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Kinetic study of Cu (II) catalysed oxidation of cysteine

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Abstract
The Kinetics and mechanism of Cu (II) catalysed oxidation of cysteine in aerobic condition was monitored spectro photometrically. All experimental sets were carried out under pseudo first order condition with [Cysteine] > [Cu^{2+}]. Appearance of the reddish brown product was monitored by measuring the absorbance at 503nm as a function of time. Kinetic data were collected by changing the concentration of substrate and the catalyst, medium pH, ionic strength one at a time at constant values of other reaction parameter and the observed phenomena are discussed.

Keywords: Cysteine, spectrophotometer, absorbance, ionic strength

1. Introduction
Metal catalysed oxidation of proteins with specific amino acids having high metal ion affinity may result in structural damage to proteins causing aging [1] and disease including neurological disorders. Sulphur chemistry and metal ion catalysis have attracted considerable attention mainly due to the multifarious involvement of these systems in metabolic pathways [2-5]. Several studies on metal catalysed oxidation (MCO) of proteins reveal that transition metal binding proteins are particularly susceptible to MCO. Such oxidations trigger an extensive structural transition and loss of enzymatic activity.
Hanaki and Kamide [6, 7] have reported the Cu(II) catalysed auto-oxidation of cysteine under various reaction conditions. It has been found that hydrogen peroxide is produced progressively during the oxidation and this indicates the possibility of four equivalent reduction of oxygen via hydrogen peroxide to water. The first step in the metal catalysed oxidation is the formation of metal-substrate complex, which decomposes subsequently to the low valence metal ion and the free radical of the substrate [8, 9]. The molecular oxygen plays an important role in the re-oxidation of the low valence metal ion, which is catalytically inactive, to the high valence metal ion. During this process hydrogen peroxide is produced. The kinetic study of L-Cysteine with 12-tungsto cobaltate(III) shows that the rate of reaction increase with the increase in pH and the reaction is first order with concentration of L-Cysteine and 12-tungsto cobaltate (III) [10, 11].
As oxidation reactions are of particular concern in biotechnology and medicine, where they can lead to protein destabilisation and inactivation. So from both biomedical and biochemical perspectives, it is important to understand the factors that influence the reactivity of metal catalysed oxidation processes. The present investigation incorporates the kinetics of Cu (II) catalysed oxidation of cysteine at and around physiological pH and the study is undertaken with a view to understand the metal ion toxicity in body system.

2. Materials and methods
All chemicals used in this work were obtained from commercial sources and were of A.R. grade, however for washing and cleaning purposes L.R. reagents were useful. Any aqueous solution used was prepared by using doubled distilled water. Stock solution (0.025 mol dm^{-3}) of L-Cysteine hydrochloride (Qualigens) was prepared by dissolving 0.1097 gm in 25 ml of distilled water. It was stored in refrigerator and used within day one or two. Accurately weighed 4.8 gm of Cu (II) nitrate trihydrate (Merck) was dissolved in 100ml water to make a solution of approximate strength 0.2 mol dm^{-3}.To find exact strength it was titrated with 0.1 mol dm^{-3} EDTA solution using fast sulphone black-F indicator. pH was maintained constant throughout the reaction using borax (Merck).

Stock solution of 0.1 mol dm^{-3} borax was prepared by dissolving 3.813 gm in 100 ml distilled water [12]. A typical Kinetic run consisted of 8ml cysteine $\left(8 \times 10^{-3}\ mol\ \ dm^{-3}\right)$ + 10ml borax $\left(4 \times 10^{-2}\ mol\ \ dm^{-3}\right)$ + 1ml Cu^{2+} $\left(8 \times 10^{-4}\ mol\ \ dm^{-3}\right)$ + 2ml water + 4ml acetone. In each experimental run the total volume was kept at 25ml. The constituents were added in the sequence as mentioned. The spectral data were recorded by the auto data processor of the instrument (shimazdu UV-visible 1700-A spectrophotometer) after making necessary correction for the solvent absorbance. All the Kinetic experiments were designed in alkaline medium.

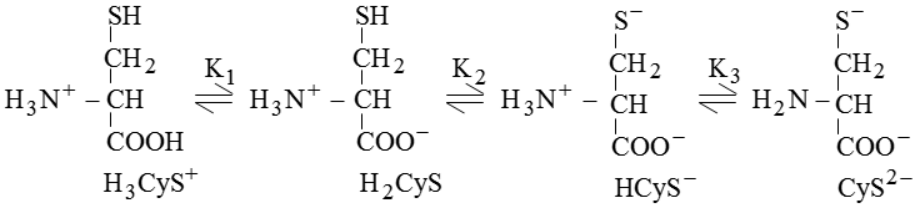
3. Result and discussion

Preliminary investigation indicates that when the constituents of the reaction are added at constant pH (in basic medium) in the sequence cysteine, copper followed by acetone an yellow colour appeared which gradually increases in intensity and after about 15-20 minutes a brownish red colouration appeared.

In acidic medium, i.e. at low pH the observations were quite different. The initial pH of the solution on mixing only Cu (II) solution with cysteine solution in the ratio 1:5 was found to remain between 2-3. The mixed solution was colourless and continued to remain the same for days together. However, when the pH was slowly increased, a yellow colour appeared and its intensity gradually increased with increasing pH. In such experiments the brownish-red colour never appeared. However, the left over solution from a pH-metric titration turned brownish red on standing for few hours. It may be mentioned here that at the end of a pH metric study, the pH is usually high (~11.0). Contrary to the above observation, in a kinetic study conducted in an alkaline medium (pH = 9-10.5) the initial transient purple colour formation soon leads to a colourless solution which slowly becomes yellow and after about 15-20 minutes the colour changes to brownish red. So it is obvious from the above analysis that the brownish red product from the reaction between Cu (II) and cysteine results only at high pH.

Since cysteine gets deprotonated differently at different pH, the deprotonated species of cysteine are possibly the reactive species which most likely form complexes with Cu(II) in the first stage (origin of yellow colour). Some or all of these metal complexes or substrate species on exposure to areal oxygen may undergo subsequent oxidation resulting in the brownish red product.

Cysteine is a bifunctional amino acid which contains three ionising groups: carboxyl, amino and thiol with pK_1, pK_2, and pK_3 as 2:12, 8:21 and 10.38 [13]. The ionisation of Cysteine can be described by following scheme.



Depending on the pH, cysteine can have a charge from +1 (H_3CyS^+) to -2 (CyS^{2-}). The pK_a of carboxyl group is low and can be identified easily. However, the ammonium and thiols have similar pK_a's and hence one cannot choose a priori between $HCyS^-$ and $CySH^-$ because of the uncertainty as to which group ionises first. Table-1 shows that variation in the percentage of different deprotonated species of cysteine as a function of pH. The species distribution presented in Table-1 has been generated using the above scheme i.e., the thial group ionises prior to the ammonium group.

As is evident from the Table-1 HCyS with deprotonated carbonyl and thiol groups but protonated amino group is the predominant deprotonated species of cysteine in the pH range 9-10. However, beyond pH = 10, the percentage of CyS^{2-} increases steadily so that by pH 10.5 both $HCyS^-$ and CyS^{2-} are present almost in equal amounts.

The absorbance time profile for the redox reaction has features of a series type reaction consisting of an initial slow and subsequent fast step. Absorbance vs time plots are sigmoidal in shape under all conditions of study. Plot of ln (A_∞ - A_t) vs time results in two intersecting lines with different slopes. Table-2 records the initial rate values, as well as the first order rate constant values for the initial slow reaction.

This discussion is limited to the initial slow reaction as the findings are consistent with the fact that the slow reaction signifies oxidation of cysteine to cystine and the subsequent fast reaction, i.e. formation of brownish red product is due to further oxidation of cysteine and other metal species to newer products.

4. Variation of cysteine

Cysteine concentration was varied from 6 x 10^{-3} mol dm^{-3} to 10 x 10^{-3} mol dm^{-3}. Linearity of Line weaver Burk plot in Fig.1 indicates a zero order dependence of [CyS] i.e., when initial concentration of Cysteine increases the half life of reaction is lengthened and the rate of oxidation is decreased. Fig. 2 shows the absorbance time plots for a number of concentrations of cysteine but at constant conditions of other reaction parameters.

5. Variation of copper

Copper concentration was varied from 4.8 x 10^{-4} mol dm^{-3} to 11.2 x 10^{-4} mol dm^{-3}. For both the initial slow and subsequent fast reaction, the ratio of the first order rate constant value to the initial concentration of Cu(II) is almost constant showing a first order dependence of rate on the concentration of Cu(II). The absorbance time profile for the formation of the product at different concentration of Cu(II) are shown in Fig. 3. The first order plots ln (A_∞ - A_t) vs t for different initial concentrations of Cu(II) are presented in Fig.4.

6. Variation of acetone

Acetone content was varied form 12 to 20%. The impact of change in acetone percentage has marginal effects on the rate of reaction. This confirms the role of acetone only as a medium and not as a reactant. Neither the dielectric constant has any effect on the rate of reaction.

7. Variation of pH

pH was varied from 8.80 to 10.49. The rate constant value increases with increase in pH. This is more pronounced for the fast reaction than for the initial slow reaction. The pH was maintained by adding different volumes of standard KOH solution to a constant volume of borax solution. From the change in pH spanning over 1.69 pH units, it is clearly evident that the reaction is favoured by the alkalinity of the medium. This is presented in Fig. 5, where the absorbance vs time plot shows a steady increase in the product formation with increase in the pH of the solution.

8. Rate Mechanism

The observations from kinetic measurements for the initial slow reaction may be summarised as follows;

1. The reaction has a complex dependence on [cysteine]
2. Linearity of Line Weaver-Burk plot indicates a zero order dependence on [cysteine].
3. The dependence of rate on Cu(II) is unity.
4. Variation of dielectric constant of the medium has no impact initial rate.
5. The rate of reaction increases with increase in pH.

Based on the observation of kinetic reaction the following mechanism is proposed.

$$2\,HCyS + Cu^{2+} + O_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Cu(CyS)_2 + H_2O_2 \quad (1)$$

$$[Cu(II)(CyS)_2] \xrightarrow{k_2} Cu(I) + 2CyS^{\bullet} \quad (2)$$

$$Cu(I) + H_2O_2 \xrightarrow{fast} Cu^{2+} + {}^{\bullet}OH + OH^{-} \quad (3)$$

$$CyS^{\bullet} + {}^{\bullet}SCy \xrightarrow{fast} CyS - SCy \quad (4)$$

$$HCyS + H_2O_2 \rightarrow CyS^{\bullet} \rightarrow CySOH \rightarrow CySO: \rightarrow CyS(=O)-OH \rightarrow CyS(=O)_2-OH \rightarrow CyS(=O)_2 \rightarrow CyS(=O)_2-OH \quad (5)$$

$$\text{Rate} = k_2\,[Cu\,(CyS)_2] \quad (6)$$

Applying steady-state condition,

$$k_1[CySH]^2\,[Cu^{2+}] = (k_{-1} + k_2)[Cu(CyS)_2]$$

$$\Rightarrow [Cu(CyS)_2] = \frac{k_1[CySH]^2\,[Cu^{2+}]}{k_{-1} + k_2} \quad (7)$$

Substituting equation (7) in equation (6) we get

$$\text{Rate} = \frac{k_2\,k_1[CySH]^2\,[Cu^{2+}]}{k_{-1} + k_2} \quad (8)$$

$$\text{Now } [Cu^{2+}]_T = Cu^{2+} + [Cu(CyS)_2]$$

$$= Cu^{2+} + K[Cu^{2+}]\,[CySH]^2$$

$$= [Cu^{2+}]\,\{1 + K[CySH]^2\}$$

$$= Cu^{2+} + K[Cu^{2+}]\,[CySH]^2$$

$$= [Cu^{2+}]\,\{1 + K[CySH]^2\}$$

$$\Rightarrow [Cu^{2+}] = \frac{[Cu^{2+}]_T}{1 + K[CySH]^2} \quad (9)$$

$$(\text{Where } K = \frac{k_1}{k_{-1}})$$

Substituting in equation (8)

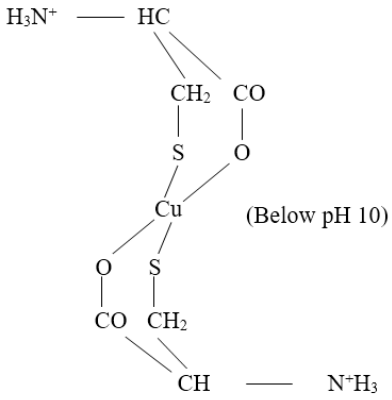
$$\text{Rate} = \frac{k_2\,k_1[CySH]^2\,[Cu^{2+}]_T}{(k_{-1} + k_2)(1 + K[CySH]^2)} \quad (10)$$

9. Conclusion

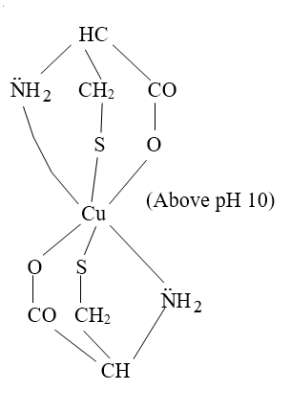
From the rate equations following explanation are observed. First order dependence of rate on $[Cu^{2+}]_T$. Since reaction of cysteine with Cu (II) is a complicating redox reaction, the log k > 10 which indicates large stability constant value (k). so the rate of reaction reduces to zero order dependence on [Cysteine]. The pH dependence of the reaction can be explained by considering the reactivity order of the various cysteine species. The first step in the auto-oxidation process is the formation of an intermediate complex involving cysteine species and the metal ion. The reactivity order for the nucleophilic attack on the metal ion is

$$CyS^{2-} > HCyS^{-} > H_2CyS > H_3CyS^{+}$$

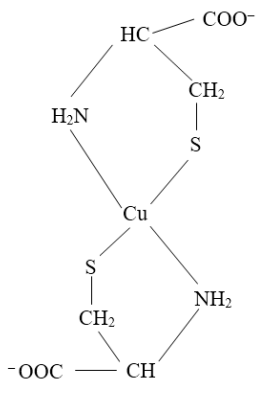
The high reactivity of CyS^{2-} results from Presence of three donor sites i.e., the carboxylate, deprotonated thiol groups, non-protonated amino nitrogen [14, 15]. Also there is formation of stable five or six membered chelate rings on complexation i.e. I, II, III. Since formation of CyS^{2-} is favoured at high pH, the rate of reaction increases with pH.



(I): [Cu(II)(HCyS)₂]



(II): [Cu(II)(CyS)₂]



(III)

Table 1: Percentage of distribution of species formed by deprotonation of cysteine at different pH.

pH	Percentage		
	CyS^{2-}	$HCyS^{-}$	H_2CyS
9.01	3.60	83.30	13.10
9.25	6.30	85.90	7.80
9.51	11.38	84.40	4.22
9.66	15.54	81.56	2.90
9.94	26.28	72.37	1.35
10.49	56.17	43.60	0.23

Table 2: Rate constant values for cu (ii) catalysed oxidation of cysteine at 30°c under different reaction condition. k_1 refers to first order rate constant values for the reaction.

[Cysteine] mol dm^{-3}	[Cu(II) mol dm^{-3}	pH	Acetone (ml)	Initial rate (k_0) × 10^5 mol dm^{-3} Sec^{-1}	$k_1 \times 10^5$ Sec^{-1}
6.0×10^{-3}	8.0×10^{-4}	8.8	4.0	10.7	12.5
7.0×10^{-3}				8.3	7.2
8.0×10^{-3}				4.3	6.8
9.0×10^{-3}				3.4	2.2
10.0×10^{-3}				1.8	1.7
8.0×10^{-3}	4.8×10^{-4}	8.8	4.0	2.8	5.2
	6.4×10^{-4}			3.6	5.3
	8.0×10^{-4}			4.3	6.8
	9.6×10^{-4}			5.6	7.0
	11.2×10^{-4}			7.1	8.3
8.0×10^{-3}	8.0×10^{-4}	8.80	4.0	4.3	6.8
		9.01		4.5	7.0
		9.25		4.8	7.7
		9.51		5.6	8.1
		9.66		6.3	8.5
		9.94		7.0	9.1
		10.49		8.2	10.4
8.0×10^{-3}	8.0×10^{-4}	8.8	3.0	4.1	6.7
			3.5	4.2	6.6
			4.0	4.3	6.8
			4.5	4.3	6.8
			5.0	4.4	6.9

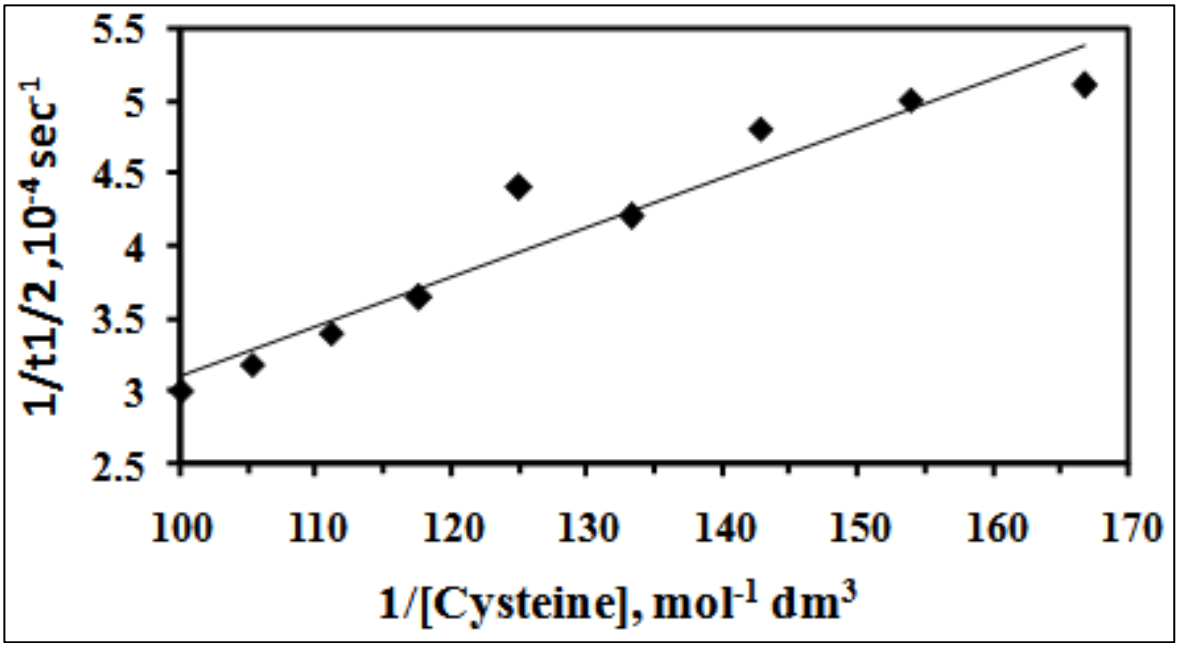


Fig 1: Line Weaver – Burk plot of $1/t_{1/2}$ vs 1/[Cysteine] for Cu(II) catalysed oxidation of cysteine. [Cu(II)] : 8 x 10^{-4} mol dm^{-3}; pH : 8.8; Acetone : 4ml; Temperature : 30 °C; [Cysteine] = 6.0, 7.0, 8.0, 9.0, 10.0 x 10^{-3} mol dm^{-3}.

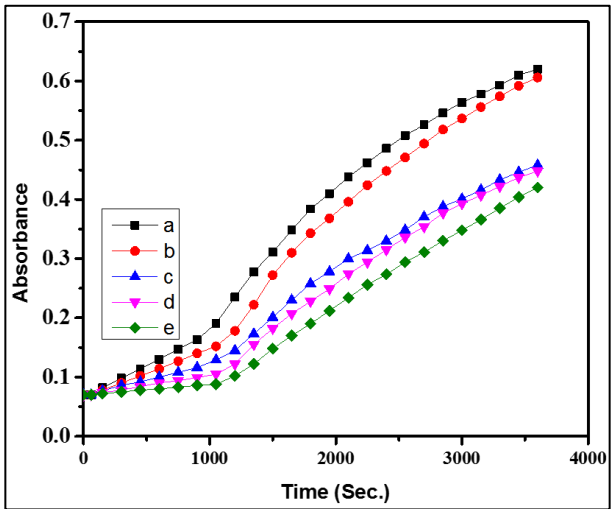


Fig 2: Absorbance – time profile for Cu(II) catalysed oxidation of cysteine. [Cu(II)] : 8 x 10^{-4} mol dm^{-3}; pH : 8.8; Acetone : 4ml; Temperature : 30°C; [Cysteine] = a : 6.0, b : 7.0, c : 8.0, d : 9.0, e : 10.0 x 10^{-3} mol dm^{-3}.

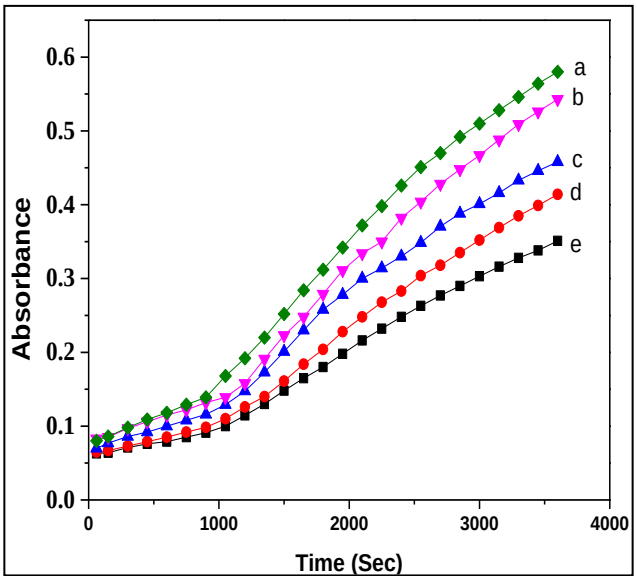


Fig 3: Absorbance – time profile for Cu(II) catalysed oxidation of cysteine. [Cysteine] : 8.0 x 10^{-3} mol dm^{-3}; pH : 8.8; Acetone: 4ml; Temperature : 30°C; [Cu(II)] = a : 11.2, b : 9.6, c : 8.0, d: 6.4, e : 4.8 x 10^{-4} mol dm^{-3}.

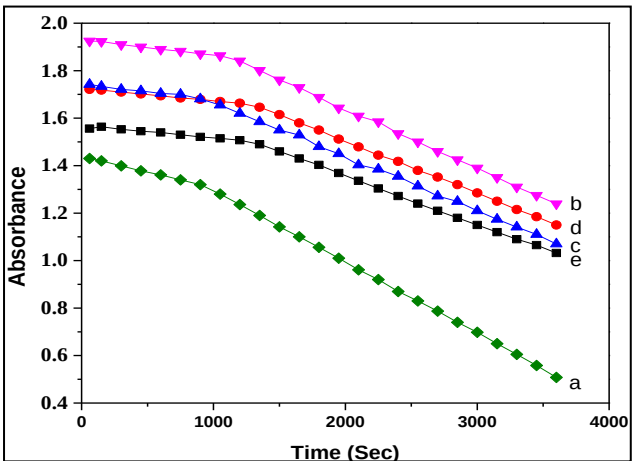


Fig 4: First order plots of $\ln(A_\infty - A_t)$ vs time at different initial concentrations of copper. Reaction conditions are same as in Fig.3. [Cu(II)] = a : 11.2, b : 9.6, c : 8.0, d : 6.4, e : 4.8 x 10^{-4} mol dm^{-3}.

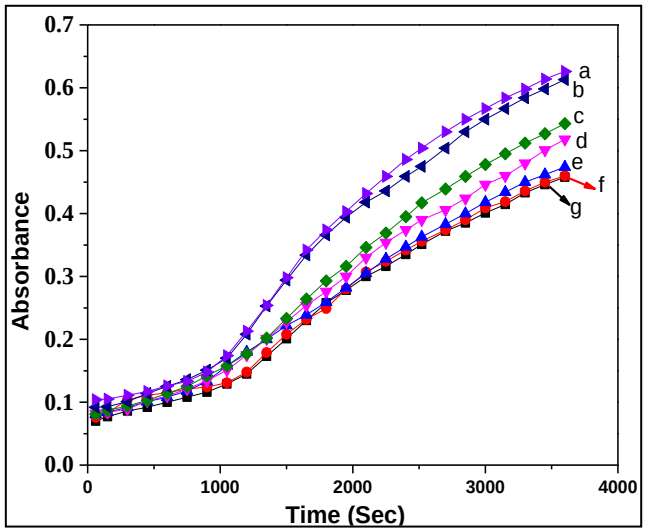


Fig 5: Absorbance – time profile for Cu (II) catalysed oxidation of cysteine. [Cysteine] : 8.0 x 10^{-3} mol dm^{-3}; [Cu(II)] : 8 x 10^{-4} mol dm^{-3}; Acetone : 4ml; Temperature : 30°C; pH = a : 10.49, b : 9.94, c : 9.66, d : 9.51, e : 9.25, f : 9.01, g : 8.80.

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