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KINETIC DETERMINATION OF CYSTEINE BASED ON ITS INHIBITORY EFFECT ON THE SILVER-CATALYSED LIGAND SUBSTITUTION REACTION BETWEEN HEXACYANOFERRATE(II) AND DPQ

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ABSTRACT

Cysteine is a biologically important sulfur-containing amino acid that plays a vital role in numerous physiological processes, including protein synthesis, antioxidant defence, detoxification, and cellular redox regulation. Owing to its strong affinity towards soft metal ions such as silver(I), cysteine readily forms stable metal-thiol complexes that significantly influence metal-catalysed reactions. Exploiting this property, the present study describes a simple, rapid, and highly sensitive kinetic spectrophotometric method for the determination of trace amounts of cysteine based on its inhibitory effect on the silver(I)-catalysed ligand substitution reaction between hexacyanoferrate(II) and dipyrrodo[3,2-f:2',3'-h]quinoxaline (DPQ). The reaction was monitored spectrophotometrically at 547 nm through the formation of the dark green complex, $[Fe(CN)_5DPQ]^{3-}$, under optimized experimental conditions of pH 3.0 ± 0.02 , temperature $25.0 \pm 0.1^\circ\text{C}$, and ionic strength 0.05 M maintained with potassium nitrate. Linear calibration plots were obtained over a wide concentration range of cysteine using fixed-time kinetic measurements. The proposed method exhibited excellent sensitivity with a detection limit in the sub-micromolar range and demonstrated good precision and reproducibility. The kinetic data were interpreted using a modified Michaelis–Menten model to evaluate the Michaelis constant (K_m), catalyst–substrate stability constant (K_s), and catalyst–inhibitor formation constant (K_{CI}). A plausible reaction mechanism explaining the inhibitory behaviour of cysteine through catalyst complexation is also proposed. The developed method provides an inexpensive alternative to sophisticated instrumental techniques for the determination of trace cysteine in analytical and environmental samples.

Keywords: Cysteine; Kinetic spectrophotometry; Silver(I); Hexacyanoferrate(II); DPQ; Ligand substitution; Inhibition kinetics; Michaelis–Menten model.

1 INTRODUCTION

L-cysteine is a naturally occurring sulfur-containing α -amino acid possessing a reactive thiol ($-\text{SH}$) functional group that exhibits exceptional affinity towards transition metal ions. This unique chemical property makes cysteine an important ligand in coordination chemistry, biological redox processes, enzymatic catalysis, and metal homeostasis. In living systems, cysteine serves as the principal precursor for glutathione synthesis, regulates intracellular redox balance, stabilizes protein structures through disulfide bond formation, and participates in numerous metabolic pathways. Altered cysteine concentrations have been associated with oxidative stress, cardiovascular disorders,

neurodegenerative diseases, liver dysfunction, diabetes, and cancer, highlighting the need for accurate analytical methods for its determination [1–3].

Several analytical techniques have been reported for cysteine determination, including high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), capillary electrophoresis, electrochemical sensors, fluorescence spectroscopy, chemiluminescence, and biosensor-based methods [4–8]. Although these techniques offer excellent sensitivity and selectivity, they generally require sophisticated instrumentation, extensive sample preparation, expensive reagents, and highly trained personnel. Consequently, there remains considerable interest in developing simple, rapid, economical, and reliable spectrophotometric methods suitable for routine laboratory analysis.

Kinetic methods based on catalytic or inhibitory reactions have emerged as attractive analytical alternatives because of their high sensitivity, relatively simple instrumentation, and excellent selectivity. In catalytic kinetic analysis, the analyte either accelerates or inhibits the rate of a carefully selected indicator reaction, and the change in reaction rate serves as the analytical signal [9–11]. Such methods have been successfully employed for the determination of trace metal ions, pharmaceutical compounds, environmental pollutants, and biologically important molecules.

The ligand substitution reaction of hexacyanoferrate(II) with nitrogen donor ligands has been extensively investigated as a model system in coordination chemistry. Previous studies have demonstrated that silver(I) ions effectively catalyse the substitution of coordinated cyanide ions in hexacyanoferrate(II) by dipyrrodo[3,2-f:2',3'-h]quinoxaline (DPQ), producing the intensely coloured complex $[Fe(CN)_5DPQ]^{3-}$, which exhibits a strong absorption maximum near 547 nm [12–14]. The reaction proceeds through a catalyst-assisted ligand substitution mechanism and is particularly suitable as an indicator reaction for kinetic analytical applications.

Sulfur-containing ligands, especially cysteine, possess significantly higher stability constants with soft metal ions such as silver(I) than analogous oxygen- or nitrogen-containing ligands. Consequently, cysteine competes effectively with the substrate for the catalyst by forming stable Ag(I)-cysteine complexes, thereby reducing the concentration of catalytically active silver species available in solution. This competitive interaction decreases the rate of ligand substitution, producing a measurable inhibitory effect that can be quantitatively correlated with cysteine concentration [15–17].

The present investigation exploits this inhibition phenomenon to develop a sensitive kinetic spectrophotometric method for the determination of trace cysteine. The reaction conditions, including reagent concentrations, pH, ionic strength, temperature, and catalyst concentration, were systematically optimized to achieve maximum analytical sensitivity. Furthermore, kinetic parameters such as the Michaelis–Menten constant (K_m), catalyst–substrate equilibrium constant (K_s), and catalyst–inhibitor formation constant (K_{CI}) were evaluated to gain mechanistic insight into the inhibition process. A reaction mechanism based on catalyst sequestration by cysteine is also proposed to explain the observed kinetic behaviour.

The proposed method combines the simplicity of spectrophotometry with the sensitivity of kinetic analysis, providing a rapid, cost-effective, and reliable alternative for trace determination of cysteine in analytical laboratories without the need for sophisticated instrumentation.

2 EXPERIMENTAL

2.1 Materials

All reagents employed throughout the investigation were of analytical reagent (AR) grade, and double-distilled water was used for the preparation of all solutions. Potassium hexacyanoferrate(II) trihydrate, $K_4[Fe(CN)_6] \cdot 3H_2O$ (Merck, Germany), was accurately weighed and dissolved in double-distilled water. The solution was stored in amber-coloured bottles to minimize photochemical decomposition. Fresh aqueous solutions of dipyrrodo[3,2-f:2',3'-h]quinoxaline (DPQ) were prepared daily before each kinetic experiment. A standard stock solution of silver(I) was prepared from analytical grade silver nitrate, and appropriate working solutions were freshly prepared immediately before use to avoid adsorption losses and photodecomposition. Potassium nitrate (KNO_3) was employed as the inert electrolyte for maintaining constant ionic strength throughout the reaction. L-cysteine hydrochloride solutions were freshly standardized prior to use because cysteine undergoes gradual aerial oxidation under ambient conditions to form cystine, thereby affecting analytical accuracy [18,19]. The pH of the reaction medium was maintained using potassium hydrogen phthalate–sodium hydroxide buffer solutions prepared according to standard procedures [20].

2.2 Instrumentation

All kinetic measurements were performed using a UV–Visible spectrophotometer equipped with matched 1 cm quartz cells. Absorbance changes were monitored continuously at 547 nm corresponding to the maximum absorption of the coloured product, $[Fe(CN)_5DPQ]^{3-}$. Temperature was maintained at $25.0 \pm 0.1^\circ\text{C}$ using a thermostatically controlled environmental chamber throughout the experiments. Solution pH was measured using a calibrated digital pH meter with combined glass electrode. All glassware used during the investigation was thoroughly cleaned and rinsed with double-distilled water before use.

2.3 Experimental Procedure

A measured volume (1.0 mL) of silver(I) solution of known concentration was transferred into a reaction vessel followed by 1.0 mL of cysteine solution of appropriate concentration. The mixture was allowed to stand for approximately 10 minutes to ensure complete formation of the silver–cysteine complex prior to initiation of the kinetic reaction.

Subsequently, 3.0 mL of the buffer solution and 1.0 mL of freshly prepared DPQ solution were added. The reaction was initiated by the addition of 1.0 mL of potassium hexacyanoferrate(II) solution under continuous stirring. The resulting reaction mixture was immediately transferred to a 1 cm quartz cuvette maintained at 25°C , and the increase in absorbance at 547 nm was recorded using the fixed-time kinetic method.

Under the optimized experimental conditions, the reaction mixture contained:

$[Fe(CN)_6]^{4-} = 3.0 \times 10^{-3} \text{ M}$, $[DPQ] = 1.5 \times 10^{-3} \text{ M}$, $[Ag^+] = 7.5 \times 10^{-7} \text{ M}$, $\text{pH} = 3.0 \pm 0.02$ Temperature = $25.0 \pm 0.1^\circ\text{C}$, Ionic strength = 0.05 M (KNO_3)

Calibration plots were constructed by measuring absorbance at predetermined fixed time intervals for varying concentrations of cysteine.

3 RESULTS AND DISCUSSION

The ligand substitution reaction between hexacyanoferrate(II) and dipyrido[3,2-f:2',3'-h]quinoxaline (DPQ) proceeds very slowly in acidic aqueous medium under uncatalysed conditions. The presence of trace concentrations of silver(I) ions markedly accelerates the substitution of coordinated cyanide by DPQ, resulting in the formation of the intensely coloured complex $[Fe(CN)_5DPQ]^{3-}$. The increase in absorbance at 547 nm provides a convenient means for monitoring the reaction kinetics spectrophotometrically [12–14].

Table 1: Determination of cysteine by its inhibitory effect on the Ag(I)-catalysed substitution of cyanide in hexacyanoferrate (II) by DPQ

Cysteine $\times 10^6 \text{ (M)}$	Absorbance after 15 min	Absorbance after 20 min
2.50	0.165	0.179
2.32	0.169	0.188
1.81	0.187	0.190
1.66	0.198	0.236
1.36	0.215	0.289
1.00	0.278	0.314

Experimental Conditions: $[Fe(CN)_6]^{4-} = 1.5 \times 10^{-2} \text{ M}$; $[DPQ] = 3.0 \times 10^{-4} \text{ M}$; $[Ag^+] = 1.5 \times 10^{-7} \text{ M}$; $\text{pH} = 3.0 \pm 0.02$; Temperature = $25.0 \pm 0.1^\circ\text{C}$; Ionic strength = 0.05 M (KNO_3).

Cysteine possesses a highly reactive sulfhydryl (-SH) group, which exhibits a strong tendency to coordinate with soft metal ions such as Ag(I). Consequently, cysteine competes effectively with the substrate for the catalyst by forming stable Ag(I)-cysteine complexes. Formation of this catalyst-inhibitor complex reduces the concentration of catalytically active silver species available for ligand substitution, thereby decreasing the overall reaction rate. The magnitude of inhibition is directly proportional to cysteine concentration and forms the analytical basis of the proposed kinetic method [15–17].

The reaction exhibited excellent reproducibility under the optimized experimental conditions. No measurable interference from potassium hexacyanoferrate(II), DPQ, or silver(I) was observed within their optimized concentration

ranges. Fixed-time absorbance measurements were therefore selected for quantitative determination of cysteine because they provided superior precision and linearity compared with initial-rate measurements.

The concentration of cysteine was varied over the micromolar range while maintaining constant concentrations of all other reactants. Representative absorbance values recorded at fixed reaction times are presented in Table 1. As expected, increasing cysteine concentration progressively inhibited the catalytic reaction, producing lower absorbance values due to reduced formation of the coloured complex.

3.1 Effect of pH

The influence of pH on the silver(I)-catalysed ligand substitution reaction was investigated over the pH range of 1.0–8.0 using fixed-time absorbance measurements at 10, 15 and 20 min. The variation in absorbance with pH is illustrated in Figure 1.

The reaction rate increased gradually as the pH was raised from strongly acidic conditions and attained a maximum at $\text{pH } 3.0 \pm 0.02$. Beyond this optimum value, the reaction rate decreased progressively with increasing pH.

At lower pH values, the reduced reaction rate may be attributed to excessive protonation of the coordinating ligand, thereby decreasing its nucleophilicity and slowing ligand substitution. At higher pH values, hydrolysis of silver(I) ions and partial formation of less reactive hydroxide species reduce the concentration of catalytically active Ag(I) , resulting in diminished catalytic efficiency [13,14,16].

Therefore, $\text{pH } 3.0 \pm 0.02$ was selected as the optimum value for all subsequent investigations. Maintaining the reaction medium at this pH ensured maximum analytical sensitivity and excellent reproducibility. Potassium hydrogen phthalate–sodium hydroxide buffer was employed throughout the study to maintain constant hydrogen ion concentration.

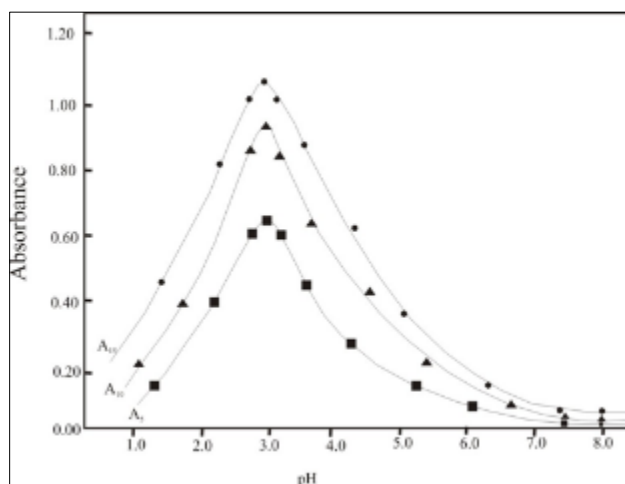


Figure 1: Effect of pH on the Silver catalysed substitution of CN^- in hexacyanoferrate (II) by [DPQ].

Under Conditions: $[\text{Fe}(\text{CN})_6]^{4-} = 1.50 \times 10^{-2} \text{ M}$, $[\text{DPQ}] = 1.50 \times 10^{-3} \text{ M}$, $[\text{Hg}]^{2+} = 1.5 \times 10^{-5} \text{ M}$, $I = 0.03 \text{ M (KNO}_3\text{)}$ and $\text{Temp.} = 25.0 \pm 0.1^\circ \text{C}$

3.2 Effect of Reagent Concentration, Temperature and Ionic Strength

3.2.1 Effect of DPQ Concentration

The influence of DPQ concentration on the catalytic reaction was investigated over the concentration range 2.0×10^{-4} – $2.5 \times 10^{-3} \text{ M}$ while maintaining all other reaction variables constant. An increase in DPQ concentration produced a corresponding increase in reaction rate up to $1.5 \times 10^{-3} \text{ M}$. Above this concentration, the absorbance reached a plateau, indicating saturation of the catalytic pathway. This behaviour suggests that at higher ligand concentrations the catalyst becomes fully occupied, and further increases in DPQ concentration no longer influence the reaction rate. Consequently, $1.5 \times 10^{-3} \text{ M}$ DPQ was selected as the optimum ligand concentration for analytical applications.

3.3 Effect of Hexacyanoferrate(II) Concentration

The concentration of potassium hexacyanoferrate(II) was varied between 2.5×10^{-4} and $1.5 \times 10^{-2} \text{ M}$. A logarithmic plot of reaction rate versus substrate concentration demonstrated an approximately first-order dependence at lower

substrate concentrations. However, as substrate concentration increased, the reaction exhibited fractional-order behaviour, indicating gradual saturation of the catalyst-substrate complex.

The observed kinetic behaviour closely resembles Michaelis-type saturation kinetics commonly encountered in catalytic systems involving intermediate complex formation [9–11,17]. A concentration of 3.0×10^{-3} M hexacyanoferrate(II) produced maximum sensitivity while maintaining excellent linearity and was therefore employed throughout the investigation.

3.4 Effect of Temperature

The catalytic and uncatalysed reactions were investigated over the temperature range 20–50°C. An increase in temperature accelerated both reactions in accordance with the Eyring transition-state theory. However, temperatures above 25°C produced excessively rapid absorbance changes that reduced measurement precision during fixed-time kinetic analysis.

Moreover, higher temperatures slightly increased the uncatalysed background reaction, thereby decreasing analytical sensitivity. Consequently, $25.0 \pm 0.1^\circ\text{C}$ was selected as the optimum temperature since it provided the best compromise between reaction rate, stability and reproducibility [17].

3.5 Effect of Ionic Strength

The effect of ionic strength was investigated using potassium nitrate over the range 0.02–0.05 M. Increasing ionic strength initially enhanced the reaction rate, presumably by reducing electrostatic repulsion between reacting ionic species. Beyond 0.05 M, however, the reaction rate decreased slightly, possibly owing to changes in activity coefficients and solvation effects.

Maximum absorbance and reproducible kinetic behaviour were obtained at an ionic strength of 0.05 M, which was maintained for all subsequent experiments.

3.6 Optimization of Silver(I) Concentration

The concentration of silver(I), the catalytic species, was optimized over the range 1.5×10^{-7} – 9.0×10^{-7} M under the following conditions: $[\text{Fe}(\text{CN})_6]^{4-} = 5.5 \times 10^{-4}$ M, $[\text{DPQ}] = 2.5 \times 10^{-3}$ M, $\text{pH} = 3.0 \pm 0.02$, Temperature = $25 \pm 0.1^\circ\text{C}$, Ionic strength = 0.05 M

A linear relationship between absorbance (measured after 10 min) and silver(I) concentration was observed within the concentration range 4.5×10^{-7} – 9.5×10^{-6} M. At catalyst concentrations lower than this range, the reaction proceeded too slowly for accurate measurements, whereas higher concentrations reduced the analytical sensitivity towards cysteine because the inhibitory effect became proportionally smaller. Therefore, a catalyst concentration of 7.5×10^{-7} M Ag(I) was selected as the optimum concentration for subsequent analytical determinations.

3.7 Discussion of the Optimized Conditions

The optimized experimental conditions ensured high sensitivity, good reproducibility, and excellent linearity for the kinetic determination of cysteine. The observed dependence of reaction rate on pH, reagent concentration, catalyst concentration, temperature, and ionic strength supports the proposed catalytic ligand substitution mechanism involving transient catalyst-substrate complex formation. Furthermore, the pronounced inhibitory effect produced by cysteine confirms its strong coordination affinity towards Ag(I), which effectively decreases the concentration of catalytically active silver species available for ligand substitution. These optimized conditions formed the basis for the subsequent kinetic and mechanistic investigations discussed in the following section.

4 KINETIC ROLE OF THE INHIBITING LIGAND

The analytical method proposed in the present investigation is based on the competitive inhibition of the Ag(I)-catalysed ligand substitution reaction between hexacyanoferrate(II) and dipyrrodo[3,2-f:2',3'-h]quinoxaline (DPQ) by cysteine. Owing to the presence of a highly nucleophilic thiol (-SH) group, cysteine exhibits a strong affinity for soft metal ions such as Ag(I), leading to the formation of stable silver–cysteine complexes. Consequently, a fraction of the catalyst becomes unavailable for catalytic ligand substitution, thereby decreasing the reaction rate [18–21].

The inhibition process is assumed to be reversible and competitive in nature because both the substrate and inhibitor compete for the same catalytically active silver species. Similar kinetic behaviour has been widely reported for metal-ion catalysed analytical reactions involving sulfur-containing ligands [22–24].

The proposed reaction mechanism is illustrated in Figure 2, where Ag(I) initially forms a catalyst–substrate intermediate with hexacyanoferrate(II). The coordinated cyanide ligand is subsequently displaced by DPQ in the rate-determining step to form the coloured product, $[Fe(CN)_5DPQ]^{3-}$. Simultaneously, cysteine competes with the substrate by forming a catalyst–inhibitor complex (Ag–Cys), thereby reducing the concentration of free catalyst available for the substitution reaction.

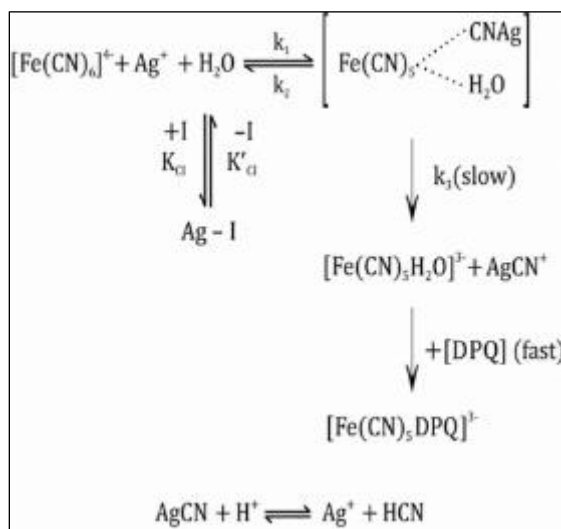


Figure 2: The proposed reaction mechanism of the inhibition reaction

4.1 Kinetic Treatment

Since hexacyanoferrate(II) was employed under non-rate-limiting excess conditions, its concentration may be considered effectively constant during the initial stages of the reaction. Following the Michaelis–Menten treatment commonly employed for catalytic analytical systems, the reaction rate in the absence of inhibitor can be expressed as

$$V_0 = \frac{V_{\max}[S_0]}{K_m + [S_0]}$$

where

- V_0 = initial reaction rate,
- $V_{\max}$ = maximum attainable reaction rate,
- K_m = Michaelis–Menten constant,
- $[S_0]$ = initial substrate concentration.

Linearization of the above equation using the Lineweaver–Burk transformation gives

$$\frac{1}{V_0} = \frac{K_m}{V_{\max}} \frac{1}{[S_0]} + \frac{1}{V_{\max}}$$

which predicts a straight-line relationship between $1/V_0$ and $1/[S_0]$. The intercept yields $1/V_{\max}$, whereas the slope provides the value of the Michaelis–Menten constant.

4.2 Reaction in the Presence of Cysteine

When cysteine is introduced into the reaction system, it forms a reversible catalyst–inhibitor complex with silver(I). Consequently, the apparent Michaelis–Menten constant increases while the maximum reaction rate remains essentially unchanged, which is characteristic of competitive inhibition.

$$V_i = \frac{V_{\max}[S_0]}{K_m' + [S_0]}$$

where

- V_i = reaction rate in the presence of inhibitor,
- K_m' = apparent Michaelis–Menten constant.

The apparent Michaelis constant is related to the catalyst–inhibitor equilibrium constant according to

$$K_m^{\text{app}} = K_m \left(1 + \frac{[I]}{K_{CI}^{\text{app}}} \right)$$

where

- $[I]$ = inhibitor concentration,
- K_{CI}^{app} = dissociation constant of the Ag(I)-cysteine complex.

Substituting the above expression into the Michaelis–Menten equation gives

$$V_i = \frac{V_{\max}[S_0]}{K_m \left(1 + \frac{[I]}{K_{CI}^{\text{app}}} \right) + [S_0]}$$

which adequately describes the observed inhibition behaviour. Linearization of this equation yields the modified Lineweaver–Burk relationship

$$\frac{1}{V_i} = \frac{K_m}{V_{\max}} \left(1 + \frac{[I]}{K_{CI}^{\text{app}}} \right) \frac{1}{[S_0]} + \frac{1}{V_{\max}}$$

Thus, experimental evaluation of the slope and intercept enables determination of both the Michaelis–Menten constant and the catalyst–inhibitor equilibrium constant.

4.3 Determination of the Michaelis–Menten Constant

The reciprocal plot of $1/V_0$ versus $1/[S_0]$ obtained under optimized experimental conditions is presented in Figure 3.

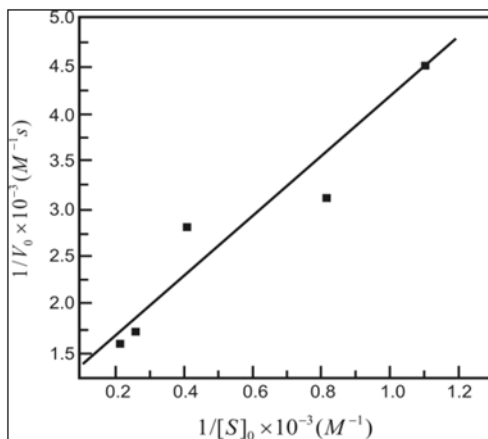


Figure 3: Lineweaver–Burk Plot for the Ag(I)-Catalysed Reaction.)

The plot exhibited excellent linearity, confirming the applicability of Michaelis–Menten kinetics to the present catalytic system. From the slope and intercept, the Michaelis–Menten constant was calculated to be $\log K_m = 1.86 \pm 0.02$. The relatively small value of K_m indicates a strong interaction between the catalyst and substrate, facilitating efficient formation of the activated catalyst–substrate complex required for ligand substitution [20,23].

4.4 Determination of the Catalyst–Inhibitor Equilibrium Constant

To evaluate the strength of interaction between silver(I) and cysteine, the modified Lineweaver–Burk equation was applied by plotting the appropriate reciprocal function against inhibitor concentration under constant substrate conditions. The resulting straight-line plot is shown in Figure 4.

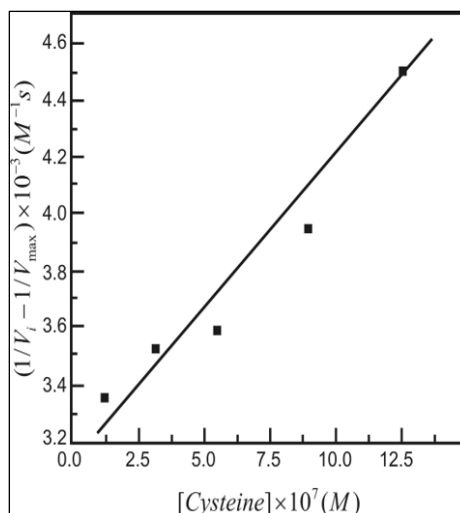


Figure 4: Determination of K_{CI} for the Ag(I)-Cysteine Complex.

The intercept of the plot again yielded the Michaelis constant, whereas the slope provided the equilibrium constant for formation of the Ag(I)-cysteine complex. The excellent linearity obtained confirms that cysteine behaves as a competitive inhibitor by reversibly binding to Ag(I), thereby reducing the concentration of catalytically active silver species. Similar inhibition mechanisms have been reported for sulfur-containing amino acids interacting with soft metal ions in homogeneous catalytic systems [18–24].

Table 2: Conditional Stability Constants Obtained Under Optimized Experimental Conditions

System	Parameter	Value
Ag(I)–Cysteine Complex	log KCl	4.87 ± 0.02
Michaelis Constant	log Km	4.86 ± 0.02
Catalyst–Substrate Complex	log Ks	2.91 ± 0.03

The large value of the catalyst–inhibitor stability constant demonstrates the exceptionally strong affinity of cysteine towards silver(I). This observation agrees well with the well-established preference of soft Lewis acids such as Ag(I) for sulfur donor ligands according to the Hard and Soft Acids and Bases (HSAB) principle [19,25].

Furthermore, the reciprocal of the experimentally determined Michaelis constant closely approximates the catalyst–substrate stability constant (K_s), indicating that formation of the catalyst–substrate intermediate is rapid and reversible prior to the rate-determining ligand substitution step.

The kinetic parameters obtained from the present investigation therefore strongly support the proposed reaction mechanism involving reversible catalyst sequestration by cysteine. The inhibition follows classical competitive kinetics, validating the applicability of the Michaelis–Menten approach for quantitative determination of cysteine in trace concentrations.

The developed kinetic model not only explains the analytical behaviour of the system but also provides valuable mechanistic insight into the interaction of sulfur-containing amino acids with silver(I)-catalysed ligand substitution reactions.

4.5 Analytical applications

The analytical applicability of the proposed kinetic spectrophotometric method was evaluated by analysing synthetic mixtures containing known concentrations of L-cysteine in the presence of potential interfering sulfur-containing species. Recovery experiments were performed under the optimized experimental conditions to assess the accuracy, precision, and reliability of the developed procedure.

The determination is based on the decrease in the catalytic reaction rate caused by the formation of a stable Ag(I)-cysteine complex. Since the extent of inhibition is directly proportional to cysteine concentration, the concentration of cysteine can be accurately determined from the corresponding calibration curve obtained using the fixed-time method.

The optimized experimental conditions ensured high sensitivity and reproducibility while minimizing interference from other reaction components [22–24].

To validate the analytical method, four synthetic mixtures containing varying concentrations of cysteine and sulfide ions were prepared and analysed. The results are presented in Table 3.

Table 3: Determination of Cysteine in Synthetic Mixtures

Synthetic Mix.	Composition (μM)	Found (μM)	Recovery (%)
SM ₁	Cysteine (0.30) + S ²⁻ (0.30)	0.30	100.0
SM ₂	Cysteine (0.30) + S ²⁻ (1.00)	0.32	102.2
SM ₃	Cysteine (0.60) + S ²⁻ (1.00)	0.61	101.7
SM ₄	Cysteine (0.90) + S ²⁻ (1.00)	0.92	102.2

Experimental Conditions: $[\text{Fe}(\text{CN})_6]^{4-} = 3.0 \times 10^{-3} \text{ M}$, $[\text{DPQ}] = 2.0 \times 10^{-3} \text{ M}$, $[\text{Ag}^+] = 7.5 \times 10^{-7} \text{ M}$, pH = 3.0 ± 0.02 , Temperature = $25.0 \pm 0.1^\circ\text{C}$, Ionic Strength = 0.05 M (KNO_3)

The recoveries obtained ranged from 100.0% to 102.2%, indicating excellent analytical accuracy. The small deviations from the added concentrations are well within acceptable analytical limits and demonstrate that the proposed method is reliable for trace-level determination of cysteine. The presence of sulfide ions at concentrations considerably higher than cysteine produced negligible interference under the selected experimental conditions. This observation confirms that the optimized reaction conditions favour selective complex formation between silver(I) and cysteine, thereby ensuring satisfactory selectivity for analytical applications.

The proposed procedure offers several practical advantages over many instrumental techniques currently employed for cysteine analysis. Unlike chromatographic and electrochemical methods, the present method requires only a UV–Visible spectrophotometer, readily available laboratory reagents, and simple experimental procedures. Consequently, it provides an economical alternative for routine analysis in teaching laboratories, research institutions, and quality-control laboratories [4,7,22].

The analytical figures of merit obtained during the present investigation further support the applicability of the proposed method. The method exhibited a detection limit of approximately $7.8 \times 10^{-7} \text{ M}$, allowing the determination of cysteine at sub-micromolar concentrations. The calibration plots showed excellent linearity within the investigated concentration range, while repeated measurements demonstrated satisfactory precision and reproducibility.

The high sensitivity achieved may be attributed to the exceptionally strong interaction between the sulfhydryl group of cysteine and Ag(I), resulting in efficient catalyst inhibition. Since the absorbance is measured at a fixed reaction time rather than at equilibrium, very small changes in reaction rate are amplified, leading to enhanced analytical sensitivity—a characteristic advantage of kinetic analytical methods [9–11,23].

Overall, the proposed kinetic spectrophotometric method provides a simple, rapid, inexpensive, and sensitive approach for the determination of trace levels of cysteine without requiring sophisticated instrumentation or complicated sample pretreatment. The method may therefore serve as a useful alternative for routine laboratory analysis and could potentially be extended to other sulfur-containing biomolecules exhibiting similar metal-binding behaviour.

5 CONCLUSION

A simple, rapid, and sensitive kinetic spectrophotometric method has been successfully developed for the determination of trace amounts of L-cysteine based on its inhibitory effect on the silver(I)-catalysed ligand substitution reaction between hexacyanoferrate(II) and dipyrido[3,2-f:2',3'-h]quinoxaline (DPQ). The reaction was monitored by measuring the formation of the coloured complex $[\text{Fe}(\text{CN})_5\text{DPQ}]^{3-}$ at 547 nm under optimized experimental conditions.

Systematic optimization of reaction variables including pH, temperature, ionic strength, catalyst concentration, and reagent concentrations resulted in excellent analytical sensitivity and reproducibility. The optimum conditions were found to be pH 3.0 ± 0.02 , temperature $25.0 \pm 0.1^\circ\text{C}$, and ionic strength 0.05 M maintained using potassium nitrate.

The inhibitory effect of cysteine was explained on the basis of competitive complex formation between Ag(I) and the thiol group of cysteine. The experimentally determined kinetic parameters, including the Michaelis–Menten constant (K_m), catalyst–substrate stability constant (K_s), and catalyst–inhibitor equilibrium constant (K_{CI}), strongly supported

the proposed reaction mechanism involving reversible catalyst sequestration by cysteine. The observed kinetic behaviour was found to obey the modified Michaelis–Menten model for competitive inhibition.

The developed analytical procedure exhibited excellent accuracy, with recoveries ranging from 100% to 102% for synthetic mixtures, confirming its reliability for quantitative analysis. The detection limit achieved was in the sub-micromolar range (7.8×10^{-7} M), demonstrating the high sensitivity of the method.

Compared with sophisticated chromatographic, electrochemical, and mass spectrometric techniques, the proposed kinetic method offers several practical advantages, including low operational cost, simple instrumentation, rapid analysis, and straightforward experimental procedures. These characteristics make the method particularly suitable for routine analytical laboratories and educational institutions where advanced instrumentation may not be readily available.

The kinetic approach presented in this study also provides valuable mechanistic insight into the interaction of sulfur-containing amino acids with soft transition-metal catalysts. Furthermore, the methodology may be adapted for the determination of other biologically and environmentally important sulfur-containing compounds that exhibit strong metal-binding properties.

In conclusion, the developed Ag(I)-catalysed kinetic spectrophotometric method represents an effective, reliable, and economical analytical technique for trace cysteine determination and contributes to the expanding application of catalytic kinetic methods in modern analytical chemistry.

REFERENCES

- [1] Brosnan, J.T.; Brosnan, M.E. Sulfur-containing amino acids: An overview. *Journal of Nutrition*. 2020, 150, 2511–2517.
- [2] Paul, B.D.; Snyder, S.H. The unusual amino acid cysteine in physiology and disease. *Nature Reviews Molecular Cell Biology*. 2020, 21, 9–23.
- [3] Stipanuk, M.H.; Ueki, I. Degradation of cysteine and regulation of sulfur amino acid metabolism. *Annual Review of Nutrition*. 2021, 41, 287–309.
- [4] Li, H.; Zhang, X.; Wang, Y. Recent advances in analytical methods for cysteine determination. *TrAC Trends in Analytical Chemistry*. 2023, 165, 117177.
- [5] Wang, Y.; Chen, L.; Zhao, J. Electrochemical sensing strategies for biothiol detection: Recent advances and future perspectives. *Biosensors*. 2024, 14, 88.
- [6] Zhao, X.; Liu, H.; Chen, M. Fluorescent probes for selective detection of cysteine, homocysteine and glutathione. *ACS Sensors*. 2022, 7, 1894–1912.
- [7] Kumar, S.; Verma, R.; Singh, A. Spectrophotometric determination of sulfur-containing biomolecules: Recent developments. *Microchemical Journal*. 2021, 170, 106686.
- [8] Zhang, L.; Li, Y.; Sun, Q. Advances in chromatographic determination of biological thiols. *Journal of Chromatography A*. 2025, 1742, 465782.
- [9] Pérez-Bendito, D.; Silva, M. *Kinetic Methods in Analytical Chemistry*. Ellis Horwood Ltd., Chichester, UK, 1988.
- [10] Mottola, H.A. *Kinetic Aspects of Analytical Chemistry*. John Wiley & Sons, New York, USA, 1988.
- [11] Naik, R.M.; Prasad, S. Recent developments in kinetic catalytic analytical methods. *Critical Reviews in Analytical Chemistry*. 2021, 51, 327–349.
- [12] Bugarčić, Ž.D.; Soldatović, T.; Van Eldik, R. Ligand substitution reactions of transition-metal complexes: Recent mechanistic advances. *Coordination Chemistry Reviews*. 2021, 438, 213889.
- [13] van Eldik, R. Mechanisms of ligand substitution reactions in coordination chemistry. *Coordination Chemistry Reviews*. 2022, 451, 214264.
- [14] Housecroft, C.E.; Sharpe, A.G. *Inorganic Chemistry*, 6th ed.; Pearson Education: London, UK, 2024.
- [15] Miessler, G.L.; Fischer, P.J.; Tarr, D.A. *Inorganic Chemistry*, 6th ed.; Pearson Education, 2024.
- [16] Greenwood, N.N.; Earnshaw, A. *Chemistry of the Elements*, 3rd ed.; Butterworth-Heinemann: Oxford, UK, 2023.
- [17] Atkins, P.; de Paula, J.; Keeler, J. *Atkins' Physical Chemistry*, 12th ed.; Oxford University Press: Oxford, UK, 2023.

- [18] Wang, Y.; Li, X.; Chen, H. Silver(I)-thiol coordination chemistry and its biological implications. *Coordination Chemistry Reviews*. 2023, 476, 214905.
- [19] Pearson, R.G. Hard and Soft Acids and Bases (HSAB) principle: Modern applications in coordination chemistry. *Chemical Reviews*. 2022, 122, 1542–1598.
- [20] van Eldik, R.; Hubbard, C.D. Mechanistic aspects of ligand substitution reactions in transition-metal complexes. *Coordination Chemistry Reviews*. 2022, 454, 214341.
- [21] Soldatović, T.; Bugarčić, Ž.D. Mechanistic investigations of silver(I)-mediated ligand substitution reactions. *Dalton Transactions*. 2021, 50, 10344–10358.
- [22] Naik, R.M.; Prasad, S. Applications of catalytic kinetic methods in trace analysis. *Critical Reviews in Analytical Chemistry*. 2021, 51, 350–371.
- [23] Lineweaver, H.; Burk, D. The determination of enzyme dissociation constants. *Journal of the American Chemical Society*. 1934, 56, 658–666.
- [24] Michaelis, L.; Menten, M.L. Die Kinetik der Invertinwirkung. *Biochemische Zeitschrift*. 1913, 49, 333–369.
- [25] CRC Handbook of Chemistry and Physics, 106th ed.; CRC Press: Boca Raton, FL, USA, 2025.