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Stoichiometry, Kinetics and Mechanism of Oxidation of L - Cysteine by Hexacyanoferrate (III) In Acidic Media

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سرعة وميكانيكية أكسدة ل . سيستين بواسطة حديدي سيانيد البوتاسيوم في وسط حامضي

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ABSTRACT

The Stoichiometry, kinetics, and mechanism of oxidation of L-cysteine by hexacyanoferrate (III) in acidic media at constant ionic strength have been studied spectrophotometrically. A 1:1 L-cysteine : hexacyanoferrate (III) has been established with L-cysteine as the only product . The reaction is zero order in hexacyanoferrate (III), first order in L-cysteine and inverse first in hydrogen ion concentration . The activation parameters were evaluated in the temperature range 15-40°C ($Ea.^{\#}$ = 28.2 kJ/mol . $\Delta S^{\#}$ = -192.4 J/mol.deg.). The oxidation was inhibited in the presence of added hexacyanoferrate (II). The effects of other added salts and composition of the solvent have been rationalized . Two mechanisms are offered to explain the kinetic data .

ملخـــص

ميكانيكية أكسدة السيستين بواسطة حديدي سيانيد البوتاسيوم في وسط حامضي .

تتناول هذه الدراسة ميكانيكية أكسدة الحامض الأميني سيستين (Cysteine) بواسطة مركب حديدي سيانيد البوتاسيوم في وسط حامضي على درجة أيونية ثابتة وذلك بمتابعة طيف $[K_3Fe(CN)_6]$ في المنطقة المرئية للضوء (Vis) .

من نتائج البحث أن نسبة العامل المؤكسد للحامض الأميني تساوي ١ : ١ كما تبين أن معدل التفاعل يعتمد على الدرجة الأولى لتركيز السيستين بينما لا يعتمد على تركيز العامل المؤكسد. كذلك اعتمد معدل التفاعل على مقلوب تركيز أيون الهيدروجين في وسط التفاعل .

تناولت هذه الدراسة أيضا اعتماد معدل التفاعل على عوامل مختلفة مثل درجة الحرارة واضافة بعض الأملاح للتفاعل . وقد تم حساب قيم كل من $Ea^{\#}$ و $\Delta S^{\#}$.

هذا وقد تم اقتراح ميكانيكية للتفاعل تتناسب مع النتائج المخبرية .

Introduction

Electron-rich organic compounds such as hydrazine[1], arylhydrazine[2], trialkylamine[3,4], aminoacids[5-10] alkyl and arylthiols[11-13] and benzoins[14-17] have been known to be oxidized by hexacyanoferrate (III) by a one-electron process. The oxidation of L-cysteine by a number of oxidizing agents such as chromium (VI)[18,19] , chloramine-B[20], chloramine-T and dichloramine- T (21,22), have been reported by several workers. In this paper a detailed investigation of the oxidation of the L-cysteine by hexacyanoferrate (III) in acidic medium is reported.

Experimental

Materials - potassium hexacyanoferrate (III) , Potassium hydrogen phthalate, hydrochloric acid, sodium chloride, potassium chloride, potassium bromide, potassium fluoride, potassium iodide, potassium chlorate, potassium iodate, and ammonium chloride were purchased from Aldrich Chemical Company in the purest form (gold labeled) and were not further purified. Potassium hydrogen phthalate and hydrochloric acid were used to prepare buffer solutions (pH 2.00-3.00). Doubly distilled water was used throughout .

Kinetics - The reaction rate was followed by monitoring the absorbance of hexacyanoferrae (III) at 414 nm with time. The required amounts of L-cysteine and hexacyanoferrae (III) were dissolved separately in thermostated portions of the buffer in separate flasks. Potassium chloride was added to adjust he ionic strength of the reaction mixture to the required value. The contents of the flasks were mixed, then a small portion of the reaction mixture was pipetted into a 1 cm spectrophotometer cell. The cell was then stoppered and returned to the sample compartment of the SP 8-100 UV recording spectrophotometer for continuous measurements of absorbance vs time. In a similar manner the effect of each of added hexacyanoferrate

(II) and other salts on the rate of the reaction was studied with adjusting the amount of buffer solution to keep the total volume constant. In the case of ethanol , the reactants were dissolved in a buffer solution already containing the desired ethanol concentration .

Stoichiometry - A reaction mixture containing five fold excess of hexacyanoferrate (III) over L-cystiene at pH 5.00 and constant ionic strength was allowed to stand for 96 hrs to ensure the completion of the reaction . The molar stoichiometry, L-cysteine : hexacyanoferrate (III) was calculated spectrophotometrically. The L-cystine product was identified qualitatively by its melting point, 260°C (lit. mp 260°C [23]) and its IR spectrum (which compared band for band with that of a pure sample of L-cystine).

Measurements - All spectra and spectrophotometric measurements were obtained using a Pye Unicam SP 8-100 UV-VIS spectrophotometer equipped with 1 cm cells and a thermostated cell holder, which in turn was connected to a circulating constant-temperature water bath. IR spectra were recorded on a Pye Unicam SP 200 infrared spectrophotometer. All pH measurements were made with a Fisher Accumet P model 230A pH/Ion Meter .

Results

The results with percentage error less than 10% were accepted and those with higher percentage error were rejected .

Stoichiometry - The experments carried out to establish the stoichiometry of the hexacyanoferrate (III) oxidation of L-cysteine at acidic pH showed that consumption of each mole of L-cysteine was accompanied by reaction of one mole of hexacyanoferrate (III).L-cystine was identified as the oxidation product, thus the overall reaction stoichiometry may be written as :

$$2\ HOOCCH(NH_2)CH_2SH + 2\ Fe(CN)_6^{-3}$$

$$[\ HOOCCH(NH_2)CH_2S]2 + 2HFe(CN)_6^{-3}$$

Effect of varying reactants - The hexacyanoferrate (III) oxidation of L-cysteine was investigated at several initial concentrations of the oxidant. The log (initial rate versus log $[Fe(CN)_6^{-3}]$ plot (Fig. 1) was found to be linear with a zero-slope indicating zero-order dependence of the rate on $[Fe(CN)_6^{-3}]$.

Plots of log(initial rate) versus log [L-cysteine] or log(initial rate) versus log [H+] show slope values of 1 and -1, respectively (Fig. 2 and 3). Therefore, it appears that the order in L-cysteine and H+ is first and inverse first respectively .

Addition of hexacyanoferrate (II) showed a positive effect on the rate up to 1.0×10^{-3} M $Fe(CN)_6^{-4}$. After the concentration of $Fe(CN)_6^{-4}$ exceeded that of $Fe(CN)_6^{-3}$, a remarkable decrease in the rate of reaction was observed (Fig. 4).

The effect of ionic strength was studied by investigating the rate in the presence of different amounts of sodium chloride. A very small positive effect was observed (e.g.on addition of 0.25, 0.50, 1.00 and 1.50×10^{-3} M NaCl the values of K_{obs} were obtained as 0.51, 0.52, 0.55 and 0.58×10^{-3} Ms^{-1} respectively at 17°C and pH = 3.0.

The results of the effect of varying anions and cations of the added salts have also been shown in figure 5 and 6 respectively. The rate of reaction increased in the order $F^- >> Br^- \geq ClO_3^- \geq I^- >> IO_3^-$.

However, changing the cation from NH_4^+ to Na^+ to K^+ showed negligible effects on the rate.

The rate dependence on solvent composition was investigated by varying ethanol/water ratio. A steady decrease in the rate of reaction with increasing the percentage of ethanol was observed which indicate a significant effect of dielectric constant of the reaction medium (Table 1).

Table (I)

Effect of varying ethanol percentage :

% Ethanol	Init. rate x 10^3 M.sec $^{-1}$	K_{obs} x 10^3 M. sec $^{-1}$
0.0	3.4	1.70
20	3.0	1.50
30	2.4	1.20
40	1.7	1.35
50	1.5	1.25

[Cysteinel] = 2.0 x 10^{-3}M; [$Fe(CN)_6^{-3}$] 1.0 x 10^{-3} M ; pH = 3.0; μ = 0.08; temperature 26° C .

Effect of temperature- The kinetic data for the reaction were obtained at 18.0, 23.0, 26.0, 33.0 and 38.0°C. The values of the activation parameters calculated from Arrhenius plot were found to be 28.2 KJ/mol, 192.5 J/mol. deg., 25.6 KJ/mol , and 83.3 KJ/mol for $E^{\#}_A$, $\Delta S^{\#}$, $\Delta H^{\#}$ and $\Delta G^{\#}$ respectively.

Discussion

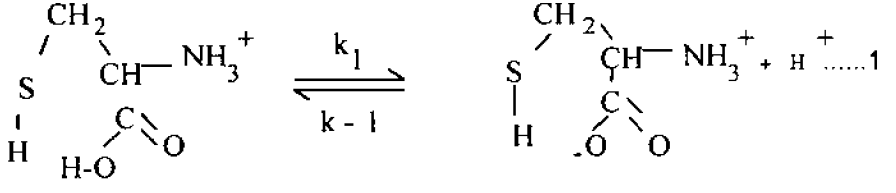
Kinetic results obtained point out that the rate of oxidation of L-cysteine by hexacyanoferrate (III) follow a zero order dependence in $Fe(CN)_6^{-3}$, first order dependence in L-cysteine and an inverse first order dependence in hydrogen ion . Thus the rate law is in the form of .

$$- d[Fe(CN)_6^{-3}]/dt = k_{obs} [L\text{-cysteine}]/[H^+]$$

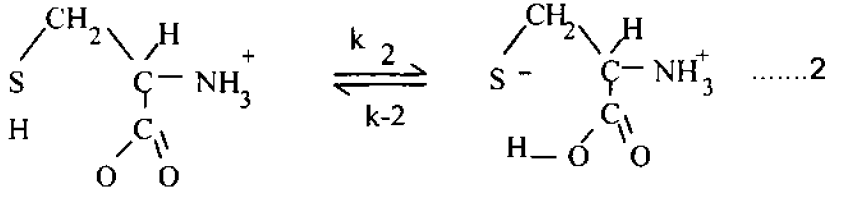
A zero order dependence in $Fe(CH)_6^{-3}$ means that the abstraction of an electron from L-cysteine by hexacyanoferrate (III) is not involved in the rate determining step. This assumption is supported by the lack of remarkable cationic salt effect in our experimental results [24-28]. L-cysteine exists as zwitter ion

$OOCCH(NH_3^+)CH_2SH$ in aqueous solution up to pH =1.0 [10]. Abstraction of an electron from an RS- species is known to be much easier than from RSH form [8,9], so we believe that L-cysteine will exist in the form of thiyl anion before it reacts with hexacyanoferrate (III). Furtheremore formation of the thiyl anion is involved in the rate determining step . Hence it is safe to assume that the slow step involves an intramolecular transfer of H- from sulfur to oxygen atom via a six-membered cyclic transition state. The calculated $\Delta E^{\#}$ = 28.2 K.J/mol (in the order of the value known for H-bonding [29] and the large negative value for $\Delta S^{\#}$= -192.5 J/mol.deg. (an indication of the existence of a rigid transition state) Support the above assumption.

A mechanism that agrees with our findings is shown in the following scheme .

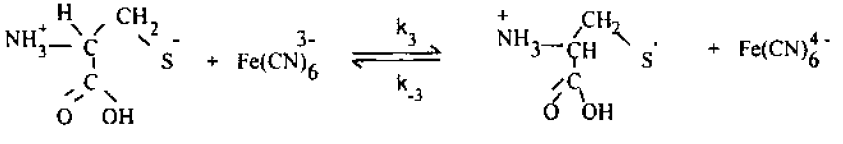


.....1



.....2

[III]

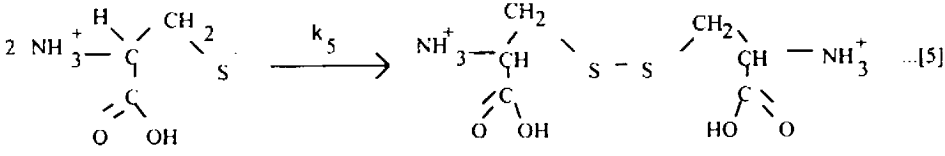


[IV] 3

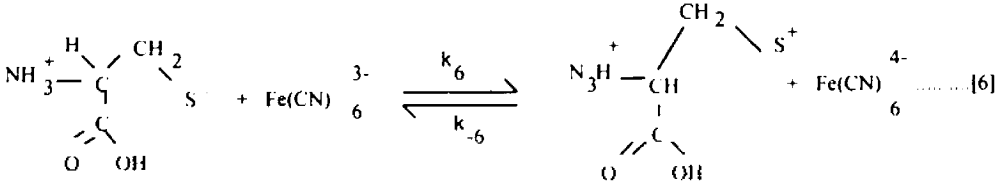
The formation of the product may occur through two different pathways; A and B similar to thiols oxidation [30,13]

Path (A):

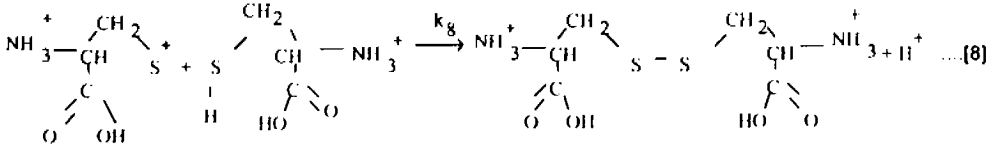
$$Fe(CN)_6^{4-} + H^+ \xrightarrow{k_4} HFe(CN)_6^{3-} \quad .....[4]$$



Path (B):



$$Fe(CN)_6^{4-} + H^+ \xrightarrow{k_7} HFe(CN)_6^{3-} \quad .....[7]$$



Both . path A and B are consistent with the found.stoichiometry of the reaction. but considering the low chance for the combination of two radicals in dilute solution and the relative ease of abstraction of another electron from the thiyl radical by hexacyanoferrate (III). it is believed that path B is more probable although path A is not totally rejected. The negative charge on sulfur atom in intermediate (III) or the higher polarity of (III) compared with (II) explains the lowering in the rate value observed on increasing ethanol percentage in the reaction medium (lower dielectric constant). The relative order in which the type of anion increases the reaction rate. F->> Br- $\geq ClO^-$ >> IO_3^-. which follows their base strength order is also explained by step (2).

The above mechanism enables us to write the rate equation of the reaction as follows .

$$-d[Fe(CN)_6^{-3}]/dt = \frac{Kk_2[L-Cysteine]}{[H+]}$$

where $K = k_1/k\text{-}1$

However two points stand against the above mechanism . First the mechanism does not give a satisfactory explanation for the sudden change in the behavior of the reaction rate when added $Fe(CH)_6^{-4}$ concentration exceeds $1x10^{-3}$ M . Second , the proposed rate- limiting step is an intramolecular proton transfer from S to O (transformation of II to III) which, one expects to be rapid. So another possible mechanism is suggested in which the electron abstraction form S atom by the oxidant precedes the proton transfer.

$$I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} II + H^+$$

$$II + Fe(CN)_6^{3-} \underset{k'_{-2}}{\overset{k'_2}{\rightleftharpoons}} \underset{A}{HS - CH_2CH(NH)_3COO^-} + Fe(CN)_6^{4-}$$

$$A \underset{\text{slow}}{\overset{k'_3}{\rightleftharpoons}} IV$$

where I, II, VI are the same as before .

The subsequent steps are the same as before as in the first mechanism, the proton transfer in this mechanism is not necessarily an intermolecular transfer.

The above mechanism enables us to derive the following rate equation .

$$\frac{-d[Fe(CN)_6^{3-}]}{dt} = K_{obs}[L-\text{cysteine}]/[H^+]$$

$$\text{where } K_{obs} = k_3K_1K_2\frac{\left[Fe(CN)_6^{3-}\right]}{\left[Fe(CN)_6^{4-}\right]}$$

$$\text{and } K_1 = \frac{k_1}{k_{-1}} \ , K_2 = \frac{k'_2}{k'_{-2}}$$

In any individual experiment $\frac{\left[Fe(CN)_6^{3-}\right]}{\left[Fe(CN)_6^{4-}\right]}$ is constant .

The formation of the free radical intermediate was detected using acrylonitrile.

However when added $\left[Fe(CN)_6^{4-}\right]$ is varied from one experiment to another the overall rate constant will change .

Both derived rate laws clearly point out that the rate of oxidation of L-cysteine by hexacyanoferrate (III) will follow a first order dependence in L-cysteine and an inverse first order dependence in hydrogen ion but independent of hexacyanoferrate (III).

Thus $Fe(CN)_6^{3-}$ is a proper oxidizing agent to cysteine as the reaction is clean, fast and goes to completion and can easily be studied spectrophotometrically.

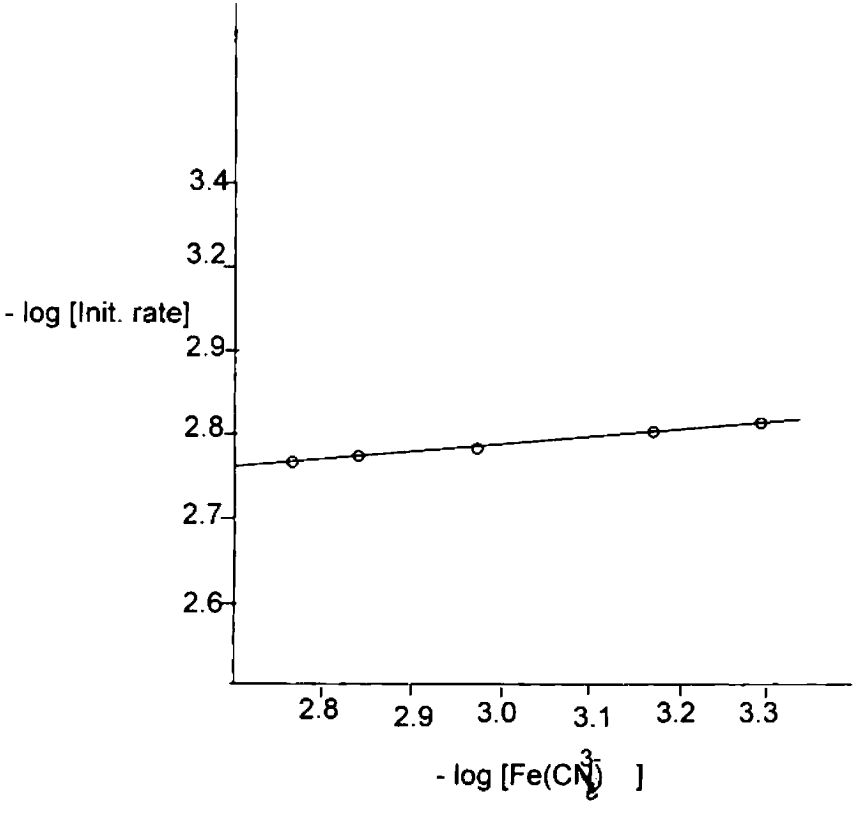


Figure 1. Plot of -log [initial rate] vs -log $\left[Fe(CN)_6^{3-} \right]$ at 18°C [L-Cysteine] = 2.0×10^{-3} M. pH = 3.2, $\mu = 0.08$.

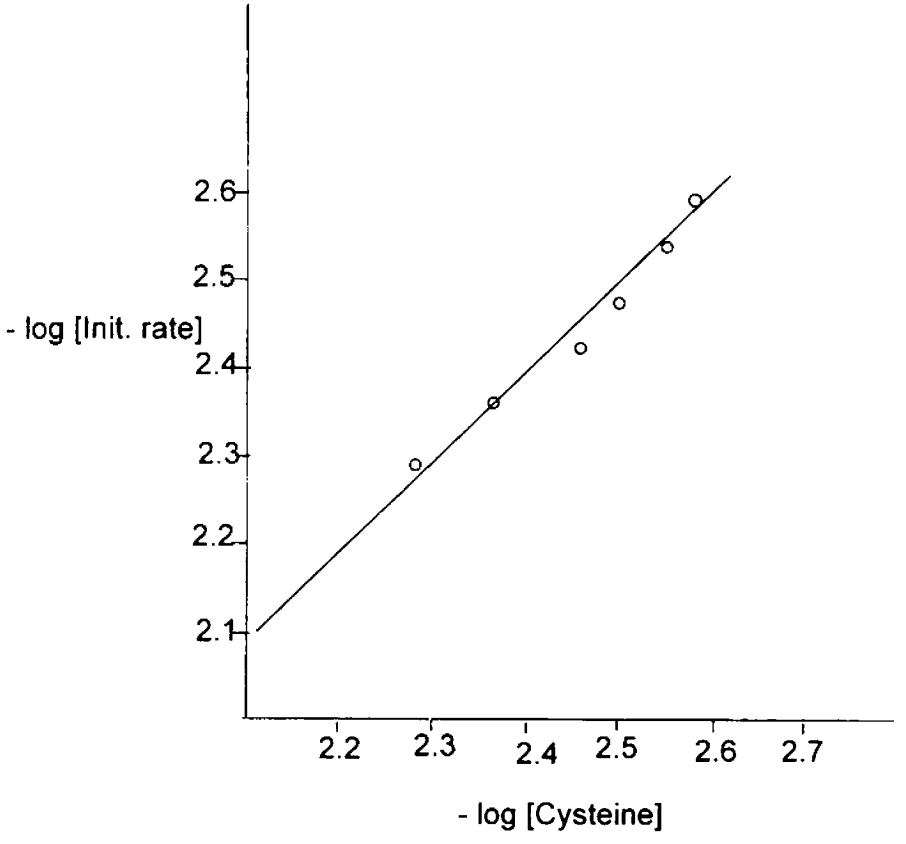


Figure 2. Plot of -log [initial rate]vs -log [L-cysteine] at 18°C $\left[Fe(CN)_6^{3-}\right]$ = 1.0 x 10^{3}-M , pH = 3.2, μ = 0.08M.

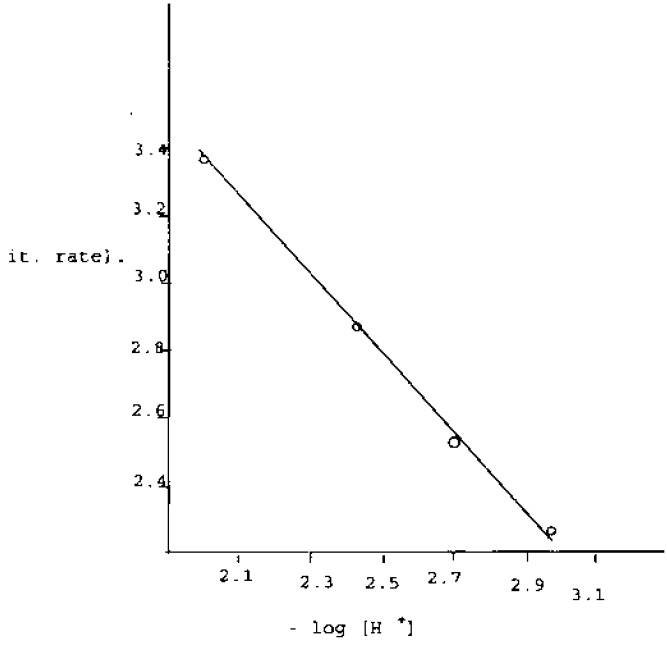


Figure 3 . Plot of -log [initial rate] versus -log $[H^+]$ at 30°C. $\left[Fe(CN)_6^{3-}\right]$ = 1.0 x 10^{3-}M [L-cysteine] = 2.0 x 10^{3-} μ = 0.08M.

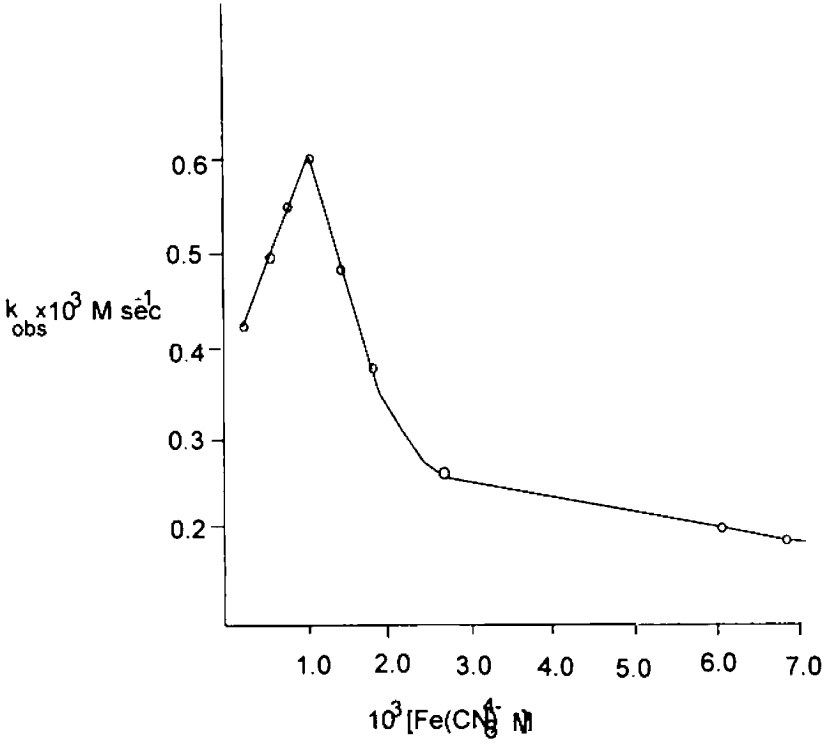


Figure 4. Plot of K_{obs} versus $\left[Fe(CN)_6^{4-}\right]$ at 16° $\left[Fe(CN)_6^{3-}\right]$ = 1.0 x 10^3M. [L-cysteine] = 2.0 x 10^3M. [L-cystein] = 2.0 x 10^3M = 3.1 .

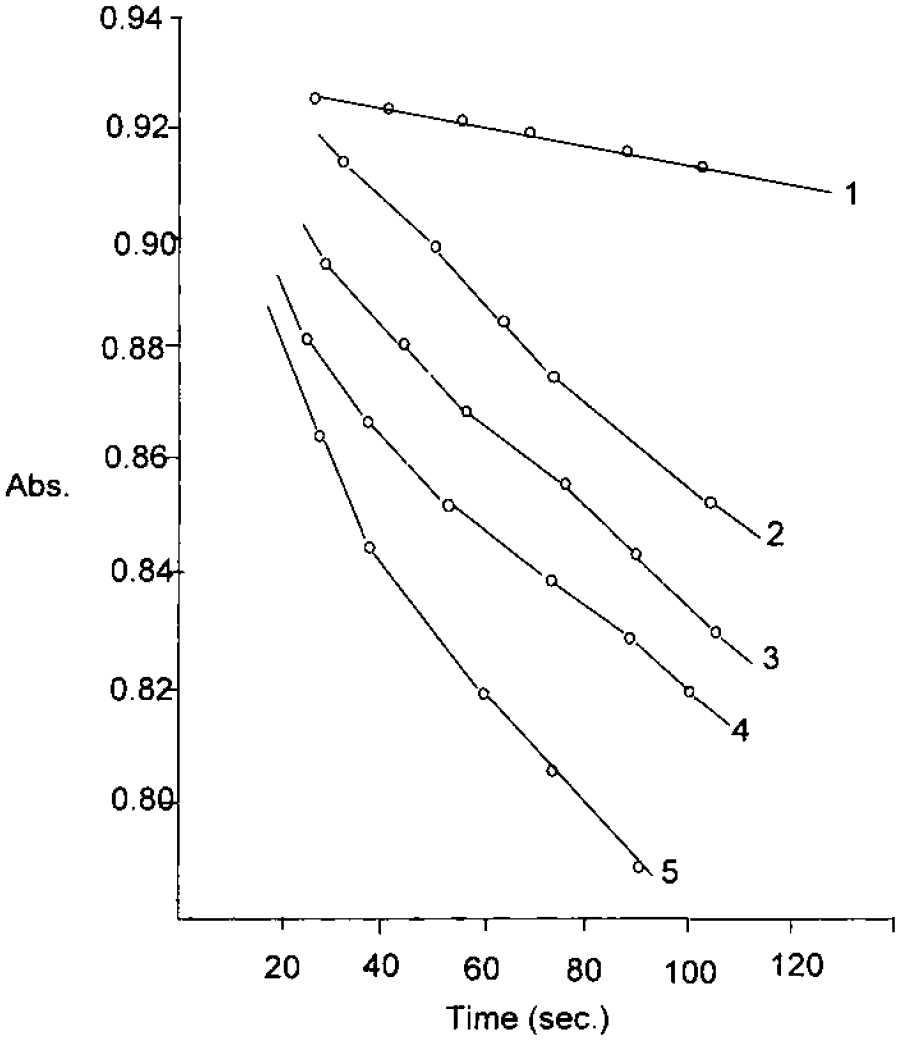


Figure 5. Plot of absorbance versus time at 16°C $\left[Fe(CN)_6^{3-}\right]$ = 1.0 x 10^3M. [L-cysteine] = 2.0 x 10^3M. , pH= 3.1, [anion] = 0.5 x 10^3M (1) - KIO_3, (2) -$KClO_3$ (3) KI; (4) KBr; (5) KF.

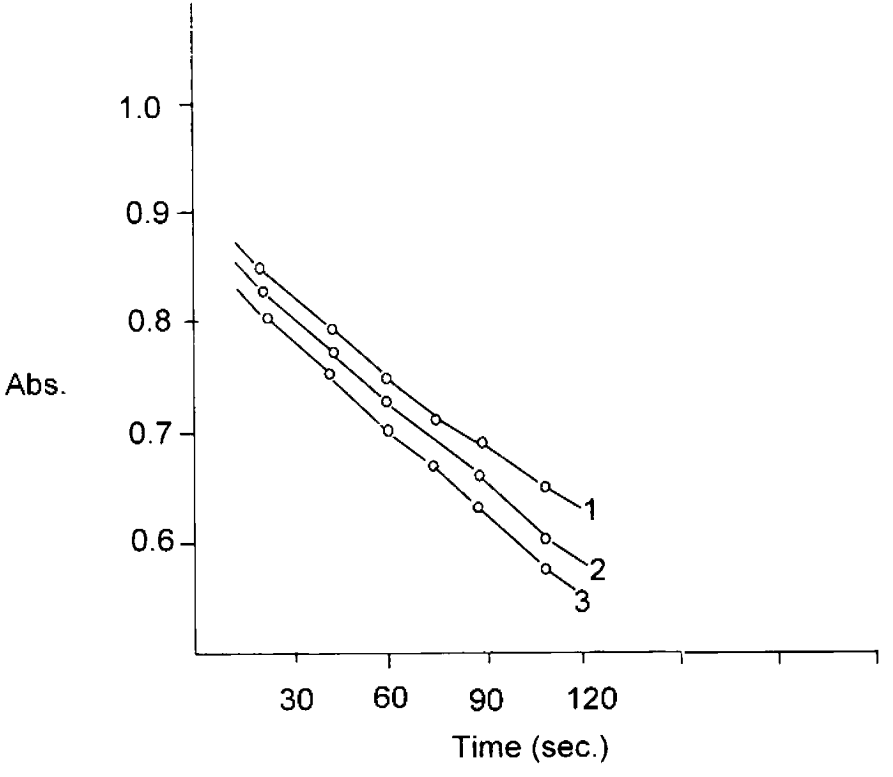


Figure 6. Plot of K_{obs} versus $\left[Fe(CN)_6^{4-}\right]$ at 16°C $\left[Fe(CN)_6^{3-}\right]$ = 1.0 x 10^3M. [L-cystein] = 2.0 x 10^3M. [L-cystein] = 2.0 x 10^3M = 3.1 . (1) NH^+, (2) Na^+, (3) K^+.

References

- M.M. Al-Subu, R. Abu-El-Halawa, and H.M. Abed, Indian J. Chem. Sect. A, **30**, 717 - 719 (1991).
- M.M. Al-Subu, R. Abu-El-Halawa, and H.M. Abed, Int. J. Chem. Kinet. **22**, 1027 - 1037 (1990).
- E.P. Burrows and D.H. Rosenblatt, J. Org. Chem. **48**, 992 - 995, (1983).
- M.M. Al-Subu, J. Ind. Chem Soc. **67** (1990) 966.
- R.C. Acharya, N.K. Saran, S.R. Rao, and M.N. Das, Int. J. of Chem. Kinet **14**, 143 - 151, (1982).
- S.K. Upadhayay, and M.C. Agrawal, Indian J. Chem. Sect. A, **15**, 416 (1977).
- S.K. Upadhayay, and M.C. Agrawal, Indian J. Chem., Sect. A. **15**, 709 (1977).
- S.K. Upadhayay, and M.C. Agrawal, Indian J. Chem., Sect. A. **16**, 39 (1978).
- R. Mehrota and R.C. Kapoor, J. Chem. Soc., Dalton Trans, 999 - 1003 (1984) .
- D.G. Lambert and M.M. Jones, J. Am. Chem. Soc. **88**, 4615 (1966).
- I.M. Kolthoff, E.J. Meehan, M.S. Tsac, Q.W. Chof, J. Am. Chem. Soc. **66**, 1233 (1962) .
- K.B. Wiberg, H. Mattz, and M. Okane, Inorg. Chem., **7**, 831 (1968).
- E.J. Meehan, I.M. Kolthoff, and H. Kakiuchi. J. Phy. Chem. **66**, 1238 (1962).
- A.A. Jarrar, R. El-Zaru and M. Mbarak, Dirasat : Nat. Sci. (Univ. Jordan) , **61** (1), 7-19 (1979).

- A. A. Jarrar, and R. El-Zaru, Chem. Ind. 17, 741 (1977).
- A.A. Jarrar, and R. El-Zaru, J. Chem. Education 54, 326, (1977).
- A.A. Jarrar, and R. El-Zaru, Egypt, J. Chem. 20,603, (1977).
- D.W.J. Kwong and D.E. Pennington, Inorg. Chem. 23, 2528 (1984).
- M.S. Yathiraian and D.S. Mahdevappa, Indian J. of Chem. 17, 62 (1979).
- M.M. Made Gowa, A.S Ananda Muthry and D.S. Makadevappa, Curr. Sci. 44, 5 (1975).
- N.M. Made Gowa, and D.S. Mahadevappa, J. Indian Chem. Sec. 73, 705 (1976).
- "CRC Hand book of Chemistry and Physics" 60th Edn. , (1979-1980), CRC press Inc. Boda Raton, Fl. 33431.
- C.A. Audeh, and J.R. Lindsay Smith, J. chem. Soc. B, 1741 (1971).
- C.A. Audeh, and J.R. Lindsay Smith, J. chem. Soc. B, 1280 (1970).
- C.A. Audeh, and J.R. Lindsay Smith, J. chem. Soc. B, 1745 (1971).
- J.R. Lindsay Smith. and L.A. V. Mead, J. Chem Soc. Perkin 11, 206 (1973).
- J.R. Lindsay Smith. and L.A. V. Mead, J. Chem Soc. Perkin 11, 1172 (1976).
- S.H. Pine, J.B. Hendrickson. D.J. Cram and G.S. Hammond. "Organic Chemistry" P. 47, Fourth Ed. ., Mc Graw - Hill, Tokyo. (1981).
- T. J. Wallace, J. Org. Chem. . 31, 3071 (1966).