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A NOVEL INHIBITORY KINETIC SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF RANITIDINE HYDROCHLORIDE

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***Abstract*— A kinetic spectrophotometric method for the determination of ranitidine hydrochloride, based on its inhibitory effect on Hg(II) catalyzed substitution of cyanide ion, by 4-cyanopyridine in hexacyanoferrate(II) is described. Ranitidine hydrochloride ions form strong complexes with Hg(II) catalyst which is used as the basis for its determination at trace level. The progress of reaction was monitored, spectrophotometrically, at 477nm (λmax of [Fe(CN)5CNpy]3−, complex) under the optimum reaction conditions at: [Fe(CN)64-] = 5 × 10-3 M, [4-CNpy] = 2.5 × 10-4 M, [Hg2+] = 2 × 10-5 M, pH = 2.8 ± 0.02, I = 0.02 M (KNO3) and temperature = 25±0.1°C. A linear relationship obtained between absorbance (measured at 477nm at different times) and inhibitor concentration, under specified conditions, has been used for the determination of [ranitidine hydrochloride] in the range of 0.2 – 2.0 × 10−5M with a detection limit of 5.2 × 10−7 M. The standard deviation and percentage relative standard deviation have been calculated and reported with each datum. A most plausible mechanistic scheme has been proposed for the reaction. The values of equilibrium constants for complex formation between catalyst–inhibitor (KCI), catalyst–substrate (Ks) and Michaelis–Menten constant (Km) have been computed from the kinetic data. The influence of possible interference by major cations and anions on the determination of ranitidine hydrochloride and their limits has been investigated.**

***Index Terms*— Credit Appraisal, Credit risk, Credibility, Financial Institution.**

I. INTRODUCTION

Ranitidine hydrochloride (RNH), chemically N, N dimethyl-5-[2-(1-methylamine-2-nitrovinyl)- thylthiomethyl]furfurylamine hydrochloride is a H2-receptor antagonist and is widely used in short term treatment of duodenal ulcer and in the management of hypersecretory conditions [1]. It acts by blocking histamine receptors which are present on the cells in the stomach lining. Ranitidine binds to H2 receptors, replacing some of the histamine. As a result, the amount of stomach acid produced by these cells is decreased. Ranitidine decreases the amount of acid in the stomach and duodenum. As a result, ranitidine helps relieve the symptoms of indigestion and aids the healing of ulcers. It is also used to depress acid production in various other conditions. Several methods have been reported for the determination of ranitidine in bulk, pharmaceutical dosage forms, and/or biological fluids. These methods include kinetic spectrophotometry [2, 3], HPLC [4-8], coulometry [9], capillary electrophoresis [10, 11], fluorimetry [12], HPTLC [13], voltammetry [14], potentiometry [15] and polarography [16]. But, such techniques are time consuming because of extensive sample pretreatment, require expensive instrumentation and beyond the reach of small laboratories, particularly in under developed and developing countries. There are several reports of the determination of RNH by spectrophotometry involving the use of Folin-Ciocalteu reagent [17], N-bromosuccinimide [18], Cerium (IV) [19], 3-methyl-2-benzothiazoline hydrazone-iron (III) [20], 7, 7, 8, 8 tetracyanoquinodimethane [21], 2, 6- dichloroquinone chlorimide [22], bromothymol blue [23], potassium dichromate [24], perchloric acid [25], DDQ [26], Hg(SCN)2 [27]. These methods are based on redox, coupling, charge-transfer complexation, and ion pair complexation reactions. Already reported spectrophotometric methods suffer from one or other deficiency such as heating or extraction step, critical dependence on acid/pH condition, use of non-aqueous medium/expensive chemicals, poor sensitivity and/or narrow range of linear response.

It has also been reported that an addition of thio-compounds to the metal ion catalyzed reaction inhibit the rate of reaction to a considerable extent [28-33]. Such rate inhibition has formed the basis of the quantitative application for the determination of thio-compounds [28, 30-33]. Few studies have been conducted on the kinetic determination of inhibitors or complexing agents based on their inhibitory effect on the rate of metal ion catalyzed reactions [30-33]. In the present paper, this fact is successfully utilized for the quantitative determination of ranitidine hydrochloride by monitoring the said indicator reaction on UV-visible spectrophotometer. For this purpose the selected indicator reaction must be such that the stability of the catalyst–inhibitor complex formed is very high so that the formation of this complex may take place even at low concentrations, hence, the kinetics and mechanism of Hg(II) catalyzed exchange of coordinated cyanide in [Fe(CN)6]4− by 4-CNpy has been chosen.

II. EXPERIMENTAL

A. Reagents and Equipments

The chemicals K4[Fe(CN)6]•3H2O (E. Merck), HgCl2 (AR, Galaxo Laboratories, India) 4-cyanopyridine (SD Fine Chemicals Ltd., India), and ranitidine hydrochloride (Aldrich Chemical Co., USA) were used for present work. All other chemicals used were of analytical grade. Deionized water was used throughout this study. The pH value was kept constant by use of potassium hydrogen phthalate buffer as described in literature [34]. The ionic strength was kept constant by adding KNO3 (E. Merck) of analytical grade.

The progress of the reaction was monitored at 477 nm at constant temperature (25±0.1°C) by following the increase of absorbance of [Fe(CN)54-CNpy]3-, the final product of the reaction, at different intervals of time with the help of DIGI-110DUV-Visible spectrophotometer having a circulatory system to maintain temperature of the cell compartment. The pH of the reaction mixture was measured on a Toshniwal digital pH-meter model, CL-46. The standard BDH buffers were used to standardize the pH meter. A fixed time procedure was adopted to record the absorbance changes as a function of varying concentration of inhibitor and the data obtained were used to plot the calibration graphs for further applications.

B. Determination of ranitidine hydrochloride

The concentration of ranitidine hydrochloride was varied by changing the mole ratio [I]/[C] from 0.10 to 1.10 keeping other variables at optimum conditions (cf. Chapter II) except the concentration of Hg(II) which was fixed at 2.0×10−5 M. The absorbance change, recorded as a function of concentration of RNH at fixed time intervals were used to achieve the calibration curves for the determination of RNH.

III. RESULT AND DISCUSSION

A. The Indicator Reaction

The cyanide substitution reaction between [Fe(CN)6]4− and 4-CNpy has been found to be catalysed by the Hg(II) ion. The product formed, [Fe(CN)5(CNpy)]3−, as a result of this reaction has wavelength of maximum absorption in the visible region at 477 nm corresponding to metal to ligand charge transfer (MLCT) transition. The RNH was found to inhibit this reaction. The inhibition of reaction rate by the addition of thio-compound i.e. RNH in the present case may be attributed to the formation of complexes of high stability between Hg(II) catalyst and the inhibitor leading to the decrease in free catalyst concentration which ultimately mask the catalytic activity of Hg(II) catalyst. The subsequent decrease in the rate of inhibited reaction is directly proportional to the concentration of inhibitor added to the reaction system. Therefore, a linear relationship between the rate of the indicator reaction and the concentration of inhibitor added may be obtained empirically by the simple treatment of rate equation/data. The inhibitory effect depends upon their [inhibitor]/ [catalyst] ratio in the reaction system.

B. Calibration graph and precision in determination of ranitidine hydrochloride

The plots of At (taken as a measure of initial rate) versus [inhibitor] were found to be linear as shown in Figure V.1, and serve as calibration curves. The linear plots provide a basis for the determination of RNH. The relevant expressions which relate the change of absorbance At (t = 5, and 10 min) to that of the concentration of inhibitor RNH in reaction mixture for these calibration curves are given by Eqs. (1) and (2).

A5=0.228–9.18×103[RNH] (1)

A10=0.275–9.24×103[RNH] (2)

The linear regression coefficients and standard deviations for A5, and A10 versus [RNH] plots are 0.9973, 0.9976 and 0.005, 0.004 respectively.

The recovery experiments were performed for the determination of RNH in aqueous solutions in the range of 0.2 - 2.0×10-5 M. The recovered amounts along with the standard deviations and the percentage errors in case of RNH are given in Table V.1. The detection limits for RNH were calculated to be and 5.2 × 10-7 M.

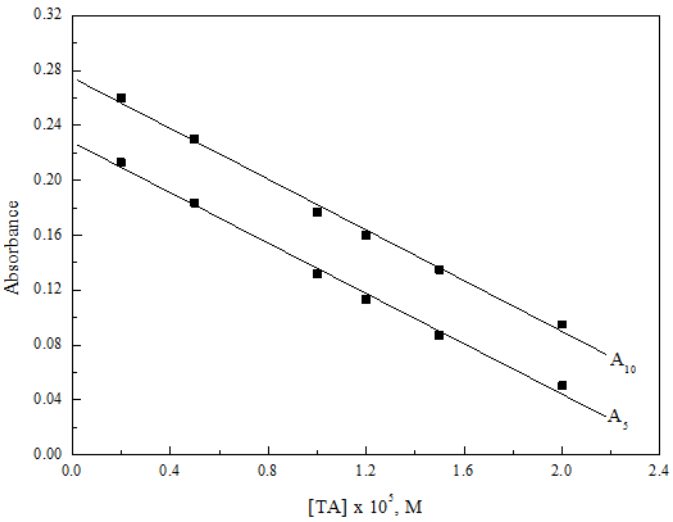


Figure V.1: Calibration curve for the determination of ranitidine hydrochloride at $[Fe(CN)_6^{4-}] = 5 \times 10^{-3}$ M, [4-CNpy] = 2.5×10^{-4} M, $[Hg^{2+}] = 2 \times 10^{-5}$ M, pH = 2.8 ± 0.02, I = 0.02 M (KNO_3) and temperature = 25±0.1°C

Table V.1: Quantitative determination of ranitidine hydrochloride (RNH) at temperature = 25±0.1°C

	A_5			A_{10}		
[RNH] × 10^5 M (taken)	[RNH] × 10^5 M (found)[a]±s.d.	RSD (%)	Recovery (%)	[RNH] × 10^5 M (found)[a]±s.d.	RSD (%)	Recovery (%)
0.25	0.26±0.01	4.00	104.00	0.24±0.01	-4.00	96.00
0.42	0.41±0.01	-2.38	97.62	0.43±0.02	2.38	102.38
0.94	0.93±0.03	-1.06	98.94	0.93±0.03	-1.06	98.94
1.24	1.25±0.02	0.81	100.81	1.26±0.04	1.61	101.61
1.56	1.57±0.03	0.64	100.64	1.54±0.02	-1.28	98.72
1.92	1.91±0.02	-0.52	99.48	1.93±0.01	0.52	100.52

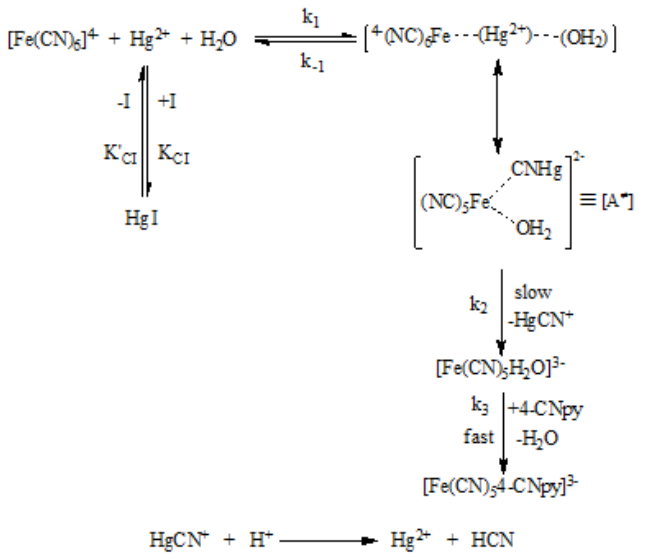
[a]mean of three determinations

±s.d values represent standard deviation of the mean for three determinations.

Reaction conditions are [Fe(CN)64-] = 5 × 10-3 M, [4-CNpy] = 2.5 × 10-4 M, [Hg2+] = 2 × 10-5 M, pH = 2.8 ± 0.02, I = 0.02 M (KNO3).

C. Mechanism of Inhibition

The inhibition caused by RNH containing sulfur donor atom to the Hg(II) catalysed CN− substitution of [Fe(CN)6]4− with 4-CNpy may be understood by modifying the mechanistic scheme for this reaction system without inhibitor and may schematically be represented as shown in Scheme V.1.



Scheme V.1

In the above mechanistic scheme, the uncatalyzed reaction path has been ignored for simplicity. If the non-rate limiting concentration of [Fe(CN)6]4− is represented by [S] and its initial concentration by [S]0, then it is simple matter to derive a kinetic formulation by a treatment similar to one followed for an enzyme catalyzed reaction involving a single substrate in the presence of an inhibitor. The reaction velocity, in the presence of catalyst only, can be given in the form of Eq. (3)

$$V_0 = \frac{V_{max}}{1 + \frac{K_m}{[S]_0}} \quad (3)$$

Double reciprocal plot of the reaction rate can also be constructed by inverting Eq. (3), which results in the Lineweaver–Burk's [35] form as shown in Eq. (4):

$$\frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \frac{1}{[S]_0} \quad (4)$$

In Eq. (4), V0 represents the initial rate in the presence of catalyst only, Vmax denotes the maximum attainable rate at a particular catalyst concentration in the presence of non-rate-limiting amount of substrate, and Km (Km = (k−1 + k2)/k1) is equivalent to the Michaelis – Menten constant, which is approximately equal to dissociation constant of the catalyst – substrate complex (vide-supra). A plot of (1/V0) versus (1/[S0]) in the absence of an inhibitor yielded a straight line (R ⩾0.9719, s⩽0.3493) as shown in Figure V. 2. From the plot in Fig. 2 (cf. Eq. (4)); it is seen that one can get Vmax from the intercept and Km from the slope intercept ratio. It is further to be noted that the values of Km and Vmax are also referred as the kinetic parameter of a catalyst and serve to characterize catalyst acting on a substrate. The values of Km of catalyst depend on particular substrate and also on

experimental conditions such as pH, ionic strength, temperature and solvent used.

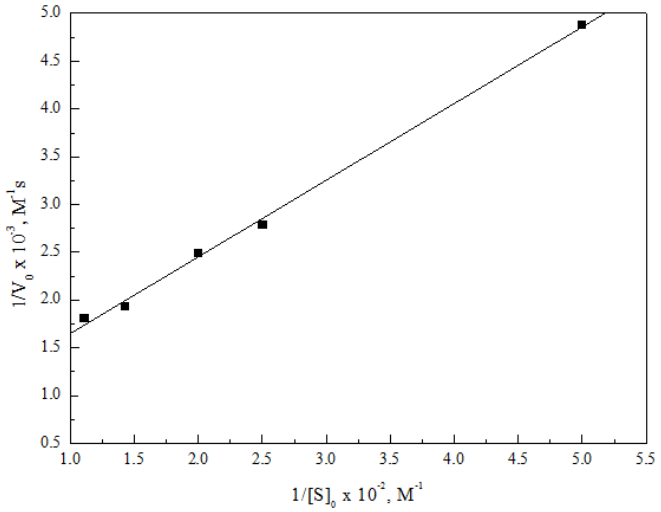


Figure V.2: The determination of Vmax by variation of substrate concentration in absence of inhibitor at [4-CNpy] = 2.5 × 10-4 M, [Hg2+] = 2 × 10-5 M, pH = 2.8 ± 0.02, I = 0.02 M (KNO3) and temperature = 25±0.1°C.

In the presence of inhibitor, the rate of reaction can be expressed in the form of Eq. (5):

$$V_i = \frac{V_{max}}{1 + \left(\frac{K_m}{[I]_0} \right) \left(1 + \frac{[I]_0}{K'_{CI}} \right)} \quad (5)$$

Comparison of Eq. (5) to the corresponding expression for the uninhibited case, i.e., Eq. (3) illustrates that with competitive inhibition, a new apparent Michaelis–Menten constant (K'm) can be defined through Eq. (6) [36]:

$$K'_m = K_m \left(1 + \frac{[I]_0}{K'_{CI}} \right) \quad (6)$$

In Eq. (5), Vi denotes the reaction rate in the presence of the inhibitor (at a fixed catalyst concentration), KCI is the dissociation constant of catalyst–inhibitor (C–I) complex. Eq. (5) was derived on the basis of assumption that [S]0 is large and [I]0 > [CI]. Eq. (3) is formally similar to the rate equation for heterogeneously catalyzed gas phase reactions proceeding by Langmuir mechanism.

A supportive reference to a similar case of inhibition was also made by Klockow et al. [37] who investigated the inhibition of zirconium catalyst for the determination of fluoride.

In order to obtain Lineweaver–Burk plot, Eq. (5) can be transformed to Eq. (7):

$$\frac{1}{V_i} - \frac{1}{V_{max}} = \frac{K_m}{V_{max}[S]_0} + \frac{K_m}{V_{max}[S]_0} \frac{[I]_0}{K'_{CI}} \quad (7)$$

Although Vmax used in Eq. (7) is not an experimental quantity, yet it can be evaluated from the intercept of the plot of 1/V0 versus 1/[S]0 using Eq. (5) in absence of inhibitor as shown in Figure V.2. The slope of this plot yields the value of Km which was found to be 9.48 mM in the present case. For the validity and good results from Eq. (7), inhibitor 'I' must form a complex of the type (C–I) with catalyst 'C'. There should be no S–I type complex formed. The plot of 1/Vi – 1/Vmax versus [I]0 for ranitidine hydrochloride was found to be linear and shown in Figure V.3. From the intercepts of the plot, the value of Km in the presence of inhibitor was evaluated. This value was again found to be in agreement with its previously calculated value from 1/V0 versus 1/[S]0 plot. The slope of this plot gives the value of K′ CI corresponding to the complexes of Hg(II) with RNH. The value of K′ CI for RNH was found to be 4.61 × 10-6.

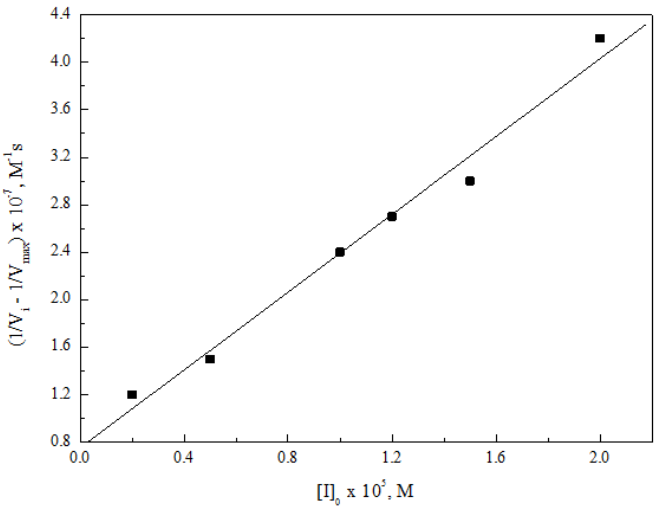
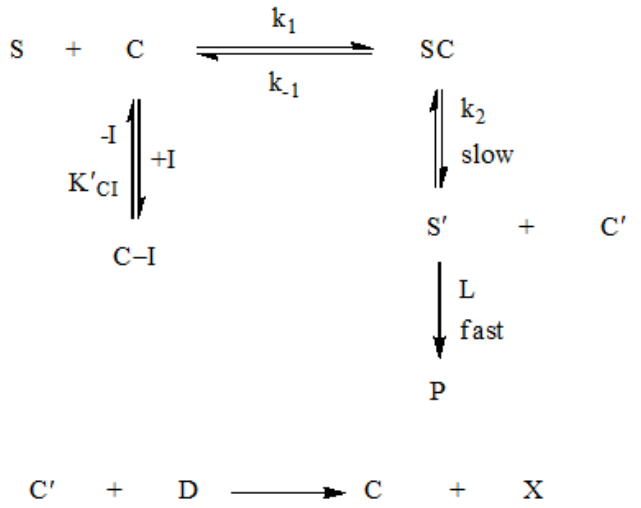


Figure V.3: Determination of K′ CI and Km in the presence of inhibitor RNH at [Fe(CN)64-] = 5 × 10-3 M, [4-CNpy] =

2.5 × 10-4 M, [Hg2+] = 2 × 10-5 M, pH = 2.8 ± 0.02, I = 0.02 M (KNO3) and temperature = 25±0.1° C

At last, a general mechanistic scheme describing the role of inhibitor may be represented as Scheme V.2



Scheme V.2

where, S, C, I, SC, L and C–I are substrate, catalyst, inhibitor, substrate–catalyst complex, ligand and catalyst–inhibitor complex respectively. S′ is a species which on reaction with a reagent gives the final product P, while C′ is a species which on reaction with D (usually H+, OH− or H2O) regenerates the catalyst C.

D. Interferences caused by foreign substances

The interferences caused due to the presence of different cations, anions and organic molecules have been studied and the maximum limits are presented in Table V.2. It is important to mention here that no complexing agent which may form a complex of high stability constant with Hg(II) should be present in the reaction system for the accuracy in the determination of these inhibitors. On the other hand, the metals which may complex with these two inhibitors more strongly than Hg(II) under these experimental conditions must also be absent.

Table V.2: Effect of different foreign ions on the determination of RNH at 25±0.1°C

Foreign ions	[Foreign ion] M, limit	Comment on interference
Sn^{2+}	6×10^{-4}	Non interfering
Pb^{2+}	3×10^{-4}	Non interfering
Sr^{2+}	3×10^{-4}	Non interfering
Cu^{2+}	4×10^{-4}	Non interfering
Co^{2+}	2×10^{-5}	Inhibition due to the formation of Co–L complex
Mg^{2+}	3×10^{-4}	Non interfering
Cd^{2+}	3×10^{-4}	Non interfering
Cr^{3+}	6×10^{-4}	Non interfering
Fe^{3+}	4×10^{-4}	Non interfering
Al^{3+}	2×10^{-4}	Non interfering
Na^{+}	6×10^{-5}	Interfering
Br^{-}	5×10^{-4}	Non interfering
I^{-}	4×10^{-4}	Non interfering
NO_3^{-}	3×10^{-4}	Almost non interfering
$(COO)_2^{2-}$	6×10^{-4}	Almost non interfering
SO_4^{2-}	4×10^{-4}	Interfering significantly
EDTA	2×10^{-4}	Interfering significantly
IDA	3×10^{-4}	Interfering significantly
NTA	2×10^{-4}	Interfering significantly
HEDTA	4×10^{-4}	Interfering significantly

IV. Analysis of Ranitidine Hydrochloride in Pharmaceutical Preparations

For the analysis of RNH in different pharmaceutical preparations, ten tablets were powdered. A weighed amount of powder was dissolved in water; the samples were centrifuged for 5 min, the supernatant was separated and water was added to prepare the solution of desired concentrations. Accordingly, the amount of RNH, contained in aqueous solutions, was determined using standard calibration equations stated above. The results obtained on three repeated determination of each sample on RNH are compiled in table V.3.

Table V.3: Analysis of RNH in pharmaceutical preparations

Tablets	RNH, mg (taken)	RNH, mg (found)	%RSD
Rantac	168.00	167.24±3.2	1.07
Zinetac	168.00	169.02±4.4	0.73

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