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Quantification of alpha-lipoic acid in pharmaceutical samples using Pd(II) catalyzed ligand substitution reaction in SLS micellar medium

Abhishek SRIVASTAVA [1] *, Neetu SRIVASTAVA [2], Ruchi SINGH [3]

[1] Department of Chemistry, GLA University, Mathura, 281406, U.P., India.
[2] Department of Chemistry, DDU Gorakhpur University, Gorakhpur, 273001, U.P., India.
[3] Department of Chemistry, B. N College of Engineering & Technology, Lucknow, U.P., India.
* Corresponding Author. E-mail: aabhichem@gla.ac.in (A.S.); Tel. +91-962-796 90 85.

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ABSTRACT: A novel, repeatable, and swift kinetic approach for determining alpha-lipoic acid (ALA) in sodium lauryl sulfate (SLS) micellar medium has been presented, and it has been connected to ALA determination in drug formulations. The approach is based on ALA inhibitory property. ALA (containing two sulfur atoms) forms a chelate with Pd^{2+}, lowering the effective [Pd(II)], and ultimately, the Pd^{2+} catalyzed cyanide substitution rate from $[Ru(CN)_6]^{4-}$ by pyrazine (Pz). Fixed times of 5 and 10 minutes were chosen under optimal reaction conditions with [Pyrazine] = 3.0×10^{-4} mole L^{-1}, pH = 4.0 ± 0.02, Temp = 318 ± 0.2 K, I = 0.1 mole L^{-1} ($NaClO_4$), $[Ru(CN)_6^{4-}] = 2.75 \times 10^{-5}$ mole L^{-1}, $[Pd^{+2}] = 6.0 \times 10^{-5}$ mole L^{-1}, and [SLS] = 8.5×10^{-3} mole L^{-1} to calculate the absorbance at 370 nm associated with the final substitution product $[Ru(CN)_5\ Pz]^{3-}$. ALA's inhibiting influence on the Pd^{2+} catalyzed cyanide substitution with pyrazine from $[Ru(CN)_6]^{4-}$, has been represented by a modified mechanistic approach. The concentration of ALA in various water specimens can be measured at the micro-level down to 1.25×10^{-6} mole L^{-1} using the established kinetic spectrophotometric approach. The suggested method is highly reproducible and has been effectively applied to accurately quantify the ALA in pharmaceutical samples. Even as much as 1000 with [ALA], typical additives used in medications do not significantly hinder the determination of ALA.

KEYWORDS: Inhibitory effect; Alpha-lipoic acid; Surfactant medium; Pharmaceutical preparations; Pd(II); Excipients.

1. INTRODUCTION

The body naturally produces alpha-lipoic acid (ALA), often referred to as thioctic acid (TA), which is necessary for the operation of certain oxidative metabolic enzymes [1,2]. Its main function is to use oxygen to turn blood sugar (glucose) into energy [3,4]. ALA is a sulfur-containing short-chain fatty acid (Fig. 1). ALA is a powerful antioxidant with numerous metabolic benefits such as antiobesity, glucose reduction, insulin sensitization, and cholesterol reduction. ALA is biologically significant and possesses anti-inflammatory, detoxifying, neuroprotective, anti-aging, and cardiovascular properties [5-9]. ALA is a good contender for metal detoxification because it can efficiently and selectively bind the majority of divalent metal ions both in vitro and vivo via different mechanistic pathways to form organometallic complexes. Due to the existence of two sulfur with lone pairs of electrons, ALA can form chelates with metal ions.

Figure 1. Structure of alpha lipoic acid

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Due to their surface-active characteristics, surfactants are frequently employed in modern industries [10-12]. Surfactants' amphiphilic structure, which includes both a tail (hydrophobic) and head (hydrophilic), is what gives them their surface activity [13]. At modest concentrations, the water-based solution of the surfactant behaves as an electrolyte. Micellization takes place in an aquatic environment because one substrate contains both hydrophilic and hydrophobic components. The critical micelle concentration (CMC) is the typical concentration of surfactant at which micellization initiates automatically [14]. Due to the repulsive and attractive interactions that exist between surfactant molecules, the molecules will self-associate beyond CMC and constitute micelles of different shapes and sizes. At 298 K, the CMC value of the anionic surfactant sodium lauryl sulfate (SLS) ranges from 8.0×10^{-3} M to 8.5×10^{-3} M [15-17]. In comparison to pure solvents, micelle-bound reactants experience a totally distinct reaction environment. The extent of substrate interaction with the micelle aggregates in a micellar medium determines the reaction rate.

Sulfur has remained the dominant heteroatom for centuries in the wide range of fungicides, insecticides, and bioactive compounds. Organosulfur compounds function specifically as structural proteins and enzymes in various metabolic processes [18-20]. Analytical chemists are constantly sought after by the pharmaceutical sector for the most effective technology for detecting and quantifying sulfur-containing bioactive compounds and medicines in various samples. The basic importance and applicability of the oxidation-reduction/ligand exchange processes of complexes containing transition metals in analytical, organometallic, and synthetic chemistry encouraged a large number of scientists to explore their kinetics [21-24]. Kinetic analyses of the Fe(II) and Co(II) complexes oxidation and metal-catalyzed cyanide substitution from [Ru/Fe(CN)6]3- by nitrogen heterocyclic ligands have been reported by a number of authors [25-27]. The aforementioned processes have also successfully been employed for the trace-level evaluation of catalysts and medications/moieties that have a profound affinity for catalysts [28-30].

Different methods are frequently employed to quantify thio compounds in analytical, biological, and pharmacological materials. The quantifica,tion methodology includes potentiometry [31], spectrophotometry [32,33], chromatography [34-36], fluorimetry [37], NMR-spectrometry [38], flow injection analysis [39], colorimetry [29], and voltammetry [40,41]. Table 1 describes the various analytical methods used for the determination of ALA with their detection limit. The aforementioned techniques may have a number of limitations, including a significant initial investment, a lengthy process, heavy equipment, and an elevated cost for the analysis of samples. A relatively small number of investigations have been conducted using the kinetic approach, which only requires a UV-Visible spectrophotometer to quantify thio compounds [28-30,42].

Table 1. Analytical methods used for quantification of ALA

Method	Linearity Range	Detection Limit	Reference
Voltammetric sensor based on CeO_2-Fe_2O_3 nanoparticles	0.075–7.5 µM	0.053 µM	31
Spectrophotometric	7.5×10^{-6} to 1×10^{-4} M	1.25×10^{-5} M	32
Spectrophotometric	------	30 ng/ml	33
HPLC-UV	2-30 µg/ml	500 ng/ml	34
HPLC	0.1-20 µmol/L	0.05 µmol/L	35
HPLC-UV	0.12-5.0 nmol/ml	0.08 nmol/ml	36
Differential pulse voltammetry	5.82×10^{-8} to 4.00×10^{-4} mol/l	1.94×10^{-8} mol/l	40
HPLC using electrochemical detection	0.01-50 µg/L	1 ng/ml	41
Our method (Spectrophotometric)	1.25×10^{-6} to 5.0×10^{-5} mole/L	1.25 µM	

Several publications have demonstrated metal-catalyzed Ag(I)/Hg(II)/Pd(II), cyanide exchange by heterocyclic ligands containing nitrogen from Fe(II)/Ru(II) cyano complexes [26,27]. The micro-level evaluation of utilized catalysts and medications or compounds with a potent affinity for a catalysts have also been effectively accomplished using these reactions [28-30]. Through the formation of a stable complex with Pd^{2+}, organosulfur compounds significantly reduce the catalytic effectiveness of Pd(II), which reduces the exchange of cyanide to a significant extent. This inhibiting property of thio moieties (containing sulfur as S-, -S-, and -SH) can be employed for their quantification at trace levels utilizing the kinetic spectrophotometric approach. ALA (containing two "S") similarly reduces Pd(II) catalytic efficacy, lowering the cyanide exchange rate from $[Ru(CN)_6]^{4-}$ by pyrazine. Since the uncatalyzed reaction between $[Ru(CN)_6]^{4-}$ and pyrazine was not

seen at the examined reaction conditions, a more accurate result will be found having the reaction at disposal [43]. Due to the ALAs' potent inhibitory effect on Pd(II)'s catalytic effectiveness, we developed a straightforward, repeatable, and quick kinetic spectrophotometric technique for the micro-level detection of ALA in various water specimens decreased to 1.25×10^{-6} mole L^{-1}. The developed procedure has also been used to quickly and accurately quantify ALA in various commercial samples with excellent consistency. The technique can be used to determine numerous medicines and biological substances at trace levels that have the potential to severely reduce Pd(II)'s catalytic efficacy.

2. RESULTS AND DISCUSSION

There are numerous approaches available for quantifying ALA. The key disadvantages of previous art include sophisticated technique, high startup costs, time-consuming processes, bulky instrumentation, and expensive sample analysis. The proposed kinetic technique will provide a solution to issues with complicated methodologies, high initial costs, lengthy processes, and heavy instrumentation for ALA quantification. The procedure is inexpensive because it only needs a visible spectrophotometer and cheap reagents. The developed method for determining ALA is straightforward, quick, and highly repeatable.

The reaction involving pyrazine and $[Ru(CN)_6]^{4-}$, which is accelerated by Pd(II), resulting in the final substitution product $[Ru(CN)_5\ Pz]^{3-}$. By analyzing the end reaction product's mole ratio and slope ratio, it was determined that the reaction between pyrazine and $[Ru(CN)_6]^{4-}$ occurs in a 1:1 mole ratio [43]. No modification to the absorption values was made because, with the exception of the ultimate reaction product, none of the reacting solutions displayed any discernible absorption at the examined wavelength. The prominent absorption band at 370 nm corresponds to the finished product $[Ru(CN)_5\ Pz]^{3-}$ (spectra not shown).

2.1. Kinetic quantification of alpha-lipoic acid (ALA)

The prior research on acetylcysteine, thioglycolic acid, D-penicillamine, and other compounds that include sulfur acknowledges that the cyanide substitution rate from $[Ru(CN)_6]^{4-}$ by nitrogen heterocyclic ligands, accelerated by Hg(II) is reduced by the sulfur compounds [28-30]. The effective concentration of Hg(II) decreases due to the stable complex formation between the Hg(II) and added sulfur compounds, which ultimately leads to a slower reaction rate. By the same token, ALAs (containing two sulfur atoms) also forms a chelate with Pd^{2+}, lowering the effective [Pd(II)], and ultimately, the substitution rate. At a set period (5 and 10 minutes after reactant mixing), the absorbance (A_t), associated with the finished substitution product $[Ru(CN)_5\ Pz]^{3-}$, with altering [ALA], was documented. ALA was quantified using absorbance against [ALA] plot (calibration curve) that was found linear in the 1.25×10^{-6} to 5.0×10^{-5} mole L^{-1} ALA concentration band (Figure 2). Eqs. 1 and 2 express the regression line linking A_t and [ALA].

$$A_5 = 0.1448 - 2.43 \times 10^3\ [ALA] \quad (1)$$

$$A_{10} = 0.1835 - 2.78 \times 10^3\ [ALA] \quad (2)$$

For the A_5 and A_{10} plots (At against [ALA]), the computed linear regression coefficient and standard deviation were 0.9978, 0.9986, and 0.0020, 0.0018, respectively. After 5 min of injecting the estimated quantity of ALA into the reaction system, the absorbance was recorded. The amount of recovered ALA, estimated using the calibration curve is documented in Table 2. The recovered ALA validates the method's repeatability and accuracy.

A modified mechanistic approach (equations 3–7) established ALA's inhibitory influence on Pd^{2+} accelerated cyanide substitution from $[Ru(CN)_6]^{4-}$ by pyrazine. The hypothesized mechanism is similar to the enzyme-catalyzed process in the presence of an inhibitor.

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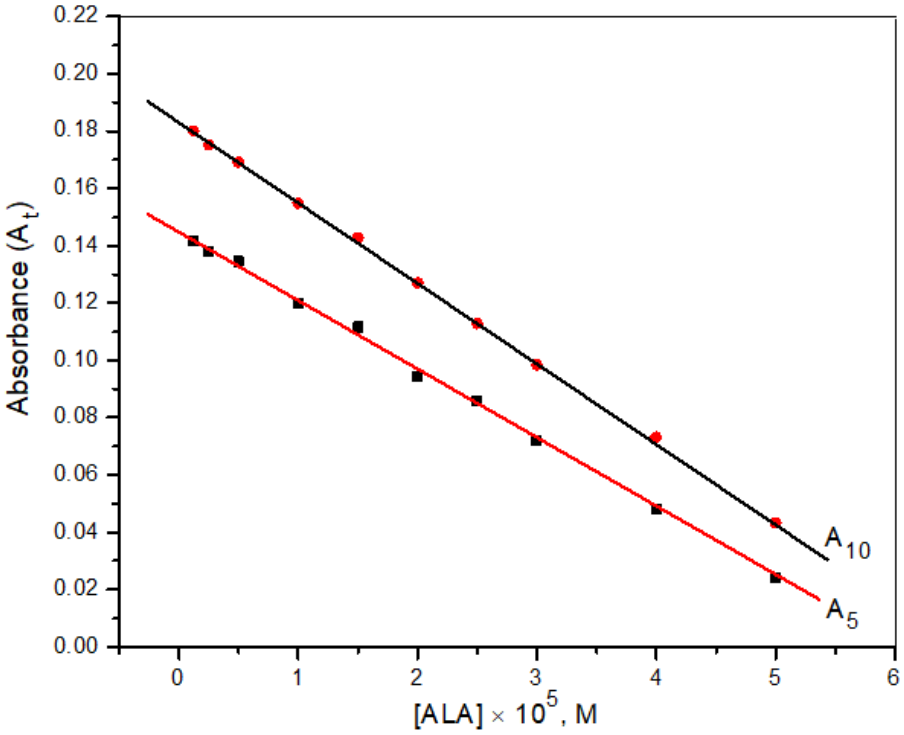

Figure 2. Calibration curve for the determination of alpha-lipoic acid at [Pyrazine] = 3.0×10^{-4} mole L^{-1}, pH = 4.0 ± 0.02, Temp = 318 ± 0.1 K, $[Ru(CN)_6^{4-}] = 2.75 \times 10^{-5}$ mole L^{-1}, I = 0.10 mole L^{-1} ($NaClO_4$), [Pd(II)] = 6.0×10^{-4} mole L^{-1}, and [SLS] = 8.5×10^{-3} mole L^{-1}

Table 2. ALA quantification versus the added ALA

[ALA]×10^5 mole L^{-1} (Taken)	A_5		A_{10}	
	[ALA]×10^5 mole L^{-1} (Found)	Error	[ALA]×10^5 mole L^{-1} (Found)	Error
0.40	0.38 ± 0.046	- 0.050	0.42 ± 0.025	+ 0.025
0.60	0.60 ± 0.051	0.000	0.58 ± 0.018	- 0.033
1.35	1.37 ± 0.068	+ 0.015	1.35 ± 0.031	0.000
1.75	1.71 ± 0.064	- 0.023	1.78 ± 0.082	+ 0.017
2.25	2.28 ± 0.082	+ 0.013	2.29 ± 0.059	+ 0.018
3.00	3.00 ± 0.074	0.000	2.94 ± 0.047	- 0.02
3.75	3.78 ± 0.066	+ 0.008	3.71 ± 0.082	- 0.011
4.50	4.46 ± 0.049	- 0.009	4.56 ± 0.063	+ 0.013

Experimental Condition: [Pyrazine] = 3.0×10^{-4} mole L^{-1}, pH = 4.0 ± 0.02, Temp = 318 ± 0.1 K, $[Ru(CN)_6^{4-}] = 2.75 \times 10^{-5}$ mole L^{-1}, I = 0.10 mole L^{-1} ($NaClO_4$), [Pd(II)] = 6.0×10^{-4} mole L^{-1}, and [SLS] = 8.5×10^{-3} mole L^{-1}

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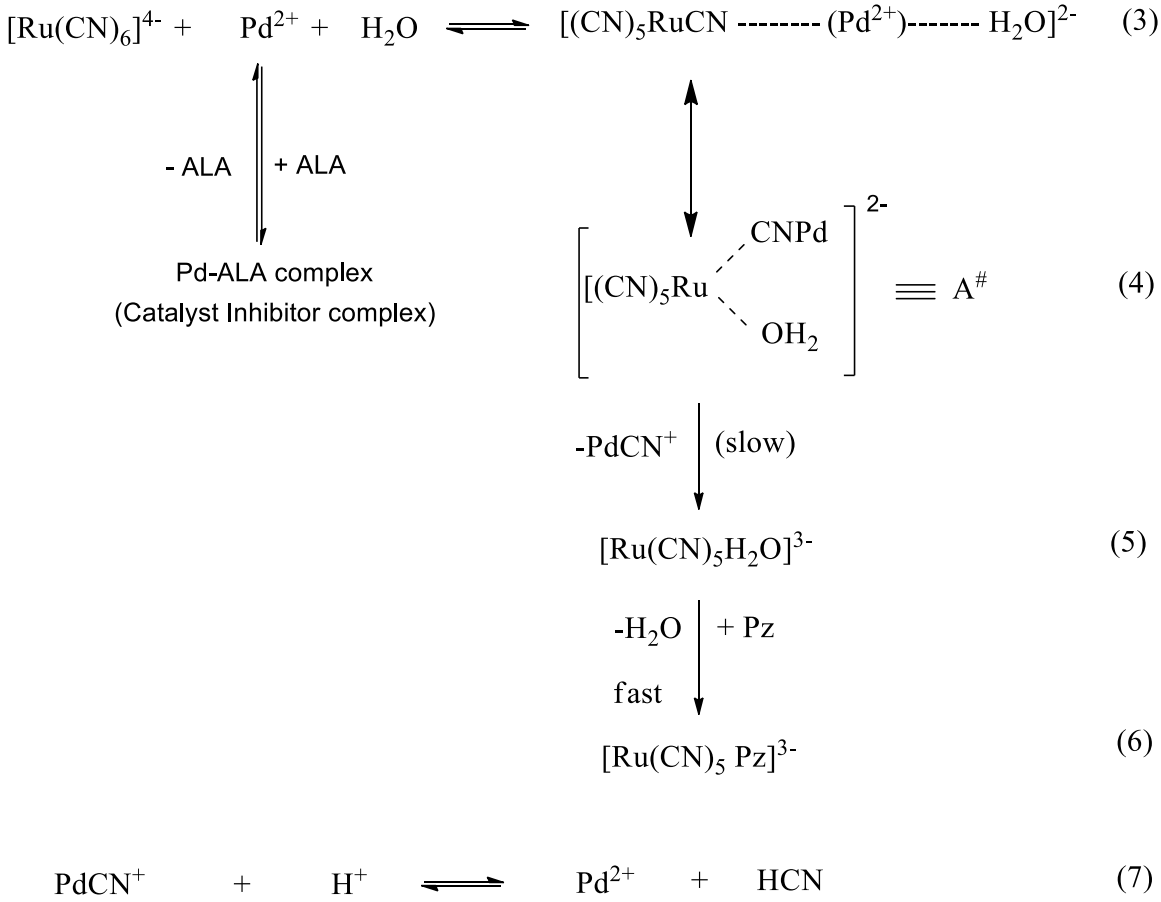

$$PdCN^{+} + H^{+} \rightleftharpoons Pd^{2+} + HCN \qquad (7)$$

Taking into account hexacyanoruthenate(II) as a sole substrate having initial concentration S_o. The catalyzed reaction rate can be developed in parallel with the enzyme-catalyzed process when an inhibitor (ALA) is present. The catalyzed reaction's rate (V_o) during ALA absence is represented by equation 8.

$$V_o = \frac{V_{max}}{1 + \frac{K_m}{[S_o]}} \qquad (8)$$

Here, V_{max} and K_m represent the optimum rate at higher reactant concentrations and, correspondingly, the Michaelis-Menten constant. The above equation's straight line version (1/Vo versus 1/[So]), given by Equation 9, is consistent with the Lineweaver-Burk expression [44]. It has a slope (K_m/V_{max}), intercept ($1/V_{max}$), and linear regression coefficient of 0.9973.

$$\frac{1}{V_o} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \frac{1}{[S_o]} \qquad (9)$$

Figure 3's slope and intercept were used to compute the Km value, which came out at 0.2453 ± 0.011 mM.

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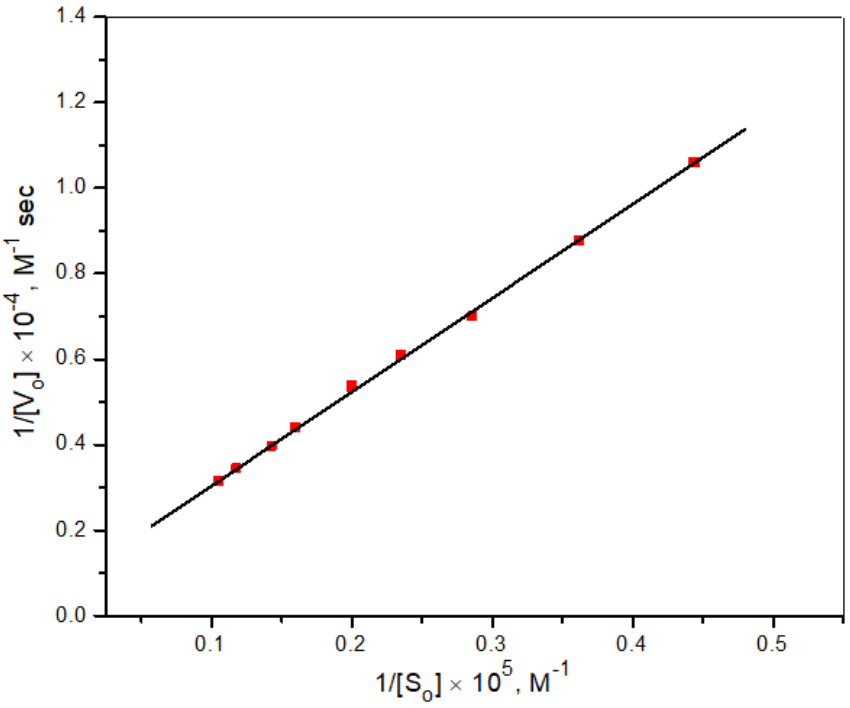

Figure 3. The Lineweaver-Burk plot at constant [Pd^{2+}] in the absence of ALA at [Pyrazine] = 3.0 × 10^{-4} mole L^{-1}, pH = 4.0 ± 0.02, Temp = 318 ± 0.1 K, I = 0.10 mole L^{-1} ($NaClO_4$), [Pd(II)] = 6.0 × 10^{-4} mole L^{-1}, and [SLS] = 8.5 × 10^{-3} mole L^{-1}

When the inhibitor is present and the catalyst concentration is constant, the apparent M-M constant "K'_m" can be expressed as:

$$K'_m = K_m\left(1 + \frac{[I_o]}{K'_{CI}}\right)$$

Where initial [ALA] is denoted by I_o, and K'_{CI} is the dissociation constant of the catalyst-inhibitor complex (C-I). Equation 10 can be used to express the initial rate (Vi) in the presence of the inhibitor at fixed Pd(II) concentration [45].

$$V_i = \frac{V_{max}}{1 + \frac{K'_m}{[S_o]}} \qquad (10)$$

$$V_i = \frac{V_{max}}{1 + \frac{K_m}{[S_o]}\left(1 + \frac{[I_o]}{K'_{CI}}\right)} \qquad (11)$$

Equation 12 represents the straight-line variant of Equation 11 according to the Lineweaver-Burk equation.

$$\frac{1}{V_i} - \frac{1}{V_{max}} = \frac{K_m}{[S_o]V_{max}} + \frac{K_m}{[S_o]V_{max}}\frac{[I_o]}{K'_{CI}} \qquad (12)$$

The K'_{CI} and K_m (in the presence of ALA) values were calculated using the slope and intercept of the linear plot between initial [ALA] and $(\frac{1}{V_i} - \frac{1}{V_{max}})$ (Figure 4) and were discovered to be 8.80 × 10^{-5} ± 0.16 and 0.2401± 0.008 mM, respectively. The computed Km value is nearly the same whether ALA is present or absent. The catalyst inhibitor complex appears to be quite stable, as indicated by the reduced dissociation constant value (8.80 × 10^{-5} ± 0.16).

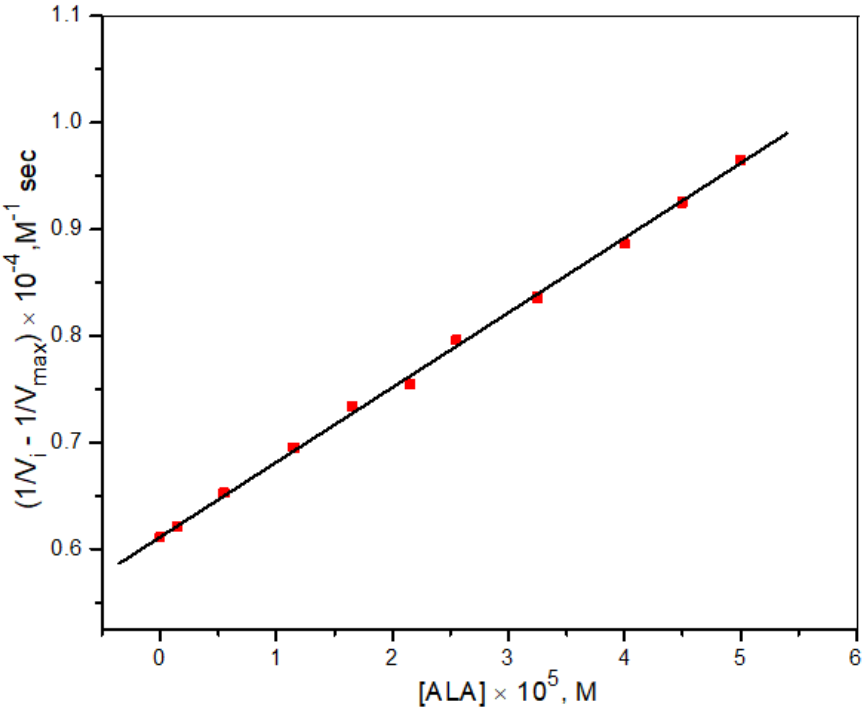

Figure 4. The plot of $(1/V_i - 1/V_{max})$ versus initial [ALA] at [Pyrazine] = 3.0×10^{-4} mole L^{-1}, pH = 4.0 ± 0.02, Temp = 318 ± 0.1 K, $[Ru(CN)_6^{4-}] = 2.75 \times 10^{-5}$ mole L^{-1}, I = 0.10 mole L^{-1} ($NaClO_4$), [Pd(II)] = 6.0×10^{-4} mole L^{-1}, and [SLS] = 8.5×10^{-3} mole L^{-1}

2.2. Interference caused by competing constituents

Excipients are inert substances used in drug formulations as fillers, coloring agents, and preservatives in addition to the active medicinal component. Recovery tests were carried out under optimized reaction conditions utilizing the A_5 calibration line, comprising 4.0 µgml^{-1} ALA and a variety of different excipients to examine the impact of excipients. The recovery findings demonstrate that regular additives included in medications do not significantly hinder the quantification of ALA, even when multiplied by 1,000 (Table 3).

Table 3. Results of ALA recovery in the presence of neutral additives

Excipients	[Excipients] / [ALA]	Recovery ± sd (%)
Maltitol	750	99.8 ± 0.6
Sorbitol	1000	100.6 ± 0.8
Citrate	750	101.6 ± 0.3
Sucrose	1000	99.6 ± 0.7
Magnesium stearate	750	101.5 ± 0.8
Lactose	1000	99.7 ± 0.9
Gelatin	750	100.3 ± 0.8

Experimental Condition: [Pyrazine] = 3.0×10^{-4} mole L^{-1}, pH = 4.0 ± 0.02, Temp = 318 ± 0.1 K, $[Ru(CN)_6^{4-}] = 2.75 \times 10^{-5}$ mole L^{-1}, [ALA] = 2.0×10^{-5} mole L^{-1}, I = 0.10 mole L^{-1} ($NaClO_4$), [Pd(II)] = 6.0×10^{-4} mole L^{-1}, and [SLS] = 8.5×10^{-3} mole L^{-1}

2.3. Application in medicinal formulations

By using the recommended kinetic spectrophotometric approach, the powdered amount of 8 capsules or tablets was dissolved in 100 ml of water to determine the amount of ALA within different medication samples. The resulting solution was then sonicated for 20 minutes, filtered through Whatman filter paper, and diluted to attain the drug concentration into the A_5 calibrating range. The recommended kinetic approach for the measurement of ALA was used for five distinct medications obtained from local pharmacies that only contained ALA and excipients. The outcome has been compared with the approved method (Table 4). The statistical analysis and average recovery (99-101) findings show that the suggested methodology for the ALA determination in pharmaceuticals and water samples is reproducible and accurate [46].

Table 4. Comparison of ALA determination in drug samples with the approved method

Samples of Medicine	Recommended Methodology [Recovery ± SD (%)]	Approved Methodology [Recovery ± SD (%)]
Alafin 600 mg Tablet (Fourrts Labs Pvt. Ltd, Chennai, India)	99.72 ± 0.56	99.92 ± 0.53
Alpha Lipoic Acid 300 mg Tablet (Bliss Lifescience LLP, Indore, India)	99.31 ± 0.21	101.05 ± 0.71
Liplac 600 mg Tablet (Apco Pharm Ltd, New Delhi, India)	100.51 ± 0.82	100.27 ± 0.28
Alpha Lipoic Acid 300 mg Capsule (Medizen Labs Pvt. Ltd, Bengaluru, India)	99.45 ± 0.40	100.27 ± 0.41
Alpha Lipoic Acid 350 mg Capsule (Pharmagenica Healthcare Pvt. Ltd, Bengaluru, India)	100.19 ± 0.35	99.58 ± 0.39
Alpha Lipoic Acid 300 mg Capsule (Inlife Pharma Pvt. Ltd, Hyderabad, India)	101.17 ± 0.46	101.38 ± 0.39
Alpha Lipoic Acid 100 mg Capsule (Zenith Nutrition, Bengaluru, India)	99.67 ± 0.73	100.55 0.66

Experimental Condition: [Pyrazine] = 3.0×10^{-4} mole L^{-1}, pH = 4.0 ± 0.02, Temp = 318 ± 0.1 K, $[Ru(CN)_6^{4-}] = 2.75 \times 10^{-5}$ mole L^{-1}, I = 0.10 mole L^{-1} ($NaClO_4$), [Pd(II)] = 6.0×10^{-4} mole L^{-1}, and [SLS] = 8.5×10^{-3} mole L^{-1}

3. CONCLUSION

For the quantitative determination of ALA, a novel, swift, and consistently repeatable kinetic approach that relies on the inhibitory behavior of the sulfur-containing molecule ALA towards Pd^{2+} has been presented. Even up to 1000 times with [ALA], the general additives used in medications do not significantly hinder the detection of ALA. The suggested kinetic spectrophotometric approach allows for the micro-level measurement of ALA in various water specimens down to 1.25×10^{-6} mole L^{-1}. The optimized methodology was also used to quickly quantify ALA in pharmaceutical samples. The suggested methodology for the determination of ALA in water samples and different drugs is reproducible and accurate, as shown by average recovery (99-101 %) and statistical analysis results. Numerous medicines and biological substances that have the potential to significantly reduce the catalytic activity of Pd(II) can be quantified at micro level using this method.

4. MATERIALS AND METHODS

4.1 Reagent Used

All chemicals that were utilized were pure and of analytical grade. The stock solution of each reagent was directly prepared by weighing its accurate amount and further dissolving it in double-distilled water. To avoid the potential photo-decomposition of $K_4[Ru(CN)_6].3H_2O$ (Sigma-Aldrich), an amber-colored bottle was used to preserve its stock solution. Alpha-lipoic acid (ALA), Pyrazine, and sodium lauryl sulfate were purchased from Himedia and utilized directly. The solution of $PdCl_2$ (Fisher Scientific) was made every day by dissolving its computed amount. $NaClO_4$ (Fisher Scientific) was utilized to control the reaction mixture's ionic strength, while Molechem's NaOH/HCl and potassium hydrogen phthalate was employed for adjusting the reaction medium's pH.

4.2 Instrumentation

Using a DD LAB (model LAB.PHM.66800620) auto digital pH meter, verified with a predefined buffer solution, the pH of the reacting solution was monitored. A double-beam UV-Visible spectrophotometer made by Electronics India, model 2375, was deployed for measuring absorbance at 370 nm.

4.3 Kinetic Procedure

The absorption values were not modified since, with the exception of the final product, none of the reacting solutions exhibit significant absorption at the pertinent wavelength. An optimum reaction setting that showed a considerable change in absorbance at 370 nm was carefully chosen from the reaction's extensive kinetic investigation. Due to the limited solubility of ALA in water, its kinetic quantification was performed

in SLS micellar medium. Following 30 minutes of thermal equilibration at 318 K, all of the reactive solutions were quickly mixed in the sequence: $PdCl_2$, buffer, SLS, pyrazine, $NaClO_4$, and $[Ru(CN)_6]^{4-}$. Immediately following a thorough shaking, the reacting mixture was poured into the spectrophotometric cell. An ingeniously constructed system of circulating water arrangement kept the cell compartment at a constant temperature. The absorbance increase related to the final product was documented. The quantification of ALA was done using a graph (calibration curve) of absorbance versus altering [ALA].

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