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An Inhibitory Kinetic Method for the Methionine Determination

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A fast and reproducible method is proposed for the kinetic determination of methionine (MET). The method depends on the inhibitory property of methionine, which reduces the Hg^{2+} catalyzed imitation of cyanide from $[Ru(CN)_6]^{4-}$ with pyrazine *via* forming a stable complex with Hg^{2+}. Spectrophotometric measurements were carried out by recording the absorbance at 370 nm (λ_{max} of $[Ru(CN)_5Pyrazine]^{3-}$ complex) at a fixed time of 12 min under the optimized reaction conditions with [Pyrazine] = 6.5×10^{-4} M, I = 0.05 M (KNO_3), Temp. = 45.0 ± 0.2 °C, pH = 4.00 ± 0.03, $[Hg^{2+}] = 7.5 \times 10^{-5}$ M and $[Ru(CN)_6^{4-}] = 5.25 \times 10^{-5}$ M [46]. With the proposed method, methionine can be determined quantitatively down to 2.5×10^{-6} M. This methodology can be effectively used for the rapid quantitative estimation of MET in the pharmaceutical samples with good accuracy and reproducibility.

Keywords: Inhibitory effect, Ligand substitution reaction, Catalyst inhibitor complex, Excipients, Pharmaceutical preparations, Hexacyanoruthenate(II)

INTRODUCTION

Methionine, a precursor for the many amino acids plays a vital role in tissue repair and human growth [1, 2]. The sulfur present in methionine is involved in several detoxifying processes, slows skin aging, and is essential for the absorption and bioavailability of selenium and zinc [3]. The complexing action of methionine is used for the excretion of lead and mercury [4]. It also helps to control strong urine odor, used in the treatment of liver disorders and improving wound healing [5-7]. Figure 1 represents the structure of methionine.

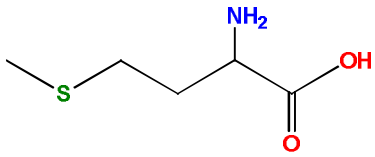

Fig. 1. Structure of methionine.

Sulfur is a vital component of numerous drugs and biological molecules. Sulfur, which is present in the proteins and enzymes of the cell, plays an important role in distinct metabolic processes [8-11]. Therefore, the development of an effective method for the quantitative determination of drugs and bioactive molecules containing sulfur in different samples is also very important and sought after by the pharmaceutical industry. The kinetic inspection and mechanistic description of oxidation/ligand substitution reaction of transition metal complexes in the aqueous environment are of paramount importance [12-15]. Such reactions attracted the attention of many chemists, biologists, and environmentalists due to their immediate applications in trace level determination of various biological molecules, drugs, and heavy metals [16-17]. In this regard, several kinetic studies on the exchange of integrated cyanide with ligand variants containing -O, -P, -S, and -N donor atoms from $[Ru(CN)_6]^{4-}$ were examined by numerous authors [18-19].

Numerous reports are available for the micro-level determination of sulfur-containing drugs and molecules in

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Table 1. Analytical Methods Used for Determination of L-Methionine

Method	Description	Detection limit	Ref.
HPLC	Enzymatic degradation using L-methionine decarboxylase	10-200 μM	[26]
Fluorimetric	Pyrophosphate detection system using ultra-performance liquid chromatography and fluorometer	0.2 μM	[30]
Electrochemical	Direct-current differential electrolytic potentiometry was used	0.1-10 mg	[25]
Potentiometry	Equivalent point is detected directly, potentiometrically, or spectrophotometrically	0.5-5 mg	[24]
HPLC-UV	The superoxide scavenging effect of methionine alone and in presence of paracetamol has been used	50 μg ml^{-1}	[28]
Cyclic voltammetry	The procedure is based on the reaction of methionine reduction to homocysteine,	10 μM	[29]
Voltammetry	Platinum electrode was used as working electrode	50 μM	[31]
Our method	Kinetic Spectrophotometric method based on ligand substitution reaction	2.5 μM	

biological samples and pharmaceutical preparations [20-23]. The determination methods includes potentiometry [24], colorimetry [20], chromatography [25-27], fluorimetry [28], voltammetry [29-31] and spectrophotometry [32, 33]. Table 1 describes the various analytical methods used for the determination of methionine with their detection limit. The high initial investment, time-consuming processes, high costs of sample analysis, and heavy instrumentation are major concerns of many reported methods. Very few kinetic studies have been performed for the determination of thio compounds using various determination methods [34-36].

Complexes of ruthenium with different drugs and bioactive molecules exhibit extensive applications as Immunosuppressant [37], Antileukemic [38], Antifungal [39]. DNA binder [40], Antiamebic [41], Antitumor [42], Anticancer [43] and Antimetastatic [44]. Hg^{2+} and Ag^{+} catalyzed substitution of cyanide with nitrogen-containing heterocyclic ligand form cyano complex of Fe(II) and Ru(II) has been reported by several authors [18, 19]. The substitution of cyanide form $[Fe(CN)_6]^{4-}$ by pyrazine, catalyzed by mercury (II) has been used successfully in the determination of low Hg(II) levels [45]. Because of the formation of a stable complex between Hg^{2+} and organo-sulfur compounds, the added sulfur compounds (having sulfur in S^{-}, SH and -S- form) significantly inhibit the catalytic efficiency of Hg^{2+}, thereby decreasing the catalyzed reaction rate to a considerable extent [34-36]. Such inhibitory properties of thio compounds formed the basis for its micro-level determination using the kinetic method. The formation and stability of various metal-ligand formed during the substitution reaction can be better justified by HSAB (hard-soft acid-base) theory. Due to the strong interaction of MET with Hg(II), the added MET reduces the substitution rate of CN^{-} from hexacyanoruthenate(II). This MET inhibitory feature motivated us to develop a simple and reproducible kinetic method for the rapid low-level determination of MET using a UV-Visible spectrophotometer. The present ligand exchange reaction may generate more authentic results in the determination of MET since the uncatalyzed exchange of CN^{-} with pyrazine does not proceed under the studied reaction condition [46]. The present communication is about the quantitative determination of carbocisteine by an inhibitory kinetic spectrophotometric method in distinct water samples. This technique can also be convincingly applied for the rapid quantitative estimation of MET in pharmaceutical samples.

EXPERIMENTAL

Reagent Used

Reagent-grade chemicals and double distilled water

were used throughout the experiment. Standard solution of $K_4[Ru(CN)_6].3H_2O$ (Sigma-Aldrich) was prepared by its calculated amount and was black covered from the exterior to prevent any photo-degradation. Pyrazine (Merck) and methionine (Sigma-Aldrich) solutions were prepared by dissolving their weighed amount in distilled water. $HgCl_2$ (Merck) solution was prepared daily and the desired dilutions were done before performing the kinetic study as the effective concentration of Hg^{2+} may get reduced by its adsorption on the glass surface. Potassium hydrogen phthalate (Sigma-Aldrich) and NaOH/HCl procured from Sigma-Aldrich were used to manage the pH while ionic strength of the reaction was regulated by 0.1 N KNO_3 (Merck) solution.

Instrumentation and Kinetic Procedure

Labman LMPH-10 digital pH meter, calibrated with predefined buffer solutions was used to check the pH of the reacting solutions. Systronics smart UV-Vis double beam spectrophotometer model-2203 was used for the kinetic study at 370 nm (absorption maximum of the substituted product) by measuring an increase in absorbance. Since the catalyst and all reacting solutions do not exhibit any significant absorption at the studied wavelength, no modification in the recorded absorbance was carried out. The exhaustive kinetic study of the ligand imitation reaction was wisely used to identify the optimum experimental condition, showing a substantial change in absorbance. The kinetic measurements were studied at 45 ℃ by sequential mixing of previously thermostatted solutions (at 45 °C for 30 min) in order: pyrazine, mercuric chloride, buffer solution, KNO_3, and MET, $[Ru(CN)_6]^{4-}$ was introduced at last. After thorough mixing the reaction mixture was promptly transferred to the spectrophotometric cell to record the absorbance value; the temperature of the spectrophotometric cell compartment was also fixed at 45 °C by circulating water arrangement. A graph plotted between the recorded absorbance with varying [MET], considered as calibration curve and was used to quantitatively determine MET.

RESULTS AND DISCUSSION

A yellow-colored complex of $[Ru(CN)_5pyrazine]^{3-}$, obtained during the reaction is due to the Hg^{2+} promoted imitation of coordinated CN^- from $[Ru(CN)_6]^{4-}$ with pyrazine [46]. It was confirmed by the slope ratio and mole ratio studies of the final product that the reactants pyrazine and $[Ru(CN)_6]^{4-}$ reacts in a 1:1 mole ratio. Since the catalyst and all reacting solutions do not exhibit any significant absorption at the studied wavelength, the strong absorption band observed at 370 nm (due to Metal to ligand charge transfer) corresponds to the final product $[Ru(CN)_5pyrazine]^{3-}$ [46]. The previous literature on sodium thiosulphate, mercaptoacetic acid, D-penicillamine, and methionine reveal that the added sulfur compound reduces the Hg^{2+} catalyzed exchange rate of cyanide by nitrogen heterocyclic ligands from $[Ru(CN)_6]^{4-}$ [34-36,47]. Methionine, an organo-sulfur compound also reduces the rate of investigated reaction by forming a stable complex with Hg^{2+}. MET reduces the effective concentration of Hg^{2+} by forming a complex with it [Hg^{2+}---- MET]. The reduced catalytic activity is responsible for the decrease in absorbance with the inclusion of MET. An optimized reaction condition with varying [MET] was used to record the absorbance at a fixed time after the mixing of reactants (12 min) [46]. The plot of absorbance *versus* [MET] displays a linear correlation in the concentration range of 2.5×10^{-6} M to 5.0×10^{-5} M (Fig. 2). The plot can be used

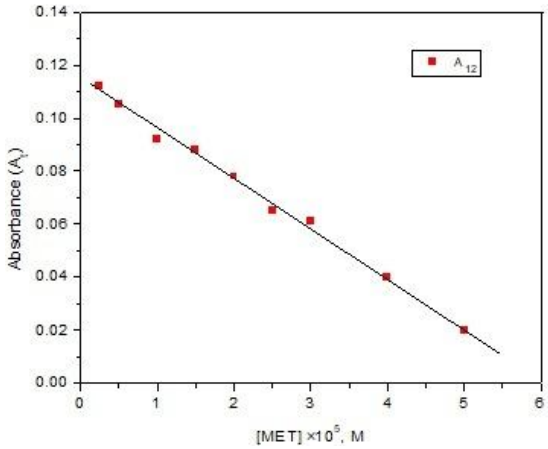


Fig. 2. Calibration curve for the determination of methionine at [pyrazine] = 6.5×10^{-4} M, I = 0.05 M (KNO_3), Temperature = 45.0 ± 0.2 °C, pH = 4.00 ± 0.03, $[Hg^{2+}] = 7.5 \times 10^{-5}$ M, and $[Ru(CN)_6^{4-}] = 5.25 \times 10^{-5}$ M.

as a calibration curve for the quantitative estimation of MET. The linear regression expressions relating to absorbance with varying methionine concentration can be described as Eq. (1).

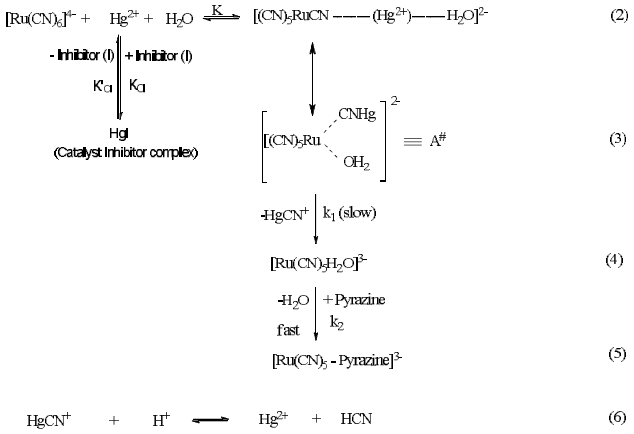
$$A_{12} = 0.131 - 2.123 \times 10^{3}[MET] \quad (1)$$

The standard deviation and linear regression coefficient obtained from the A_{12} calibration curve (Fig. 2) are found to be 0.0025 ± 0.0002 and 0.9863 ± 0.0031, respectively. To verify the accuracy and reproducibility of the proposed method, a calculated amount of MET was added to the reaction mixture and recovered MET was calculated by the calibration curve. The recovered MET against the added MET with percentage error and the standard deviation is compiled in Table 2. Results show that with the proposed method methionine can be determined quantitatively down to 2.5×10^{-6} M.

Table 2. MET Determination Against the Added MET Experimental Condition: [Pyrazine] = 6.5×10^{-4} M, I = 0.05 M (KNO_3), Temperature = 45.0 ± 0.2 °C, pH = 4.00 ± 0.03, $[Hg^{2+}] = 7.5 \times 10^{-5}$ M, and $[Ru(CN)_6^{4-}] = 5.25 \times 10^{-5}$ M

[MET] × 10^5 M (Taken)	A_{12}	
	[MET] × 10^5 M (Found)	% Error
0.20	0.21 ± 0.016	+ 0.04
0.55	0.55 ± 0.02	0.000
1.15	1.13 ± 0.06	− 0.045
1.35	1.39 ± 0.08	+ 0.051
1.70	1.70 ± 0.00	0.000
2.35	2.31 ± 0.05	− 0.038
2.60	2.59 ± 0.01	− 0.019
2.95	2.97 ± 0.08	+ 0.027

The inhibition action of MET towards the catalytic activity of Hg^{2+} for the CN^- substitution from $[Ru(CN)_6]^{4-}$ by pyrazine can be schematically be represented by the modified mechanism (Eqs. (2)-(6)). The present ligand exchange reaction may generate more authentic results in the determination of MET since the uncatalyzed exchange of CN^- with pyrazine is insignificant under the studied reaction condition [46].



The proposed inhibition mechanism is analogous to the enzyme-catalyzed. The rate of catalyzed reaction, considering $Ru(CN)_6^{4-}$ as a single substrate, in the absence of MET can be governed by Eq. (7).

$$V_0 = \frac{V_{max}}{1 + \frac{K_m}{[S_0]}} \quad (7)$$

Where V_o and S_o correspond to the initial rate of the mercury(II) catalyzed imitation reaction and the concentration of $[Ru(CN)_6]^{4-}$ respectively. At higher substrate concentration, the maximum rate is represented by V_{max} while K_m is the Michaelis-Menten constant.

To calculate the value of V_{max} and K_m Eq. (7) can be represented in the form of a straight-line equation (Eq. (8)).

$$\frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \frac{1}{[S_0]} \quad (8)$$

Equation (9) is in agreement with the Lineweaver-Burk expression [48]. The graph of $1/V_o$ *vs.* $1/[S_o]$ (Fig. 3) gives a straight line having intercept and slope of $1/V_{max}$ and K_m/V_{max} respectively. In the defection of inhibitor, the calculated K_m value was found to be 0.2232 ± 0.016 mM. If I_o and K'_{CI} represent the initial [MET] and the dissociation constant of the catalyst inhibitor complex (C-I)

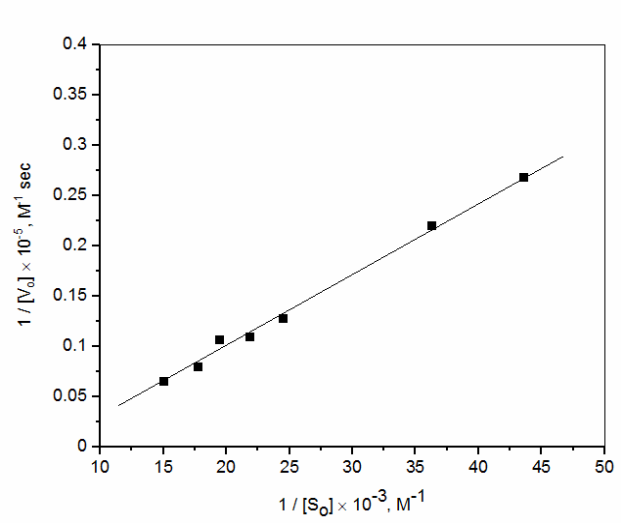


Fig. 3. The Line weaver-Burk plot ($1/V_o$ *vs.* $1/[S_o]$) at constant $[Hg^{2+}]$ in absence of inhibitor at [Pyrazine] = 6.5×10^{-4} M, I = 0.05 M (KNO_3), Temperature = 45.0 ± 0.1 °C, pH = 4.00 ± 0.03 and $[Hg^{2+}] = 7.5 \times 10^{-5}$ M.

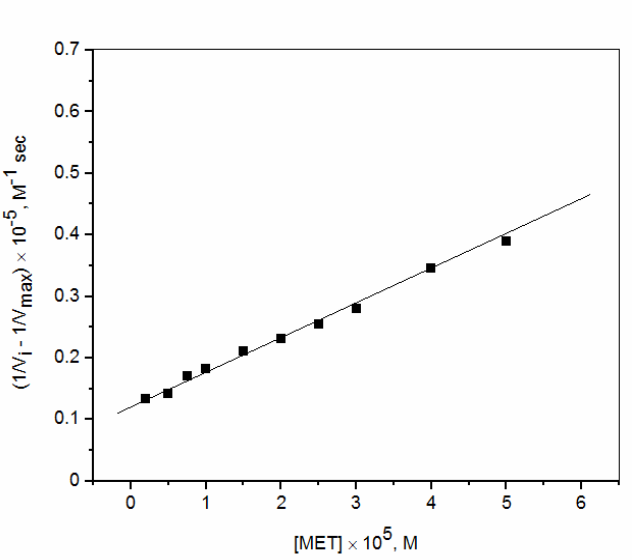


Fig. 4. The plot of $(1/V_i - 1/V_{max})$ *vs.* initial [MET] at [pyrazine] = 6.5×10^{-4} M, I = 0.05 M (KNO_3), temperature = 45.0 ± 0.1 °C, pH = 4.00 ± 0.03, $[Hg^{2+}] = 7.5 \times 10^{-5}$ M, and $[Ru(CN)_6^{4-}] = 5.25 \times 10^{-5}$ M.

respectively then at fixed $[Hg^{2+}]$, in the presence of inhibitor, the apparent M-M constant "K'_m" can be given as:

$$K'_m = K_m(1 + \frac{[I_0]}{K'_{Cl}})$$

Then from Eq. (7):

$$V_i = \frac{V_{max}}{1 + \frac{K'_m}{[S_0]}} \quad (9)$$

Equation (9) shows the rate of substitution reaction (V_i) in the presence of MET (inhibitor) when catalyst concentration was kept constant [49].

$$V_i = \frac{V_{max}}{1 + \frac{K_m}{[S_0]}(1 + \frac{[I_0]}{K'_{Cl}})} \quad (10)$$

Equation (10), transformed to the Lineweaver-Burk form can be represented by Eq. (11).

$$\frac{1}{V_i} - \frac{1}{V_{max}} = \frac{K_m}{[S_0]V_{max}} + \frac{K_m}{[S_0]V_{max}}\frac{[I_0]}{K'_{Cl}} \quad (11)$$

Equation (11) produces good results when MET only inhibits the catalytic activity of Hg^{2+} through complex formation.

The plot of $(\frac{1}{V_i} - \frac{1}{V_{max}})$ *versus* initial [MET] is in alignment with the straight-line equation (Fig. 4). K'_{Cl} and K_m (in the presence of MET) calculated from the slope and intercept of the graph were $15.93 \times 10^{-5} \pm 0.02$ and 0.2235 ± 0.0016 mM respectively. K_m has approximately the same value in the defection and presence of inhibitor. The lower K'_{Cl} value ($15.93 \times 10^{-5} \pm 0.02$) exhibits that the Hg-(MET) complex is highly stable.

To analyze the possible interference of cations, complexing agents, and anions in the quantitative estimation of MET, the A_{12} calibration curve was used. Table 3 exhibits the ultimate limit of complexing agents and various ions. Complexing agents must be absent during kinetic examination as they significantly interfere in MET determination by forming a stable complex with Hg(II).

Application in Pharmaceutical Preparations

The proposed inhibitory kinetic study was effectively

Table 3. Examination of Interference of Complexing Agents and Ions in MET Determination Experimental Condition: [Pyrazine] = 6.5×10^{-4} M, I = 0.05 M (KNO_3), Temperature = 45.0 ± 0.1 °C, pH = 4.00 ± 0.03, $[Hg^{2+}] = 7.5 \times 10^{-5}$ M, and $[Ru(CN)_6^{4-}] = 5.25 \times 10^{-5}$ M

Complexing agents/ion	[Complexing agents/ion] M, limit	Possible interference
Na^+	6.5×10^{-5}	Non-interfering
Mg^{2+}	5.5×10^{-4}	Non-interfering
Cd^{2+}	7.5×10^{-5}	Non-interfering
Zn^{2+}	3.5×10^{-4}	Non-interfering
Fe^{3+}	7.5×10^{-4}	Non-interfering
Al^{3+}	4.5×10^{-4}	Non-interfering
I^-	6.0×10^{-4}	Non-interfering
NO_3^-	2.5×10^{-4}	Almost non-interfering
$C_2O_4^{2-}$	3.5×10^{-4}	Interfering significantly
SO_4^{2-}	2.5×10^{-4}	Almost non-interfering
IDA	5.5×10^{-4}	Interfering significantly
EDTA	7.5×10^{-4}	Interfering significantly

Table 4. Determination of MET in Pharmaceutical Samples and Statistical Comparison with the Official Method Experimental Condition: [Pyrazine] = 6.5×10^{-4} M, I = 0.05 M (KNO_3), Temperature = 45.0 ± 0.1 °C, pH = 4.00 ± 0.03, $[Hg^{2+}] = 7.5 \times 10^{-5}$ M, and $[Ru(CN)_6^{4-}] = 5.25 \times 10^{-5}$ M

Pharmaceutical samples	Proposed method recovery ± RSD (%)	Official method recovery ± RSD (%)
L-Methionine 500 mg Capsule (West-Coast Pharmaceutical Works Ltd.)	99.46 ± 0.52	99.72 ± 0.68
Methionine 200 mg Tablet (Debco Pharma Pvt. Ltd.)	100.76 ± 0.72	99.54 ± 0.51
Methio-Form 500 mg Tablet (Lloyd Inc.)	99.94 ± 0.73	100.24 ± 0.38
L-Methionine 500 mg Capsule (Larthborn Elements)	101.08 ± 0.59	99.91 ± 0.49
L-Methionine 1000 mg Capsule (Horbaach)	99.69 ± 0.68	100.98 ± 0.75

used for the quantitative determination of MET in pharmaceutical preparations. The content of MET from 10 capsules/tablets was finally grounded and dissolved in 100 ml of de-ionized distilled water, which after sonication for 20 min was filtered off using Whatman filter paper. The solution was further diluted with de-ionized distilled water to bring [MET] within the calibration range. Five different pharmaceutical samples of MET (capsules/tablets) were subjected to the spectrophotometric determination of MET. The statistical comparison of the result obtained by the

designed method with the standard method indicates the precision and accuracy of the developed method for MET determination (Table 4) [50]. The mean recovery (99-101) demonstrates that the present kinetic method can be effectively used for the quick quantitative determination of MET in pharmaceutical samples with good accuracy and reproducibility.

CONCLUSIONS

Using the inhibition action of MET towards the catalytic activity of Hg^{2+} for the CN^- substitution from $[Ru(CN)_6]^{4-}$ by pyrazine, a reproducible inhibitory kinetic method was proposed for the quick estimation of methionine. The present ligand exchange reaction may generate more authentic results in the determination of MET since the uncatalyzed exchange of CN^- with pyrazine is insignificant under the studied reaction condition. The compounds having sulfur in S^-, SH and -S- form can be efficiently determined at a micro level by the proposed method as they significantly inhibit the rate of studied substitution reaction. MET decreases the rate of substitution reaction by reducing the active concentration of Hg^{2+} through the formation of a stable complex with it. With the prop
osed method, MET can be determined quantitatively down to 2.5×10^{-6} M. The proposed methodology can be convincingly adopted for the rapid quantitative determination of MET in pharmaceutical samples with good accuracy and reproducibility. The next step of this work is to check other metal ions, which are able to mimic the property of Hg^{2+} and are lesser toxic in the biological system.

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