Kinetic oxidation of L-cysteine by \([\text{Fe}(\text{Fz})_2]^+\) complex was carried out in acidic medium under pseudo rate conditions. The molar ratios between iron(III), iron(II) and Ferrozine (HFz) complexes in absence and presence of L-cysteine were individually determined using job's method. The formation of \([\text{Fe}^{2+}-\text{Fz}]\) at \(\lambda_{\text{max}} = 562\text{nm}\) was spectrophotometrically followed during this kinetic study. The reaction is found to be first-order with respect to iron(III) and L-cysteine, second-order with respect to HFz\(^{-1}\) ligand and reversed second-order with respect to hydrogen ion concentrations. The salts effect was determined and no radical species have been detected. The \(k_{\text{obs}}\) rose when the temperature was increased which empowered the activation parameters of the rate-determining step calculations. A reaction mechanism and rate law derivation are proposed with a pre-equilibrium of an adduct formation between L-cysteine and \([\text{Fe}^{3+}-\text{Fz}]\) complex.

**Keywords:** Iron(II) complexes, Kinetic determination, Oxidation, L-Cysteine, Ferrozine ligand.

**INTRODUCTION**

Transition-metal complexes with ligand systems containing nitrogen-donor atoms have been used successfully to promote the transformation of organic compounds and also to act as structural mimics of metal centers in enzymes [1-5]. The chemistry of metallacycles using multinitrogen ligand components has received growing interest during recent years, as a result of their involvement in catalytic processes [6-8]. A significant progress in the stabilization, isolation,
and examination of undercoordinated species was achieved by the introduction of multifunctional ligand. Moreover the sterical and electronic properties of these kinds of ligands can be varied in wide range by the employments of different substituents at the coordinated atoms which are in most case phosphorus or nitrogen. Such of these complexes which are in general containing multidentate pure nitrogen or phosphine-donor atoms served as novel catalysts [9-14]. On the other hand it used as good oxidizing reagents in a medium with an appropriate pH value [15-18].

L-Cysteine (C₅H₇NO₂S) as sulfur-containing amino acid is a tri-basic compound, it linked together by peptide bonds to form proteins or it can bind through disulfide bridge to synthesize sulfur-organic compounds such as cystine (RS-RS). It is considered as the primary source of organic sulfur containing compounds in the environment, it can be found in human blood, urine and tissues in very small amounts. Micrograms of L- or D-cysteine are essential to control the daily biochemical bodies activities [15-18]. Due to the presence of multi-atoms (S, O and N) which are ready to coordinate metal center as well as one chiral center in the backbone of L-cysteine compound, it will act as mono or bidentate ligand in coordination chemistry [19-22] as an example see Scheme 1.

Reactions of L-cysteine appears to play key roles in the biological chemistry of Pt(II) and Pt(IV), Fe(III) and Ru(III) anticancer agents. The reigo-binding selectivity of L-cysteine with transition metal ions which affects the chemical and biological properties depends directly on the ionization state of cysteine molecule and the reactions medium. Cysteine can be ionized to different forms [22-24] as in Scheme 2.

Scheme 1: L-Cysteine as bidentate (S, N) ligand as in complex A and monodentate (S or O) ligands as in complexes B and C respectively.

Scheme 2: Cysteine ionization forms.
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) species [19-22].

Many efforts have been constructed to develop what so called the radiolabeled bioactive peptides which found to be attractive vectors for targeting a variety of diseases through interaction with specific cell surface receptors [25,26]. Current approaches make use of tripeptides (such as L-cysteine) that can form chelate complexes with the radiometals [26-29].

In this work, Ferrozine (HFz) has been used as water soluble nitrogen multi-donor ligand. It ionized in water to Fz$^{2-}$ anion. The method is based on mixing HFz ligand with iron(III) to form yellowish Fe$^{3+}$-Fz complex which showed a maximum absorbance at 344 nm. The Fe$^{3+}$-Fz complex in acidic medium served as a good oxidizing agent. Herein, we wish to report a helpful study of reaction kinetics, comparative, mechanism and rate law derivation of L-cysteine oxidation by Fe$^{3+}$-Fz in aqueous acidic medium.

**EXPERIMENTAL**

**Apparatus:**

Absorbance measurements in this study were performed using A UNICAM UV2_Shimadzou computerized UV-visible spectrophotometer with a 1 cm length quartz cell. It has got vision software and a circulation thermostat water bath (C-85A) for temperature control. 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 test [30]. Computerized Fourier transform infrared spectrophotometer (Shimadzou FTIR 820PC), with Hypride software were used to characterize the products. Standard buffer solutions were used 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. FeCl$_3$·6H$_2$O and Ferrozine were obtained from Aldrich Company. $1 \times 10^{-5}$ M of Fe$^{3+}$ solution and $1 \times 10^{-3}$ M of Ferrozine were prepared in distilled-deionized water and used as stock solutions; other concentrations from Fe$^{3+}$ solution were obtained by dilution. The stock solutions were sealed in brown bottles in black cold place. L-cysteine was pursed from Aldrich, solutions of $1 \times 10^{-3}$ M was daily prepared using deionized oxygen-removed water. The sodium acetate and acetic acid buffer system was prepared according to the literature [31].

**Synthesis and procedures:**

The structure of Ferrozine [3-(2-pyridyl)-5,6-bis-(4-phenylsulfonic acid)-1,2,4-triazine] as electrons multitdentate ligand is shown in Scheme 3.

![Scheme 3: The structure of Ferrozine [3-(2-pyridyl)-5,6-bis-(4-phenylsulfonic acid)-1,2,4-triazine] as multifunctional atoms ligand.](image)

The reaction was carried out in the quartz cell of the UV-visible spectrophotometer directly to save the reaction time, care also was taken to exclude walls air bubbles formation. Fixed general procedure was adjusted to get the best reproducibility, taking in consideration the sequence of order which found to be: buffer-water system, Ferrozine ligand, Fe$^{3+}$ followed by L-cysteine respectively. Together by following this sequence of addition and using a suitable acidic medium, absolutely no direct reaction between Fe$^{3+}$ and L-cysteine was observed. The total reaction mixture volume was 10 ml. These reagents concentrations were carefully calculated to be fit with the acceptable absorption range (beer's law) [32].
RESULTS AND DISCUSSION

General investigations under the condition of pseudo-first-order rate, the plots of ln(A∞ - A_t) versus time (min.) for more than 3 half lives of the reaction were found to be linear (with slope 0.99 or 1.98 depending on the reagents studied). The pseudo-first-order rate constants, k_{obs}, were calculated by the least-squares method. The rate constants reported here are the averages of 3 independent runs. Deviations in duplicate determinations are generally less than ± 5%. The complex formation between Fe(III) and HFz was performed in aqueous acidic medium. The absorption spectra of the produced complex showed λ_{max} = 344 nm which revealed [Fe(Fz)_2]_1^+ formation.

When this complex was treated with L-cysteine a blue unstable color was detected by eyes for a very short time (< 1 sec.), this is attributed to [Fe^{3+}-Fz-Cysteine] flash-intermediate formation before it is reduced to the stable new violet color of Fe^{2+}-Fz complex. The study of L-cysteine oxidation by Fe^{3+}-Fz complex was performed spectrophotometrically at λ_{max} = 562 nm of the violet [Fe(Fz)_3]^+ complex, λ_{max} was selected from the spectra scanned at suitable pH. The rate of reaction was modified by suitable hydrogen ions concentration to be measurable. The absorption spectra of [Fe(Fz)_3]^+ complex at λ_{max} = 562 nm as a function of wavelength (nm) at different time periods were illustrated in Figure 1.

![Figure 1: Absorption spectra of the violet [Fe^{2+}-Fz] complex at λ_{max} = 562 nm, t=28°C, [Fe^{2+}] = 3×10^{-4} M, [HFz] = 6×10^{-4} M, pH = 4. (a) after 2 min; (b) after 3 min; (c) after 5 min and (d) after 7 min. from Fe^{2+} addition.](image)

**Complexes stoichiometries:**

The molar ratios of Fe^{3+}-Fz, Fe^{2+}-Fz and Fe^{3+}-Fz in the presence of cysteine have been studied individually, by using job's continuous variation method [33] to determine the stoichiometry of the iron(III)/(II)-Fz complexes.
The result showed the existence of [1:3] [Fe$^{2+}$:Fz], [1:2] [Fe$^{3+}$:Fz] and [1:2:1] [Fe$^{3+}$:Fz:Cys] respectively, the result which summarized in Figure 2 and figure 3 are in agree with the literature [15, 22,34-36].

**Optimum conditions:**

The oxidation rates of L-cysteine to cystine using Fe$^{3+}$-Fz 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 2.5 and 7.0 at 28 °C as shown in Figure 4. The optimum pH lied between 3.9 and 4.1. The absorption-time curves of the reaction in different H$^+$ concentration (0.1-3.1 × 10^{-4} M, 2.5-4.0 pH) indicated that the reaction rate was increased by increasing pH. The acids acted as inhibitor in this pH range the best straight line was recorded when 1/k$^{obs}$ vs. [H$^+$]$^2$ was plotted (Figures 5).

Effect of ionic strength:

The effect of NaClO$_4$ concentration on the rate of the reaction was studied and the result is presented in Table 1 and Figure 6. It can be seen that the k$^{obs}$ values decreased with increasing the [NaClO$_4$]. Plot of - ln k$^{obs}$ vs. [(I)$^{1/2}$] / (1+ (I)$^{1/2}$] was linear with a slope = -2.27.

![Figure 2: Job's plots to determination the molar ratios at t = 28 °C, a) Plot of absorbance vs. [Fe$^{2+}$/HFz] at $\lambda$$_{max}$ = 562 nm, [HFz] = 3 × 10^{-4} M, [Fe$^{2+}$] = 0.1 - 4.0 × 10^{-4} M and pH = 2.50; b) Plot of absorbance vs. [Fe$^{3+}$/HFz] at $\lambda$$_{max}$ = 344 nm, [HFz] = 3 × 10^{-4} M, [Fe$^{3+}$] = 0.10 - 3.0 × 10^{-4} M and pH = 2.50.](image)
Figure 3: Job’s plots to determine the molar ratios at $t = 28^\circ C$; a) Plot of absorbance vs. [$Fe^{3+}$] at $\lambda_{\text{max}} = 562\text{ nm}$, $\text{pH} = 2.50$, [$HFz^-] = 3 \times 10^{-4}$ M, [Cysteine] = $1.5 \times 10^{-4}$ M, and [$Fe^{3+}] = 0.1 - 4.0 \times 10^{-4}$ M; b) Plot of absorbance vs. [Cysteine] at $\lambda_{\text{max}} = 344\text{ nm}$ pH = 2.50, [$HFz^-] = 3 \times 10^{-4}$ M, [$Fe^{3+}] = 1.5 \times 10^{-4}$ M and [Cysteine] = $0.10 - 3.0 \times 10^{-4}$ M.

Figure 4: Influence of $H^+$ concentrations on the initial rate at $\lambda_{\text{max}} = 562$ nm, $t = 28^\circ C$, [$HFz^-] = 3 \times 10^{-4}$ M, [$Fe^{3+}] = 5 \times 10^{-5}$ M and [Cysteine] = $1 \times 10^{-4}$ M.
Figure 5: Plot of $1/k_{obs}$ vs. $[H^+]^2$ at $\lambda_{max} = 562$ nm, $t = 28$ °C, $[HF_2^-] = 3 \times 10^{-4}$ M, $[Fe^{3+}] = 5 \times 10^{-5}$ M, [Cysteine] = 1 $\times$ 10$^{-4}$ M.

Figure 6: Plot of $-\ln k_{obs}$ vs. $[(I)^{1/2}/(1 + (I)^{1/2})$ at $\lambda_{max} = 562$ nm, $t = 28$ °C, $[HF_2^-] = 3 \times 10^{-4}$ M, $[Fe^{3+}] = 5 \times 10^{-5}$ M, [Cysteine] = 1 $\times$ 10$^{-4}$ M and pH = 2.80.
The dependence of the rate on the reagents concentrations:

**Effect of Iron(III) concentration**

At constant temperature 28 °C, and by fixing all the other reaction conditions, the rate increased by raising the concentration of Fe$^{3+}$. The plot of $1/k_{obs}$ vs. $1/[Fe^{3+}]$ revealed the best straight line (Figure 7).

**Effect of ferrozine ligand concentration**

The effect of HFz$^{-}$ concentration on the rate of the reaction mixture was presented in Figure 8. A straight line was exhibited when $1/k_{obs}$ was plotted against $[HFz]^{2}$.

**Effect of L-Cysteine concentration**

Figure 9 showed an increase in the rate when L-cysteine concentration was increased; the plot of $1/k_{obs}$ vs. $1/[Cysteine]$ gave a straight line (Figure 9).

Table 1: The effect of ionic strength on the reaction at $\lambda_{max} = 562$ nm, $t = 28$ °C, $[HFz] = 3 \times 10^{-4}$ M, $[Fe^{3+}] = 5 \times 10^{-5}$ M, $[Cysteine] = 1 \times 10^{-4}$ M and pH = 2.80.

<table>
<thead>
<tr>
<th>[NaClO$_4$] / M</th>
<th>I / M</th>
<th>(I)$^{1/2}$</th>
<th>$[(I)^{1/2}/(1+(I)^{1/2})]$</th>
<th>$k_{obs}$ (min)$^{-1}$</th>
<th>- ln $k_{obs}$</th>
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<tr>
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</tr>
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<td>0.046</td>
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<td>1.898</td>
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</tbody>
</table>

**Figure 7:** Plot of $1/k_{obs}$ vs. $1/[Fe^{3+}]$ at $\lambda_{max} = 562$ nm, $[HFz] = 5 \times 10^{-4}$ M, $[Cysteine] = 1 \times 10^{-4}$ M, $t = 28$ °C and pH = 2.50.
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Figure 8: Plot of $1/k_{\text{obs}}$ vs. $1/[HFz^2]$ at $\lambda_{\text{max}} = 562$ nm, $[Fe^{3+}] = 1 \times 10^{-4}$ M, [Cysteine] = $1 \times 10^{-4}$ M, $t = 28 ^\circ C$ and pH = 2.70.

Figure 9: Plot of $1/k_{\text{obs}}$ vs. $1/[Cysteine]$ at $\lambda_{\text{max}} = 562$ nm, $[HFz^2] = 3 \times 10^{-4}$ M, $[Fe^{3+}] = 1 \times 10^{-4}$ M, $t = 28 ^\circ C$ and pH = 2.70.
The Dependence of reaction rate on temperature:

The dependence of the reaction rate on temperature was investigated between 32 °C and 68 °C, to control the reaction rate at such high temperature in acidic medium (pH = 2.1). The values of $k_{obs}$, were increased linearly by increasing temperature in the above temperature range (Table 2). The data fulfilled Arrhenius equation and allowed us to calculate the thermodynamic parameters (Table 3 and Figure 10).

Table 2: The effect of temperature on the rate of the reaction.

<table>
<thead>
<tr>
<th>Temp. °C</th>
<th>Temp. K</th>
<th>$1 \times 10^3/ \text{T (K}^{-1})$</th>
<th>$k_{obs}$ (min)$^{-1}$</th>
<th>$- \ln k_{obs}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>68</td>
<td>341</td>
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<td>0.097</td>
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<td>62</td>
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<td>0.063</td>
<td>2.757</td>
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<td>42</td>
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<td>0.047</td>
<td>3.051</td>
</tr>
<tr>
<td>32</td>
<td>305</td>
<td>3.279</td>
<td>0.035</td>
<td>3.352</td>
</tr>
</tbody>
</table>

Figure 10: Plot of $- \ln k_{obs}$ vs. $1 / \text{T (K}^{-1}$ at $\lambda_{max} = 562$ nm, $[\text{HFz}^-] = 3 \times 10^{-4}$ M, $[\text{Fe}^{3+}] = 5 \times 10^{-3}$ M, [Cysteine] = $1 \times 10^{-4}$ M, $I = 0.05$ M and pH = 2.05.
Effect of acrylonitrile as free radical scavenger:
The addition of acrylonitrile to the reaction mixture at 36°C and 60°C did not alter the rate and there was absolutely no polymer detection, showing the absence of free radicals in the reaction mechanism.

The proposed mechanism:
We made every effort depending on the literature and the kinetic observed result to suggest steps illustrated in Scheme 4 formed the mechanism of this oxidation process.

\[ \text{Fe}^{3+} \text{(aq)} + 2\text{HFz} \xrightarrow{K_1} [\text{Fe(HFz)}]_2^{1+} + 2\text{H}^+ \quad (1) \]
\[ [\text{Fe(HFz)}]_2^{1+} + \text{RS}^{1-} \xrightarrow{K_2} [\text{Fe(HFz)SH}]^{2-} \quad (2) \]
\[ [\text{Fe(HFz)SH}]^{2-} \xrightarrow{K_D, S \text{ slow}} [\text{Fe(HFz)S}]^{3+} + \text{RS}^{1+} \quad (3) \]
\[ [\text{Fe(HFz)S}]^{3+} + [\text{Fe(HFz)}]^{1+} \xrightarrow{\text{very fast}} 2[\text{Fe(HFz)}]^{2+} \quad (4) \]
\[ [\text{Fe(HFz)}]^{2+} + \text{Fz}^{2-} \xrightarrow{\text{very fast}} [\text{Fe(HFz)}]^4^{\text{e}} \quad (5) \]
\[ \text{RS}^{1+} \text{(aq)} + \text{RS}^{1-} \text{(aq)} \xrightarrow{\text{very fast}} \text{RS} - \text{SR} \quad (6) \]

Scheme 4: The proposed mechanism of L-cysteine oxidation processes by [Fe\textsuperscript{3+}-HFz] A complex.

The first step is the formation of [Fe(HFz)]\textsuperscript{2+} (A) [1:2] complex between Fe\textsuperscript{3+} and HFz ligand. This step was proved stoichiometrically and has been confirmed by separation, solubility and extraction tests. The concentration of Fe\textsuperscript{3+} was low enough to prevent any dimers or hydroxyls complexes formation. The second step involved the flash charge-transfer complex [Fe(HFz)SR]\textsuperscript{2-} formation [1:2:1] B, between Fe\textsuperscript{3+}, Fz and Cysteine respectively. This step was proved also stoichiometrically and provided to be an intermediate. The intermediate B complex in step three decomposed slowly to give the RS\textsuperscript{1+} and complex product [Fe(HFz)]\textsuperscript{3+} C. The charges transfer steps in this mechanism were provided by no free radical detections. The absorbance of C complex formation at \(\lambda\text{max} = 562\) nm by function of time has been followed spectrophotometrically in this study. Step six was very fast and provided the disulfide bridge formation (cystine).

Derivation of the rate law:
The following equations have been suggested to derive the rate law of this oxidation process.

\[ K_1 = [\text{A}[\text{H}^+]^2]/[\text{Fe}^{3+}][\text{HFz}]^2 \quad (1) \]
\[ [\text{A}] = K_k[\text{Fe}^{3+}][\text{HFz}]^2/\text{[H}^+]^2 \quad (2) \]
\[ [\text{B}] = K_k[\text{A}][\text{RS}^-] \quad (3) \]
\[ [\text{A}] = K_k[\text{Fe}^{3+}][\text{HFz}]^2 - K_k[\text{A}][\text{RS}^-] \quad (4) \]
Rate = $k_{\text{obs}}[\text{Fe}^{3+}]$  
(5)

The rate of the reaction is the rate of the slowest step.

Rate = $k_0[B]$  
(6)

Rate = $k_0K_2[A][RS']$  
(7)

$$= k_0K_2[RS'] \text{Fe}^{3+} K_1[HFz]^2/[H^+]^2$$

$$+ K_1[HFz]^2 + K_1K_2[HFz]^{-1}[RS']$$  
(8)

$$k_{\text{obs}} = k_0 K_2 [RS'] K_1[HFz]^2/[H^+]^2 + K_1 K_2[HFz]^{-1}[RS']$$  
(9)

$$1/k_{\text{obs}} = \frac{1}{[RS']} \frac{1}{k_0 K_2} \frac{1}{K_1[HFz]^2} + \frac{1}{k_0}$$  
(10)

$$1/k_{\text{obs}} = \left[ \frac{[H^+]}{[RS']} \right] + \frac{1}{k_0 K_2 [RS']}$$  
(11)

$$1/k_{\text{obs}} = \left[ \frac{[H^+]}{[RS']} \right] + \frac{1}{k_0 K_2 [RS']}$$  
(12)

1) A plot of $1/k_{\text{obs}}$ vs. $1/[RS']$ as in equation (11) was linear with a slope $\frac{1}{k_0 K_2 [RS']}$ and an intercept $= \frac{1}{k_0}$. From Figure (9) $k_0$ was calculated, it equal 0.024 (min)$^{-1}$.

2) A plot of $1/[HFz]^2$ as in equation (12) was linear with a slope $\frac{1}{k_0 K_2 [RS']}$. From Figure (8) $K_2$ and $k_0$ were calculated and it equal 140 and 14.80 respectively.

**Conclusion:**

Complex $[\text{Fe(Fz)}_2]^{+}$ has been used as an oxidizing agent in the kinetic oxidation of L-cysteine study in acidic medium under pseudo rate conditions. The molar ratios between iron(III), iron(II) and HFz complexes in absence and presence of L-cysteine were determined. The reaction was found to be first-order with respect to iron(III), second-order with respect to HFz ligand and negative second-order with respect to hydrogen ion concentration. The salts affected the reaction rate while no radical species have been detected when free radical detector was added. The rate-determining step was suggested and the activation parameters were calculated using Arrhenius equation. With a pre-equilibrium of an adduct formation between L-cysteine and $[\text{Fe}^{3+}-\text{Fz}]$ complex a mechanism and rate law derivation were proposed.

**Acknowledgments:**

Dr. I. Warad wishes to thank An-Najah National University Palestine for allowing the use of chemistry department labs and instruments. The referees for their important notes, Dr. S. AlResayes for his continuous help.

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