometrically at room temperature. The reaction was found to be of first order with respect to amino acid, zero order with respect to oxidant and fractional order dependence on Alkali. It was found that the rate of the reaction increases with increase in concentration of [OH<sup>-</sup>]. There is a good agreement between observed and calculated rate constant under experimental conditions. The activation parameters are computed with respect to the slow step of the reaction. Cysteine exhibits relatively consistent kinetic behavior than Methionine. The oxidation product of the reaction was found to be cysteine and Methionine sulfoxide. A plausible reaction mechanism has been proposed for the experimental results.

**INTRODUCTION:** Amino acids are organic compounds of biological importance composed of both amine and carboxylate groups along with a side chain. Study of oxidation of amino acid by various oxidants has become important because of their biological significance and selectivity towards the oxidants <sup>1-2</sup>. The study of amino acids is one of the most exciting and interesting fields of organic chemistry as they play a significant role in biosynthesis of polypeptides, nucleotides, protein and a number of metabolic reactions of living organisms. The study of the mechanism of oxidation of amino acids is gaining importance in order to understand some aspects of enzyme kinetics.

Amino acids find a number of applications in biochemical research, pharmaceuticals, metabolism, nutrition, and microbiology. Generally, only the carboxyl and amino functional group undergo chemical transformation whereas hydrocarbon moiety remains unaffected during oxidation <sup>3-4</sup>. The kinetic investigations of oxidation of biologically important various amino acids by variety of oxidants have been carried out under different experimental conditions. In many cases amino acids undergo oxidative decarboxylation.

Oxidation of amino acids by diperioatoargentate in alkaline medium involves two electron transfers in which imino acid is formed in a rate determining step, the acid thus formed subsequently undergoes hydrolysis to yield keto acids. One electron oxidant such as peroxomonosulphate and Hexacyanoferrate also oxidizes amino acid to keto acids. In most of the oxidation studies corresponding aldehydes have been reported as oxidation products. Although a variety of organic and inorganic substrates are oxidized by alkaline potassium permanganate but

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there are a few reports on the oxidation of amino acids by alkaline potassium permanganate<sup>5-7</sup>.

Cysteine is known for playing an important role in protein synthesis and participating in electron transfer reactions. There are three active donor sites N, O and S in Cysteine, in which electron rich sulfur is most susceptible to oxidation attacks. Depending on the pH and redox potential of the environment, Cysteine side chains can exist in distinct oxidation states. Cysteine plays an important role in various cellular responses to change in the redox environment such as those, which are result of oxidative stress with extensive links to pathological conditions like neurodegradation<sup>7-9</sup>.

The oxidation of Cysteine by some oxidants like hexacyanoferrate (III), Chromium (VI), Chloramine-T, Bromamine-B, Pyridinium Chlorochromate (PCC) has been studied. In most of the oxidations Cystine is the main oxidation product and in some cases cysteic acid and acetaldehyde are also reported. Aldehydes are tested by spot test analysis. L- cystine is one of the nonessential amino acids. It is a covalently linked and dimeric compound. The formation of disulfide bond between two cysteine residue is of biological importance. Cystine is required for proper utilization of vitamin B6 and helpful in healing of wounds and burns, breaking down mucus deposits in bronchitis patients. The reduction capability of cystine is responsible for all these beneficiary effects where it acts as scavengers to the oxidants and free radicals in situ. Oxidation of Cysteine by Pyridinium Chlorochromate exhibits first order dependence with respect to PCC and fractional order dependence on Cysteine and Cysteic acid was reported to be oxidation product<sup>10-12</sup>. The literature survey reveals that kinetic study on oxidation of L- cysteine is limited, this study helps to understand the mechanism of several biological redox reactions. So, in order to gain further insight in its oxidation mechanism we have taken this as substrate.

Methionine is an essential sulfur containing amino acid which plays an important role in human metabolism, antioxidant, building block of proteins and important sources of sulfur. Several inborne diseases result due to disturbances in its

biochemistry. Individuals with methionine malabsorption syndrome suffer fatty liver mainly due to accumulation of triacyl glycerol<sup>13-15</sup>. In our body N-Acetyl DL-Methionine is a methyl donor and this process is activated by adenosine triphosphate and enzymes such as phosphatase or dehydrogenase. Extensive studies on Methionine report that it behaves differently in comparison to other amino acids towards many oxidants, which may be due to presence of an electron rich sulphur centre which is easily oxidisable<sup>16-18</sup>. Literature survey does not reveal the clear mechanism of oxidation of L-cysteine and Methionine by any oxidant thus in order to explore the kinetics and mechanism of oxidation of cysteine and Methionine and investigate oxidation of structurally related amino acids under identical conditions.

Among all oxidizing agents, Potassium permanganate is the most widely and commonly used oxidant and disinfectant. It is strong, colored, and serves as its own indicator. It is the most important polyelectron oxidant in organic and inorganic chemistry. It is applied extensively in organic synthesis and has the ability to interact with many active groups of organic compounds including alcohols, aldehydes, ketones, amines and amino acids<sup>19</sup>. There are six oxidation states of manganese (+2 to +7), of which Mn(+7) is most potent in acidic, alkaline and neutral medium. The manganese chemistry is involved in these reactions because they have sufficiently long life time intermediates which are easy to identify and useful conclusions can be drawn. Oxidation is affected by many factors such as concentration, temperature, pH and Catalyst<sup>20</sup>.

### Experimental:

**Materials:** The stock solution of L-cysteine, DL-Methionine, Sodium Hydroxide, Sodium Chlorate and permanganate is prepared by dissolving requisite amount of amino acid in double distilled water. Potassium permanganate (BDH) solution was standardized against oxalate. All other reagents were of analytical grade and their solutions were prepared by dissolving the requisite amount of compound in double distilled water. Sodium Hydroxide was used to provide Alkalinity and sodium chlorate to maintain ionic strength<sup>21</sup>.

**Kinetic Measurements:** All kinetic measurements were performed under pseudo first order conditions where concentration of amino acid is tenfold excess over concentration of oxidant. Previously thermostated solutions of Potassium permanganate and Cysteine along with alkali and sodium chlorate were mixed to initiate the reaction. The temperature was uniformly maintained at  $25 \pm 0.1^\circ\text{C}$  through Thermostat. The course of the reaction was

monitored by observing decrease in the absorbance of potassium permanganate at its absorption maxima (526 nm). The same pattern was repeated for DL-Methionine. The first order rate constants were evaluated by plotting graph between  $\log [\text{permanganate}]$  versus time. In almost all cases first order plots were linear and reproducible within  $\pm 5\%$ .

**TABLE 1: EFFECT OF VARIATIONS OF POTASSIUM PERMANGANATE ( $\text{KMnO}_4$ ), AMINO ACIDS AND SODIUM HYDROXIDE ( $\text{NaOH}$ ) ON OXIDATION OF CYSTEINE IN ALKALINE MEDIUM AT ROOM TEMPERATURE**

S. no.	$[\text{MnO}_4^-] 10^3$ $\text{dm}^3/\text{mol}^3$	$[\text{AA}] 10^2$ $\text{dm}^3/\text{mol}^3$	$[\text{OH}^-] 2 \times 10^1$ $\text{dm}^3/\text{mol}^3$	$10^4 k_{\text{obs}} (\text{s}^{-1})$	
				Cysteine	Methionine
1	3.5	3.1	0.5	25.36	1.74
2	3.5	3.2	0.5	32.33	2.31
3	3.5	3.3	0.5	33.94	2.57
4	3.5	3.4	0.5	34.57	2.68
5	3.5	3.5	0.5	35.14	3.53
6	3.5	3.6	0.5	36.17	3.93
7	3.5	3.7	0.5	36.65	4.32
8	3.5	3.8	0.5	37.55	4.44
9	3.5	3.9	0.5	38.37	5.16
10	3.5	4	0.5	40.65	6.27
11	3.1	4	0.5	34.86	3.99
12	3.2	4	0.5	34.91	4.01
13	3.3	4	0.5	34.94	4.03
14	3.4	4	0.5	34.97	4.04
15	3.5	4	0.5	35.00	4.07
16	3.6	4	0.5	35.02	4.09
17	3.7	4	0.5	35.06	4.1
18	3.8	4	0.5	35.08	4.11
19	3.9	4	0.5	35.11	4.13
20	4	4	0.5	35.13	4.14
21	3.5	4	0.1	28.87	1.31
22	3.5	4	0.2	30.74	1.34
23	3.5	4	0.3	31.16	1.57
24	3.5	4	0.4	32.67	1.62
25	3.5	4	0.6	33.01	1.65
26	3.5	4	0.7	37.02	1.83
27	3.5	4	0.8	38.93	2.03
28	3.5	4	0.9	40.67	2.34
29	3.5	4	1	41.08	3.34
30	3.5	4	1.1	42.6	4.16

### Result Analysis:

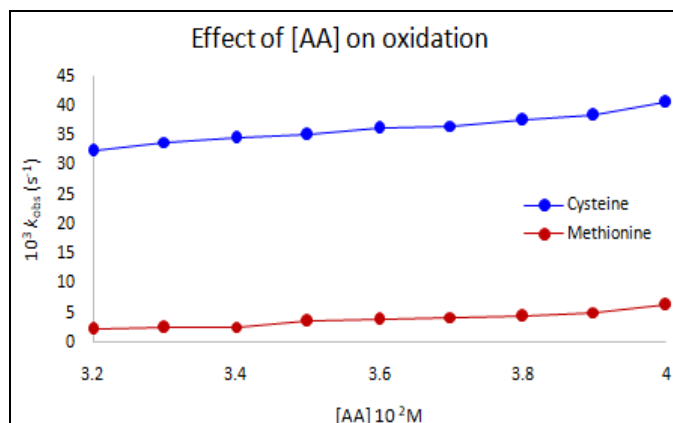
**Effect of Amino Acid Concentration:** The oxidation of cysteine and Methionine by potassium permanganate ( $\text{MnO}_4^-$ ) in an alkaline medium was investigated by adjusting the concentrations of amino acids  $[\text{AA}]$  ( $[\text{MnO}_4^-]$ ,  $[\text{OH}^-]$ ). The results suggest that the oxidation rates are significantly influenced by the concentration of amino acids **Fig. 1**. A sustained increase in oxidation was observed as  $[\text{AA}]$  increased from  $3.1 \times 10^{-1} \text{ M}$  to  $4.0 \times 10^{-1} \text{ M}$ ,

with cysteine increasing from 25.36 to 40.65 and Methionine from 1.744 to 6.27.

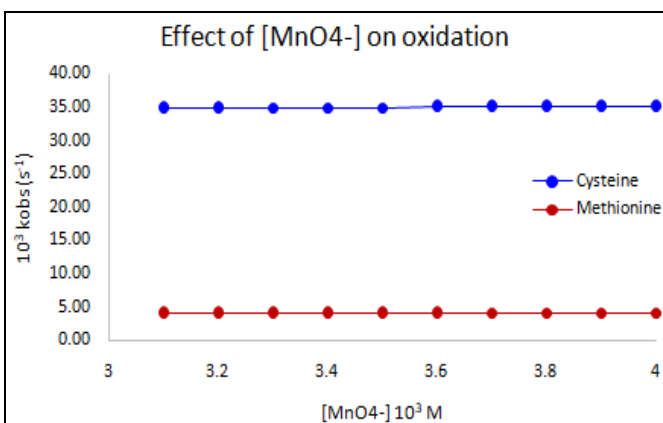
**Effect of Permanganate Ion Concentration:** Conversely, the oxidation rates were not significantly affected by varying  $[\text{MnO}_4^-]$  between  $3.1 \times 10^{-2} \text{ M}$  and  $4.0 \times 10^{-2} \text{ M}$  while maintaining  $[\text{AA}]$  and  $[\text{OH}^-]$  at a constant level, indicating a zero-order dependence on permanganate **Fig. 2**.

**Impact of Hydroxide Ion Concentration:** In the interim, the oxidation rates were significantly improved by an increase in  $[\text{OH}^-]$  from  $0.1 \times 10^{-2}$  M to  $1.1 \times 10^{-2}$  M. Cysteine increased from 28.87 to 42.59 and Methionine from 0.93 to 4.16, suggesting a positive dependence on hydroxide ions **Fig. 3**.

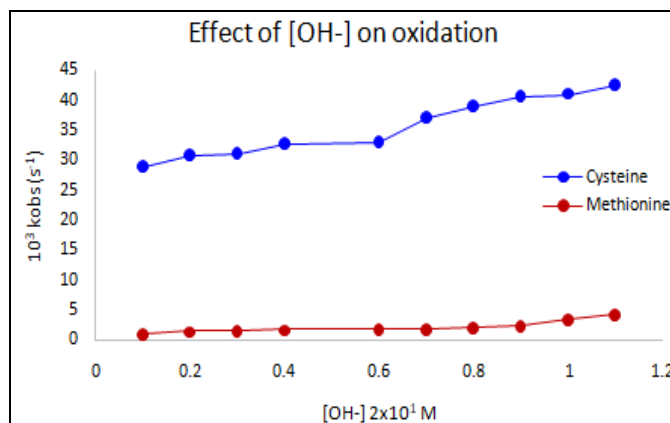
These results indicate that the oxidation reaction is governed by first-order kinetics with respect to amino acid concentration, zero-order kinetics with respect to permanganate, and a potential first or fractional order in relation to hydroxide ion concentration.



**FIG. 1: EFFECT OF AMINO ACID CONCENTRATION ON OXIDATION OF CYSTEINE AND METHIONINE**



**FIG. 2: EFFECT OF POTASSIUM PERMANGANATE CONCENTRATION ON OXIDATION OF CYSTEINE AND METHIONINE**



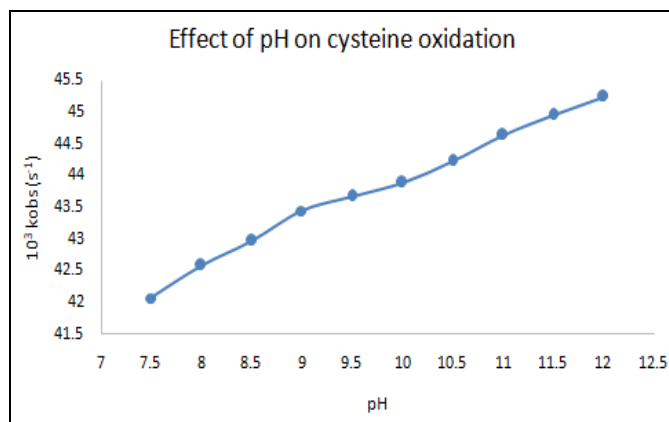
**FIG. 3: EFFECT OF SODIUM HYDROXIDE CONCENTRATION ON OXIDATION OF CYSTEINE AND METHIONINE**

**Effect of pH:** At various pH levels, the oxidation of cysteine and methionine was investigated, and the observed rate constants ( $k_{\text{obs}}$ ) were estimated **Fig. 6 & 7**.

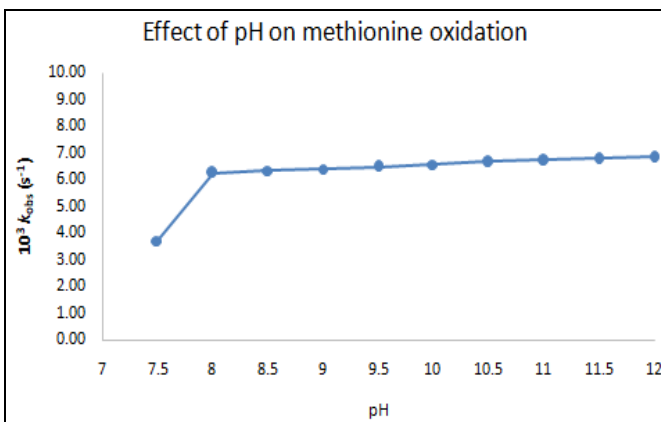
The results indicate a distinct pH-dependent trend, in which the oxidation rates of cysteine and methionine increase as the medium becomes more alkaline. The reactivity of cysteine increases steadily as the concentration of  $\text{OH}^-$  increases, from  $42.07 \times 10^4 \text{ s}^{-1}$  at pH 7.5 to  $45.24 \times 10^4 \text{ s}^{-1}$  at pH 12. In the same vein, the rate of  $k_{\text{obs}}$  increases from  $3.72 \times 10^4 \text{ s}^{-1}$  at pH 7.5 to  $6.85 \times 10^4 \text{ s}^{-1}$  at pH 12. Nevertheless, the oxidation rate of cysteine is

substantially higher than that of methionine at all pH levels, indicating that the thiol ( $-\text{SH}$ ) group in cysteine is more reactive than the thioether ( $-\text{S}-$ ) group in methionine. The consistent increase in  $k_{\text{obs}}$  with pH indicates that hydroxide ions ( $\text{OH}^-$ ) are essential for oxidation, potentially by deprotonating the amino acids and increasing their susceptibility to the oxidizing agent.

In general, the research affirms that the alkalinity of the medium significantly influences the oxidation reactions of these amino acids, with cysteine being more reactive than methionine under identical conditions.



**FIG. 4: EFFECT OF PH ON OXIDATION OF CYSTEINE**



**FIG. 5: EFFECT OF PH ON OXIDATION OF METHIONINE**

**Effect of Temperature:** Across the temperature range of 300–315 K, the kinetic data for cysteine and methionine oxidation demonstrate distinct trends in their rate constants, activation energies, enthalpy ( $\Delta H$ ), and entropy ( $\Delta S$ ) properties **Table 2**. The reaction rate of cysteine oxidation is comparatively stable, as evidenced by the slight increase in the rate constant ( $k$ ) from  $45.45 \times 10^4 k_{\text{obs}} \text{s}^{-1}$  at 300 K to  $107.25 \times 10^4 k_{\text{obs}} \text{s}^{-1}$  at 315 K. Reinforcing the stability of cysteine's oxidation kinetics, the Arrhenius plot ( $\ln(k)$  vs.  $1/T$ ) exhibits a modest decrease.

The Arrhenius plot for cysteine oxidation illustrates a strong linear relationship between  $\ln(k)$  and  $1/T$  ( $1/\text{K}$ ), confirming that the reaction follows Arrhenius kinetics **Fig. 8**. The equation of the best-fit line,  $y = -5328.x + 21.62$  provides valuable kinetic parameters. The slope ( $-5328$ ) corresponds to  $-E_a/R$ , where  $E_a$  is the activation energy and  $R=8.314 \text{ J/mol. K}$  is the universal gas constant. The Arrhenius analysis of cysteine oxidation provided key kinetic parameters essential for understanding the reaction mechanism. The activation energy ( $E_a$ ) was determined to be  $44.296 \text{ kJ/mol}$ , indicating the minimum energy required for the reaction to proceed. The enthalpy of activation ( $\Delta H$ ) was calculated as  $41.78 \text{ kJ/mol}$ , which represents the actual energy needed to reach the transition state after accounting for thermal energy contributions. Additionally, the entropy of activation ( $\Delta S$ ) was found to be  $-73.56 \text{ J/mol. K}$ , suggesting that the transition state is more ordered than the reactants. This negative entropy value implies possible solvation effects or specific molecular interactions that restrict the degrees of freedom in the transition state. The high correlation coefficient ( $R^2=0.961$ ) in

the Arrhenius plot confirms a strong linear relationship, validating the temperature dependence of the reaction rate. These results suggest that cysteine oxidation follows a reaction pathway similar to methionine oxidation, with comparable activation parameters and a slightly more ordered transition state.

The rate constant ( $k$ ) for methionine increases from  $5.69 \text{ min}^{-1}$  at 300 K to  $19.25 \text{ min}^{-1}$  at 315 K, indicating a more pronounced temperature dependence than that of cysteine. The Arrhenius plot in figure 9 for methionine oxidation illustrates a linear relationship between the reciprocal of temperature ( $1/T$ ) and the natural logarithm of the rate constant ( $\ln(k)$ ). A high coefficient of determination ( $R^2 = 0.979$ ) is indicative of an outstanding fit to the Arrhenius equation, as the fitted line's equation is  $y = -7885.x + 28.01$ .  $-E_a/R$  is the activation energy divided by the universal gas constant, as indicated by the negative slope ( $-31.262$ ). This implies that the oxidation of methionine exhibits an Arrhenius-type behaviour, and the high linearity corroborates the reliability of the kinetic data. The Arrhenius analysis of methionine oxidation revealed an activation energy ( $E_a$ ) of  $65.55 \text{ kJ mol}^{-1}$ , indicating a moderate energy barrier for the reaction. The enthalpy of activation ( $\Delta H$ ) was calculated as  $62.25 \text{ kJ mol}^{-1}$ , reflecting the energy required to reach the transition state. The entropy of activation ( $\Delta S$ ) was found to be  $-23.09 \text{ JK}^{-1} \text{ mol}^{-1}$ , suggesting a highly ordered transition state, likely due to solvation effects or specific molecular interactions. The high correlation coefficient ( $R^2=0.979$ ) confirms the strong linear relationship in the Arrhenius plot, indicating that the reaction follows classical



temperature dependence. These findings suggest that methionine oxidation is an energetically feasible reaction with a structured transition stat. In general, cysteine demonstrates a more consistent and stable reaction profile, whereas methionine demonstrates a greater degree of variability in activation parameters, suggesting the potential for complexities in its reaction mechanism and cysteine exhibits a relatively consistent kinetic behaviour.

TABLE 2: EFFECT OF TEMPERATURE ON RATE CONSTANT THE ACTIVATION ENERGY, ENTHALPY, AND ENTROPY WERE CALCULATED FROM GIVEN PLOT

Amino acid	Temperature	Rate constant(k)*10 <sup>4</sup>	Ea (kJ mol <sup>-1</sup> )	ΔH (kJ mol <sup>-1</sup> )	ΔS (JK <sup>-1</sup> mol <sup>-1</sup> )
Cysteine	300	45.45	44.29	41.78	-73.56
	305	67.25			
	310	88.45			
	315	107.25			
Methionine	300	5.69	65.55	62.25	-23.09
	305	8.25			
	310	14.25			
	315	19.25			

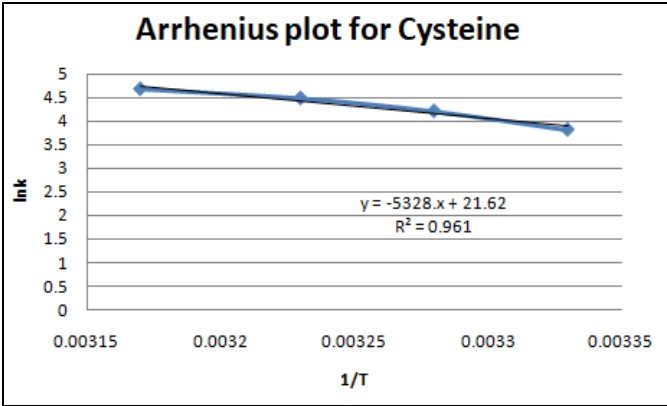


FIG. 6: THE PLOT SHOWS EFFECT OF TEMPERATURE ON CYSTEINE OXIDATION AS OBSERVED BY ARRHENIUS CALCULATION

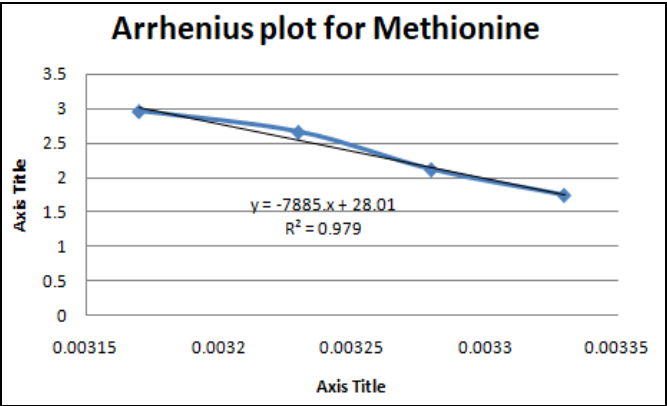


FIG. 7: THE PLOT SHOWS EFFECT OF TEMPERATURE ON METHIONINE OXIDATION AS OBSERVED BY ARRHENIUS CALCULATION

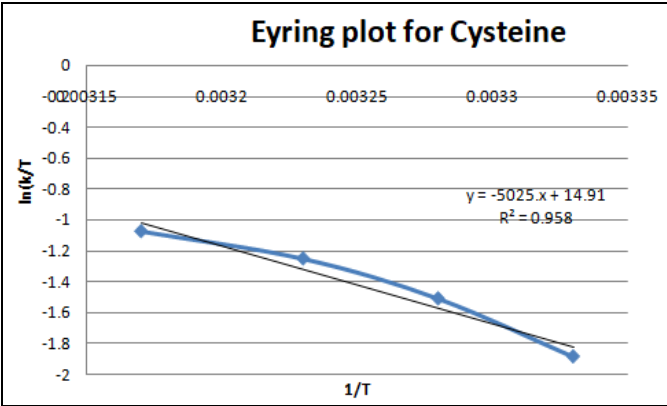


FIG. 8: THE PLOT SHOWS EFFECT OF TEMPERATURE ON CYSTEINEE OXIDATION AS OBSERVED BY EYRING CALCULATION

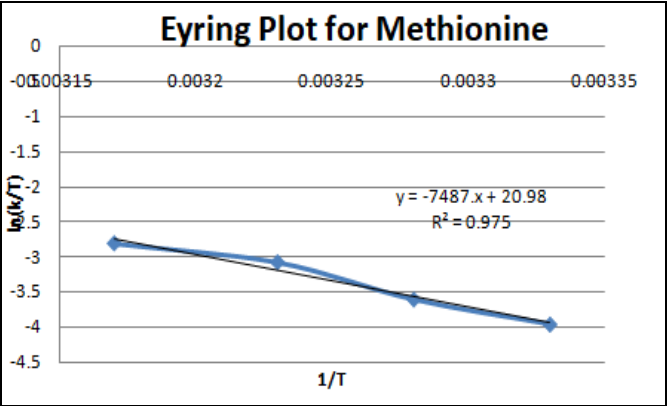


FIG. 9: THE PLOT SHOWS EFFECT OF TEMPERATURE ON METHIONINE OXIDATION AS OBSERVED BY EYRING CALCULATION

**FT-IR Analysis of Oxidative Products of Cysteine and Methionine:** The FT-IR spectrum of cysteine exhibits characteristic peaks corresponding to its functional groups, confirming its molecular structure as observed in Fig. 10. A broad peak observed at 3583 cm<sup>-1</sup> corresponds to the O–H and

N–H stretching vibrations, indicating the presence of hydroxyl (-OH) and amine (-NH<sub>2</sub>) groups. The broadness of this peak suggests strong hydrogen bonding, which is common in amino acids. Another significant peak at 2917 cm<sup>-1</sup> corresponds to C–H stretching vibrations, arising from the aliphatic

groups present in the cysteine structure. A prominent peak at  $1639\text{ cm}^{-1}$  is assigned to the C=O stretching vibration (Amide I band), confirming the presence of the carboxyl (-COOH) or amide (-CONH) functional groups. Additionally, a peak at  $1462\text{ cm}^{-1}$  corresponds to N-H bending (Amide II band), further supporting the presence of the amine (-NH<sub>2</sub>) group. The presence of a distinct peak at  $1255\text{ cm}^{-1}$  is characteristic of C-S stretching, which is absent in spectrum, confirming the absence of the thiol (-SH) group, which is unique to cysteine. This finding indicates that cysteine has undergone to oxidation and formed cystine. Overall, the FT-IR spectrum of cysteine effectively demonstrates the presence of its key functional groups, including hydroxyl (-OH), amine (-NH<sub>2</sub>), carboxyl (-COOH), and thiol (-SH). The broad O-H/N-H stretching, strong C=O stretching, and distinct thiol peak provide strong evidence of the molecular identity of cysteine. This analysis confirms that the sample aligns with the expected spectral features of cysteine, validating its structural composition.

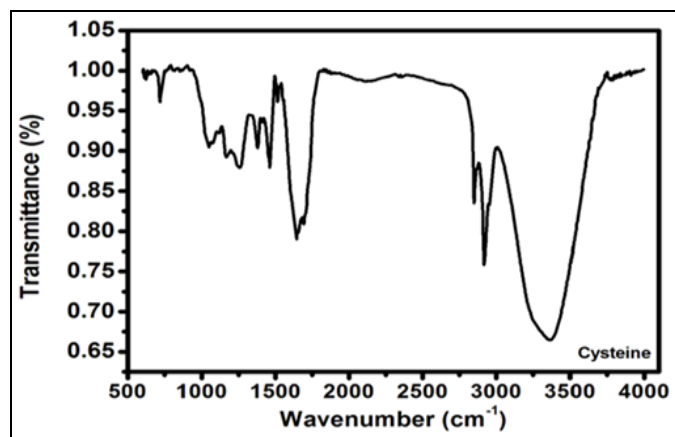


FIG. 10: THE FT-IR ANALYYSIS OF CYSTEINE AFTER THE COMPLETION OF OXIDATION PROCESS

The oxidation of methionine is confirmed by the FTIR analysis, which reveals critical vibrational bands that correspond to various functional groups **Fig. 11**. The carboxyl functional group is indicated by the presence of a robust absorption peak at  $1794.0\text{ cm}^{-1}$ , which is ascribed to the carbonyl (C=O) stretching vibration. The oxidized molecule's retention of the amino group is further confirmed by the observation of the primary amine (NH<sub>2</sub>) bending vibration at  $1008.6\text{ cm}^{-1}$ . The spectrum is characterized by the presence of sulfoxide (S=O) stretching vibrations, which are

represented by two distinct peaks at  $1134.7\text{ cm}^{-1}$  and  $1044.3\text{ cm}^{-1}$ . These peaks indicate the successful oxidation of methionine's sulfur group to sulfoxide. Furthermore, the spectrum displays a peak at  $1218.2\text{ cm}^{-1}$ , which corresponds to the C-C bond stretching between the  $\alpha$ -carbon and carboxyl group, as well as another peak at  $1237.6\text{ cm}^{-1}$ , which is attributed to the stretching and bending of  $\alpha$ ,  $\beta$ , and  $\gamma$  carbon-hydrogen Bonds. The  $492.4\text{ cm}^{-1}$  peak is indicative of hydroxyl (O-H) stretching and vibrations associated with the methylthio (-S-CH<sub>2</sub>-) group, while a significant low-wavenumber peak at  $205.5\text{ cm}^{-1}$  suggests interactions between nitrogen, carbon, and sulfur. The peak at  $1190.3\text{ cm}^{-1}$  confirms the presence of O-H, H-N-H,  $\alpha$ -C-H, and  $\gamma$ -C-H stretching vibrations, while the bending of the H-N-H functional group is indicated by another peak at  $1565.6\text{ cm}^{-1}$ . In general, the FTIR spectrum confirms the oxidation of methionine to methionine sulfoxide, as evidenced by the retention of the amine and carboxyl functional groups and the presence of characteristic sulfoxide (S=O) bands. FTIR is a valuable instrument for the analysis of biochemical modifications in amino acids, as these findings confirm the structural integrity of the oxidized molecule.

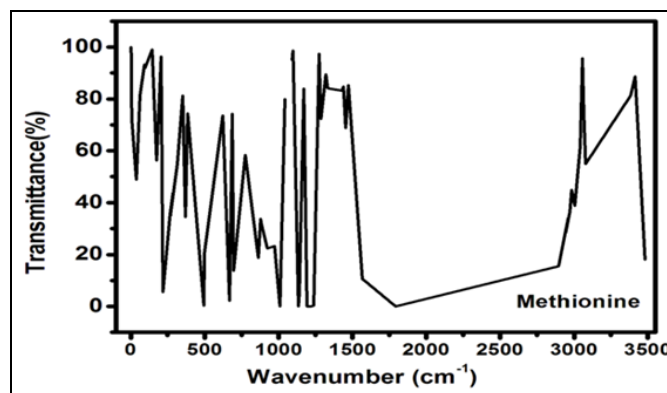


FIG. 11: THE FT-IR ANALYYSIS OF METHIONINE AFTER THE COMPLETION OF OXIDATION PROCESS

**CONCLUSION:** Overall, the kinetic analysis highlights the distinct oxidation behavior of cysteine and methionine. Cysteine oxidation is strongly influenced by amino acid and hydroxide ion concentrations, showing a clear increase in rate. Methionine oxidation also follows a regular pattern, but the oxidation rate is relatively less than cysteine. Which is likely attributable to the fact that its thiol (-SH) group is more reactive than methionine's thioether (-S-) group in alkaline

environments. These findings provide valuable insights into the reactivity of sulfur-containing amino acids under oxidative conditions. In general, the kinetic study emphasizes a significant correlation between the oxidation rate and the structure of the amino acid, the concentration of permanganate, and the pH of the alkaline medium. This information offers a deeper understanding of the oxidation mechanisms of sulfur-containing amino acids.

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