UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

FINAL REPORT OF THE MAJOR RESEARCH PROJECT WORK DONE

1	Project Report No	FINAL
2	UGC Reference No. & Date	F.NO.42-303/2013(SR) Dated 12.03.2013
3	Period of report	3 years from 01-04-2014 to 01-04-2016 then extended upto 31.03.2017
4	Title of the project	Kinetic, Mechanistic and Spectral studies on the oxidation of beta blockers
5	Name of the principal Investigator	DR.N.ANNAPURNA Associate Professor Dept. Of engineering chemistry A.U.College of Engineering Andhra University, Visakhapatnam 530003
	Name of the Co Investigator	Prof.P.Vani
	Department and University/ college where the project has undertaken	Dept. Of engineering chemistry A.U.College of Engineering Andhra University
6	Date of Implementation	01-04-2013
7	Grant approved and expenditure incurred during the period of report	
	Total amount approved	Rs.2,60,000.00
	Total expenditure	Rs,2,47,311.00

Annexure-IX

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SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1	Name and Address of the principal Investigator	DR.N.ANNAPURNA Associate Professor Dept. Of engineering chemistry A.U.College of Engineering Andhra University, Visakhapatnam 530003
2	Name and Address of the Institution	Dept. Of engineering chemistry A.U.College of Engineering Andhra University, Visakhapatnam 530003
3	UGC Reference No. & Date	F.NO.42-303/2013(SR) Dated 12.03.2013
4	Date of Implementation	01-04-2013
5	Tenure of the project	3 years
6	Total grant allocated	Rs.2,60,000.00
7	Total grant received	Rs.2,30,000.00
8	Total expenditure	Rs.2,47,311.00
9	Title of the project	Kinetic, Mechanistic and Spectral studies on the oxidation of beta blockers
10	Objectives of the project	1.To .investigate the detailed kinetics of oxidation of beta blockers using various oxidants in absence as well as in presence of transition metal ions as catalysts. 2. To find the selectivity of these drugs towards various oxidants 3. To establish a rate law through kinetic measurements 4. To identify the products of oxidation. 5. To propose a suitable mechanism basing on the kinetic results
11	Whether objectives were achieved	All the objectives were achieved
12	Achievements from the project	• Detailed kinetic investigationswere made on the selected reactions by spectrophotometric techniques. • Establishment of stoichiometry for the

		reaction. • Identification and confirmation of the products of oxidation by FTIR and H^1NMR. • Evaluation and computation of thermo dynamic parameters. • Proposing a plausible mechanism of the oxidation basing on the kinetic results obtained. • One candidate has been awarded Ph.D from the work • Three research papers have been published in reputed journals
13	Summary of the findings	Annexure -1

Kinetic, Mechanistic and Spectral studies on the oxidation of beta blockers

Introduction

The proposal originated from the recent literature reports on the kinetic and mechanistic studies of oxidation of various types of drugs.

Although there were recent studies on the kinetics of oxidation of many drugs over the past few years, the detailed investigations on the kinetics and mechanism of oxidation of Beta-blocker potent drugs for the regulation of blood pressure has met only a limited attention and however, despite of the importance of these drugs, relatively less information is available in the literature about their oxidation kinetics. Thus we believe that a systematic study on the mechanism of oxidation of these drugs with various oxidants like cerium(IV), chromium(VI), osmium(VIII), diperiodatocuprate(III), hexachloroiridate(IV), hexacyanoferrate(III) and manganese(III) will be helpful not only for the development of physiologically more relevant mimics of their oxidations but also for understanding the oxidation mechanism of these bioactive compounds.

Significance of the study

Hypertension or high blood pressure is a cardiac chronic medical condition in which the systemic arterial blood pressure is elevated. It increases hardening of the arteries thus predisposes individuals to heart disease, peripheral vascular disease and strokes. Types of heart disease that may occur include myocardial infarction, heart failure, and left ventricular hypertrophy other complications.

The goal of hypertension treatment is to lower high blood pressure and protect important organs, like the brain, heart, and kidneys from damage. Treatment for hypertension has been associated with reductions in stroke (reduced an average of 35%-40%), heart attack (20%-25%), and heart failure (more than 50%), according to research.

Drugs to Treat High Blood Pressure

There are several types of drugs used to treat high blood pressure, including:

- Angiotensin-converting enzyme (ACE) inhibitors
- Angiotensin || receptor blockers (ARBs)
- Diuretics
- Beta-blockers
- Calcium channel blockers
- Alpha-blockers
- Alpha-agonists
- Renin inhibitors
- Combination medications

Out of the above drugs Beta-adrenergic antagonists (ie, beta-blockers) have been in use for nearly 50 years. In addition to their traditional role in treating hypertension and other cardiovascular disorders, beta-blockers are also used for additional purposes such as migraine headaches, hyperthyroidism, glaucoma, anxiety, and various other disorders.

Beta-blockers reduce the stress on the heart by decreasing its workload. They work by blocking the effects of the hormone epinephrine, also known as adrenaline. As a result, the heart beats slowly and with less force, thereby reducing blood pressure. Beta blockers help the blood vessels to relax and open up that improves the flow of the blood. Studies show the value of beta-blockers in avoiding second heart attacks.

Beta Blockers Include

- Atenolol (Tenormin)
- Propranolol (Inderal, Inderal LA
- Metoprolol (Lopressor, Toprol-XL)
- Labetalol
- Nebivolol (Bystolic)
- Bisoprolol (Zebeta) etc..

1. We believe that a systematic and detailed investigation on the kinetics and mechanism of oxidation of these drugs with various oxidants like cerium(IV), chromium(VI), osmium(VIII), diperiodatocuprate(III), hexachloroiridate(IV), hexacyanoferrate(III) and manganese(III) will be helpful not only for the development of physiologically more relevant mimics of their oxidations but also for understanding the oxidation mechanism of these bioactive compounds.

2. Further, as a result of expanded use of these drugs, the incidence of overdose with these agents has also increased. Thus the study could throw some light on the fate of these compounds in biological systems.

3. From the reports in literature survey and from the preliminary investigations that we have carried out in our lab, it is clear that these drugs are able to react with oxidants in vitro. So considerable interest has been expressed in the possibility that oxidant scavenging contributes to the action of these drugs in vivo.

Objectives

The objectives of the work are

- To investigate the detailed kinetics of oxidation of beta blockers using various oxidants in absence as well as in presence of transition metal ions as catalysts.
- To find the selectivity of these drugs towards various oxidants
- To establish a rate law through kinetic measurements .
- To study the relative reactivity of the species.
- To identify the products of oxidation.
- To propose a suitable mechanism basing on the kinetic results and to characterize the intermediates involved
- To find the application of the derivatives of the oxidation products

Methodology

1. Preliminary studies on the oxidations of a few β-blockers using various oxidants in absence and presence of transition metal ions as catalysts.

2. Detailed kinetic investigations by spectrophotometric techniques.
3. Establishment of stoichiometry for the reaction.
4. Identification and confirmation of the products of oxidation by FTIR and H^1NMR.
5. Identification of free radicals during the course of the reaction by making use of their ability to induce polymers with some reagents and characterization of the intermediates involved in the course of reaction
6. Evaluation and computation of thermo dynamic parameters.
7. Proposing a plausible mechanism of the oxidation basing on the kinetic results obtained.

Work done

Labetalol and Metoprolol are selective β-blockers used in the treatment of several diseases like hypertension, angina, acute myocardial infarction, supraventicular tachycardia, ventricular tachycardia, congestive heart failure and prevention of migraine headaches. Although there were recent studies on the kinetics of oxidation of many drugs over the past few years, the detailed investigations on the kinetics and mechanism of oxidation of labetalol and metoprolol, has met only a limited attention. These two drugs were selected for the study.

A detailed investigation is carried out on the kinetics and mechanism of the oxidation of labetalol by alkaline hexacyanoferrate(III), [HCF(III)] spectrophotometrically at 30 ± 0.1^0C. Requisite amounts of all reagents except HCF(III) are taken in a borosil glass reaction bottle and equilibrated in the thermostat. The reaction is initiated by transferring a calculated amount of thermostated HCF(III) into the reaction mixture. The rate measurements are made under the conditions $[OH^-]$ >> [Labetalol] > [HCF(III)] and following [HCF(III)] which is being isolated. The course of the reaction is followed by measuring the absorbance at regular time intervals at 420nm at which no other species except hexacyanoferrate(III) has significant absorption under the conditions employed. The absorbance of the solution is taken as a measure of the residual concentration of hexacyanoferrate(III) at time 't'. plots of log(absorbance) versus time were found to be linear beyond 85% completion of the reaction indicting that the reaction is first order with respect to [HCF(III)]. The

pseudo first order rate constants calculated from these plots are denoted by k^{I} and the rate constants are found to be reproducible within an error of ± 4%.

The stoichiometry of the reaction was established spectrophotometrically and found to correspond to the equation

$$\underset{\text{labetalol}}{C_{19}H_{24}N_2O_3} + 2\,HCF(III) + 2\,OH^- \longrightarrow$$

$$\underset{\text{1-methyl-3-phenyl propylamine}}{C_{10}H_{15}N} + \underset{\text{2-hydroxy-5-formyl benzamide}}{C_8H_7NO_2} + \underset{\text{formaldehyde}}{HCHO} + 2HCF(II) + H_2O$$

The effect of the [HCF(III)], [labetalol], [OH-], ionic strength and [HCF(II)] on the reaction rate were studied by varying the concentration of the respective species and keeping the concentration of the all other species constant.

The following significant features were observed from the experimental results in the kinetic investigation on the oxidation of labetalol by hexacyanoferrate(III) in alkaline medium.

1. The reaction is first order in [HCF(III)].

2. The reaction is first order in [labetalol].

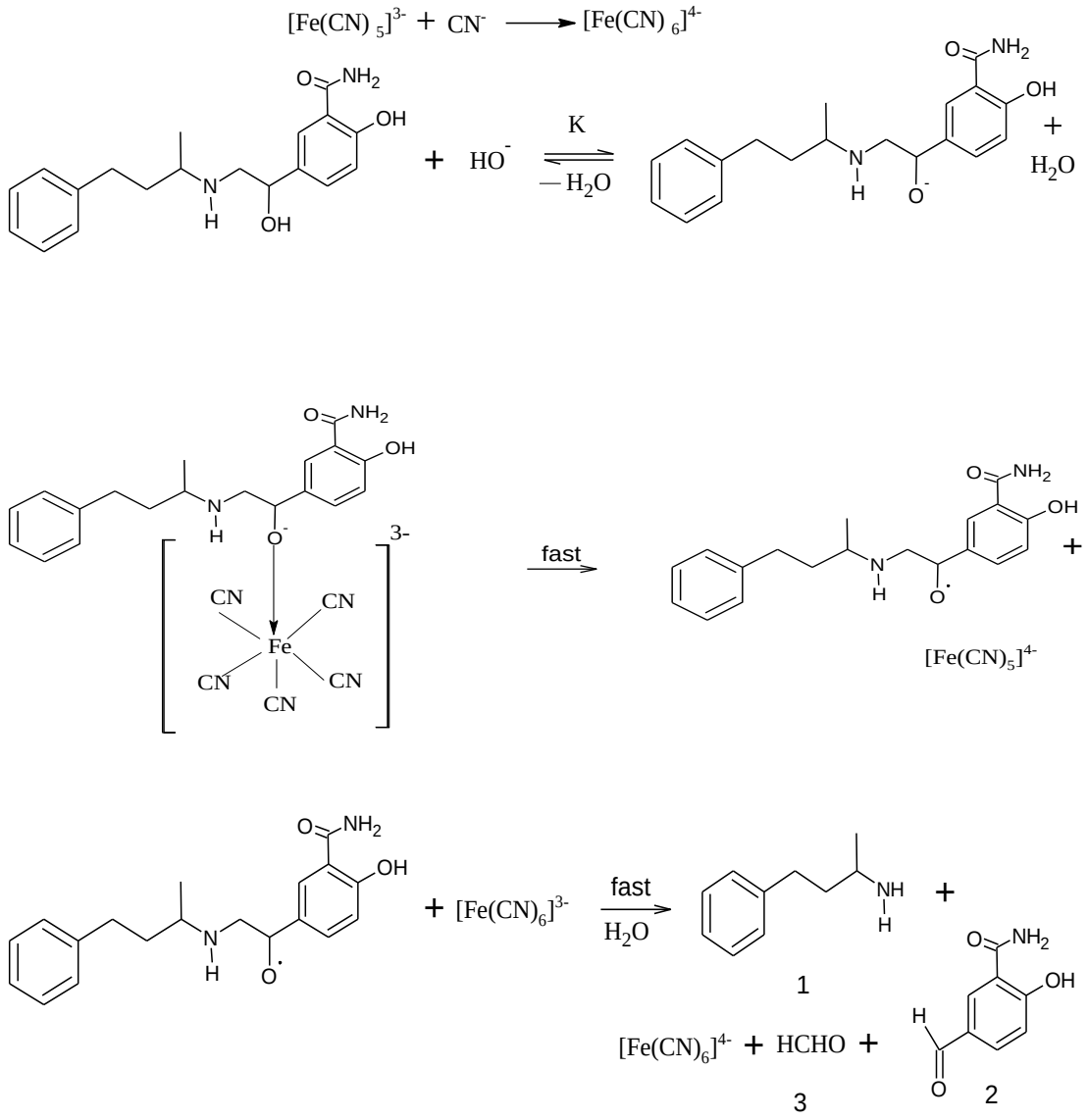
3. HCF(II), one of the products was found to have no effect on the rate of the reaction.

4. Ionic strength has negligible effect on rate of the reaction.

5. The rate of the reaction increases with increase in [OH-] and the order with respect to [OH-] was found to be less than one.

Temperature variations were carried out at four different temperatures and it was observed that rate of reaction increases with increase of temperature and the reaction obeys Arrhenius temperature dependence. The thermodynamic parameters of the reaction , energy of activation,$E_a$and entropy of activation,$\Delta S^{\neq}$ are computed using linear least squares method and are found to be 69.04 ± 2.0 kJ $mole^{-1}$ and -314.43 ±

3.2 $JK^{-1}mole^{-1}$ respectively. A plausible mechanism has been proposed based on the results obtained.



1. 4-phenylbutan-2-amine, 2. 5-formyl-2-hydroxybenzamide, 3. Formaldehyde

A rate equation was deduced from the above mechanism

$$\text{rate} = \frac{Kk[LAB]_t\ [OH^-]\ [HCF(III)]}{1+K[OH^-]}$$

Kinetics and mechanism of oxidation of Metoprolol by Chromium(VI), a versatile reagent capable of oxidizing all the oxidisable organic functional groups has been studied in perchloricacid medium at 30 ± 0.1^0C Requisite amounts of all

reagents except Cr(VI) are taken in a borosil glass reaction bottle and equilibrated in the thermostat. The reaction is initiated by transferring a calculated amount of thermostatedCr(VI) solution into the reaction mixture. The rate measurements are carried out at $[H^+]$ >> [Metoprolol] >Cr(VI)] by measuring the absorbance at regular time intervals at 360nm at which other species except chromium(VI) has a significant absorption. The absorbance of the solution is taken as a measure of the residual concentration of chromium(VI) at time 't'. plots of log absorbance versus time are found to be linear upto 85% completion of the reaction indicting that the reaction is first order with respect to [Cr(VI)]. The pseudo first order rate constants, k^{I} were calculated from these plots and are found to be reproducible within ± 4%.

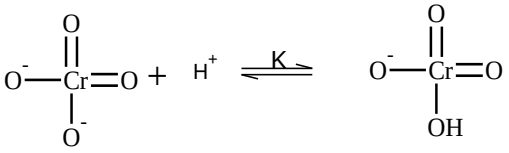
Variation studies were carried out to know the effect of concentration of chromium(VI), metoprolol, acid, sodium perchlorate and one of the products, chromium(III) on the rate of reaction by varying the concentration of the respective species and keeping the concentration of the all other species constant at 30 ± 0.1^0C. The significant features observed in the kinetic study on the oxidation of labetalol by chromium(VI) in perchloric acid medium are

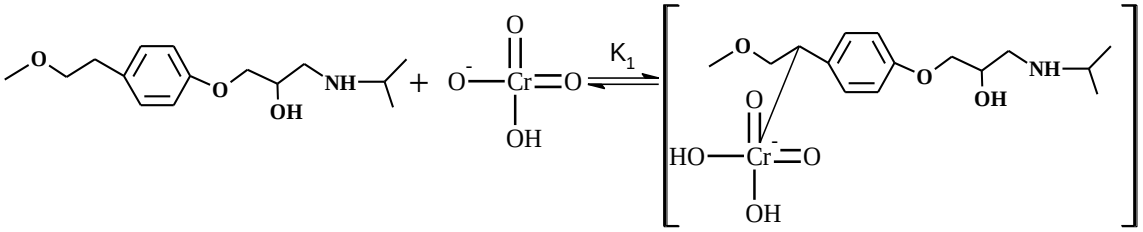
1.The reaction rate is first order with respect to [Chromium(VI)]
2. The reaction is fractional order dependent on [Metoprolol].
3. The rate of reaction is fractional order dependent on $[H^+]$.
4. Cr(III), one of the products was found to have no effect on the rate of the reaction.
5. Ionic strength has negligible effect on the reaction rate

To know the effect of temperature on rate of the reaction the kinetic runs at four different temperatures 25, 30, 35, and 40^0C has been carried out by theauthor taking the concentrations of all the reactants constant. The pseudo first order rate constants calculated from the log absorbance versus time plots. The plot of log k^{I} versus 1/T is a straight line showing that the reaction obeys Arrhenius temperature dependence. The activation parameters of the reaction, energy of activation (E_a) and entropy of activation($\Delta S^{\#}$) are computed using linear squares method and are found to be 16.86 ± 2.0 $kJmole^{-1}$ and -310.43 ± 3.2 $JK^{-1}mole^{-1}$ respectively.

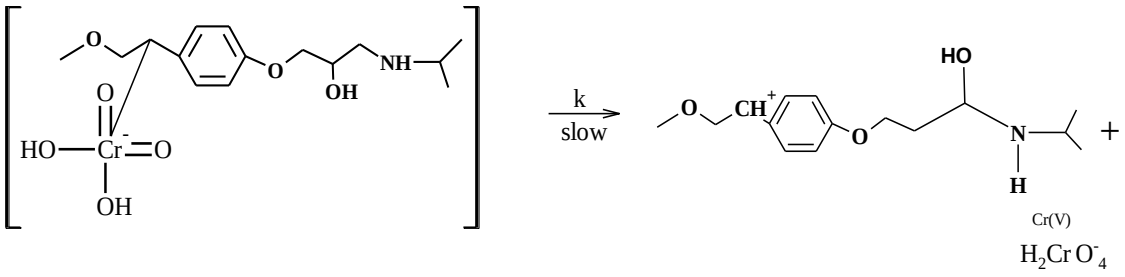
The stoichiometry of the reaction is established spectrophotometrically and the main products of oxidation are analysed by IR and NMR studies.

A plausible mechanism is proposed basing on the observed kinetic results



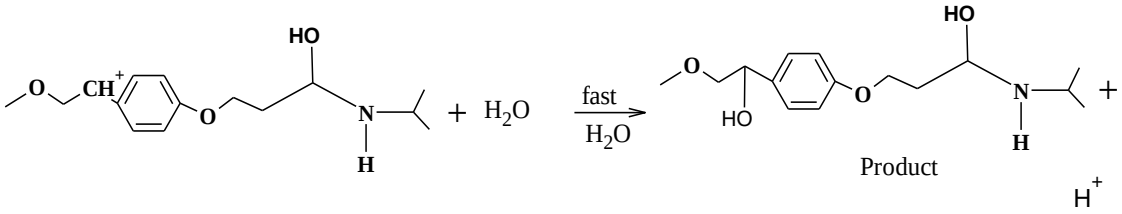


Complex



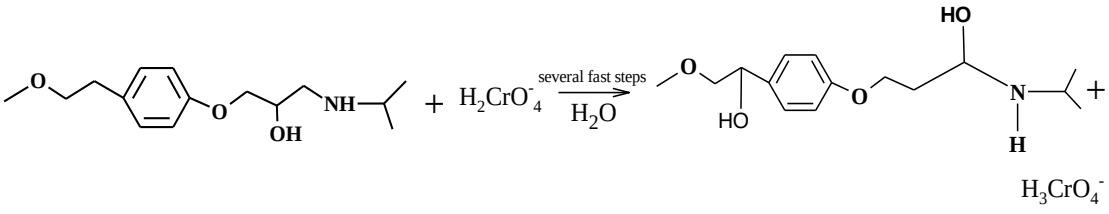
Cr(V)

$H_2CrO_4^-$



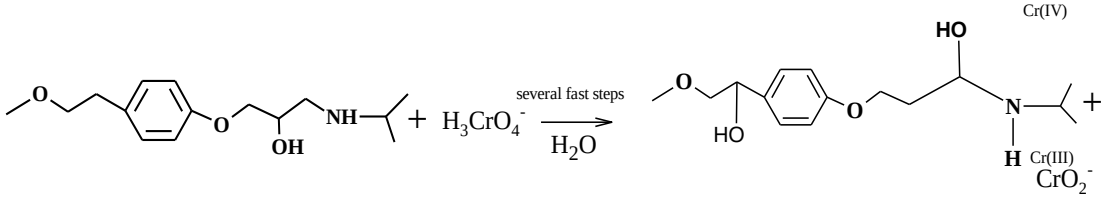
Product

H^+



$H_3CrO_4^-$

Cr(IV)



Cr(III)

CrO_2^-

and a rate law was deduced from the mechanism

$$\text{Rate (r)} = \frac{kKK_1[Cr(VI)]_t[H^+][MET]_t}{1 + K[H^+] + KK_1[MET]_t[H^+]}$$

The equation explains first order dependence on [Cr(VI)] and fractional order dependence on both [MET] and $[H^+]$.

Further, a successful investigations were also carried out on the kinetics and mechanism of oxidation of metoprolol by hexacyanoferrate(III) in alkaline medium at 30 ± 0.1^0C. Requisite amounts of all reagents except HCF(III) are taken in a glass reaction bottle and equilibrated in the thermostat. The reaction is initiated by transferring a calculated amount of thermostated HCF(III) into the reaction mixture. The rate measurements are carried out under the conditions $[OH^-]$ >> [Metoprolol] > [HCF(III)] and following [HCF(III)] which is being isolated. The course of the reaction is followed by measuring the absorbance at regular time intervals at 420nm as no other species except hexacyanoferrate(III) has significant absorption at this wavelength. The absorbance of the solution is taken as a measure of the residual concentration of hexacyanoferrate(III) at time 't'. Plots of log absorbance versus time are found to be linear upto 85% completion of the reaction indicting that the reaction is first order with respect to HCF(III). The pseudo first order rate constants calculated from these plots.

Kinetic orders with respect to the species HCF(III), metoprolol, OH^- and HCF(II), one of the products were established by carrying variation studies at constant temperature. significant observations from the kinetic investigation on the oxidation of metoprolol by hexacyanoferrate(III) in alkaline medium are

1. The reaction is first order in [HCF(III)].

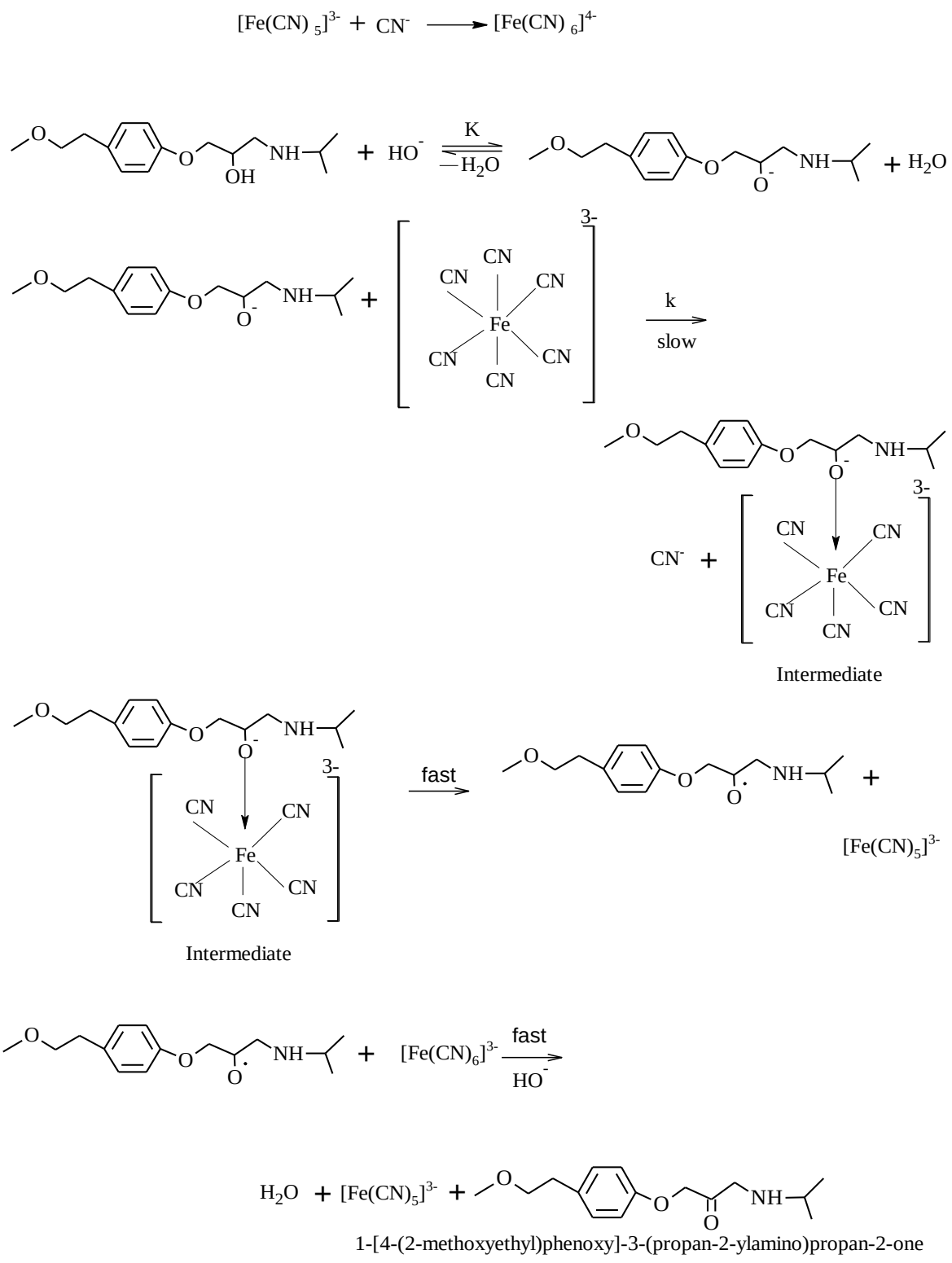
2. The reaction is first order in [metoprolol].

3. HCF(II), one of the products was found to have no effect on the rate of the reaction.

4. Ionic strength has negligible effect on rate of the reaction.

5. The rate of the reaction increases with increase in $[OH^-]$ and the order with respect to [OH-] was found to be less than one.

Aplausible mechanism has been proposed basing on the results



Rate equation for the above mechanism is

$$\text{rate} = \frac{Kk[MET]_t\,[OH^-]\,[HCF(III)]}{1+K[OH^-]}$$

The above equation explains first order dependence on [Met], [HCF(III)] and fractional order dependence on [OH^-]

Temperature variations were carried out at four different temperatures 25, 30, 35, and 40^0C and the pseudo first order rate constants were calculated from the log absorbance versus time plots. The plot of log k' versus 1/T is a straight line showing that the reaction obeys Arrhenius temperature dependence. The activation parameters of the reaction were computed using linear least squares method and the values of the energy of activation 'Ea' and the entropy of activation 'ΔS#' were calculated and were found to be 44.67kJ/mole -309.51 $JK^{-1}mole^{-1}$ respectively.

14	Contribution to the society	The results have been incorporated in many other studies
15	Whether any Ph.D enrolled /produced out of the project	YES D.Apparao Babu was awarded Ph.D from Andhra University on the thesis entitled "some new analytical and kinetic aspects metoprolol and labetalol" (copy of the proceedings enclosed)
16	No of publications out of the project conference	1 paper presened at international 3 papers published in journals

The research investigations were presented at the International seminar on Emerging Trends in Synthetic Organic Medicinal Chemistry(ESMC-2013), Nov 2013, Vikrama Simhapuri University, Nellore.

1. The research paper "Kinetics and mechanism of oxidation of labetalol by hexacyanoferrate (III) in alkaline medium" has been published in International Journal of Scientific Research, vol 3, issue2, page no 131, Feb 2014
2. A research paper "Oxidemetric determination of labetalol hydrochloride with potassium permanganate in acid medium" has been published in Res. J.Pharm. Bio.Chem.Sci.7(2)1158-1166(2016)
3. A research paper "Spectrophotometer Aided Kinetic, Mechanistic and Thermodynamic Study of Ruthenium(III) Catalysed Oxidation of Esmolol by Sulphate of Cerium(IV) in Aqueous Sulphuric Acid Medium" has been published in Current Physical Chemistry, 2018, 8, 22-36

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