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Kinetics Of L-Threonine Oxidation In Acidic Medium Using TMGCC: A Comprehensive Study

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Abstract: The kinetics of L-threonine oxidation in an acidic medium using tetramethylguanidinium chlorochromate (TMGCC) as an oxidant was systematically investigated. The study explores the impact of various reaction parameters, including substrate concentration, oxidant concentration, acidity, and temperature on the rate of oxidation. A first-order dependence on both L-threonine and TMGCC was observed, while the reaction showed a fractional-order dependence on hydrogen ion concentration. Activation parameters such as the enthalpy, entropy, and the energy of activation were calculated from temperature-dependent data using the Arrhenius equation. A plausible reaction mechanism has been proposed based on experimental results, supported by the formation of intermediate complexes. This study provides comprehensive insights into the oxidation process of L-threonine, which could contribute to a deeper understanding of amino acid oxidation reactions in biological systems.

Keywords - L-Threonine, kinetics, oxidation, mechanism.

1 INTRODUCTION

The oxidation of amino acids has gained significant attention due to its relevance in biological systems and industrial applications. Amino acids play crucial roles in metabolic processes, and their oxidative degradation is important in the regulation of biochemical pathways. L-threonine, an essential amino acid, is particularly involved in protein synthesis, immune function, and metabolic regulation. Understanding the mechanisms of its oxidation can provide insights into various biological processes, including energy production and cellular signaling.

The oxidation of L-threonine has been studied using various oxidizing agents[1-7]; however, the use of tetramethylguanidinium chlorochromate (TMGCC) as an oxidant in acidic media remains largely unexplored. TMGCC is a versatile, mild oxidant that has shown efficacy in the oxidation of organic substrates under controlled conditions[8]. Its selective oxidizing properties make it an attractive candidate for studying the oxidation behavior of amino acids like L-threonine.

In this study, we present a detailed kinetic investigation of L-threonine oxidation using TMGCC in an acidic medium. The influence of several factors such as substrate concentration, oxidant concentration, acidity, and temperature on the reaction rate was explored. By determining activation parameters and proposing a mechanistic pathway, this research aims to enhance the understanding of amino acid oxidation and contribute valuable data to the field of chemical kinetics.

This work not only sheds light on the mechanistic aspects of L-threonine oxidation but also offers potential applications in fields such as pharmaceutical chemistry and bioengineering, where controlled oxidation processes are essential.

2 EXPERIMENTAL

2.1 Materials and methods:

The chemicals utilized in this research were of high purity. TMGCC was freshly prepared in the laboratory following the procedure outlined in the literature[8], and its purity was confirmed using the iodometric method[9]. L-threonine was used as supplied by Merck, without further modification. Solvent purification was carried out using established methods[10].

2.2 Kinetic measurements:

The kinetics studies were conducted under standard pseudo-first-order conditions, ensuring that the concentration of L-threonine remained significantly higher than that of TMGCC. The reaction temperature was precisely maintained at 25°C with a margin of ±0.2°C. Both the TMGCC solution and a mixture of threonine and sulfuric acid were independently thermostated for approximately two hours. Following this period, the solutions were combined, and placed in the spectrophotometer cell, and experimental readings were taken every 3 to 4 minutes. The decrease in Cr(VI) concentration, monitored through absorption reduction at its peak absorption wavelength (λ_{max} = 350 nm), was measured over time using a UV-Vis spectrophotometer with a constant-temperature cell. The observed pseudo-first-order rate constant, k_{obs}, was determined from the slope of the plot between ln(A) and time, as outlined by the following equation.

$$\log_e(A_t - A_\infty) = \log_e(A_0 - A_\infty) - K_{obs} \cdot T \quad \text{------ (1)}$$

{A_t = the absorbance of the reaction mixture measured at time t.
and A_∞ = the absorbance of the mixture measured at equilibrium.}
A_∞ was measured as soon as the reaction got completed.
Then the k_2 (second-order rate constant), was evaluated using the relation given below:

$$K_2 = K_{obs} / [\text{L- threonine}] \quad \text{------ (2)}$$

3 RESULTS AND DISCUSSION

3.1 Stoichiometry

The stoichiometry of the reaction between L-threonine and TMGCC in a sulfuric acid medium was determined to be 1:1. TMGCC underwent a two-electron transfer, which aligns with previous observations for structurally similar halochromates.
Thus, the overall equation for the oxidation of L-threonine by TMGCC in an acidic (H_2SO_4) medium can be represented as follows:

$$\text{L- Threonine} + \text{TMGCC} \xrightarrow[H_2O]{H^+} \text{2-Hydroxy propanal} + \text{Cr(IV)} + NH_3 + CO_2 \quad \text{------ (3)}$$

3.2 Product analysis

In the oxidation of L-threonine by TMGCC, the primary product formed was a carbonyl compound, which was confirmed using the 2,4-DNP test[11]. Adding 2,4-dinitrophenylhydrazine to the reaction mixture produced a yellow precipitate, indicating the quantitative presence of a carbonyl compound. The main product identified was 2-hydroxypropanal, confirmed through a spot test[12]. Cr(IV) was detected using the iodometric method. The reaction also produced carbon dioxide and ammonia as byproducts; carbon dioxide was confirmed by the limewater test, and ammonia by Nessler's reagent[13]. Similar oxidation products have been observed under varying experimental conditions, as reported in previous studies[14-17]. The IR spectrum of 2-hydroxy propanal exhibits characteristic absorption peaks due to its functional groups. 3500 cm^{-1} (O-H Stretch), 1710 cm^{-1} (C=O Stretch), 1050 cm^{-1} (C-O Stretch), 2720 & 2820 cm^{-1} (C-H Stretch), 2950 cm^{-1} (C-H Stretch).

3.3 Rate-laws

A typical kinetic analysis demonstrated that the reaction rate with respect to TMGCC follows first-order kinetics (Figure 1). The observed pseudo-first-order rate constant, k_{obs}, remains unaffected by the initial TMGCC concentration. A plot between $1/k_{obs}$ and 1/[L- threonine] yielded a linear relationship with an intercept on the y-axis and a correlation coefficient with $r > 0.995$, supporting this finding. To further validate, the Michaelis-Menten model was applied to L-threonine, leading to the development of the reaction mechanisms (4 and 5) that correspond to the rate law illustrated by Equation (6).

$$\text{L- Threonine} + \text{TMGCC} \underset{}{\overset{K,\ H^+}{\rightleftharpoons}} [\text{Complex}] \qquad \text{-----(4)}$$

$$[\text{Complex}] \longrightarrow \text{Products} \qquad \text{-----(5)}$$

$$\text{Rate} = k_2[\text{Threonine}][\text{TMGCC}] \ / (1+K[\text{Threonine}]) \qquad \text{-----(6)}$$

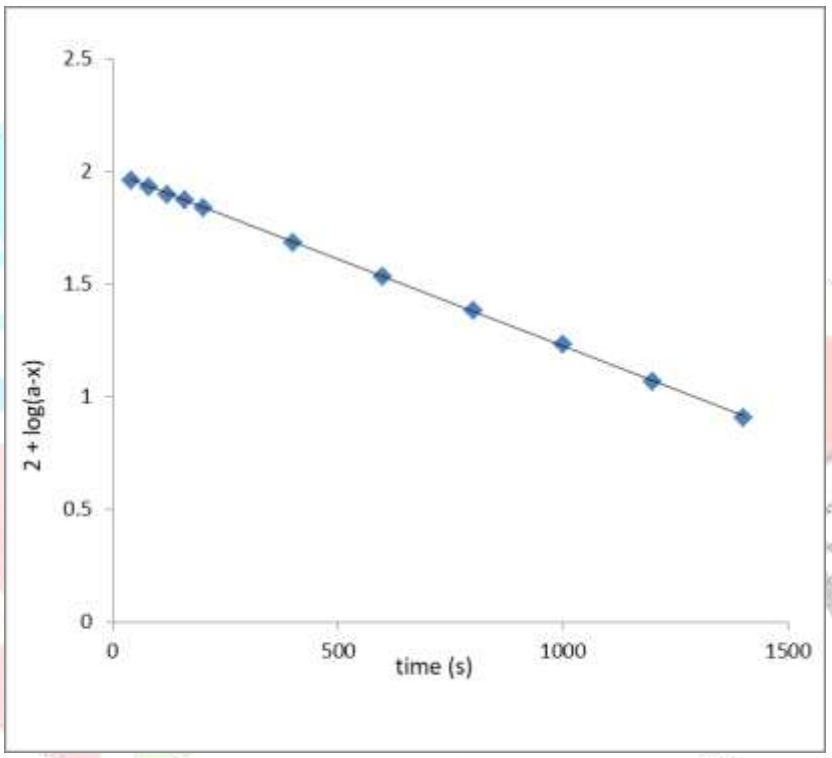


FIGURE 1: Oxidation of threonine by TMGCC: A typical kinetic run

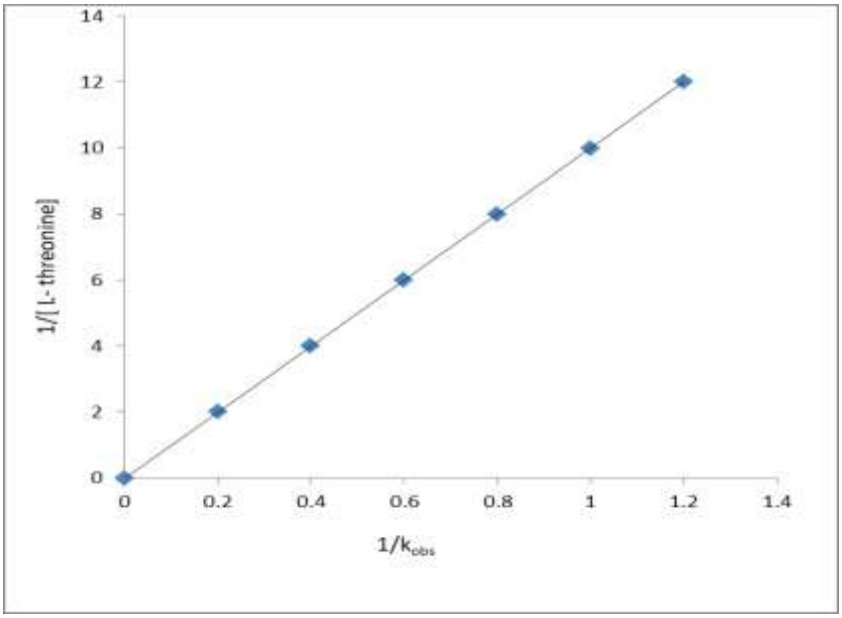


FIGURE 2: Oxidation of threonine by TMGCC: A double reciprocal plot

TABLE 1: Effect of variation of [TMGCC)], [Thr], on the observed first-order rate constant in the oxidation of L- threonine by TMGCC in sulphuric acid solutions at 298K.

[TMGCC] (mol/dm^3)	**10^3 [Thr] (mol/dm^3)**	**[H^+] (mol/dm^3)**	**10^4 k_{obs} (1/s)**
1.0	0.02	0.10	3.75
1.0	0.03	0.10	4.71
1.0	0.05	0.10	6.9
1.0	0.10	0.10	9.6
1.0	0.20	0.10	12.45
1.0	0.30	0.10	14.25
0.3	0.05	0.10	7.2
0.5	0.05	0.10	7.05
0.8	0.05	0.10	7.005
1.0	0.05	0.10	6.9
2.0	0.05	0.10	7.35
1.0	0.10	0.10	9.705*
*Contained Acrylonitrile (0.001 mol/dm^3)			

The reaction rate was analyzed across different temperatures to determine its dependence on L-threonine concentration. The collected data allowed for the calculation of k_2 and K values. Using these values at various temperatures, the thermodynamic parameters for complex decomposition and the activation parameters for the formation of Thr-TMGCC complexes were calculated (Tables 2 and 3).

TABLE 2: Activation Parameters for the oxidation of Thr by TMGCC.

$\Delta H^{\#}$ (kJ/mol)	**$-\Delta S^{\#}$ (J/mol K)**	**$\Delta G^{\#}$ (kJ/mol)**
45.6 ± 0.8	62 ± 3	60.2 ± 0.8

TABLE 3: Thermodynamic Parameters for decomposition of Thr-TMGCC complexes.

−ΔH (kJ/mol)	**−ΔS (J/mol K)**	**−ΔG (kJ/mol)**
15.4 ± 0.3	12 ± 2	6.4 ± 0.3

3.4 Order of reaction

The reaction order(n) was calculated using the slope of the plots between logk_{obs} and log(concentration) by changing the concentrations (C) of substrate and acid, and making other conditions constant. The [TMGCC] was varied by keeping others at a fixed concentration. The pseudo-first-order rate constants, k_{obs}, were calculated using a gradient from ln(A) versus time plots. It was confirmed from the above data that reaction is of order first with respect to [TMGCC]. The k_{obs} was also calculated at diverse initial concentrations of the threonine by keeping concentrations of others at constant. A linear plot of k_{obs} against [L- threonine] indicated that the reaction's order was less than one with respect to [L- threonine] and its intercept was positive.

3.5 Effect of [L- threonine] on the oxidation rate

As reported in the literature survey that the amino acid exists as a zwitter ion at Ph of 7 and predominantly tends to protonate at Ph<7. The employment of a high concentration of hydrogen ion during the reaction and also the observed augmentation of the reaction's rate on increasing acid's concentration recommended the protonation of L- threonine in the step before equilibrium, whereas the protonated form (Thr^+) appeared as reactive in the slow step.

3.6 Effect of temperature

Temperature's effect on the reaction's rate had been studied at four different temperatures i.e. at 288, 298, 308, and 318 K with changeable concentrations of sulfuric acid and other conditions being kept constant. It further suggested that the reaction's rate is directly proportional to temperature i.e. it increased with increasing temperature (Table 4). By studying the reactions, at diverse temperatures, the thermodynamic and activation parameters were calculated (Tables 2, 3).

TABLE 4: Rate constants (k_2) for the oxidation of Threonine by TMGCC.

10^4 k_2(dm^3/mol s) at			
288 K	**298 K**	**308 K**	**318 K**
6.92	14.8	32.4	73.5

TABLE 5: Formation constants for the decomposition of the Thr-TMGCC complexes.

K (dm^3/mol)			
288 K	**298 K**	**308 K**	**318 K**
31.5	27.4	21.2	15.4

3.7 Test for free radicals

The polymerization test was employed to check the presence of the free radical species formed during the reaction. It was performed by taking a known quantity of acrylonitrile with the mixture of the reaction and was thus kept for approximately 3 hours. Mixture's dilution using methanol does not result in any white precipitate which supported the absence of free radicals in the reaction mixture. Further, it was reinforced by the fact that the reaction's rate does not change with the addition of acrylonitrile (table 1). This completely ruled out the prospect of oxidation of only a single electron giving rise to the involvement of free radicals.

3.8 Effect of acidity

Varying the concentration of sulfuric acid and keeping others constant, was used to study the acidity effect on the reaction's rate. Any increase in the concentration of acid was seen to catalyze the rate of reaction. It was indicated by Table 6, that the process of oxidation was catalyzed by acid.

TABLE 6: Dependence of reaction rate on hydrogen ion concentration at 298K.

[TMGCC] (mol/dm³)	10^3 [Thr] (mol/dm³)	[H^+] (mol/dm³)	10^4 k_{obs} (1/s)
1.0	0.10	0.02	1.875
1.0	0.10	0.03	2.85
1.0	0.10	0.04	3.87
1.0	0.10	0.06	5.55
1.0	0.10	0.10	9.69
1.0	0.10	0.20	18.45

3.9 Solvent effect

The effect of solvent can be described in terms of solvation as solvent plays an important role during reactions. Here, L- threonine's oxidation has been considered in various types of solvents. In all of the selected solvents the same type of kinetics has been observed and the values of second-order rate constants, k_2, have been presented in Table (7).

TABLE 7: Effect of solvent at 298K.

Solvents	K (dm³/mol)	k_2 (1/s)
Chloroform	23.52	4.59
1,2-dichloroethane	24.08	5.43
DCM	23.24	5.28
DMSO	24.36	16.2
Acetophenone	23.80	4.87
DMF	24.50	8.67
Butanone	23.38	3.57
Nitrobenzene	23.94	6.40
Benzene	23.10	1.80
Cyclohexane	24.22	0.19
Toluene	22.82	1.35
Acetophenone	24.64	7.53
Tetrahydrofuran	25.20	2.40
tert-Butyl alcohol	23.38	1.91
1,4-Dioxane	23.66	2.57
1,2-Dimethoxyethane	24.92	1.28
Acetic acid	23.24	0.90
Ethyl acetate	24.78	1.95
Carbon disulphide	24.50	0.68

Kamlet presented equation[18] for the rate constant in terms of linear solvation energy relationship which has been given below (Equation) where the π, α, β (solvatochromic parameters) are characteristic of various solvents.

$$\log k_2 = A_0 + p\pi + a\alpha + b\beta \quad \text{------ (7)}$$

Here π indicates the polarity of solvent (a measure of the ability of solvent to stabilize a charge or dipole due to its dielectric effect), β indicates the hydrogen bond acceptor basicity (ability of the solvent to donate an electron pair or in a hydrogen bond to accept a proton) and α indicates the hydrogen bond donor acidity (where solvent either donate a proton or accept electron pair in hydrogen bond which is in between solute to solvent) and A_0 is the term of intercept. Here the coefficient of determination (r^2), standard deviation (SD), and Exner's statistical parameter[19], (ψ) have been used to correlate analyses. Results thus obtained from parametric equation (7) have been given by the following equations (8-11):

$\log k_2 = -6.81 + (2.61 \pm 0.91)\Pi - (0.16 \pm 0.15)\ \alpha + (0.20 \pm 0.16)\ \beta$ ------ (8)

$r^2 = 0.8754$, SD = 0.16, n= 18, $\psi = 0.40$

$\log k_2 = -6.54 + (2.70 \pm 0.18)\Pi - (0.13 \pm 0.16)\ \beta$ ------ (9)

$r^2 = 0.8572$, SD = 0.17, n= 18, $\psi = 0.41$

$\log k_2 = -6.50 + (2.70 \pm 0.17)\Pi$ ------ (10)

$r^2 = 0.8574$, SD = 0.18, n= 18, $\psi = 0.41$

$\log k_2 = -6.38 + (0.43 \pm 0.35)\ \beta$ ------ (11)

$r^2 = 0.0850$, SD = 0.45, n= 18, $\psi = 0.98$

here, the number of data points considered in analysis has been represented by 'n'.

Swain equation has also been used for the solvent effect examination. Swain equation[20] has given the following data on the concept of cation-anion solvation:

$\log k_2 = aA + bB + C$ ------ (12)

In equation (12), the anion-solvating power of solvent A has been indicated by 'A', the anion-solvating power indicated by 'B', and 'C' is the term of intercept. Solvent polarity is indicated by (A+B). Equation (12) has been used to analyze rates in various solvents.

$\log k_2 = (0.63 \pm 0.02)\ A + (1.72 \pm 0.01)\ B - 4.74$ ------ (13)

$r^2 = 0.9998$, SD = 0.01, n= 19, $\psi = 0.01$

$\log k_2 = (0.38 \pm 0.58)\ A - 3.54$ ------ (14)

$r^2 = 0.0274$, SD = 0.45, n= 19, $\psi = 1.00$

$\log k_2 = (1.67 \pm 0.12)\ B - 4.50$ ------ (15)

$r^2 = 0.9379$, SD = 0.11, n= 19, $\psi = 0.24$

$\log k_2 = 1.36 \pm 0.16\ (A + B) - 4.70$ ------ (16)

$r^2 = 0.8558$, SD = 0.17, n= 19, $\psi = 0.40$

Swain's equation (13) correlation for rates of oxidation of threonine in diverse solvents has given brilliant results. In equation (15) only 'B' parameter gives a major contribution. In equation (16) the term (A+B) shows the solvent polarity parameter and also contributed for ca. 84% of the data.

4 REACTION MECHANISM

A possible mechanism for the oxidation of L- threonine with TMGCC in sulphuric acid media can be given on the basis of other previously reported mechanisms[15]. The absence of free radicals in the respective test ruled out any possibility of intermediate Cr(V) species in the reaction involving Cr(VI) as an oxidant, which involved one-electron transfer.

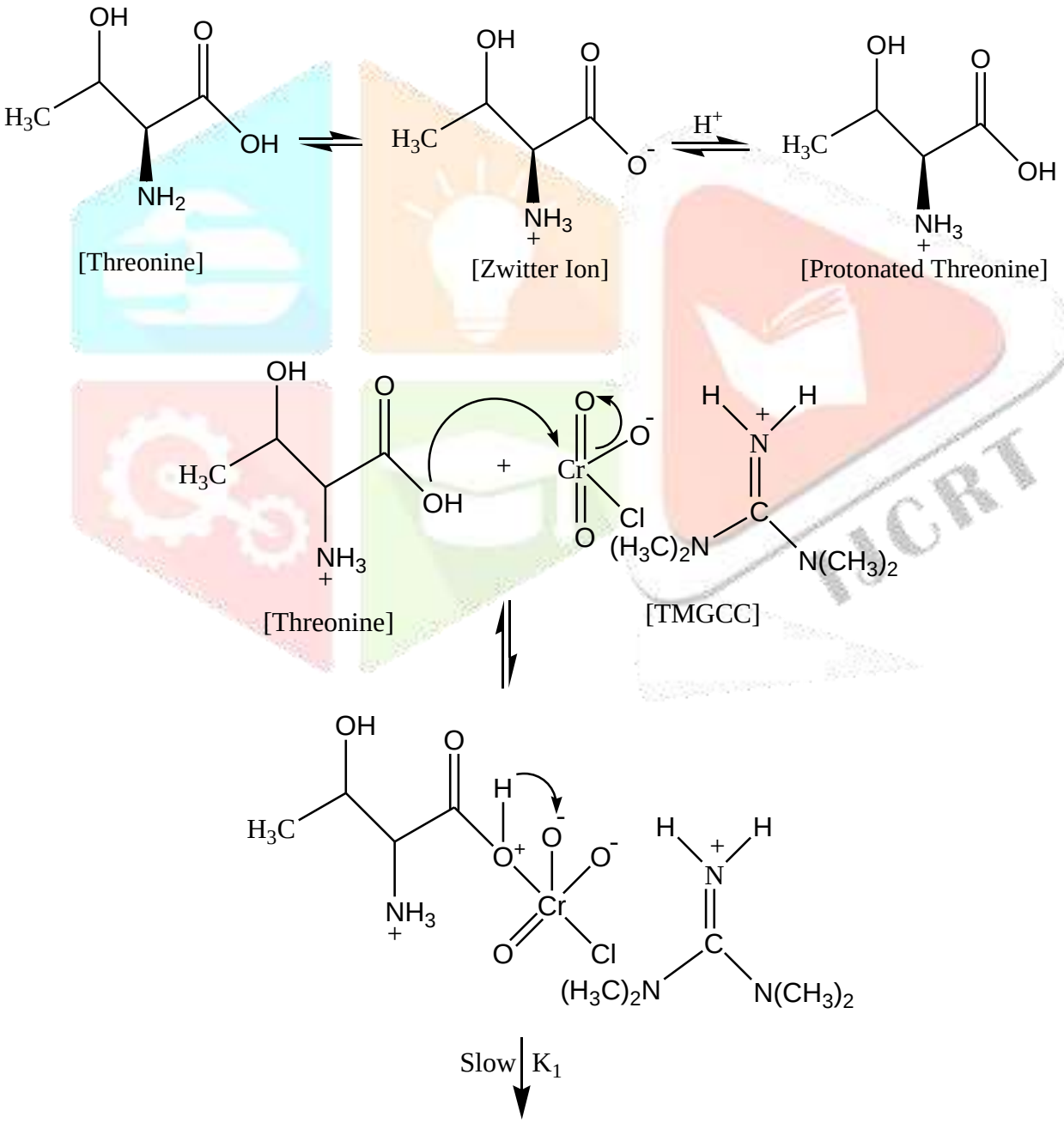
The existence of Amino acids in the form of zwitter ions is known[21,22]. Mainly they tend to protonate in an acidic medium which is in accordance to the below equilibria:

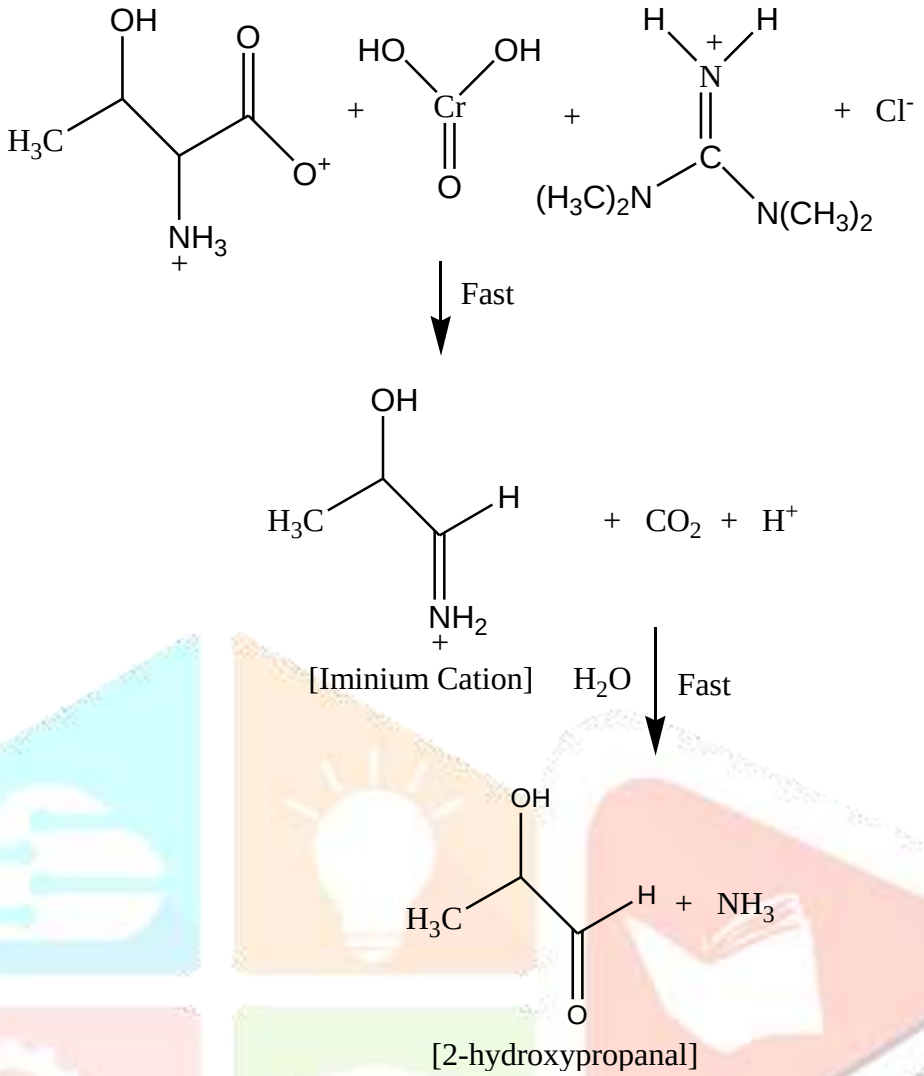
$$Thr + H^+ \rightleftharpoons Thr^+ \quad (17)$$

Also, the fractional-second order rate constant for the concentration of hydrogen ion was elucidated on basis of polar nature of both TMGCC and threonine in acidic media as they are more reactive species which have a major impact on the kinetics of redox reactions.

The 1:1 stoichiometry for TMGCC and L- threonine reaction in H_2SO_4 media, i.e. 1 Thr:1 TMGCC, with a fractional-first order dependence on [Thr] and a first-order dependence on [TMGCC)]. The formation of complex before the slow step supported fractional order dependence of threonine concentration.

The mechanism for the oxidation of threonine by TMGCC in H_2SO_4 medium may be suggested by mechanism (1), which involved a complex formation with fast step between the protonated Threonine and TMGCC leading to the formation of an intermediate complex (C). Further decomposition of the intermediate complex during the slow step, with successive fast steps, gave final products of oxidation.





SCHEME 1: Mechanism for L-threonine's oxidation by TMGCC in H_2SO_4 medium.

5 CONCLUSIONS

The kinetics of L-threonine's oxidation by TMGCC was studied in H_2SO_4 medium, which proceeded through a complex formation. It showed a stoichiometry of 1:1, i.e., a single mole of threonine was used with one mole of TMGCC. The reaction is of first order with respect to TMGCC, fractional first order with respect to threonine, and fractional second order with respect to acid. The final products of the oxidation of threonine were recognized as 2-hydroxypropanal, NH_3 ion with CO_2.

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