OXIDATIVE KINETIC STUDY OF FLUOROQUINOLONE PHARMACEUTICALS IN ACIDIC/BASIC AQUEOUS SOLUTIONS

A THESIS

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Submitted by:

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2017

Dedicated to
My Parents,
My Husband
Amit &
My Daughter Aarna

University of Kota, Kota

M. B. S. Marg, Near Kabir Circle, Rawatbhata Road, Kota (Raj.)

Certificate

It is to certify that,

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(ii) Literary presentation is satisfactory and the thesis is in a form suitable for publication.

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A Word of Gratitude

"Words are powerless to express my gratitude."

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Ankita Jain

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Chapter – 1

Introduction

ABSTRACT

The present chapter describes the introduction of chemical kinetics, potassium permanganate, its oxidizing properties and includes a brief discussion on fluoroquinolones − powerful antibiotics used in human and veterinary medicine. The fate of antibiotic parent and metabolite compounds entering environmental ecosystems through various pathways raise environmental impact concerns. The current investigation was undertaken with the intent of computing reaction kinetics and clarifying the reaction pathways involved in oxidative degradation of the environmentally significant fluoroquinolone antibiotics by potassium permanganate in aqueous acidic/alkaline medium.

1.1. CHEMICAL KINETICS

Chemical kinetics is the study of rate of chemical processes, it includes investigation of how different experimental conditions can influence the speed of a chemical reaction and yield information about the reaction's mechanism and transition states, as well as the construction of mathematical models that can describe the characteristics of a chemical reaction. The mathematical models that describe chemical reaction kinetics provide chemists and chemical engineers with tools to better understand and describe chemical processes and the complex chemistry of biological systems. These models also used in the design or modification of chemical reactors to optimize product yield, more efficiently separate products, and eliminate environmentally harmful by-products.

One reason for the importance of kinetics is that it provides evidence for the mechanism of chemical processes. Many reactions of great commercial importance can proceed by more than one reaction path; knowledge of the reaction mechanism involved may make it possible to choose reaction conditions favouring one path over another, thereby giving maximum amounts of desired products and minimum amounts of undesired products.

Reaction mechanism describes each reactive intermediate, activated complex, transition states, which bonds are broken, and which are formed. A complete mechanism must also explain the reason for the reactants and catalyst used, the stereochemistry observed in reactants, products and the amount of each. The rate law gives additional information about the individual steps that might be involved in the reaction mechanism. Determining the rate law begins with setting up a kinetics experiment for the chemical reaction. A kinetics experiment is carefully controlled so that measurements are made in time intervals in order to determine the change in concentration of a species over time. That species can be either a reactant (decreasing concentration with time) or a product (increasing concentration with time). If multiple reactants are involved, it is also very important that the concentration of only one reactant change with time.

While determining rate law variables can be involved mathematically. As long as the disappearance of a reactant or appearance of a product measured, rate plots can used to calculate the rate constant. An extension of this method used frequently to determine the activation energy of a reaction, E_a, by measuring the rate and calculating the rate constant at a variety of temperatures. This method involves using the Arrhenius equation, $k = Ae^{(-Ea/RT)}$. Combining the rate law, including reaction order, with the activation energy of a reaction provides a full kinetic profile for how fast or slows a reaction progress and provides information on how factors like temperature and concentration can affect that reaction.

The present investigation deals with the kinetic and mechanistic aspects of the title reaction.

1.2. FLUOROQUINOLONES

Quinolones and fluoroquinolones (FQs) comprises a relatively large, growing and most interesting group of antibacterial drugs which have made a major impact on the field of antimicrobial chemotherapy, particularly in the past few years [1-3]. Fluoroquinolones are relatively a new class of synthetic antibiotics with potent bactericidal, broad spectrum activity against many clinically important pathogens which are responsible for variety of infections including urinary tract infections, gastrointestinal infections [4], respiratory tract infections, sexually transmitted diseases and skin infections [5, 6]. The newer FQs have a wider clinical use and a broader spectrum of antibacterial activity including gram-positive and gram-negative aerobic and anaerobic organisms. Some of the newer fluoroquinolones have an important role in the treatment of community-acquired pneumonia and intra-abdominal infections [7].

Nalidixic acid was the first member of the quinolone class of antimicrobials described in 1962 [8], generated as a by-product of an antimalarial agent synthesis, chloroquine [9]. The nalidixic acid was the first 4-quinolone marketed for clinical use. It was active against some Gram-negative bacteria and possessed pharmacokinetic properties for treating urinary tract infections. It had minor significance because of their limited therapeutic utility and the rapid development of resistance [10, 11].

Consequently, other quinolones have been synthesized and tested that broaden the antibacterial spectrum and the usefulness of these drugs.

The breakthrough for this class of antibacterial agents came when a fluorine atom and a piperazine ring were attached to the 6 and 7 positions of the basic quinolone nucleus. **Figure 1.1** shows the basic fluoroquinolone molecule or 'pharmacore'. These substitutions increased absorption, increased antibacterial activity and reduced toxicities.

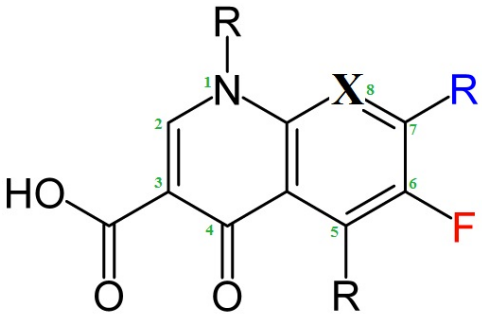


Figure 1.1: General structure of 4-Quinolones.
Quinolones: X = CH or C-R_8; Naphthyridones: X = N.

Two major groups have developed from the basic structure: quinolones and naphthyridones [12-16]. The presence of nitrogen at position 8 identifies the naphthyridones, a carbon and associated group at position 8 identifies the quinolones. Flumequine was the first compound developed with a fluoro- group at position 6 [9]. After that, many FQs have patented and still used today. Thus, continuous efforts were directed to further modify the quinolone pharmacophore with more complex newer fluoroquinolones.

Fluoroquinolones consist of a bicyclic ring structure in which there is a substitution at position N-1, with an alkyl group. Carboxylic acid at position 3 is required for antimicrobial activity, similarly like a keto group at position 4. A fluorine atom at position 6 on the quinolone carboxylic acid nucleus enhances the efficacy of these compounds against gram-negative pathogens and broadens the spectrum of activity against gram-positive pathogens: a basic nitrogen-containing moiety enhances tissue penetration and reduces the central nervous system toxicity.

Modifications of the basic structure at positions 2, 5 and 7 alter the pharmacokinetics of the compound. The presence of carboxylic acid and one or several basic amines functional groups make these antibacterial agents amphoteric and considered as zwitterionic. Both functions are weak and give a good solubility for the quinolones in acidic or basic media. Water solubility at functional pH varies across these compounds, depending on the substitutions on the quinolone carboxylic acid nucleus.

Fluoroquinolones can be classified into four generations based on their antibacterial spectrum [17, 18] **(Figure 1.2)**. The first-generation is rarely used today. Various alterations were done to improve antibacterial activity [4, 9, 19-33]. Second-generation drugs with the addition of fluorine at position 6 of quinolone have improved gram-negative activity and tissue distribution but limited gram-positive activity. Ciprofloxacin and Ofloxacin are the most widely used of the second-generation FQs [34]. They are antibiotics useful for the treatment of a number of bacterial infections.

Third-generation FQs have increased activity against gram-positive bacteria and some anaerobic bacteria. Levofloxacin, a chiral fluorinated carboxyquinolone, is the pure (−)-(*S*)-enantiomer of the racemic ofloxacin [35, 36]. It is a third generation fluoroquinolone antibiotic, usually results in death of the bacteria. Fourth-generation FQs are also unique structurally with a five-member pyrrolidine group at position 7, resulting in improved activity against anaerobic and gram-positive organisms.

Fluoroquinolones have been widely used in animal husbandry, and several agents have veterinary-specific applications. Among them, Enrofloxacin was the first fluoroquinolone introduced into veterinary medicine. It is a bactericidal agent having antibacterial activity against a broad spectrum of Gram-negative and Gram-positive bacteria.

Human Application:

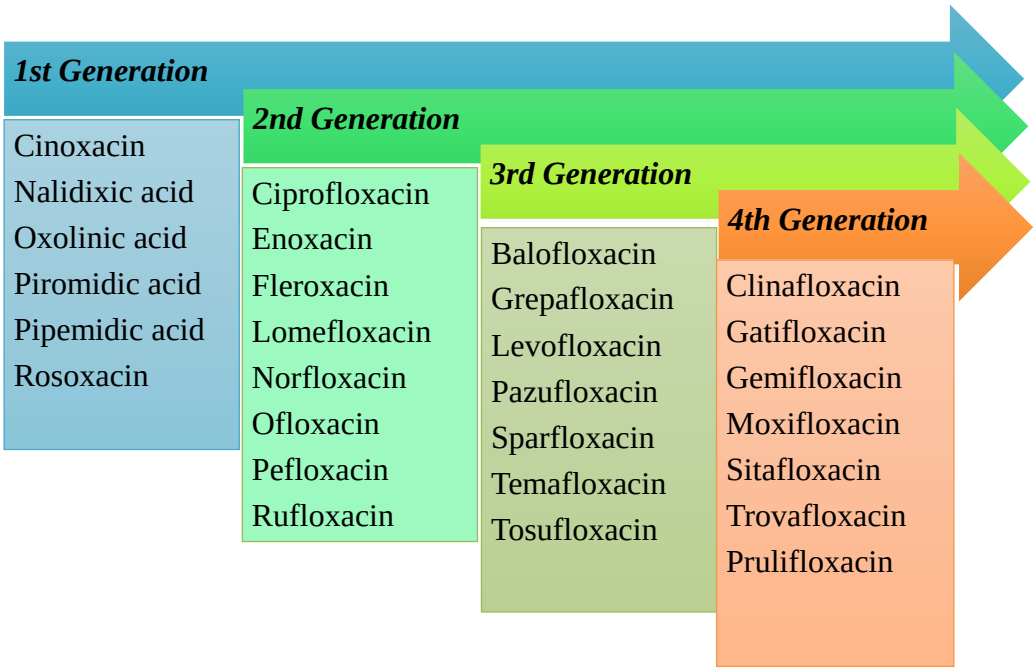


Figure 1.2: Classification of Fluoroquinolones.

Antibiotics are among the emerging micro-contaminants in water because of concerns of their potential adverse effects on the ecosystem and possibly on human health. Antibiotics are released into the aquatic environment via wastewater effluent and agricultural runoff [37] because of incomplete metabolism [38], ineffective treatment removal because large quantities of antibiotics are used annually in human therapy and in agriculture [39]. FQs are of interest, since they are wide spectrum antibacterial with an increasing use in hospitals, households, and veterinary applications [40, 41].

Effective removal of FQs by water treatment processes is important to minimize the possibility of antibiotic resistance development and other potential health risks that may be associated with FQ residues in drinking water. Recently, concern has been raised regarding public health issues over the presence of antibiotics in the environment and by indications of increased bacteria resistance in waste effluents from hospitals, pharmaceutical plants and animal husbandry. The potential toxicities of these antibacterial micro contaminants in the environment necessitate further investigation on their oxidative transformation in order to properly evaluate their risks.

1.3. OXIDATION OF FLUOROQUINOLONES

The presence of fluoroquinolones (FQs) in the aquatic environment is a matter of great concern. Research is on-going for effective removal or transformation of FQs into less hazards species. Transformation by oxidation of fluoroquinolone is an effective process for the purpose. Oxidation techniques have application in water treatment because of their ability to oxidize organics to inactive intermediates or CO_2, H_2O, and salts. The mechanism by which this occurs is called a “redox” couple because an oxidation reaction is coupled with a reduction reaction. Within water treatment, the oxidizing compound reacts with the contaminants to produce less harmful compounds.

Numerous studies have investigated the interactions of FQs with metal oxides [42-50] in acidic as well as in alkaline medium. Zhang et al. [42] studied the adsorption and oxidation of seven fluoroquinolones (ciprofloxacin, enrofloxacin,

norfloxacin, ofloxacin, lomefloxacin, pipemidic acid and flumequine) and structurally related amines with goethite. The carboxylic group of fluoroquinolone is critical for adsorption while the piperazinyl ring is susceptible to oxidation. In comparison to the work of Zhang et al. [43] on the interactions of FQs with manganese oxide, exhibited reactivity in the order of ciprofloxacin ≈ enrofloxacin ≈ norfloxacin ≈ ofloxacin > lomefloxacin > pipemidic acid >> flumequin. FQs have different adsorption sites (piperazine ring versus carboxylic group, respectively) but the same oxidation site (the piperazine ring) toward manganese and iron oxides. Although different adsorption behaviour is involved with these two oxides, the same radical-based oxidation mechanism is present in both cases. In another study, Zhang et al. [44] demonstrated that the reaction kinetics of antibacterial with MnO_2 is controlled by the rate of electron transfer within the precursor complex. This new model improves the ability to quantitatively evaluate the kinetics of oxidative transformation of organic contaminants by manganese oxides in well-defined systems.

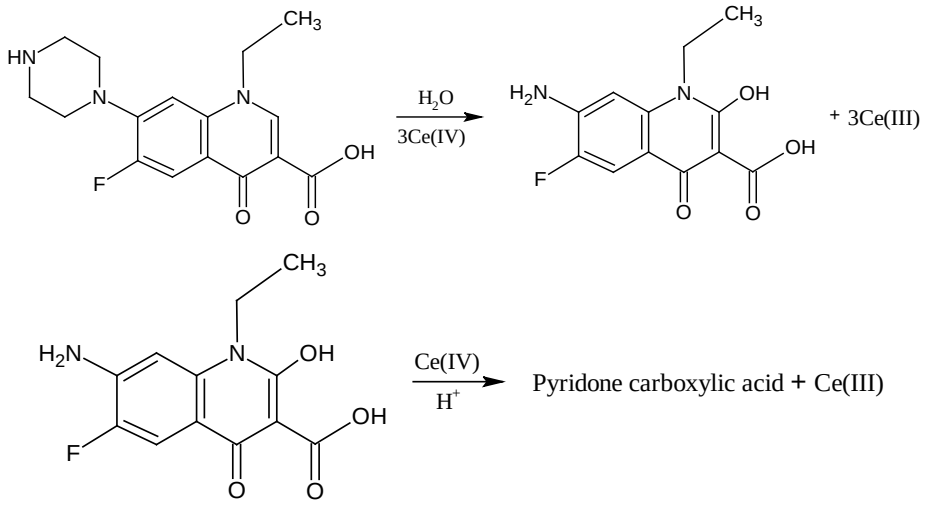
Xiao et al. [51] reports the degradation of ciprofloxacin (m/z 332) by cryptomelane-type manganese (III/IV) oxides. Five major peaks with $[M + H]^+$ molecular ions of m/z 364, 362, 334, 306, and 263 were observed. The study confirms that the quinolone ring is relatively inert to oxidation in the Mn(III/IV) oxide system. Such an observation is consistent with the previous literature where only FQ antibiotics with piperazine substituents are reactive [43].

Advanced oxidation processes (AOPs) [52] have been explored as potential transformation processes for fluoroquinolones. Several studies have examined the formation of degradation products upon the oxidation of fluoroquinolone antibiotics by AOPs [53-63] and oxidants including, Cl_2 [64] and ClO_2 [65]. De Witte et al. [62, 63] identified twelve degradation products formed in the case of the oxidation of ciprofloxacin by ozone while eleven species were observed for levofloxacin. However, the degradation products identified in these studies do not necessarily deplete the range of species formed in these processes, and more information needs to be obtained about their yields and formation kinetics.

Research investigating the reaction of FQs with chlorine has indicated that the piperazine moiety in secondary-amine containing FQs such as ciprofloxacin (CIP) was more readily transformed than that in tertiary-amine-containing FQs such as enrofloxacin (ENR) [64]. In contrast, ENR reacted faster than CIP with chlorine dioxide [65]. Wang in his study [65] of oxidation of seven FQs (ciprofloxacin, enrofloxacin, norfloxacin, ofloxacin, lomefloxacin, pipemidic acid and flumequine) and three structurally related amines by ClO_2 investigated that FQs with piperazine groups are more reactive to ClO_2. FQs follow the trend of OFL > ENR > CIP ~ NOR ~ LOM > > PIP in reactivity. Oxidation leads to dealkylation, hydroxylation and intramolecular ring closure at the piperazine moiety, while quinolone ring remain mostly intact. Similarly, during ozone oxidation, ENR reacted at higher rates than CIP at neutral pH [66]. However, ENR and CIP had comparable reaction rates with manganese oxide [43]. The reactions of ozone and hydroxyl radicals with enrofloxacin and ciprofloxacin lead to stoichiometric elimination of antibacterial activity, indicating that the oxidation products retained significantly less antibacterial potency [67].

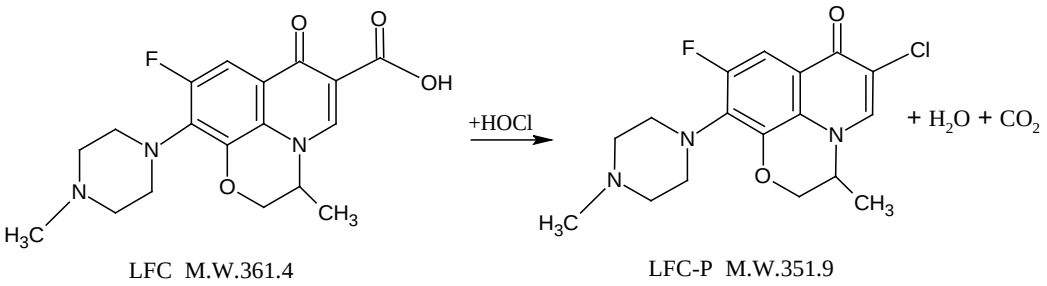
Nanda et al. studied kinetic and mechanistic studies on the oxidation of norfloxacin (NRF) by chloramine-B (CAB) and N-Chlorobenzotriazole (CBT) [68] and on the oxidation of ciprofloxacin by chloramine-B in acidic medium [69]. Electrophilic attack by the oxidant (RNHCl of CAB and R′NCl of CBT) through its positive chlorine, results in the formation of products. Reaction was retarded by the presence of H^+ ions. While in the oxidation of NRF by N-Chlorosuccinimide (NCS) in aqueous HCl medium [70], rate of the reaction increased with the presence of H^+ ions. The active species of NCS was N^+HCS.

Cerium(IV) sulphate is also used as an oxidising agent to oxidise ofloxacin and levofloxacin [71]; ciprofloxacin, Pefloxacin and sparfloxacin [72]; ciprofloxacin [73]; ofloxacin [74] in acidic medium. Oxidation of norfloxacin with cerium(IV) was studied in 0.3 M hydrochloric acid media [75] through the following reaction **(Scheme- I)**. Stoichiometry of the reaction was 1:4 (norfloxacin: Ce^{4+}).



Scheme- I: Oxidation of Norfloxacin with Ce(IV) in 0.3 M HCl [75].

The oxidation of levofloxacin (LFC) was reported by different oxidants like O_3 and hydroxyl radical [76], Cl_2 [77-79], chloramine-T [80], N- Bromosuccinimide [81], hexacyanoferrate(III) [82] in acidic/alkaline media. A Kinetic study of chlorination of LFC was performed at pH 7.2 [77]. It was proposed that the identified transformation products were come from a successive halodecarboxylation and piperazine fragmentation of LFC. Similar product was obtained during the kinetics and mechanism of LFC by free available chlorine **(Scheme- II)** between the pH values 4.2 and 8.2 [78, 79].

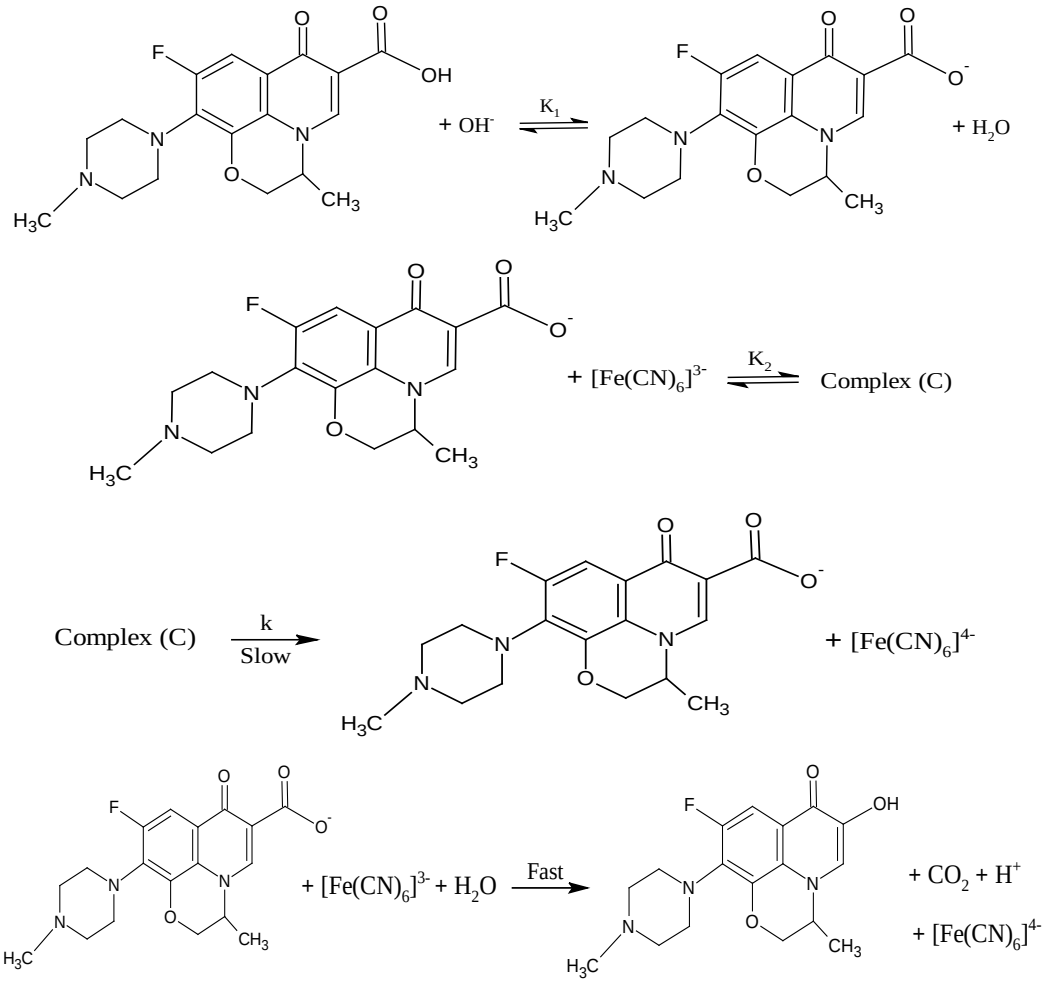


LFC M.W.361.4 LFC-P M.W.351.9

Scheme- II: Formation of Product during Chlorination of LFC [79].

Khan et al. [80] used Sodium-N-chloro-4-methyl benzenesulfonamide, p-$CH_3{-}C_6H_4SO_2NClNa{\cdot}3H_2O$, commonly known as chloramine-T (CAT) and N-bromosuccinimide (NBS) [81] to oxidize levofloxacin in acidic medium. The active oxidizing species of CAT and NBS was TsNHCl (where Ts represents the $CH_3C_6H_4SO_2$-group) and $(CH_2CO)_2N{+}HBr$, respectively. NBS undergoes a simple two-electron reduction in the reaction.

Patgar et al. [82] studied the kinetics of oxidation of levofloxacin (LF) by hexacyanoferrate(III) (HCF) in aqueous alkaline medium. The oxidant and reductant change their oxidation state by different number of units hence this oxidation is a non-complementary reaction with oxidant undergoing one equivalent change **(Scheme-III).**



Scheme- III: Detailed Scheme for the Oxidation of LF by HCF(III) [82].

In comparison, alkaline HCF(III) showed almost no reaction with the FQs, ciprofloxacin (CIP), norfloxacin (NOR), enrofloxacin (ENR) and nalidixic acid (NAL) in the absence of the catalyst [83]. NAL showed no reaction with $K_3Fe(CN)_6$ even in the presence of the catalyst suggesting that piperazine ring is the active site for oxidation of FQs by HCF(III). The rate of oxidation of the studied fluoroquinolones is followed the order: CIP > NOR > ENR.

Oxidation of CIP and ENR, by Fe(VI) were investigated by Yang et al. [84]. Structural changes to the CIP and ENR molecule included dealkylation, formation of alcohols and amides in piperazine ring and oxygen transfer to the double bond in quinolone structure. Mechanism involving the formation of enamine was proposed. Similarly Zhou et al [85] studied kinetics of CIP with Fe(VI) at pH 8 and pH 9. The attack on the piperazinyl ring of the CIP by Fe(VI) appeared to lead to the cleavage or hydroxylation of the rings, and the attack on the quinolone moiety by ferrate(VI) might lead to the cleavage of the double bond at the six-member heterocyclic ring and converted into end products.

1.4. Mn(VII) – AN OXIDANT

Potassium permanganate is an inorganic chemical compound with the chemical formula $KMnO_4$. It is a salt consisting of K^+ and MnO_4^- ions. The manganese is in the +7 oxidation state. It is also known as permanganate of potash and Condy's crystals being named after its discoverer Henry Bollman Condy. It is a strong oxidizing agent, which means it has a tendency to take electrons from other chemicals [86]. It dissolves in water to give purple solutions. If it is evaporated, it makes purple-black shiny crystals [87]. It has a sweet taste and is odorless [88].

Permanganate is a powerful agent in neutral, acidic and alkaline media. The nature of reaction is different in each medium. In an acidic solution, Mn(VII) is reduced to the colourless +2 oxidation state of the Mn^{2+} ion.

$$8\ H^+ + MnO_4^- + 5\ e^- \rightarrow Mn^{2+} + 4\ H_2O,\ E^o = 1.51\ V$$

In a strongly basic solution, permanganate(VII) is reduced to the green +6 oxidation state of the manganate ion, MnO_4^{2-}.

$$MnO_4^- + e^- \rightarrow MnO_4^{2-},\ E^o = 0.56\ V$$

In a neutral medium however, it is reduced to the brown +4-oxidation state of manganese dioxide, MnO_2.

$$2\ H_2O + MnO_4^- + 3\ e^- \rightarrow MnO_2 + 4\ OH^-,\ E^o = 1.23\ V$$

Manganese shows variable oxidation state from +7 to +2. Among them, the most stable oxidation states are +2, +4 and +7. Manganese(III) ions known as mangenic ion, exist in strong concentration acidic media. It undergoes dispropornation to give Mn^{2+} and Mn^{4+}. The Mn is stable in +4 forms as MnO_2. It is grey-to-grey white black solid. In potassium hypomanganate, (K_3MnO_4) manganese is in +5 states, which is unstable and decomposes to give Mn^{4+} or Mn^{3+}. It slowly decomposes to MnO_2. The Mn in +6 states exists only in basic solution as deep green manganese ion. The permanganate i.e. Mn in +7 oxidation state and has intense purple colour.

The permanganate ion can oxidize organic compounds through several pathways that include hydrogen abstraction, electron abstraction, incorporation of oxygen atom into structure, and hydride-ion abstraction. Mn(VII) can oxidize all hydrocarbons or any oxidisable functional group. This is because the standard reduction potential of most of the inorganic oxidants is usually around 1.0 V, whereas standard reduction potential of most of the organic compounds are much less than this value.

In acidic medium active species of Mn(VII) exists in different forms as $HMnO_4$, $H_2MnO_4^+$, $HMnO_3$ and Mn_2O_7. Among them MnO_4^- ion is powerful oxidizing agent in aqueous alkaline as well as in acidic medium. The stable reduction product of MnO_4^- in acid medium is Mn(II). In the alkaline medium, permanganate shows various oxidation states, such as Mn(VII), Mn(V), and Mn(VI). In a strongly alkaline medium, the stable reduction product [89, 90] of permanganate is manganate ion, MnO_4^{2-}. The process can divided into a number of partial steps and examined separately. The MnO_2 appears only after a long time, that is, after the complete consumption of MnO_4^-. The mechanisms for different organic substrates suggested by various authors are not similar, indicating that varieties of mechanisms are possible, depending upon the nature of the reactive manganese species, the reaction environment, and the nature of the substrate [91].

1.5. KINETIC STUDIES WITH Mn(VII)

As a green and inexpensive oxidant, Mn(VII) has been widely used in water treatment for enhancing coagulation and controlling cyanotoxins and micro pollutants [92-94]. Although Mn(VII) reactivity with the antibiotics was lower than that reported for ozone and free chlorine, its high selectivity and stability suggests a promising oxidant for treating sensitive micro-pollutants in organic-rich matrices [95].

In view of synthetic & mechanistic aspects, oxidation by permanganate researched by Sukalyan Dash and co-workers [96]. Permanganate oxidizes most of the substrates [97-113], viz., alkenes, alcohols, aldehydes, ketones, carboxylic acids, esters, sulphides, thiols, sugar, steroids, etc. and it finds extensive applications in organic synthesis [114-117]. Many researchers [118-125] have reported the oxidation of amino acids by permanganate ion in varieties of media under different conditions. Recently, permanganate has been successfully used for the in situ degradation of many organic contaminants [126, 127].

Considerable amount of work has been done on the oxidation of various drugs by potassium permanganate in aqueous acidic/alkaline medium. In earlier report on oxidation by Mn(VII) in acidic media, some investigators have observed the induction period [128] and some have noticed autocatalytic nature either by Mn(II) or by one of the products obtained by substrates [129]. Rajeshwari et al. [130] observed an autocatalysis reaction due to the formation of one of the products, Mn(II) in the reaction kinetics of oxidation of etophylline (bronchodilator) by permanganate ions in sulphuric acid medium at 25 °C and at constant ionic strength, 1.60 mol dm^{-3}. Based on the results a free radical mechanism was proposed. The rate law was given as:

$$\frac{[\mathrm{ETO}]}{k_{\mathrm{autocat}}} = \frac{1}{k_2[\mathrm{Mn(II)}]} + \frac{1}{k_2 K_2[\mathrm{Mn(II)}]} + \frac{1}{k_2}$$

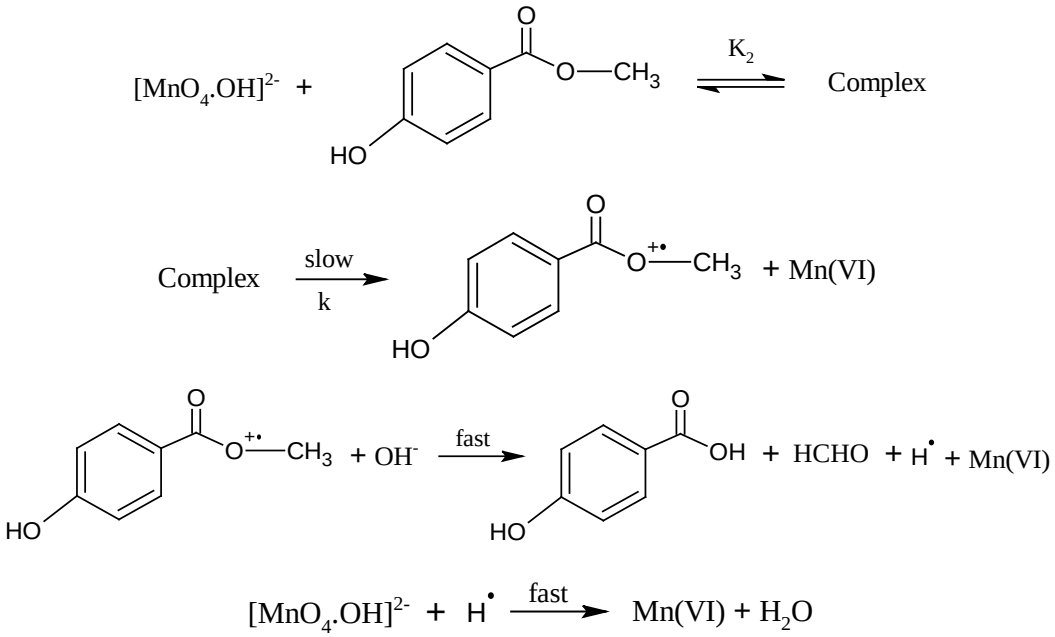
While in autocatalytic pathways in which Mn(II) ions have been determined responsible for the effect, a mechanism with no free radicals has been suggested [131,132].

During the kinetic study of oxidation of imidazole by Mn(VII) in aqueous sulphuric acid medium [133], the rate of the reaction increased with increase in ionic strength of reaction medium, suggesting positive salt effect. This means that both species at the activated complex are of the same charges in the rate determine step. The enhancement of the rate by added ions and absence of the evidence of intermediate complex formation suggests that the reaction most probably occurred by the outer-sphere mechanism. Similarly, during oxidation of cetirizine hydrochloride (an anti-allergic drug) in acidic medium [134], there is no shifting of λ_{max} = 525 nm during oxidation reaction indicating there is no intermediate complex formation suggesting outer sphere mechanism. While Patgar et al. [135] reported that as the ionic strength increases, the rate of reaction also increases but the reaction proceeds by inner-sphere mechanism. In other reported reactions, increasing ionic strength had no effect on the rate of reaction and oxidation occurs via inner-sphere mechanism [136, 137].

Recently, Kulkarni et al. [138] studied the uncatalysed and Pd(II)-catalysed oxidation of linezolid by permanganate in acidic medium in the pH range from 3.0 to 6.0. The rate constants were decreases with increase in pH of the reaction medium. While during the oxidation of ranitidine (H_2-receptor antagonist) by $KMnO_4$ [139] rate decreases with increase in H^+ ions. It clearly indicates that, the role of hydrogen ion is important in the reaction.

The oxidant, manganese(VII) exist in alkali media as alkali-permanganate species $[MnO_4.OH]^{2-}$, which takes part in the chemical reaction. Naik et al. [140] studied the kinetics of the oxidation of metronidazole by alkaline permanganate. The dealkylated products of metronidazole have reduced antimicrobial activities after oxidation. Ariga et al. [141] studied the free-radical-induced oxidative degradation of methylparaben (antibacterial drug) by permanganate in alkaline medium. In the slow step of the mechanism, complex (formed between $[MnO_4.OH]^{2-}$ and methylparaben) decomposed into a cation free-radical intermediate, which react with alkali to form products **(Scheme- IV)**.

$$MnO_4^- + OH^- \overset{K_1}{\rightleftharpoons} [MnO_4.OH]^{2-}$$



Scheme- IV: Mechanism for the Oxidation of Methylparaben by Permanganate in Aqueous Alkaline Medium [141].

The uncatalysed and ruthenium(III)-catalysed oxidation of D-panthenol (provitamin B_5) by MnO_4^- was studied in alkaline medium at 298 K [142]. The stoichiometry in both the cases was [panthenol]: [MnO_4^-] = 1:4 and the same oxidation products were observed for both the reactions. Mulla et al. [143] studied the ruthenium(III) catalyzed oxidation of atenolol (an antihypertensive drug) by permanganate in alkaline medium. Reaction exhibit 1: 8 stoichiometry (atenolol: $KMnO_4$). A mechanism involving the formation of a complex between catalyst and substrate was proposed. Under the conditions studied, the reaction occurs as two successive one-electron reductions rather than a successive one-electron reduction in a single step [144].

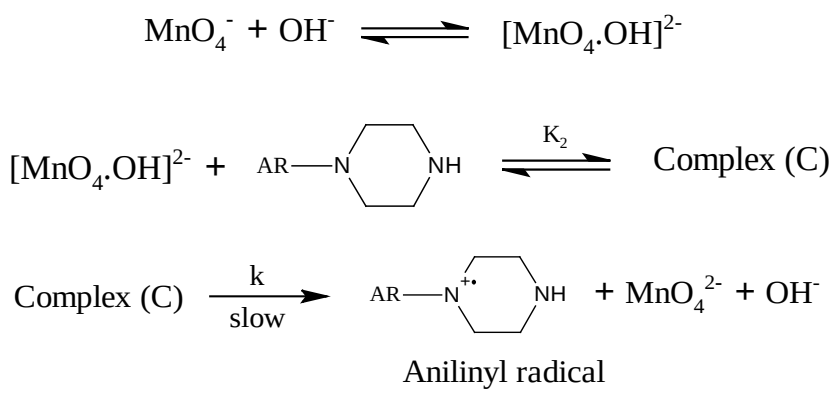
Many researchers investigate the oxidation of FQs by permanganate in aqueous acidic/alkaline medium. Hu et al. [95] examined the oxidation of ciprofloxacin (CIP) by permanganate. The observed small pH dependence suggested that Mn(VII) was mainly targeting the tertiary aromatic amine (N1) on the piperazine ring instead of the aliphatic amine (N4), similar to the mechanism proposed for fluoroquinolone reactions with MnO_2 [53]. Hu et al. [145] examined the oxidation

pathway of CIP, observed that the oxidation target sites were the tertiary aromatic and secondary aliphatic amine groups on the piperazine ring and cyclopropyl group, and identified twelve oxidation products for CIP.

Mn(VII) has been proved to be fairly effective in treating several FQs, ciprofloxacin, difloxacin, lomefloxacin, norfloxacin, and ofloxacin. Study reported the susceptibility for oxidation process that followed the subsequent rank order: CIP = NOR > OFL > DIF > LOM. Oxidation of FQs proceeded at piperazine moiety yielding respective hydroxy and oxo analogs, and the quinolone fragment remains intact. Structures of products were characterized based on UPLC/MS/MS fragmentation pathways [146]. Similar oxidation and characterization process was observed for danofloxacin, enrofloxacin, marbofloxacin, orbifloxacin and pefloxacin under permanganate treatment in acidic conditions at pH from 3.0 to 6.0 [147].

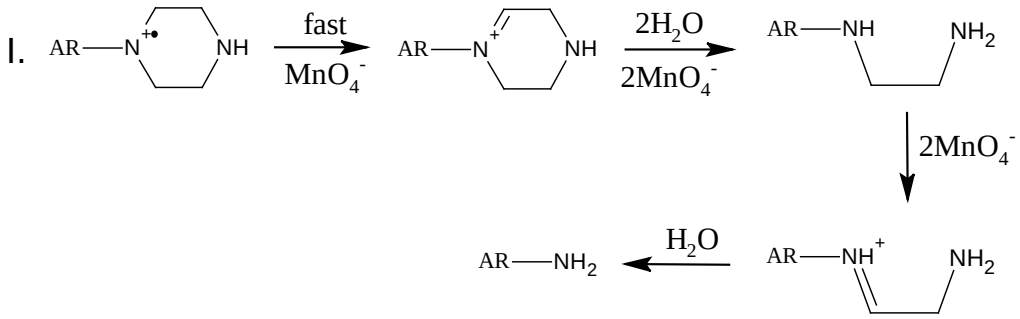
Recently Xu et al. [148] investigated oxidation of enrofloxacin (ENR) by permanganate in water with respect to the kinetics, pH effect, and the evaluation of residual antibacterial activity after oxidative treatment. Four main oxidation products were identified at different pH values. Autocatalysis taking place at slightly acidic pH promotes the reaction but has no effect on the product types. The oxidation took place at the piperazine ring, include N-dealkylation, hydroxylation, and hydrolysis. While in another study, Xu et al. [149] identified nine products for the oxidation of ENR by $KMnO_4$ at neutral pH, one of which was an N-oxide product formed from the oxidation of tertiary amines.

The kinetics and degradation pathways of oxidation of ciprofloxacin (CIP) by permanganate in alkaline medium at constant ionic strength of 0.04 mol dm^{-3} has been studied by Thabaj et al. which reveal that the piperazine moiety of CIP is the predominant oxidative site to $KMnO_4$ [150]. Product characterization of reaction mixtures indicates the formation of three major products corresponding to m/z 263, 306, and 348 (corresponding to full or partial dealkylation of the piperazine ring). The reaction kinetics and product characterization point to a reaction mechanism that likely begins with formation of a complex between CIP and the $KMnO_4$, followed by oxidation at the aromatic N1 atom of piperazine moiety to generate an anilinyl radical intermediate **(Scheme- V).**

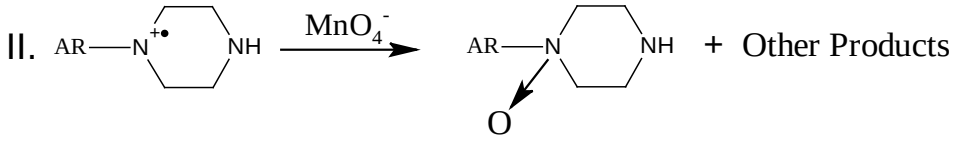


Scheme- V (Followed by other Fast Steps).

Based on formation of oxidation products, two pathways were reported. Pathway I is an *N*-dealkylation process in which one electron is transformed from the radical N1 to Mn(VIII), yielding an iminium ion. Similar oxidation and hydrolysis occurs at the N4 atom, and form partially dealkylated M– 26 product. In a similar fashion, the M– 26 product was converted to the fully dealkylated M– 69 product.



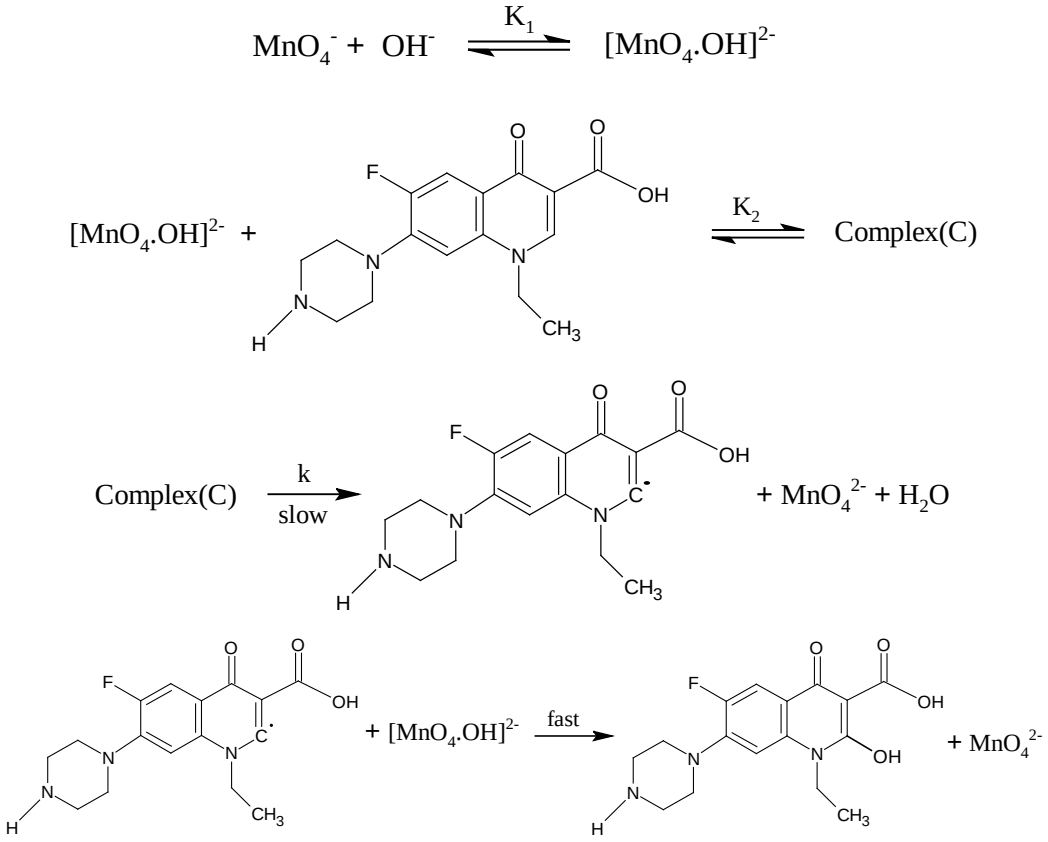
In Pathway II, Anilinyl radical on reaction with $KMnO_4$ forms *N*-oxide product.



Scheme- V: Oxidation Products of CIP by Alkaline $KMnO_4$ [150].

Naik et al. [151] studied the kinetics of the oxidation of norfloxacin (NF) by alkaline permanganate at 25 °C. The stoichiometry was found to be 1:2 with respect to NF and Mn(VII). The results imply that the alkali-permanganate species $[MnO_4{\cdot}OH]^{2-}$ combines with NF to form an intermediate complex. The complex

breaks forming NF radical intermediate and gets converted into the final products, hydroxylated NF and Mn(VI) **(Scheme- VI)**.



Scheme- VI: Mechanism for the Oxidation of NF by Alkaline Mn(VII) [151].

Similar mechanism was reported by Khan et al. [152] for the oxidation of levofloxacin by permanganate in alkaline medium, involving free radicals and decomposes into hydroxylated LF and Mn(VI) as products.

Kinetics of the oxidation of Moxifloxacin (MOX) by Mn(VII) in alkali followed the mechanism involving free radical generated from MOX [153]. The main product was identified as 1-cyclopropyl -6-fluoro -1,4- dihydro -7- (octahydro -2-oxopyrrolo [3,4-b] pyridin -6-yl)-8-methoxy-4-oxoquinoline-3-carboxylic acid. The other three oxidative products were similar to the oxidative products of other fluoroquinolones [65, 154]. However, the abnormally high values of m/z of products were assigned to the permanganate complexes of the products, which are unusual in the non-metallic [78] oxidation of MOX.

The kinetic and mechanistic investigation of oxidation of emerging contaminant lomefloxacin (LMF) by alkaline permanganate carried out spectrophotometrically [155]. Results showed that permanganate attacks the piperazinyl moiety of LMF, degrades completely, and forms the final product that has a lomefloxacin structure, in which the piperazine ring was replaced with $–NH_2$ group. The result is consistent with previously reported literature for ciprofloxacin and norfloxacin [150, 151]. The same oxidation product was reported for lomefloxacin oxidation by manganese oxide and by TiO_2 photo catalysis [43, 156]. The observed higher rate constant of LMF as compared to norfloxacin and ciprofloxacin is due to the presence of two highly electronegative fluorine atoms on oxoquinoline moiety.

Kinetic results of the oxidation of fluoroquinolones by various oxidants in aqueous acidic/alkaline medium have given in **Table 1.1**.

TABLE: 1.1

Oxidation of Fluoroquinolones in aqueous acidic/alkaline medium by different Oxidants

S. No.	Fluoroquinolone	Oxidant	Medium	Products	Activation Parameters				Ref.
					E_a (kJ mol^{-1})	$\Delta H^{\#}$ (kJ mol^{-1})	$\Delta S^{\#}$ (JK^{-1} mol^{-1})	$\Delta G^{\#}$ (kJ mol^{-1})	
1.	Norfloxacin ($C_{16}H_{18}N_3O_3F$)	CAB CBT	$HClO_4$	3-fluoro-4-piperazinyl-6-Nethylaminophenylglyoxalic acid ($C_{14}H_{18}N_3O_3F$)	115.9 32.59	112.5 29.8	52.22 − 208.2	97.20 37.12	68
2.	Norfloxacin ($C_{16}H_{18}N_3O_3F$)	NCS	HCl	3-fluoro-4-piperazinyl-6-N-ethylaminoglyoxylic acid ($C_{14}H_{18}N_3O_3F$)	8.83	6.23	− 132.95	41.62	70
3.	Levofloxacin (m/z = 361.4)	HOCl	Aqueous	m/z = 351.9 Decarboxylation, Chlorination	28.6	26.1	− 12.4	29.8	79
4.	Levofloxacin (m/z = 361.4)	CAT	$HClO_4$	Oxo derivative of LF	22.4	42.6	− 123.4	92.12	80
5.	Levofloxacin (m/z = 361.4)	NBS	HCl	Hydroxylated LF (m/z = 330), Succinimide	88.55	85.22	− 76.12	90.14	81
6.	Levofloxacin (m/z = 361.4)	HCF(III)	KOH	m/z = 333 Decarboxylation, hydroxylation	47	44	− 151	89	82

7.	Ciprofloxacin (m/z = 332)	$KMnO_4$	KOH	m/z = 263 (Full dealkylation) m/z = 306 (Partial dealkylation) m/z = 348 (*N*- oxide product)	13.4 ± 0.3	10.9 ± 0.2	− 247 ± 6	84.5 ± 3.0	150
8.	Norfloxacin (NF) (m/z = 319.33)	$KMnO_4$	NaOH	Hydroxylated NF (m/z = 335), Mn(VI)	58.6 ± 2.0	56.0 ± 2.0	− 29.8 ± 3.0	58.2 ± 2.0	151
9.	Levofloxacin(LF) (m/z = 361.4)	$KMnO_4$	KOH	Hydroxylated LF, Mn(VI)	14.9	11.9	− 143.2	63.10	152
10.	Moxifloxacin (m/z = 400.42)	$KMnO_4$	KOH	Oxo derivative (m/z = 415.41), Mn(VI)	37.4 ± 1	34.9 ± 1	− 166 ± 4	85 ± 2	153
11.	Lomefloxacin (m/z = 351)	$KMnO_4$	NaOH	Full dealkylation (m/z = 268), Mn(VI)	27.6 ± 2.7	25.5 ± 2.6	−0.87 ±0.10	25.8 ± 2.5	155
12.	Norfloxacin (m/z = 319.33)	DPA*	KOH	Hydroxylated NF (m/z = 335), Ag(I)	28.72	26.24	− 115	36.9	157

* Diperiodoargentate(III).

1.6. SCOPE OF THE WORK

Antibiotics are more and more a focus point of research due to their high detection frequency in the environment and the increasing bacterial resistance formation. Among various antibiotics, fluoroquinolones are of great interest, since they are wide spectrum antibacterials with an increasing use in hospitals, households, and veterinary applications. There is major concern that antibiotics emitted into the aquatic environment can reach drinking water. Contamination with FQs has been widely reported all around the world in different aquatic matrices including surface water, ground water, sewage or sediment samples. Attractive treatment technologies for the degradation of FQs in aqueous solution are needed because under oxidation–degradation process most of produced intermediates can be finally mineralized into CO_2, water, and mineral species.

$KMnO_4$ has been extensively used in drinking water and wastewater treatment practices for decades. Mn(VII) oxidize a wide range of emerging environmental micropollutants, such as carbamazepine, cefazoline, dichlorvos, chlorophenol, lincomycin, triclosan, bisphenol A and sulfamethoxazole. It also has been widely used by water utilities to control dissolved manganese, taste/odour/color, and biological growth. It is a potent oxidising agent at all pH values.

Since the present atmosphere is oxidative in nature, mostly drug transformations under the natural environment are most likely to follow oxidation path. Present investigation is based upon the kinetic and mechanistic study of oxidation-reduction reactions between commonly used oxidant permanganate and few of the most frequently used fluoroquinolone drugs. The proposed work will gives a novel application in the field of pharmaceuticals as well as kinetics. The dealkylated oxidation products of fluoroquinolones have reduced antimicrobial activity. Since dealkylated products were obtained in the present study, it is evident that the products of the title reaction have reduced antimicrobial activity after oxidation. So this study will be effectively used in waste-water treatment at the sites contaminated by fluoroquinolone antibiotics.

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Chapter - 2

Experimental

ABSTRACT

The current chapter describes the experimental information of the work accompanied in this thesis. The chapter divides into two sections. The first section describes the details of numerous reagents, chemicals and preparation of their solutions. The second section deals with the explanation of instruments, characterization techniques, and analytical procedures which are used to study the kinetics of oxidation of fluoroquinolones by permanganate in aqueous acidic/alkaline medium.

The present chapter includes the instrumental details of all the characterization tools, details of the reagents, chemicals and their solutions used to study the kinetics of the numerous oxidation reactions. This chapter is divided into two sections:

SECTION – I

2.1. CHEMICALS

The details of the reagents, chemicals and their solutions used in kinetic study of the different oxidation reactions are as follows:

2.1.1. Ciprofloxacin ($C_{17}H_{18}FN_3O_3$).

The solution of ciprofloxacin (KORES India Limited) was prepared by dissolving the requisite amount of its salt in doubly distilled water to obtain the solution of desired concentration. The drug was used as received. Freshly prepared solution of ciprofloxacin was always employed.

2.1.2. Ofloxacin ($C_{18}H_{20}FN_3O_4$).

Standard solution of ofloxacin (KORES India Limited) was prepared by dissolving the calculated quantity of pure drug in 0.1 M H_2SO_4. The acid present in the substrate solution is also taken into account in the calculation of the total acid present in each case of the proposed reaction. Solution of ofloxacin was always prepared freshly before experiment.

2.1.3. Levofloxacin Hemihydrate ($C_{18}H_{20}FN_3O_4.1/2\,H_2O$).

Levofloxacin hemihydrate sample (Dr. Reddy's Laboratories Limited) was used as supplied and the solution of desired concentration was prepared by dissolving appropriate quantity of the drug in doubly distilled water. Freshly prepared solution of levofloxacin was always employed.

2.1.4. Enrofloxacin ($C_{19}H_{22}FN_3O_3$).

Solution of required concentration of enrofloxacin (Cipla Limited) was prepared by dissolving the known amount of drug in doubly distilled water.

Enrofloxacin solution was always prepared freshly and the drug was used as received.

2.1.5. Potassium Permanganate ($KMnO_4$).

The solution of permanganate was made by dissolving the required quantity of $KMnO_4$ (BDH AnalaR) crystals in double-distilled water. Commercially available potassium permanganate generally contains impurity. Thus it cannot be used as a primary standard. In order to make standard potassium permanganate solution it is standardized by titrating against oxalic acid [1]. Freshly prepared & standardized permanganate solutions were always used in kinetics experiments.

2.1.6. Manganese Sulphate ($MnSO_4$).

The Mn(II) solution was made by dissolving appropriate amount of manganese sulphate (BDH AnalaR) in known volume of water to obtain a solution of desired concentration. The solution of manganese sulphate was quite stable for longer periods.

2.1.7. Potassium Manganate (K_2MnO_4).

Potassium manganate, Mn(VI) solution was prepared as described by Carrington and Symons [2] The green-coloured solution of K_2MnO_4 formed is characterized by its visible spectrum. The solution was standardized spectrophotometrically at 608 nm.

2.1.8. Sodium Sulphate (Na_2SO_4).

Solution of sodium sulphate (BDH AnalaR) was prepared by dissolving requisite quantity of the salt in doubly distilled water to obtain the solution of desired concentration.

2.1.9. Sodium Nitrate ($NaNO_3$).

The solution of sodium nitrate (Merck) was prepared by dissolving the required amount of the salt in doubly distilled water. The solution is quite stable and does not show any deterioration even on long standing at ambient temperature.

2.1.10. Sodium Hydroxide (NaOH).

The pellets of sodium hydroxide (BDH AnalaR) were dissolved in doubly distilled water and the solution was standardized by titrating its known aliquot sample against the oxalic acid solution using phenolphthalein [3] as an indicator. The solutions of sodium hydroxide were always used after standardizing as these solutions deteriorate on standing.

2.1.11. Oxalic Acid [$(COOH)_2$].

Oxalic acid (BDH AnalaR) is a primary standard and its aqueous solution exhibits longer stability. The solution of oxalic acid was kept in dark at ambient temperature to avoid photolytic decomposition.

2.1.12. Sulphuric Acid (H_2SO_4).

The solution of sulphuric acid (Merck) was prepared by dissolving the required volume of sulphuric acid in doubly distilled water. Then the solution was standardized against prestandardized sodium hydroxide solution using phenolphthalein as an indicator. The standardized solution was kept stoppered as a stock solution.

2.1.13. Sodium Acetate (CH_3COONa).

Solution of sodium acetate (E. Merck, G.R.) was prepared by dissolving the required quantity of the salt in doubly distilled water. The solution does not show any decomposition even in diffused light for a longer period.

2.1.14. Sodium Fluoride (NaF).

Solution of sodium fluoride (S. D. Fine Chem., A.R.) was prepared by dissolving the requisite amount of the salt in doubly distilled water.

2.1.15. 2, 4 – Dinitrophenylhydrazine ($C_6H_3(NO_2)_2NHNH_2$).

The reagent was prepared by means of Brady's method. Requisite amount of 2, 4 – dinitrophenylhydrazine was mixed with 10 ml conc. HCl and 12.5 ml water, warming on a water bath, then diluted with deionized water. This reagent is more suitable for water soluble aldehydes.

2.1.16. Phenolphthalein Indicator ($C_{20}H_{14}O_4$).

Phenolphthalein (BDH, AnalaR) was used as an end point indicator for acid base titration.

2.1.17. Nessler's Reagent [$K_2(HgI_4)$].

The reagent was prepared by dissolving 100gm of mercury (II) iodide and 70gm of potassium iodide in 100 ml of deionized water. The resulting solution was then added to a solution of 160gm of sodium hydroxide in 700 ml deionized water with stirring. This solution was diluted up to one litre with deionized water. The precipitate was allowed to settle for three days and the supernatant liquid was pour out and kept in a brown bottle.

All other reagents such as, hydrochloric acid, acrylonitrile, methanol, *t*-butyl alcohol, acetic acid etc. were either of (BDH AnalaR) grade or (E. Merck) guaranteed grade and used as supplied.

Doubly distilled water, second distillation being from alkaline permanganate solution in a glass assembly, was employed in all the preparations and kinetic studies.

The glass vessels employed both for storing the solutions and for kinetic studies were either of Corning or Borosil makes.

SECTION – II

2.2. INSTRUMENTS

This section describes the basic theories and principles of the numerous instrumental techniques such as LC-MS, FT-IR spectroscopy, UV-VIS spectroscopy, etc. and details of analytical techniques used to study the oxidation of fluoroquinolones in aqueous acidic/alkaline medium.

2.2.1. Liquid Chromatography - Mass Spectroscopy.

Liquid chromatography–mass spectrometry (LC-MS) is an analytical chemistry technique that combines the physical separation capabilities of liquid

chromatography (LC) with the mass analysis capabilities of mass spectrometry (MS). LC-MS is a powerful technique that has very high sensitivity and selectivity and so is useful in many applications. This technique allows for the structural elucidation of unknown molecules through fragmentation. Liquid chromatography generally utilizes very small particles packed and operating at relatively high pressure, and is referred to as high performance liquid chromatography (HPLC). Mass spectrometry is an analytical technique that works by ionizing molecules and then sorting and identifying the ions according to their mass-to-charge (*m/z*) ratios.

Two key components in this process are the ion source, which generates the ions, and the mass analyzer, which sorts the ions. Several different types of ion sources are commonly used for LC-MS. Each is suitable for different classes of compounds. In electron spray ionization, the analyte molecules are ionized first, at atmospheric pressure. The analyte ions are then mechanically and electrostatically separated from neutral molecules. There are many different mass analyzers that can be used in LC-MS. Single quadrupole, triple quadrupole, ion trap, time of flight (TOF) and quadrupole-time of flight (Q-TOF). In a time-of-flight (TOF) mass analyzer, a uniform electromagnetic force is applied to all ions at the same time, causing them to accelerate down a flight tube. Lighter ions travel faster and arrive at the detector first, so the mass-to-charge ratios of the ions are determined by their arrival times. Time-of flight mass analysers have a wide mass range and can be very accurate in their mass measurements.

One fundamental application of LC-MS is the determination of molecular weights. This information is a key to determining identity. Another fundamental application of LC-MS is the determination of information about molecular structure. The major advantages of this technology include sensitivity, specificity and precision as analysis is done at molecular level.

LC-MS was suitable to detect fluoroquinolones and their products. The molecular ions of unknown products were easily recognized by the soft electrospray ionization technique. Increasing fragmentation voltage of LC-MS yielded more fragments to facilitate product identification. In the present study, an LC-ESI-MS,

(Q-TOF Micromass, WATERS Company, UK), was used for product analysis over a mass scan range of 50 – 1000 m/z. The characterization was done from SAIF/CIL, Panjab University, Chandigarh.

2.2.2. Fourier Transform Infrared (FT-IR) Spectrophotometer.

Fourier Transform-Infrared Spectroscopy (FT-IR) [4] is a technique which is used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. An infrared spectrum represents a fingerprint of a sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. Because each different material is a unique combination of atoms, no two compounds produce the exact same infrared spectrum. Therefore, infrared spectroscopy can result in a positive identification (qualitative analysis) of every different kind of material. In addition, the size of the peaks in the spectrum is a direct indication of the amount of material present. With modern software algorithms, infrared is an excellent tool for quantitative analysis.

The improved sensitivity and speed have opened up new areas of application. The sensitivity benefits enable identification of even the smallest of contaminants. This makes FT-IR an invaluable tool for quality control or quality assurance applications. Spectra can be measured in situations where very little energy reaches the detector and scan rates can exceed 50 spectra a second. Fourier transform infrared spectroscopy is used in geology, chemistry, materials and biology for and research fields.

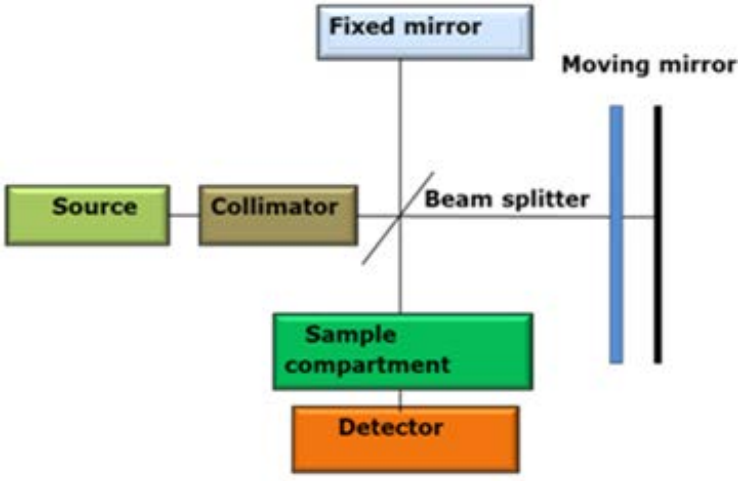

Figure 2.1: Block diagram of an FT-IR spectrometer [5].

In the present study, FT-IR spectra were recorded using ALPHA-T model, Bruker, Germany spectrometer. Spectra are recorded in the range of 400-4000 cm^{-1} by mixing the sample with dried KBr with a resolution of 4 cm^{-1}.

2.2.3. Ultraviolet - Visible (UV-VIS) Spectroscopy.

Ultraviolet-Visible (UV-VIS) spectroscopy is an important tool in analytical chemistry for the quantitative determination of different analytes, such as transition metal ions, highly conjugated organic compounds, and biological macromolecules. It also refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible and adjacent (near-UV and near-infrared). The absorption or reflectance in the visible range directly affects the perceived colour of the chemicals involved. In this region of the electromagnetic spectrum, atoms and molecules undergo electronic transitions. Spectroscopic analysis is commonly carried out in solutions but solids and gases may also be studied.

UV-Vis spectroscopy can be applied to determine the kinetics and the rate constant of a chemical reaction. The reaction, occurring in solution, must present color or brightness shifts from reactants to products in order to use UV-Vis spectrophotometer for this application. The rate constant of a particular reaction can be determined by measuring the UV-Vis absorbance spectrum at specific time intervals. If the reaction is first order, it would have the integral first order rate law: ln[A](time t) = −kt + ln[A](initial). Therefore, graphing the natural log (ln) of the concentration [A] versus time will graph a line with slope − k, or negative the rate constant. Different rate orders have different integrated rate laws depending on the mechanism of the reaction. In order to study the kinetics of the reaction UV-Vis radiation is passed through the reaction cell and the absorbance changes can be observed.

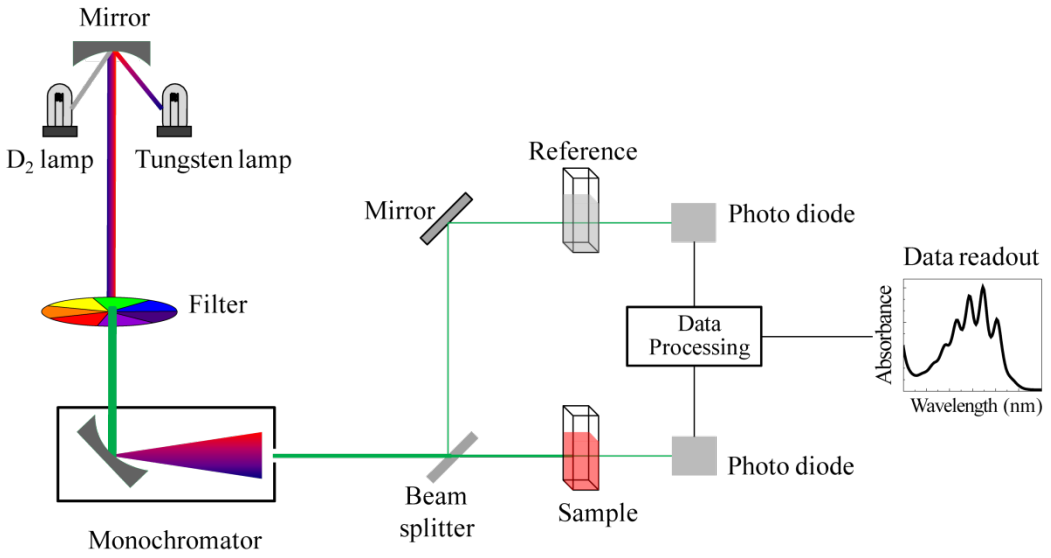

Figure 2.2: Schematic of a double beam UV-Vis spectrophotometer.

In the kinetic studies, a double beam 3000+ LABINDIA, UV-Vis spectrophotometer with a cell of 1.0 cm path length in the spectral range of 200-800 nm, was used to analyse the reactions. A Peltier accessory (temperature-Controller) model PTC-2 is attached with UV-Visible spectrophotometer and is used for the variations at different temperature.

2.2.4. pH - Meter.

Systronic digital pH meter, model MAC (MSW- 552) was used for the determination of pH of the reaction mixtures with the maximum uncertainty in pH of ±0.01 unit.

2.2.5. Electronic Balance.

Citizon electronic balance, CX 220 was used for weighing purposes. The least and maximum count of balance is 0.0001 mg and 220gm, respectively.

Procedure and methodology [6] used for the kinetic study are given in concerned chapters.

2.3. REFERENCES

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Chapter - 3

Mechanistic and Kinetic Study of Oxidation of Ciprofloxacin by Permanganate in Aqueous Sulphuric Acid Medium

ABSTRACT

Mechanistic and kinetic study of oxidation of ciprofloxacin by permanganate ion has been studied in aqueous sulphuric acid medium. Order with respect to substrate, oxidant and acid concentrations were determined. Product characterization of reaction mixture indicates the formation of major product m/z 263 corresponding to dealkylation of the piperazine ring of ciprofloxacin. The piperazine moiety of ciprofloxacin is the predominant oxidative site to $KMnO_4$. A suitable mechanism is proposed and rate law is derived.

$$k_{obs} = \frac{k_1 K_1 [CIP]\,[H^+]}{1 + K_1 [H^+]}$$

The activation parameters with respect to the slow step of the mechanism were computed and thermodynamic quantities were also determined.

3.1. INTRODUCTION

Fluoroquinolones currently represent one of the most important class of antibacterial agent's worldwide, on the basis of annual global sale and therapeutic versatility [1]. They are a family of synthetic, broad spectrum antibacterial compounds, used in a huge number of human and veterinary applications [2]. Ciprofloxacin (CIP) {1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazine-1-yl)-quinolone-3-carboxylic acid} is a second generation fluoroquinolone antimicrobial agent with a wide spectrum of activity against many gram positive and gram negative aerobic and anaerobic bacteria. Ciprofloxacin has been use in the treatment of a wide range of infections. Due to their extensive usage, fluoroquinolones may pass in the environment through waste water discharge and bio solids from sewage treatment plants. Continuous exposure of antibiotic drugs to bacterial communities, promotes the bacteria to develop antibiotic resistance power. The possible induction of antibiotic resistance in bacteria is directly related to human health. The behaviour of fluoroquinolones moiety containing drugs plays an important role during water treatment process [3, 4]**.** There are studies on the modified pharmacological and toxicological properties of these drugs in the form of metallic complexes [5-7]. The structure of Ciprofloxacin **(Figure 3.1)** is shown below which consist of piperazine and pyridone moieties.

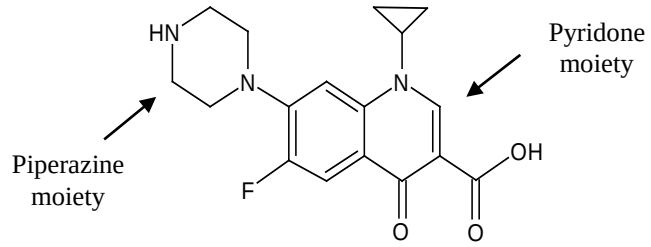


Figure 3.1: Structure of Ciprofloxacin.

Potassium permanganate is widely used as an oxidizing agent as well as in analytical chemistry. The mechanism of oxidation depends on the nature of the substrate and pH of the system [8]. Among six oxidation states of manganese from +2 to+7, permanganate, Mn(VII) is the most potent oxidant in acid as well as is alkaline media. Permanganate oxidation finds extensive applications in organic synthesis [9, 10], especially since the advent of phase transfer catalysis [11, 12].In

general, the reduction of permanganate in slightly basic or neutral solution and in acid media goes through Mn(IV) and Mn(II) with reduction potentials of 1.695 V for Mn(VII)/Mn(IV) and 1.51 V for Mn(VII)/Mn(II) [13]. In acid medium, permanganate exists in different forms namely $HMnO_4$ and $H_2MnO_4^+$ and depending on the nature of the reductant, the oxidant has been assigned both inner sphere and outer sphere mechanism pathways in their redox reactions [14, 15].

The literature survey reveals that the oxidation of CIP by many oxidants such as hexacyanoferrate(III), chloramine-B, Cl_2, ClO_2, $CeSO_4$, Fe(VI) [16-22] have been carried out in either alkaline or acidic medium. Studies reveal that the piperazine moiety of CIP is the predominant oxidative site for oxidation [23-27]. In view of the potential pharmaceutical importance of ciprofloxacin and lack of reported kinetic and mechanistic study on the oxidation of this drug, a detailed oxidation study might elucidate the mechanism of conversion of such compounds. The present study deals to investigate the redox chemistry of permanganate in acid media and establishing a possible mechanism for oxidation of CIP by permanganate on the basis of experimental results.

3.2. EXPERIMENTAL

3.2.1. Chemicals and Reagents.

The methods of preparation of the reagents are given in chapter 2 (Experimental section). However, all other reagents were either of AnalaR grade or guaranteed reagent grade and were used as supplied without any further treatment. A fresh solution of potassium permanganate was prepared before running the experiment every time. All the glass vessels and apparatus used in this investigation were of corning or Pyrex makes. Doubly distilled water was used throughout the study for dilution.

3.2.2. Kinetic Procedure.

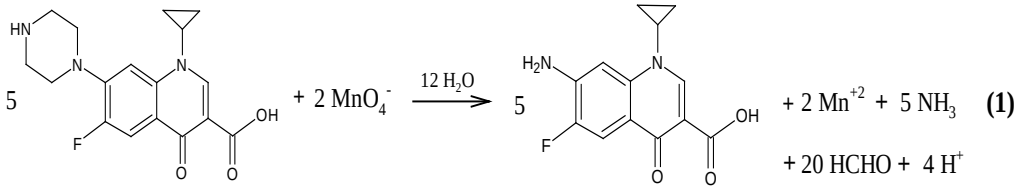
All kinetic measurements were carried out under pseudo-first-order conditions, where the concentration of ciprofloxacin was much greater than permanganate ion concentration at constant temperature at 25 ± 0.1°C unless otherwise stated. The reaction was initiated by mixing thermostated solution of

permanganate and ciprofloxacin with the required amount of sulphuric acid and sodium sulphate. The progress of the reaction was followed by measuring the absorbance of permanganate in the reaction mixture at 525 nm as a function of time on UV- Visible spectrophotometer. It was verified that other components of the reaction mixture do not absorb considerably at this wavelength. Application of Beer's law under the reaction condition was verified in permanganate concentration range $5.0 \times 10^{-5} – 5.0 \times 10^{-4}$ mol dm^{-3} at 525 nm. The molar absorptivity index of permanganate was found to be 2260 ±60 dm^3 mol^{-1} cm^{-1} as a function of time (compared to the literature, ε = 2389 [28]).

The kinetics reactions were followed more than 75% completion of the reaction. The pseudo-first-order rate constant (k_{obs}) were calculated from the slopes of plots of the logarithm of absorbance versus time, which were linear. The values of rate constants, k_{obs} were reproducible within ± 5%.

3.2.3. Stoichiometry and product analysis.

Different sets of concentration of reactants in 0.01 mol dm^{-3} sulphuric acid at constant ionic strength, 2.0×10^{-2} mol dm^{-3}, were kept over 24 hours at 25°C in a closed container. When [permanganate] > [ciprofloxacin], the remaining permanganate concentration was assayed by measuring the absorbance at 525 nm. Estimation of unreacted [MnO_4^-] indicates that 5 moles of CIP consumed 2 moles of Permanganate; the Stoichiometry of the reaction is given in **equation (1)**.



(1)

The main reaction products were identified and isolated with the help of TLC and characterized by LC-MS and FT-IR. Product Mn^{2+} was confirmed by spot test [29]. LC/MS analysis of ciprofloxacin reaction indicates the formation of product with molecular ion of **m/z 263** corresponds to 7-amino-1-cyclopropyl-6-fluoro-4-oxo-quinolone-3-carboxylic acid **(Figure 3.2)**. The molecular ion of ciprofloxacin is m/z 332. The m/z 263 corresponds to full dealkylation of the

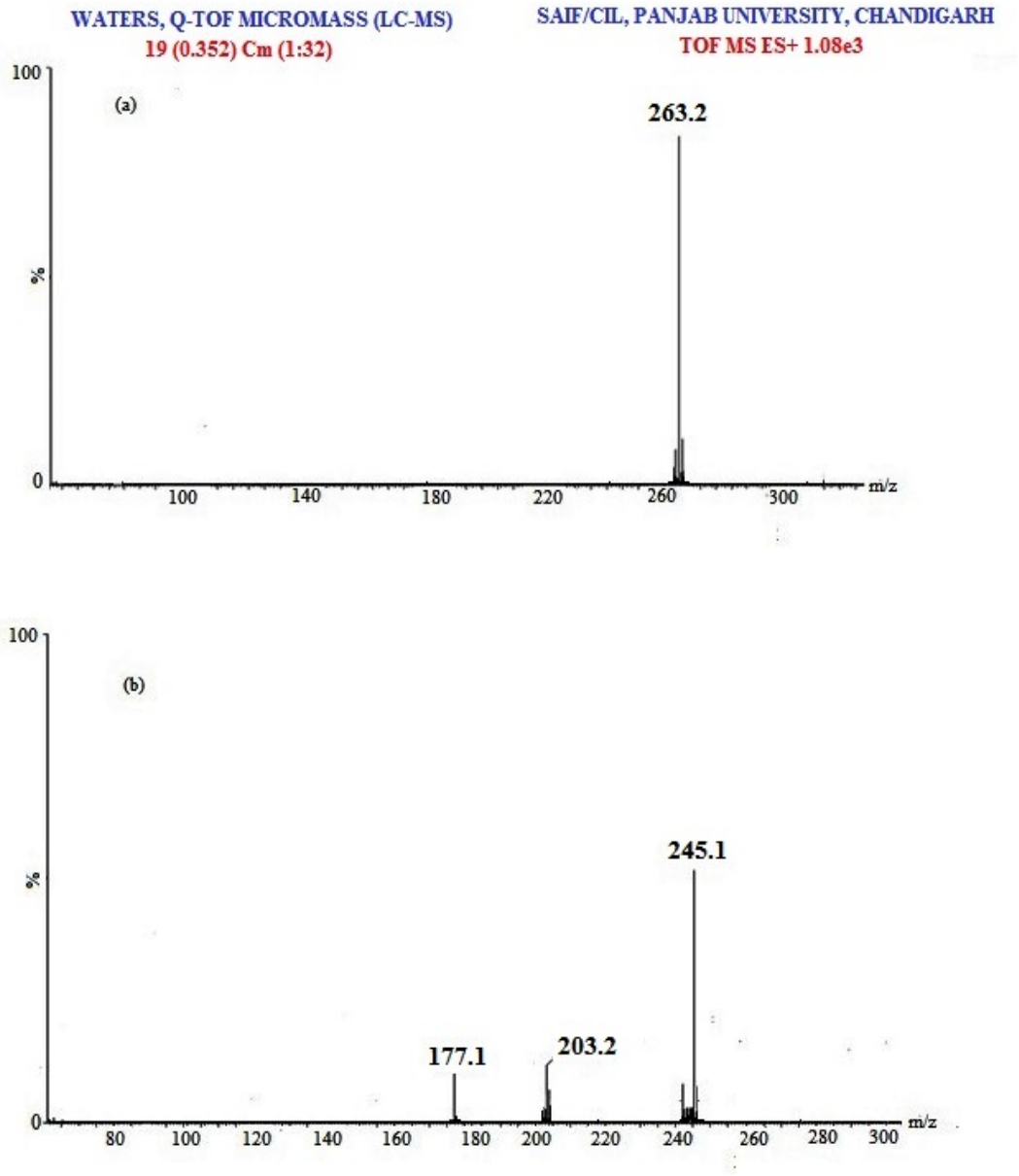


Figure 3.2: LC-ESI-MS spectra of oxidation product of ciprofloxacin.

(a) moiecular ion peak of m/z 263 (M-69).

(b) Fragmentation of (M-69) product.

piperazine ring (i.e. the $–NH_2$ product). It is worth noting, that oxidation of piperazine moiety of ciprofloxacin between oxidized centres and nitrogen atoms lead to distinctive mass loss m/z = 69 and m/z = 83. This was attributed to ring opening, dealkylation and deamination process, which finally yielded 7-amino fluoroquinolone product. The product was also short written as M-69, indicating the net mass loss of the product from the parent ciprofloxacin. This product was also identified previously as oxidation product of ciprofloxacin [30].

IR Spectroscopy analysis also confirmed the presence of $–NH_2$ group in the oxidation product **(Figure 3.3)**. The IR spectroscopy shows a peak at **3324 cm^{-1}** which is due to − NH stretching of the $–NH_2$ group and the remaining peaks are of the parent compound (quinolone ring) [31]. The by-product formaldehyde, formed in the reaction mixture was detected quantitatively by 2, 4-DNP test [32]. The yellow precipitate of 2, 4 dinitrophenylhydrazone of aldehyde product was obtained. It was also observed that aldehyde does not undergo further oxidation in the same experimental conditions and the test for the corresponding acid was negative. The other product ammonia was detected by Nesseler's reagent test [33].

3.3. RESULTS

3.3.1. Reaction Orders.

The reaction orders were determined from the slopes of log k_{obs} versus log [concentration] plots by varying the concentration of CIP, permanganate and acid in turn, keeping all other concentration and conditions constant.

3.3.2. Permanganate Dependence.

The oxidant $KMnO_4$ concentration varied from $5.0 \times 10^{-5} - 4.0 \times 10^{-4}$ mol dm^{-3}, at three concentrations of [CIP] = 3.0×10^{-3}, 4.0×10^{-3} and 5.0×10^{-3} mol dm^{-3} respectively, at constant concentration of $[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3}, I = 2.0×10^{-2} mol dm^{-3} at temperature = 25°C. The plot of log absorbance versus time was linear **(Figure 3.4)** indicating that the reaction is first order with respect to [$KMnO_4$]. This was also confirmed by the constant value of pseudo-first-order rate constant, k_{obs}, for variable concentration of manganese(VII). Results are given in **Tables 3.1, 3.2 and 3.3**.

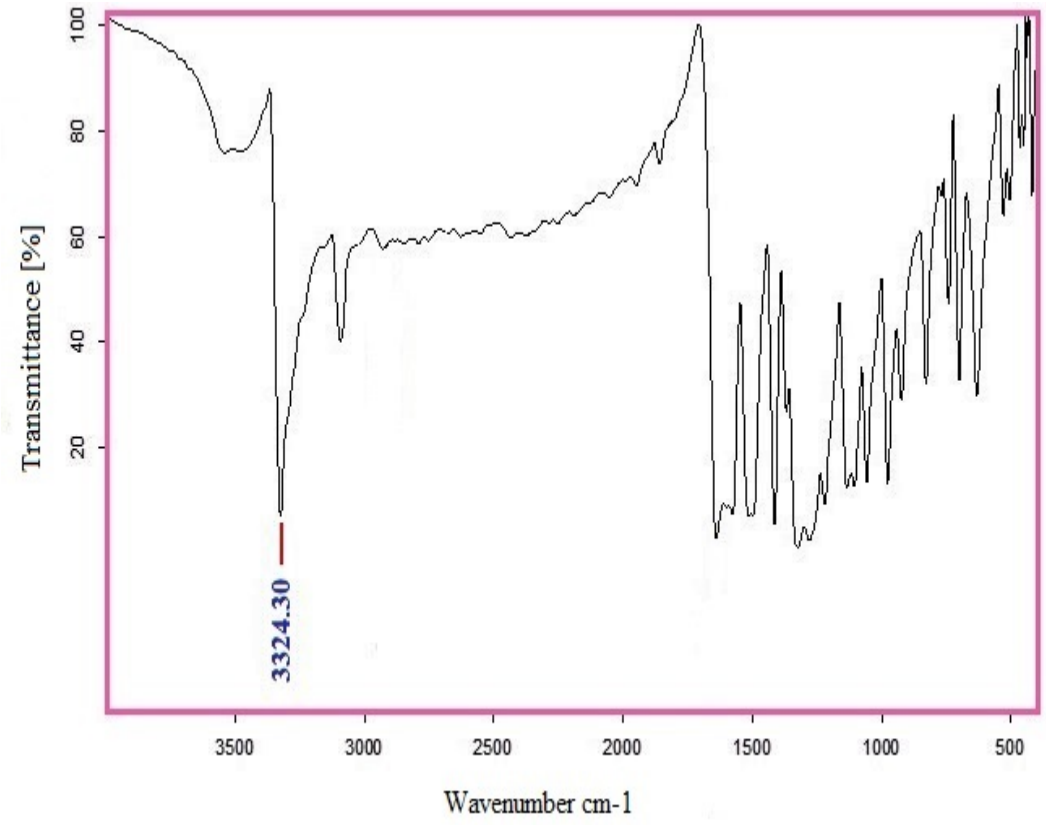


Figure 3.3: FTIR spectra of the oxidative product of ciprofloxacin by permanganate in acidic aqueous medium.

TABLE: 3.1

VARIATION OF $KMnO_4$

[CIP] = 3.0 x 10^{-3} mol dm^{-3} **Temp. = 25°C**

[H^+] = 1.0 x 10^{-2} mol dm^{-3} **I = 2.0 x 10^{-2} mol dm^{-3}**

10^4[$KMnO_4$], mol dm^{-3}	**0.5**	**0.75**	**1.0**	**2.0**	**2.5**	**3.0**	**4.0**
Time in minutes				**Absorbance**			
0	0.116	0.178	0.248	0.482	0.616	0.719	0.974
1	0.092	0.148	0.199	0.398	0.501	0.552	0.776
2	0.078	0.115	0.165	0.323	0.417	0.488	0.660
3	0.064	0.102	0.131	0.263	0.355	0.386	0.549
4	0.054	0.087	0.112	0.226	0.288	0.330	0.449
5	0.044	0.071	0.087	0.181	0.234	0.269	0.380
6	0.037	0.059	0.076	0.151	0.200	0.210	0.309
7	0.031	0.046	0.058	0.125	0.158	0.182	0.245
8	0.025	0.036	0.048	0.102	0.134	0.149	0.204
$10^3(k_{obs})$, sec^{-1}	**3.11**	**3.12**	**3.13**	**3.13**	**3.13**	**3.11**	**3.13**

TABLE: 3.2

VARIATION OF $KMnO_4$

[CIP] = 4.0 x 10^{-3} mol dm^{-3} **Temp. = 25°C**

[H^+] = 1.0 x 10^{-2} mol dm^{-3} **I = 2.0 x 10^{-2} mol dm^{-3}**

10^4[$KMnO_4$], mol dm^{-3}	**0.5**	**0.75**	**1.0**	**2.0**	**2.5**	**3.0**	**4.0**
Time in minutes				**Absorbance**			
0	0.115	0.186	0.244	0.481	0.618	0.720	0.972
1	0.088	0.138	0.173	0.389	0.479	0.530	0.630
2	0.070	0.104	0.138	0.295	0.372	0.428	0.501
3	0.056	0.083	0.109	0.229	0.288	0.338	0.426
4	0.043	0.068	0.089	0.177	0.229	0.252	0.354
5	0.034	0.053	0.066	0.141	0.182	0.198	0.295
6	0.025	0.043	0.053	0.112	0.141	0.156	0.239
7	0.020	0.033	0.041	0.026	0.110	0.120	0.199
$10^3(k_{obs})$, sec^{-1}	**4.12**	**4.14**	**4.12**	**4.11**	**4.12**	**4.14**	**4.12**

TABLE: 3.3

VARIATION OF $KMnO_4$

[CIP] = 5.0 x 10^{-3} mol dm^{-3} **Temp. = 25°C**

[H^+] = 1.0 x 10^{-2} mol dm^{-3} **I = 2.0 x 10^{-2} mol dm^{-3}**

10^4[$KMnO_4$], mol dm^{-3}	**0.5**	**0.75**	**1.0**	**2.0**	**2.5**	**3.0**	**4.0**
Time in minutes				**Absorbance**			
0	0.116	0.187	0.243	0.483	0.620	0.717	0.973
1	0.085	0.134	0.159	0.367	0.447	0.491	0.691
2	0.061	0.102	0.120	0.251	0.324	0.374	0.501
3	0.044	0.071	0.092	0.182	0.238	0.265	0.363
4	0.032	0.050	0.063	0.134	0.170	0.189	0.257
5	0.024	0.036	0.043	0.073	0.126	0.138	0.194
6	0.017	0.025	0.033	0.065	0.048	0.096	0.141
$10^3(k_{obs})$, sec^{-1}	**5.29**	**5.31**	**5.28**	**5.29**	**5.29**	**5.31**	**5.29**

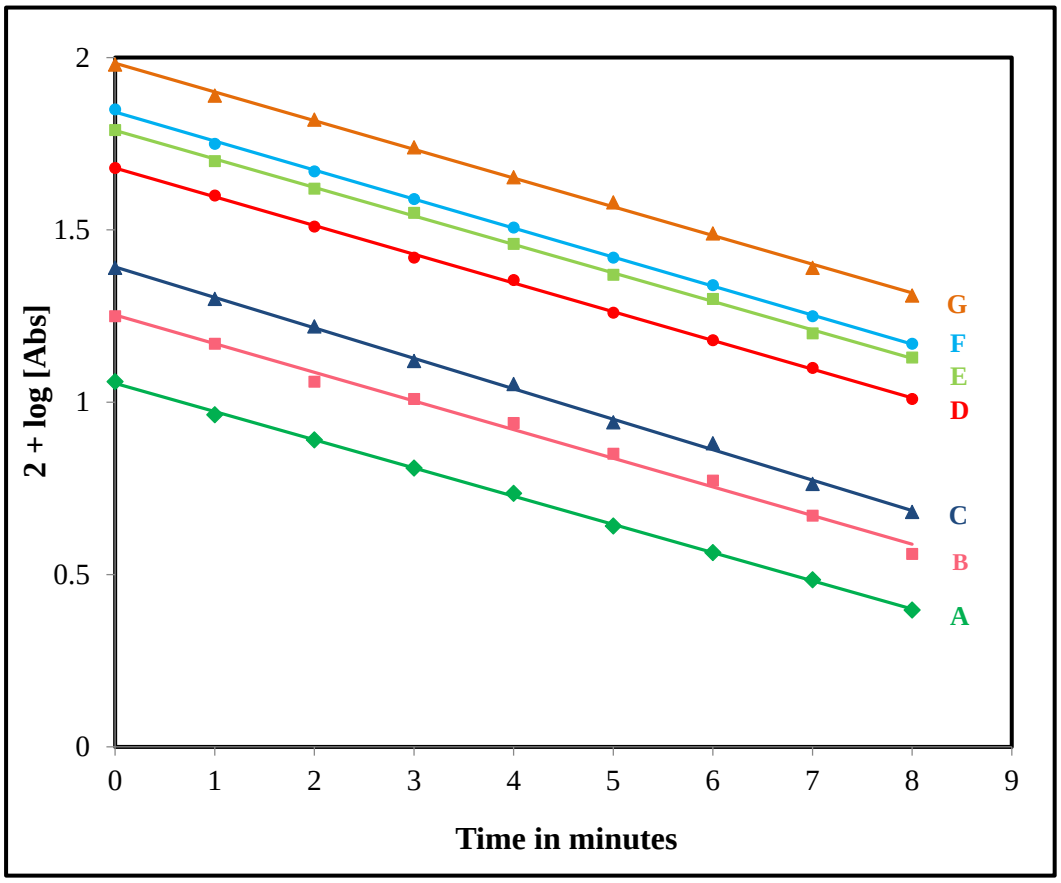


Figure 3.4: First order plots of the variation of permanganate concentration.

[CIP] = 3.0×10^{-3} mol dm^{-3}; $[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3};

I = 2.0×10^{-2} mol dm^{-3}; Temp. = 25°C;

[Mn(VII)] = (A) 0.50×10^{-4} mol dm^{-3} (B) 0.75×10^{-4} mol dm^{-3}

(C) 1.0×10^{-4} mol dm^{-3} (D) 2.0×10^{-4} mol dm^{-3}

(E) 2.5×10^{-4} mol dm^{-3} (F) 3.0×10^{-4} mol dm^{-3}

(G) 4.0×10^{-4} mol dm^{-3}.

(Ref. Table 3.1)

3.3.3. Ciprofloxacin Dependence.

The effect of variation of ciprofloxacin on the rate of reaction was studied in the concentration range 1.0×10^{-3} – 7.0×10^{-3} mol dm^{-3} at constant concentration of $[KMnO_4] = 2.5 \times 10^{-4}$ mol dm^{-3}, $[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3} and $I = 2.0 \times 10^{-2}$ mol dm^{-3} at three temperature viz. 25°C, 30°C and 35°C respectively. The rate of reaction increases with increasing concentration of ciprofloxacin. The plot of k_{obs} versus ciprofloxacin concentration **(Figure 3.5)** gives a straight line passing through the origin which confirms the reaction is first order with respect to ciprofloxacin concentration. Results are given in **Tables 3.4, 3.5 and 3.6**.

3.3.4. Hydrogen ion dependence.

The effect of variation of sulphuric acid on the rate of reaction was studied in the concentration range 0.01 – 0.07 mol dm^{-3} at fixed concentrations of $[KMnO_4] = 2.5 \times 10^{-4}$ mol dm^{-3}, $[CIP] = 2.0 \times 10^{-3}$ mol dm^{-3} and $I = 2.0 \times 10^{-2}$ mol dm^{-3} at three temperature viz. 25°C, 30°C and 35°C respectively. The rate constants were found to increase with increasing $[H^+]$ concentration **(Figure 3.6)**. From the plot of log k_{obs} versus log $[H^+]$, the reaction order was found to be less than unity (0.68). Results are given in **Tables 3.7, 3.8 and 3.9**.

3.3.5. Effect of Ionic Strength and Dielectric Constant.

The ionic strength was varied by varying concentration of sodium sulphate between 0.01 – 0.1 mol dm^{-3} at constant concentration of $[KMnO_4] = 2.5 \times 10^{-4}$ mol dm^{-3}, $[CIP] = 2.0 \times 10^{-3}$ mol dm^{-3} and $[H^+] = 1.0 \times 10^{-2}$ moldm^{-3} at 25°C. Variation of ionic strength has negligible effect on the rate of reaction. Results are given in **Table 3.10**.

The effect of the dielectric constant (*D*) was studied by varying the acetic acid - water content (v/v) in the reaction mixture at constant concentration of $[KMnO_4] = 2.5 \times 10^{-4}$ mol dm^{-3}, $[CIP] = 2.0 \times 10^{-3}$ mol dm^{-3}, $[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3} and $I = 2.0 \times 10^{-2}$ mol dm^{-3}at 25°C. Increase in the acetic acid content in the reaction, has negligible effect on the rate of reaction. Results are given in **Table 3.11**.

TABLE: 3.4

VARIATION OF CIPROFLOXACIN

$[KMnO_4]$ = 2.5 x 10^{-4} mol dm^{-3} **Temp. = 25°C**

$[H^+]$ = 1.0 x 10^{-2} mol dm^{-3} **I = 2.0 x10^{-2} mol dm^{-3}**

10^3[CIP], mol dm^{-3}	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**	**7.0**
Time in minutes				**Absorbance**			
0	(0)0.617	(0)0.616	(0)0.616	0.618	0.618	0.619	0.616
1	(3)0.500	(2)0.447	(2)0.423	0.443	0.448	0.425	0.389
2	(6)0.382	(4)0.347	(4)0.296	0.361	0.351	0.309	0.251
3	(9)0.288	(6)0.269	(6)0.189	0.288	0.240	0.218	0.162
4	(12)0.223	(8)0.204	(8)0.138	0.223	0.181	0.150	0.105
5	(15)0.181	(10)0.155	(10)0.067	0.164	0.144	0.099	0.052
6	(18)0.141	(12)0.120	(12)0.015	0.138	0.102	0.042	–
7	(21)0.112	(14)0.064	–	0.114	0.069	–	–
$10^3(k_{obs})$, sec^{-1}	**1.06**	**2.27**	**3.13**	**4.14**	**5.29**	**6.21**	**7.48**

Figures in parentheses denote time in minutes.

TABLE: 3.5

VARIATION OF CIPROFLOXACIN

$[KMnO_4]$ = 2.5 x 10^{-4} mol dm^{-3} **Temp. = 30°C**

$[H^+]$ = 1.0 x 10^{-2} mol dm^{-3} **I = 2.0 x 10^{-2} mol dm^{-3}**

10^3[CIP], mol dm^{-3}	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**	**7.0**
Time in minutes				**Absorbance**			
0	(0)0.616	(0)0.617	0.617	0.616	0.619	0.618	0.616
1	(3)0.483	(2)0.447	0.483	0.446	0.423	0.401	0.409
2	(6)0.385	(4)0.339	0.381	0.319	0.287	0.255	0.256
3	(9)0.288	(6)0.251	0.299	0.230	0.198	0.156	0.151
4	(12)0.229	(8)0.182	0.238	0.171	0.130	0.096	0.069
5	(15)0.177	(10)0.138	0.184	0.127	0.053	0.033	0.026
6	(18)0.138	(12)0.100	0.143	0.035	0.029	–	–
7	(21)0.112	(14)0.068	0.115	0.006	–	–	–
$10^3(k_{obs})$, sec^{-1}	**1.34**	**2.50**	**4.03**	**5.36**	**6.51**	**7.94**	**9.10**

Figures in parentheses denote time in minutes.

TABLE: 3.6

VARIATION OF CIPROFLOXACIN

$[KMnO_4] = 2.5 \times 10^{-4}$ mol dm^{-3} **Temp. = 35°C**

$[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3} **I = 2.0×10^{-2} mol dm^{-3}**

10^3[CIP], mol dm^{-3}	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**	**7.0**
Time in minutes				**Absorbance**			
0	(0)0.616	(0)0.618	0.619	0.616	0.616	(0)0.617	(0)0.616
1	(2)0.511	(2)0.451	0.465	0.414	0.398	(0.5)0.468	(0.5)0.457
2	(4)0.398	(4)0.320	0.356	0.296	0.241	(1)0.352	(1)0.333
3	(6)0.316	(6)0.234	0.272	0.204	0.154	(1.5)0.263	(1.5)0.234
4	(8)0.257	(8)0.169	0.208	0.143	0.096	(2)0.208	(2)0.176
5	(10)0.204	(10)0.132	0.161	0.092	0.042	(2.5)0.151	(2.5)0.129
6	(12)0.162	(12)0.115	0.117	0.048	–	(3)0.122	(3)0.057
7	(14)0.135	(14)0.070	0.036	–	–	(3.5)0.037	(3.5)0.018
$10^3(k_{obs})$, sec^{-1}	**1.55**	**2.61**	**4.60**	**6.12**	**7.70**	**9.21**	**10.54**

Figures in parentheses denote time in minutes.

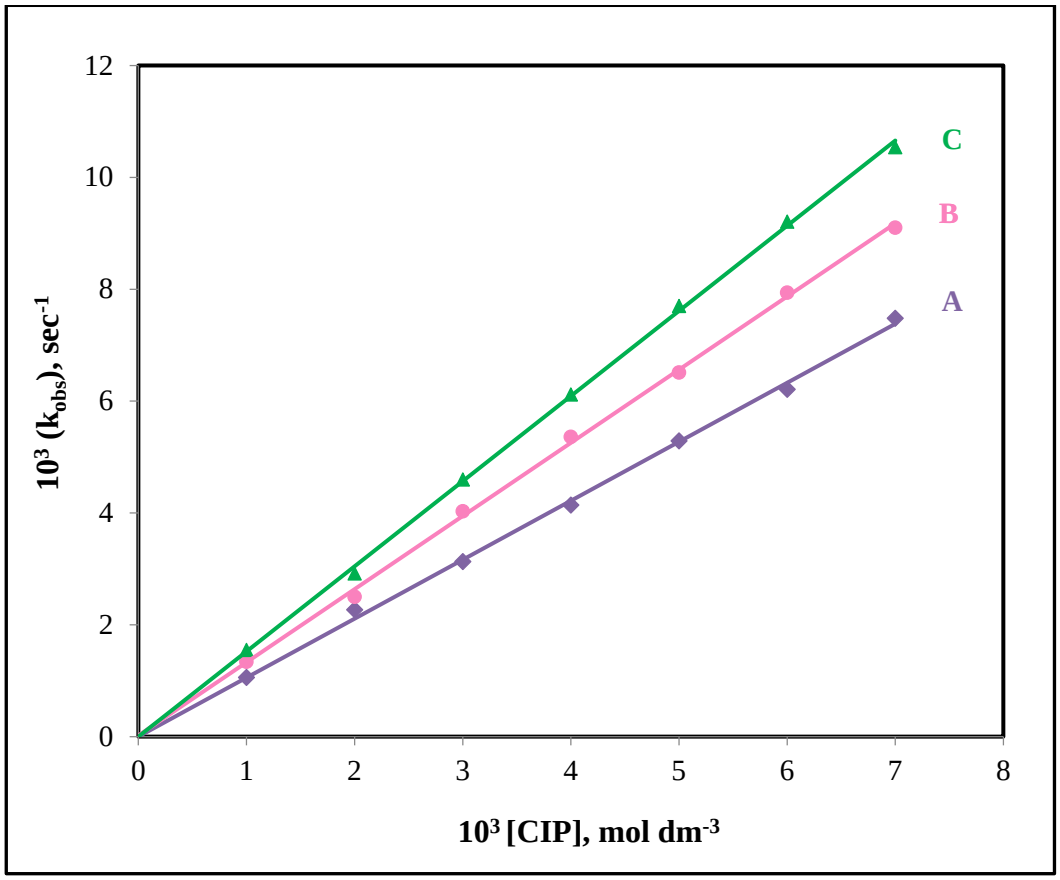


Figure 3.5: Variation of Ciprofloxacin at different temperature

(A) 25°C, (B) 30°C, (C) 35°C.

$[KMnO_4] = 2.5 \times 10^{-4}$ mol dm^{-3};

$[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3};

$I = 2.0 \times 10^{-2}$ mol dm^{-3}.

(Ref. Table: 3.4, 3.5, 3.6)

TABLE: 3.7

VARIATION OF HYDROGEN ION

[$KMnO_4$] = 2.5 x 10^{-4} mol dm^{-3} **Temp. = 25°C**

[CIP] = 2.0 x 10^{-3} mol dm^{-3} **I = 2.0 x 10^{-2} mol dm^{-3}**

10^2 [H^+], mol dm^{-3}	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**	**7.0**
Time in minutes				**Absorbance**			
0	(0)0.616	(0)0.619	0.619	0.618	0.617	0.616	0.616
1	(2)0.484	(2)0.417	0.497	0.459	0.450	0.446	0.444
2	(4)0.357	(4) 0.270	0.369	0.347	0.321	0.316	0.260
3	(6)0.281	(6)0.181	0.283	0.255	0.231	0.219	0.204
4	(8)0.200	(8)0.120	0.218	0.197	0.175	0.163	0.150
5	(10)0.148	(10)0.051	0.170	0.145	0.125	0.116	0.107
6	(12)0.115	(12)0.009	0.126	0.107	0.068	0.024	0.014
$10^3(k_{obs})$, sec^{-1}	**2.27**	**3.44**	**4.34**	**4.76**	**5.26**	**5.55**	**5.88**

Figures in parentheses denote time in minutes.

TABLE: 3.8

VARIATION OF HYDROGEN ION

[$KMnO_4$] = 2.5 x 10^{-4} mol dm^{-3} **Temp. = 30°C**

[CIP] = 2.0 x 10^{-3} mol dm^{-3} **I = 2.0 x 10^{-2} mol dm^{-3}**

10^2 [H^+], mol dm^{-3}	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**	**7.0**
Time in minutes				**Absorbance**			
0	(0)0.616	0.619	0.619	0.618	0.616	0.617	0.616
1	(2)0.448	0.500	0.460	0.448	0.427	0.417	0.404
2	(4)0.338	0.390	0.345	0.321	0.288	0.282	0.275
3	(6)0.256	0.311	0.256	0.228	0.204	0.191	0.182
4	(8)0.184	0.236	0.195	0.160	0.141	0.132	0.126
5	(10)0.138	0.189	0.145	0.115	0.084	0.048	0.032
6	(12)0.103	0.141	0.107	0.025	0.017	–	–
$10^3(k_{obs})$, sec^{-1}	**2.50**	**4.01**	**4.80**	**5.62**	**6.14**	**6.40**	**6.62**

Figures in parentheses denote time in minutes.

TABLE: 3.9

VARIATION OF HYDROGEN ION

$[KMnO_4] = 2.5 \times 10^{-4}$ mol dm^{-3} **Temp. = 35°C**

[CIP] = 2.0×10^{-3} mol dm^{-3} **I = 2.0×10^{-2} mol dm^{-3}**

10^2 [H^+], mol dm^{-3}	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**	**7.0**
Time in minutes				**Absorbance**			
0	(0)0.616	0.617	0.616	0.616	0.618	0.619	0.618
1	(2)0.451	0.461	0.417	0.414	0.407	0.397	0.380
2	(4)0.320	0.333	0.286	0.266	0.253	0.242	0.230
3	(6)0.231	0.249	0.204	0.172	0.166	0.145	0.142
4	(8)0.170	0.170	0.126	0.117	0.109	0.078	0.046
5	(10)0.120	0.133	0.053	0.035	0.023	–	–
$10^3(k_{obs})$, sec^{-1}	**2.61**	**5.12**	**6.41**	**7.02**	**7.42**	**7.80**	**8.21**

Figures in parentheses denote time in minutes.

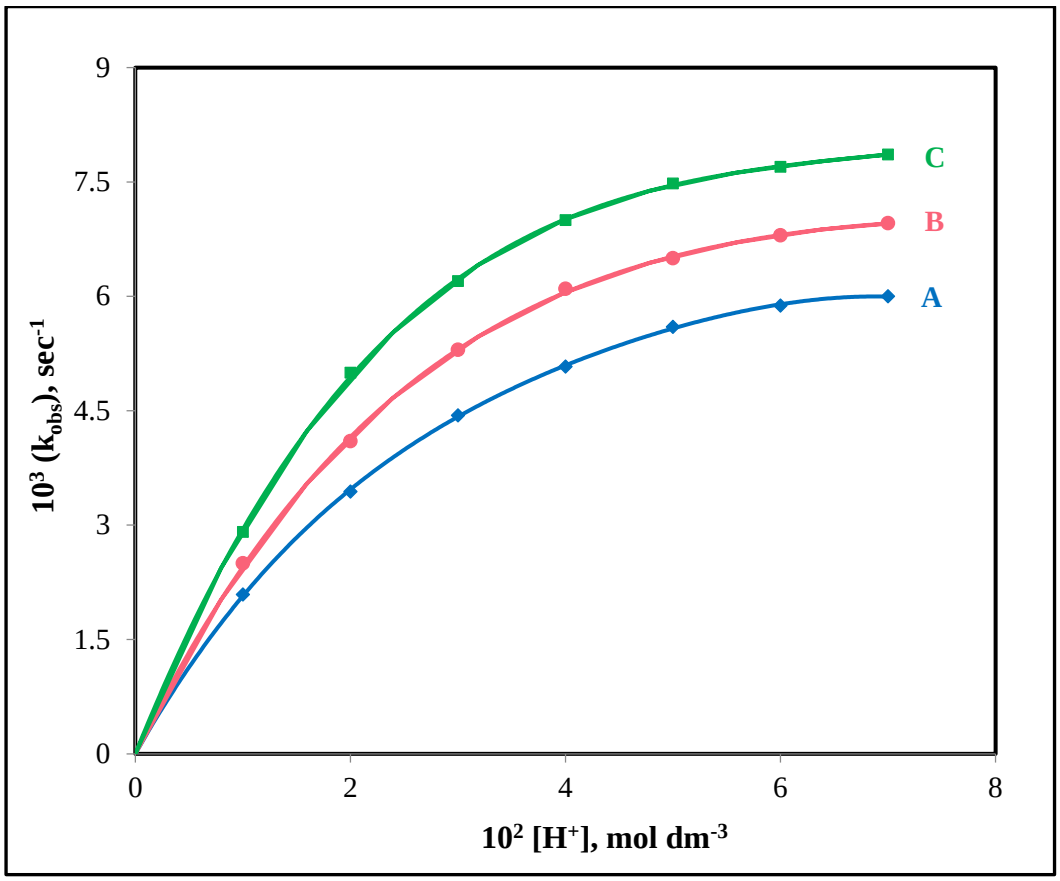


Figure 3.6: Variation of Hydrogen Ion at different temperature

(A) 25°C, (B) 30°C, (C) 35°C.

$[KMnO_4] = 2.5 \times 10^{-4}$ mol dm^{-3};

[CIP] = 2.0×10^{-3} mol dm^{-3};

I = 2.0×10^{-2} mol dm^{-3}.

(Ref. Table: 3.7, 3.8, 3.9)

TABLE: 3.10

VARIATION OF SODIUM SULPHATE

$[KMnO_4]$ = 2.5 x 10^{-4} mol dm^{-3} **Temp. = 25°C**

[CIP] = 2.0 x 10^{-3} mol dm^{-3} **$[H^+]$ = 1.0 x 10^{-2} mol dm^{-3}**

10^2 $[Na_2SO_4]$, mol dm^{-3}	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0
Time in minutes	**Absorbance**									
0	0.616	0.619	0.618	0.617	0.617	0.616	0.618	0.616	0.619	0.616
2	0.473	0.500	0.477	0.484	0.466	0.477	0.487	0.483	0.481	0.468
4	0.362	0.358	0.355	0.359	0.357	0.360	0.357	0.356	0.354	0.357
6	0.275	0.267	0.278	0.270	0.274	0.268	0.272	0.270	0.273	0.274
8	0.201	0.205	0.208	0.212	0.204	0.211	0.206	0.203	0.210	0.208
10	0.165	0.160	0.154	0.155	0.158	0.161	0.168	0.169	0.165	0.160
12	0.121	0.118	0.113	0.119	0.115	0.116	0.114	0.116	0.115	0.117
$10^3(k_{obs})$, sec^{-1}	**2.24**	**2.26**	**2.30**	**2.25**	**2.27**	**2.25**	**2.27**	**2.29**	**2.31**	**2.27**

TABLE: 3.11

EFFECT OF DIELECTRIC CONSTANT

$[KMnO_4] = 2.5 \times 10^{-4}$ mol dm^{-3} **Temp. = 25°C**

[CIP] = 2.0×10^{-3} mol dm^{-3} **I = 2.0×10^{-2} mol dm^{-3}**

$[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3}

[acetic acid], %	**5**	**10**	**15**	**20**
Time in minutes		**Absorbance**		
0	0.616	0.619	0.618	0.616
2	0.457	0.448	0.468	0.451
4	0.357	0.350	0.359	0.354
6	0.269	0.263	0.275	0.266
8	0.204	0.200	0.208	0.202
10	0.155	0.149	0.159	0.152
12	0.115	0.112	0.117	0.113
$10^3(k_{obs})$, sec^{-1}	**2.27**	**2.23**	**2.29**	**2.25**

3.3.6. Effect of Initially Added Products.

The effect of Mn(II) ion was studied in the range of 5.0×10^{-5} to 5.0×10^{-4} mol dm^{-3} at constant concentration of $[KMnO_4] = 2.5 \times 10^{-4}$ mol dm^{-3}, [CIP] = 2.0×10^{-3} mol dm^{-3}, $[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3} and $I = 2.0 \times 10^{-2}$ mol dm^{-3} at 25°C. It was found that initially added product Mn^{2+} has no effect on the rate of reaction. Results are given in **Table 3.12**.

3.3.7. Test for Free Radicals.

The reaction mixture (10ml), to which a known quantity (2ml) of acrylonitrile (scavenger) has been added and kept in an inert atmosphere for 5 hours at room temperature. When the reaction mixture was diluted with methanol, a white precipitate was formed, indicating the intervention of free radicals in the reaction. The blank experiment of reacting either $KMnO_4$ or ciprofloxacin alone with acrylonitrile did not induce polymerisation under the same conditions.

3.4. DISCUSSION

The expected oxidizing species of permanganate in acid media are $HMnO_4$, $H_2MnO_4^+$, $HMnO_3$ and Mn_2O_7. Among them MnO_4^- ion is powerful oxidizing agent in aqueous alkaline as well as in acidic medium. The stable reduction product of MnO_4^- in acid medium is Mn(II). **Figure 3.7** illustrates the spectroscopic changes during the oxidation of ciprofloxacin by acid permanganate at 25°C with scanning interval of 3 minutes.

The active species of permanganate in aqueous acid solution may be deduced from the dependence of the rate on $[H^+]$, in the reaction medium. The order of $[H^+]$ is less than unity, which may be indicate the formation of permanganate acid from permanganate ion. Permanganate acid $HMnO_4$ is more efficient oxidant species of Manganese(VII) then permanganate ion [34]. It has been observed that the rate of reaction was tending to attain a limiting value at higher concentration of $[H^+]$ ion, which indicates that only the protonated form is active then acid permanganate [35]. Equilibrium can be represented by **equation (2)**.

$$MnO_4^- + H^+ \underset{}{\overset{K_1}{\rightleftharpoons}} HMnO_4 \qquad \textbf{(2)}$$

TABLE: 3.12

EFFECT OF Mn(II) ION

$[KMnO_4] = 2.5 \times 10^{-4}$ mol dm^{-3} **Temp. = 25°C**

[CIP] = 2.0×10^{-3} mol dm^{-3} **I = 2.0×10^{-2} mol dm^{-3}**

$[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3}

10^4 $[Mn]^{2+}$	**0.5**	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**
Time in minutes			**Absorbance**			
0	0.617	0.619	0.616	0.616	0.618	0.616
2	0.489	0.468	0.474	0.480	0.473	0.474
4	0.361	0.356	0.362	0.354	0.362	0.360
6	0.264	0.273	0.275	0.270	0.272	0.268
8	0.202	0.204	0.203	0.211	0.202	0.210
10	0.156	0.151	0.162	0.165	0.163	0.161
12	0.115	0.112	0.120	0.116	0.120	0.115
$10^3(k_{obs})$, sec^{-1}	**2.23**	**2.29**	**2.24**	**2.31**	**2.27**	**2.25**

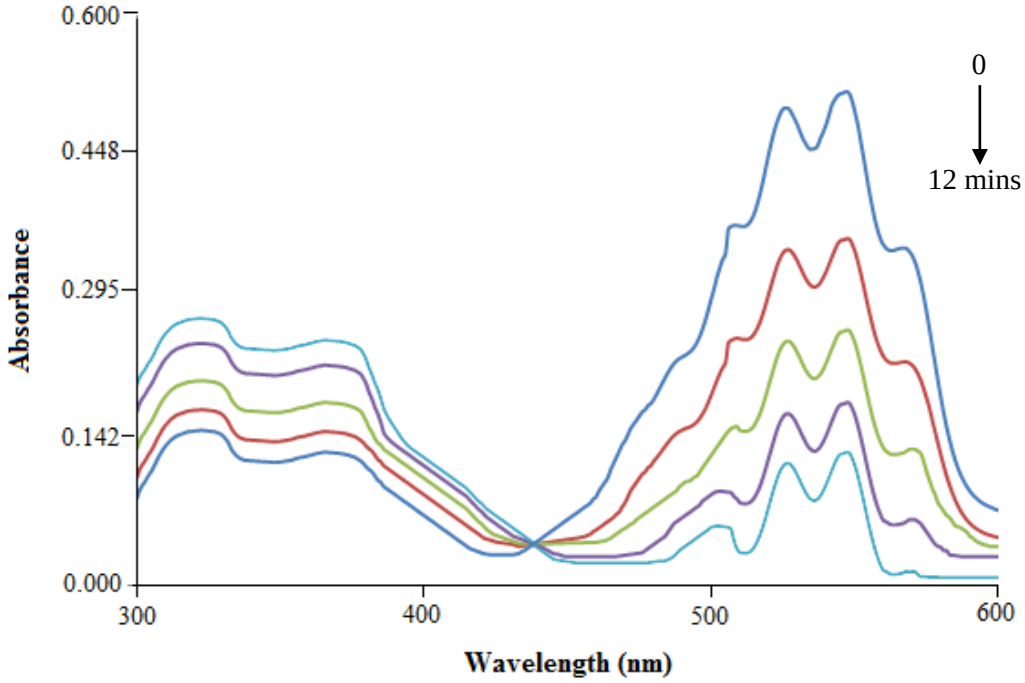


Figure 3.7: Spectral changes during the oxidation of ciprofloxacin (CIP) by permanganate in acidic medium at 25°C.

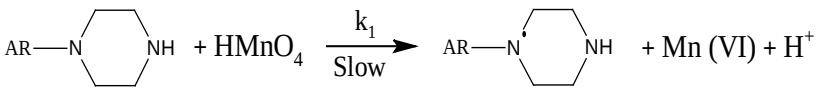
$[KMnO_4] = 2.0 \times 10^{-4}$ mol dm^{-3}; $[CIP] = 2.0 \times 10^{-3}$ mol dm^{-3};

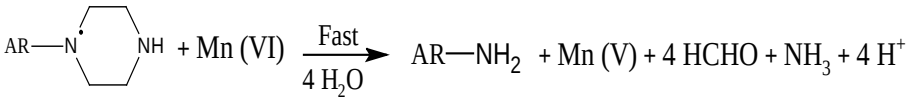
$[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3}; $I = 2.0 \times 10^{-2}$ mol dm^{-3}.

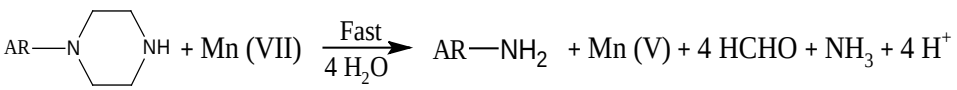
The reaction between permanganate and ciprofloxacin in sulphuric acid has Stoichiometry 5:2, having first order dependence with permanganate and ciprofloxacin and less than unit order with H^+ concentration. The oxidation products were Mn(II), 7-amino fluoroquinolone, NH_3 and HCHO. On the basis of experimental results, the mechanism can be proposed. In view of increasing the rate with increase in $[H^+]$ ion, in the prior equilibrium step, H^+ reacts with MnO_4^- to form $HMnO_4$, which reacts with the one mole of ciprofloxacin in the rate determining step to give a free radical derived from ciprofloxacin and an intermediate Mn(VI). In further fast steps the intermediate Mn(VI) reacts with a free radical to produce the product 7-amino fluoroquinolone, NH_3, HCHO and intermediate Mn(V). In further fast steps Mn(V) subsequently reduced to the end product Mn(II).

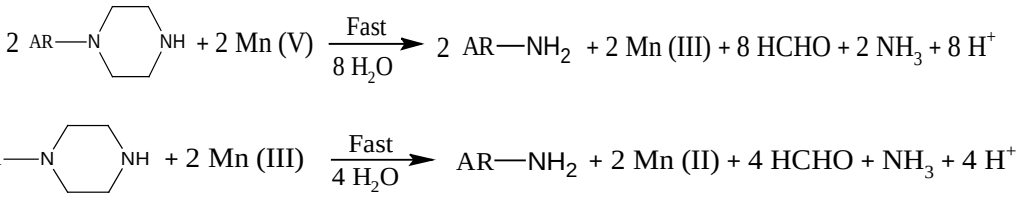
Although Mn(VI) and Mn(IV) are the final reduced species of MnO_4^- in alkaline and neutral media, it was observed that Mn(II) was the only reduced species of MnO_4^- in acid medium. Since none of the intermediate could be detected, scheme-1 is the only possible mechanism for the reaction in the presence of free radical. Attempts were made to allow spectroscopic detection of intermediate Mn(V) and Mn(III) as the reaction proceeded in the oxidation of ciprofloxacin by permanganate. Unfortunately the low concentration of Mn(V) and Mn(III) intermediate obtained under our experimental conditions made the spectroscopic detection failure. However, the evidence for intermediate such as Mn(V) and Mn(III) is as presented in the literature [35, 36]. The results are accommodated in the **Scheme- 1**.

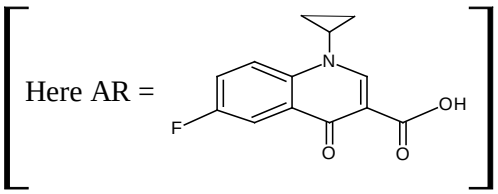
$$MnO_4^- + H^+ \overset{K_1}{\rightleftharpoons} HMnO_4$$











Scheme- 1

From the scheme-1, the following rate law can be derived as follows:

$$\text{Rate} = \frac{-d[MnO_4^-]}{dt} = k_1[HMnO_4][CIP] \quad \textbf{(3)}$$

$$= k_1K_1[MnO_4^-]_f[CIP]_f[H^+]_f \quad \textbf{(4)}$$

The total concentration of permanganate is given by:

$$[MnO_4^-]_t = [MnO_4^-]_f + [HMnO_4]_f$$

$$= [MnO_4^-]_f + K_1[H^+][MnO_4^-]$$

$$= [MnO_4^-]_f\ (1 + K_1[H^+])$$

$$\text{So } [MnO_4^-]_f = \frac{[MnO_4^-]_t}{(1 + K_1[H^+])} \quad \textbf{(5)}$$

Where “t” and “f” stands for total and free concentration.

$$[H^+]_f = \frac{[H^+]_t}{(1 + K_1[MnO_4^-])} \quad \textbf{(6)}$$

Putting equation (5) and (6) in equation (4) and omitting “t” and “f” subscripts

$$\text{Rate} = \frac{-d[MnO_4^-]}{dt} = \frac{k_1K_1[MnO_4^-]\,[CIP]\,[H^+]}{(1 + K_1[H^+])\,(1 + K_1[MnO_4^-])} \quad \textbf{(7)}$$

$$= \frac{k_1 K_1 [MnO_4^-]\,[CIP]\,[H^+]}{1 + K_1[H^+] + K_1[MnO_4^-] + K_1^2[H^+][MnO_4^-]} \quad \textbf{(8)}$$

$K_1[MnO_4^-]$ and $K_1^2[H^+][MnO_4^-] << 1$ or neglected due to low concentration of $[MnO_4^-]$ used in the experiment so equation (8) change into equation (9)

$$\text{Rate} = \frac{-d[MnO_4^-]}{dt} = \frac{k_1 K_1 [MnO_4^-]\,[CIP]\,[H^+]}{1 + K_1[H^+]} \quad \textbf{(9)}$$

$$\frac{\text{Rate}}{[MnO_4^-]} = k_{obs} = \frac{k_1 K_1 [CIP]\,[H^+]}{1 + K_1[H^+]} \quad \textbf{(10)}$$

$$\frac{k_{obs}}{[CIP]} = \frac{k_1 K_1\,[H^+]}{1 + K_1[H^+]} \quad \textbf{(11)}$$

Equation (11) can be rearranged as:

$$\frac{[CIP]}{k_{obs}} = \frac{1}{k_1 K_1\,[H^+]} + \frac{1}{k_1} \quad \textbf{(12)}$$

According to equation (12) the plot of $[CIP]/k_{obs}$ versus $1/[H^+]$ is linear with positive intercept and slope **(Figure 3.8)** at three different temperature. The rate constant k_1, of the slow step and the equilibrium constant of $HMnO_4$ (K_1), scheme-1 was obtained from the intercept and slope of the plots $[CIP]/k_{obs}$ versus $1/[H^+]$ **(Table 3.13)**. The energy of activation was determined by the plot of log k_1 versus 1/T **(Figure 3.9)** from which activation parameters were calculated. The value of K_1 (40.6) is in good agreement with earlier work [28].Thermodynamic quantities were calculated from the Van't Hoff plot i.e. log K_1 versus 1/T **(Figure 3.10)**.The moderate values of $\Delta H^{\#}$ and $\Delta S^{\#}$ were favourable for electron transfer process. The value of $\Delta H^{\#}$ was due to energy of solution changes in the transition state. The negative value of $\Delta S^{\#}$ within the range of radical reaction has been ascribed [37] to the nature of electron pairing and electron unpairing process. The negligible effect of ionic strength and dielectric constant is consistent with reaction between two neutral molecules which supports the proposed mechanism [38]. There is no evidence of intermediate complex formation, thus, the outer-sphere mechanism is proposed as the mechanism for this reaction [39].

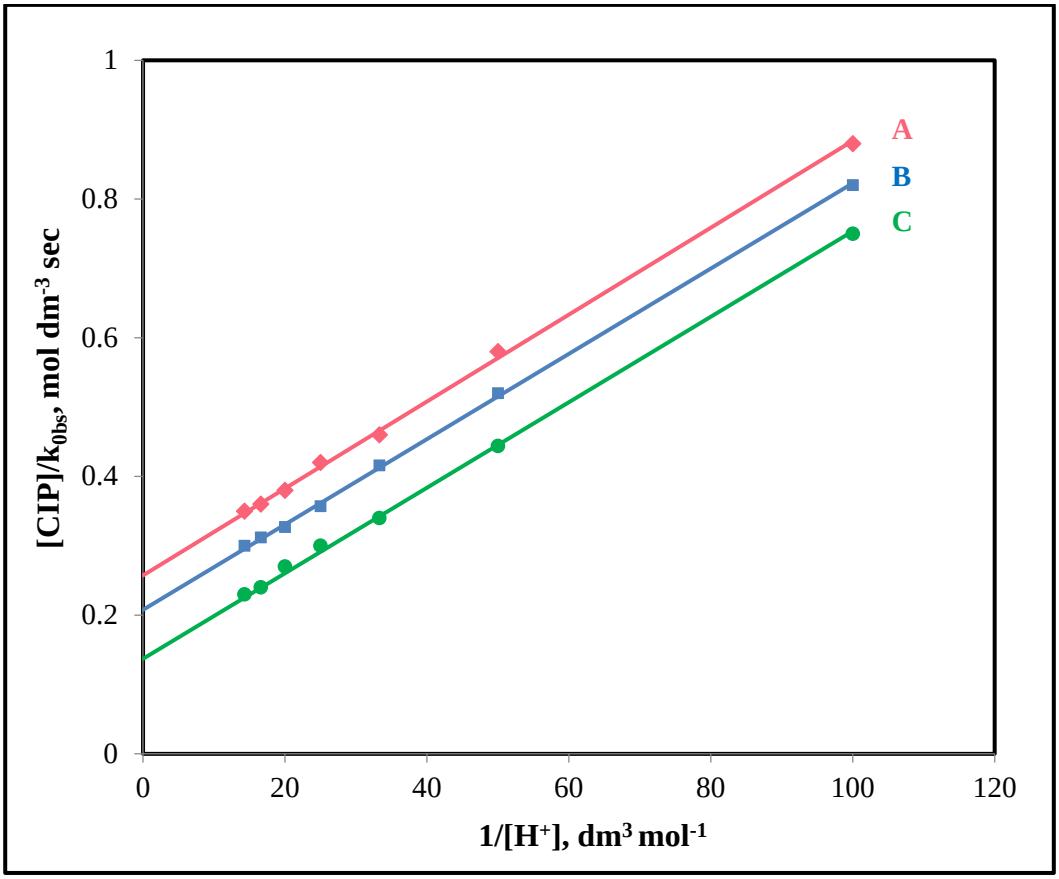


Figure 3.8: Plots of [CIP]/k_{obs} versus 1/[H^+]at different temperature

(A) 25°C, (B) 30°C, (C) 35°C.

$[KMnO_4] = 2.5 \times 10^{-4}$ mol dm^{-3};

$[CIP] = 2.0 \times 10^{-3}$ mol dm^{-3};

$I = 2.0 \times 10^{-2}$ mol dm^{-3}.

TABLE: 3.13

ACTIVATION PARAMETERS AND THERMODYNAMIC QUANTITIES EVALUATED FROM SCHEME 1.

Temperature (Kelvin)	**k_1 ($dm^3\ mol^{-1}\ s^{-1}$)**	**Activation Parameters**	**K_1 ($dm^3\ mol^{-1}$)**	**Thermodynamic Quantities**
298	3.88	E_a= 34.84 ($kJ\ mol^{-1}$)	40.6	$\Delta H = -30.63$ ($kJ\ mol^{-1}$)
303	4.81	$\Delta H^{\#}$= 32.37 ($kJ\ mol^{-1}$)	34.61	$\Delta S = -99.13$ ($J\ K^{-1}\ mol^{-1}$)
308	7.29	$\Delta S^{\#} = -116.6$ ($JK^{-1}\ mol^{-1}$)	22.8	$\Delta G = -1.62$ ($kJ\ mol^{-1}$)
		$\Delta G^{\#}$ = 69.61 ($kJ\ mol^{-1}$)		

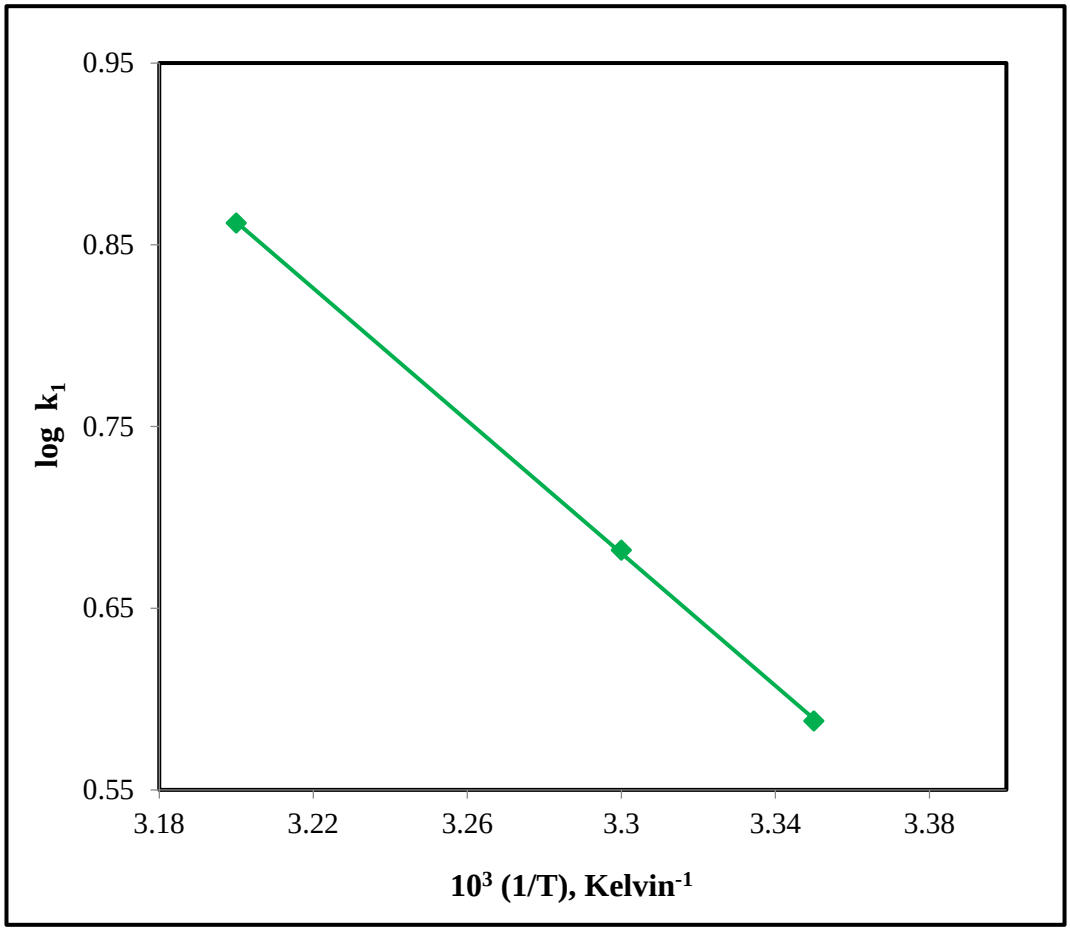


Figure 3.9: Plot of log k_1 versus 1/T.

(Ref. Table: 3.13)

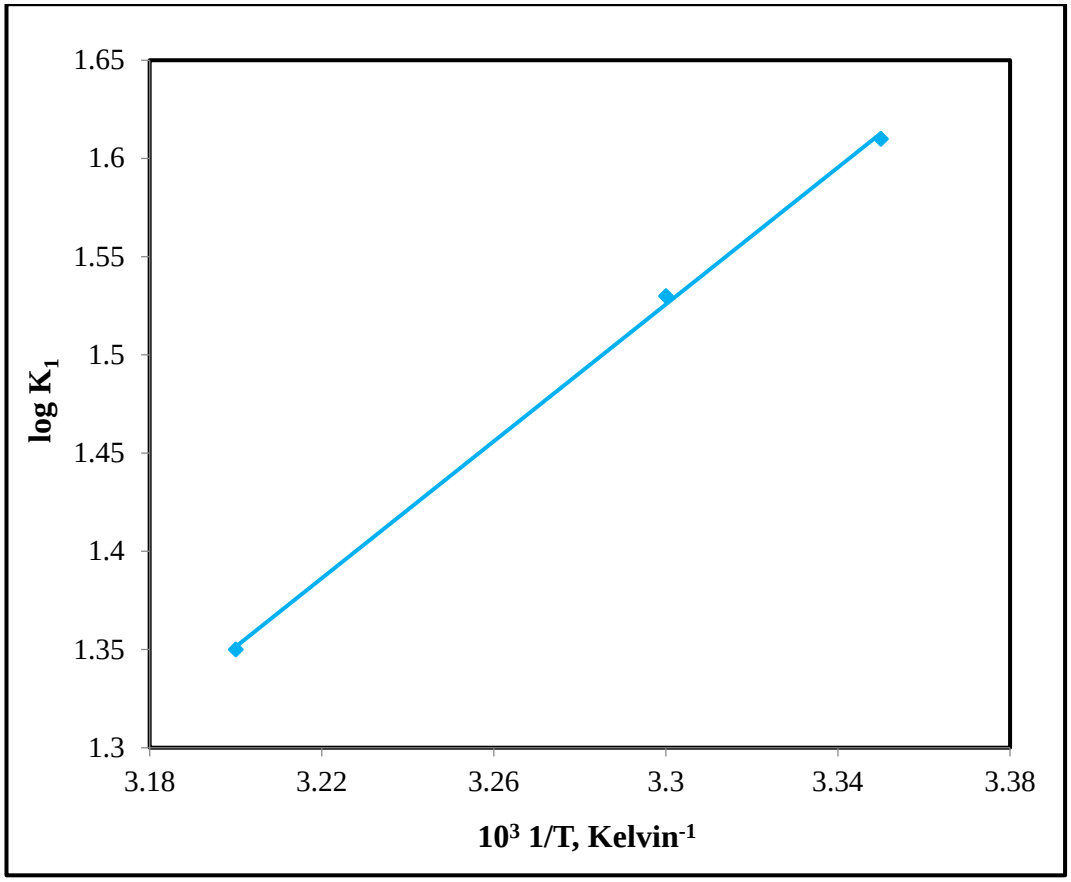


Figure 3.10: Plot of log K_1 versus 1/T.

(Ref. Table: 3.13)

3.5. CONCLUSION

The Kinetic study of oxidation of ciprofloxacin by permanganate in acidic medium has been studied. The oxidation products were identified (7-amino-1-cyclopropyl-6-fluoro-4-oxo-quinolone-3-carboxylic acid) by spot test, FT-IR, and LC-MS. The results demonstrate the role of pH in the reaction medium is crucial. The literature [40] reports that dealkylated products of ciprofloxacin have reduced antimicrobial activity. Since dealkylated products are obtained in the present study, it is evident that the products of the title reaction have reduced antimicrobial activity after oxidation. So this study will be effectively used in waste water treatment at the sites contaminated by fluoroquinolone antibiotics. The proposed mechanism is consistent with product, mechanism and kinetic studies.

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Chapter - 4

Mechanistic and Kinetic Study of Oxidation of Ofloxacin by Permanganate in Aqueous Sulphuric Acid Medium

ABSTRACT

The kinetics and mechanism of oxidation of ofloxacin by permanganate ion in acidic medium have been studied at 30 ± 1°C. The Stoichiometry was observed to be 2:5 in terms of mole ratio of permanganate ion and ofloxacin consumed. The reaction shows first order with respect to oxidant and fractional order in both the substrate and hydrogen ion concentration. The effect of added products and ionic strength has also been investigated. The main product was identified as 7-amino quinolone by FT-IR, and LC-MS analysis. From above results a suitable mechanism is proposed and rate law is derived.

$$k_{obs} = \frac{kK_1K_2[H^+][OFL]}{1+K_1[H^+]+K_1K_2[H^+][OFL]}$$

Investigation of the reaction at different temperature allowed the determination of the activation parameters with respect to the slow step of the proposed mechanism.

4.1. INTRODUCTION

Ofloxacin (OFL) [9-fluoro-2, 3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido-[1, 2, 3-de]-1, 4-benzoxazine-6-carboxylic acid] belongs to the fluoroquinolone class of antibiotics. They are synthetic broad spectrum antibacterial drugs that exhibit significant activity against both gram-positive and gram-negative bacteria [1]. They act as specific inhibitors of the bacterial DNA-Gyrase, the enzyme responsible for converting double stranded DNA into a negative super-helical form. Ofloxacin **(Figure 4.1)** possess two relevant ionisable functional groups: a basic piperazinyl group and a carboxylic group. The carboxylic group and the carbonyl groups are required for antimicrobial activity.

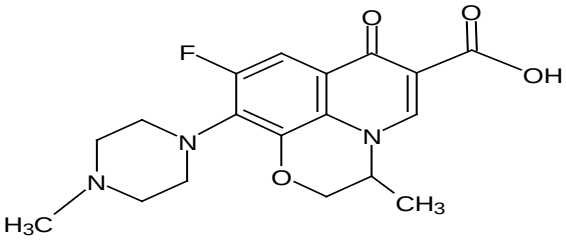


Figure 4.1: Structure of Ofloxacin (OFL).

Potassium permanganate is widely used as an oxidizing, disinfectant and also as analytical reagent [2]. The oxidation by Mn(VII) ions finds extensive applications in organic synthesis [3], especially since the advent of phase transfer catalysis [4-6]. Kinetic studies are important sources of mechanistic information on such reactions, as demonstrated by the results referring to unsaturated acids both in aqueous [4-7] and in non-aqueous media [8]. During oxidation by Mn(VII) it is evident that Mn(VII) is reduced to various oxidation states in acid, alkaline and neutral media. Among six oxidation states of manganese from +2 to+7, permanganate, Mn(VII) is the most potent oxidation state in acid medium with reduction potentials 1.69V of Mn(VII)/Mn(IV) couple and 1.51V of Mn(VII)/Mn(II) couple [9]. In acidic medium active species of Mn(VII) exists in different forms as $HMnO_4$, $H_2MnO_4^+$, $HMnO_3$ and Mn_2O_7 depending on the nature of the reductant, the oxidant has been assigned both inner sphere and outer sphere mechanism pathways in their redox reactions [10, 11].

The literature survey reveals that there are few study reports on the oxidation of ofloxacin by $CeSO_4$ [12, 13] and MnO_2 followed by evaluation of the reaction

kinetics and analysis of chemical structure of degradation products [14, 15]. Other studies reported that FQs with piperazine groups are more reactive to oxidation and leads to dealkylation, hydroxylation and intramolecular ring closure at the piperazine moiety, while quinolone ring remain mostly intact [16, 17]. Interaction of ofloxacin with various metal ions was studied for the determination of ofloxacin spectrophotometrically and polarographically in pharmaceutical formulation [18-21]. Hence, ofloxacin finds extensive application in pharmaceutical industry. It is noted that despite the importance of the drug, the literature survey reveals that there is no information about the oxidation kinetics. This prompted us to undertake the title reaction. The present study deals to investigate the redox chemistry of permanganate in acid media and establishing a plausible mechanism for oxidation of ofloxacin by permanganate on the basis of experimental results.

4.2. EXPERIMENTAL

4.2.1. Chemicals and Reagents.

The method of preparation and standardization of the reagents are given in chapter 2 (Experimental). All chemicals used were of analytical reagent grade, commercially available, and were used without further purification. Always freshly prepared and standardized $KMnO_4$ solutions were used in the kinetics. All the solutions were prepared by using double distilled water; the second distillation was done in presence of potassium permanganate.

4.2.2. Kinetic Procedure.

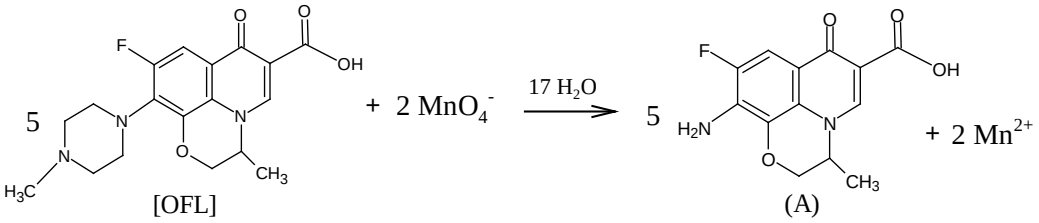
Kinetic measurements were performed on a U.V. 3000^+ UV-Visible spectrophotometer (LABINDIA). All kinetic measurements were carried out under pseudo-first-order conditions with [ofloxacin]:[$KMnO_4$] $\geq$ 10:1 at constant ionic strength (2.0×10^{-2} mol dm^{-3}). The temperature was consistently maintained at 30 ± 0.1°C. The reaction was initiated by adding previously thermostated solutions of $KMnO_4$ and ofloxacin which also contained the required quantities of H_2SO_4 and Na_2SO_4 to maintain the required acidity and ionic strength respectively. The progress of the reaction was monitored by measuring decrease in the absorbance of permanganate on UV-Visible spectrophotometer at its absorption maximum of 525

nm as a function of time. It was verified that other components of the reaction mixture do not absorb significantly at this wavelength. The application of Beer's law to permanganate was verified in concentration range $5.0 \times 10^{-5} – 5.0 \times 10^{-4}$ mol dm^{-3} at 525 nm. The molar extinction coefficient was found to be $\varepsilon = 2260 \pm 60$ dm^3 mol^{-1} cm^{-1} (Literature, $\varepsilon = 2389$ dm^3 mol^{-1} cm^{-1}) [22].

The first order plots in almost all the cases were linear to more than 75% completion of the reaction and k_{obs} values were reproducible within ± 5%.

4.2.3. Stoichiometry and product analysis.

The reaction mixture containing varying ratios of the OFL to $KMnO_4$ were mixed in the presence of 1.0×10^{-2} mol dm^{-3} $[H^+]$ maintaining to a constant ionic strength of 0.02 mol dm^{-3}, then equilibrated for 24 hours at 30°C. After the completion of reaction excess of permanganate was measured spectrophotometrically at 525 nm. The stoichiometry of the reaction was found as 5:2. Oxidative product was formed by reacting 5 moles of OFL with 2 moles of $KMnO_4$. The reaction can be represented as follows:



$+ 5\ NH_3 + 25\ HCHO + 14\ H^+$

The oxidation products were isolated using the thin-layer chromatography (TLC) separation technique and characterized by physicochemical spectral studies. The reaction products were identified as Mn^{2+}, (A), and formaldehyde as a by-product. Mn^{2+} was confirmed by spot test [23]. Product A was confirmed by LC-MS and IR spectra. The observed peaks of LC/MS spectra **(Figure 4.2)** interpreted in accordance with the proposed structure of the product (A). The mass spectrum showed the molecular ion peak at **m/z 279** consistent with dealkylation and deamination process from the molecular ion of ofloxacin (m/z 362). Oxidation following dealkylation and deamination yielded 7-amino fluoroquinolones analogues. The product was also short written as (M – 69) because of its structural similarity to the (M – 69) product of

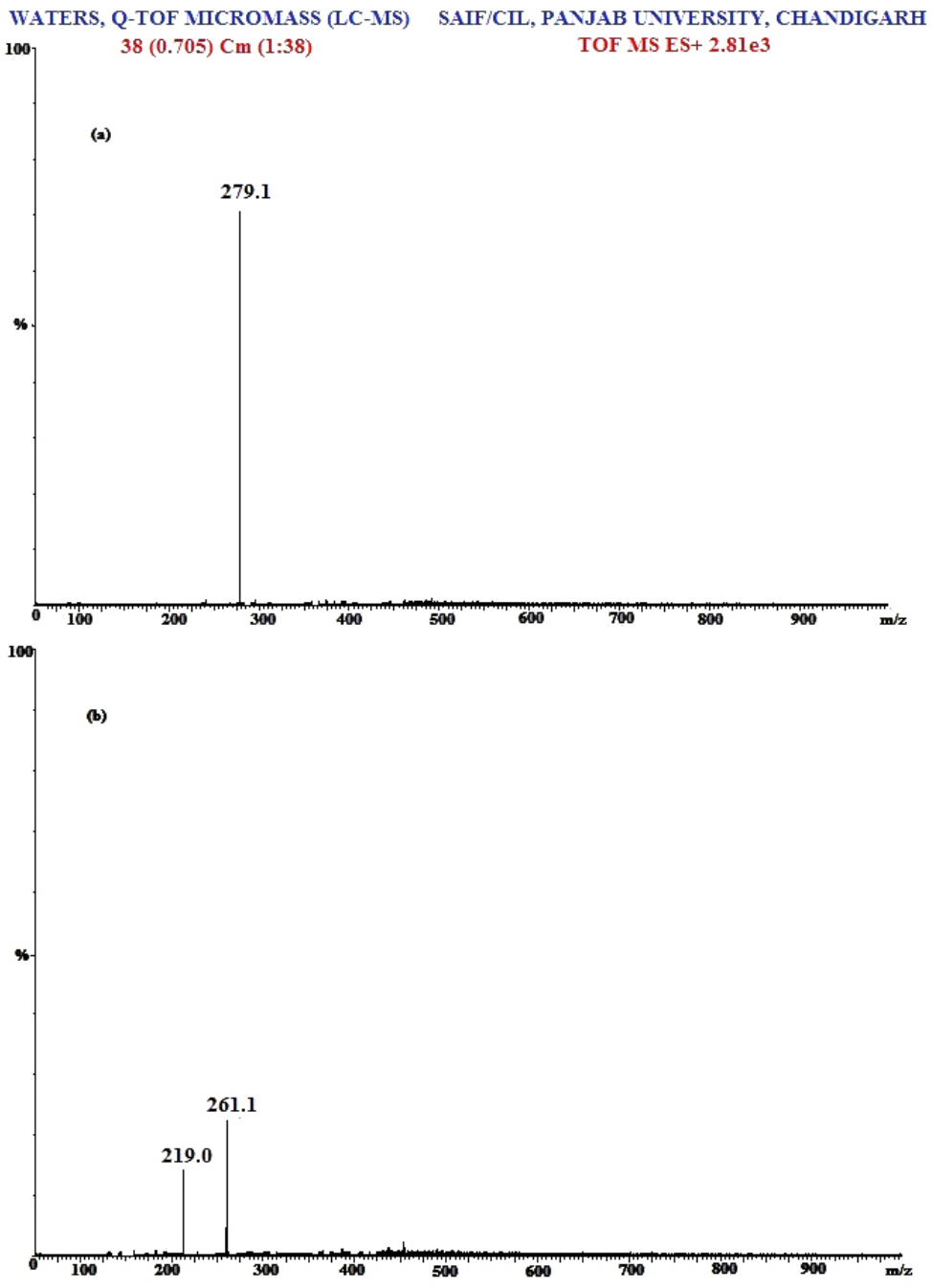


Figure 4.2: LC-ESI-MS spectra of oxidation product of ofloxacin.

(a) moiecular ion peak of m/z 279 (M-69).

(b) Fragmentation of (M-69) product.

ciprofloxacin [24] indicating the net mass loss of the product from the parent ofloxacin. This product was also in good agreement with earlier literature [17].

The presence of $–NH_2$ group in the major identified product (A) is also confirmed by IR spectroscopy **(Figure 4.3)** which shows a band at **3353.85 cm^{-1}** which is due to − NH stretching of the $–NH_2$ group [25]. Remaining peaks corresponds to the parent compound (quinolone ring). An addition of 2,4-dinitrophenylhydrazine in the reaction mixture yield yellow precipitate of hydrazone derivative of aldehyde [26]. The other product ammonia was detected by Nesseler's reagent test [27].

4.3. RESULTS

4.3.1. Reaction Orders.

The reaction orders were obtained from the slopes of log k_{obs} versus log [concentration] plots by changing the concentrations of OFL, permanganate and acid, while maintaining all other experimental conditions constant.

4.3.2. Permanganate Dependence.

The effect of permanganate concentration on the oxidation of OFL has been studied by varying its concentration from $7.5 \times 10^{-5} – 6.0 \times 10^{-4}$ mol dm^{-3}, at three different concentration of [OFL] = 1.0×10^{-3}, 2.5×10^{-3} and 4.0×10^{-3} mol dm^{-3} respectively, at constant concentration of $[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3}, I = 2.0×10^{-2} mol dm^{-3} at temperature = 30°C. The linear plot of log absorbance versus time **(Figure 4.4)** shows that the order with respect to [$KMnO_4$] was unity. This fact was also confirmed by varying [MnO_4^-] which did not show any change in values of pseudo- first-order rate constants (k_{obs}). Results are given in **Tables 4.1, 4.2 and 4.3**.

4.3.3. Ofloxacin Dependence.

The effect of concentration variation of ofloxacin on the rate of reaction was studied in the concentration range $2.0 \times 10^{-3} – 7.0 \times 10^{-3}$ mol dm^{-3} at constant concentration of [$KMnO_4$] = 2.0×10^{-4} mol dm^{-3}, $[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3} and I = 2.0×10^{-2} mol dm^{-3} at three temperature viz. 20°C, 25°C and 30°C respectively. It was

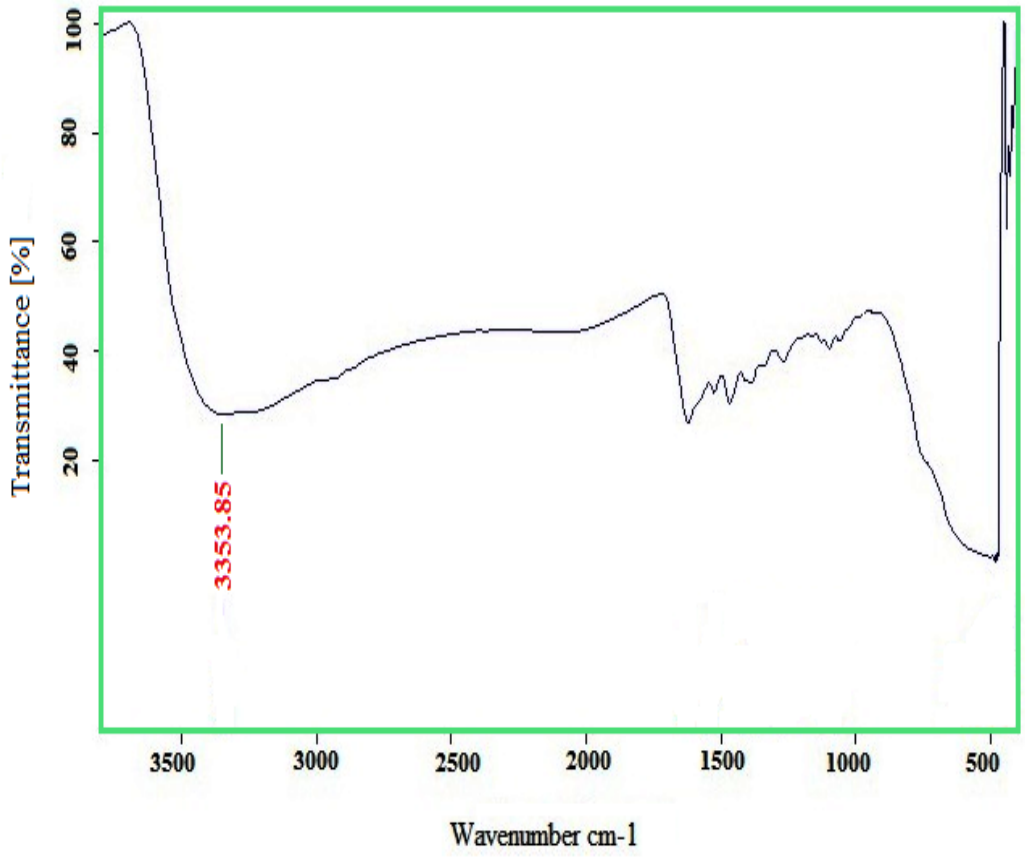


Figure 4.3: FTIR spectra of the oxidative product of ofloxacin by permanganate in acidic aqueous medium.

TABLE: 4.1

VARIATION OF $KMnO_4$

[OFL] = 1.0 x 10^{-3} mol dm^{-3} **Temp. = 30°C**

[H^+] = 1.0 x 10^{-2} mol dm^{-3} **I = 2.0 x 10^{-2} mol dm^{-3}**

10^4 [$KMnO_4$], mol dm^{-3}	**0.75**	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**
Time in minutes				**Absorbance**			
0	0.187	0.243	0.483	0.721	0.973	1.21	1.46
0.5	0.145	0.191	0.398	0.525	0.759	0.955	1.14
1	0.124	0.152	0.309	0.446	0.617	0.759	0.872
1.5	0.092	0.120	0.234	0.363	0.479	0.603	0.724
2	0.079	0.100	0.201	0.295	0.399	0.502	0.604
2.5	0.062	0.080	0.162	0.219	0.295	0.407	0.468
3	0.050	0.064	0.132	0.186	0.252	0.317	0.399
3.5	0.040	0.048	0.105	0.145	0.204	0.251	0.324
4	0.032	0.042	0.081	0.124	0.159	0.205	0.252
10^3 (k_{obs}), sec^{-1}	**7.29**	**7.28**	**7.29**	**7.27**	**7.30**	**7.27**	**7.29**

TABLE: 4.2

VARIATION OF $KMnO_4$

[OFL] = 2.5 x 10^{-3} mol dm^{-3} **Temp. = 30°C**

[H^+] = 1.0 x 10^{-2} mol dm^{-3} **I = 2.0 x 10^{-2} mol dm^{-3}**

10^4 [$KMnO_4$], mol dm^{-3}	**0.75**	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**
Time in minutes				**Absorbance**			
0	0.186	0.244	0.485	0.721	0.972	1.21	1.47
0.5	0.126	0.158	0.331	0.468	0.646	0.832	0.933
1	0.087	0.110	0.229	0.316	0.457	0.550	0.646
1.5	0.060	0.081	0.162	0.240	0.316	0.398	0.457
2	0.043	0.055	0.110	0.163	0.219	0.276	0.316
2.5	0.029	0.039	0.077	0.115	0.151	0.191	0.219
3	0.020	0.028	0.056	0.084	0.102	0.138	0.166
3.5	0.015	0.018	0.036	0.055	0.073	0.088	0.110
$10^3(k_{obs})$, sec^{-1}	**12.26**	**12.28**	**12.27**	**12.26**	**12.28**	**12.29**	**12.28**

TABLE: 4.3

VARIATION OF $KMnO_4$

[OFL] = 4.0 x 10^{-3} mol dm^{-3} **Temp. = 30°C**

[H^+] = 1.0 x 10^{-2} mol dm^{-3} **I = 2.0 x 10^{-2} mol dm^{-3}**

10^4 [$KMnO_4$], mol dm^{-3}	**0.75**	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**
Time in minutes				**Absorbance**			
0	0.183	0.245	0.484	0.723	0.974	1.21	1.47
0.5	0.105	0.138	0.275	0.427	0.550	0.692	0.776
1	0.064	0.080	0.170	0.263	0.347	0.398	0.478
1.5	0.038	0.048	0.102	0.145	0.195	0.155	0.288
2	0.023	0.030	0.059	0.088	0.119	0.149	0.172
2.5	0.013	0.017	0.034	0.050	0.073	0.092	0.107
3	0.004	0.008	0.020	0.033	0.041	0.053	0.064
10^3 (k_{obs}), sec^{-1}	**17.38**	**17.39**	**17.36**	**17.35**	**17.38**	**17.38**	**17.36**

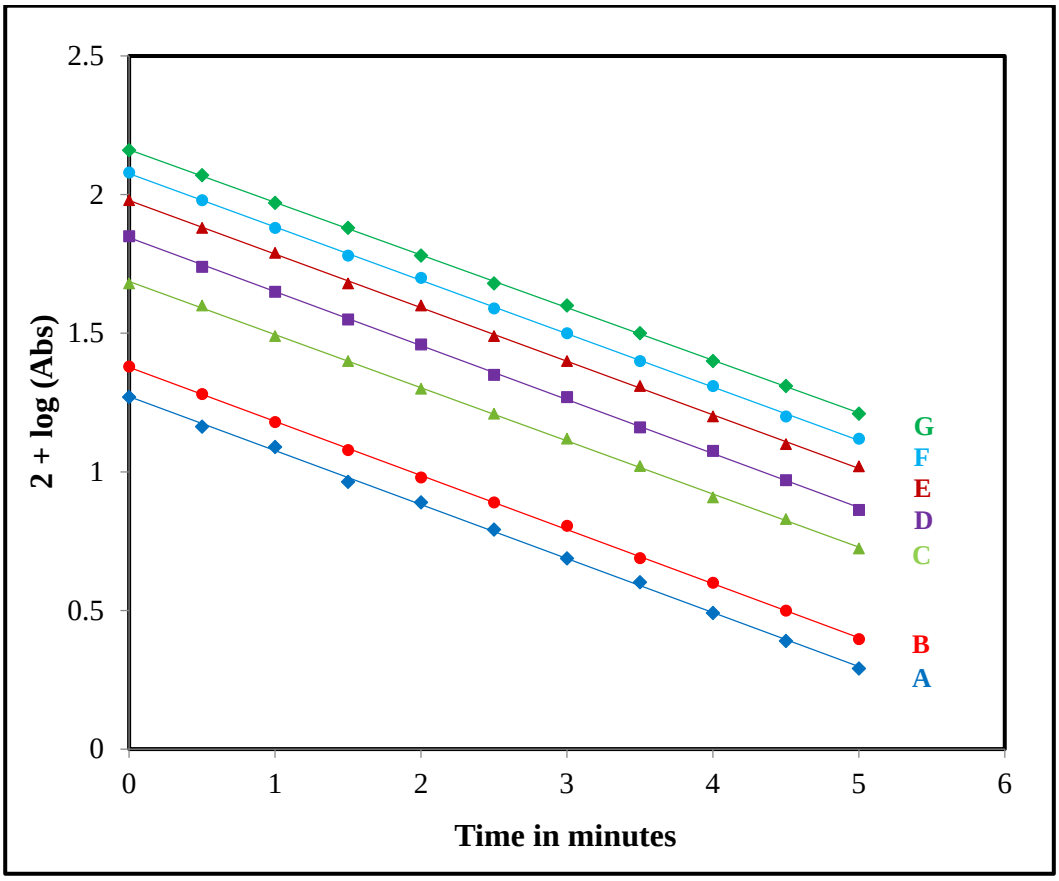


Figure 4.4: First order plots of the variation of permanganate concentration.

[OFL] = 1.0×10^{-3} mol dm^{-3};

I = 2.0×10^{-2} mol dm^{-3};

[Mn(VII)] = (A) 0.75×10^{-4} mol dm^{-3}

(C) 2.0×10^{-4} mol dm^{-3}

(E) 4.0×10^{-4} mol dm^{-3}

$[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3};

Temp. = 30°C;

(B) 1.0×10^{-4} mol dm^{-3}

(D) 3.0×10^{-4} mol dm^{-3}

(F) 5.0×10^{-4} mol dm^{-3}

(G) 6.0×10^{-4} mol dm^{-3}.

(Ref. Table 4.1)

observed that as the concentration of ofloxacin increased, rate of the reaction also increased **(Figure 4.5)**. The plot of log k_{obs} versus log [OFL] was linear with a slope of 0.63, thus indicating a fractional-order dependence on ofloxacin concentration. Results are given in **Tables 4.4, 4.5 and 4.6**.

4.3.4. Hydrogen ion dependence.

The effect of hydrogen ion was studied employing different concentration of sulphuric acid in the concentration range 2.0×10^{-3} to 2.0×10^{-2} mol dm^{-3}, keeping all other reactant concentration and conditions constant viz. $[KMnO_4] = 2.0 \times 10^{-4}$ mol dm^{-3}, [OFL] = 2.0×10^{-3} mol dm^{-3} and I = 2.0×10^{-2} mol dm^{-3} (Ionic strength was adjusted employing sodium sulphate) at three temperature viz. 20°C, 25°C and 30°C respectively. The values of pseudo-first-order rate constants (k_{obs}) were found to be increased with increase in $[H^+]$ **(Figure 4.6)**. A plot of log k_{obs} versus log $[H^+]$ was linear with a fractional slope of 0.75. Results are given in **Tables 4.7, 4.8 and 4.9**.

4.3.5. Effect of Ionic Strength and Dielectric Constant.

To study the effect of ionic strength on the rate of the reaction, concentration of sodium sulphate was varied from 0.01 to 0.1 mol dm^{-3} at constant concentrations of $[KMnO_4] = 2.0 \times 10^{-4}$ mol dm^{-3}, [OFL] = 2.0×10^{-3} mol dm^{-3} and $[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3} at 30°C. Change in ionic strength did not show any significant effect on the rate of the reaction. Results are given in **Table 4.10**.

The effect of dielectric constant (*D*) on the rate of the reaction was studied by employing different ratios of acetic acid - water content (v/v) in the reaction mixture at fixed concentration of $[KMnO_4] = 2.0 \times 10^{-4}$ mol dm^{-3}, [OFL] = 2.0×10^{-3} mol dm^{-3}, $[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3} and I = 2.0×10^{-2} mol dm^{-3} at 30°C. It was found that the rate constants did not change with increase in the dielectric constant of the medium. Results are given in **Table 4.11**.

4.3.6. Effect of Initially Added Products.

The effect of Mn(II) ion was studied in the range of 5.0×10^{-5} to 5.0×10^{-4} mol dm^{-3} at constant concentration of $[KMnO_4] = 2.0 \times 10^{-4}$ mol dm^{-3}, [OFL] = 2.0×10^{-3} mol dm^{-3}, $[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3} and I = 2.0×10^{-2} mol dm^{-3} at 30°C. It was

TABLE: 4.4

VARIATION OF OFLOXACIN

$[KMnO_4]$ = 2.0 x 10^{-4} mol dm^{-3} **Temp. = 20°C**

$[H^+]$ = 1.0 x 10^{-2} mol dm^{-3} **I = 2.0 x 10^{-2} mol dm^{-3}**

10^3 [OFL], mol dm^{-3}	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**	**7.0**
Time in minutes			**Absorbance**			
0	0.483	0.484	0.482	0.483	0.484	0.482
0.5	0.363	0.347	0.316	0.302	0.288	0.263
1	0.295	0.251	0.209	0.182	0.166	0.170
1.5	0.219	0.191	0.132	0.120	0.105	0.073
2	0.185	0.144	0.094	0.073	0.063	0.055
2.5	0.145	0.100	0.064	0.046	0.038	0.032
3	0.110	0.080	0.042	0.028	0.023	0.019
3.5	0.088	0.058	0.027	0.017	–	–
10^3 (k_{obs}), sec^{-1}	**7.93**	**10.03**	**13.53**	**15.72**	**16.94**	**18.04**

TABLE: 4.5

VARIATION OF OFLOXACIN

$[KMnO_4]$ = 2.0 x 10^{-4} mol dm^{-3} **Temp. = 25°C**

$[H^+]$ = 1.0 x 10^{-2} mol dm^{-3} **I = 2.0 x 10^{-2} mol dm^{-3}**

10^3 [OFL], mol dm^{-3}	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**	**7.0**
Time in minutes			**Absorbance**			
0	0.482	0.484	0.483	0.483	0.484	0.482
0.5	0.347	0.331	0.288	0.275	0.257	0.267
1	0.251	0.229	0.195	0.158	0.138	0.132
1.5	0.204	0.155	0.115	0.092	0.080	0.070
2	0.150	0.113	0.075	0.053	0.042	0.039
2.5	0.105	0.082	0.048	0.029	0.024	0.021
3	0.084	0.053	0.029	0.018	0.012	0.009
10^3 (k_{obs}), sec^{-1}	**9.68**	**12.04**	**15.48**	**18.34**	**20.27**	**20.86**

TABLE: 4.6

VARIATION OF OFLOXACIN

$[KMnO_4]$ = 2.0 x 10^{-4} mol dm^{-3} **Temp. = 30°C**

$[H^+]$ = 1.0 x 10^{-2} mol dm^{-3} **I = 2.0 x 10^{-2} mol dm^{-3}**

10^3 [OFL], mol dm^{-3}	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**	**7.0**
Time in minutes			**Absorbance**			
0	0.482	0.482	0.484	0.483	0.484	0.483
0.5	0.347	0.302	0.273	0.257	0.263	0.240
1	0.245	0.209	0.169	0.145	0.126	0.115
1.5	0.174	0.126	0.101	0.081	0.067	0.065
2	0.133	0.088	0.059	0.042	0.032	0.030
2.5	0.102	0.056	0.032	0.025	0.018	0.016
3	0.073	0.036	0.020	0.013	–	–
10^3 (k_{obs}), sec^{-1}	**10.65**	**14.14**	**17.36**	**20.26**	**22.43**	**22.98**

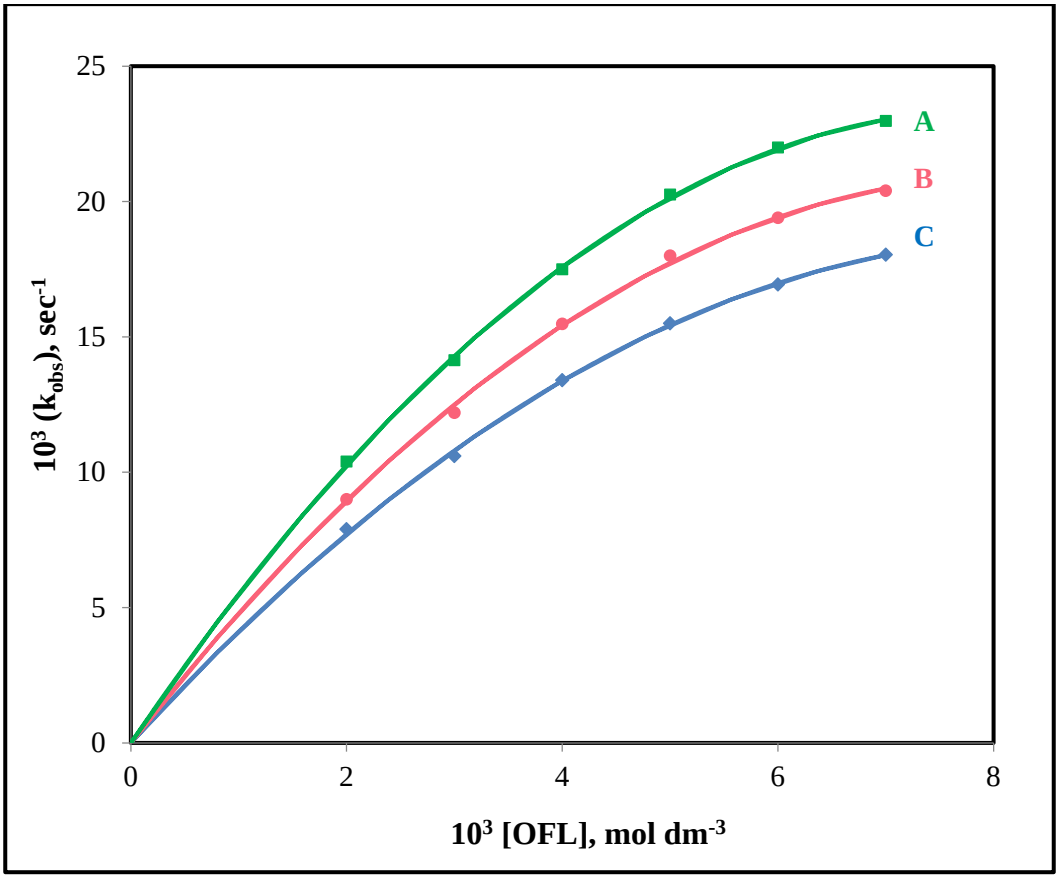


Figure 4.5: Variation of Ofloxacin at different temperature

(A) 20°C, (B) 25°C, (C) 30°C.

$[KMnO_4] = 2.0 \times 10^{-4}$ mol dm^{-3};

$[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3};

$I = 2.0 \times 10^{-2}$ mol dm^{-3}.

(Ref. Table: 4.4, 4.5, 4.6)

TABLE: 4.7

VARIATION OF HYDROGEN ION

$[KMnO_4]$ = 2.0 x 10^{-4} mol dm^{-3} **Temp. = 20°C**

[OFL] = 2.0 x 10^{-3} mol dm^{-3} **I = 2.0 x 10^{-2} mol dm^{-3}**

10^2 $[H^+]$, mol dm^{-3}	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**	**1.5**	**2.0**
Time in minutes						**Absorbance**					
0	(0)0.483	(0)0.483	0.482	0.481	0.483	0.482	0.481	0.484	0.483	0.483	0.482
1	(2)0.359	(2)0.327	0.380	0.347	0.324	0.316	0.309	0.316	0.297	0.269	0.275
2	(4)0.263	(4)0.229	0.297	0.274	0.248	0.224	0.213	0.200	0.185	0.158	0.145
3	(6)0.182	(6)0.151	0.234	0.200	0.174	0.155	0.138	0.132	0.110	0.084	0.085
4	(8)0.135	(8)0.107	0.182	0.162	0.126	0.105	0.088	0.084	0.076	0.051	0.042
5	(10)0.096	(10)0.076	0.151	0.120	0.098	0.075	0.062	0.056	0.041	0.028	0.025
6	(12)0.067	(12)0.052	0.110	0.086	0.065	0.050	0.042	0.034	0.028	0.016	0.014
10^3 (k_{obs}), sec^{-1}	**2.39**	**3.18**	**3.96**	**4.66**	**5.47**	**6.32**	**6.75**	**7.29**	**7.93**	**9.25**	**9.94**

Figures in parentheses denote time in minutes.

TABLE: 4.8

VARIATION OF HYDROGEN ION

$[KMnO_4]$ = 2.0 x 10^{-4} mol dm^{-3} **Temp. = 25°C**

[OFL] = 2.0 x 10^{-3} mol dm^{-3} **I = 2.0 x 10^{-2} mol dm^{-3}**

10^2 $[H^+]$, mol dm^{-3}	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**	**1.5**	**2.0**
Time in minutes						**Absorbance**					
0	(0)0.483	(0)0.483	0.481	0.484	0.482	0.482	0.483	0.484	0.483	0.484	0.483
1	(2)0.328	(2)0.290	0.339	0.331	0.326	0.288	0.282	0.269	0.253	0.251	0.229
2	(4)0.219	(4)0.174	0.249	0.214	0.205	0.187	0.169	0.157	0.150	0.118	0.104
3	(6)0.158	(6)0.112	0.158	0.145	0.135	0.117	0.102	0.086	0.084	0.063	0.052
4	(8)0.110	(8)0.069	0.115	0.102	0.092	0.074	0.058	0.051	0.048	0.028	0.024
5	(10)0.078	(10)0.042	0.084	0.067	0.063	0.044	0.034	0.028	0.025	0.016	–
6	(12)0.051	(12)0.024	0.058	0.046	0.042	0.030	0.022	0.017	0.014	–	–
10^3 (k_{obs}), sec^{-1}	**3.14**	**4.16**	**5.43**	**6.69**	**7.08**	**7.85**	**8.67**	**9.26**	**9.68**	**11.69**	**12.75**

Figures in parentheses denote time in minutes.

TABLE: 4.9

VARIATION OF HYDROGEN ION

$[KMnO_4]$ = 2.0 x 10^{-4} mol dm^{-3} **Temp. = 30°C**

[OFL] = 2.0 x 10^{-3} mol dm^{-3} **I = 2.0 x 10^{-2} mol dm^{-3}**

10^2 $[H^+]$, mol dm^{-3}	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**	**1.5**	**2.0**
Time in minutes						**Absorbance**					
0	(0)0.483	0.482	0.481	0.483	0.482	0.480	0.481	0.482	0.483	0.482	0.483
1	(2)0.315	0.363	0.324	0.309	0.288	0.275	0.263	0.251	0.245	0.209	0.200
2	(4)0.191	0.264	0.229	0.200	0.173	0.158	0.147	0.142	0.133	0.093	0.087
3	(6)0.115	0.191	0.158	0.135	0.107	0.092	0.082	0.076	0.073	0.042	0.039
4	(8)0.079	0.145	0.110	0.088	0.063	0.053	0.044	0.041	0.035	0.019	0.017
5	(10)0.048	0.107	0.076	0.055	0.040	0.032	0.024	0.021	0.018	0.011	–
6	(12)0.030	0.081	0.053	0.038	0.023	0.018	0.014	0.012	0.009	–	–
10^3 (k_{obs}), sec^{-1}	**3.49**	**4.98**	**6.14**	**7.28**	**8.46**	**9.21**	**9.83**	**10.16**	**10.65**	**13.66**	**14.20**

Figures in parentheses denote time in minutes.

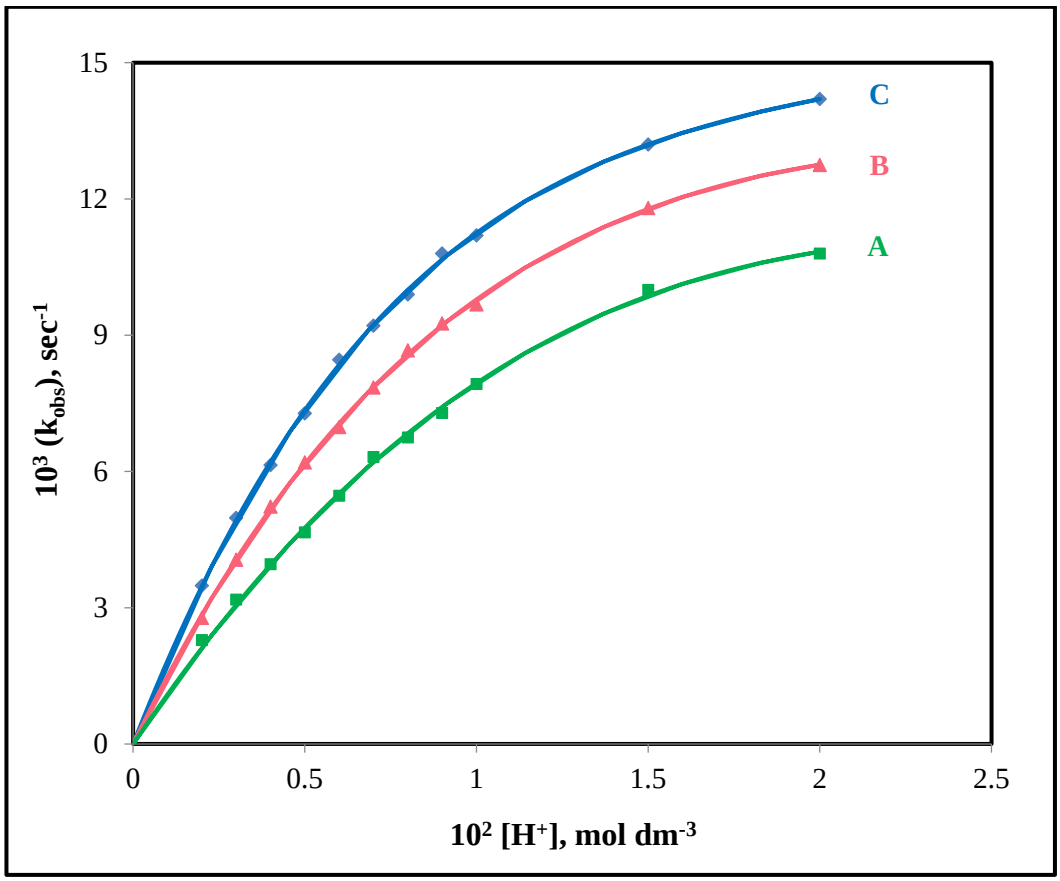


Figure 4.6: Variation of Hydrogen Ion at different temperature

(A) 20°C, (B) 25°C, (C) 30°C.

$[KMnO_4] = 2.0 \times 10^{-4}$ mol dm^{-3};

$[OFL] = 2.0 \times 10^{-3}$ mol dm^{-3};

$I = 2.0 \times 10^{-2}$ mol dm^{-3}.

(Ref. Table: 4.7, 4.8, 4.9)

TABLE: 4.10

VARIATION OF SODIUM SULPHATE

$[KMnO_4]$ = 2.0 x 10^{-4} mol dm^{-3} **Temp. = 30°C**

[OFL] = 2.0 x 10^{-3} mol dm^{-3} **$[H^+]$ = 1.0 x 10^{-2} mol dm^{-3}**

10^2 $[Na_2SO_4]$, mol dm^{-3}	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0
Time in minutes	**Absorbance**									
0	0.481	0.482	0.480	0.482	0.483	0.482	0.482	0.481	0.483	0.482
0.5	0.344	0.348	0.345	0.346	0.349	0.348	0.348	0.340	0.343	0.347
1	0.243	0.246	0.244	0.243	0.245	0.246	0.243	0.245	0.240	0.243
1.5	0.178	0.173	0.177	0.175	0.175	0.171	0.173	0.176	0.176	0.173
2	0.134	0.133	0.134	0.133	0.133	0.133	0.133	0.134	0.134	0.133
2.5	0.108	0.105	0.107	0.105	0.102	0.106	0.103	0.106	0.107	0.104
3	0.078	0.074	0.077	0.073	0.072	0.075	0.074	0.078	0.076	0.073
10^3 (k_{obs}), sec^{-1}	**10.62**	**10.65**	**10.63**	**10.64**	**10.68**	**10.65**	**10.64**	**10.62**	**10.63**	**10.65**

TABLE: 4.11

EFFECT OF DIELECTRIC CONSTANT

$[KMnO_4] = 2.0 \times 10^{-4}$ mol dm^{-3} **Temp. = 30°C**

[OFL] = 2.0×10^{-3} mol dm^{-3} **I = 2.0×10^{-2} mol dm^{-3}**

$[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3}

[Acetic acid], %	**5**	**10**	**15**	**20**
Time in minutes		**Absorbance**		
0	0.482	0.483	0.482	0.481
0.5	0.348	0.349	0.348	0.346
1	0.246	0.245	0.243	0.245
1.5	0.173	0.175	0.173	0.171
2	0.133	0.134	0.133	0.133
2.5	0.105	0.105	0.103	0.104
3	0.074	0.077	0.074	0.072
10^3 (k_{obs}), sec^{-1}	**10.65**	**10.63**	**10.64**	**10.67**

found that externally added product Mn^{2+} did not show any significant effect on the rate of the reaction. Results are given in **Table 4.12**.

4.3.7. Test for Free Radicals.

To the reaction mixture a known quantity of CH_2=CHCN (acrylonitrile) scavenger, had been added initially, was kept in an inert atmosphere for 5 hours. On diluting the reaction mixture with methanol, precipitate appears in the reaction mixture, indicating the presence of free radical in the reaction.

4.4. DISCUSSION

The expected oxidizing species of permanganate in acid media are $HMnO_4$, $H_2MnO_4^+$, $HMnO_3$ and Mn_2O_7. Permanganate ion, MnO_4^- ion is powerful oxidizing agent in acidic medium. The stable oxidation product of MnO_4^- in acid medium is Mn(II). **Figure 4.7** illustrates the spectroscopic changes occurring in the oxidation of ofloxacin by acid permanganate at 30°C with scanning interval of 1 minute.

The active species of permanganate in aqueous acid solution may be deduced from the dependence of the rate on $[H^+]$, in the reaction medium. The order of $[H^+]$ is less than unity, which may indicate the formation of permanganate acid from permanganate ion. Permanganic acid $HMnO_4$ is more efficient oxidant species of Manganese (VII) then permanganate ion [28]. Equilibrium can be represented by **equation (1).**

$$MnO_4^- + H^+ \underset{}{\overset{K_1}{\rightleftharpoons}} HMnO_4 \qquad \textbf{(1)}$$

The reaction between ofloxacin and permanganate in sulphuric acid has Stoichiometry 5:2, having first order dependence with permanganate and less than unit order with H^+ concentration and ofloxacin concentration. The oxidation products were Mn(II), 7-amino fluoroquinolone, NH_3 and HCHO. In view of increasing the rate with increase in $[H^+]$ ion, in the prior equilibrium step, H^+ reacts with MnO_4^- to form $HMnO_4$, which reacts with the one mole of ofloxacin to form a complex. Complex formed is dissociate in the rate determining step to give a free

TABLE: 4.12

EFFECT OF Mn(II) ION

$[KMnO_4] = 2.0 \times 10^{-4}$ mol dm^{-3} **Temp. = 30°C**

[OFL] = 2.0×10^{-3} mol dm^{-3} **I = 2.0×10^{-2} mol dm^{-3}**

$[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3}

10^4 $[Mn]^{2+}$	**0.5**	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**
Time in minutes			**Absorbance**			
0	0.483	0.482	0.480	0.483	0.482	0.481
0.5	0.343	0.346	0.345	0.348	0.347	0.345
1	0.244	0.246	0.243	0.244	0.243	0.243
1.5	0.177	0.170	0.178	0.170	0.173	0.175
2	0.134	0.133	0.134	0.133	0.133	0.133
2.5	0.109	0.105	0.107	0.102	0.104	0.104
3	0.078	0.075	0.076	0.071	0.073	0.073
10^3 (k_{obs}), sec^{-1}	**10.62**	**10.65**	**10.63**	**10.68**	**10.65**	**10.67**

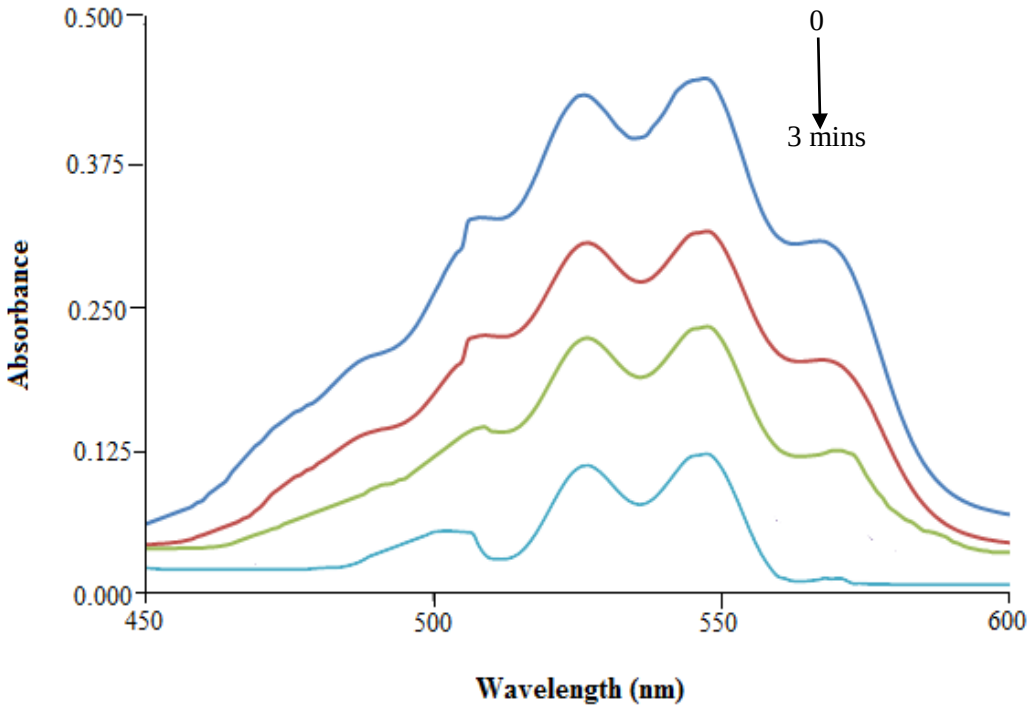
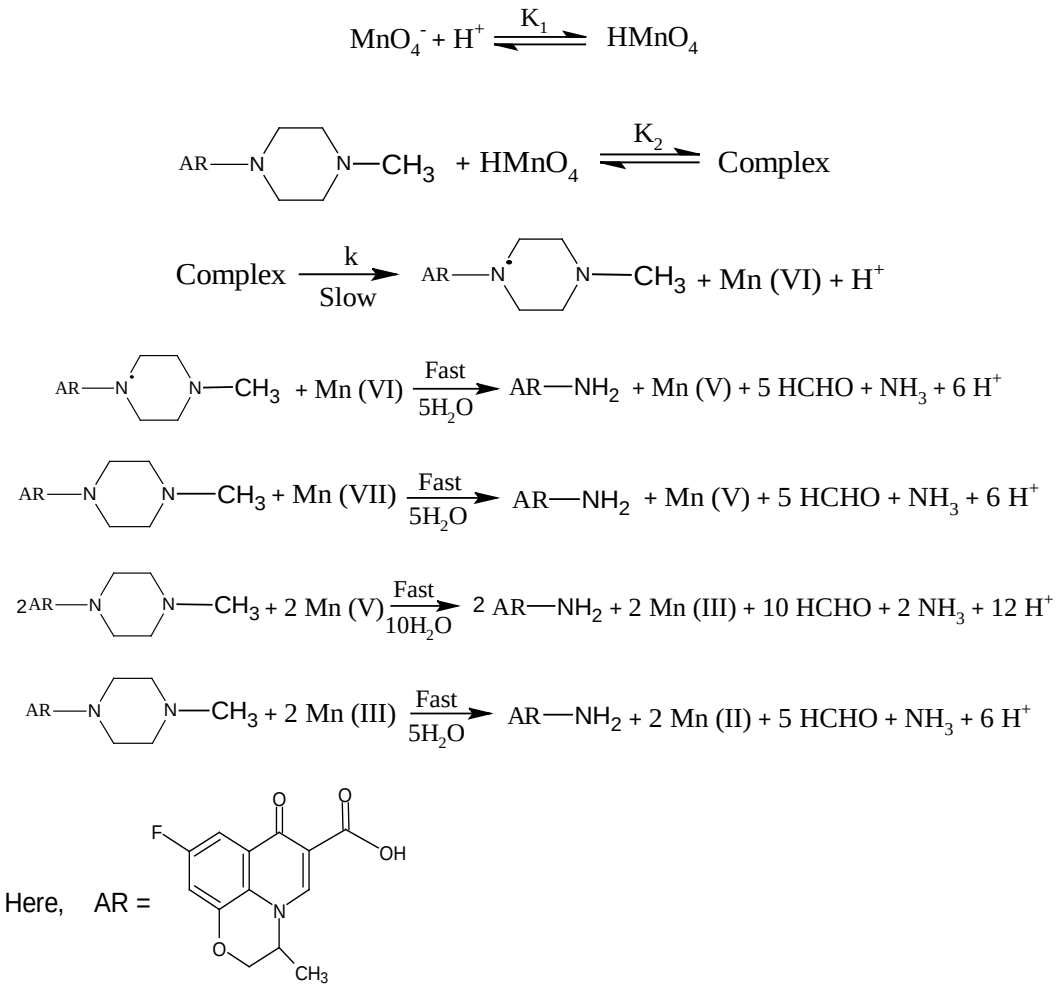


Figure 4.7: Spectral changes during the oxidation of ofloxacin (OFL) by permanganate in acidic medium at 30°C.

$[KMnO_4] = 2.0 \times 10^{-4}$ mol dm^{-3}; $[OFL] = 2.0 \times 10^{-3}$ mol dm^{-3};
$[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3}; $I = 2.0 \times 10^{-2}$ mol dm^{-3}.

radical derived from ofloxacin and an intermediate Mn(VI). In further fast steps the intermediate Mn(VI) reacts with a free radical to produce the product 7-amino fluoroquinolone, NH_3, HCHO and intermediate Mn(V). In further fast steps Mn(V) subsequently reduced to the end product Mn(II). Although Mn(VI) and Mn(IV) are the final reduced species of MnO_4^- in alkaline and neutral media, it was observed that Mn(II) was the only reduced species of MnO_4^- in acid medium.

Attempts were made to allow spectroscopic detection of intermediate Mn(V) and Mn(III) as the reaction proceeded in the oxidation of ofloxacin by permanganate. Unfortunately the low concentration of Mn(V) and Mn(III) intermediate obtained under our experimental conditions made the spectroscopic detection failure. However, the evidence for intermediate such as Mn(V) and Mn(III) are reported in the literature [29, 30].The results are accommodated in the **Scheme-1**.



Scheme-1

From the scheme-1, the following rate law can be derived as follows:

$$\text{Rate} = \frac{-d[MnO_4^-]}{dt} = k[\text{Complex}]$$

$$= kK_2[HMnO_4][OFL]$$

$$= kK_1K_2[MnO_4^-]_f[H^+]_f[OFL]_f \quad \textbf{(2)}$$

Total concentration of permanganate is given by:

$$[MnO_4^-]_t = [MnO_4^-]_f + [HMnO_4] + [\text{Complex}]$$

$$= [MnO_4^-]_f + K_1[MnO_4^-]_f[H^+]_f + K_2[HMnO_4][OFL]$$

$$= [MnO_4^-]_f + K_1[MnO_4^-]_f[H^+]_f + K_1K_2[MnO_4^-]_f[H^+]_f[OFL]$$

$$= [MnO_4^-]_f\{1+ K_1[H^+]_f + K_1K_2[H^+]_f[OFL]\}$$

$$[MnO_4^-]_f = \frac{[MnO_4^-]_t}{\{1+ K_1[H^+]_f + K_1K_2[H^+]_f[OFL]} \quad \textbf{(3)}$$

$[MnO_4^-]_t$ and $[MnO_4^-]_f$ are total and free concentration of Mn (VII) respectively.

Total concentration of ofloxacin is given by:

$$[OFL]_t = [OFL]_f + [\text{Complex}]$$

$$= [OFL]_f + K_2[OFL]_f[HMnO_4]$$

$$=[OFL]_f\{1+K_2[HMnO_4]\}$$

$$[OFL]_f = \frac{[OFL]_t}{1+ K_2[HMnO_4]}$$

Very low concentration of $[MnO_4^-]$ were used in the experiment, so $K_2[HMnO_4] << 1$

$$[OFL]_f = [OFL]_t \quad \textbf{(4)}$$

Total concentration of $[H^+]$ is given by:

$$[H^+]_t = [H^+]_f + [HMnO_4]$$

$$= [H^+]_f + K_1[MnO_4^-]_f[H^+]_f$$

$$= [H^+]_f\{1+ K_1[MnO_4^-]_f\}$$

So, $[H^+]_t = [H^+]_f$ **(5)**

Substituting equation (3), (4) and (5) in equation (2) and omitting "t" and "f" subscripts,

$$\text{Rate} = \frac{-d[MnO_4^-]}{dt} = \frac{kK_1K_2[MnO_4^-][H^+][OFL]}{1+ K_1[H^+] + K_1K_2[H^+][OFL]} \quad \textbf{(6)}$$

$$\frac{\text{Rate}}{[MnO_4^-]} = k_{obs} = \frac{kK_1K_2[H^+][OFL]}{1+ K_1[H^+] + K_1K_2[H^+][OFL]} \quad \textbf{(7)}$$

Equation (7) can be rearranged as:

$$\frac{1}{k_{obs}} = \frac{1}{kK_1K_2[H^+][OFL]} + \frac{1}{kK_2[OFL]} + \frac{1}{k} \quad \textbf{(8)}$$

According to equation (8) the plot of $1/k_{obs}$ versus 1/[OFL] **(Figure 4.8)** is linear with positive intercept and slope at three different temperature viz. 20°C, 25°C and 30°C. The rate constant k, of the slow step of scheme-1 was obtained from the intercept of the plots $1/k_{obs}$ versus 1/[OFL]. The energy of activation was determined by the plot of log k versus 1/T **(Figure 4.9)** from which activation parameters was calculated. The equilibrium constant of $HMnO_4$ (K_1) and the equilibrium constant of complex (K_2) in scheme-1 were calculated from the intercept and slope of the plot $1/k_{obs}$ versus $1/[H^+]$ **(Figure 4.10)**. The value of K_1 (42.5) is in good agreement with earlier work [22] at 30°C. Thermodynamic quantities **(Table 4.13)** were calculated from the Van't Hoff plots i.e. log K_1 versus 1/T **(Figure 4.11)** and log K_2 versus 1/T **(Figure 4.12)**.

The values of $\Delta H^{\#}$ and $\Delta S^{\#}$ are both favourable for electron transfer process [31]. The value of $\Delta S^{\#}$ within the range of radical reaction has been ascribed [32] to the nature of electron pairing and unpairing process. The negative value of $\Delta S^{\#}$ indicates that complex is more ordered than the reactants [33]. The observed modest enthalpy of activation and a relatively low value of the Entropy of activation as well as a higher rate constant of the slow step indicate that the oxidation presumably occurs via inner-sphere mechanism [34]. The negligible effect of ionic strength and dielectric constant is consistent with reaction between two neutral molecules which supports the proposed mechanism [35].

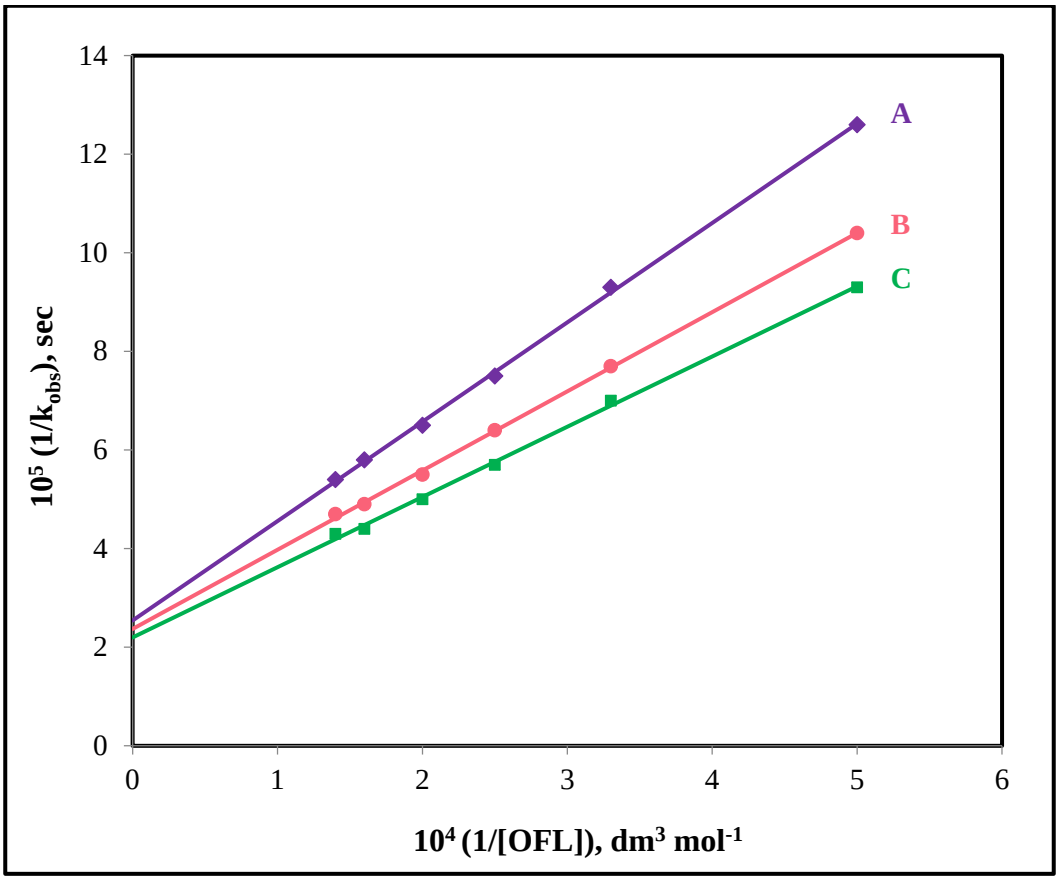


Figure 4.8: Plots of 1/k_{obs} versus 1/[OFL] at different temperature

(A) 20°C, (B) 25°C, (C) 30°C.

$[KMnO_4] = 2.0 \times 10^{-4}$ mol dm^{-3};

$[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3};

$I = 2.0 \times 10^{-2}$ mol dm^{-3}.

TABLE: 4.13

ACTIVATION PARAMETERS AND THERMODYNAMIC QUANTITIES EVALUATED FROM SCHEME 1.

Temperature (Kelvin)	**10^2 k (sec^{-1})**	**Activation Parameters**	**10^{-1} K_1 (dm^3 mol^{-1})**	**Thermodynamic Quantities (From K_1)**	**10^{-2} K_2 (dm^3 mol^{-1})**	**Thermodynamic Quantities (From K_2)**
293	4.00	E_a = 12.98 ($kJ\ mol^{-1}$)	4.80	$\Delta H = -10.30$ ($kJ\ mol^{-1}$)	3.57	$\Delta H = 29.75$ ($kJ\ mol^{-1}$)
298	4.34	$\Delta H^{\#}$ = 10.47 ($kJ\ mol^{-1}$)	4.62	$\Delta S = -31.0$ ($JK^{-1}\ mol^{-1}$)	4.61	$\Delta S = 100.4$ ($JK^{-1}\ mol^{-1}$)
303	4.76	$\Delta S^{\#} = -171.74$ ($JK^{-1}\ mol^{-1}$)	4.25	$\Delta G = -1.30$ ($kJ\ mol^{-1}$)	5.25	$\Delta G = -1.42$ ($kJ\ mol^{-1}$)
		$\Delta G^{\#}$ = 68.02 ($kJ\ mol^{-1}$)				

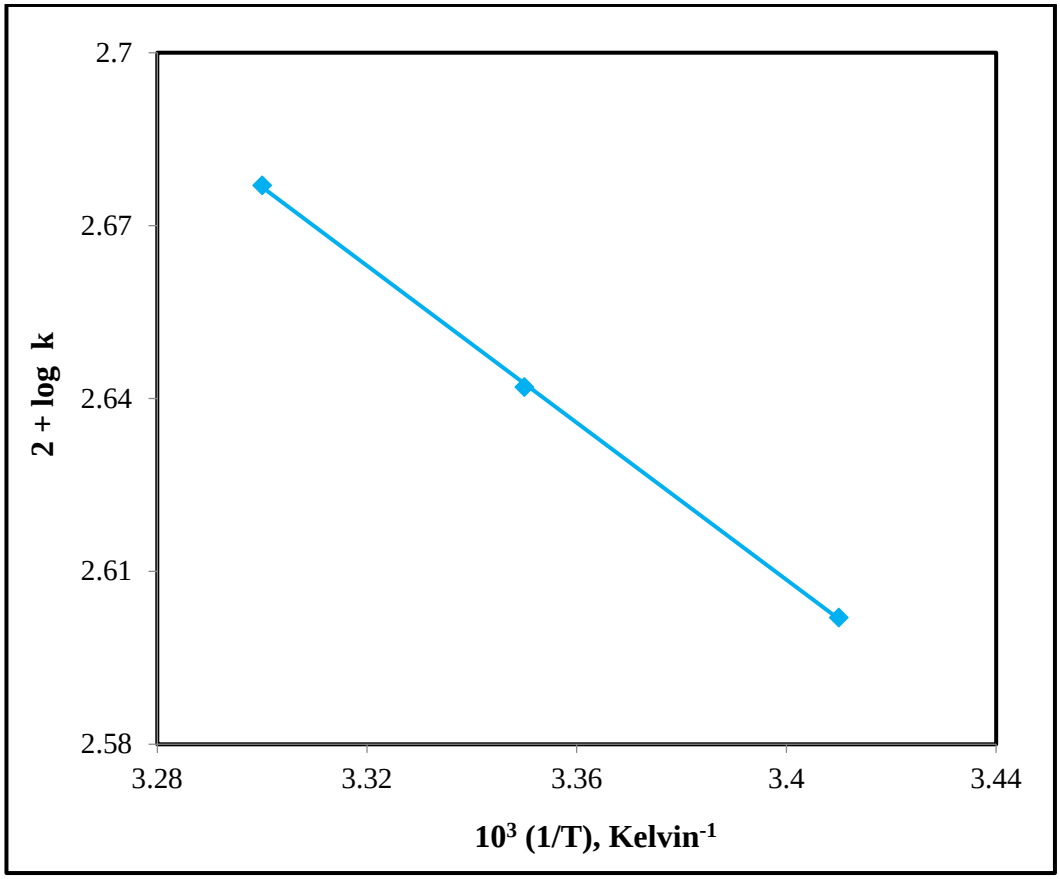


Figure 4.9: Plot of log k versus 1/T.

(Ref. Table: 4.13)

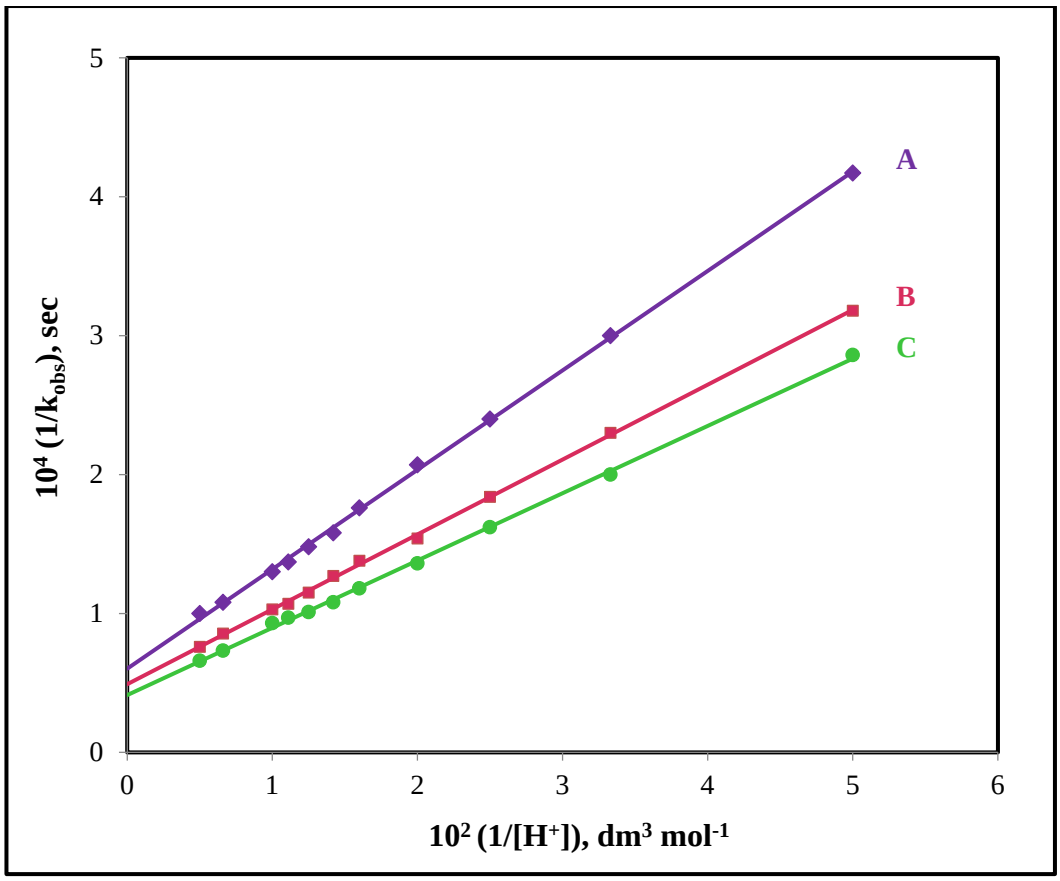


Figure 4.10: Plots of $1/k_{obs}$ versus $1/[H^+]$ at different temperature

(A) 20°C, (B) 25°C, (C) 30°C.

$[KMnO_4] = 2.0 \times 10^{-4}$ mol dm^{-3};

$[OFL] = 2.0 \times 10^{-3}$ mol dm^{-3};

$I = 2.0 \times 10^{-2}$ mol dm^{-3}.

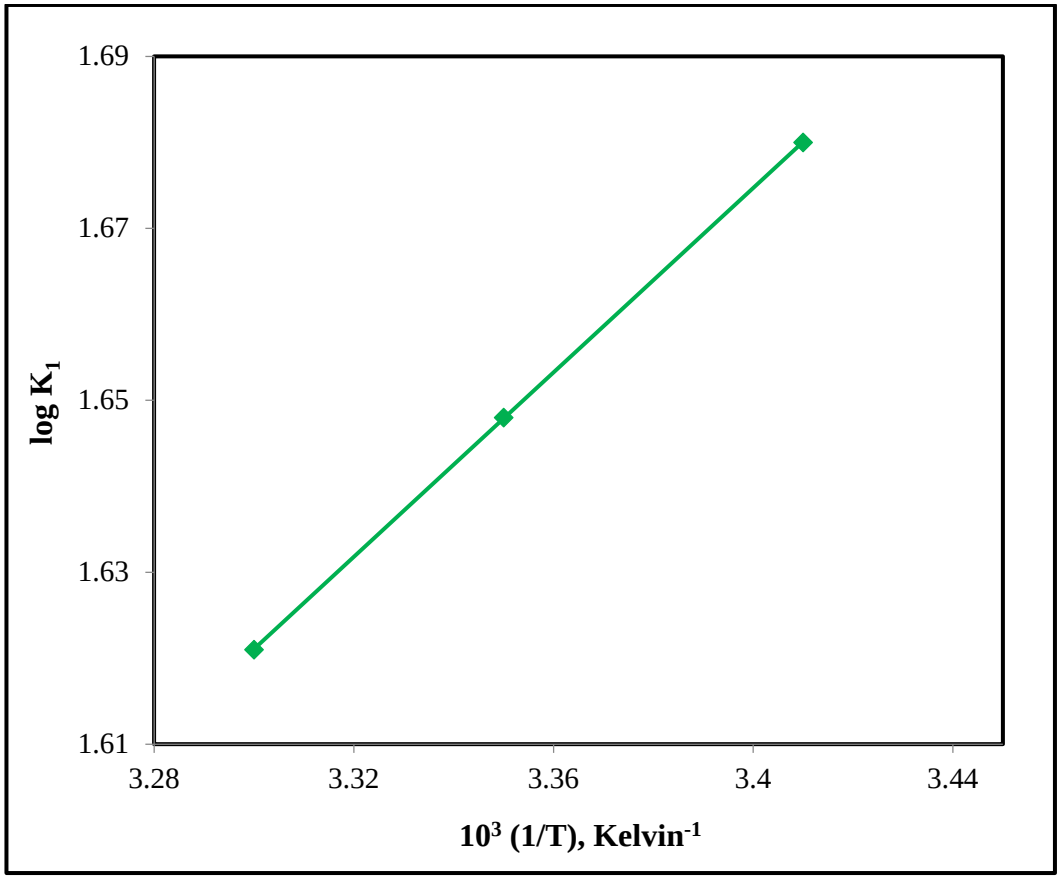


Figure 4.11: Plot of log K_1 versus 1/T.

(Ref. Table: 4.13)

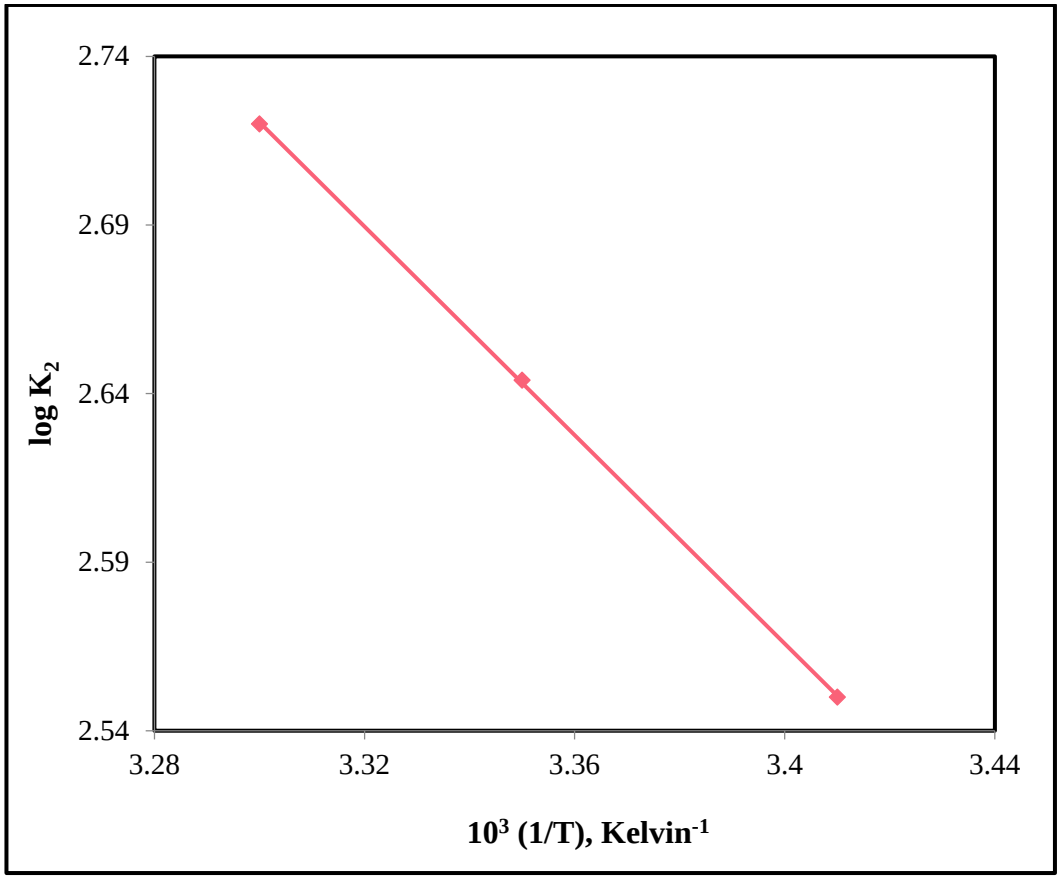


Figure 4.12: Plot of log K_2 versus 1/T.

(Ref. Table: 4.13)

4.5. CONCLUSION

The Kinetic study of oxidation of ofloxacin by permanganate in acidic medium has been studied. The oxidation products were identified by spot test, FT-IR, and LC-MS analysis. The results demonstrate that the role of H^+ in the reaction medium is crucial. The literature [36] reports that dealkylated products of ofloxacin have antimicrobial activity. Since dealkylated products are obtained in the present study, it is evident that the products of the title reaction have antimicrobial activity after oxidation. So this study will be effectively used in waste water treatment at the sites contaminated by fluoroquinolone antibiotics. Chemical oxidation using Mn(VII) has been widely used for treatment of pollutants in drinking water and waste water applications. The proposed mechanism is consistent with product, mechanism and kinetic studies.

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Chapter - 5

Mechanistic and Kinetic Study of Oxidation of Levofloxacin by Permanganate in Aqueous Sulphuric Acid Medium

ABSTRACT

The kinetic and mechanistic investigation of oxidation of levofloxacin (LF) has been studied by permanganate ion in aqueous sulphuric acid medium at 25°C. The reaction followed first-order kinetics with respect to [LF], and [H^+] in their lower concentrations range, tending to zero-order at their higher concentrations. The effect of added products and dielectric constant of the medium was studied on the rate of reaction. Effect of varying salt electrolyte concentration was insignificant showing that the molecular species was involved in the rate determining step. The products were identified by spot test, FT-IR, and LC-MS analysis. A mechanism was proposed on the basis of experimental results and rate law is derived.

$$k_{obs} = \frac{kK_1K_2[H^+][LF]}{1+ K_1[H^+] + K_1K_2[H^+][LF]}$$

The activation parameters with respect to the slow step of the mechanism were evaluated, and the thermodynamic parameters were also determined and discussed.

5.1. INTRODUCTION

Levofloxacin (LF), (−)-(S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H pyrido [1,2,3-de]-1,4-benzoxazine-6-carboxylic acid hemihydrate **(Figure 5.1)**, is one of the commonly used third-generation fluoroquinolone antimicrobials, being the active S-isomer isolated from racemic ofloxacin and is twice as active as the parent drug.

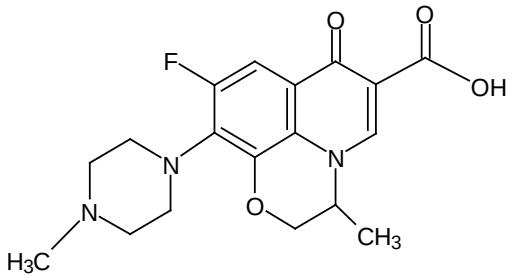


Figure 5.1: Structure of levofloxacin (LF).

Levofloxacin is a broad spectrum drug of activity against various bacteria, including gram-positive and gram-negative microorganisms [1, 2]. Because of its effective antibacterial activity and low frequency of adverse effects on oral administration, levofloxacin has been widely used for the treatment of infectious diseases, such as community-acquired pneumonia and acute exacerbation of chronic bronchitis [3]. The interaction of fluoroquinolone with metal ions is of interest not only for the development of analytical techniques but also to afford information about the mechanism of action of the pharmaceutical preparation [4]. Since the metal ions cause fluorescence quenching of the drug, the spectrofluorimetric method for quantitative determination of the quinolone type drugs has been developed [5] along with titrimetric [6], spectrophotometric [7, 8], electrochemical [9], electrophoretic [10] and chromatographic [11] techniques. The increase of fluoroquinolone in aquatic environments, even in low concentration, may cause intimidation to the ecosystem and human health by including the multiplication of drug resistance bacteria owing to long term exposure [12]. A number of kinetic study on oxidation of levofloxacin in aqueous, alkaline and acidic medium have been reported by different oxidants like $CeSO_4$, O_3, hydroxyl radical, Cl_2, chloramine-T, N-Bromosuccinimide, hexacyanoferrate(III) and MnO_2, in acidic/alkaline media [7, 13-21]. In view of

potential pharmaceutical importance of levofloxacin and complexity of the reaction, a detail study of the reaction becomes important.

Potassium permanganate widely used as oxidizing agent play key role in the kinetics of number of organic and biological active compounds [22-26]. Literature survey reveals that permanganate ions are widely used as oxidizing agent in synthetic, analytical chemistry [27, 28] and also as a disinfectant [29, 30]. It has been used in the determination of content of pharmaceutical formulation, [31, 32] as oxidizing agent for removal of organic molecules and heavy metals from the nuclear waste [33]. Oxidation reactions by Potassium permanganate are of considerable academic and technological importance because of variable oxidation states. Permanganate is one such powerful multi-electron oxidant which can exist in various oxidation states, among which +7 is its highest oxidation state, which occurs in the Oxo compounds like MnO_4^-, Mn_2O_7, MnO_3F. Out of which MnO_4^- is the most commonly used well known oxidant species to carry out kinetic studies in acidic, neutral and alkaline media. In acidic medium it exists in different forms as $HMnO_4$, $HMnO_4^+$, $HMnO_3$, Mn_2O_7 and one depending on the nature of the reductant [34].

So this study is concerned with the identity of the redox reaction and to discover a suitable mechanism for oxidation of levofloxacin by $KMnO_4$ in acidic medium on the basis of kinetics parameters.

5.2. EXPERIMENTAL

5.2.1. Chemicals and Reagents.

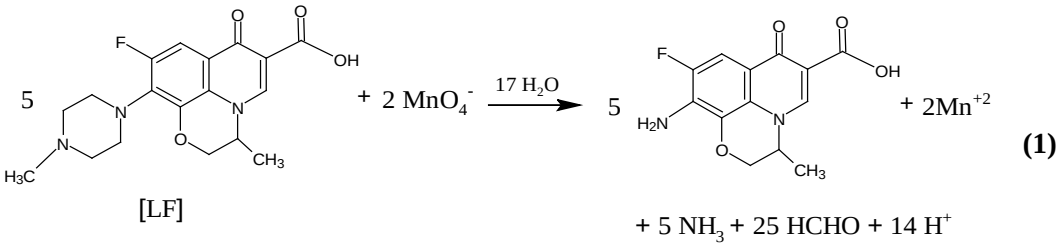
Preparation and standardization of the reagent solutions have already discussed in chapter 2 (Experimental section). All Chemicals and reagents used, levofloxacin, potassium permanganate, H_2SO_4 and Na_2SO_4, were of analytical grade and solutions were prepared using double distilled water, free from dissolved oxygen. Freshly prepared and standardized permanganate solutions were always used in kinetics experiments. Corning glass vessels were employed for kinetic study.

5.2.2. Kinetic Procedure.

Kinetic measurements were performed on a U.V. 3000^{+} UV-Visible spectrophotometer connected to a Peltier accessory (temperature-controlled). Kinetics was allowed at 25 ± 0.1°C and ionic strength 2.0×10^{-2} mol dm^{-3}. The oxidation reaction was initiated by adding previously thermostated reactant solution of $KMnO_4$ in levofloxacin which also contained required volume of sulphuric acid and sodium sulphate. The progress of the reaction was followed by measuring the absorbance of $KMnO_4$ in the reaction mixture at 525 nm in different interval of time by the UV-Visible spectrophotometer. The Beer's law was verified and the coefficient was found to be $\varepsilon = 2260 \pm 60$ dm^3 mol^{-1} cm^{-1} (Literature, $\varepsilon = 2389$ dm^3 mol^{-1} cm^{-1}) [34]. The kinetics was studied under pseudo-first-order conditions where [LF] >> [$KMnO_4$]. The rate of reaction followed first order kinetics and rate constant k_{obs} was calculated from the linear plots of log (absorbance) versus time.

5.2.3. Stoichiometry and product analysis.

The reaction mixtures containing an excess permanganate concentration over levofloxacin at concentrations of 0.01 mol dm^{-3} H_2SO_4 and adjusted to ionic strength of 0.02 mol dm^{-3} was allowed to react for 24 hours at 25 ± 1.0 °C. The remaining Mn(VII) was then analysed spectrophotometrically. Results showed that six moles of Mn(VII) were reduced for two moles of LF oxidized according to stoichiometric **equation (1).**



[LF] **(1)**

The oxidation product of levofloxacin was detected by TLC and characterized by LC-MS and FT-IR analysis. Reduction product of $KMnO_4$, Mn^{2+} was confirmed by spot test [35]. LC-ESI-MS analysis of levofloxacin reaction indicates that major product is of molecular ion **m/z 279 (Figure 5.2)**. This has an LF (m/z 361.4)

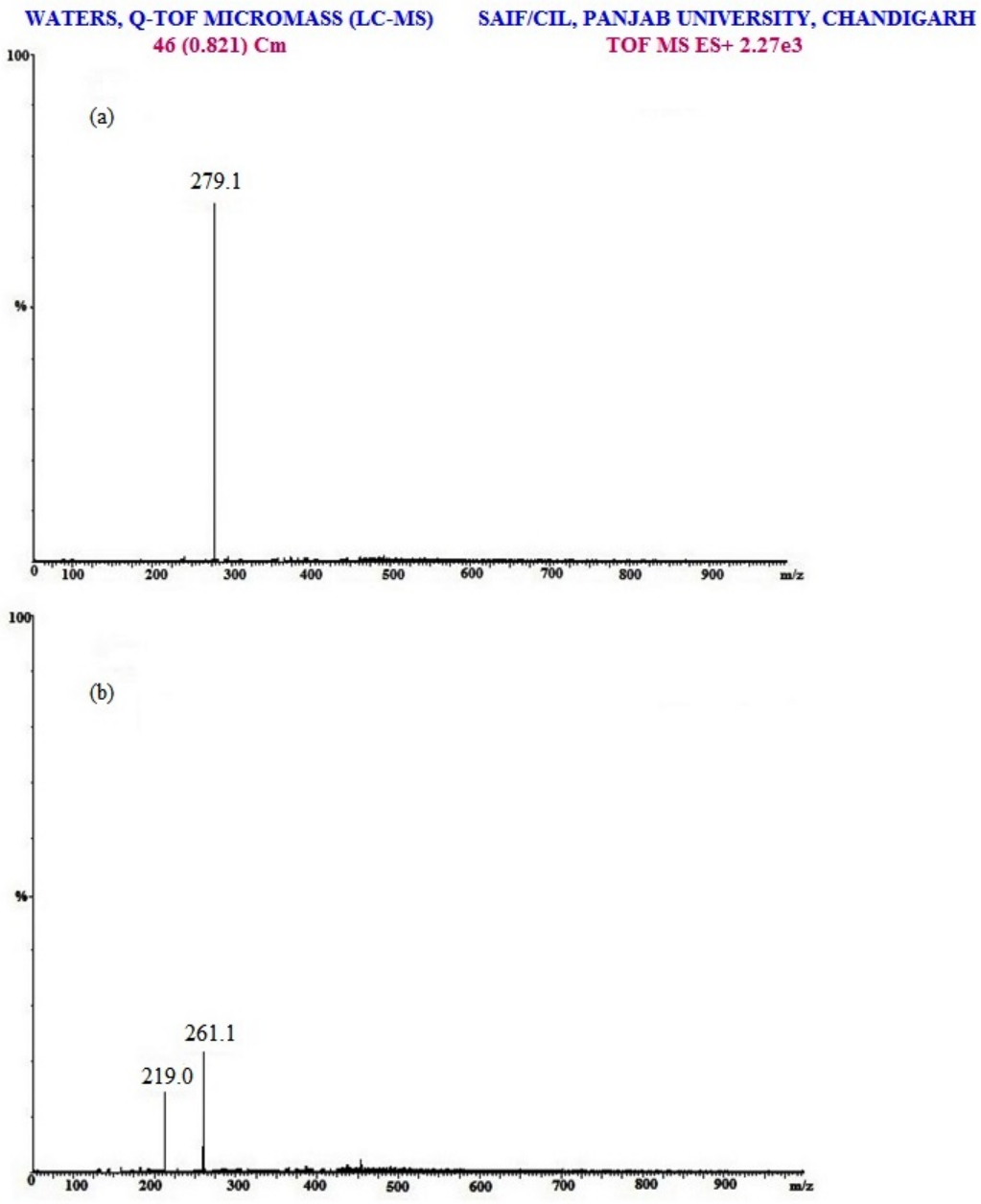


Figure 5.2: LC-ESI-MS spectra of oxidation product of levofloxacin.

(a) moiecular ion peak of m/z 279 (M-69).

(b) Fragmentation of (M-69) product.

structure, in which the piperazine group was displaced by an amino group through dealkylation and deamination process. The oxidation process was found to mainly affect 4-methyl piperazine, while the fluoroquinolone core, responsible for antibacterial activity, remained unchanged.

Analysis by Infrared Spectroscopy also confirmed the presence of $–NH_2$ group in the major oxidation product **(Figure 5.3)**. The IR spectrum shows a peak at **3412.70 cm^{-1}** which is due to − NH stretching of the $–NH_2$ group [36]. All other peaks observed in the IR spectrum can be interpreted in accordance with the structure of levofloxacin. Reaction mixture was tested quantitatively by 2, 4-DNP for presence of aldehyde. Test for aldehyde was positive and yields yellow precipitate of 2, 4 dinitrophenylhydrazone of aldehyde product [37]. The other product ammonia was detected by Nesseler's reagent test [38].

5.3. RESULTS

5.3.1. Reaction Orders.

Reaction orders for the oxidation of levofloxacin by potassium permanganate in acidic medium were determined from the slopes of log k_{obs} vs. log [concentration] plots by varying the concentration of LF, permanganate and acid in turn, while keeping other conditions constant.

5.3.2. Permanganate Dependence.

The concentration of permanganate was varied in the range $7.5 \times 10^{-5} – 6.0 \times 10^{-4}$ mol dm^{-3}, at three but fixed concentrations of [LF] to be 1.0×10^{-3}, 1.5×10^{-3} and 2.0×10^{-3} mol dm^{-3} respectively, keeping fixed concentrations of other reaction ingredients viz. $[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3}, $I = 2.0 \times 10^{-2}$ mol dm^{-3} at 25°C. All kinetic runs exhibited identical characteristics. The linearity of plots of log (absorbance) vs. time, for different concentrations of permanganate indicates order in manganese (VII) concentration as unity (**Figure 5.4**). This was also confirmed by the constant values of pseudo-first order rate constants, k_{obs} for different manganese (VII) concentrations. Results are given in **Tables 5.1, 5.2 and 5.3**.

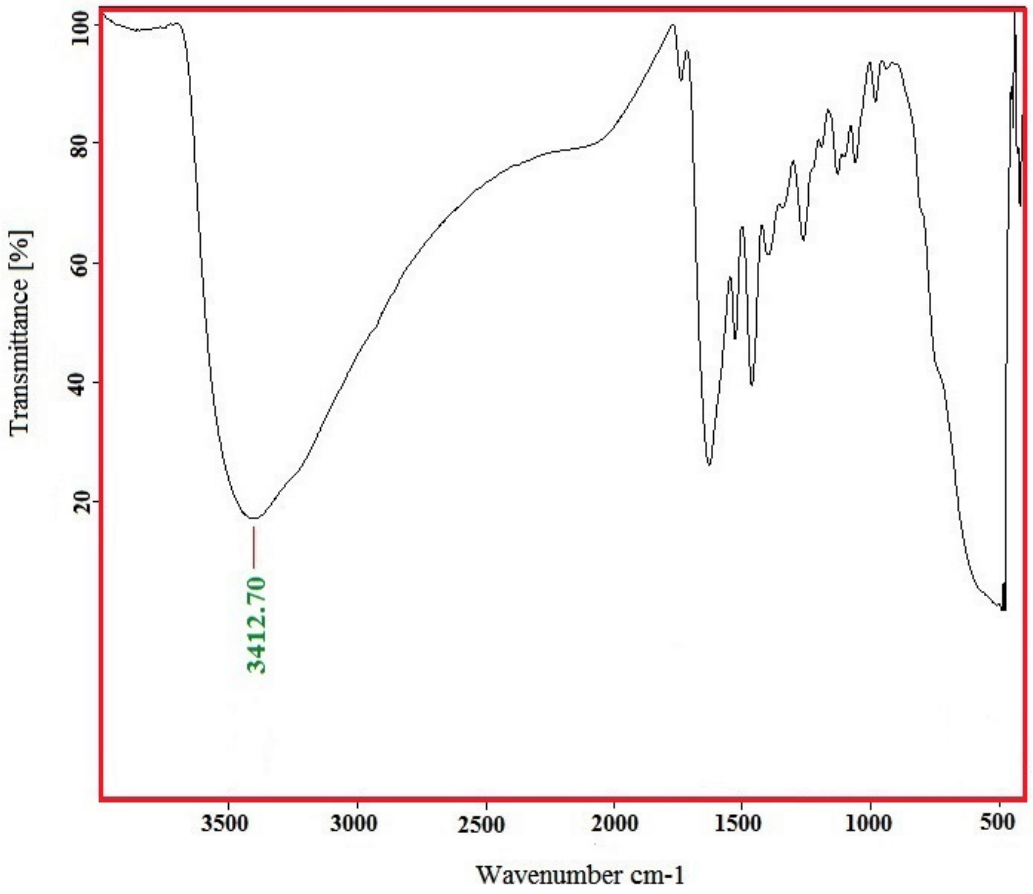


Figure 5.3: FTIR spectra of the oxidative product of levofloxacin by permanganate in acidic aqueous medium.

TABLE: 5.1

VARIATION OF $KMnO_4$

[LF] = 1.0 x 10^{-3} mol dm^{-3} **Temp. = 25°C**

[H^+] = 1.0 x 10^{-2} mol dm^{-3} **I = 2.0 x 10^{-2} mol dm^{-3}**

10^4 [$KMnO_4$], mol dm^{-3}	**0.75**	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**
Time in minutes				**Absorbance**			
0	0.187	0.243	0.483	0.721	0.973	1.202	1.445
0.5	0.138	0.182	0.363	0.550	0.741	0.933	1.122
1	0.115	0.138	0.289	0.437	0.550	0.723	0.871
1.5	0.087	0.112	0.224	0.331	0.457	0.550	0.692
2	0.069	0.089	0.178	0.264	0.355	0.447	0.538
2.5	0.055	0.070	0.135	0.204	0.282	0.355	0.427
3	0.042	0.053	0.105	0.159	0.209	0.252	0.317
3.5	0.034	0.044	0.082	0.126	0.174	0.204	0.251
10^3 (k_{obs}), sec^{-1}	**8.25**	**8.23**	**8.25**	**8.25**	**8.26**	**8.24**	**8.23**

TABLE: 5.2

VARIATION OF $KMnO_4$

[LF] = 1.5 x 10^{-3} mol dm^{-3} **Temp. = 25°C**

[H^+] = 1.0 x 10^{-2} mol dm^{-3} **I = 2.0 x 10^{-2} mol dm^{-3}**

10^4 [$KMnO_4$], mol dm^{-3}	**0.75**	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**
Time in minutes				**Absorbance**			
0	0.187	0.244	0.483	0.721	0.975	1.202	1.445
0.5	0.145	0.178	0.372	0.525	0.724	0.912	1.096
1	0.110	0.135	0.282	0.398	0.550	0.692	0.832
1.5	0.085	0.110	0.219	0.331	0.437	0.525	0.676
2	0.066	0.085	0.170	0.245	0.337	0.427	0.501
2.5	0.051	0.066	0.126	0.191	0.245	0.324	0.407
3	0.039	0.050	0.105	0.135	0.182	0.263	0.288
3.5	0.030	0.038	0.073	0.105	0.145	0.195	0.234
$10^3(k_{obs})$, sec^{-1}	**8.63**	**8.65**	**8.63**	**8.67**	**8.69**	**8.63**	**8.65**

TABLE: 5.3

VARIATION OF $KMnO_4$

[LF] = 2.0 x 10^{-3} mol dm^{-3} **Temp. = 25°C**

[H^+] = 1.0 x 10^{-2} mol dm^{-3} **I = 2.0 x 10^{-2} mol dm^{-3}**

10^4 [$KMnO_4$], mol dm^{-3}	**0.75**	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**
Time in minutes				**Absorbance**			
0	0.183	0.245	0.484	0.723	0.974	1.204	1.473
0.5	0.145	0.182	0.347	0.525	0.692	0.912	1.047
1	0.112	0.135	0.295	0.407	0.537	0.661	0.794
1.5	0.086	0.107	0.209	0.316	0.417	0.513	0.646
2	0.063	0.081	0.161	0.240	0.316	0.405	0.486
2.5	0.048	0.064	0.123	0.182	0.240	0.302	0.363
3	0.038	0.046	0.093	0.135	0.182	0.234	0.282
3.5	0.029	0.035	0.068	0.105	0.138	0.166	0.214
10^3 (k_{obs}), sec^{-1}	**9.02**	**9.05**	**9.06**	**9.02**	**9.05**	**9.07**	**9.06**

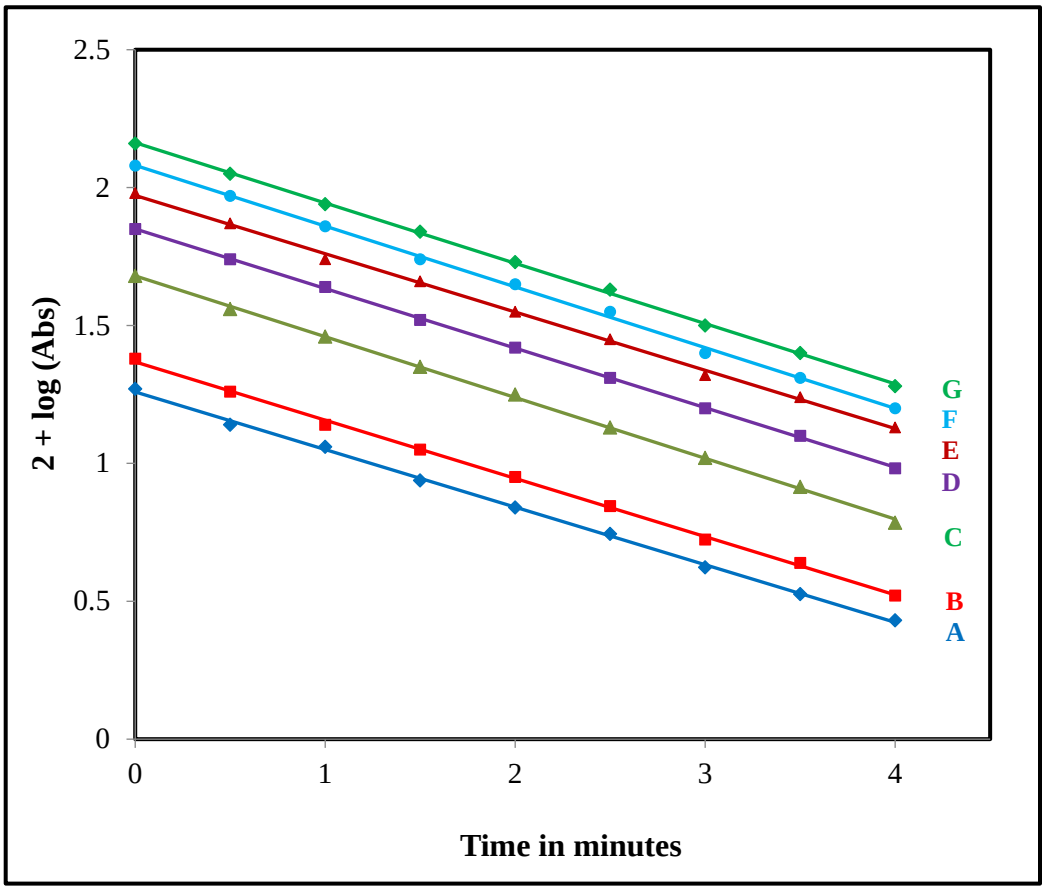


Figure 5.4: First order plots of the variation of permanganate concentration.

[LF] = 1.0×10^{-3} mol dm^{-3};

I = 2.0×10^{-2} mol dm^{-3};

[Mn(VII)] = (A) 0.75×10^{-4} mol dm^{-3}

(B) 1.0×10^{-4} mol dm^{-3}

(C) 2.0×10^{-4} mol dm^{-3}

(D) 3.0×10^{-4} mol dm^{-3}

(E) 4.0×10^{-4} mol dm^{-3}

(F) 5.0×10^{-4} mol dm^{-3}

(G) 6.0×10^{-4} mol dm^{-3}.

$[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3};

Temp. = 25°C;

(Ref. Table 5.1)

5.3.3. Levofloxacin Dependence.

Reactions were carried out by varying concentration of levofloxacin from 2.0 × 10^{-3} – 7.0 × 10^{-3} mol dm^{-3} at constant concentration of [$KMnO_4$] = 2.0 × 10^{-4} mol dm^{-3}, [H^+] = 1.0 × 10^{-2} mol dm^{-3} and I = 2.0 × 10^{-2} mol dm^{-3} at three temperature viz. 20°C, 25°C and 30°C respectively. It was observed that as the concentration of levofloxacin increased, rate of the reaction also increased **(Figure 5.5)**. The plot of log k_{obs} versus log [LF] was made that yielded straight line with a slope of 0.64, thus indicating a less than unit order dependence with respect to concentration of levofloxacin. Results are given in **Tables 5.4, 5.5 and 5.6**.

5.3.4. Hydrogen ion dependence.

The effect of hydrogen ion concentration was studied in the range of 5.0 × 10^{-3} – 2.0 × 10^{-2} mol dm^{-3} at fixed concentrations of [$KMnO_4$] = 2.0 × 10^{-4} mol dm^{-3}, [LF] = 2.0 × 10^{-3} mol dm^{-3} and I = 2.0 × 10^{-2} mol dm^{-3} at three temperature viz. 20°C, 25°C and 30°C respectively. With increase in concentration of acid, prominent increase in reaction rate was observed **(Figure 5.6)**. The order with respect to [H^+] was obtained from the plot of log k_{obs} versus log [H^+] and was found to be less than unit order (0.60). Results are given in **Tables 5.7, 5.8 and 5.9**.

5.3.5. Effect of Ionic Strength and Dielectric Constant.

Effect of change in varying electrolyte concentration was monitored to establish the nature of intermediate species in the rate determining step by varying concentration of Na_2SO_4 at fixed concentration of [$KMnO_4$] = 2.0 × 10^{-4} mol dm^{-3}, [LF] = 2.0 × 10^{-3} mol dm^{-3} and [H^+] = 1.0 × 10^{-2} mol dm^{-3} at 25 °C. It was observed that the change in an ionic strength of the medium does not alter the rate constants. Results are given in **Table 5.10**.

The dielectric constant (*D*) of the medium was studied by varying the acetic acid – water percentage (v/v) in the range of 5 – 25% at constant concentration of [$KMnO_4$] = 2.0 × 10^{-4} mol dm^{-3}, [LF] = 2.0 × 10^{-3} mol dm^{-3}, [H^+] = 1.0 × 10^{-2} mol dm^{-3} and I = 2.0 × 10^{-2} mol dm^{-3} at 25°C. It was found that increase in the dielectric constant has negligible effect on reaction rate. Results are given in **Table 5.11**.

TABLE: 5.4

VARIATION OF LEVOFLOXACIN

$[KMnO_4]$ = 2.0 x 10^{-4} mol dm^{-3} **Temp. = 20°C**

$[H^+]$ = 1.0 x 10^{-2} mol dm^{-3} **I = 2.0 x 10^{-2} mol dm^{-3}**

10^3 [LF], mol dm^{-3}	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**	**7.0**
Time in minutes			**Absorbance**			
0	0.483	0.482	0.485	0.487	0.488	0.483
0.5	0.372	0.324	0.309	0.302	0.275	0.269
1	0.309	0.240	0.209	0.182	0.166	0.155
1.5	0.234	0.166	0.138	0.112	0.097	0.089
2	0.196	0.119	0.086	0.067	0.057	0.052
2.5	0.148	0.086	0.057	0.041	0.035	0.031
3	0.115	0.060	0.035	0.026	0.020	0.016
3.5	0.092	0.042	0.024	0.016	0.012	0.010
10^3 (k_{obs}), sec^{-1}	**7.45**	**11.63**	**14.26**	**16.37**	**17.67**	**18.54**

TABLE: 5.5

VARIATION OF LEVOFLOXACIN

$[KMnO_4]$ = 2.0 x 10^{-4} mol dm^{-3} **Temp. = 25°C**

$[H^+]$ = 1.0 x 10^{-2} mol dm^{-3} **I = 2.0 x 10^{-2} mol dm^{-3}**

10^3 [LF], mol dm^{-3}	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**	**7.0**
Time in minutes			**Absorbance**			
0	0.482	0.484	0.484	0.483	0.489	0.486
0.5	0.347	0.316	0.302	0.282	0.257	0.251
1	0.282	0.219	0.195	0.166	0.151	0.145
1.5	0.209	0.145	0.151	0.102	0.088	0.079
2	0.161	0.098	0.074	0.059	0.048	0.041
2.5	0.123	0.067	0.049	0.035	0.025	0.024
3	0.093	0.044	0.031	0.022	0.015	0.012
10^3 (k_{obs}), sec^{-1}	**9.06**	**13.22**	**15.53**	**17.38**	**9.23**	**20.40**

TABLE: 5.6

VARIATION OF LEVOFLOXACIN

$[KMnO_4]$ = 2.0 x 10^{-4} mol dm^{-3} **Temp. = 30°C**

$[H^+]$ = 1.0 x 10^{-2} mol dm^{-3} **I = 2.0 x 10^{-2} mol dm^{-3}**

10^3 [LF], mol dm^{-3}	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**	**7.0**
Time in minutes			**Absorbance**			
0	0.485	0.482	0.483	0.483	0.484	0.486
0.5	0.347	0.309	0.275	0.257	0.251	0.245
1	0.269	0.204	0.174	0.145	0.138	0.132
1.5	0.191	0.132	0.107	0.084	0.074	0.067
2	0.144	0.086	0.063	0.048	0.039	0.034
2.5	0.107	0.053	0.037	0.027	0.021	0.019
3	0.078	0.038	0.023	0.015	0.012	0.010
10^3 (k_{obs}), sec^{-1}	**10.02**	**14.28**	**16.96**	**19.23**	**20.83**	**22.06**

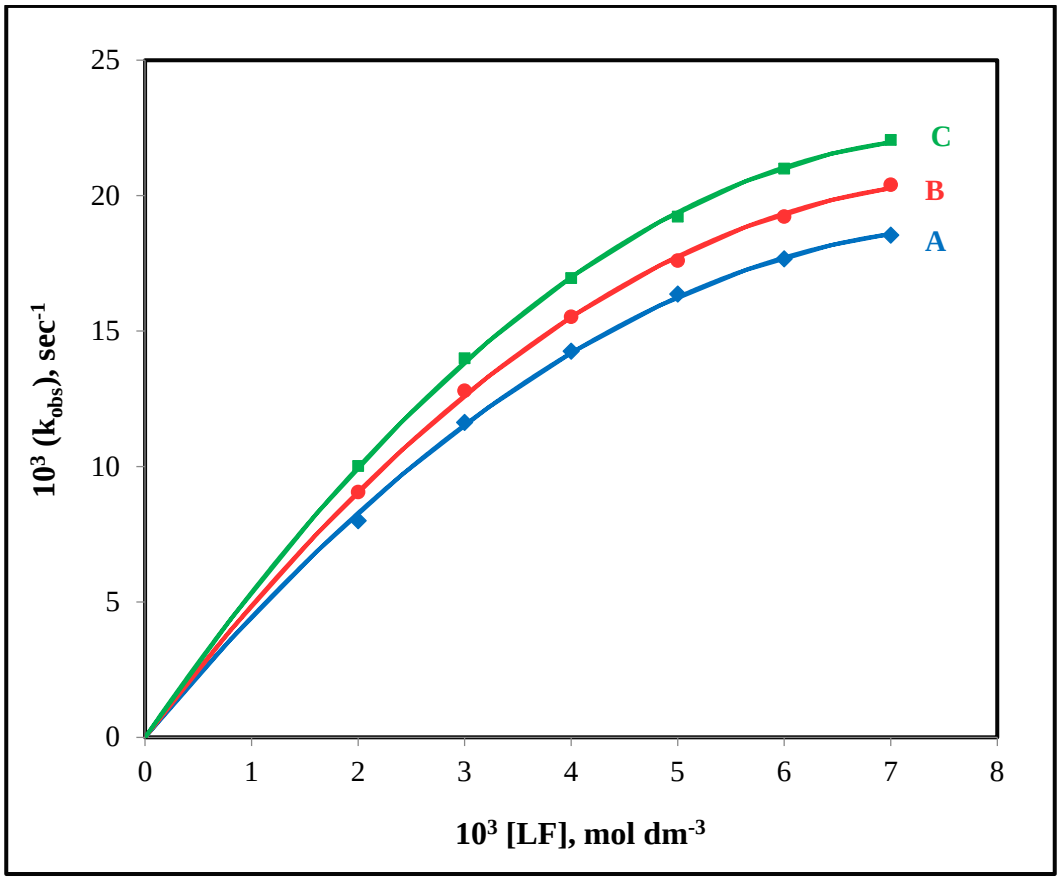


Figure 5.5: Variation of Levofloxacin at different temperature

(A) 20°C, (B) 25°C, (C) 30°C.

$[KMnO_4] = 2.0 \times 10^{-4}$ mol dm^{-3};

$[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3};

$I = 2.0 \times 10^{-2}$ mol dm^{-3}.

(Ref. Table: 5.4, 5.5, 5.6)

TABLE: 5.7

VARIATION OF HYDROGEN ION

$[KMnO_4] = 2.0 \times 10^{-4}$ mol dm^{-3} **Temp. = 20°C**

[LF] = 2.0×10^{-3} mol dm^{-3} **I = 2.0×10^{-2} mol dm^{-3}**

10^2 $[H^+]$, mol dm^{-3}	0.5	0.6	0.7	0.8	0.9	1.0	1.5	2.0
Time in minutes				**Absorbance**				
0	0.483	0.483	0.482	0.486	0.485	0.483	0.484	0.488
1	0.398	0.380	0.347	0.331	0.316	0.310	0.302	0.282
2	0.290	0.273	0.248	0.230	0.216	0.196	0.165	0.154
3	0.229	0.195	0.174	0.158	0.145	0.117	0.105	0.088
4	0.178	0.145	0.115	0.105	0.099	0.074	0.061	0.051
5	0.135	0.107	0.084	0.073	0.067	0.045	0.034	0.028
6	0.105	0.082	0.060	0.050	0.044	0.032	0.021	0.017
10^3 (k_{obs}), sec^{-1}	**4.16**	**4.68**	**5.48**	**6.13**	**6.63**	**7.45**	**8.86**	**9.46**

TABLE: 5.8

VARIATION OF HYDROGEN ION

$[KMnO_4]$ = 2.0 x 10^{-4} mol dm^{-3} **Temp. = 25 °C**

[LF] = 2.0 x 10^{-3} mol dm^{-3} **I = 2.0 x 10^{-2} mol dm^{-3}**

10^2 $[H^+]$, mol dm^{-3}	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**	**1.5**	**2.0**
Time in minutes				**Absorbance**				
0	0.485	0.487	0.484	0.483	0.484	0.483	0.483	0.484
1	0.389	0.363	0.347	0.316	0.302	0.297	0.275	0.257
2	0.272	0.249	0.226	0.195	0.176	0.163	0.146	0.134
3	0.204	0.162	0.155	0.123	0.110	0.091	0.088	0.070
4	0.158	0.115	0.107	0.079	0.067	0.053	0.049	0.039
5	0.115	0.080	0.073	0.048	0.041	0.033	0.026	0.021
6	0.088	0.056	0.050	0.032	0.026	0.018	0.016	0.012
10^3 (k_{obs}), sec^{-1}	**4.71**	**5.46**	**6.24**	**7.49**	**8.33**	**9.06**	**9.89**	**10.62**

TABLE: 5.9

VARIATION OF HYDROGEN ION

$[KMnO_4]$ = 2.0 x 10^{-4} mol dm^{-3} **Temp. = 30°C**

[LF] = 2.0 x 10^{-3} mol dm^{-3} **I = 2.0 x 10^{-2} mol dm^{-3}**

10^2 $[H^+]$, mol dm^{-3}	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**	**1.5**	**2.0**
Time in minutes				**Absorbance**				
0	0.482	0.487	0.484	0.484	0.483	0.485	0.483	0.484
1	0.355	0.339	0.324	0.316	0.282	0.269	0.263	0.251
2	0.248	0.218	0.195	0.179	0.160	0.144	0.117	0.111
3	0.174	0.155	0.132	0.112	0.098	0.078	0.064	0.059
4	0.123	0.105	0.086	0.070	0.053	0.040	0.032	0.029
5	0.090	0.070	0.053	0.042	0.032	0.022	0.016	0.014
6	0.061	0.046	0.035	0.026	0.018	0.013	0.010	0.006
10^3 (k_{obs}), sec^{-1}	**5.49**	**6.57**	**7.46**	**8.18**	**9.15**	**10.02**	**11.76**	**12.18**

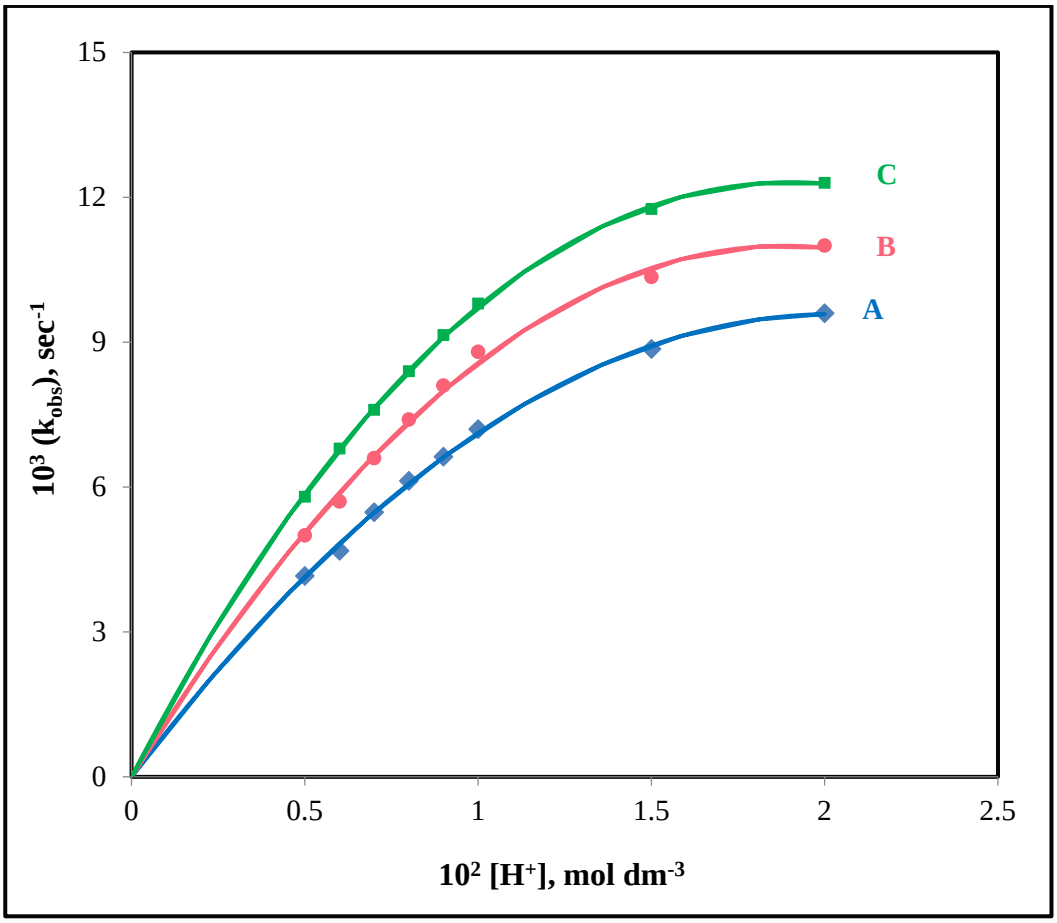


Figure 5.6: Variation of Hydrogen Ion at different temperature

(A) 20°C, (B) 25°C, (C) 30°C.

$[KMnO_4] = 2.0 \times 10^{-4}$ mol dm^{-3};

$[LF] = 2.0 \times 10^{-3}$ mol dm^{-3};

$I = 2.0 \times 10^{-2}$ mol dm^{-3}.

(Ref. Table: 5.7, 5.8, 5.9)

TABLE: 5.10

VARIATION OF SODIUM SULPHATE

$[KMnO_4]$ = 2.0 x 10^{-4} mol dm^{-3} **Temp. = 25°C**

[LF] = 2.0 x 10^{-3} mol dm^{-3} **$[H^+]$ = 1.0 x 10^{-2} mol dm^{-3}**

10^2 [Na2SO4], mol dm^{-3}	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**	**7.0**	**8.0**	**9.0**	**10.0**
Time in minutes					**Absorbance**					
0	0.481	0.482	0.480	0.482	0.483	0.482	0.482	0.481	0.483	0.482
0.5	0.348	0.340	0.344	0.342	0.347	0.346	0.348	0.340	0.345	0.346
1	0.284	0.277	0.278	0.276	0.284	0.282	0.283	0.273	0.282	0.280
1.5	0.216	0.209	0.208	0.206	0.208	0.214	0.215	0.203	0.206	0.211
2	0.162	0.160	0.159	0.158	0.160	0.163	0.163	0.157	0.159	0.161
2.5	0.124	0.127	0.118	0.116	0.126	0.122	0.122	0.112	0.123	0.120
3	0.101	0.091	0.094	0.093	0.094	0.098	0.100	0.089	0.093	0.096
10^3 (k_{obs}), sec^{-1}	**9.03**	**9.10**	**9.09**	**9.11**	**9.06**	**9.05**	**9.03**	**9.13**	**9.06**	**9.06**

TABLE: 5.11

EFFECT OF DIELECTRIC CONSTANT

$[KMnO_4] = 2.0 \times 10^{-4}$ mol dm^{-3} **Temp. = 25°C**

[LF] = 2.0×10^{-3} mol dm^{-3} **I = 2.0×10^{-2} mol dm^{-3}**

$[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3}

[Acetic acid], %	**5**	**10**	**15**	**20**
Time in minutes		**Absorbance**		
0	0.482	0.483	0.484	0.481
1	0.295	0.298	0.301	0.296
2	0.159	0.161	0.162	0.160
3	0.087	0.090	0.092	0.088
4	0.048	0.052	0.054	0.049
5	0.032	0.036	0.037	0.034
6	0.016	0.018	0.021	0.017
10^3 (k_{obs}), sec^{-1}	**9.09**	**9.06**	**9.04**	**9.08**

5.3.6. Effect of Added Neutral Salts.

The effect of added neutral salts on the rate of reaction was studied by varying concentration of $NaNO_3$, CH_3COONa and NaF from 1.0×10^{-2} – 4.0×10^{-2} mol dm^{-3} at fixed concentration of $[KMnO_4] = 2.0 \times 10^{-4}$ mol dm^{-3}, $[LF] = 2.0 \times 10^{-3}$ mol dm^{-3}, $[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3} and $I = 2.0 \times 10^{-2}$ mol dm^{-3} at 25°C. Addition of different sodium salts has no effect on the reaction rates. Results are given in **Tables 5.12, 5.13 and 5.14**.

5.3.7. Effect of Initially Added Product.

The effect of Mn^{2+} on the rate of the reaction was studied by employing it from 5.0×10^{-5} – 5.0×10^{-4} mol dm^{-3} in the reaction mixture at constant concentration of $[KMnO_4] = 2.0 \times 10^{-4}$ mol dm^{-3}, $[LF] = 2.0 \times 10^{-3}$ mol dm^{-3}, $[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3} and $I = 2.0 \times 10^{-2}$ mol dm^{-3} at 25°C. No significant effect on the rate of reaction was observed. Results are given in **Table 5.15.**

5.3.8. Test for Free Radicals.

The possibility of free radicals in the reaction was examined as follows: a known quantity of acrylonitrile (scavenger) had been added initially in the reaction mixture, and then kept in an inert atmosphere for 5 hours. Upon diluting the reaction mixture with methanol, white precipitate was found, suggesting the involvement of free radicals in the reaction.

5.4. DISCUSSION

Permanganate ion, MnO_4^- ion is powerful oxidizing agent in acidic medium. The stable oxidation product of MnO_4^- in acid medium is Mn(II). **Figure 5.7** illustrates the spectroscopic changes occurring in the oxidation of levofloxacin by acid permanganate at 25°C with scanning interval of 1 minute.

The reaction between levofloxacin and permanganate in sulphuric acid has Stoichiometry 5:2, having first order dependence with permanganate and less than unit order with H^+ concentration and levofloxacin concentration. The fact that Mn(II) is the reduced product of Mn(VII) in the reaction might indicate that levofloxacin shows a strong reducing character in H_2SO_4 medium. In view of the presence of

TABLE: 5.12

EFFECT OF SODIUM NITRATE

$[KMnO_4] = 2.0 \times 10^{-4}$ mol dm^{-3} **Temp. = 25°C**

[LF] = 2.0×10^{-3} mol dm^{-3} **I = 2.0×10^{-2} mol dm^{-3}**

$[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3}

10^2 [$NaNO_3$], mol dm^{-3}	**1**	**2**	**3**	**4**
Time in minutes		**Absorbance**		
0	0.481	0.482	0.482	0.480
0.5	0.346	0.340	0.347	0.344
1	0.280	0.277	0.282	0.278
1.5	0.206	0.209	0.209	0.208
2	0.160	0.160	0.161	0.159
2.5	0.120	0.127	0.123	0.118
3	0.092	0.089	0.093	0.094
10^3 (k_{obs}), sec^{-1}	**9.08**	**9.10**	**9.06**	**9.09**

TABLE: 5.13

EFFECT OF SODIUM ACETATE

$[KMnO_4] = 2.0 \times 10^{-4}$ mol dm^{-3} **Temp. = 25°C**

[LF] = 2.0×10^{-3} mol dm^{-3} **I = 2.0×10^{-2} mol dm^{-3}**

[H^+] = 1.0×10^{-2} mol dm^{-3}

10^2 [CH_3COONa], mol dm^{-3}	**1**	**2**	**3**	**4**
Time in minutes		**Absorbance**		
0	0.487	0.484	0.486	0.480
0.5	0.350	0.347	0.348	0.346
1	0.298	0.295	0.297	0.282
1.5	0.216	0.209	0.215	0.214
2	0.162	0.161	0.163	0.163
2.5	0.126	0.123	0.125	0.122
3	0.102	0.093	0.100	0.098
10^3 (k_{obs}), sec^{-1}	**9.02**	**9.06**	**9.03**	**9.05**

TABLE: 5.14

EFFECT OF SODIUM FLUORIDE

$[KMnO_4] = 2.0 \times 10^{-4}$ mol dm^{-3} **Temp. = 25 °C**

[LF] = 2.0×10^{-3} mol dm^{-3} **I = 2.0×10^{-2} mol dm^{-3}**

$[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3}

10^2 [NaF], mol dm^{-3}	**1**	**2**	**3**	**4**
Time in minutes		**Absorbance**		
0	0.482	0.484	0.483	0.481
0.5	0.347	0.349	0.345	0.346
1	0.282	0.310	0.280	0.280
1.5	0.209	0.211	0.208	0.206
2	0.161	0.162	0.160	0.160
2.5	0.123	0.124	0.122	0.120
3	0.093	0.096	0.094	0.092
10^3 (k_{obs}), sec^{-1}	**9.06**	**9.04**	**9.06**	**9.08**

TABLE: 5.15

EFFECT OF Mn(II) ION

$[KMnO_4] = 2.0 \times 10^{-4}$ mol dm^{-3} **Temp. = 25°C**

[LF] = 2.0×10^{-3} mol dm^{-3} **I = 2.0×10^{-2} mol dm^{-3}**

$[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3}

10^4 $[Mn]^{2+}$	**0.5**	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**
Time in minutes			**Absorbance**			
0	0.480	0.482	0.482	0.486	0.481	0.482
0.5	0.344	0.346	0.347	0.348	0.340	0.346
1	0.278	0.280	0.282	0.286	0.277	0.282
1.5	0.206	0.208	0.209	0.213	0.209	0.207
2	0.159	0.160	0.161	0.163	0.160	0.161
2.5	0.118	0.120	0.123	0.125	0.121	0.120
3	0.090	0.092	0.093	0.098	0.091	0.094
10^3 (k_{obs}), sec^{-1}	**9.09**	**9.07**	**9.06**	**9.03**	**9.10**	**9.06**

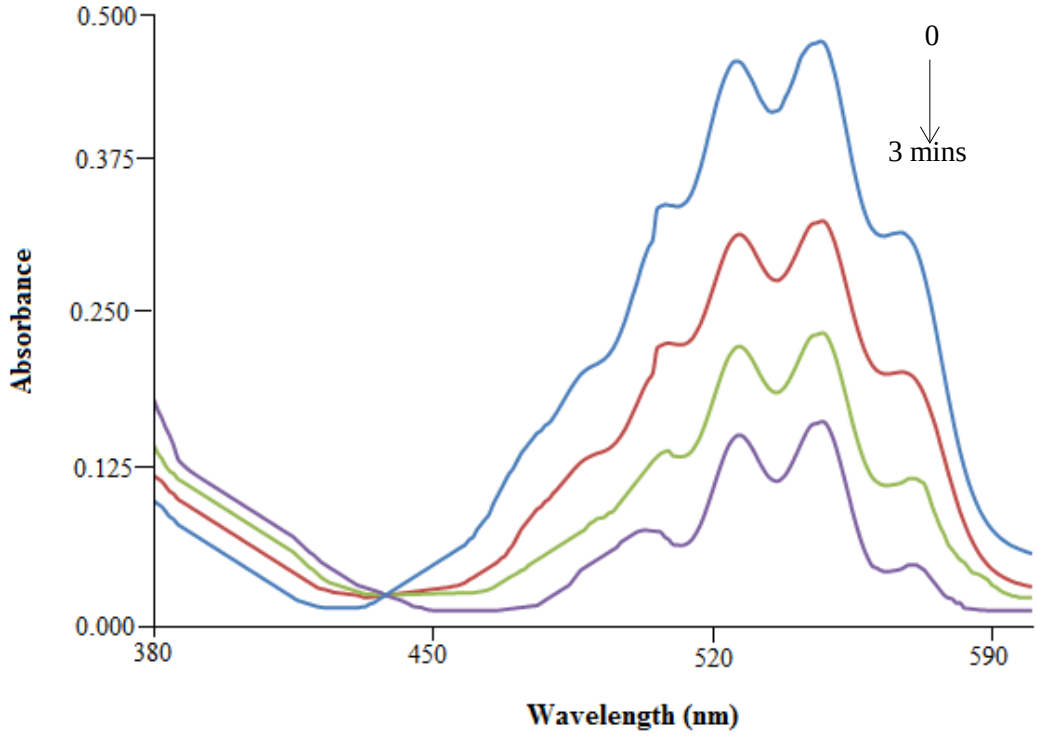


Figure 5.7: Spectral changes during the oxidation of levofloxacin (LF) by permanganate in acidic medium at 25°C.

$[KMnO_4] = 2.0 \times 10^{-4}$ mol dm^{-3}; $[LF] = 2.0 \times 10^{-3}$ mol dm^{-3};

$[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3}; $I = 2.0 \times 10^{-2}$ mol dm^{-3}.

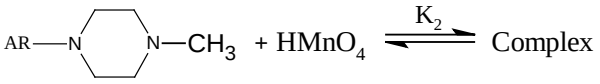
sulphuric acid in the reaction mixture, the oxidation of LF by sulphuric acid was checked, and it was found to be negligible compared to the oxidation of LF by permanganate. The active species of permanganate in aqueous acid solution may be deduced from the dependence of the rate on [H^+], in the reaction medium. The order of [H^+] is less than unity, which may be indicate the formation of permanganate acid from permanganate ion. Permanganic acid $HMnO_4$ is more efficient oxidant species of Manganese (VII) then permanganate ion [39]. The negligible effect of ionic strength on the rate of reaction also confirms that $HMnO_4$ is the active species of MnO_4^-, and can be represented by **equation (2).**

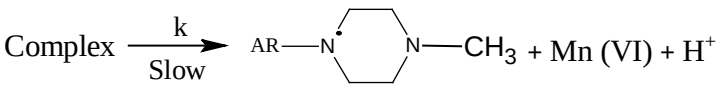
$$MnO_4^- + H^+ \underset{}{\overset{K_1}{\rightleftharpoons}} HMnO_4 \qquad \textbf{(2)}$$

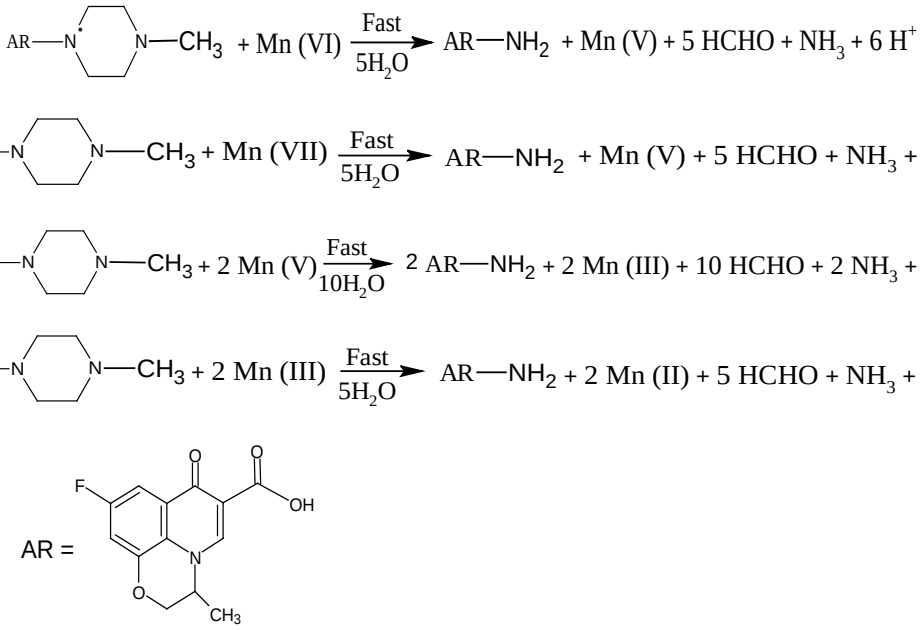
Here K_1 is the equilibrium constant of $HMnO_4$.

Based on the experimental results, a mechanism is proposed for which all the observed orders in each constituent such as [oxidant], [reductant] and [H^+] may be well accommodated. In view of increasing the rate with increase in [H^+] ion, in the prior equilibrium step, H^+ reacts with MnO_4^- to form $HMnO_4$, which reacts with the one mole of levofloxacin to form a complex. Complex formed is dissociate in the rate determining step to give a free radical derived from levofloxacin and an intermediate Mn(VI). In further fast steps the intermediate Mn(VI) reacts with a free radical to produce the product 7-amino fluoroquinolone, NH_3, HCHO and intermediate Mn(V), subsequently reduced to the end product Mn(II). The low concentration of intermediates obtained under experimental conditions made the spectroscopic detection failure. However, the evidence for intermediates such as Mn(V) and Mn(III) are reported in the literature [40, 41]. A reasonable mechanism is proposed in **Scheme-1.**

$$MnO_4^- + H^+ \underset{}{\overset{K_1}{\rightleftharpoons}} HMnO_4$$







Scheme-1

From the scheme-1, the following rate law can be derived as follows:

$$\text{Rate} = \frac{-d[MnO_4^-]}{dt} = k\,[\text{Complex}]$$

$$= kK_2[HMnO_4][LF]$$

$$= kK_1K_2[MnO_4^-]_f[H^+]_f[LF]_f \qquad \textbf{(3)}$$

Total concentration of permanganate is given by:

$$[MnO_4^-]_t = [MnO_4^-]_f + [HMnO_4] + [\text{Complex}]$$

$$= [MnO_4^-]_f + K_1[MnO_4^-]_f[H^+]_f + K_2[HMnO_4][LF]$$

$$= [MnO_4^-]_f + K_1[MnO_4^-]_f[H^+]_f + K_1K_2[MnO_4^-]_f[H^+]_f[LF]$$

$$= [MnO_4^-]_f\{1 + K_1[H^+]_f + K_1K_2[H^+]_f[LF]\}$$

$$[MnO_4^-]_f = \frac{[MnO_4^-]_t}{\{1 + K_1[H^+]_f + K_1K_2[H^+]_f[LF]} \qquad \textbf{(4)}$$

$[MnO_4^-]_t$ and $[MnO_4^-]_f$ are total and free concentration of Mn (VII) respectively.

Total concentration of levofloxacin is given by:

$$[LF]_t = [LF]_f + [\text{Complex}]$$

$$= [LF]_f + K_2[LF]_f[HMnO_4]$$

$$= [LF]_f \{1+ K_2[HMnO_4]\}$$

$$[LF]_f = \frac{[LF]_t}{1+ K_2[HMnO_4]}$$

Very low concentration of $[MnO_4^-]$ were used in the experiment, so $K_2 [HMnO_4] << 1$

$$[LF]_f = [LF]_t$$

Total concentration of $[H^+]$ is given by: **(5)**

$$[H^+]_t = [H^+]_f + [HMnO_4]$$

$$= [H^+]_f + K_1[MnO_4^-]_f[H^+]_f$$

$$= [H^+]_f \{1+ K_1[MnO_4^-]_f\}$$

So, $[H^+]_t = [H^+]_f$ **(6)**

Substituting equation (4), (5) and (6) in equation (3) and omitting "t" and "f" subscripts,

$$\text{Rate} = \frac{-d[MnO_4^-]}{dt}$$

$$= \frac{kK_1K_2[MnO_4^-][H^+][LF]}{1+ K_1[H^+] + K_1K_2[H^+][LF]} \quad \textbf{(7)}$$

$$\frac{\text{Rate}}{[MnO_4^-]} = k_{obs} = \frac{kK_1K_2[H^+][LF]}{1+ K_1[H^+] + K_1K_2[H^+][LF]} \quad \textbf{(8)}$$

Equation (8) can be rearranged as

$$\frac{1}{k_{obs}} = \frac{1}{kK_1K_2[H^+][LF]} + \frac{1}{kK_2[LF]} + \frac{1}{k} \quad \textbf{(9)}$$

Equation 9, indicates that the linear plots of $1/k_{obs}$ versus 1/[LF] **(Figure 5.8)** and $1/k_{obs}$ versus $1/[H^+]$ **(Figure 5.9)** were obtained with a straight line and positive intercept on the y-axis at three different temperature viz. 20°C, 25°C and 30°C respectively. This proves the validity of rate law, and the proposed reaction scheme has been derived. The formation of complex was proved kinetically by Michaelis-Menten plot, a non-zero intercept of the plot $1/k_{obs}$ versus 1/ [LF]. The slow step rate constant k of the scheme 1 was obtained from the intercept of the plots $1/k_{obs}$ versus

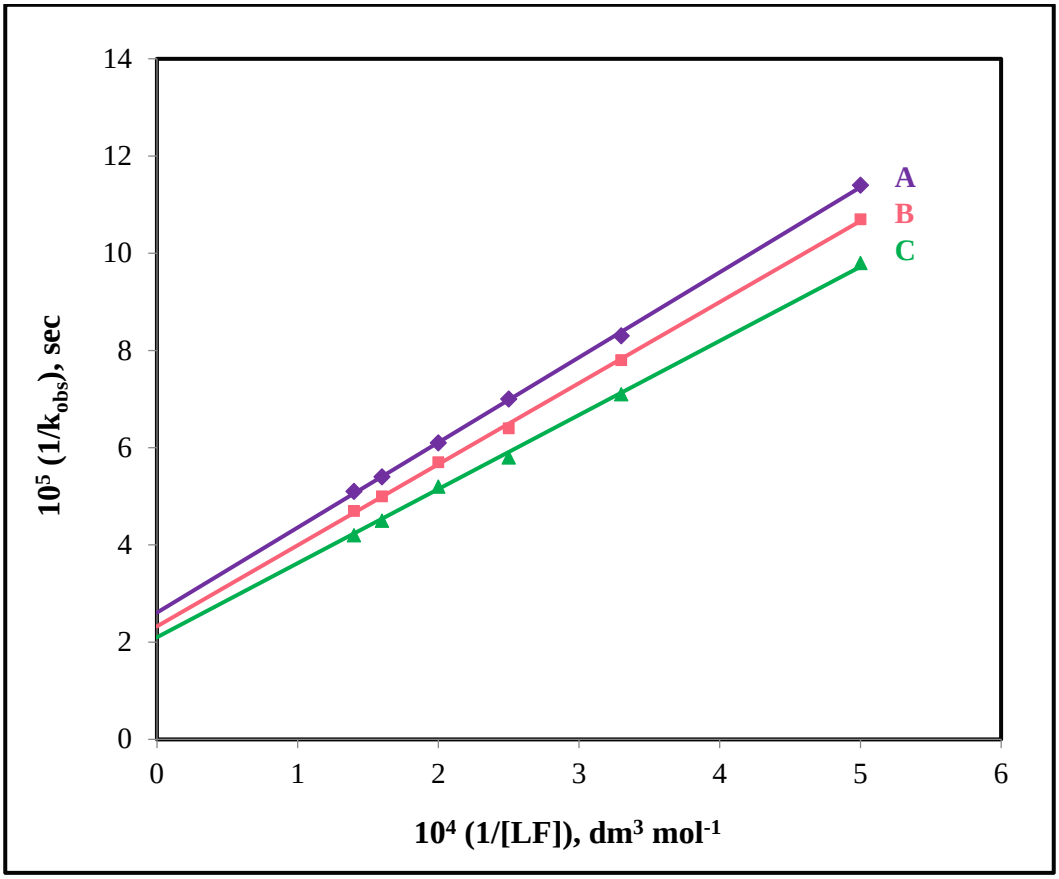


Figure 5.8: Plots of $1/k_{obs}$ versus 1/[LF] at different temperature

(A) 20°C, (B) 25°C, (C) 30°C.

$[KMnO_4] = 2.0 \times 10^{-4}$ mol dm^{-3};

$[H^+] = 1.0 \times 10^{-2}$ mol dm^{-3};

$I = 2.0 \times 10^{-2}$ mol dm^{-3}.

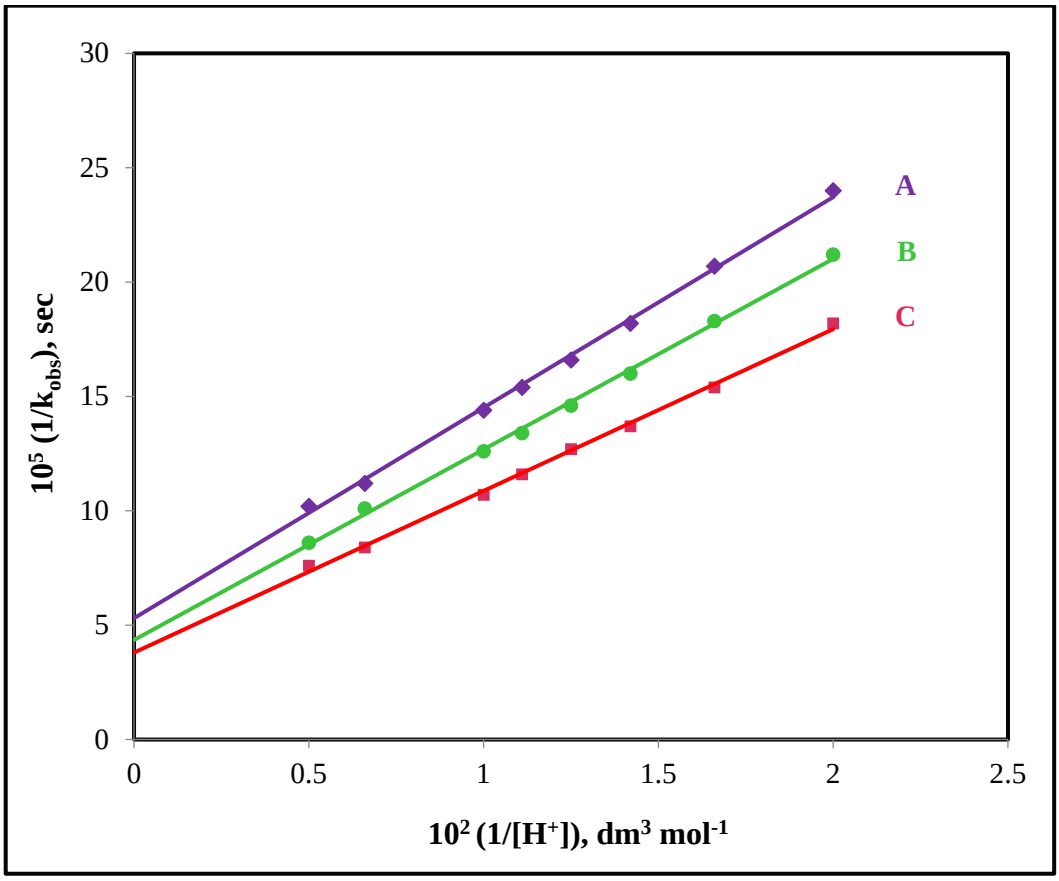


Figure 5.9: Plots of $1/k_{obs}$ versus $1/[H^+]$ at different temperature

(A) 20°C, (B) 25°C, (C) 30°C.

$[KMnO_4] = 2.0 \times 10^{-4}$ mol dm^{-3};

[LF] = 2.0×10^{-3} mol dm^{-3};

I = 2.0×10^{-2} mol dm^{-3}.

1/ [LF]. The activation parameters were calculated from the plot of log k versus 1/T **(Figure 5.10)**. The intercept and slope of the plot $1/k_{obs}$ versus 1/ $[H^+]$ gives the value of equilibrium constant of $HMnO_4$ (K_1) and the equilibrium constant of complex (K_2). The value of K_1 (40.7) is in good agreement with earlier work [34] at 25°C. Van't Hoff plots was drawn for the variations of K_1 and K_2 with temperature i.e. log K_1 vs. 1/T **(Figure 5.11)** and log K_2 vs. 1/T **(Figure 5.12)** and thermodynamic quantities were calculated **(Table 5.16)**.

According to the rate determining step in Scheme 1, the change in the ionic strength and dielectric constant of the medium does not alter the reaction rate, which suggests the involvement of non-ionic species at the rate-determining step [42]. The values of $\Delta H^{\#}$ and $\Delta S^{\#}$ are both favourable for electron transfer process [43]. The value of $\Delta S^{\#}$ within the range of radical reaction has been ascribed [44] to the nature of electron pairing and unpairing process. The negative value of $\Delta S^{\#}$ indicates that complex is more ordered than the reactants [45]. The observed modest enthalpy of activation and a comparatively low value of the entropy of activation in addition to a higher rate constant of the slow step point out that the oxidation most probably occurs via inner-sphere mechanism [46].

5.5. CONCLUSION

The oxidant MnO_4^- exists in acid medium as $HMnO_4$, which takes part in the chemical reaction. The oxidation of levofloxacin by permanganate in acidic medium has a Stoichiometry of 5:2. The oxidation products were identified as Mn(II), 7-amino fluoroquinolone, NH_3 and HCHO. Dealkylated products of levofloxacin have antimicrobial activity. Since dealkylated products are obtained in the present study, it is evident that the products of the title reaction have antimicrobial activity after oxidation. So this study will be effectively used in waste water treatment at the sites contaminated by fluoroquinolone antibiotics. The rate constant of the slowest step and other equilibrium constants involved in the mechanism are evaluated, and activation parameters with respect to slowest step were computed. The total mechanism described here is consistent with mechanism, product, and kinetic studies.

TABLE: 5.16

ACTIVATION PARAMETERS AND THERMODYNAMIC QUANTITIES EVALUATED FROM SCHEME 1.

Temperature (Kelvin)	**10^2 k (sec^{-1})**	**Activation Parameters**	**10^{-1} K_1 (dm^3 mol^{-1})**	**Thermodynamic Quantities (From K_1)**	**10^{-2} K_2 (dm^3 mol^{-1})**	**Thermodynamic Quantities (From K_2)**
293	3.84	E_a = 16.19 ($kJ\ mol^{-1}$)	4.22	$\Delta H = -6.93$ ($kJ\ mol^{-1}$)	4.82	$\Delta H = 23.16$ ($kJ\ mol^{-1}$)
298	4.34	$\Delta H^{\#}$ = 13.72 ($kJ\ mol^{-1}$)	4.07	$\Delta S = -\ 20.11$ ($JK^{-1}\ mol^{-1}$)	5.77	$\Delta S = 80.55$ ($JK^{-1}\ mol^{-1}$)
303	4.76	$\Delta S^{\#} = -224.78$ ($JK^{-1}\ mol^{-1}$)	3.81	$\Delta G = -1.10$ ($kJ\ mol^{-1}$)	6.56	$\Delta G = -1.00$ ($kJ\ mol^{-1}$)
		$\Delta G^{\#}$ = 80.77 ($kJ\ mol^{-1}$)				

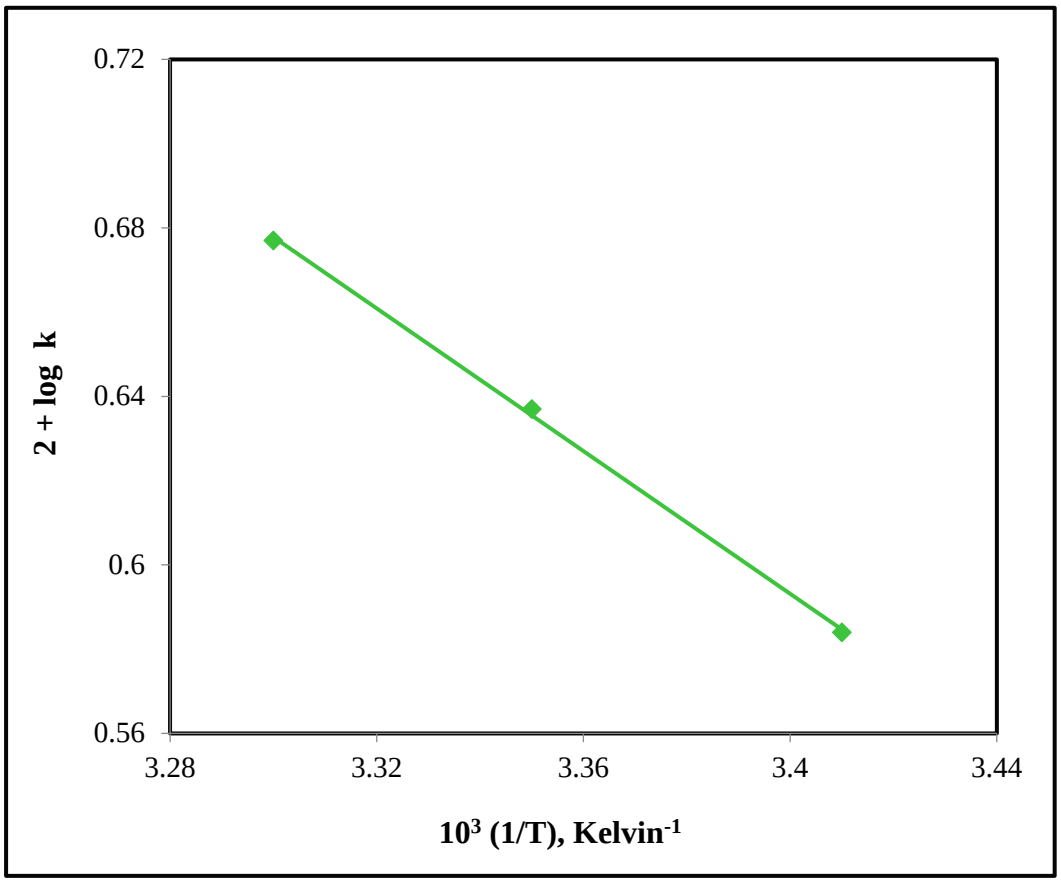


Figure 5.10: Plot of log k versus 1/T.

(Ref. Table: 5.16)

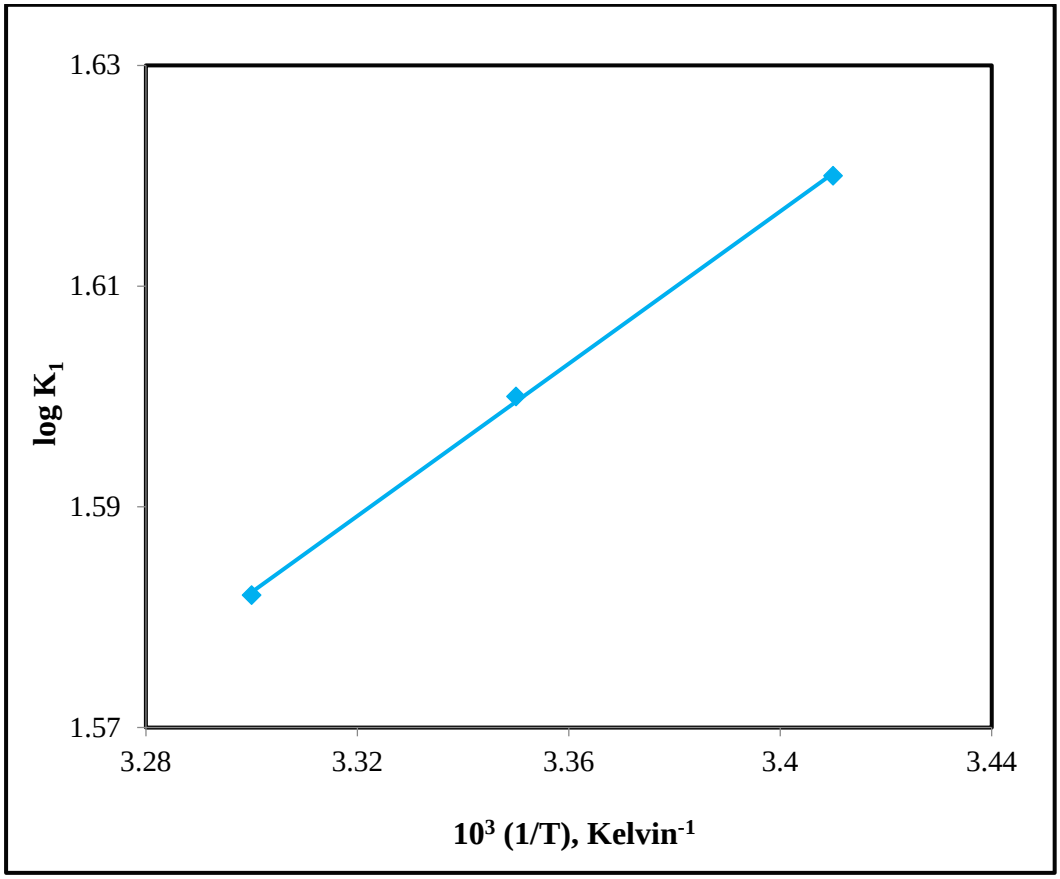


Figure 5.11: Plot of log K_1 versus 1/T.

(Ref. Table: 5.16)

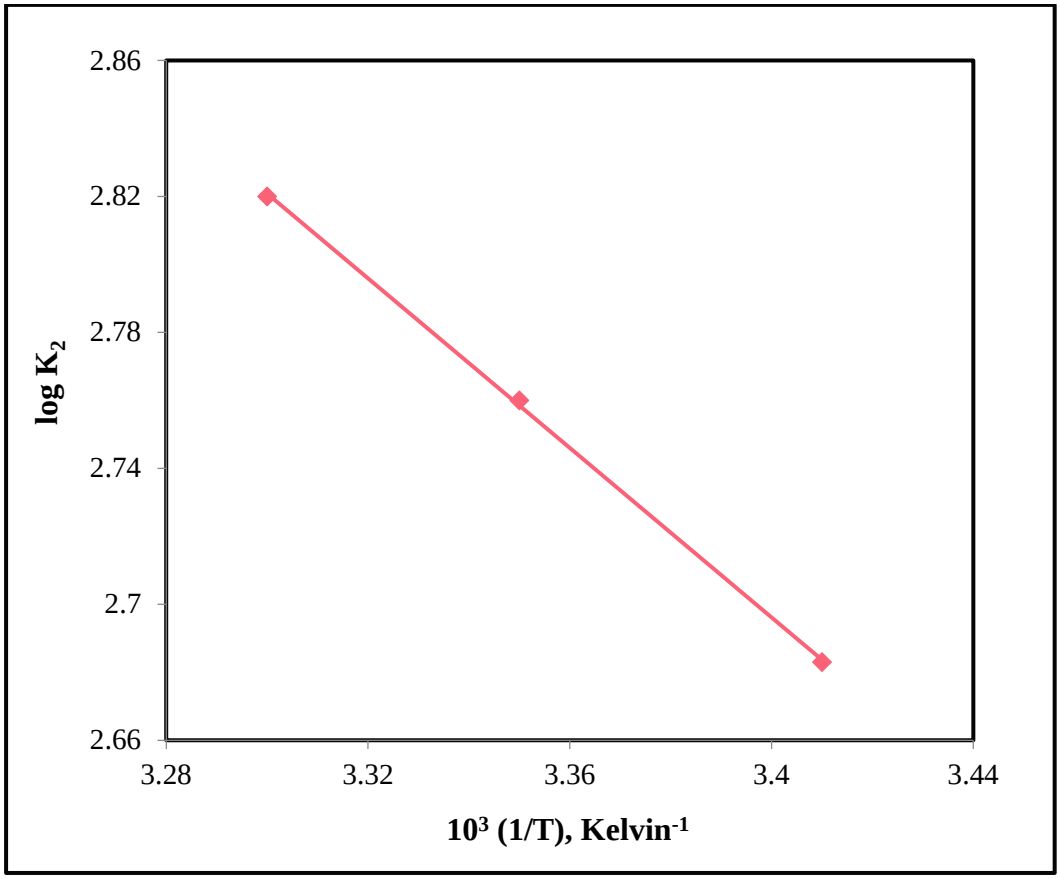


Figure 5.12: Plot of log K_2 versus 1/T.

(Ref. Table: 5.16)

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Chapter - 6

Mechanistic and Kinetic Study of Oxidation of Enrofloxacin by Permanganate in Aqueous Alkaline Medium

ABSTRACT

The kinetics and mechanism of oxidation of enrofloxacin (ENR) by permanganate ion in alkaline medium have been studied at 30 ± 1ºC. The Stoichiometry was observed to be 2:1 in terms of mole ratio of permanganate ion and enrofloxacin consumed. The reaction shows first order with respect to oxidant and substrate and fractional order with respect to alkali concentration. Product characterization of reaction mixture indicates the formation of major product m/z 263 corresponding to dealkylation of the piperazine ring of enrofloxacin. The effects of added products and ionic strength have also been investigated. A mechanism was proposed on the basis of experimental results and rate law is derived.

$$k_{obs} = \frac{k_1 K_1 [ENR]\,[OH^-]}{1 + K_1 [OH^-]}$$

There is no evidence of intermediate complex formation, thus, the outer-sphere mechanism is proposed as the mechanism for this reaction.

6.1. INTRODUCTION

Potassium permanganate used widely as oxidizing agent and play a dynamic role in the kinetics of number of organic and biological active compounds [1-5]. Oxidation reactions by Potassium permanganate are of great academic and technological importance because of its variable oxidation states. Permanganate is a powerful multi-electron oxidant which can exist in numerous oxidation states, among which +7 is its highest oxidation state, which occurs in the Oxo compounds like MnO_4^-, Mn_2O_7, MnO_3F. Out of which MnO_4^- is the most commonly used oxidant species to carry out kinetic studies in acidic, neutral and alkaline media. Oxidations by permanganate ion find widespread applications in organic syntheses [1, 6-11] especially as the introduction of phase transfer catalysis [8, 9, 11] which permits the use of solvents like methylene chloride and benzene. An important sources of mechanistic information on these reactions are kinetic studies, as certified by result stating to unsaturated acids in both aqueous [1, 6-12] and non-aqueous media [12]. Previous studies reveals that the permanganate ion oxidizes a number of organic compounds in aqueous alkaline medium, which are very slowly, attacked in acidic or neutral medium [2, 3, 13].

The oxidation mechanism depends on the nature of the substrate and pH of the reaction mixture [14]. In strongly alkaline medium, permanganate ion gives the manganate ion, MnO_4^{2-} as the stable reduction product [15–17]. No mechanistic information is available to differentiate between a direct one-electron reduction to Mn(VI) and a mechanism in which a hypomanganate ion formed in a two-electron reduction followed by its rapid re-oxidation [18, 19]. The multistep redox reactions are major source of information as when the manganese intermediates have sufficiently long life time, it's quite easy to identify them and the possible reaction mechanism were presumed by the oxidation states of the intermediates.

Fluoroquinolones are broad-spectrum antibacterial agents used to treat the bacterial infections in human beings. Pharmaceuticals, of which antibacterial groups are important, have been identified as growing environmental pollutants [20]. Fluoroquinolones are partially metabolised in human body due to which a major

fraction of it pass into the domestic sewage. This signifies the main route for entry of such pharmaceutical compounds into natural aquatic environment. So the transformations of fluoroquinolone in suitable water treatment process definitely play a major role [21]. Enrofloxacin (ENR) with molecular formula $C_{19}H_{22}FN_3O_3$,{1-Cyclopropyl-7-(4-ethyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo- 3- quinolone carboxylic acid} **(Figure 6.1)** is a broad-spectrum antibacterial agent from the class of fluoroquinolones, is the antibiotic most frequently used for the treatment of domestic animals.

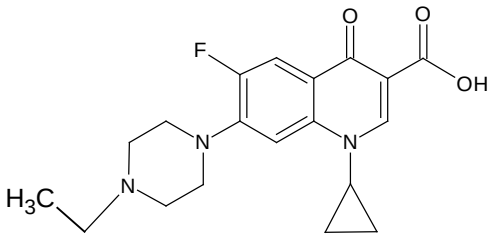


Figure 6.1: Structure of Enrofloxacin (ENR).

The structure of ENR is similar to the fluoroquinolone ciprofloxacin, with an additional ethyl substituent in the N4 atom on the piperazine ring, which contains the tertiary aromatic group and tertiary aliphatic amine groups. A previous investigation has shown that minor substitutions on the piperazine ring might affect the degradation products [22]. ENR might illustrate a different degradation pathway with Mn(VII).The literature survey reveals that there are few study reports [22-28] on the oxidation of enrofloxacin in either alkaline or acidic medium. Due to pharmaceutical importance and lack of literature on the kinetic and mechanistic study of oxidation of this drug, prompted us to study the kinetics and mechanism of the enrofloxacin by permanganate in aqueous alkaline medium.

6.2. EXPERIMENTAL

6.2.1. Chemicals and Reagents.

The method of preparation and standardization of the reagents are given in chapter 2 (Experimental). All reagents were of analytical grade and their solutions

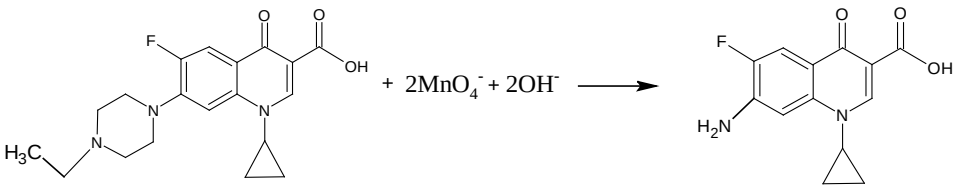
were prepared by dissolving the necessary amounts of the samples in doubly distilled water. Solution of Potassium permanganate was prepared freshly before running the experiment all the time. Doubly distilled water was employed throughout the work. The second distillation was from alkaline permanganate solution in a glass assembly.

6.2.2. Kinetic Procedure.

All the chemical reactants were placed in a thermostatic bath at 30.0 ± 0.1°C for at least 30 minutes to attain thermal equilibrium. The kinetic measurement were followed under pseudo- first-order condition where concentration of ENR is at least ten times greater than concentration of $KMnO_4$ at a constant ionic strength 0.5 mol dm^{-3}. The reaction was initiated by mixing permanganate solution to ENR with the required volume of NaOH and $NaNO_3$. The course of the reaction was followed by monitoring decrease in the absorbance of $KMnO_4$ at 525 nm as a function of time in a 1 cm path length quartz cell of UV-Visible spectrophotometer. The application of Beer's law of $KMnO_4$ at λ_{max} 525 nm had been verified giving $\varepsilon = 2260 \pm 60$ dm^3 mol^{-1} cm^{-1} (Literature, $\varepsilon = 2389$ dm^3 mol^{-1} cm^{-1}) [29]. The first order rate constants, k_{obs} were evaluated by plots of log absorbance versus time. The plots were linear to more than 75% completion of the reaction and rate constants were reproducible within ± 6%.

6.2.3. Stoichiometry and product analysis.

A reaction mixture containing ten-fold molar excess of Mn(VII) over enrofloxacin in the presence of constant amounts of alkali and ionic strength was allowed to stand for 24 hours at 30 ± 1°C. The experiments at alkaline pH showed that two moles of permanganate consume one mole of enrofloxacin. Thus the overall reaction stoichiometry may be written as:-



ENR + $2MnO_4^{2-}$ + NH_3 + Other produts

The reaction products were identified as manganese(VI) and 7-amino-1-cyclopropyl-6-fluoro-4-oxo-quinolone-3-carboxylic acid. The main reaction product of ENR was isolated with the help of TLC (Thin-Layer Chromatography) and characterized by LC-MS and FT-IR. LC-MS analysis of the reaction mixture indicated the presence of product with molecular ion peak of **m/z 263 (Figure 6.2)**. The m/z 263 corresponds to full dealkylation of the piperazine ring and classified as (M – 69) product because of its structural similarity to the (M – 69) product of ciprofloxacin [30]. However, the product does not have the same mass loss of 69 from the parent molecule. This product was also identified previously as oxidation product of enrofloxacin [27, 28].

The product was also confirmed by its IR spectrum which showed the band at **3327.10 cm^{-1}** indicating the presence of the $–NH_2$ group in the major oxidation product **(Figure 6.3)**. [31]. All other peaks observed in the IR spectrum correspond to the parent compound. The other product ammonia was detected by Nesseler's reagent test [32].

6.3. RESULTS

6.3.1. Reaction Orders.

The reaction orders were determined from the slopes of log k_{obs} versus log concentration plots, by varying the concentration of oxidant, substrate and alkali, while keeping others constant.

6.3.2. Permanganate Dependence.

At three but fixed concentrations of enrofloxacin, 3.0×10^{-3}, 5.0×10^{-3} and 7.5×10^{-3} mol dm^{-3}, constant concentration of alkali, 0.05 mol dm^{-3}, and ionic strength, 0.10 mol dm^{-3}, the permanganate concentration was varied in the concentration range of 1.0×10^{-4} – 7.0×10^{-4} mol dm^{-3} at 30°C. The linear plot of log absorbance versus time **(Figure 6.4)** shows that the order with respect to [$KMnO_4$] was unity. This fact was also confirmed by the fairly constant values of k_{obs} for varying [MnO_4^-]. Results are given in **Tables 6.1, 6.2 and 6.3**.

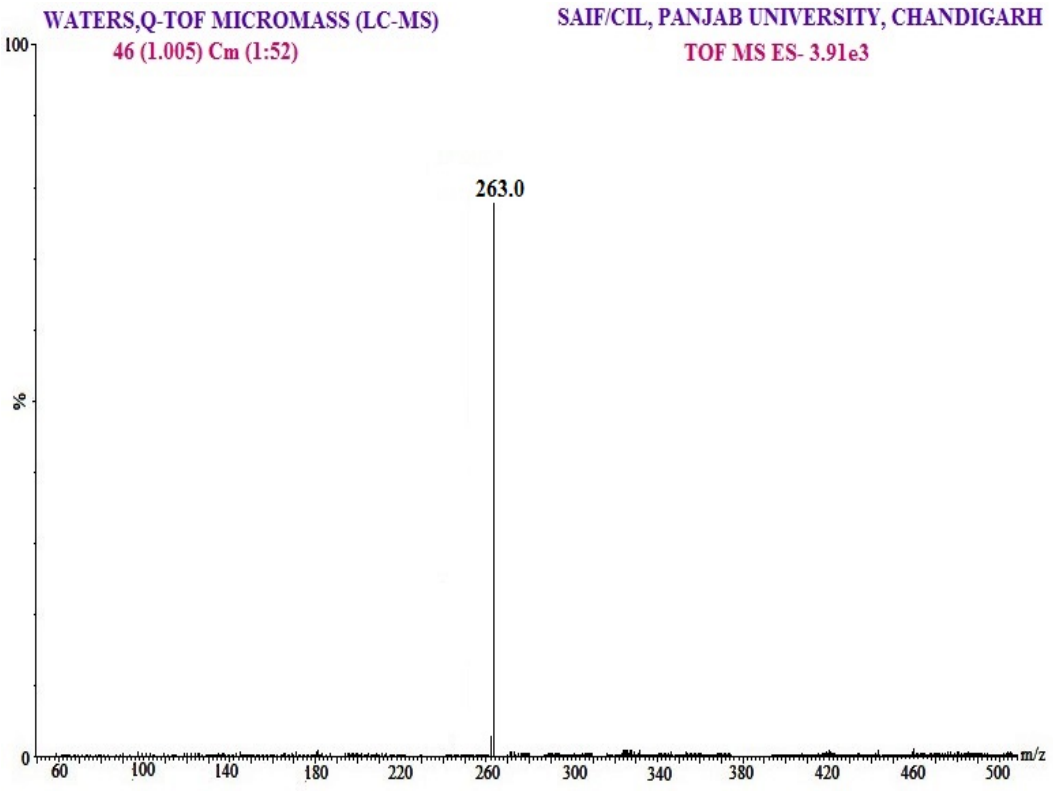


Figure 6.2: LC-ESI-MS spectra of oxidation product of Enrofloxacin.

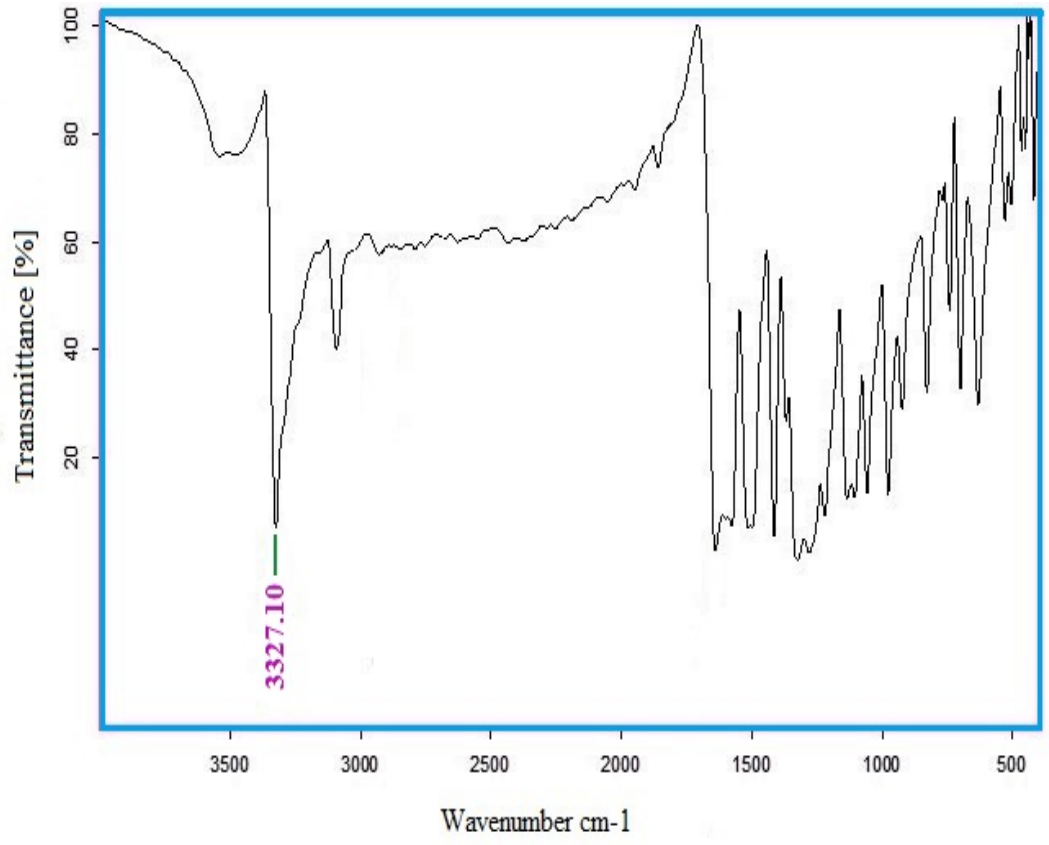


Figure 6.3: FTIR spectra of the oxidative product of Enrofloxacin by permanganate in aqueous alkaline medium.

TABLE: 6.1

VARIATION OF $KMnO_4$

[ENR] = 3.0 x 10^{-3} mol dm^{-3} **Temp. = 30°C**

[OH^-] = 5.0 x 10^{-2} mol dm^{-3} **I = 10.0 x 10^{-2} mol dm^{-3}**

10^4 [$KMnO_4$], mol dm^{-3}	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**	**7.0**
Time in minutes				**Absorbance**			
0	0.243	0.483	0.721	0.970	1.211	1.462	1.713
1	0.186	0.363	0.550	0.741	0.933	1.096	1.318
2	0.155	0.310	0.457	0.618	0.778	0.933	1.094
3	0.123	0.240	0.363	0.468	0.575	0.692	0.851
4	0.095	0.182	0.275	0.380	0.468	0.525	0.692
5	0.081	0.145	0.234	0.302	0.363	0.427	0.562
6	0.066	0.120	0.182	0.245	0.302	0.331	0.427
7	0.051	0.092	0.148	0.182	0.229	0.263	0.347
10^3 (k_{obs}), sec^{-1}	**3.71**	**3.68**	**3.72**	**3.68**	**3.71**	**3.74**	**3.72**

TABLE: 6.2

VARIATION OF $KMnO_4$

[ENR] = 5.0 x 10^{-3} mol dm^{-3} **Temp. = 30°C**

[OH^-] = 5.0 x 10^{-2} mol dm^{-3} **I = 10.0 x 10^{-2} mol dm^{-3}**

10^4 [$KMnO_4$], mol dm^{-3}	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**	**7.0**
Time in minutes				**Absorbance**			
0	0.240	0.483	0.721	0.970	1.211	1.462	1.713
1	0.162	0.331	0.490	0.661	0.813	0.977	1.148
2	0.115	0.230	0.339	0.457	0.578	0.689	0.818
3	0.081	0.158	0.240	0.324	0.410	0.468	0.550
4	0.057	0.114	0.170	0.234	0.263	0.316	0.380
5	0.041	0.078	0.123	0.158	0.191	0.229	0.251
6	0.028	0.055	0.088	0.110	0.132	0.151	0.174
$10^3(k_{obs})$, sec^{-1}	**6.12**	**6.10**	**6.16**	**6.14**	**6.10**	**6.18**	**6.09**

TABLE: 6.3

VARIATION OF $KMnO_4$

[ENR] = 7.5 x 10^{-3} mol dm^{-3} **Temp. = 30°C**

[OH^-] = 5.0 x 10^{-2} mol dm^{-3} **I = 10.0 x 10^{-2} mol dm^{-3}**

10^4 [$KMnO_4$], mol dm^{-3}	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**	**7.0**
Time in minutes				**Absorbance**			
0	0.241	0.481	0.720	0.973	1.204	1.461	1.712
1	0.138	0.282	0.407	0.550	0.692	0.851	1.148
2	0.082	0.164	0.242	0.327	0.410	0.495	0.575
3	0.047	0.102	0.141	0.182	0.240	0.275	0.347
4	0.028	0.058	0.080	0.110	0.138	0.166	0.200
5	0.016	0.035	0.048	0.061	0.082	0.088	0.115
6	0.011	0.020	0.026	0.036	0.050	0.054	0.070
10^3 (k_{obs}), sec^{-1}	**8.95**	**8.92**	**8.96**	**8.94**	**8.96**	**8.92**	**8.96**

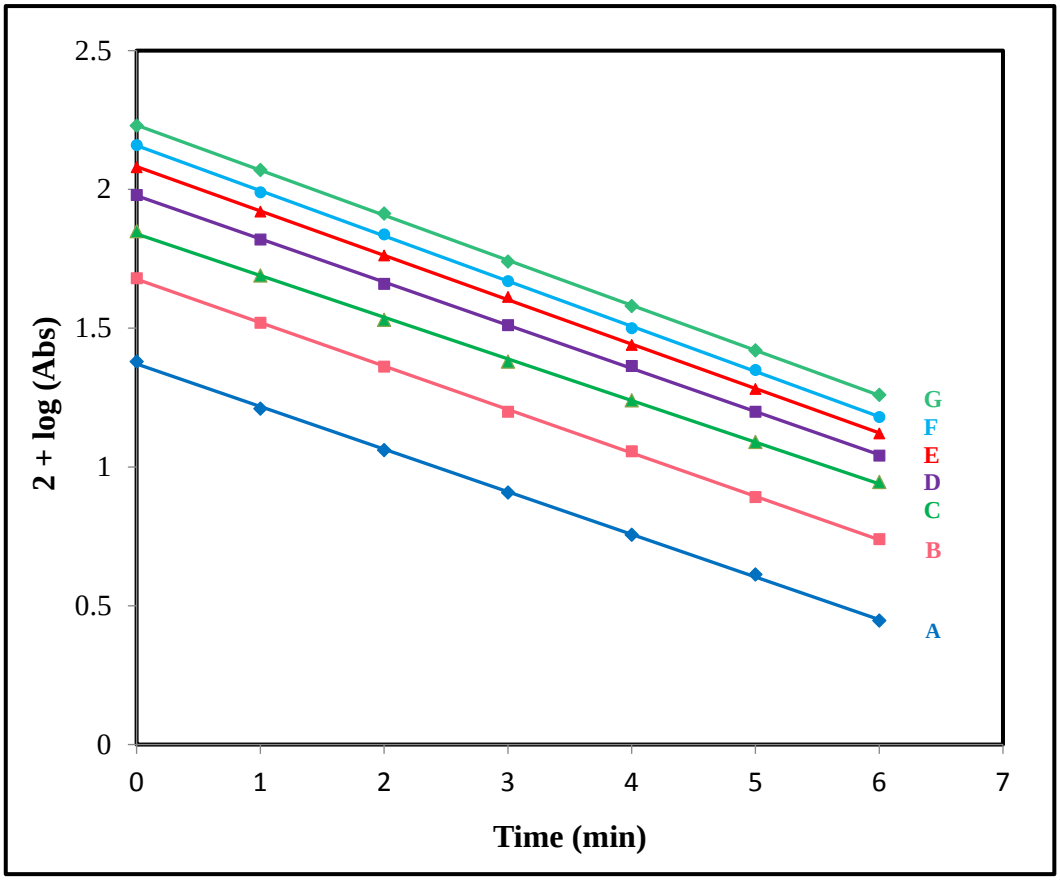


Figure 6.4: First order plots of the variation of permanganate concentration.

[ENR] = 5.0×10^{-3} mol dm^{-3};

I = 10.0×10^{-2} mol dm^{-3};

[Mn(VII)] = (A) 1.0×10^{-4} mol dm^{-3}

(C) 3.0×10^{-4} mol dm^{-3}

(E) 5.0×10^{-4} mol dm^{-3}

$[OH^-]$ = 5.0×10^{-2} mol dm^{-3};

Temp. = 30°C;

(B) 2.0×10^{-4} mol dm^{-3}

(D) 4.0×10^{-4} mol dm^{-3}

(F) 6.0×10^{-4} mol dm^{-3}

(G) 7.0×10^{-4} mol dm^{-3}.

(Ref. Table 6.2)

6.3.3. Enrofloxacin Dependence.

The effect of enrofloxacin concentration on the reaction was studied at constant concentrations of $[KMnO_4] = 5.0 \times 10^{-4}$ mol dm^{-3}, $[OH^-] = 5.0 \times 10^{-2}$ mol dm^{-3} and at a constant ionic strength of 10.0×10^{-2} mol dm^{-3} at three temperatures viz. 25°C, 30°C and 35°C . The substrate, ENR concentration was varied in the range of $2.0 \times 10^{-3} - 10.0 \times 10^{-3}$ mol dm^{-3}. The order with respect to ENR concentration was found to be unity which was confirmed by the plot of k_{obs} versus enrofloxacin concentration **(Figure 6.5)** which gives a straight line passing through the origin. Results are given in **Tables 6.4, 6.5 and 6.6**.

6.3.4. Alkali dependence.

The effect of hydroxyl ion concentration on the reaction was studied at constant concentration of $[KMnO_4] = 5.0 \times 10^{-4}$ mol dm^{-3}, $[ENR] = 5.0 \times 10^{-3}$ mol dm^{-3}, and $I = 10.0 \times 10^{-2}$ mol dm^{-3} at three temperatures viz. 25°C, 30°C and 35°C. Concentration of $[OH^-]$ was varied in the range of $2.0 \times 10^{-2} - 10.0 \times 10^{-2}$ mol dm^{-3}. The values of pseudo-first order rate constants (k_{obs}) were found to be increased with increase in $[OH^-]$ **(Figure 6.6)**. A plot of log k_{obs} versus log $[OH^-]$ was linear with less than unit order (0.68). Results are given in **Tables 6.7, 6.8 and 6.9**.

6.3.5. Effect of Ionic Strength and Dielectric Constant.

Ionic strength was studied by varying concentration of $NaNO_3$ from 0.01 – 0.10 mol dm^{-3} keeping concentration of $[KMnO_4] = 5.0 \times 10^{-4}$ mol dm^{-3}, $[ENR] = 5.0 \times 10^{-3}$ mol dm^{-3} and $[OH^-] = 5.0 \times 10^{-2}$ mol dm^{-3} at 30°C as constants. It was found that change in ionic strength has no significant effect on reaction rate. Results are given in **Table 6.10**. The effect of dielectric constant (*D*) was studied by varying the *t*-butanol water content (v/v) in the reaction mixture at fixed concentration of $[KMnO_4] = 5.0 \times 10^{-4}$ mol dm^{-3}, $[ENR] = 5.0 \times 10^{-3}$ mol dm^{-3}, $[OH^-] = 5.0 \times 10^{-2}$ mol dm^{-3} and $I = 10.0 \times 10^{-2}$ mol dm^{-3} at 30°C. The *D* values were calculated from the equation, $D = D_W V_W + D_B V_B$, where D_W and D_B are dielectric constants of pure water and *t*-butyl alcohol, respectively, and V_W and V_B are the volume fractions of water and *t*-butyl alcohol, respectively, in the total volume of the mixture. The rate of reaction increases with increasing *t*-butanol volume. The plot of log k_{obs} versus 1/*D* was linear with positive slope **(Figure 6.7)**. Results are given in **Table 6.11**.

TABLE: 6.4

VARIATION OF ENROFLOXACIN

$[KMnO_4]$ = 5.0 x 10^{-4} mol dm^{-3} **Temp. = 25°C**

$[OH^-]$ = 5.0 x 10^{-2} mol dm^{-3} **I = 10.0 x 10^{-2} mol dm^{-3}**

10^3 [ENR], mol dm^{-3}	**2.0**	**3.0**	**4.0**	**5.0**	**7.5**	**10.0**
Time in minutes			**Absorbance**			
0	(0)1.202	1.204	1.208	1.206	1.202	1.205
1	(2)0.935	0.977	0.912	0.871	0.759	0.661
2	(4)0.741	0.826	0.738	0.649	0.470	0.347
3	(6)0.589	0.661	0.550	0.479	0.316	0.191
4	(8)0.447	0.550	0.437	0.363	0.195	0.105
5	(10)0.363	0.457	0.339	0.269	0.123	0.055
6	(12)0.282	0.355	0.251	0.200	0.081	0.025
7	(14)0.229	0.269	0.214	0.151	0.051	0.018
10^3 (k_{obs}), sec^{-1}	**2.09**	**3.12**	**4.06**	**5.14**	**7.83**	**10.37**

Figures in parentheses denote time in minutes.

TABLE: 6.5

VARIATION OF ENROFLOXACIN

[$KMnO_4$] = 5.0 x 10^{-4} mol dm^{-3} **Temp. = 30°C**

[OH^-] = 5.0 x 10^{-2} mol dm^{-3} **I = 10.0 x 10^{-2} mol dm^{-3}**

10^3 [ENR], mol dm^{-3}	**2.0**	**3.0**	**4.0**	**5.0**	**7.5**	**10.0**
Time in minutes			**Absorbance**			
0	(0)1.204	1.202	1.207	1.206	1.203	1.204
1	(2)0.893	0.934	0.871	0.812	0.691	0.589
2	(4)0.617	0.776	0.667	0.574	0.410	0.285
3	(6)0.437	0.572	0.479	0.412	0.240	0.135
4	(8)0.316	0.463	0.347	0.262	0.136	0.064
5	(10)0.229	0.361	0.257	0.191	0.081	0.030
6	(12)0.170	0.304	0.195	0.131	0.052	0.016
7	(14)0.123	0.226	0.145	0.092	0.029	0.010
10^3 (k_{obs}), sec^{-1}	**2.48**	**3.71**	**4.92**	**6.10**	**8.96**	**11.99**

Figures in parentheses denote time in minutes.

TABLE: 6.6

VARIATION OF ENROFLOXACIN

$[KMnO_4] = 5.0 \times 10^{-4}$ mol dm^{-3} **Temp. = 35°C**

$[OH^-] = 5.0 \times 10^{-2}$ mol dm^{-3} **I = 10.0×10^{-2} mol dm^{-3}**

10^3 [ENR], mol dm^{-3}	**2.0**	**3.0**	**4.0**	**5.0**	**7.5**	**10.0**
Time in minutes			**Absorbance**			
0	(0)1.201	1.202	1.206	1.204	1.206	1.205
1	(2)0.838	0.912	0.832	0.794	0.646	0.513
2	(4)0.589	0.713	0.610	0.513	0.343	0.228
3	(6)0.417	0.537	0.407	0.347	0.182	0.073
4	(8)0.288	0.398	0.288	0.229	0.100	0.039
5	(10)0.200	0.324	0.209	0.158	0.053	0.017
6	(12)0.148	0.240	0.151	0.105	0.029	0.011
10^3 (k_{obs}), sec^{-1}	**3.02**	**4.36**	**5.66**	**7.11**	**10.46**	**13.88**

Figures in parentheses denote time in minutes.

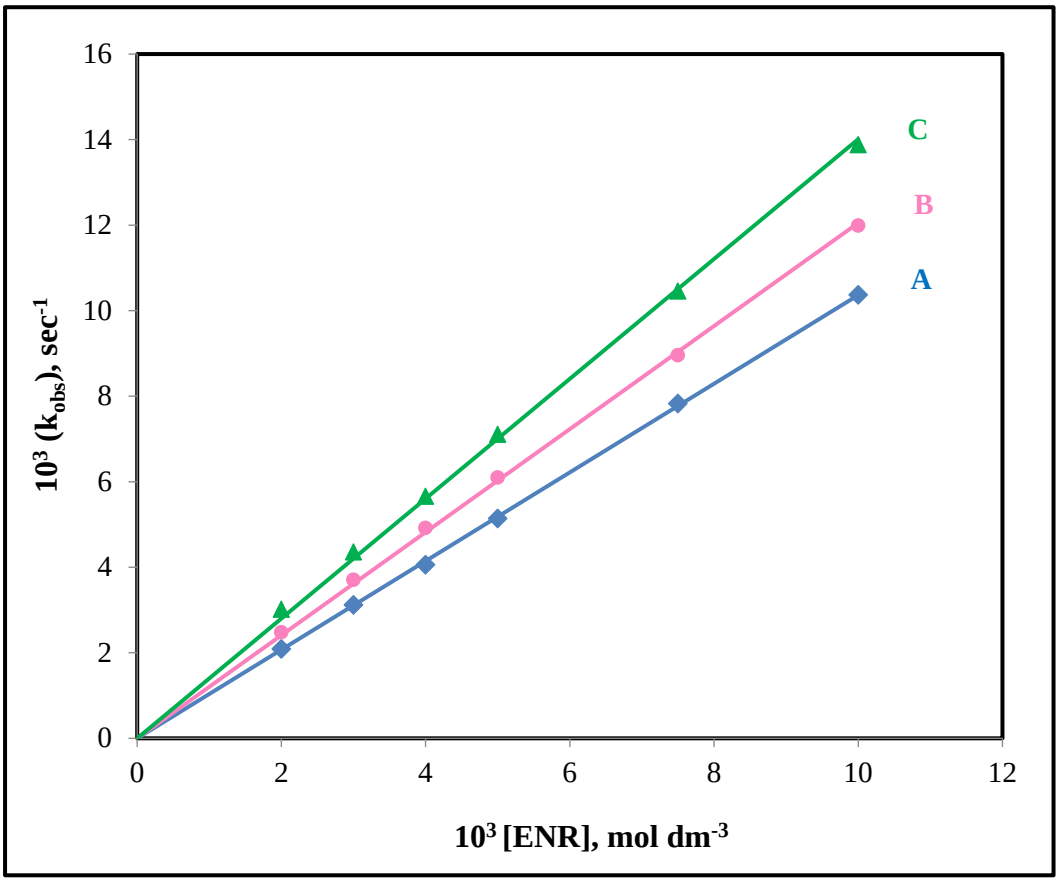


Figure 6.5: Variation of enrofloxacin at different temperatures

(A) 25°C, (B) 30°C, (C) 35°C.

$[KMnO_4] = 5.0 \times 10^{-4}$ mol dm^{-3};

$[OH^-] = 5.0 \times 10^{-2}$ mol dm^{-3};

$I = 10.0 \times 10^{-2}$ mol dm^{-3}.

(Ref. Table: 6.4, 6.5, 6.6)

TABLE: 6.7

VARIATION OF HYDROXYL ION

$[KMnO_4] = 5.0 \times 10^{-4}$ mol dm^{-3} **Temp. = 25°C**

[ENR] = 5.0×10^{-3} mol dm^{-3} **I = 10.0×10^{-2} mol dm^{-3}**

10^2 [OH^-], mol dm^{-3}	**2.0**	**3.0**	**4.0**	**5.0**	**7.5**	**10.0**
Time in minutes			**Absorbance**			
0	(0)1.202	1.202	1.205	1.206	1.207	1.203
1	(2)0.885	1.023	0.933	0.870	0.832	0.759
2	(4)0.646	0.798	0.723	0.649	0.552	0.498
3	(6)0.479	0.644	0.575	0.476	0.380	0.295
4	(8)0.347	0.525	0.437	0.365	0.251	0.182
5	(10)0.251	0.437	0.355	0.265	0.170	0.117
6	(12)0.186	0.339	0.269	0.203	0.115	0.073
7	(14)0.138	0.263	0.214	0.150	0.079	0.045
10^3 (k_{obs}), sec^{-1}	**2.55**	**3.42**	**4.25**	**5.14**	**6.48**	**7.35**

Figures in parentheses denote time in minutes.

TABLE: 6.8

VARIATION OF HYDROXYL ION

$[KMnO_4] = 5.0 \times 10^{-4}$ mol dm^{-3} **Temp. = 30°C**

[ENR] = 5.0×10^{-3} mol dm^{-3} **I = 10.0×10^{-2} mol dm^{-3}**

10^2 [OH^-], mol dm^{-3}	**2.0**	**3.0**	**4.0**	**5.0**	**7.5**	**10.0**
Time in minutes			**Absorbance**			
0	(0)1.208	1.207	1.204	1.202	1.202	1.206
1	(2)0.822	0.955	0.851	0.814	0.741	0.708
2	(4)0.562	0.729	0.646	0.573	0.481	0.441
3	(6)0.380	0.575	0.501	0.411	0.288	0.263
4	(8)0.269	0.457	0.380	0.265	0.174	0.166
5	(10)0.191	0.355	0.282	0.189	0.120	0.100
6	(12)0.129	0.251	0.204	0.133	0.072	0.060
10^3 (k_{obs}), sec^{-1}	**3.12**	**4.16**	**5.18**	**6.10**	**7.64**	**8.37**

TABLE: 6.9

VARIATION OF HYDROXYL ION

$[KMnO_4] = 5.0 \times 10^{-4}$ mol dm^{-3} **Temp. = 35°C**

[ENR] = 5.0×10^{-3} mol dm^{-3} **I = 10.0×10^{-2} mol dm^{-3}**

10^2 [OH^-], mol dm^{-3}	**2.0**	**3.0**	**4.0**	**5.0**	**7.5**	**10.0**
Time in minutes			**Absorbance**			
0	1.202	1.203	1.207	1.202	1.206	1.209
1	0.977	0.891	0.832	0.792	0.708	0.676
2	0.774	0.662	0.579	0.511	0.425	0.394
3	0.603	0.501	0.398	0.345	0.251	0.209
4	0.490	0.372	0.275	0.230	0.145	0.120
5	0.380	0.269	0.191	0.156	0.088	0.068
6	0.263	0.204	0.132	0.105	0.051	0.039
10^3 (k_{obs}), sec^{-1}	**3.66**	**4.98**	**6.08**	**7.11**	**8.68**	**9.30**

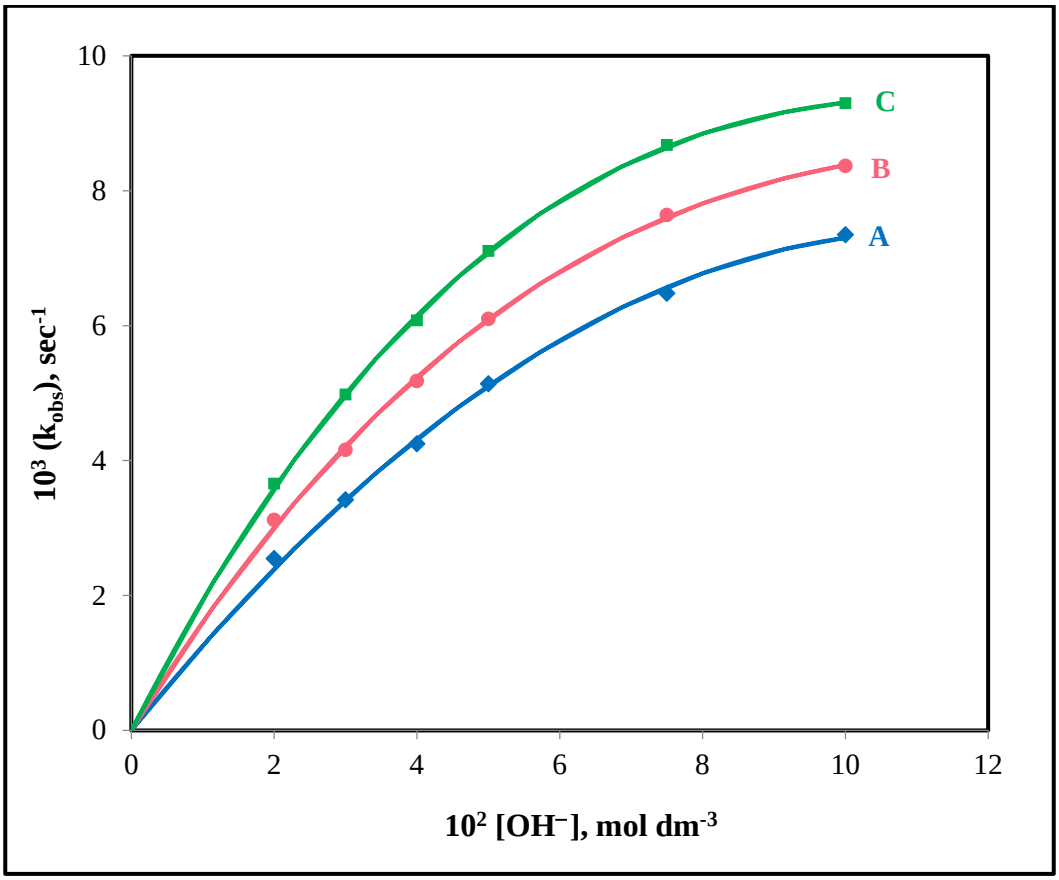


Figure 6.6: Variation of alkali at different temperatures

(A) 25°C, (B) 30°C, (C) 35°C.

$[KMnO_4] = 5.0 \times 10^{-4}$ mol dm^{-3};

[ENR] = 5.0×10^{-3} mol dm^{-3};

I = 10.0×10^{-2} mol dm^{-3}.

(Ref. Table: 6.7, 6.8, 6.9)

TABLE: 6.10

VARIATION OF SODIUM NITRATE

$[KMnO_4] = 5.0 \times 10^{-4}$ mol dm^{-3} **Temp. = 30°C**

[ENR] = 5.0×10^{-3} mol dm^{-3} **$[OH^-] = 5.0 \times 10^{-2}$ mol dm^{-3}**

10^2 $[NaNO_3]$, mol dm^{-3}	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0
Time in minutes	**Absorbance**									
0	1.202	1.206	1.204	1.202	1.206	1.203	1.204	1.203	1.202	1.204
1	0.816	0.812	0.810	0.812	0.812	0.814	0.815	0.813	0.813	0.810
2	0.582	0.578	0.577	0.578	0.578	0.579	0.582	0.578	0.579	0.578
3	0.416	0.411	0.410	0.411	0.412	0.413	0.415	0.412	0.414	0.412
4	0.266	0.262	0.260	0.261	0.262	0.264	0.265	0.262	0.265	0.261
5	0.193	0.190	0.188	0.189	0.191	0.192	0.194	0.191	0.190	0.193
6	0.133	0.131	0.28	0.129	0.131	0.130	0.132	0.131	0.133	0.131
10^3 (k_{obs}), sec^{-1}	**6.05**	**6.10**	**6.12**	**6.11**	**6.10**	**6.09**	**6.05**	**6.11**	**6.09**	**6.10**

TABLE: 6.11

EFFECT OF DIELECTRIC CONSTANT

$[KMnO_4] = 5.0 \times 10^{-4}$ mol dm^{-3} **Temp. = 30°C**

[ENR] = 5.0×10^{-3} mol dm^{-3} **I = 10.0×10^{-2} mol dm^{-3}**

$[OH^-] = 5.0 \times 10^{-2}$ mol dm^{-3}

[*t*-butyl alcohol], %	**5**	**10**	**15**	**20**
Time in minutes		**Absorbance**		
0	1.202	1.205	1.202	1.206
1	0.794	0.759	0.724	0.692
2	0.514	0.485	0.473	0.442
3	0.339	0.316	0.282	0.257
4	0.229	0.166	0.160	0.158
5	0.151	0.129	0.110	0.099
6	0.100	0.081	0.070	0.058
10^3 (k_{obs}), sec^{-1}	**7.08**	**7.56**	**7.78**	**8.35**

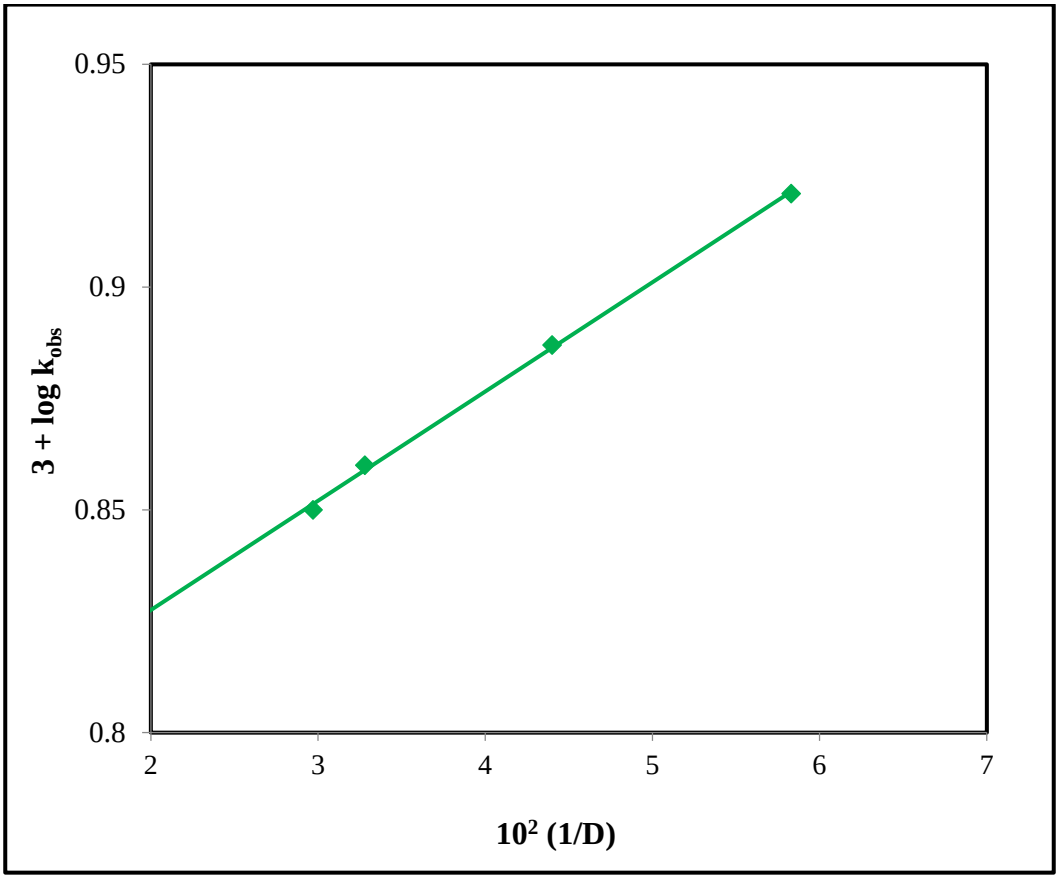


Figure 6.7: Effect of dielectric constant on the oxidation of Enrofloxacin by alkaline permanganate at 30°C.

(Ref. Table: 6.11)

6.3.6. Effect of Initially Added Product.

The effect of manganate ion concentration on the reaction was studied at constant concentrations of [$KMnO_4$] = 5.0×10^{-4} mol dm^{-3}, [ENR] = 5.0×10^{-3} mol dm^{-3}, [OH^-] = 5.0×10^{-2} mol dm^{-3} and at a constant ionic strength of 10.0×10^{-2} mol dm^{-3} at temperature 30°C. Mn(VI) concentration was varied in the range of 4.0×10^{-5} to 4.0×10^{-4} mol dm^{-3}. Initially added Mn(VI) ions have no influence on the rate constant. Results are given in **Table 6.12**.

6.3.7. Test for Free Radicals.

Free radical involvement in oxidation of enrofloxacin by alkaline permanganate was studied by adding acrylonitrile (CH_2=CHCN) in the reaction mixture for 5 hours in an inert atmosphere followed by methyl alcohol dilution which involves precipitate formation indicating that reaction path is restricted by radical mechanism.

6.4. DISCUSSION

Permanganate ion is a strong oxidant in an aqueous alkaline media. Since it shows various oxidation states, the stoichiometric results and the pH of reaction medium play a significant role. Under the present experimental conditions at pH > 12, the reduction product of Mn(VII) is stable and further reduction of Mn(VI) might be stopped [15,16]. However, prolong standing, green Mn(VI) is reduced to Mn(IV) under experimental conditions. The permanganate shows various oxidation states, such as Mn(VII), Mn(V), and Mn(VI) in the alkaline medium. The colour of the reaction mixture changes from violet Mn(VII) to dark green through blue Mn(IV) were observed. It is plausible that blue colour originated from the violet of permanganate and the green from manganate, excluding the accumulation of hypomanganate. It is clear from **Figure 6.8** that the concentration of MnO_4^- decreases at wavelength 526 nm, while increases at 610 and 460 nm are due to Mn(VI).

The reaction between Mn(VII) and enrofloxacin in alkaline medium has Stoichiometry 2:1, having first order dependence with permanganate and enrofloxacin and less than unit order with OH^- concentration. The results shows that OH^- ions first

TABLE: 6.12

EFFECT OF Mn(VI) ION

[$KMnO_4$] = 5.0 x 10^{-4} mol dm^{-3} **Temp. = 30°C**

[ENR] = 5.0 x 10^{-3} mol dm^{-3} **I = 10.0 x 10^{-2} mol dm^{-3}**

[OH^-] = 5.0 x 10^{-2} mol dm^{-3}

10^4 [Mn(VI)]	**0.4**	**1.0**	**2.0**	**3.0**	**4.0**
Time in minutes			**Absorbance**		
0	1.208	1.206	1.207	1.205	1.204
1	0.810	0.812	0.815	0.813	0.812
2	0.577	0.578	0.581	0.579	0.578
3	0.410	0.412	0.415	0.414	0.411
4	0.260	0.262	0.265	0.265	0.262
5	0.188	0.191	0.194	0.190	0.190
6	0.28	0.131	0.132	0.133	0.131
10^3 (k_{obs}), sec^{-1}	**6.12**	**6.10**	**6.06**	**6.09**	**6.10**

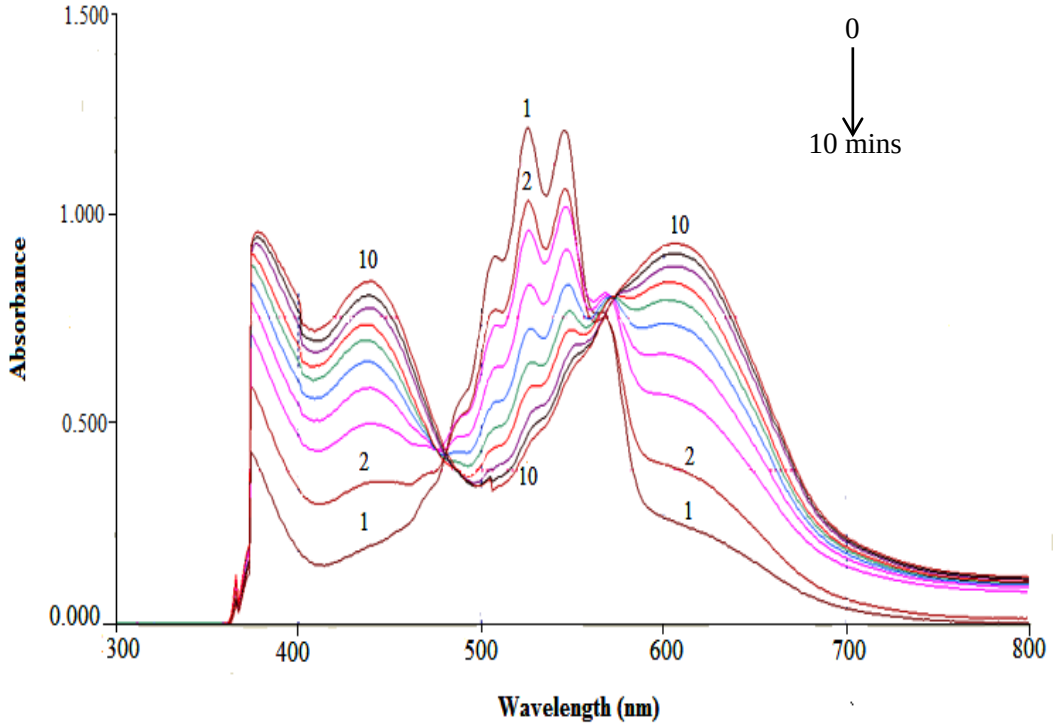


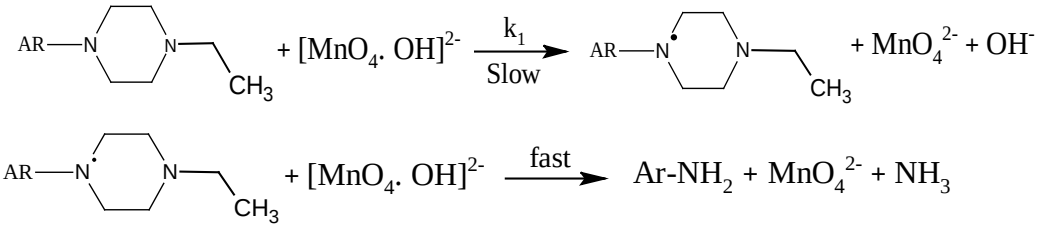
Figure 6.8: Spectral changes during the oxidation of enrofloxacin (ENR) by permanganate in alkaline medium at 30°C.

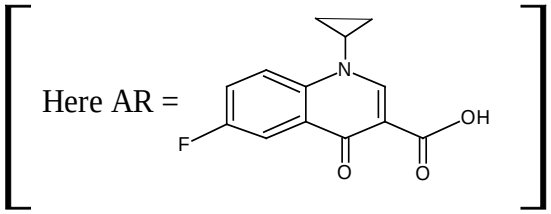
$[KMnO_4] = 5.0 \times 10^{-4}$ mol dm^{-3}; $[ENR] = 5.0 \times 10^{-3}$ mol dm^{-3};

$[OH^-] = 5.0 \times 10^{-2}$ mol dm^{-3}; $I = 10.0 \times 10^{-2}$ mol dm^{-3}.

combined with permanganate to form a basic permanganate reactive species $[MnO_4 \cdot OH]^{2-}$ [30, 33]. Then $[MnO_4 \cdot OH]^{2-}$ reacts with the one mole of enrofloxacin in the rate determining step to give a free radical derived from enrofloxacin and Mn(VI). In further fast step, formed free radical reacts with another molecule of $[MnO_4 \cdot OH]^{2-}$ to produce the product 7-amino fluoroquinolone, Mn(VI), NH_3 and other products **(Scheme- 1)**. The effect of ionic strength and dielectric constant on the rate explains qualitatively the involvement of a neutral molecule in the reaction.

$$MnO_4^- + OH^- \underset{}{\overset{K_1}{\rightleftharpoons}} [MnO_4.OH]^{2-}$$





Scheme- 1

From the scheme-1, the following rate law can be derived as follows:

$$\text{Rate} = \frac{-d[MnO_4^-]}{dt} = k_1[MnO_4.OH]^{2-}\ [ENR] \qquad \textbf{(1)}$$

$$= k_1K_1[MnO_4^-]_f[ENR]_f[OH^-]_f \qquad \textbf{(2)}$$

The total concentration of permanganate is given by:

$$[MnO_4^-]_t = [MnO_4^-]_f + [MnO_4.OH]^{2-}_f$$

$$= [MnO_4^-]_f + K_1[OH^-]_f[MnO_4^-]_f$$

$$= [MnO_4^-]_f\ (1 + K_1[OH^-]_f)$$

$$\text{So, } [MnO_4^-]_f = \frac{[MnO_4^-]_t}{(1 + K_1[OH\text{-}]_f)} \qquad \textbf{(3)}$$

Here "t" and "f" stands for total and free concentration.

$$[OH^-]_f = \frac{[OH\text{-}]_t}{(1 + K_1[MnO_4^-]_f)} \quad \textbf{(4)}$$

Putting equation (3) and (4) in equation (2) and omitting "t" and "f" subscripts

$$\text{Rate} = \frac{-d[MnO_4^-]}{dt} = \frac{k_1K_1[MnO_4^-]\,[ENR]\,[OH^-]}{(1 + K_1[OH^-])\,(1 + K_1[MnO_4^-])} \quad \textbf{(5)}$$

$$= \frac{k_1K_1[MnO_4^-]\,[ENR]\,[OH^-]}{1 + K_1[OH^-] + K_1[MnO_4^-] + K_1^2[OH^-][MnO_4^-]} \quad \textbf{(6)}$$

$K_1[MnO_4^-]$ And $K_1^2[OH^-][MnO_4^-] << 1$ or neglected due to low concentration of $[MnO_4^-]$ used in the experiment so equation (6) change into equation (7)

$$\text{Rate} = \frac{-d[MnO_4^-]}{dt} = \frac{k_1K_1[MnO_4^-]\,[ENR]\,[OH^-]}{1 + K_1[OH^-]} \quad \textbf{(7)}$$

$$\frac{\text{Rate}}{[MnO_4^-]} = k_{obs} = \frac{k_1K_1[ENR]\,[OH^-]}{1 + K_1[OH^-]} \quad \textbf{(8)}$$

$$k' = \frac{k_{obs}}{[ENR]} = \frac{k_1K_1\,[OH^-]}{1 + K_1[OH^-]} \quad \textbf{(9)}$$

Equation (9) can be rearranged as

$$\frac{1}{k'} = \frac{[ENR]}{k_{obs}} = \frac{1}{k_1K_1\,[OH^-]} + \frac{1}{k_1} \quad \textbf{(10)}$$

According to Equation (10) the plot of $1/k'$ versus $1/[OH^-]$ is linear with positive intercept and slope **(Figure 6.9)** at three different temperature 25°C, 30°C and 35°C. The rate constant k_1, of the slow step and the equilibrium constant K_1 of scheme-1 was obtained from the intercept and slope of the plots $1/k'$ versus $1/[OH^-]$. The energy of activation was determined by the plot of log k_1 versus 1/T **(Figure 6.10)** from which activation parameters was calculated. The value of K_1 (9.38) is in good agreement with that derived in earlier work [30] (literature value is 9.3 dm^3mol^{-1} at 30°C). Van't Hoff's plot of log K_1 versus 1/T **(Figure 6.11)** give the values of enthalpy of reaction ΔH, entropy of reaction ΔS and free energy of reaction ΔG **(Table 6.13)**.

The ionic strength had no effect on rate of reaction which is in the correct way of involvement of neutral species. The effect of solvent on the rate of reaction has

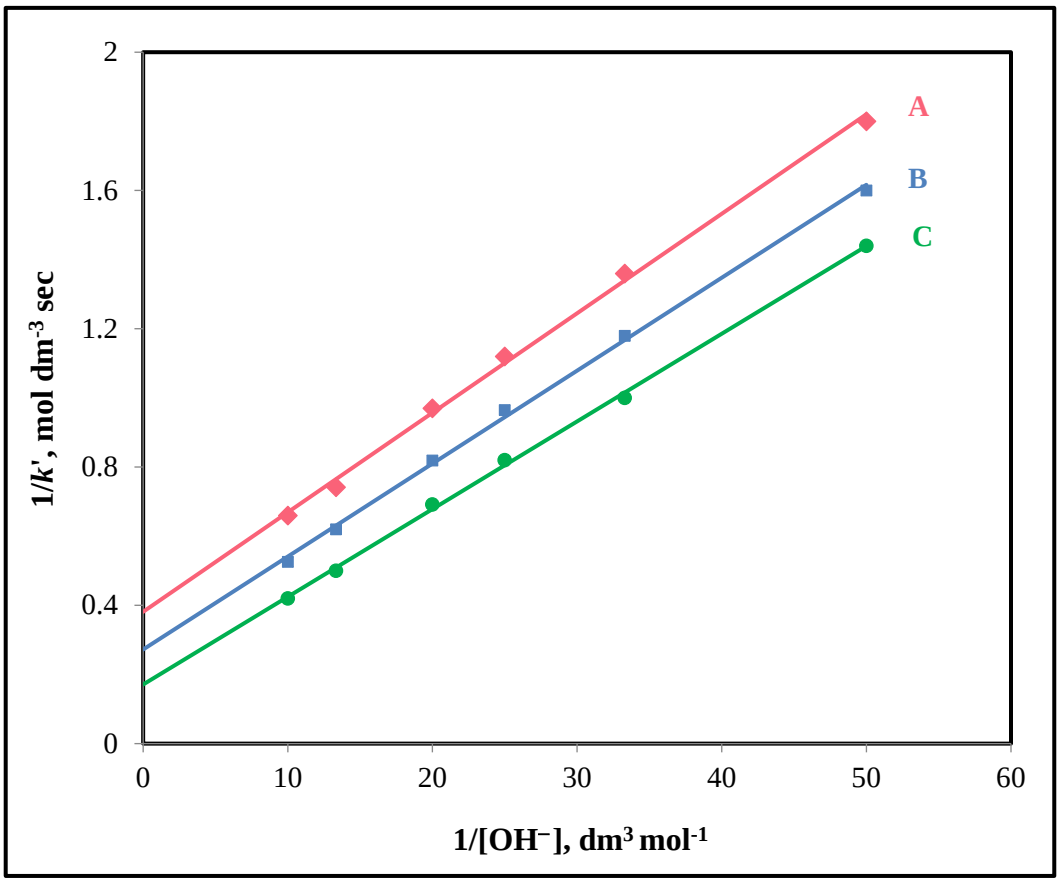


Figure 6.9: Plots of 1/k' versus 1/[OH⁻] at different temperatures
(A) 25°C, (B) 30°C, (C) 35°C.

$[KMnO_4] = 5.0 \times 10^{-4}$ mol dm^{-3};
$[OH^-] = 5.0 \times 10^{-2}$ mol dm^{-3};
$I = 10.0 \times 10^{-2}$ mol dm^{-3}.

TABLE: 6.13

ACTIVATION PARAMETERS AND THERMODYNAMIC QUANTITIES EVALUATED FROM SCHEME 1.

Temperature (Kelvin)	**k_1 ($dm^3\ mol^{-1}\ s^{-1}$)**	**Activation Parameters**	**K_1 ($dm^3\ mol^{-1}$)**	**Thermodynamic Quantities**
298	3.09	$E_a = 34.08$ ($kJ\ mol^{-1}$)	11.22	$\Delta H = -28.72$ ($kJ\ mol^{-1}$)
303	4.30	$\Delta H^{\#} = 31.57$ ($kJ\ mol^{-1}$)	9.38	$\Delta S = -92.8$ ($JK^{-1}\ mol^{-1}$)
308	5.84	$\Delta S^{\#} = -133.03$ ($JK^{-1}\ mol^{-1}$)	6.73	$\Delta G = -1.72$ ($kJ\ mol^{-1}$)
		$\Delta G^{\#} = 70.19$ ($kJ\ mol^{-1}$)		

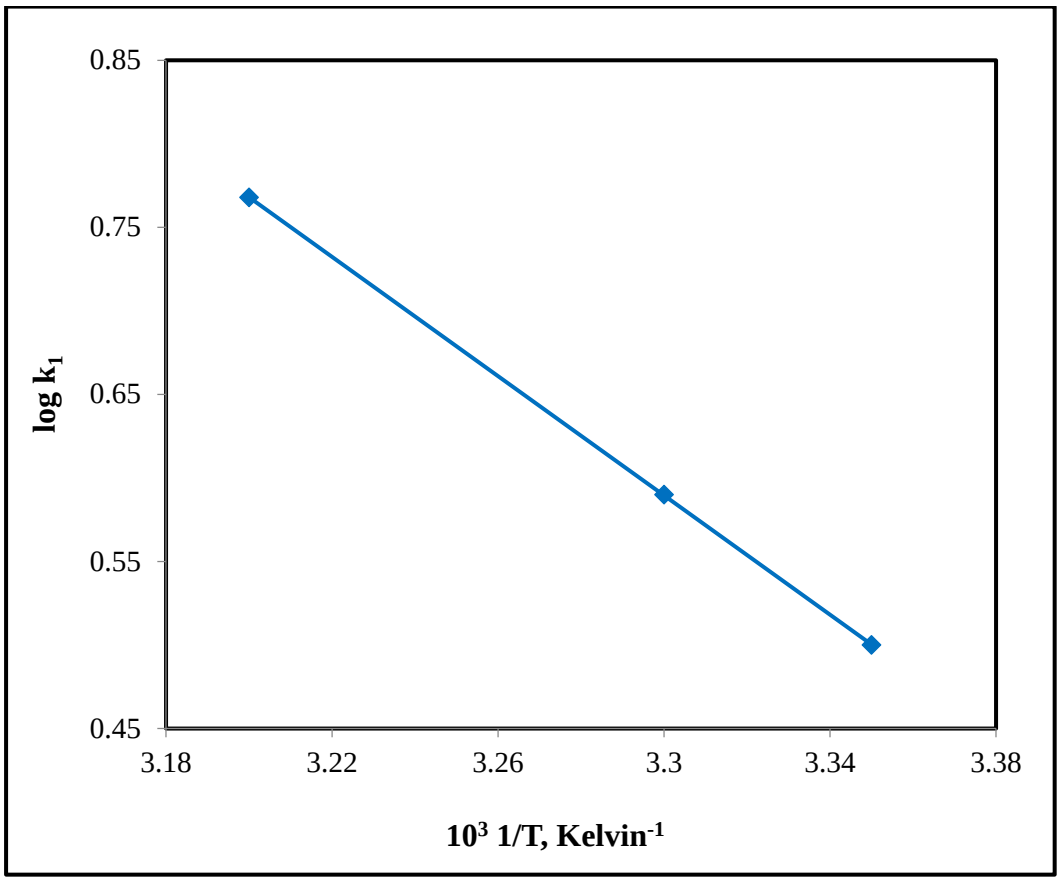


Figure 6.10: Plot of log k_1 versus 1/T.

(Ref. Table: 6.13)

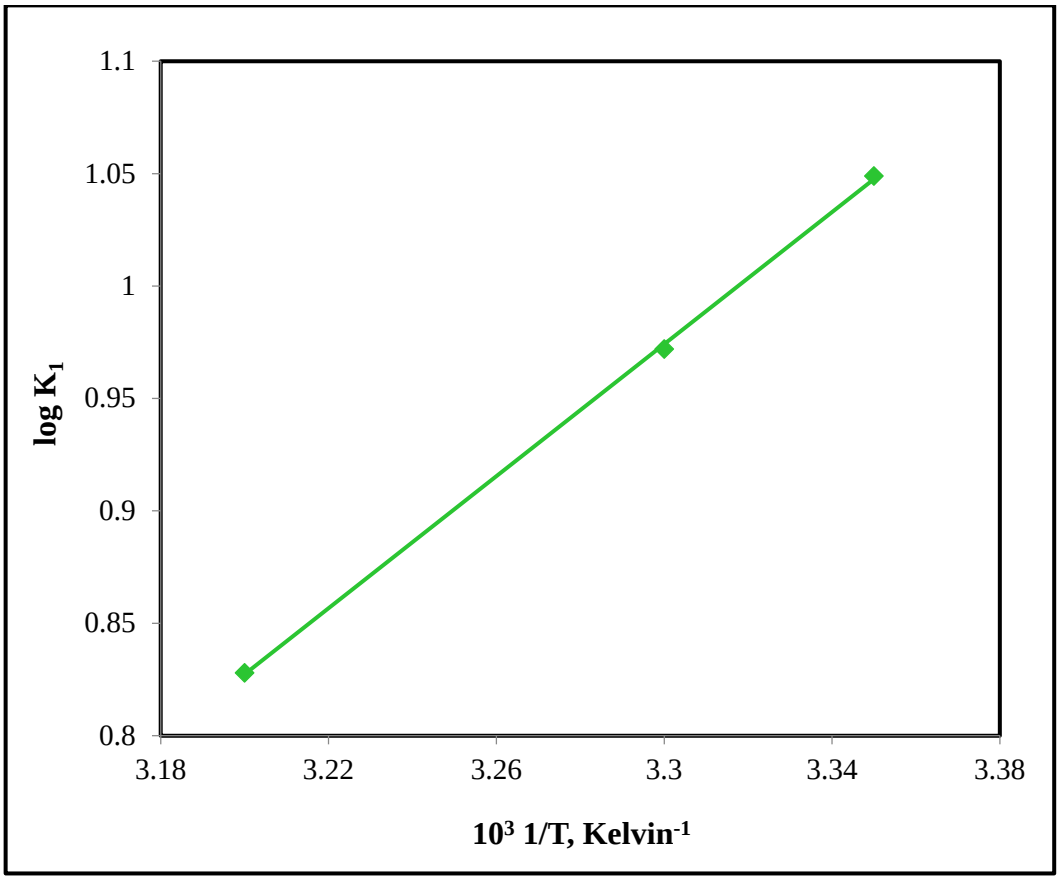


Figure 6.11: Plot of log K_1 versus 1/T.

(Ref. Table: 6.13)

been described in detail in the literature [34]. For the interaction between a negative ion and dipole or two dipoles, a plot of log k_{obs} versus 1/*D* gives a straight line with negative slope while for interaction between a positive ion and dipole a positive slope results. In the present study, rate of the reaction increases with decrease in the dielectric constant of the medium, which cannot be explained by Amis theory [34] because the existence of a positive ion is improbable in the alkaline medium. Since permanganate is a one electron oxidant in alkaline medium, the reaction between substrate and oxidant would give rise to a radical intermediate. However, an increase in the rate of reaction with decreasing dielectric constant may be due to the increased formation of active reactant species at low dielectric constant.

The values of $\Delta H^{\#}$ and $\Delta S^{\#}$ are both favourable for electron transfer process [35].The value of $\Delta S^{\#}$ within the range of radical reaction has been ascribed [36] to the nature of electron pairing and unpairing process. Absence of the evidence of intermediate complex formation suggests that the reaction most probably occurs through outer-sphere mechanism [37].

6.5. CONCLUSION

The Kinetic study of oxidation of enrofloxacin by permanganate in alkaline medium has been studied. It is interesting to note that the oxidant species [MnO_4^-] requires pH > 12, below which the system becomes disturbed and the reaction proceeds further to give a reduced oxidation product as manganese(IV), which slowly develops a yellow turbidity. Hence, the role of pH in the reaction medium is important. The oxidant, manganese(VII), exists in alkali media as alkali-permanganate species $[MnO_4{\cdot}OH]^{2-}$, which takes part in the chemical reaction. The main reaction product was characterized by LC-MS and FT-IR techniques. Chemical oxidation using Mn(VII) has been widely used for treatment of pollutants in drinking water and waste water applications. The proposed mechanism is consistent with product, mechanism and kinetic studies.

6.6. REFERENCES

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ANNEXURE – I

LIST OF PAPERS PUBLISHED: 12

1. "Oxidation of Ciprofloxacin by Hexacyanoferrate(III) in the presence of Cu(II) as a catalyst: A Kinetic Study", Gajala Tazwar, **Ankita Jain,** Naveen Mittal, Vijay Devra, **Int. J. Chem. Kinet. (John Wiley & Sons, Inc.),** Accepted in March 2017.

2. "Oxidative Degradation of Levofloxacin by Water-Soluble Manganese Dioxide in Aqueous Acidic medium: A Kinetic Study", Gajala Tazwar, **Ankita Jain,** Vijay Devra, **Chemical Papers (Springer),** Accepted in March, 2017.

3. "Kinetics and Mechanism of Permanganate Oxidation of Nalidixic Acid in Aqueous alkaline Medium", **Ankita Jain,** Gajala Tazwar, Vijay Devra, **Journal of Applied Pharmaceutical Science,** 2017; 7: 135-143.

4. "Catalytic Oxidation of Levofloxacin by Hexacyanoferrate(III) in Aqueous Alkaline Medium: A Kinetic Study", Gajala Tazwar, **Ankita Jain,** Vijay Devra, **International Conference on Innovative Research in Science, Technology and Management,** 2017: 528-539.

5. "Copper Nanoparticles Catalyzed Oxidation of Threonine by Peroxomonosulphate", Shikha Jain, **Ankita Jain,** Vijay Devra, **Journal of Saudi Chemical Society (Elsevier),** 2016; 20.

6. "Oxidation of Levofloxacin by Acidic Permanganate: A Kinetic and Mechanistic Study", **Ankita Jain,** Gajala Tazwar, Vijay Devra, **Int. J. Res. Pharm. Sci.,** 2015; 5; 1 – 8.

7. "Synthesis and Size Control of Copper Nanoparticles and their Catalytic Application", Shikha Jain, **Ankita Jain,** Pranav Kachhawah, Vijay Devra, **Trans. Nonferrous Met. Soc. China (Elsevier),** 2015; 25: 3995-4000.

8. "A Kinetic Study on Copper Nanocatalysis in the Oxidation of Serine by Peroxomonosulphate", Shikha Jain, **Ankita Jain,** Vijay Devra, **International Journal of Advanced Research in Engineering and Applied Sciences,** 2015; 4: 1-16.

9. "Kinetics and Mechanism of Permanganate Oxidation of Ciprofloxacin in Aqueous Sulphuric Acid Medium", **Ankita Jain,** Shikha Jain, Vijay Devra, **International Journal of Pharmaceutical Sciences and Drug Research,** 2015; 7: 205-210.

10. "Kinetic Analysis of Oxidation of Ofloxacin by Permanganate Ion in Sulphuric Acid Medium: A Mechanistic Approach", Vijay Devra, **Ankita Jain,** Shikha Jain, **World Journal of Pharmaceutical Research,** 2015; 4: 963-977.

11. "Experimental Investigation on the Synthesis of Copper Nanoparticles by Chemical Reduction Method", Shikha Jain, **Ankita Jain,** Vijay Devra, **International Journal of Scientific & Engineering Research,** 2014; 5: 973-978.

12. "Correlation Analysis of Physico-Chemical Parameters and Water Quality of Chambal River: A case study of Kota City", **Ankita Jain,** Shikha Jain, Niharika Nagar, Pankaj Kachhawah, Vijay Devra, **International Journal of Engineering, Research and Technology,** (ETWQQM -2014 Conference Proceedings), 2014: 52-55.

ANNEXURE – II

Papers Presented In International and National Conferences/Seminars

1. **Ankita Jain,** Vijay Devra. Oral Presentation of Research Work of Thesis in **Annual Research Seminar Organised by Centre For Excellence (Model College)** at J. D.B. Govt. Girls College, Kota on 2nd March, 2013.

2. **Ankita Jain,** Vijay Devra. Participated in **1st Rajasthan Science Congress** held at Tagore International School Campus, Jaipur, Rajasthan during 11th to 13th May, 2013.

3. **Ankita Jain,** Vijay Devra. Presented a Poster in **"National Conference on Global Environmental Changes and Disaster Management for sustainable Life on Earth – A Burning Issue"** at Maharishi Arvind College of Engineering and Technology, Ranpur, Kota, Rajasthan held on 21st October, 2013.

4. **Ankita Jain,** Vijay Devra. Participated in **"5th National Academic Workshop on Organic Reaction Mechanism and Analytical Techniques used in Chemical Sciences"**, organized by Department of Pure and Applied Chemistry, University of Kota, Kota during 21st to 25th October, 2013.

5. "Kinetic Analysis of Oxidation of Ofloxacin by Permanganate Ion in Sulphuric Acid Medium: A Mechanistic Approach", **Ankita Jain,** Shikha Jain, Vijay Devra. Paper Presentation in **"National Seminar on Pure and Applied Chemical Sciences (Current Trends and Future Prospects)"** in Association with Indian Chemical Society, Kolkata NSPACS-2014 Organised by Department of Chemistry, Faculty of Science, Jai Narayan Vyas University, Jodhpur (Rajasthan) India held on 10th and 11th January, 2014.

6. **Ankita Jain,** Vijay Devra. Paper Presentation in National Seminar and Science Exhibition on **"Innovations in Science and Technology for Inclusive Development"** organised by Dr. B. Lal Institute of

Biotechnology and Indian Science Congress Association, Jaipur, held on 16th and 17th January, 2014.

7. "Correlation Analysis of Physico-Chemical Parameters and Water Quality of Chambal River: A case study of Kota City", **Ankita Jain,** Shikha Jain, Niharika Nagar, Pankaj Kachhawah, Vijay Devra. Paper Presentation in National Conference on **"Emerging Trends in water Quantity and Quality Management"** organised by Department of Chemistry and Department of Civil Engineering, Poornima University, Jaipur during 19th to 20th December, 2014.

8. "Synthesis of Dispersed Copper Nanoparticles by Chemical Reduction Method", Shikha Jain, **Ankita Jain,** Vijay Devra. Paper Presentation in 3rd International Conference on **"Advance Trends in Engineering, Technology and Research" (ICATETR-2014),** at Bal Krishna Institute of Technology, Kota, Rajasthan during 22nd to 24th December, 2014.

9. **Ankita Jain,** Vijay Devra. Oral Presentation of Research Work of Thesis in **Annual Research Seminar Organised by Centre For Excellence (Model College)** at J. D.B. Govt. Girls College, Kota on 30th January, 2015.

10. "Catalytic Oxidation of Levofloxacin by Hexacyanoferrate(III) in Aqueous Alkaline Medium: A Kinetic Study", Gajala Tazwar, **Ankita Jain,** Vijay Devra. Paper Presentation in **"International Conference on Innovative Research in Science, Technology and Management"** at Modi Institute of Management and Technology, Dadabari, Kota, Rajasthan during 22nd to 23rd January, 2017.

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Kinetics and mechanism of permanganate oxidation of nalidixic acid in aqueous alkaline medium

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ABSTRACT

The kinetics and mechanism of oxidation of nalidixic acid (NA) by permanganate ion in alkaline medium have been studied at 40 ± 1°C. The Stoichiometry was observed to be 2:1 in terms of mole ratio of permanganate ion and nalidixic acid consumed. The reaction shows first order with respect to oxidant and fractional order in both the substrate and alkali concentration. The oxidation reaction proceeds via an alkali permanganate species that forms a complex with nalidixic acid and the complex then decomposes to give the product. The effects of added products and ionic strength have also been investigated. The main products identified were hydroxylated NA and Mn(VI). A mechanism was proposed on the basis of experimental results. Investigation of the reaction at different temperature allowed the determination of the activation parameters with respect to the slow step of the proposed mechanism.

INTRODUCTION

Potassium permanganate widely used as oxidizing agent play vital role in the kinetics of number of organic and biological active compounds (Fatiadi, 1987; Ladbury and Cullis, 1958; William, 1958; Banerji, 1988; Baljeet and Kothari, 1997). Oxidation reactions by Potassium permanganate are of considerable academic and technological importance because of variable oxidation states. Permanganate is one such powerful multi-electron oxidant which can exist in various oxidation states, among which +7 is its highest oxidation state, which occurs in the Oxo compounds like MnO_4^-, Mn_2O_7, MnO_3F. Out of which MnO_4^- is the most commonly used well known oxidant species to carry out kinetic studies in acidic, neutral and alkaline media. Oxidation by permanganate ion find extensive applications in organic syntheses (Fatiadi, 1987; Stewart and Wiberg, 1965; Freeman, 1976; Lee, 1980; Lee and Tranhanovsky, 1982; Simandi *et al*., 1983; Lee *et al*., 1987) especially since the introduction of phase transfer catalysis (Lee, 1980; Lee and Tranhanovsky, 1982; Lee *et al*., 1987) which permits the use of solvents like methylene chloride and benzene. Kinetic studies are vital sources of mechanistic information on these reactions, as validated by result stating to unsaturated acids in both aqueous (Fatiadi, 1987; Stewart and Wiberg, 1965; Freeman, 1976; Lee, 1980; Lee and Tranhanovsky, 1982; Simandi *et al*., 1983; Lee *et al*., 1987; Wiberg *et al.* 1973) and non-aqueous media (Wiberg *et al.* 1973). As is known, in aqueous alkaline medium the permanganate ion oxidizes a number of organic compounds, which are not, or only very slowly, attacked in acidic or neutral medium (Ladbury and Cullis, 1958; William, 1958), (Drummond and Waters, 1935). The mechanism of oxidation depends on the nature of the substrate and pH of the reaction mixture (Stewart *et al.* 1997). In strongly alkaline medium, the stable reduction product (Simandi *et al.*, 1985; Timmanagoudar *et al.*, 1997; Nadimpalli *et al*., 1993) of permanganate is manganate ion, MnO_4^{2-}. MnO_2 appears only after long time, i.e., after the complete consumption of MnO_4^-.

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No mechanistic information is available to discriminate between a direct one-electron reduction to Mn(VI) and a mechanism in which a hypomanganate ion formed in a two-electron reduction followed by its rapid re-oxidation (Panari *et al*., 1998; Bohn *et al*., 1992).

The manganese chemistry involved in these multistep redox reactions is a significant source of information as the manganese intermediates are relatively easy to identify when they have sufficiently long life time and oxidation states of the intermediates permit useful deductions as to the possible reaction mechanisms including the nature of intermediates.

Fluoroquinolones are broad-spectrum antibacterial agents used to treat the bacterial infections in human beings. Pharmaceuticals, of which antibacterial groups are important, have been identified as evolving environmental contaminants (Johnson *et al*., 2003). A major fraction of fluoroquinolones pass into the domestic sewage due to partial metabolism in the human body. This represents the main route for entry of such pharmaceutical compounds into natural aquatic environment. In this perception, transformations of fluoroquinolone antibacterial agents in suitable water treatment process definitely play a major role (Halling-Sorensen *et al*., 1998). Nalidixic acid (NA) with molecular formula $C_{12}H_{12}N_2O_3$ (1-ethydm-3, 4-dihydro-7-methyl-4-oxo-1, 8-naphthyridine-3-carboxylic acid) (Figure 1) is the first synthesized antimicrobial quinolone. NA is an ionizable, non-biodegradable photosensitive molecule (Mascolo *et al.*, 2010; Ge *et al.*, 2010) with a carboxylic acid function having a pKa of 5.95 (Ross and Riley, 1990).

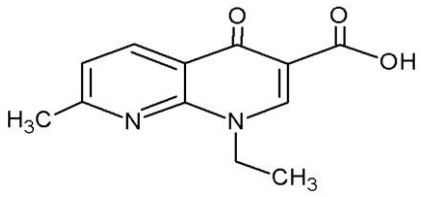


Fig. 1: Structure of Nalidixic acid.

NA is an antibacterial drug still widely used for urinary tract infections (Barlow, 1963). Its two major metabolites are 7-hydroxynalidixic acid (HNA), which exhibits antibacterial properties equal to NA (Mcchesney *et al*., 1964; Moore *et al*., 1965; Portmann *et al.*, 1966) and 7-carboxynalidixic acid (CNA), which is inactive. Permanganate has been widely used for the water and wastewater treatment from last five decades (Hicks, 1976). The oxidation of nalidixic acid by permanganate was studied to investigate the kinetics and mechanism.

MATERIALS AND METHODS

Chemicals

All chemicals used were of analytical grade and doubly distilled water was used throughout this study. Standard solution of nalidixic acid (KORES India Limited) was prepared by dissolving calculated quantity of pure drug in double distilled water. Permanganate solution was obtained by dissolving potassium permanganate (BDH Analar) in water and standardized by titrating against oxalic acid (Vogel, 1967). Freshly prepared & standardized permanganate solutions were always used in kinetics experiments. The Mn(II) solution was made by dissolving manganese sulphate (BDH) in water. NaOH (BDH) and $NaNO_3$ (MERCK) were used to provide required alkalinity and ionic strength respectively.

Instrumentation

For kinetic measurements, a Peltier accessory (temperature-Controlled) attached to a U.V. 3000^+ UV-Visible spectrophotometer (LABINDIA) was used. For product analysis, LC-ESI-MS, (Q-TOF Micromass, WATERS Company, UK), alpha-T FTIR spectrophotometer (BRUKER, Germany), and for pH measurements MSW-552 pH meter were used.

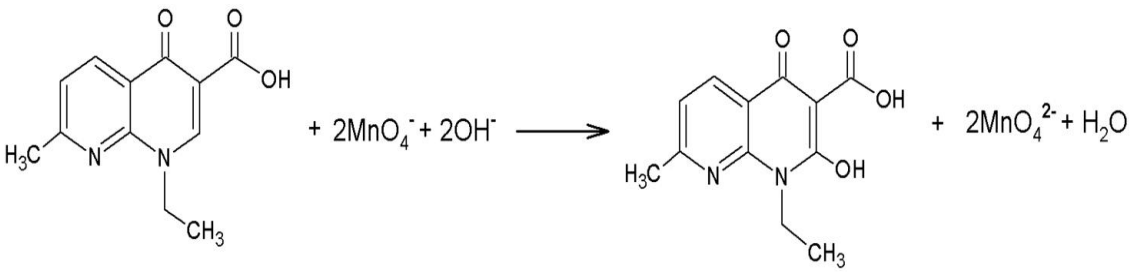
Kinetic Measurements

All kinetic measurements were conducted under pseudo-first-order conditions, where the concentration of nalidixic acid was much greater than permanganate ion concentration at constant temperature 40 ± 0.1°C unless otherwise stated. The reaction was initiated by mixing thermostated solution of permanganate and nalidixic acid; in addition to that required quantities of NaOH and $NaNO_3$ are added to provide required alkalinity and ionic strength of reaction. The progress of the reaction was followed spectrophotometrically at 525nm. The application of Beer's law to permanganate at 525nm had been verified. The molar absorptivity index of permanganate was found to be ε = 2260 ±50 $dm^3\,mol^{-1}\,cm^{-1}$ as a function of time (compared to the literature, ε = 2200, Simandi *et al*., 1985). The kinetics reactions were followed more than 85 % completion of the reaction. The pseudo-first-order rate constants k_{obs} were calculated from the plots of the logarithm of absorbance versus time, which were linear. The values of k_{obs} were reproducible within ± 5%.

Stoichiometry and Product Analysis

Different sets of concentration of reactants in 0.5 mol dm^{-3} of OH^- ion and at constant ionic strength, 0.5mol dm^{-3}, were kept over 24 hours at 40°C in a closed container. When [permanganate] > [nalidixic acid], the remaining permanganate concentration was assayed by measuring the absorbance at 525 nm. Estimation of unreacted [MnO_4^-] indicates that 1 mole of nalidixic acid consumed 2 moles of Permanganate; the Stoichiometry of the reaction is given in Scheme 1. The main reaction products were identified as manganese (VI) and 1-ethyl-2-hydroxy-1, 4-dihydro-7-methyl-4-oxo-1, 8-naphthyridine-3-carboxylic acid.

LC/MS analysis of the reaction indicated the presence of a product with molecular ion of m/z 248 corresponds to 1-ethyl-2-hydroxy-1, 4-dihydro-7-methyl-4-oxo-1, 8-naphthyridine-3-carboxylic acid (Figure 2). The molecular ion of nalidixic acid is m/z 232.2. The IR spectroscopy shows a broad peak at 3382.39 cm^{-1}which is due to -OH stretching (Figure 3) and the remaining peaks are of the parent compound.



Scheme 1: Formation of hydroxylated NA and Mn(VI).

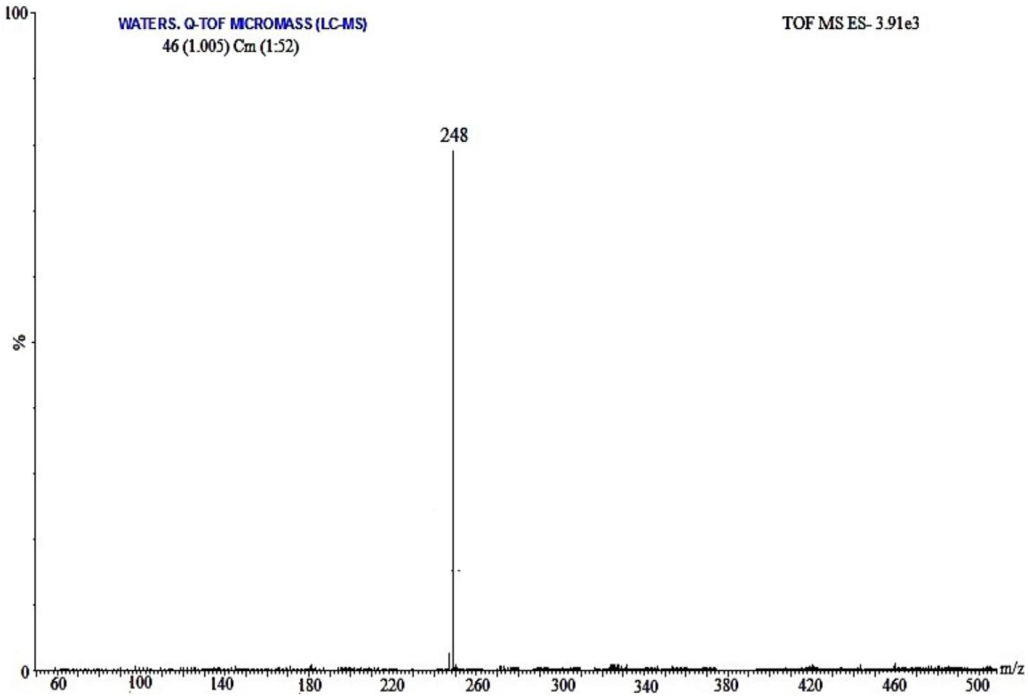


Fig. 2: LC-MS spectra of oxidation product of nalidixic acid.

RESULTS

The reaction orders were determined from the slopes of log k_{obs} versus log [concentration] plots by different concentration of nalidixic acid, permanganate and alkali in turn, keeping all other concentration and conditions constant.

Effect of Concentration of Manganese(VII)

The oxidant permanganate [MnO_4^-] concentration varied from 1×10^{-4} to 7×10^{-4} mol dm^{-3}, and all other concentrations and conditions were constant. The plot of log absorbance versus time was linear (Figure 4) indicating that the reaction is first order with respect to [$KMnO_4$]. The observed pseudo first order rate constant (k_{obs}) were independent of the concentration of $KMnO_4$.

Effect of Concentration of Nalidixic acid

The effect of concentration variation of nalidixic acid on the rate of reaction was studied in the range 2×10^{-3} to 10×10^{-3}mol dm^{-3} at constant concentration of permanganate, alkali and ionic strength at 35°, 40°, 45°C respectively. The rate of reaction increases with increasing concentration of nalidixic acid (Table 1). A plot of log k_{obs} versus log [NA] was linear with a slope of 0.52, thus indicating a fractional-order dependence on nalidixic acid concentration. This was confirmed by the plot of $1/k_{obs}$ versus 1/ [NA] (Figure 5) which was also linear with a positive intercept.

Effect of Concentration of Alkali

The effect of concentration variation of alkali on the rate of reaction was studied in the concentration range 2.0×10^{-1} to 10×10^{-1}mol dm^{-3} at fixed concentration of permanganate, nalidixic acid and ionic strength at three temperatures viz. 35°, 40°, 45°C respectively.

Pseudo first-order rate constant (k_{obs}) was found to be increased with increase in [OH^-] (Table 1). A plot of log k_{obs} versus log [OH^-] was linear with a fractional slope of 0.56. This was confirmed by the plot of $1/k_{obs}$ versus 1/ [OH^-] (Figure 6) which was also linear with a positive intercept.

Table 1: Effects of variation of $[MnO_4^-]$, [NA] and $[OH^-]$ on the oxidation of nalidixic acid by alkaline permanganate at 40°C and I = 0.5 mol dm^{-3}.

10^4 $[MnO_4^-]$ (mol dm^{-3})	10^3 [NA] (mol dm^{-3})	10^1 $[OH^-]$ (mol dm^{-3})	$10^3 k_{obs}$ (s^{-1})
1.0	5.0	5.0	7.25
2.0	5.0	5.0	7.25
3.0	5.0	5.0	7.27
4.0	5.0	5.0	7.24
5.0	5.0	5.0	7.24
6.0	5.0	5.0	7.27
7.0	5.0	5.0	7.24
5.0	2.0	5.0	4.23
5.0	3.0	5.0	5.61
5.0	4.0	5.0	6.57
5.0	5.0	5.0	7.24
5.0	7.5	5.0	8.47
5.0	10.0	5.0	8.78
2.0	2.0	2.0	4.09
2.0	2.0	3.0	5.43
2.0	2.0	4.0	6.56
2.0	2.0	5.0	7.24
2.0	2.0	7.5	8.54
2.0	2.0	10.0	8.92

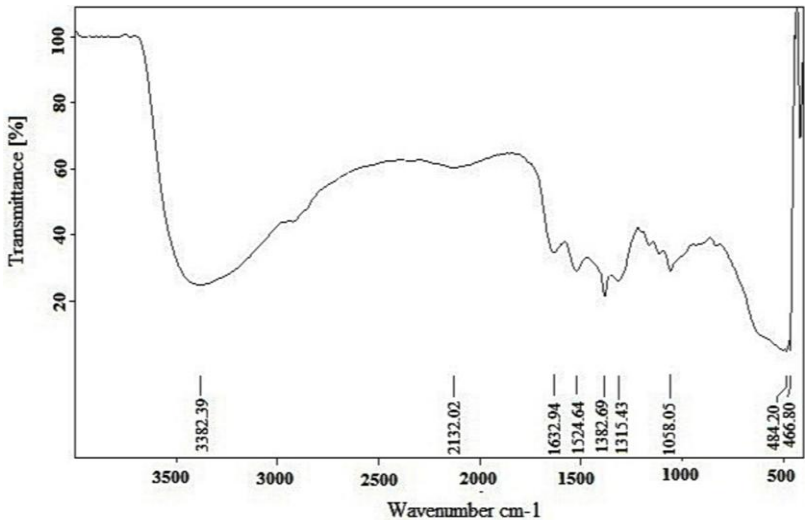


Fig. 3: FT-IR spectra of the product of oxidation of Nalidixic acid by permanganate.

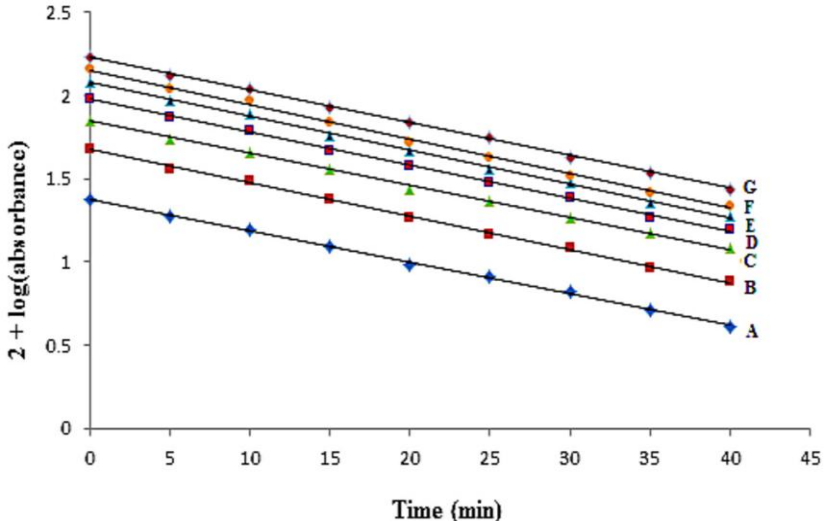


Fig. 4: First order plots of the variation of permanganate concentration at 40°C. [NA]=5.0×10^{-3}, $[OH^-]$ = 0.5 and I = 0.5/ mol dm^{-3}. $[MnO_4^-]\times10^{-4}$ mol dm^{-3} = (A) 1.0, (B) 2.0,(C) 3.0, (D) 4.0, (E) 5.0, (F) 6.0, (G) 7.0

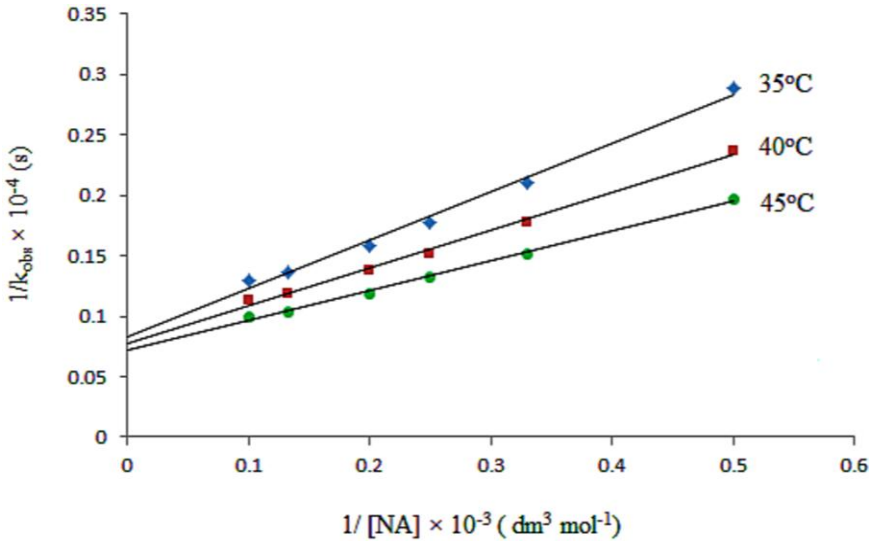


Fig. 5: Plots of $1/k_{obs}$ versus 1/ [NA] at three different temperatures.

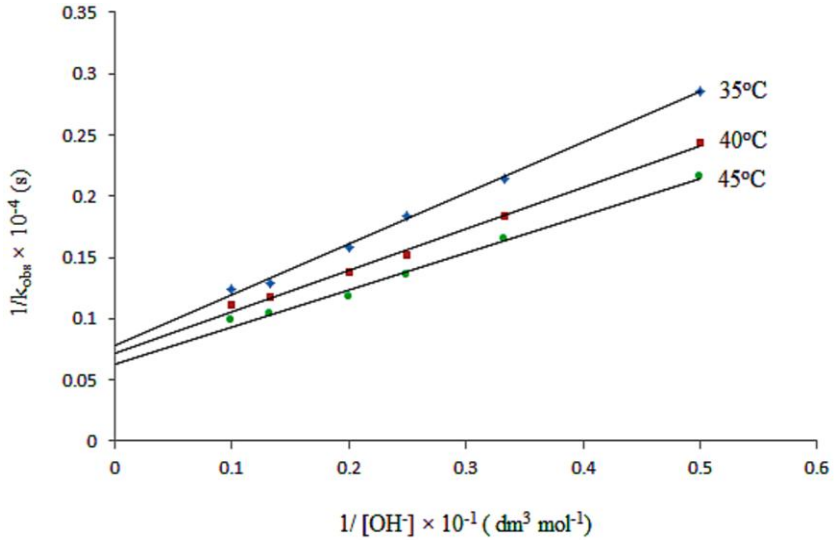


Fig. 6: Plots of $1/k_{obs}$ versus 1/ $[OH^-]$ at three different temperatures.

Effect of Ionic Strength and Dielectric Constant

At constant concentration of reactants and other conditions constant, the ionic strength was varied by varying concentration of sodium nitrate from 0.75 - 1.75 mol dm^{-3}. Ionic strength had negligible effect on the rate of reaction. The effect of the dielectric constant (D) was studied by varying the *t*-butanol–water content (v/v) in the reaction mixture with all other conditions being maintained constant. The rate of reaction increases with increasing t-butanol volume. The plot of log k_{obs} versus 1/D was linear with positive slope (Figure 7).

Effect of Added Products

The manganate ion concentration was varied from 4.0×10^{-5} to 4.0×10^{-4} mol dm^{-3} at constant concentrations of permanganate, nalidixic acid, alkali, and ionic strength. It was found that initially added manganate ion had no effect on the rate of reaction.

Tests for Free Radical

The reaction mixture (10ml) in which known quantity (2ml) of acrylonitrile has been added and kept in an inert atmosphere for 5 hours then diluted with methanol, white precipitate was formed, indicating the intervention of free radicals in the reaction.

The blank experiment of reacting either $KMnO_4$ or nalidixic acid alone with acrylonitrile did not induce polymerisation under the same conditions.

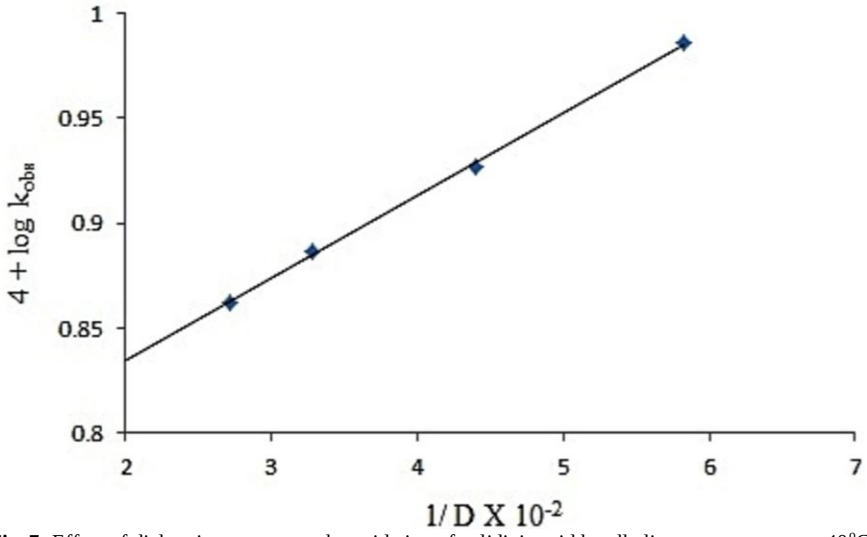


Fig. 7: Effect of dielectric constant on the oxidation of nalidixic acid by alkaline permanganate at 40°C.

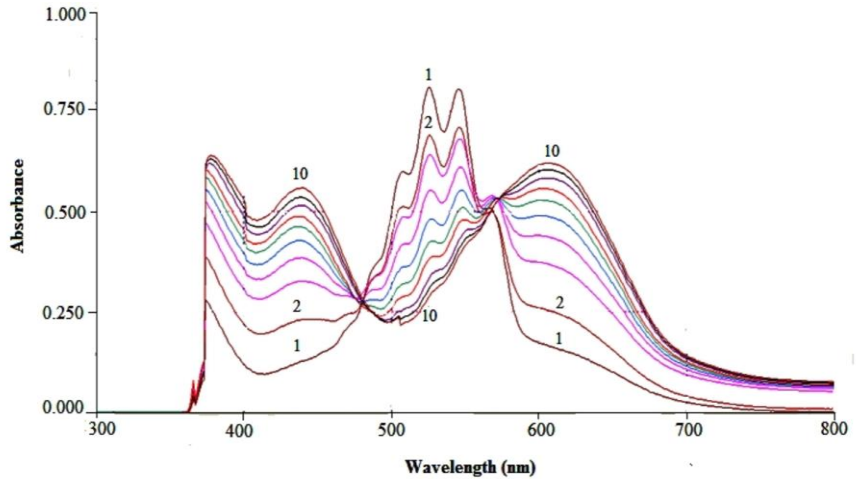


Fig. 8: Spectral changes during the oxidation of nalidixic acid (NA) by permanganate in alkaline medium at 40°C: $[MnO_4^-] = 5.0 \times 10^{-4}$, [NA] = 5.0×10^{-3}, $[OH^-] = 5.0 \times 10^{-1}$ and *I* = 0.5/ mol dm^{-3}.

Table 2: Activation and thermodynamic quantities for the oxidation of nalidixic acid by alkaline permanganate.

Temperature (Kelvin)	10^3 k (s^{-1})	
Effect of temperature with respect to the slow step of figure 10.		
308	1.22	
313	1.29	
318	1.40	
Activation parameters	Value	
E_a (kJ mol^{-1})	11.48	
$\Delta H^{\neq}$ (kJ mol^{-1})	8.88	
$\Delta S^{\neq}$ ± (J K^{-1} mol^{-1})	-289.27	
$\Delta G^{\neq}$ ± (kJ mol^{-1})	88.13	
Equilibrium constants at different temperatures		
Temperature (Kelvin)	$10^{-2}K_1$ (dm^3 mol^{-1})	$10^{-3}K_2$ (dm^3 mol^{-1})
308	12.09	3.30
313	16.77	2.81
318	29.76	1.69
Thermodynamic quantities	Using K_1 values	Using K_2values
ΔH (kJ mol^{-1})	76.58	-55.52
ΔS ± (J K^{-1} mol^{-1})	256.04	-168.03
ΔG ± (kJ mol^{-1})	-3.64	-3.0

DISCUSSION

Permanganate ion is a strong oxidant in an aqueous alkaline media. Since it shows various oxidation states, the stoichiometric results and the pH of reaction medium play a significant role. Under the present experimental conditions at pH > 12, the reduction product of Mn(VII) is stable and further reduction of Mn(VI) might be stopped (Simandi *et al*., 1985; Timmanagoudar *et al*., 1987). However, prolong standing, green Mn(VI) is reduced to Mn(IV) under experimental conditions. The permanganate shows various oxidation states, such as Mn(VII), Mn(V), and Mn(VI) in the alkaline medium. The colour of the reaction mixture changes from violet Mn(VII) to dark green Mn(VI) through blue Mn(IV) were observed. It is plausible that blue colour originated from the violet of permanganate and the green from manganate, excluding the accumulation of hypomanganate. It is clear from Figure 8 that the concentration of MnO_4^- decreases at wavelength 526 nm, while increases at 610 and 460 nm are due to Mn(VI). As the reaction proceeds, a yellow turbidity slowly develops, and after keeping for a long time the solution decolourises and forms a brown precipitate. This suggests that the initial products might have undergone further oxidation resulting in a lower oxidation state of manganese.

The results shows that OH^- ions first combined with permanganate to form a basic permanganate reactive species $[MnO_4{\cdot}OH]^{2-}$ (Thabaj *et al*., 2007), (Panari *et al*., 1998). Then $[MnO_4{\cdot}OH]^{2-}$ reacts with NA to form a complex (C) (Intermediate).

The less than unit order with respect to NA may be due to the complex formation between the $[MnO_4{\cdot}OH]^{2-}$ and NA before the rate determining step. A plot of $1/k_{obs}$ versus 1/ [NA] (Figure 5) shows an intercept in agreement with complex formation. Further evidence for complex formation was obtained from the UV–VIS spectra of reaction mixtures. Two isosbestic points were observed for this reaction (Figure 8), indicating the presence of an equilibrium before the slow step of the mechanism (Chang, 1981; Sathyanarayana, 2001). Within the complex one electron is transferred from nalidixic acid to Mn(VII). The breaking of this complex (C) is assigned as the slowest step, leading to the formation of an NA radical intermediate and Mn(VI). The radical intermediate reacts with another Mn(VII) species, $[MnO_4{\cdot}OH]^{2-}$, to give the final products (Scheme 2). The effect of ionic strength and dielectric constant on the rate explains qualitatively the involvement of a neutral molecule in the reaction.

From the above mechanism the following rate law, eqn. (1) - (8) can be derived.

$$\text{Rate} = \frac{-d[MnO_4^-]}{dt} = kK_1K_2[MnO_4^-]_f[NA]_f[OH^-]_f \quad (1)$$

Total concentration of permanganate is given by

$$[MnO_4^-]_t = [MnO_4^-]_f + [MnO_4 \cdot OH]^{2-} + [Complex]$$

$$= [MnO_4^-]_f + [MnO_4^-][OH^-] + kK_1K_2[MnO_4^-]_f[NA][OH^-]$$

$$= [MnO_4^-]_f (1 + K_1[OH^-]_f + K_1K_2[OH^-]_f[NA])$$

$$[MnO_4^-]_f = \frac{[MnO_4^-]_t}{1 + K_1[OH^-] + K_1K_2[NA][OH^-]} \quad ..2$$

$[MnO_4^-]_t$ and $[MnO_4^-]_f$ are total and free concentration of Mn (VII) respectively.

Total concentration of $[OH^-]$ is given by:

$$[OH^-]_t = [OH^-]_f + [MnO_4 \cdot OH]^{2-} + [Complex]$$

$$[OH^-]_f = \frac{[OH^-]_t}{1 + K_1[MnO_4^-] + K_1K_2[NA][MnO_4^-]} \quad ..3$$

In view of low concentration of MnO_4^- and nalidixic acid used, above equation can be written as:

$$[OH^-]_f = [OH^-]_t \quad \ldots 4$$

Similarly,

$$[NA]_f = [NA]_t \quad \ldots 5$$

Substituting equation (2), (4) and (5) in equation (1) and omitting "t" and "f" subscripts

$$\text{Rate} = \frac{kK_1K_2[MnO_4^-][OH^-][NA]}{1 + K_1[OH^-] + K_1K_2[OH^-][NA]} \quad \ldots 6$$

$$\frac{\text{Rate}}{[MnO_4^-]} = k_{obs} = \frac{kK_1K_2[OH^-][NA]}{1 + K_1[OH^-] + K_1K_2[OH^-][NA]} \quad \ldots 7$$

Equation (7) can be rearranged as

$$\frac{1}{k_{obs}} = \frac{1}{k\,K_1K_2[OH^-][NA]} + \frac{1}{kK_2[NA]} + \frac{1}{k} \quad \ldots 8$$

According to Eqn (8) the plot of $1/k_{obs}$ versus 1/ [NA] (Figure 5) is linear with positive intercept and slope at three different temperatures. The rate constant k, of the slow step, (Scheme 2) was obtained from the intercept of the plots $1/k_{obs}$ versus 1/ [NA] (Table 2). The energy of activation was determined by the plot of log k versus 1/T from which activation parameters were calculated (Table 2). The equilibrium constant (K_1) and the equilibrium constant of complex (K_2) in Scheme 2 were calculated from the intercept and slope of the plot 1/ k_{obs} versus 1/ $[OH^-]$ (Figure 6) (Table 2).

The value of K_1 is in good agreement with earlier work (Thabaj *et al*., 2007) at 40°C. Van't Hoff's plots of log K_1 versus 1/T and log K_2 versus 1/T gave the values of enthalpy of reaction ΔH, entropy of reaction ΔS and free energy of reaction ΔG, calculated for the first, and second equilibrium steps (Table 2).

The values of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ are both favourable for electron transfer process (Farokhi and Nandibewoor, 2004). The value of $\Delta S^{\neq}$ within the range of radical reaction has been ascribed (Walling, 1957) to the nature of electron pairing and unpairing process. The negative value of $\Delta S^{\neq}$ indicates that complex is more ordered than the reactants (Rangappa *et al*., 2001; Bugarcic *et al*.,

2006). The observed modest enthalpy of activation and a relatively low value of the entropy of activation as well as a higher rate constant of the slow step indicate that the oxidation probably occurs via inner-sphere mechanism (Farokhi and Nandibewoor, 2003).

CONCLUSION

It is interesting that the oxidant species [MnO_4^-] requires pH > 12, below which the system becomes disturbed and the reaction proceeds further to give a reduced oxidation product as manganese(IV), which slowly develops a yellow turbidity. Hence, the role of pH in the reaction medium is crucial. The oxidant, manganese(VII), exists in alkali media as alkali-permanganate species [$MnO_4 \cdot OH]^{2-}$, which takes part in the chemical reaction. Chemical oxidation using Mn(VII) has been widely used for treatment of pollutants in drinking water and waste water applications. The proposed mechanism is consistent with product, mechanism and kinetic studies.

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Research Article

Oxidation of levofloxacin by acidic permanganate: a kinetic and mechanistic study

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ABSTRACT

The kinetic and mechanistic investigation of oxidation of levofloxacin (LF) has been studied by permanganate ion in aqueous sulphuric acid medium at 25°C. The reaction followed first-order kinetics with respect to [LF], and [H+] in their lower concentrations range, tending to zero-order at their higher concentrations. The effect of added products and dielectric constant of the medium was studied on the rate of reaction. Effect of varying salt electrolyte concentration was insignificant showing that the molecular species was involved in the rate determining step. The main products were identified by spot test, FT-IR, and LC-MS. A mechanism was proposed on the basis of experimental results. The activation parameters with respect to the slow step of the mechanism were evaluated, and the thermodynamic parameters were also determined and discussed.Potassium permanganate widely used as oxidizing agent in the kinetics of number of organic and biological active compounds. Permanganate is multi-electronoxidant, which can exist in various oxidation states, among which +7 is its highest oxidation state. In acidic medium it exists in different forms as HMnO4, HMnO4+, HMnO3, Mn2O7 and one depending on the nature of the reductant. Levofloxacin is a broad spectrum drug of activity against various bacteria, including gram-positive and gram-negative microorganisms. Kinetic measurements were performed on a Peltier accessory (temperature-Controlled) attached to a U.V.3000+ UV-Visible spectrophotometer (LABINDIA).The product analysis is characterized by LC-ESI-MS and FT-IR studies. The reaction stoichiometry indicates that 5 moles of levofloxacin require 2 moles of Mn(VII). The oxidation products were identified as 7-amino fluoroquinolone and Mn (II). The reaction shows first order kinetics with respect to MnO4- and fractional order with respect to levofloxacin and hydrogen ion concentration. The effect of added product, varying salt electrolyte were studied on the rate of reaction. The rate constant of the slowest step and other equilibrium constants involved in the mechanism are evaluated. Overall mechanistic sequence described here is consistent with product, mechanistic and kinetic studies.

Key words: *Kinetics, Oxidation, Mechanism, Levofloxacin, Permanganate ion, Sulphuric acid medium..*

INTRODUCTION

Levofloxacin (LF), (-)-(S)-9-fluoro-2,3-dihydro-3-methyl- 10-(4-methyl-1-piperazinyl)-7-oxo-7H pyrido [1,2,3-de]- 1,4-benzoxazine-6-carboxylic acid hemihydrates (Figure. 1), is one of the commonly used third-generation fluoroquinolone antimicrobials, being the active S-isomer isolated from racemic ofloxacin and is twice as active as the parent drug.

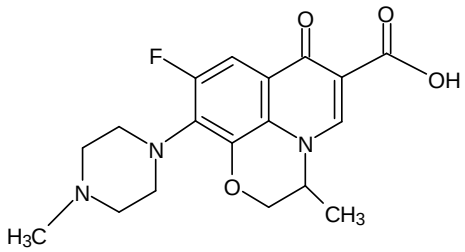


Figure 1 - Structure of levofloxacin (LF).

Levofloxacin is a broad spectrum drug of activity against various bacteria, including gram-positive and gram-negative microorganisms[1, 2]. Because of its effective antibacterial activity and low frequency of adverse effects on oral administration, levofloxacin has been widely used for the treatment of infectious diseases, such as community-acquired pneumonia and acute exacerbation of chronic bronchitis[3].The interaction of fluoroquinolone with metal ions is of interest not only for the development of analytical techniques but also to afford information about the mechanism of action of the pharmaceutical preparation[4]. Since the metal ions cause fluorescence quenching of the drug, the spectrofluorimetric method for quantitative determination of the quinolone type drugs has been developed[5] along with titrimetric[6], spectrophotometric[7,8], electrochemical[9],electrophoretic[10] and chromatographic[11]techniques. The increase of fluoroquinolone in aquatic environments, even in low concentration, may cause intimidation to the ecosystem and human health by including the multiplication of drug resistance bacteria owing to long term exposure[12]. Chemical oxidation of pollutant in drinking water and waste water by Chloramine-T[13] has been widely done. A number of kinetic study on oxidation of levofloxacin in alkaline, aqueous and acidic medium have been reported[7,13-16] . In view of potential pharmaceutical importance of levofloxacin and lack of literature on the oxidation of this drug and complexity of the reaction, a detail study of the reaction become important. Potassium permanganate widely used as oxidizing agent play key role in the kinetics of number of organic and biological active compounds[17-21]. Literature survey reveals that permanganate ions are widely used as oxidizing agent in synthetic, analytical chemistry[22,23]and also as a disinfectant[24,25]. It has been used in the determination of content of pharmaceutical formulation, [26-27] as oxidizing agent for removal of organic molecules and heavy metals from the nuclear waste[25]. Oxidation reactions by Potassium permanganate are of considerable academic and technological importance because of variable oxidation states. Permanganate is one such powerful multi-electronoxidant which can exist in various oxidation states, among which +7 is its highest oxidation state, which occurs in the Oxo compounds like MnO_4^-, Mn_2O_7, MnO_3F. Out of which MnO_4^- is the most commonly used well known oxidant species to carry out kinetic studies in acidic, neutral and alkaline media. In acidic medium it exists in different forms as $HMnO_4$, $HMnO_4^+$, $HMnO_3$, Mn_2O_7 and one depending on the nature of the reductant[29]. So this study is concerned with the identity of the redox reaction and to explore a suitable mechanism for oxidation oflevofloxacin by $KMnO_4$in acidic medium on the basis of kinetics parameters.

MATERIALS AND METHODS

Chemicals

All chemicals used were of analytical grade and doubly distilled water was used throughout this study. Standard solution of levofloxacinwas prepared by dissolving known amount of the drug in double distilled water.Permanganate solution was obtained by dissolving potassium permanganate (BDH Analar) in water and standardized by titrating against oxalic acid[30].Freshly prepared & standardized permanganate solutions were always used in kinetics experiments. The Mn (II) solution was made by dissolving manganese sulphate (BDH) in water. Na_2SO_4 (BDH) and H_2SO_4 (MERCK) were used to provide required ionic strength and acidity respectively.

Instrumentation

For kinetic measurements, a Peltier accessory (temperature-Controlled) attached to a U.V.3000$^+$ UV-Visible spectrophotometer (LABINDIA) was used.For product analysis, LC-ESI-MS, (Q-TOF Micromass, WATERS Company, UK), alpha-T FTIR spectrophotometer (BRUKER, Germany), and for pH measurementsMSW-552 pH meter were used.

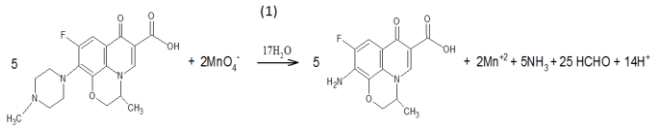
Kinetic Measurements

All kinetic measurements were conducted under pseudo-first-order conditions, where the concentration of levofloxacin was much greater than permanganate ion concentration at constant temperature 25± 0.1^{o}C unless otherwise stated. The reaction was initiated by mixing thermostated solution of permanganate and levofloxacin; in addition to that required quantities of H_2SO_4, Na_2SO_4 are added to provide required acidity and ionic strength of reaction. The progress of the reaction was followed spectrophotometrically at 525nm. The Beer's law verified in permanganate concentration range $(0.50 – 5.0) \times 10^{-4}$ $moldm^{-3}$ at 525nm. The molar absorptivity index of permanganate was found to be $\varepsilon = 2260 \pm 50$ $dm^3mol^{-1}cm^{-1}$ as a function of time (compared to the literature, $\varepsilon = 2200$ [31]). The kinetics reactions were followed more than 85 % completion of the reaction. The pseudo-first-order rate constants k_{obs}were calculated from the plots of the logarithm ofabsorbance versus time, which were linear. The values of k_{obs}were reproducible within ±5%.

RESULTS AND DISCUSSION

Stoichiometry and Product Analysis

Different sets of concentration of reactants at constant concentration of sulphuric acid and ionic strength were kept over 24 hrsat25^{o}C in a closed container. When [permanganate] > [levofloxacin], the remaining permanganate concentration was assayed by measuring the absorbance at 525 nm. Estimation of unreacted[MnO_4^-] indicates that 5 moles of levofloxacin consumed 2 moles of Permanganate; the Stoichiometry ofthe reaction is given in equation (1).



[LF]

LC/MS analysis of levofloxacin oxidation reaction indicates the formation of product with molecular ions of m/z 279 (Figure. 2). The molecular ion of levofloxacin is m/z 361.4. The m/z 279corresponds to full dealkylation of the piperazine ring (i.e. the $–NH_2$ product). It is worth noting, that oxidation of piperazine moiety of levofloxacin between oxidized centres and nitrogen atoms lead to distinctive mass loss m/z = 69 and m/z = 83. This was attributed to ring opening, dealkylation and deamination process, which finally yielded 7-amino fluoroquinolone product.Theproduct was also short written as M-69, indicating the net mass loss of the product from the parent levofloxacin. Infrared Spectroscopy analysis confirmed the presence of $–NH_2$ group in the oxidation product (Figure. 3). The Infrared spectrum shows a peak at 3412.70 cm^{-1}which is due to -NH stretching of the $–NH_2$ group and the remaining peaks are of the parent compound (quinolone ring).Theby-product formaldehyde was identified by spot test[32]. The other product ammonia was detected by Nesseler's reagent test[33].

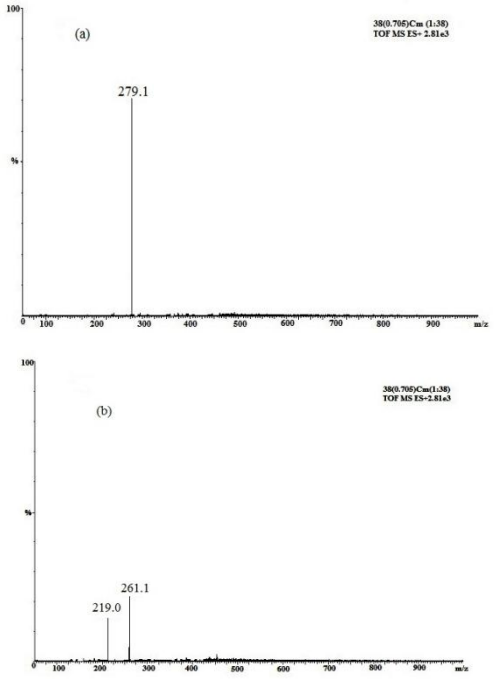


Figure 2 - LC-ESI-MS spectra of oxidation product of levofloxacin. (a) Molecular ion peak of m/z 279 (M-69). (b) Fragmentation of (M-69) product.

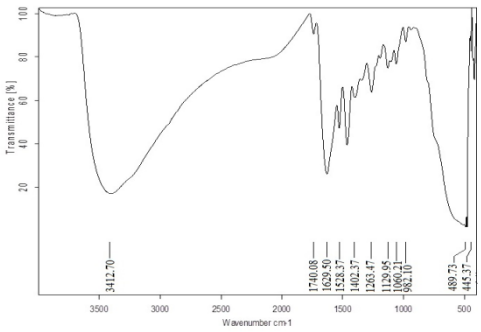


Figure3 - FT-IR spectra of the product of oxidation of levofloxacin by permanganate.

Reaction Orders

The reaction orders were determined from the slopes of log k_{obs} versus log [concentration] plots by different concentration of levofloxacin, permanganate and acid in turn, keeping all other concentration and conditions constant.

Dependence of Rate on the Concentration of Permanganate

The oxidant permanganate [MnO_4^-] concentration varied from7.5×10^{-5} to 6×10^{-4}moldm^{-3}, and all other concentrations and conditions were constant. The plot of log absorbance versus time was linear (Figure. 4) indicating that the reaction is first order with respect to [$KMnO_4$]. The observed pseudo first order rate constant (k_{obs}) were independent of the concentration of $KMnO_4$ (Table-1).

Table 1:"Effect of variation of [MnO_4^-], [LF] and [H^+] on the oxidation of levofloxacin by acidic permanganate at 25°C and I = 0.02 mol dm^{-3}"

S. No.	10^4MnO_4(mol dm^{-3})	10^3 [LF] (mol dm$^-$)	10^2 [H^+] (mol dm^{-3})	10^3k_{obs} (s^{-1})
1	0.75	1.0	1.0	8.25
2	1.0	1.0	1.0	8.23
3	2.0	1.0	1.0	8.25
4	3.0	1.0	1.0	8.25
5	4.0	1.0	1.0	8.25
6	5.0	1.0	1.0	8.23
7	6.0	1.0	1.0	8.23
8	2.0	2.0	1.0	9.06
9	2.0	3.0	1.0	13.22
10	2.0	4.0	1.0	15.53
11	2.0	5.0	1.0	17.38
12	2.0	6.0	1.0	19.23
13	2.0	7.0	1.0	20.4
14	2.0	2.0	0.5	4.71
15	2.0	2.0	0.6	5.46
16	2.0	2.0	0.7	6.24
17	2.0	2.0	0.8	7.49
18	2.0	2.0	0.9	8.33
19	2.0	2.0	1.0	9.06
20	2.0	2.0	1.5	9.89
21	2.0	2.0	2.0	10.62

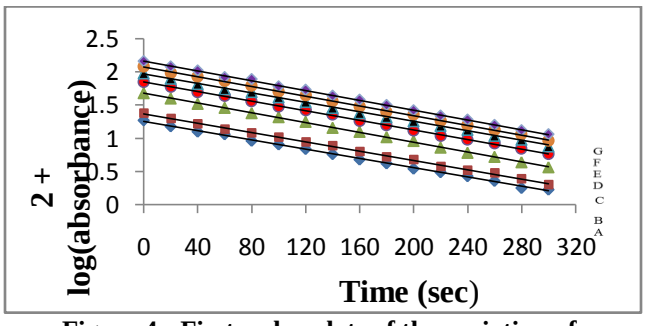


Figure 4 - First order plots of the variation of permanganate concentration at 25oC.

[LF]=1.0×10-3mol dm-3,[H+] = 1.0×10-2 mol dm-3and I = 0.02mol dm-3. [MnO4-]×10-4 mol dm-3 = (A) 0.75, (B) 1.0,(C) 2.0, (D) 3.0, (E) 4.0, (F)5.0, (G) 6.0

Dependence of Rate on the Concentration of Levofloxacin

The effect of concentration variation of levofloxacin on the rate of reaction was studied in the range 2×10^{-3} to 7×10^{-3}mol dm^{-3} at constant concentration of permanganate, acid and ionic strengthat three temperatures viz. 20°, 25°,30°Crespectively. The rate of reaction increases with increasing concentration of levofloxacin (Table 1). A plot of log k_{obs} versus log [LF] was linear with a slope of 0.64, thus indicating a fractional-order dependence on levofloxacin concentration. This was confirmed by the plot of $1/k_{obs}$ versus 1/ [LF] (Figure.5) which was also linear with a positive intercept.

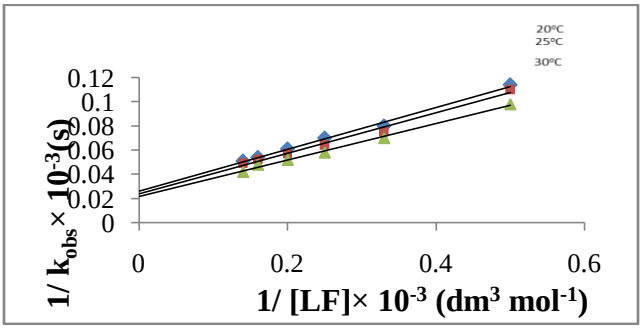


Figure 5 - Plots of 1/kobs versus 1/ [LF] at three different temperatures. [KMnO4] = 2.0 x 10-4 mol dm-3, [H+] = 1.0 x 10-2 mol dm-3 and I = 0.02 mol dm-3.

Dependence of Rate on the Concentration of Sulphuric Acid

The effect of concentration variation of sulphuric acid on the rate of reaction was studied in the concentration range 5×10^{-3} to 2×10^{-2}moldm^{-3} at fixed concentration of permanganate,levofloxacin and ionic strength at three temperaturesviz. 20°, 25°, 30°C respectively. Pseudo first-order rate constant(k_{obs})was found to be increased with increase in [H^+] (Table 1). A plot of log k_{obs} versus log [H^+] was linear with a fractional slope of 0.60. This was confirmed by the plot of $1/k_{obs}$ versus 1/ [H^+] (Figure.6) which was also linear with a positive intercept.

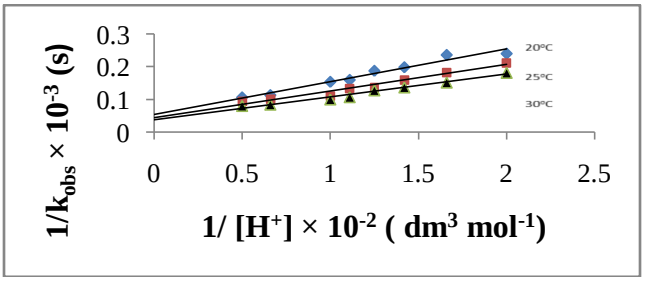


Figure 6 - Plots of 1/kobs versus 1/ [H+] at three different temperatures. [KMnO4] = 2.0 x 10-4 mol dm-3, [LF] = 2.0 x 10-3 mol dm-3 and I = 0.02 mol dm-3.

Dependence of Rate on Ionic Strength and Dielectric Constant

Effect of change in varying electrolyte concentration was monitored to establish the nature of intermediate species in the rate determining step by Na_2SO_4. It was observed that the change in an ionic strength of the medium does not alter the rate constant. The absence of salt effect indicates that the reaction does not take place between ionic species. The slope of plots between log k_{obs} against $\sqrt{\mu}$was zero, which confirms the presence of the molecular species in the rate determining step. At constant acidity and other constant conditions, as the t-butyl alcohol content increase from 0 to 50% (v/v) in the reaction, change in dielectric constant had negligible effect on the rate of reaction.

Neutral Salts Dependence

The effect of added neutral salt on the rate of reaction has been studied at varying concentration 1×10^{-2} - 4×10^{-2} mol $dm^{-3}$of $NaNO_3$, CH_3COONa and NaF at fixed concentration of other reactant and constant conditions. Addition of different sodium salts has no effect on the reaction rates.

Effect of Initially Added Products

The initial added products, Mn(II) was studied in the range of 5×10^{-5} to 5×10^{-4} mol dm^{-3} while other reactants concentration and conditions constant, does not change the rate of reaction.

Test for Free Radical

The reaction mixture (10ml) in which known quantity (2ml) of acrylonitrile has been added and kept in an inert atmosphere for 5 hours then diluted with methanol, white precipitate was formed, indicating the intervention of free radicals in the reaction. The blank experiment of reacting either $KMnO_4$ or levofloxacin alone with acrylonitrile did not induce polymerisation under the same conditions.

Permanganate ion, MnO_4^- ion is powerful oxidizing agentin acidic medium. The stable oxidation product of MnO_4^-in acid medium is Mn(II). Figure 7 illustrates the spectroscopic changes occurring in the oxidation of levofloxacin by acid permanganate at 25°C with scanning interval of 1minute. The literature survey reveals that[34]Mn(IV) ion absorbs in region 400-600 nm. Figure 7 shows no features in this wavelength area indicating that MnO_2 is not a reaction product.

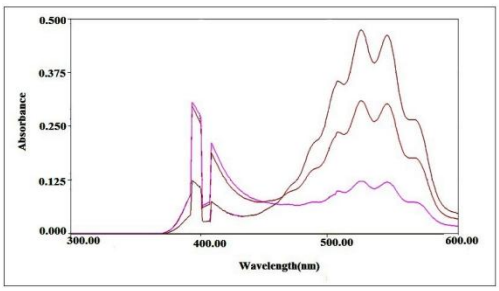


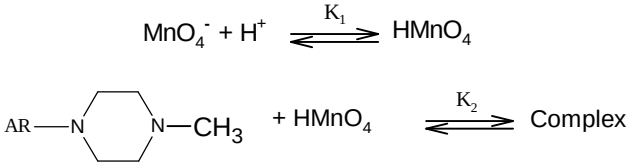
Figure 7 - Spectral changes during the oxidation of levofloxacin (LF) by permanganate in acidic medium at 25°C. [$KMnO_4$] = 2.0×10^{-4}mol dm^{-3}, [LF] = 2.0×10^{-3}mol dm^{-3}, [H^+] = 1.0×10^{-2}mol $dm^{-3}$and I = 0.02 mol dm^{-3}

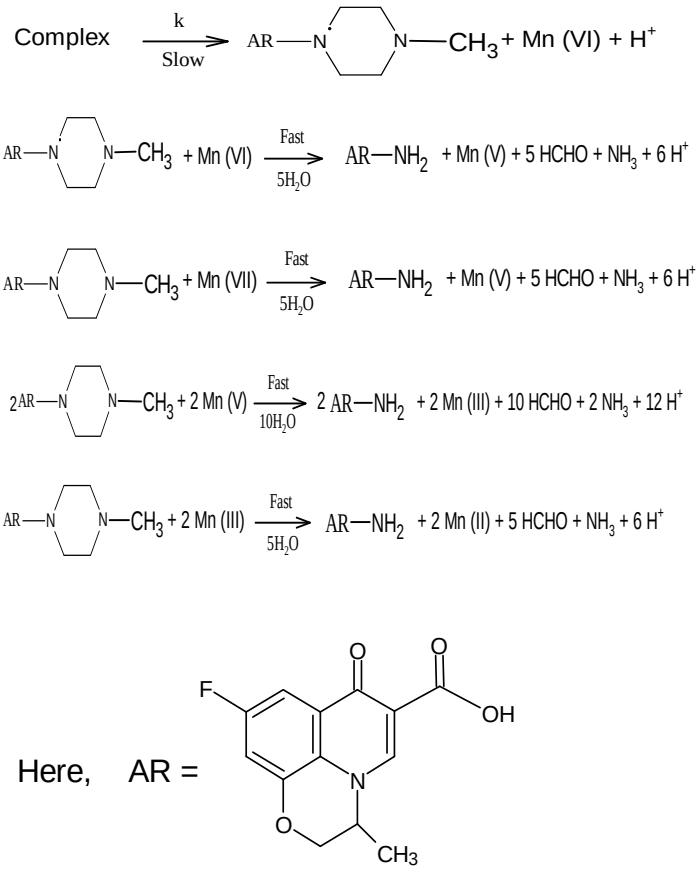
The reaction between levofloxacin and permanganate in sulphuric acid has Stoichiometry 5:2, with first order dependence with permanganate and less than unit order with H^+ concentration and levofloxacin concentration. The fact that Mn(II) is the reduced product of Mn(VII) in the reaction might indicate that levofloxacin shows a strong reducing character in H_2SO_4 medium. In view of the presence of sulphuric acid in thereactionmixture,theoxidationof LF by sulphuric acidwas checked, and it was found to be negligible compared to the oxidation of LF by permanganate. The active species of permanganate in aqueous acid solution may be deduced from the dependence of the rate on $[H^+]$, in the reaction medium. The order of $[H^+]$ is less than unity, which may be indicate the formation of permanganate acid from permanganate ion. Permanganate acid $HMnO_4$ is more efficient oxidant species of Manganese (VII) then permanganate ion[35]. It has been observed that the rate of reaction was tending to attain a limiting value at higher concentration of $[H^+]$ ion, which indicates that only the protonated form is active then acid permanganate[36].Thenegligibleeffectofionic strength ontherateofreactionalso confirmsthat$HMnO_4$istheactive species of MnO_4^-, can be represented by equation-(2)

$$MnO_4^- + H^+ \underset{}{\overset{K_1}{\rightleftharpoons}} HMnO_4 \qquad (2)$$

Where K_1 is the equilibriumconstant of $HMnO_4$.

In view of increasing the rate with increase in $[H^+]$ ion, in the prior equilibrium step, H^+ reacts with MnO_4^- to form $HMnO_4$, which reacts with the one mole of levofloxacin to form a complex. Complex formed is dissociate in the rate determining step to give a free radical derived from levofloxacin and an intermediate Mn(VI). In further fast steps the intermediate Mn(VI) reacts with a free radical to produce the product 7-amino fluoroquinolone, NH_3, HCHO and intermediate Mn(V), subsequently reduced to the end product Mn(II). Although Mn(VI) and Mn(IV) are the final reduced species of MnO_4^- in alkaline and neutral media, it was observed that Mn(II) was the only reduced species of MnO_4^- in acid medium. Attempts were made to allow spectroscopic detection of intermediate Mn(V) and Mn(III) as the reaction proceeded in the oxidation of levofloxacin by permanganate. Unfortunately the low concentration of Mn(V) and Mn(III) intermediate obtained under our experimental conditions made the spectroscopic detection failure. However, the evidence for intermediate such as Mn(V) and Mn(III) are reported in the literature[37, 38].The results are accommodated in the following mechanism (Scheme 1).





Scheme-1Proposed mechanism for the oxidation of Levofloxacin by acidic permanganate.

Following rate law can be derived from scheme 1:

$$\text{Rate} = \frac{-d[MnO_4^-]}{dt} = k[\text{Complex}]$$

$$= kK_2[HMnO_4][LF]$$

$$= kK_1K_2[MnO_4^-]_f[H^+]_f[LF]_f \quad (3)$$

Total concentration of permanganate is given by

$$[MnO_4^-]_t = [MnO_4^-]_f + [HMnO_4] + [\text{Complex}]$$

$$= [MnO_4^-]_f + K_1[MnO_4^-]_f[H^+]_f + K_2[HMnO_4][LF]$$

$$= [MnO_4^-]_f + K_1[MnO_4^-]_f[H^+]_f + K_1K_2[MnO_4^-]_f[H^+]_f[LF]$$

$$= [MnO_4^-]_f \{1 + K_1[H^+]_f + K_1K_2[H^+]_f[LF]\}$$

$$[MnO_4^-]_f = \frac{[MnO_4^-]_t}{\{1 + K_1[H^+]_f + K_1K_2[H^+]_f[LF]} \quad (4)$$

$[MnO_4^-]_t$ and $[MnO_4^-]_f$are total and free concentration of Mn (VII) respectively.

Total concentration of levofloxacin is given by:

$$[LF]_t = [LF]_f + [\text{Complex}]$$

$$= [LF]_f + K_2[LF]_f[HMnO_4]$$

$$= [LF]_f \{1 + K_2[HMnO_4]\}$$

$$[LF]_f = \frac{[LF]_t}{1+K_2[HMnO_4]}$$

Very low concentration of $[MnO_4^-]$ were used in the experiment, so $K_2[HMnO_4] << 1$

$$[LF]_f = [LF]_t \quad (5)$$

Total concentration of $[H^+]$ is given by:

$$[H^+]_t = [H^+]_f + [HMnO_4]$$
$$= [H^+]_f + K_1[MnO_4^-]_f[H^+]_f$$
$$= [H^+]_f\{1+K_1[MnO_4^-]_f\}$$

$$\text{So, } [H^+]_t = [H^+]_f \quad (6)$$

Substituting equation (4), (5) and (6) in equation (3) and omitting "t" and "f" subscripts

$$\text{Rate} = \frac{-d[MnO_4^-]}{dt} = \frac{kK_1K_2[MnO_4^-][H^+][LF]}{1+K_1[H^+]+K_1K_2[H^+][LF]} \quad (7)$$

$$\frac{\text{Rate}}{[MnO_4^-]} = k_{obs} = \frac{kK_1K_2[H^+][LF]}{1+K_1[H^+]+K_1K_2[H^+][LF]} \quad (8)$$

Equation (8) can be rearranged as

$$\frac{1}{k_{obs}} = \frac{1}{kK_1K_2[H^+][LF]} + \frac{1}{kK_2[LF]} + \frac{1}{k} \quad (9)$$

Equation 9, indicates that the linear plots of $1/k_{obs}$ versus $1/[LF]$ and $1/k_{obs}$ versus $1/[H^+]$ were obtained with a straight line and positive intercept on the y-axis (Figure. 5, 6) at three different temperatures.This proves the validity of rate law, and the proposed reaction scheme has been derived. The rate constant k, of the slow step, scheme 1 was obtained from the intercept of the plots $1/k_{obs}$ versus $1/[LF]$ (Table 2). The energy of activation was determined by the plot of log kversus 1/T from which activation parameters were calculated (Table 2). The equilibrium constant of $HMnO_4$ (K_1) and the equilibrium constant of complex (K_2) in scheme-1 were calculated from the intercept and slope of the plot $1/k_{obs}$versus $1/[H^+]$ respectively (Figure.6) (Table 2). The value of K_1 is in good agreement with earlier work[37] at 25°C. Thermodynamic quantities were calculated from the Van't Hoff plot (Table 2). According to the rate determining step in Scheme 1, thechange in the ionic strength and dielectric constant of the medium does not alter thereaction rate, which suggests the involvement of non-ionic species at the rate-determining step[39]. The values of$\Delta H^{\neq}$and$\Delta S^{\neq}$arebothfavourable for electron transferprocess[40].The value of $\Delta S^{\neq}$ within the range of radical reaction has been ascribed[41] to the nature of electron pairing and unpairing process. The negative value of $\Delta S^{\neq}$ indicates that complex is more ordered than the reactants[42]. The observed modest enthalpy of activation and a relatively low value of the entropy of activation as well as a higher rate constant of the slow step indicate that the oxidation presumably occurs via inner-sphere mechanism[43].

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Table 2: "Activation and thermodynamic quantities for the oxidation of levofloxacin by acidic permanganate from scheme 1"

Temperature (Kelvin)	**10^2 k (s^{-1})**	
293	3.84	
298	4.34	
303	4.76	
Activation parameters	**Value**	
E_a(kJ mol^{-1})	16.19	
$\Delta H^{\neq}$ (kJ mol^{-1})	13.72	
$\Delta S^{\neq}$ ± (J K^{-1} mol^{-1})	45.05	
$\Delta G^{\neq}$ ± (kJ mol^{-1})	80.77	
Equilibrium constants at different temperatures		
Temperature (Kelvin)	**$10^{-1}K_1$ (dm^3 mol^{-1})**	**$10^{-2}K_2$ (dm^3 mol^{-1})**
293	4.22	4.82
298	4.07	5.77
303	3.81	6.56
Thermodynamic quantities	**Using K_1 values**	**Using K_2values**
ΔH (kJ mol^{-1})	-19.14	23.16
ΔS ± (J K^{-1} mol^{-1})	-63.53	80.55
ΔG ± (kJ mol^{-1})	-1.14	-1.0

CONCLUSION

The oxidant MnO_4^- exists in acid medium as $HMnO_4$, which takes part in the chemical reaction. The oxidation of levofloxacin by permanganate in acidic medium has a Stoichiometry of 5:2.The oxidation products were identified as Mn(II), 7-amino fluoroquinolone, NH_3 and HCHO. Dealkylated products of levofloxacin have antimicrobial activity. Since dealkylated products are obtained in the present study, it is evident that the products of the title reaction haveantimicrobial activity after oxidation. So this study will be effectively used in waste water treatment at the sites contaminated by fluoroquinolone antibiotics.The rate constant of the slowest step and other equilibrium constants involved in the

mechanism are evaluated, and activation parameters with respect to slowest step were computed.

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Research Article

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Kinetics and Mechanism of Permanganate Oxidation of Ciprofloxacin in Aqueous Sulphuric Acid Medium

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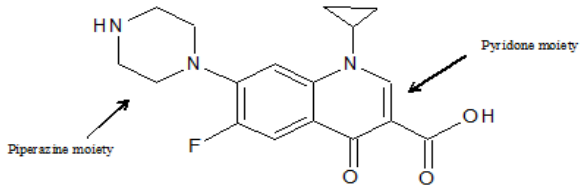
ABSTRACT

The oxidation of ciprofloxacin (CIP) by permanganate ion in aqueous sulphuric acid medium at constant ionic strength (I = 0.05 mol dm^{-3}) has been investigated spectrophotometrically at 525 nm. Order with respect to substrate, oxidant and acid concentrations were determined. Product characterization of reaction mixture indicates the formation of major product m/z 263 corresponding to dealkylation of the piperazine ring of ciprofloxacin. The piperazine moiety of ciprofloxacin is the predominant oxidative site to $KMnO_4$. Product analysis indicates that oxidation of permanganate results in dealkylation at the piperazine moiety of ciprofloxacin, with the quinolone ring essentially intact. The reaction constants involved in different steps of the mechanism were calculated at different temperatures. The activation parameters with respect to the slow step of the mechanism were computed and thermodynamic quantities were also determined.

Keywords: Permanganate, ciprofloxacin, sulphuric acid, oxidation, kinetics.

INTRODUCTION

Fluoroquinolones currently represent one of the most important classes of antibacterial agents worldwide, on the basis of annual global sales and therapeutic versatility. [1] They are a family of synthetic, broad spectrum antibacterial compounds, used in a multitude of human and veterinary applications. [2] Ciprofloxacin(CIP){1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazine-1-yl)-quinolone-3-carboxylic acid} is a second generation fluoroquinolone antimicrobial agent with a wide spectrum of activity against many gram positive and gram negative aerobic and anaerobic bacteria. Ciprofloxacin has been use in the treatment of a wide range of infections. Due to their extensive usage, fluoroquinolones may enter in the environment via



waste water effluent and bio solids from sewage treatment plants. There are studies on the modified pharmacological and toxicological properties of these drugs in the form of metallic complexes. [3-5] The structure of Ciprofloxacin is shown below which consist of piperazine and pyridone moieties.

Potassium permanganate is widely used as an oxidizing agent as well as in analytical chemistry. These reactions are governed by the pH of the medium. Among six oxidation states of manganese from +2 to +7, permanganate, Mn(VII) is the most potent oxidant in acid as well as is alkaline media. Permanganate oxidation finds extensive applications in organic synthesis [6-7], especially since the advent of phase transfer catalysis. [8-9] In general, the reduction of

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permanganate in slightly basic or neutral solution and in acid media goes through Mn(IV) and Mn(II) with reduction potentials [10] of 1.695 V for Mn(VII)/Mn(IV) and 1.51V for Mn(VII)/Mn(II). In acid medium, permanganate exists in different forms namely $HMnO_4$ and $H_2MnO_4^+$ and depending on the nature of the reductant, the oxidant has been assigned both inner sphere and outer sphere mechanism pathways in their redox reactions. [11-12]

A literature survey reveals that there are few study reports [13-15] on the oxidation of ciprofloxacin in either alkaline or acidic medium. In view of the potential pharmaceutical importance of ciprofloxacin and lack of reported kinetic & mechanical data on the oxidation of this drug, a detailed oxidation study might elucidate the mechanism of conversion of such compounds. The present study deals to investigate the redox chemistry of permanganate in acid media and establishing a plausible mechanism for oxidation of ciprofloxacin by permanganate on the basis of experimental results.

MATERIALS AND METHODS

Experimental

All chemicals used were of analytical grade and doubly distilled water was used throughout this study. An aqueous solution of ciprofloxacin (KORES India Limited) was prepared by dissolving known amount of its hydrochloride salt in double distilled water. Permanganate solution was obtained by dissolving potassium permanganate (BDH Analar) in water and standardized by titrating against oxalic acid. [16] Freshly prepared & standardized permanganate solutions were always used in kinetics experiments. The Mn(II) solution was made by dissolving manganese sulphate (BDH) in water. Na_2SO_4 (BDH) and H_2SO_4 (MERCK) were used to provide required ionic strength & acidity respectively.

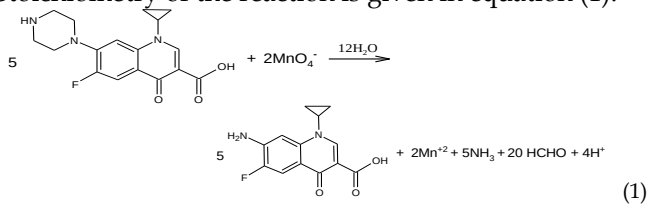
For kinetic measurements, a Peltier accessory (temperature-Controlled) attached to a U.V. 3000+ UV-Visible spectrophotometer (LABINDIA) was used.For product analysis, an LC-ESI-MS, (Q-TOF Micromass, WATERS Company, UK), an alpha-T FTIR spectrophotometer (Bruker, Germany), and for pH measurements MSW-552 pH meter were used.

Kinetic measurements

All kinetic measurements were conducted under pseudo first order conditions, where the concentration of ciprofloxacin was much greater than permanganate ion concentration at constant temperature at 25 ± 0.1°C unless otherwise stated. The reaction was initiated by mixing thermostated solution of permanganate and ciprofloxacin with the required amount of sulphuric acid and sodium sulphate. The progress of the reaction was followed spectrophotometrically at 525nm. The Beer's law verified in permanganate concentration range (0.50 – 5.0) × 10^{-4} $moldm^{-3}$ at 525 nm. The molar absorptivity index of permanganate was found to 2260 ± 50 $dm^3mol^{-1}cm^{-1}$ as a function of time. The kinetics reactions were followed more than 85 % completion of the reaction. The pseudo first order rate constant k_{obs}were calculated from the plots of log(abs) versus time, which were linear. The values of k_{obs} were reproducible within ± 5%.

Stoichiometry and product analysis

Different sets of concentration of reactants in 0.01 mol dm^{-3} sulphuric acid at constant ionic strength, 0.05mol dm^{-3}, were kept over 24 hours at 25°C in a closed container. When [permanganate] > [ciprofloxacin], the remaining permanganate concentration was assayed by measuring the absorbance at 525 nm. Estimation of unreacted [MnO_4^-] indicates that 5 moles of ciprofloxacin consumed 2 moles of Permanganate; the Stoichiometry of the reaction is given in equation (1).



(1)

LC/MS analysis of ciprofloxacin reaction indicates the formation of product with molecular ions of m/z 263 (Fig. 1). The molecular ion of ciprofloxacin is m/z 332. The m/z 263 corresponds to full dealkylation of the piperazine ring (i.e. the $–NH_2$ product). It is worth noting, that oxidation of piperazine moiety of ciprofloxacin between oxidized centres and nitrogen atoms lead to distinctive mass loss m/z = 69 and m/z = 83. This was attributed to ring opening, dealkylation and deamination process, which finally yielded 7-amino fluoroquinolone product. The product was also short written as M-69, indicating the net mass loss of the product from the parent ciprofloxacin. This product was also identified previously as oxidation product of ciprofloxacin [17] and IR Spectroscopy analysis confirmed the presence of $–NH_2$ group in the oxidation product (Fig. 2). The IR spectroscopy shows a peak at 3324 cm^{-1}which is due to -NH stretching of the $–NH_2$ group and the remaining peaks of the parent compound (quinolone ring). The by-product formaldehyde was identified by spot test. [18] The other product ammonia was detected by Nesseler's reagent test. [19]

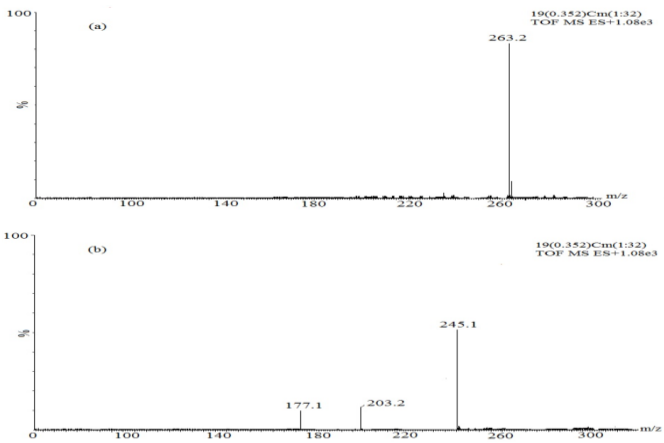


Fig. 1: LC-ESI-MS spectra of oxidation product of ciprofloxacin. (a) Molecular ion peak of m/z 263 (M-69). (b) Fragmentation of (M-69) product.

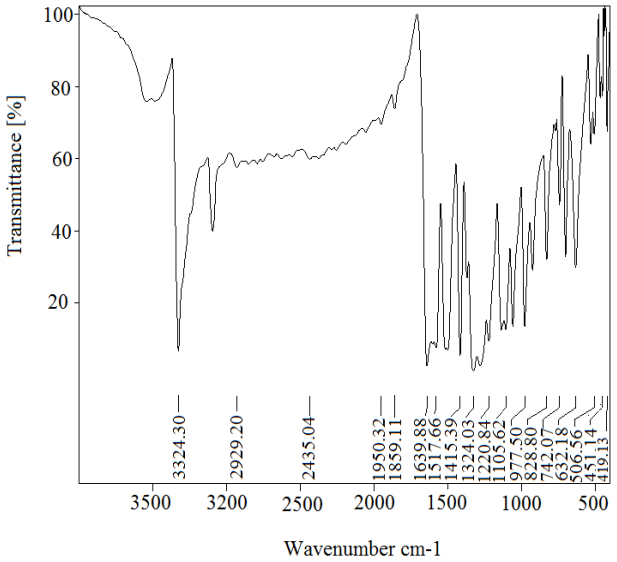


Fig. 2: FTIR spectra of the product of oxidation of ciprofloxacin by permanganate.

RESULTS AND DISCUSSION

Permanganate dependence

The reaction orders were determined from the slopes of log k_{obs} versus log [concentration] plots by different concentration of ciprofloxacin, permanganate and acid in turn, keeping all other concentration and conditions constant. The oxidant permanganate [MnO_4^-] concentration varied from 5×10^{-5} to 4×10^{-4} mol dm^{-3}, and all other concentrations and conditions were constant (Fig. 3). The plot of log absorbance versus time was linear (Fig. 3) indicating that the reaction is first order with respect to [$KMnO_4$]. The observed pseudo first order rate constant k_{obs} were independent of the concentration of $KMnO_4$.

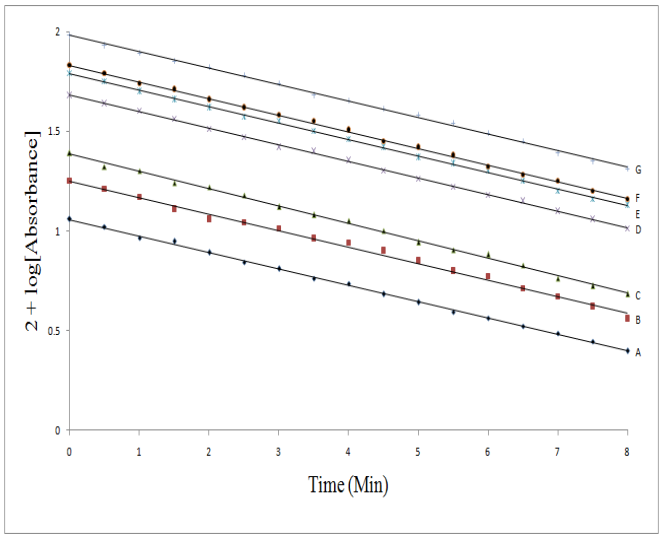


Fig. 3: First order plots of the variation of permanganate concentration at 25°C. [CIP] = 3.0×10^{-3}, [H^+] = 1.0×10^{-2}, I = 0.05/ mol dm^{-3}. [MnO_4^-] × 10^{-4} mol dm^{-3} = (A) 0.75, (B) 0.50, (C) 1.0, (D) 2.0, (E) 2.5, (F) 3.0, (G) 4.0.

Ciprofloxacin dependence

The effect of variation of ciprofloxacin on the rate of reaction was studied in the concentration range 1×10^{-3} to 7×10^{-3} mol dm^{-3} at constant concentration of permanganate, acid and constant ionic strength at 25°C. The rate of reaction increases with increasing concentration of ciprofloxacin. The value of slope of the plot of log k_{obs} versus log [CIP] was found to be unity, which confirms the reaction is first order with respect to ciprofloxacin concentration. This was also confirmed by the plot of k_{obs} versus ciprofloxacin concentration (Fig. 4) which is a straight line passing through the origin.

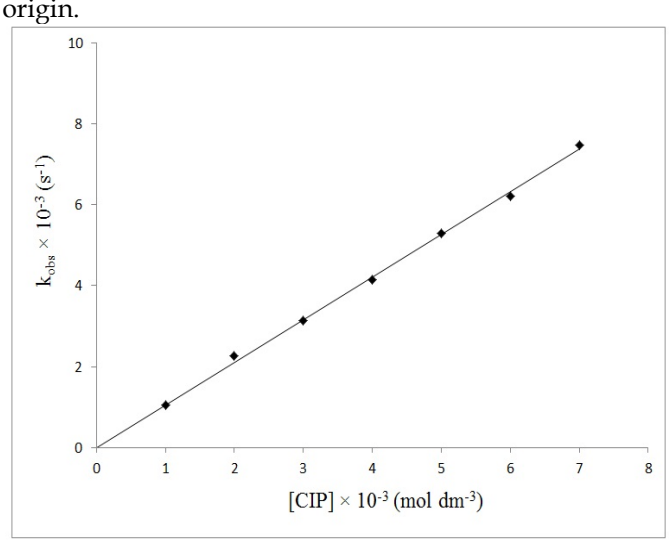


Fig. 4: Plot of [CIP] versus k_{obs}.
[$KMnO_4$] = 2.5×10^{-4}, [H^+] = 1.0×10^{-2}, I = 5×10^{-2}/mol dm^{-3} at 25°C.

Hydrogen ion dependence

The effect of variation of sulphuric acid on the rate of reaction was studied in the concentration range 0.01 to 0.07 mol dm^{-3}at fixed concentrations of permanganate, ciprofloxacin and constant ionic strength at three temperatures viz. 25°C, 30°C, 35°C respectively and other conditions were constant. k_{obs} was found to be increased with increase [H^+] concentration (Table 1). The order with respect to [H^+] was found to be less than unity (0.68).

Table 1: Observed rate constants for the reaction of ciprofloxacin and permanganate at different hydrogen ion concentration at three temperatures. [CIP] = 2.0×10^{-3} mol dm^{-3}, [$KMnO_4$] = 2.5×10^{-4} mol dm^{-3}, I = 0.05 mol dm^{-3}.

	$10^3 k_{obs}(s^{-1})$		
[H^+] (mol dm^{-3})	25°C	30°C	35°C
0.01	2.27	2.50	2.61
0.02	3.44	4.01	5.12
0.03	4.34	4.80	6.41
0.04	4.76	5.62	7.02
0.05	5.26	6.14	7.42
0.06	5.55	6.40	7.80
0.07	5.88	6.62	8.21

Effect of ionic strength and dielectric constant

At constant concentration of reactants and other conditions constant, the ionic strength was varied by varying concentration of sodium sulphate 0.01 to 0.1mol dm^{-3}. Ionic strength had negligible effect on the rate of reaction. At constant acidity and other constant conditions, as the t-butyl alcohol content increase from 0 to 50% (v/v) in the reaction, change in dielectric constant had negligible effect on the rate of reaction.

Effect of added products

The initial added products, Mn(II) was studied in the range of 5×10^{-5} to 5×10^{-4}mol dm^{-3} while other reactants concentration and conditions constant and aldehyde does not change the rate of reaction.

Test for free radical

The reaction mixture(10 ml) to which a known quantity (2 ml) of acrylonitrile has been added and kept in an inert atmosphere for 5 hours then diluted with methanol, white precipitate was formed, indicating the intervention of free radicals in the reaction. The blank experiment of reacting either $KMnO_4$or ciprofloxacin alone with acrylonitrile did not induce polymerisation under the same conditions.

The expected oxidizing species of permanganate in acid media are $HMnO_4$, $H_2MnO_4^+$, $HMnO_3$ and Mn_2O_7. Among them MnO_4^- ion is powerful oxidizing agent in aqueous alkaline as well as in acidic medium. The stable reduction product of MnO_4^- in acid medium is Mn(II). Figure 5 illustrates the spectroscopic changes occurring in the oxidation of ciprofloxacin by acid permanganate at 25°C with scanning interval of 3 minutes. The literature survey reveals that [20] Mn(IV) ion absorbs in region 400-600 nm. Figure 5 shows no features in this wavelength area indicating that MnO_2 is not a reaction product.

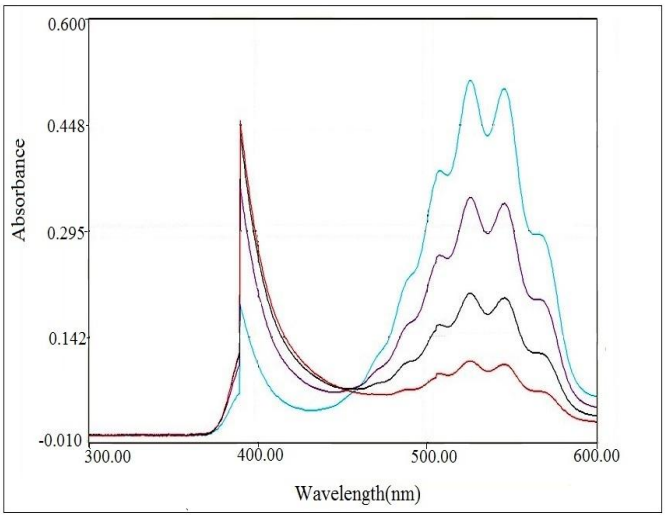
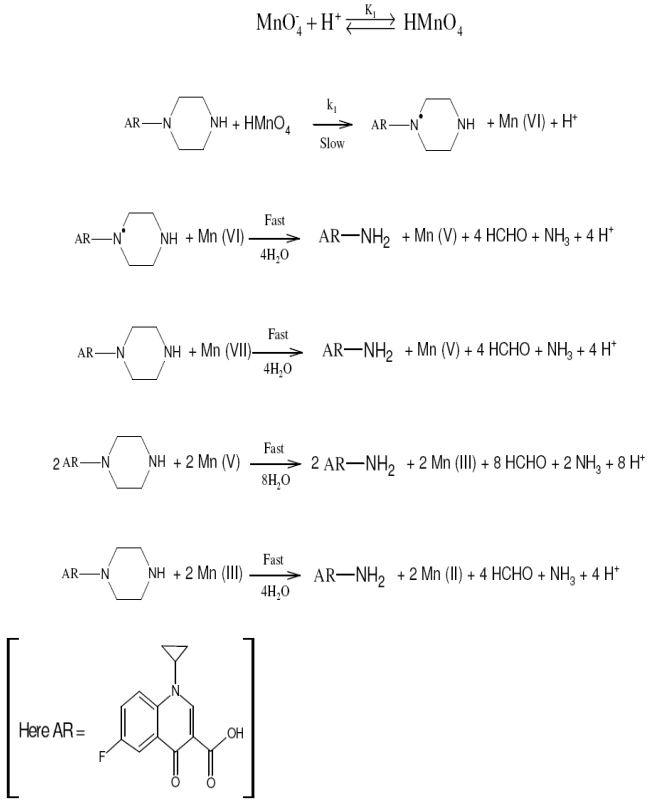


Fig. 5: Spectral changes during the oxidation of ciprofloxacin (CIP) by permanganate in acidic medium at 25°C: $[MnO_4^-] = 2.0 \times 10^{-4}$, $[CIP] = 2.0 \times 10^{-3}$, $[H^+] = 1.0 \times 10^{-2}$ and $I = 0.05$/ mol dm^{-3}.

The active species of permanganate in aqueous acid solution may be deduced from the dependence of the rate on $[H^+]$, in the reaction medium. The order of $[H^+]$ is less than unity, which may be indicate the formation of permanganate acid from permanganate ion. Permanganate acid $HMnO_4$ is more efficient oxidant species of Manganese(VII) then permanganate ion[21]. It has been observed that the rate of reaction was tending to attain a limiting value at higher concentration of $[H^+]$ ion, which indicates that only the protonated form is active then acid permanganate. [22] Equilibrium can be represented by equation-(2)

$$MnO_4^- + H^+ \underset{}{\overset{K_1}{\rightleftharpoons}} HMnO_4 \qquad (2)$$

The reaction between permanganate and ciprofloxacin in sulphuric acid has Stoichiometry 5:2, with first order dependence with permanganate and ciprofloxacin and less than unit order with H^+ concentration. The oxidation products were Mn(II), 7-amino fluoroquinolone, NH_3 and HCHO. On the basis of experimental results, the mechanism can be proposed. In view of increasing the rate with increase in $[H^+]$ ion, in the prior equilibrium step, H^+ reacts with MnO_4^- to form $HMnO_4$, which reacts with the one mole of ciprofloxacin in the rate determining step to give a free radical derived from ciprofloxacin and an intermediate Mn(VI). In further fast steps the intermediate Mn(VI) reacts with a free radical to produce the product 7-amino fluoroquinolone, NH_3, HCHO and intermediate Mn(V). In further fast steps Mn(V) subsequently reduced to the end product Mn(II). Although Mn(VI) and Mn(IV) are the final reduced species of MnO_4^- in alkaline and neutral media, it was observed that Mn(II) was the only reduced species of MnO_4^- in acid medium. Since none of the intermediate could be detected, scheme-1 is the only possible mechanism for the reaction in the presence of free radical. Attempts were made to allow spectroscopic detection of intermediate Mn(V) and Mn(III) as the reaction proceeded in the oxidation of ciprofloxacin by permanganate. Unfortunately the low concentration of Mn(V) and Mn(III) intermediate obtained under our experimental conditions made the spectroscopic detection failure. However, the evidence for intermediate such as Mn(V) and Mn(III) is as presented in the literature. [23-24] The results are accommodated in the following mechanism.



Scheme 1. Proposed mechanism for the oxidation of ciprofloxacin by acidic permanganate.

From the scheme-1, the following rate law can be derived as follows:

$$\text{Rate} = \frac{-d[MnO_4^-]}{dt} = k_1[HMnO_4][CIP] \quad (3)$$

$$= k_1K_1[MnO_4^-]_f[CIP]_f[H^+]_f \quad (4)$$

The total concentration of permanganate is given by:

$$[MnO_4^-]_t = [MnO_4^-]_f + [HMnO_4]_f$$

$$= [MnO_4^-]_f + K_1[H^+][MnO_4^-]$$

$$= [MnO_4^-]_f\ (1 + K_1[H^+])$$

So

$$[MnO_4^-]_f = \frac{[MnO_4^-]_t}{(1 + K_1[H^+])} \quad (5)$$

Where "t" and "f" stands for total and free

$$[H^+]_f = \frac{[H^+]_t}{(1 + K_1[MnO_4^-])} \quad (6)$$

Putting equation (5) and (6) in equation (4) and omitting "t" and "f" subscripts

$$\text{Rate} = \frac{-d[MnO_4^-]}{dt} = \frac{k_1K_1[MnO_4^-]\,[CIP]\,[H^+]}{(1 + K_1[H^+])\,(1 + K_1[MnO_4^-])} \quad (7)$$

$$= \frac{k_1K_1[MnO_4^-]\,[CIP]\,[H^+]}{1 + K_1[H^+] + K_1[MnO_4^-] + K_1^2[H^+][MnO_4^-]} \quad (8)$$

$K_1[MnO_4^-]$ And $K_1^2[H^+][MnO_4^-]$ << 1 or neglected due to low concentration of $[MnO_4^-]$ used in the experiment so equation (8) change into equation (9)

$$\text{Rate} = \frac{-d[MnO_4^-]}{dt} = \frac{k_1K_1[MnO_4^-]\,[CIP]\,[H^+]}{1 + K_1[H^+]} \quad (9)$$

$$\frac{\text{Rate}}{[MnO_4^-]} = k_{obs} = \frac{k_1K_1[CIP]\,[H^+]}{1 + K_1[H^+]} \quad (10)$$

(Where k_{obs} = First order rate constant)

$$\frac{k_{obs}}{[CIP]} = \frac{k_1K_1\,[H^+]}{1 + K_1[H^+]} \quad (11)$$

Equation (11) can be rearranged as

$$\frac{[CIP]}{k_{obs}} = \frac{1}{k_1K_1\,[H^+]} + \frac{1}{k_1} \quad (12)$$

According to equation (12) the plot of $[CIP]/k_{obs}$ versus $1/[H^+]$ is linear with positive intercept and slope (Fig. 6) at three different temperatures. The rate constant k_1, of the slow step, scheme-1 was obtained from the intercept of the plots $[CIP]/k_{obs}$ versus $1/[H^+]$ (Table 2). The energy of activation was determined by the plot of log k_1 versus 1/T from which activation parameters were calculated (Table 2). The equilibrium constant of $HMnO_4$ (K_1) was calculated from the intercept and slope of the plot [CIP] / k_{obs} versus 1/ $[H^+]$ (Table 2). The value of K_1 is in good agreement with earlier work [23] (literature value is 40 $dm^3\ mol^{-1}$ at 25°C). Thermodynamic quantities were calculated from the Van't Hoff plot (Table 2).

Table 2: Activation and thermodynamic parameters for the oxidation of ciprofloxacin by acidic permanganate from scheme 1.

Temperature (Kelvin)	k_1 ($dm^3\ mol^{-1}\ s^{-1}$)
Effect of temperature with respect to the slow step of Scheme 1.	
298	3.88
303	4.81
308	7.29
Activation parameters	Value
E_a(kJ mol^{-1})	34.8
$\Delta H^{\neq}$ (kJ mol^{-1})	32.3
$\Delta S^{\neq}$ ± (J K^{-1} mol^{-1})	-116.6
$\Delta G^{\neq}$ ± (kJ mol^{-1})	69.6
Temperature (Kelvin)	Equilibrium constant K_1 ($dm^3\ mol^{-1}$)
298	40.6
303	34.6
308	22.8
Thermodynamic quantities	Value
ΔH (kJ mol^{-1})	-25
ΔS ± (J K^{-1} mol^{-1})	-81
ΔG ± (kJ mol^{-1})	-1.6

The moderate values of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ were favourable for electron transfer process. The value of $\Delta H^{\neq}$ was due to energy of solution changes in the transition state. The negative value of $\Delta S^{\neq}$ within the range of radical reaction has been ascribed [25] to the nature of electron pairing and electron unpairing process. The negligible effect of ionic strength and dielectric constant is consistent with reaction between two neutral molecules which supports the proposed mechanism. [26]

The study of oxidation of ciprofloxacin by permanganate in acidic medium, results demonstrate the role of pH in the reaction medium is crucial. The literature [27] reports that dealkylated products of ciprofloxacin have reduced antimicrobial activity. Since dealkylated products are obtained in the present study, it is evident that the products of the title reaction have reduced antimicrobial activity after oxidation. So this study will be effectively used in waste water treatment at the sites contaminated by fluoroquinolone antibiotics. The proposed mechanism is consistent with product, mechanism and kinetic studies.

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KINETIC ANALYSIS OF OXIDATION OF OFLOXACIN BY PERMANGANATE ION IN SULPHURIC ACID MEDIUM: A MECHANISTIC APPROACH

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ABSTRACT

The kinetics and mechanism of oxidation of ofloxacin by permanganate ion in acidic medium have been studied at 30 ±1^{o}C. The Stoichiometry has been observed to be 2:5 in terms of mole ratio of permanganate ion and ofloxacin consumed. The reaction shows first order with respect to oxidant and fractional order in both the substrate and hydrogen ion concentration. The effect of added products and ionic strength has also been investigated. The main products identified were 7-amino quinolone and Mn(II). Investigation of the reaction at different temperature allowed the determination of the activation parameters with respect to the slow step of the proposed mechanism.

Keywords: Kinetics, Oxidation, Mechanism, Ofloxacin, Permanganate ion, Sulphuric acid medium.

INTRODUCTION

Ofloxacin (OFL) [9-fluoro-2, 3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido-[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid] belongs to the fluoroquinolone class of antibiotics. They are synthetic broad spectrum antibacterial drugs that exhibit significant activity against both gram-positive and gram-negative bacteria. [1] They act as specific inhibitors of the bacterial DNA-Gyrase, the enzyme responsible for converting double stranded DNA into a negative super-helical form. Ofloxacin possess two relevant ionisable functional groups: a basic piperazinyl group and a carboxylic group. The carboxylic group and the carbonyl groups are required for antimicrobial activity.

Potassium permanganate is widely used as an oxidizing, disinfectant and also as an analytical reagent. [2] The oxidation by Mn(VII) ions finds extensive applications in organic synthesis [3], especially since the advent of phase transfer catalysis. [4-6] Kinetic studies are important sources of mechanistic information on such reactions, as demonstrated by the results referring to unsaturated acids both in aqueous [4-7] and in non-aqueous media. [8] During oxidation by Mn(VII) it is evident that Mn(VII) is reduced to various oxidation states in acid, alkaline and neutral media. Among six oxidation states of manganese from +2 to+7, permanganate, Mn(VII) is the most potent oxidation state in acid medium with reduction potentials [9] 1.69V of Mn(VII)/Mn(IV) couple and 1.51V of Mn(VII)/Mn(II) couple. In acidic medium active species of Mn(VII) exists in different forms as $HMnO_4$, $H_2MnO_4^+$, $HMnO_3$ and Mn_2O_7 depending on the nature of the reductant, the oxidant has been assigned both inner sphere and outer sphere mechanism pathways in their redox reactions[10, 11].

The literature survey reveals that there are few study reports [12, 13] on the oxidation of ofloxacin by MnO_2 followed by evaluation of the reaction kinetics and analysis of chemical structure of degradation products formed. Interaction of ofloxacin with various metal ions was studied for the determination of ofloxacin spectrophotometrically and polarographically in pharmaceutical formulation. [14-17] Hence, ofloxacin finds extensive application in pharmaceutical industry. It is noted that despite the importance of the drug, the literature survey reveals that there is no information about the oxidation kinetics. Thus prompted us to undertake the title reaction. The present study deals to investigate the redox chemistry of permanganate in acid media and establishing a plausible mechanism for oxidation of ofloxacin by permanganate on the basis of experimental results.

MATERIALS AND METHODS

Chemicals

All chemicals used were of analytical grade and doubly distilled water was used throughout this study. Standard solution of ofloxacin (KORES India Limited) was prepared by dissolving calculated quantity of pure drug in 0.1 M H_2SO_4. The acid present in the substrate solution is also taken into account in the calculation of the total acid present in each case of the present reaction. Permanganate solution was obtained by dissolving potassium permanganate (BDH Analar) in water and standardized by titrating against oxalic acid. [18] Freshly prepared & standardized permanganate solutions were always used in kinetics experiments. The Mn(II) solution was made by dissolving manganese sulphate (BDH) in water. Na_2SO_4 (BDH) and H_2SO_4 (MERCK) were used to provide required ionic strength & acidity respectively.

Instrumentation

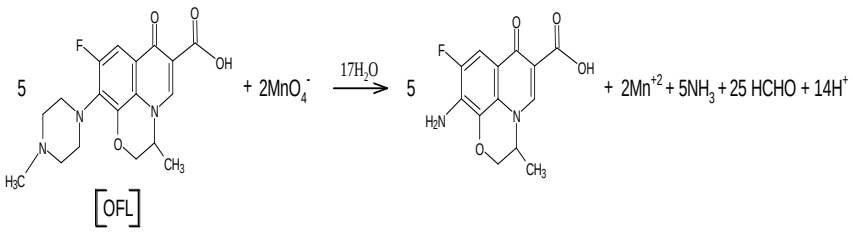
For kinetic measurements, a Peltier accessory (temperature-Controlled) attached to a U.V.3000$^+$ UV-Visible spectrophotometer (LABINDIA) was used. For product analysis, LC-ESI-MS, (Q-TOF Micromass, WATERS Company, UK), alpha-T FTIR spectrophotometer (BRUKER, Germany), and for pH measurements MSW-552 pH meter were used.

Kinetic Measurements

All kinetic measurements were conducted under pseudo-first-order conditions, where the concentration of ofloxacin was much greater than permanganate ion concentration at constant temperature 30 ± 0.1^{o}C unless otherwise stated. The reaction was initiated by mixing thermostated solution of permanganate and ofloxacin; in addition to that required quantities of H_2SO_4, Na_2SO_4 are added to provide required acidity and ionic strength of reaction. The progress of the reaction was followed spectrophotometrically at 525nm. The Beer's law verified in permanganate concentration range (0.50 – 5.0) × 10^{-4} moldm^{-3} at 525nm. The molar absorptivity index of permanganate was found to be ε = 2260 ±50 dm^{3}mol^{-1}cm^{-1} as a function of time. The kinetics reactions were followed more than 85 % completion of the reaction. The pseudo-first-order rate constants k_{obs} were calculated from the plots of the logarithm of absorbance versus time, which were linear. The values of k_{obs} were reproducible within ± 5%.

Stoichiometry and Product Analysis

Different sets of concentration of reactants in 0.01 mol dm^{-3} sulphuric acid at constant ionic strength, 0.02mol dm^{-3}, were kept over 24 hrs at 30^{o}C in a closed container. When [permanganate] > [ofloxacin], the remaining permanganate concentration was assayed by measuring the absorbance at 525 nm. Estimation of unreacted [MnO_4^-] indicates that 5 moles of ofloxacin consumed 2 moles of Permanganate; the Stoichiometry of the reaction is given in equation (1).



(1)

LC/MS analysis of ofloxacin oxidation reaction indicates the formation of product with molecular ions of m/z 279 "Fig.1". The molecular ion of ofloxacin is m/z 362. The m/z 279 corresponds to full dealkylation of the piperazine ring (i.e. the –NH_2 product).. It is worth

noting, that oxidation of piperazine moiety of ofloxacin between oxidized centres and nitrogen atoms lead to distinctive mass loss m/z = 69 and m/z = 83. This was attributed to ring opening, dealkylation and deamination process, which finally yielded 7-amino fluoroquinolone product. The product was also short written as M-69, indicating the net mass loss of the product from the parent ofloxacin. This product was also identified previously as oxidation product of ofloxacin [19] and IR Spectroscopy analysis confirmed the presence of $–NH_2$ group in the oxidation produc "Fig.2". The IR spectroscopy shows a peak at 3353.85 cm^{-1} which is due to -NH stretching of the $–NH_2$ group and the remaining peaks are of the parent compound (quinolone ring).The by-product formaldehyde was identified by spot test [20]. The other product ammonia was detected by Nesseler's reagent test. [21]

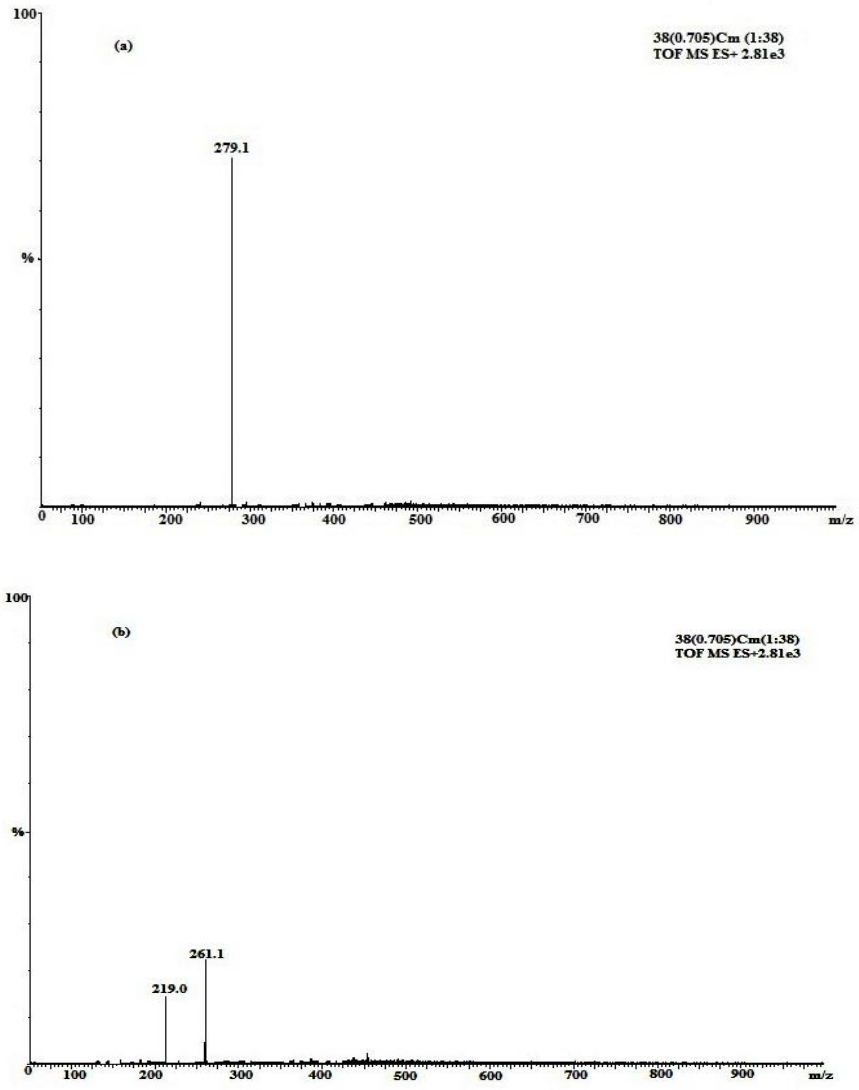


Fig. 1LC-ESI-MS spectra of oxidation product of ofloxacin. (a) Molecular ion peak of m/z 279 (M-69). (b) Fragmentation of (M-69) product.

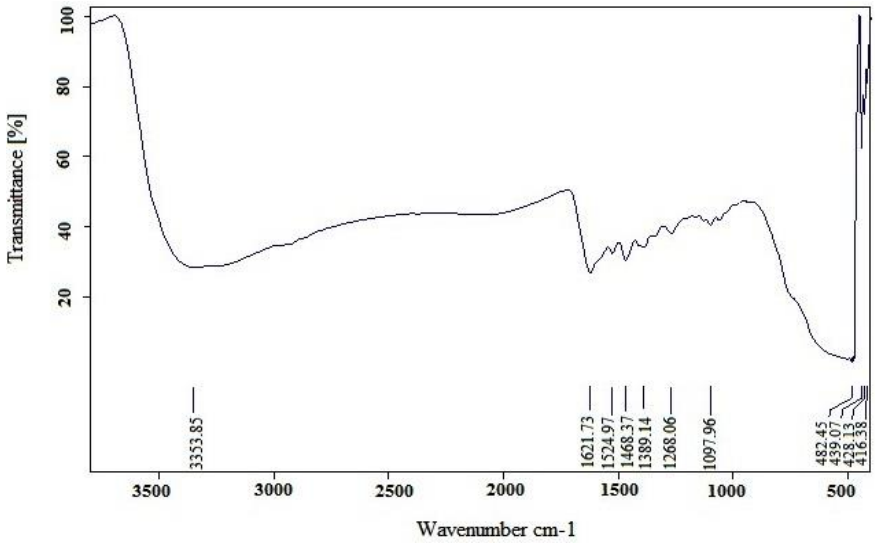


Fig. 2 FT-IR spectra of the product of oxidation of ofloxacin by permanganate.

RESULTS

The reaction orders were determined from the slopes of log k_{obs} versus log [concentration] plots by different concentration of ofloxacin, permanganate and acid in turn, keeping all other concentration and conditions constant.

Permanganate Dependence

The oxidant permanganate [MnO_4^-] concentration varied from7.5×10^{-5} to 6×10^{-4}moldm^{-3}, and all other concentrations and conditions were constant. The plot of log absorbance versus time was linear "Fig. 3" indicating that the reaction is first order with respect to [$KMnO_4$]. The observed pseudo first order rate constant (k_{obs}) were independent of the concentration of $KMnO_4$.

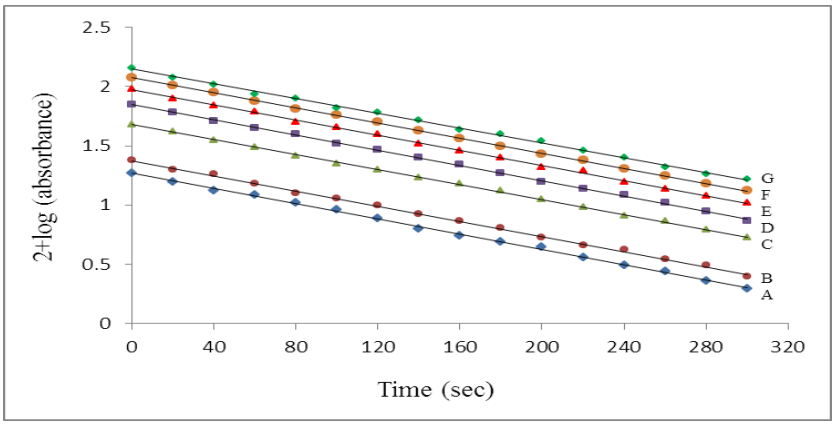


Fig. 3 First order plots of the variation of permanganate concentration at 30^{o}C. [OFL]=1.0×10^{-3}, [H^+] = 1.0×10^{-2} and I = 0.02/ mol dm^{-3}. [MnO_4^-]$\times10^{-4}$ mol dm^{-3} = (A) 0.75, (B) 1.0,(C) 2.0, (D) 3.0, (E) 4.0, (F) 5.0, (G) 6.0

Ofloxacin Dependence

The effect of concentration variation of ofloxacin on the rate of reaction was studied in the range 2×10^{-3} to 7×10^{-3} mol dm^{-3} at constant concentration of permanganate, acid and ionic strengthat20^{o}, 25^{o}, 30^{o}C respectively.. The rate of reaction increases with increasing concentration of ofloxacin (Table 1). A plot of log k_{obs} versus log [OFL] was linear with a slope of 0.63, thus indicating a fractional-order dependence on ofloxacin concentration. This was confirmed by the plot of $1/k_{obs}$ versus 1/ [OFL] "Fig.4" which was also linear with a positive intercept.

Hydrogen Ion Dependence

The effect of concentration variation of sulphuric acid on the rate of reaction was studied in the concentration range 2×10^{-3} to 2×10^{-2} moldm^{-3} at fixed concentration of permanganate, ofloxacin and ionic strength at three temperatures viz. 20^{o}, 25^{o}, 30^{o}C respectively.. Pseudo first-order rate constant (k_{obs}) was found to be increased with increase in [H^{+}] (Table 1). A plot of log k_{obs} versus log [H^{+}] was linear with a fractional slope of 0.75. This was confirmed by the plot of $1/k_{obs}$ versus 1/ [H^{+}] "Fig.5" which was also linear with a positive intercept.

Table 1: Effects of variation of [MnO_4^{-}], [OFL] and [H^{+}] on the oxidation of ofloxacin by acidic permanganate at 30^{o}C and I = 0.02 mol dm^{-3}.

10^4 [MnO_4^{-}] (mol dm^{-3})	10^3 [OFL] (mol dm^{-3})	10^2 [H^{+}] (mol dm^{-3})	10^3k_{obs} (s^{-1})
0.75	1.0	1.0	7.29
1.0	1.0	1.0	7.29
2.0	1.0	1.0	7.29
3.0	1.0	1.0	7.27
4.0	1.0	1.0	7.29
5.0	1.0	1.0	7.27
6.0	1.0	1.0	7.29
2.0	2.0	1.0	10.65
2.0	3.0	1.0	14.14
2.0	4.0	1.0	17.36
2.0	5.0	1.0	20.26
2.0	6.0	1.0	22.43
2.0	7.0	1.0	22.98
2.0	2.0	0.2	3.49
2.0	2.0	0.3	4.98
2.0	2.0	0.4	6.14
2.0	2.0	0.5	7.28
2.0	2.0	0.6	8.46
2.0	2.0	0.7	9.21
2.0	2.0	0.8	9.83

2.0	2.0	0.9	10.16
2.0	2.0	1.0	10.65
2.0	2.0	1.5	13.66
2.0	2.0	2.0	14.20

Effect of Ionic Strength and Dielectric Constant

At constant concentration of reactants and other conditions constant, the ionic strength was varied by varying concentration of sodium sulphate 0.01 to 0.1mol dm^{-3}. Ionic strength had negligible effect on the rate of reaction. At constant acidity and other constant conditions, as the t-butyl alcohol content increase from 0 to 50% (v/v) in the reaction, change in dielectric constant had negligible effect on the rate of reaction.

Effect of Added Products

The initial added products, Mn(II) was studied in the range of 5×10^{-5} to 5×10^{-4} mol dm^{-3} while other reactants concentration and conditions constant and by-product aldehyde does not change the rate of reaction.

Test for Free Radical

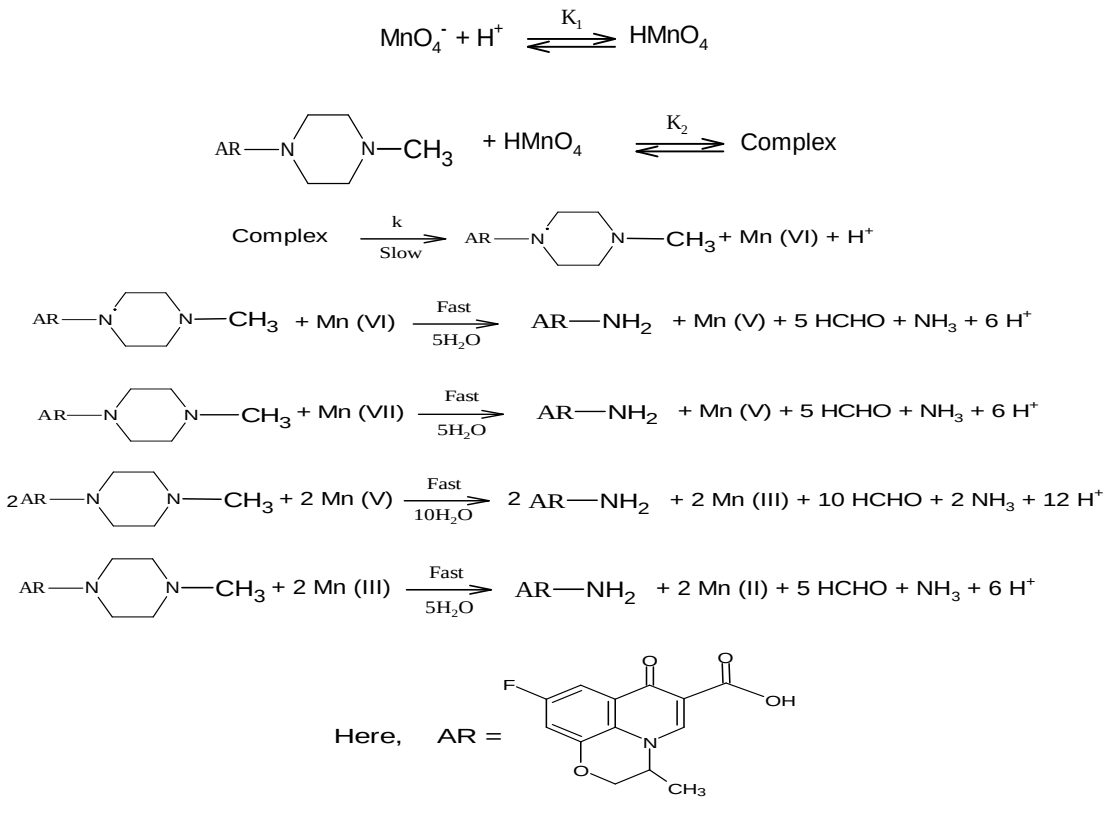
The reaction mixture (10ml) in which known quantity (2ml) of acrylonitrile has been added and kept in an inert atmosphere for 5 hours then diluted with methanol, white precipitate was formed, indicating the intervention of free radicals in the reaction. The blank experiment of reacting either $KMnO_4$ or ofloxacin alone with acrylonitrile did not induce polymerisation under the same conditions.

DISCUSSION

The expected oxidizing species of permanganate in acid media are $HMnO_4$, $H_2MnO_4^+$, $HMnO_3$ and Mn_2O_7. Permanganate ion, MnO_4^- ion is powerful oxidizing agent in acidic medium. The stable oxidation product of MnO_4^-in acid medium is Mn(II). The active species of permanganate in aqueous acid solution may be deduced from the dependence of the rate on $[H^+]$, in the reaction medium. The order of $[H^+]$ is less than unity, which may be indicate the formation of permanganate acid from permanganate ion. Permanganate acid $HMnO_4$ is more efficient oxidant species of Manganese (VII) then permanganate ion [22]. It has been observed that the rate of reaction was tending to attain a limiting value at higher concentration of $[H^+]$ ion, which indicates that only the protonated form is active then acid permanganate [23]. Equilibrium can be represented by equation-(2)

$$MnO_4^- + H^+ \underset{}{\overset{K_1}{\rightleftharpoons}} HMnO_4 \qquad (2)$$

The reaction between ofloxacin and permanganate in sulphuric acid has Stoichiometry 5:2, with first order dependence with permanganate and less than unit order with H^+ concentration and ofloxacin concentration. The oxidation products were Mn(II), 7-amino fluoroquinolone, NH_3 and HCHO. In view of increasing the rate with increase in $[H^+]$ ion, in the prior equilibrium step, H^+ reacts with MnO_4^- to form $HMnO_4$, which reacts with the one mole of ofloxacin to form a complex. Complex formed is dissociate in the rate determining step to give a free radical derived from ofloxacin and an intermediate Mn(VI). In further fast steps the intermediate Mn(VI) reacts with a free radical to produce the product 7-amino fluoroquinolone, NH_3, HCHO and intermediate Mn(V). In further fast steps Mn(V) subsequently reduced to the end product Mn(II). Although Mn(VI) and Mn(IV) are the final reduced species of MnO_4^- in alkaline and neutral media, it was observed that Mn(II) was the only reduced species of MnO_4^- in acid medium. Attempts were made to allow spectroscopic detection of intermediate Mn(V) and Mn(III) as the reaction proceeded in the oxidation of ofloxacin by permanganate. Unfortunately the low concentration of Mn(V) and Mn(III) intermediate obtained under our experimental conditions made the spectroscopic detection failure. However, the evidence for intermediate such as Mn(V) and Mn(III) are reported in the literature. [24, 25] The results are accommodated in the following mechanism (Scheme 1).



Scheme 1 Proposed mechanism for the oxidation of ofloxacin by acidic permanganate.

Following rate law can be derived from scheme 1:

$$\text{Rate}=\frac{-d[MnO_4^-]}{dt}=k[\text{Complex}]$$

$$=kK_2[HMnO_4][OFL]$$

$$=kK_1K_2[MnO_4^-]_f[H^+]_f[OFL]_f \quad (3)$$

Total concentration of permanganate is given by

$$[MnO_4^-]_t=[MnO_4^-]_f+[HMnO_4]+[\text{Complex}]$$

$$=[MnO_4^-]_f+K_1[MnO_4^-]_f[H^+]_f+K_2[HMnO_4][OFL]$$

$$=[MnO_4^-]_f+K_1[MnO_4^-]_f[H^+]_f+K_1K_2[MnO_4^-]_f[H^+]_f[OFL]$$

$$=[MnO_4^-]_f\{1+K_1[H^+]_f+K_1K_2[H^+]_f[OFL]\}$$

$$[MnO_4^-]_f=\frac{[MnO_4^-]_t}{\{1+K_1[H^+]_f+K_1K_2[H^+]_f[OFL]} \quad (4)$$

$[MnO_4^-]_t$ and $[MnO_4^-]_f$ are total and free concentration of Mn (VII) respectively.

Total concentration of ofloxacin is given by:

$$[OFL]_t=[OFL]_f+[\text{Complex}]$$

$$=[OFL]_f+K_2[OFL]_f[HMnO_4]$$

$$=[OFL]_f\{1+K_2[HMnO_4]\}$$

$$[OFL]_f=\frac{[OFL]_t}{1+K_2[HMnO_4]}$$

Very low concentration of $[MnO_4^-]$ were used in the experiment, so $K_2[HMnO_4] << 1$

$$[OFL]_f=[OFL]_t \quad (5)$$

Total concentration of $[H^+]$ is given by:

$$[H^+]_t=[H^+]_f+[HMnO_4]$$

$$=[H^+]_f+K_1[MnO_4^-]_f[H^+]_f$$

$$=[H^+]_f\{1+K_1[MnO_4^-]_f\}$$

$$\text{So, } [H^+]_t=[H^+]_f \quad (6)$$

Substituting equation (4), (5) and (6) in equation (3) and omitting "t" and "f" subscripts

$$\text{Rate}=\frac{-d[MnO_4^-]}{dt}=\frac{kK_1K_2[MnO_4^-][H^+][OFL]}{1+K_1[H^+]+K_1K_2[H^+][OFL]} \quad (7)$$

$$\frac{\text{Rate}}{[MnO_4^-]}=k_{obs}=\frac{kK_1K_2[H^+][OFL]}{1+K_1[H^+]+K_1K_2[H^+][OFL]} \quad (8)$$

Equation (8) can be rearranged as

$$\frac{1}{k_{obs}}=\frac{1}{kK_1K_2[H^+][OFL]}+\frac{1}{kK_2[OFL]}+\frac{1}{k} \quad (9)$$

According to equation (9) the plot of $1/k_{obs}$ versus 1/ [OFL] "Fig.4"is linear with positive intercept and slope at three different temperatures. The rate constant k, of the slow step, scheme 1 was obtained from the intercept of the plots $1/k_{obs}$ versus 1/ [OFL] (Table 2). The energy of activation was determined by the plot of log k versus 1/T from which activation parameters were calculated (Table 2). The equilibrium constant of $HMnO_4$ (K_1) and the equilibrium constant of complex (K_2) in scheme-1 were calculated from the intercept and slope of the plot $1/k_{obs}$ versus 1/ $[H^+]$ "Fig.5" (Table 2). The value of K_1 is in good agreement with earlier work [24] at 30°C. Thermodynamic quantities were calculated from the Van't Hoff plot (Table 2).

Table 2:Activation and thermodynamic quantities for the oxidation of ofloxacin by acidic permanganate from scheme 1.

Temperature (Kelvin)	**10^2 k (s^{-1})**
Effect of temperature with respect to the slow step of Scheme 1.	
293	4.0
298	4.34
303	4.76
Activation parameters	Value
E_a(kJ mol^{-1})	12.98
$\Delta H^{\neq}$ (kJ mol^{-1})	10.47
$\Delta S^{\neq}$ ± (J K^{-1} mol^{-1})	-171.74
$\Delta G^{\neq}$ ± (kJ mol^{-1})	68.02
Equilibrium constants at different temperatures	

Temperature (Kelvin)	$10^{-1}K_1$ (dm^3 mol^{-1})
293	4.80
298	4.62
303	4.25
Thermodynamic quantities	Using K_1 values
ΔH (kJ mol^{-1})	-10.3
ΔS ± (J K^{-1} mol^{-1})	-31.0
ΔG ± (kJ mol^{-1})	-1.3
Temperature (Kelvin)	$10^{-2}K_2$ (dm^3 mol^{-1})
293	3.57
298	4.61
303	5.25
Thermodynamic quantities	Using K_2values
ΔH (kJ mol^{-1})	29.75
ΔS ± (J K^{-1} mol^{-1})	100.4
ΔG ± (kJ mol^{-1})	-1.42

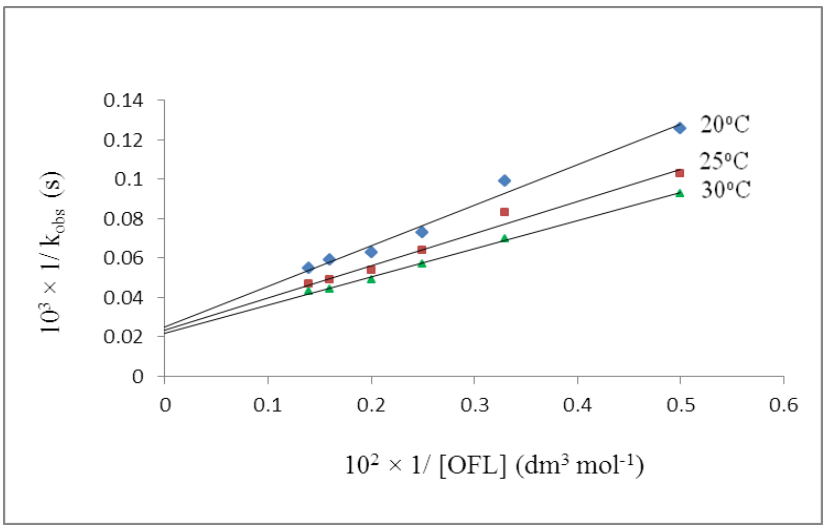


Fig. 4 Plots of $1/k_{obs}$ versus 1/ [OFL] at three different temperatures.

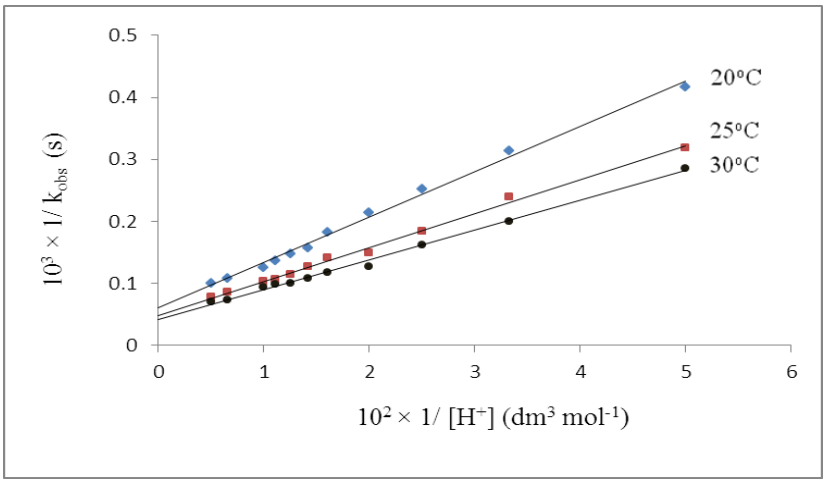


Fig. 5 Plots of $1/k_{obs}$ versus $1/ [H^+]$ at three different temperatures.

The values of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ are both favourable for electron transfer process [26].The value of $\Delta S^{\neq}$ within the range of radical reaction has been ascribed [27] to the nature of electron pairing and unpairing process. The negative value of $\Delta S^{\neq}$ indicates that complex is more ordered than the reactants [28]. The observed modest enthalpy of activation and a relatively low value of the entropy of activation as well as a higher rate constant of the slow step indicate that the oxidation presumably occurs via inner-sphere mechanism. [29] The negligible effect of ionic strength and dielectric constant is consistent with reaction between two neutral molecules which supports the proposed mechanism. [30]

CONCLUSION

The study of oxidation of ofloxacin by permanganate in acidic medium, the results demonstrate the role of H^+ in the reaction medium is crucial. The literature [31] reports that dealkylated products of ofloxacin have antimicrobial activity. Since dealkylated products are obtained in the present study, it is evident that the products of the title reaction have antimicrobial activity after oxidation. So this study will be effectively used in waste water treatment at the sites contaminated by fluoroquinolone antibiotics.Chemical oxidation using Mn(VII) has been widely used for treatment of pollutants in drinking water and waste water applications. The proposed mechanism is consistent with product, mechanism and kinetic studies.

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