



Kinetics and Mechanism of Oxidation of *L*-Cysteine by Bis-3-di-2-pyridylketone-2-thiophenylhydrazone-iron(III) Complex in Acidic Medium

ISMAIL WARAD^{#*}, MOHAMMED AL-NURI[§], MAHER ABU EID[§],
ZEID AL-OTHMAN[#], SAUD AL-RESAYES[#] and NIZAM DIAB

[#]Department of Chemistry
King Saud University P.O Box 2455, Riyadh-11451, Saudi Arabia

[§]Chemistry Department
AN-Najah National University, P.O. Box 7, Nablus, Palestine

Department of Chemistry
Arab American University-Jenin, P.O. Box 240, Jenin, Palestine
warad@ksu.edu.sa

Received 25 February 2010; Accepted 1 May 2010

Abstract: The kinetics of oxidation of *L*-cysteine by 3-di-2-pyridylketone-2-thiophenylhydrazone-iron(III), [Fe(DPKTH)₂]³⁺ complex in acidic medium was studied spectrophotometrically at 36 °C temperature. The molar ratios of DPKTH to iron(III) and iron(II) individually, were found to be [2:1] [DPKTH : iron(III)/(II)]. The reaction was stroked to be first-order with respect to iron(III) and *L*-cysteine, second-order with respected to DPKTH ligand and reversed second-order with respected to hydrogen ion concentration. Added salts did not affect the rate and no free radical was detected when radical detector was placed in the reaction mixture. Ethanol solvent ratio was found to effect both the initial rate and the maximum absorbance (λ_{max}) of [Fe(DPKTH)₂]²⁺ complex. The initial rate rose when the temperature was increased which empowered to calculate the activation parameters. A suitable reaction mechanism was proposed.

Keywords: Iron(II), Kinetics, *L*-cysteine, 3-*Di*-2-pyridylketone-2-thiophenylhydrazone ligand.

Introduction

In recent years, the study of the highest oxidation state of transition metals has become a central topic in the development of inorganic and organometallic chemistry^{1,2}. Transition metals in a higher oxidation state can generally be stabilized by suitable chelation of polydentate ligands to establish the proper complexes. Such of these complexes with ligand

systems containing multidentate nitrogen or/ and phosphine-donor atoms have been used successfully to promote the transformation of organic compounds³⁻⁸. As oxidizing reagents metal chelates complexes such as hexacyanoferrate(III), dihydroxydiperdatonickelate(IV), bisenylendiamine-cobalt(II), chloramines-B and biphenyl-diamineruthenium(II)⁹⁻¹² have been used to oxidize cysteine in a medium with an appropriate pH value. The relation between the structure of the ligands and the physicochemical properties of the corresponding metal complexes has been the subject of many investigations in order to understand and enhance the reactivity as well as find new applications of such of these complexes¹³. Reactions of sulfur-containing amino acids such as *L*-cysteine and *L*-methionine appear to play key roles in the biological chemistry of Pt(II) and Pt(IV), Fe(III) and Ru(III) anticancer agents¹⁴⁻¹⁶. The concentration of the cysteine-containing tripeptide glutathione is elevated in some platinum-resistant cell lines and thiols appear to be involved in the reduction of Pt(IV), Ru(III) and Fe(III) complexes to active Pt(II), Ru(II) and Fe(II) species¹⁴⁻¹⁶.

In this work, $[\text{Fe}(\text{DPKTH})_2]^{3+}$ complex as an oxidizing agent using 3-di-2-pyridylketone-2-thiophenylhydrazone (DPKTH) as nitrogen electrons bidentate-donor ligand was employed to oxidize *L*-cysteine amino acids under mild acidic condition. Herein, we aspired to perform a helpful kinetic study of oxidation of amino acid using Fe^{3+} -DPKTH as new oxidation reagent.

Experimental

Absorbance measurements of the kinetics were performed using AUNICAM UV2_Shimadzu computerized UV-visible single-beam spectrophotometer with a 1 cm length quart cell. It has got vision software and a circulation thermostat water bath (C-85A) to the control temperature. All pH measurements were made using a calibrated HANNA E 18521 pH meter with combined glass electrode. Oxidation product (cystine) was identified as the corresponding amino acid by spot test¹⁷. Computerized Fourier transform infrared spectrophotometer (Shimadzu FTIR 820PC), with Hypride software was used to characterize the product. Standard buffer solutions were made available to standardize the set before any runs have been carried out.

Reagents

All the chemicals used were analytical reagents or of pure grade; distilled water and 95-100% redistilled ethanol were also used. $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ was obtained from Aldrich Company. 1×10^{-2} M was prepared in distilled water and served as stock solution; other concentrations were obtained from it by dilution. The stock solution was sealed in brown bottle to protect it from light and stored in cold place. *L*-cysteine was purchased from Aldrich, solution of 1×10^{-3} M was daily prepared using deionized oxygen removed water. A stock solution of 1×10^{-2} M of DPKTH^{18,19} was prepared using ethanol as solvent. The Buffer sodium acetate and acetic acid buffer system was prepared using the manual²⁰.

Synthesis and procedures

Preparation of nitrogen multi-electrons donor (DPKTH) ligand 3-di-2-pyridylketone-2-thiophenylhydrazone ($\text{C}_{16}\text{H}_{12}\text{N}_4\text{OS}$, DPKTH) was synthesized by treating of 3-di-2-pyridylketone with of 2-thiophenylhydrazone in equivalent amount^{18,19}. All the reactions runs were carried out in the 12 mL quartz cell of the UV-spectrophotometer directly to save the reaction time; care also was taken to exclude walls air bubbles formation. Fixed general procedure was adopted to achieve the best reproducibility, taking in consideration the best sequence of order which found to be: buffer-water (EtOH) solution, DPKTH ligand, Fe^{3+}

followed by *L*-cysteine, respectively. 1 mL of (5×10^{-3} M) DPKTH was added to 7.5 mL of buffer-water solution, 0.5 mL of Fe^{3+} (2×10^{-3} M), the complex was shaken for 1 minute, which provided to be enough for Fe^{3+} -DPKTH complex-formation, finally the mixture was treated with 1 mL of (2×10^{-3} M) *L*-cysteine. The total reaction mixture volume was 10 mL. By this sequence of addition and by employment of suitable acidic medium care was taken to prevent any expected direct reaction between Fe^{3+} and *L*-cysteine. These reagents concentrations were carefully calculated to be fit with the acceptable absorption range through trial and error technique. Together the appearing of green color (Fe^{2+} -DPKTH) formed complex at $\lambda_{\text{max}} = 656$ nm parallel to the disappearing of the yellowish color of (Fe^{3+} -DPKTH) consumed complex at $\lambda_{\text{max}} = 364$ nm indicated the oxidation reaction launching, directly by *L*-cysteine addition.

Results and Discussion

General investigations

Because of poor solubility of DPKTH in water and high solubility in ethanol, the complexation processes between Fe(III) and DPKTH and Fe(II) and DPKTH, respectively were performed individually in water-alcohol medium. The absorption spectra of the products showed $\lambda_{\text{max}} = 364$ nm exhibited the yellowish Fe^{3+} -DPKTH, while $\lambda_{\text{max}} = 656$ nm revealed the green Fe^{2+} -DPKTH complexes formation. When the yellowish complex Fe^{3+} -DPKTH was treated with *L*-cysteine in ethanol-water solvent λ_{max} at 364 nm was granularly disappeared due to reduce of iron(III) to iron(II) which formed the green Fe^{2+} -DPKTH complex with $\lambda_{\text{max}} = 656$ nm. The study of *L*-cysteine oxidation by Fe^{3+} -DPKTH complex has been carried out at 656nm (λ_{max} of Fe^{2+} -DPKTH), selected from the spectra scanned at suitable pH. The rate of reaction was modified to be measurable by suitable hydrogen ions concentration.

The molar ratios at their proper corresponding absorptions maxima of Fe^{3+} -DPKTH, Fe^{2+} -DPKTH complexes and Fe^{3+} -DPKTH complex in the presence of *L*-cysteine individually, have been carried out using job's continuous variation method²⁰ to determine the stoichiometry of the Iron(III)/(II)-DPKTH complexes as well as to predict the real complexes structures formation. The result showed the existence of [1:2] [Iron(III)/(II): DPKTH] respectively as in Figure 1 and 3.

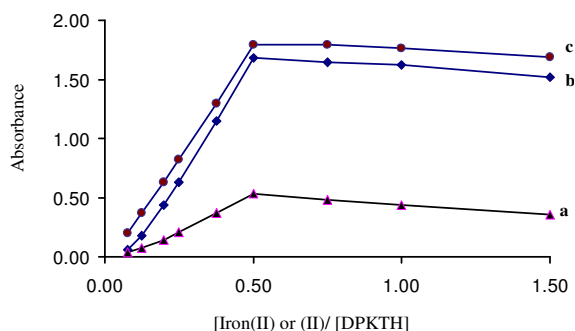


Figure 1. Job's plots to determination the molar ratios at $T = 36$ °C; (a) plot of absorbance vs. $[\text{Fe}^{2+}/\text{DPKTH}]$ at $\lambda_{\text{max}} = 656$ nm, $[\text{DPKTH}] = 2 \times 10^{-4}$ M, $[\text{Fe}^{2+}] = 0.15 - 4.5 \times 10^{-4}$ M, 10% ethanol and $\text{pH} = 4.7$; (b) Plot of absorbance vs. $[\text{Fe}^{3+}/\text{DPKTH}]$ at $\lambda_{\text{max}} = 364$ nm, $[\text{DPKTH}] = 2 \times 10^{-4}$ M, $[\text{Fe}^{3+}] = 0.15 - 4.5 \times 10^{-4}$ M, 10% ethanol and $\text{pH} = 4.9$; (c) Plot of absorbance vs. $[\text{Fe}^{3+}/\text{DPKTH}]$ at $\lambda_{\text{max}} = 656$ nm, $[\text{DPKTH}] = 6 \times 10^{-4}$ M, $[\text{Cysteine}] = 6 \times 10^{-4}$ M, $[\text{Fe}^{3+}] = 0.15 - 4.5 \times 10^{-4}$ M, 10% ethanol and $\text{pH} = 5$

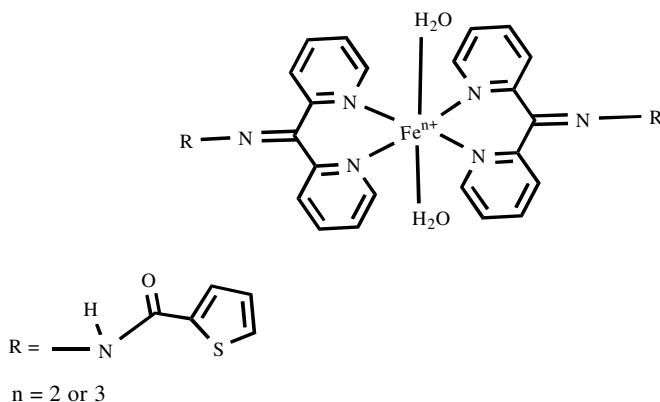


Figure 2. Proposed structure of bis(3-di-2-pyridylketone-2-thiophenylhydrazone) iron(II)/(III) complexes

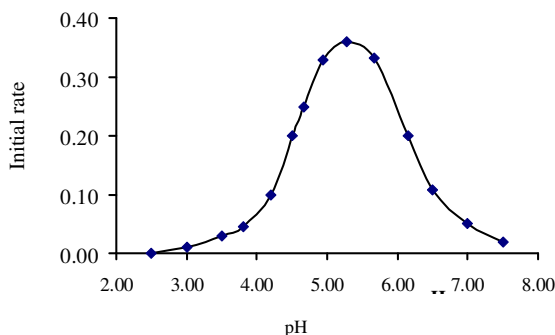


Figure 3. Influence of H^+ concentrations on the reaction to determine the optimum pH condition at $\lambda_{max} = 656 \text{ nm}$, $T = 36 \text{ }^\circ\text{C}$, $[\text{DPKTH}] = 5 \times 10^{-4} \text{ M}$, $[\text{Fe}^{3+}] = 1 \times 10^{-4} \text{ M}$, $[\text{Cysteine}] = 2 \times 10^{-4} \text{ M}$ and 10% ethanol

Optimum conditions

The oxidation rates of *L*-cysteine to cystine using Fe^{3+} -DPKTH complex as an oxidizing agent were influenced by the pH of the buffer medium, reagents concentrations, ionic strength, ethanol ratio and temperature. The optimum conditions of the method were investigated as follows.

Effect of pH on the rate of the reaction

The effect of pH on the initial rate of the reaction was studied at different pH values between 2.2 and 8.0 at $36 \text{ }^\circ\text{C}$ temperature, the optimum pH lies between 4.5 and 5.5 shown in Figure 3. The absorption-time curves of the reaction in different H^+ concentration (0.18 - $1.6 \times 10^{-4} \text{ M}$, 3.8 - 4.7 pH) indicated that the reaction rate was increased by pH increasing (Figure 4). In this pH range the acid acted as inhibitor. A straight linear with slope = -1.94 was recorded (Figures 5) when $\log [\text{initial rate}]$ vs. $\log [H^+]$ was plotted.

Effect of ionic strength

It was observed that no sufficient effect on the initial rate was detected when NaClO_4 as selected salt was employed; the initial rate remained constant with NaClO_4 concentrations were increased even when more of 1000 fold was tried.

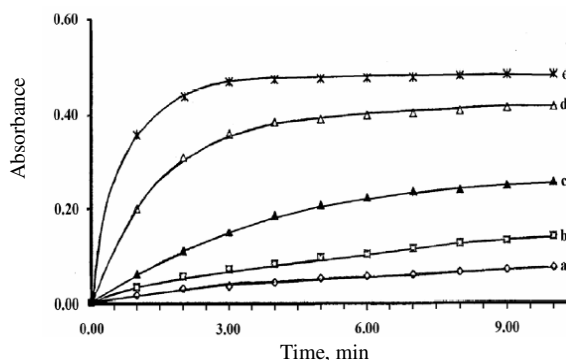


Figure 4. Effect of H^+ concentrations (a-e, $1.6 \times 10^{-4} M$, $1.0 \times 10^{-4} M$, $6.0 \times 10^{-5} M$, $3.2 \times 10^{-5} M$ and $1.8 \times 10^{-5} M$, respectively) on the absorbance of the reaction at $\lambda_{max} = 656 \text{ nm}$, $T = 36 \text{ }^\circ\text{C}$, $[\text{DPKTH}] = 5 \times 10^{-4} M$, $[\text{Fe}^{3+}] = 1 \times 10^{-4} M$, $[\text{Cysteine}] = 2 \times 10^{-4} M$ and 10% ethanol

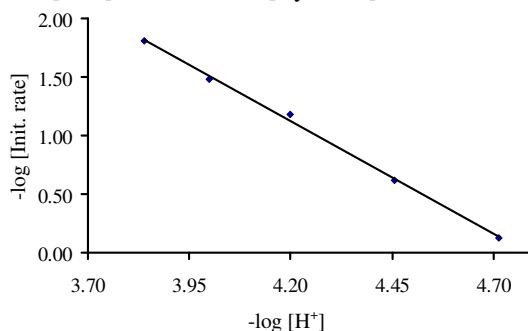


Figure 5. Plot of $\log [\text{Initial rate}]$ vs. $\log [H^+]$ at $\lambda_{max} = 656 \text{ nm}$, $T = 36 \text{ }^\circ\text{C}$, $[\text{DPKTH}] = 5 \times 10^{-4} M$, $[\text{Fe}^{3+}] = 1 \times 10^{-4} M$, $[\text{Cysteine}] = 2 \times 10^{-4} M$, 10% ethanol and $\text{pH} = 3.8 - 4.7$

The dependence of initial rate on the reagents concentration

Effect of iron(III) concentration

At constant temperature $36 \text{ }^\circ\text{C}$ and by fixing all the reaction conditions, initial rate increased by raising the concentration of Fe^{3+} . The plot of $\log [\text{initial rate}]$ vs. $\log [\text{Fe}^{3+}]$ was found to be a straight linear with slope = 0.99 (Figure 6).

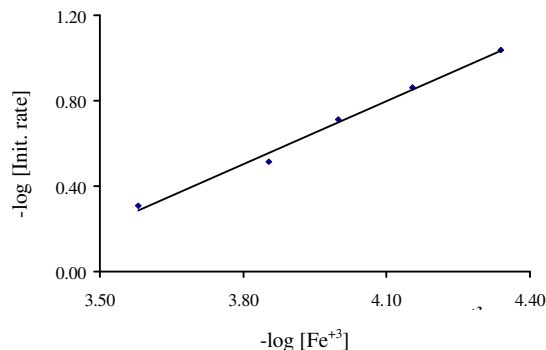


Figure 6. Plot of $\log [\text{Initial rate}]$ vs. $\log [\text{Fe}^{3+}]$ at $\lambda_{max} = 656 \text{ nm}$, $T = 36 \text{ }^\circ\text{C}$, $[\text{DPKTH}] = 5 \times 10^{-4} M$, $[\text{Fe}^{3+}] = 0.15 - 2 \times 10^{-4} M$, $[\text{Cysteine}] = 2 \times 10^{-4} M$, 10% ethanol and $\text{pH} = 5$

Effect of DPKTH ligand concentration

Positive effect of DPKTH concentration on the initial rate was noted and presented in Figure 7. A straight line with slope = 2.02 was obtained when \log [initial rate] was plotted against \log [DPKTH].

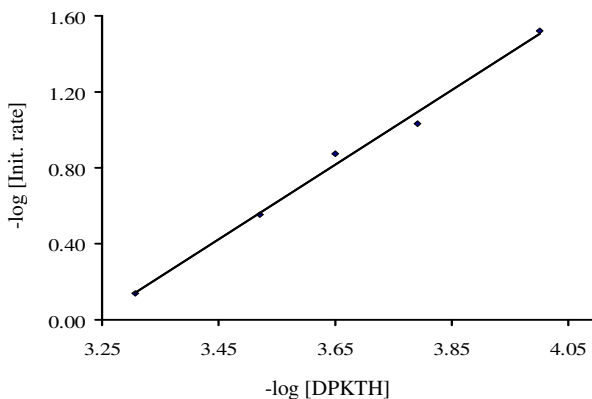


Figure 7. Plot of \log [Initial rate] vs. \log [DPKTH] at $\lambda_{\max} = 656$ nm, $T = 36$ °C, $[\text{Fe}^{3+}] = 1 \times 10^{-4}$ M, $[\text{Cysteine}] = 2 \times 10^{-4}$ M, $[\text{DPKTH}] = 0.5\text{--}2 \times 10^{-4}$ M, 10% ethanol and $\text{pH} = 5$

Effect of L-cysteine concentration

Figure 8 shows an increase in the initial rate when *L*-cysteine concentration was increased; the plot of \log [initial rate] vs. \log [cysteine] revealed a straight linear with slope = 0.98 (Figure 8).

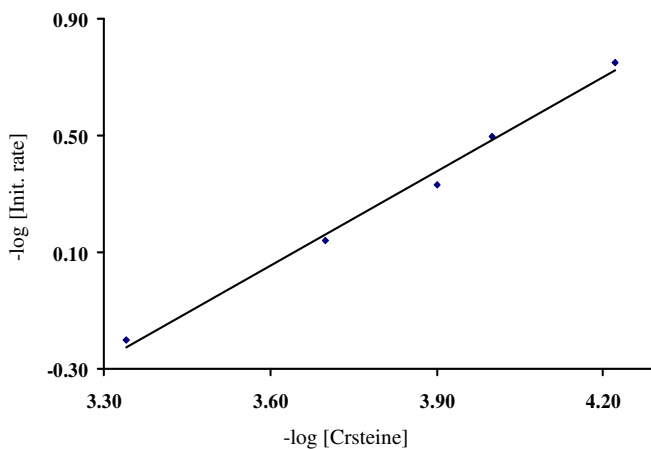


Figure 8. Plot of \log [Initial rate] vs. \log [Cysteine] at $\lambda_{\max} = 656$ nm, $T = 36$ °C, $[\text{Fe}^{3+}] = 1 \times 10^{-4}$ M, $[\text{DPKTH}] = 5 \times 10^{-4}$ M, $[\text{Cysteine}] = 0.6\text{--}4 \times 10^{-4}$ M, 10% ethanol and $\text{pH} = 5$

Effect of ethanol as solvent on the reaction

The effect of ethanol on the initial rate of reaction was studied, it can be seen that the initial rate was increased with increasing in EtOH %. Due to best salve of the ligand the λ_{\max} of belong to Fe^{2+} -DPKTH was shifted positively from 656 nm to 667 nm when ethanol ratios were raised from 10-50% the result are summarized in Table 1.

Table 1. Effect of ethanol % (v/v) on the maximum absorbance (λ_{\max}) of $[\text{Fe}(\text{DPKTH})_2]^{2+}$ complex and initial rate of the reaction at $T = 36^\circ\text{C}$, $[\text{DPKTH}] = 5 \times 10^{-4} \text{ M}$, $[\text{Fe}^{3+}] = 1 \times 10^{-4} \text{ M}$, $[\text{Cysteine}] = 2 \times 10^{-4} \text{ M}$ and $\text{pH} = 5$

EtOH %	Init. rate, M min^{-1}	λ_{\max} , nm
10	0.284	656
15	0.405	657
30	0.526	661
40	0.713	665
50	0.886	667

The dependence of reaction rate on temperature

The dependence of the reaction rate on temperature was investigated between 36 and 64 °C, higher temperatures were not attempted for fear of decomposition of the complex. The reaction initial rate was increased linearly by the temperature increasing, which fulfilled Arrhenius equation and empowered to calculate the activation parameters (Table 2).

Table 2. Activation parameters of the rate-determining step calculated from Arrhenius equation by plotting \ln initial rate vs. $1/T \text{ K}^{-1}$, at $\lambda_{\max} = 656 \text{ nm}$, 10% v/v ethanol, $T = 36\text{-}64^\circ\text{C}$, $[\text{DPKTH}] = 5 \times 10^{-4} \text{ M}$, $[\text{Fe}^{3+}] = 1 \times 10^{-4} \text{ M}$, $[\text{Cysteine}] = 2 \times 10^{-4} \text{ M}$ and $\text{pH} = 3.5$

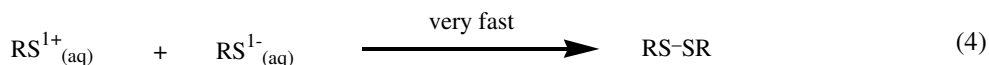
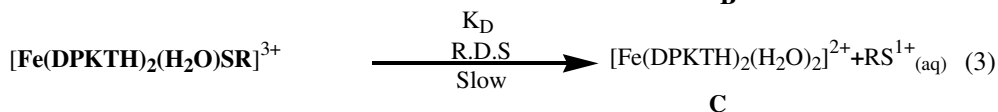
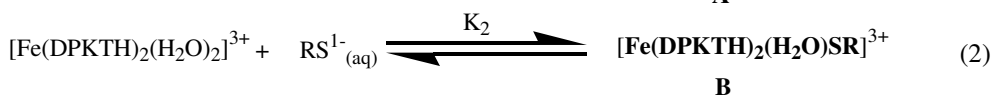
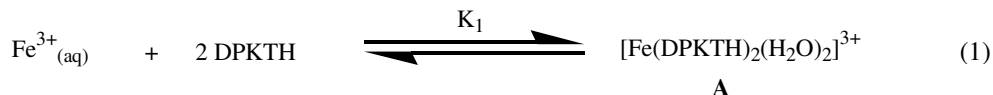
The Activation Parameters	Founded value
Activation Energy (Ea.)*	17.08 kJ/mol
Entropy (ΔS)*	- 182.68 J/mol
Enthalpy (ΔH)*	14.59 kJ/mol
Gibbs Energy (ΔG)*	69.03 kJ/mol
The equilibrium (K)*	$7.96 \times 10^{-13} \text{ kJ/mol}$

Effect of acrylonitrile as free radical detector

The addition of acrylonitrile to the reaction mixture at 36 and 60 °C did not alter the rate and there was absolutely no polymer or change in the viscosity was detected, showing the absence of free radicals in the reaction mechanism.

The suggested mechanism

We made every effort depending on the literature and the above observed result to suggest steps formed the mechanism of this oxidation process which illustrated in Scheme 1.



Scheme 1. The proposed mechanism of *L*-cysteine oxidation process by Fe^{3+} -DPKTH complex

The first step is the formation of $[\text{Fe}(\text{DPKTH})_2]^{3+}$ A [1:2] complex between Fe^{3+} and DPKTH ligand this step was proved stoichiometrically and have been confirmed by separation, solubility and extraction tests. Fe^{3+} concentration was low enough to prevent any dimmers or hydroxyl complexes formation. The second step involved the charge transfer intermediate complex formation $[\text{Fe}(\text{DPKTH})_2\text{SR}]^{2+}$ [1:2:1] B between Fe^{3+} , DPKTH and *L*-cysteine, respectively this step was proved also stoichiometrically. The slowly intermediate B complex decomposition which gave the final complex product $[\text{Fe}(\text{DPKTH})_2]^{2+}$ C was suggested to be the rate-determining step. Two electrons were transferred from the *L*-cysteine anion to form C complex during the charge-transfer intermediate B complex desiccation. This fact was provided by no free radicals effects were detected by acrylonitrile addition. The absorbance of C complex formation by function of time found to be very suitable stoichiometrically. Step four is very fast and provided to form Disulfide Bridge (RS-SR) cystine as final product formation.

Conclusion

Kinetic of oxidation of *L*-cysteine by $[\text{Fe}(\text{DPKTH})_2]^{3+}$ complex was carried out in acidic medium. The differential rate method was found to be suitable for such of these investigations. The method is based on mixing DPKTH ligand with iron(III) to form yellowish Fe^{3+} -DPKTH complex with $\lambda_{\text{max}} = 364$ nm. $[\text{Fe}(\text{DPKTH})_2]^{3+}$ complex was reduced to $[\text{Fe}(\text{DPKTH})_2]^{2+}$ at $\lambda_{\text{max}} = 656$ nm in the presence of *L*-cysteine, this change in the color was followed spectrophotometrically in order to study the kinetic relations. The reaction found to be first-order with respect to iron(III) and *L*-cysteine, second-order with respect to DPKTH ligand and reversed second-order with respect to concentrations hydrogen ion. No salts effect or radical species have been detected. Ethanol solvent ratio was found to increase both the initial rate and the absorbance maxima. The activation parameters of the rate-determining step have been calculated, the mechanism was proposed to contained a pre-equilibrium of an adduct formation between *L*-cysteine and $[\text{Fe}(\text{DPKTH})_2]^{3+}$ complex.

Acknowledgments

Dr. Warad would like to thank the Research Center /Science College /King Saud University for financial support and use of some measurement facilities.

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