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Kinetics and mechanistic studies of oxidation of fluoroquinolone antibacterial agent norfloxacin by diperiodatocuprate(III) in aqueous alkaline medium

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Abstract: The kinetics of the oxidation of norfloxacin by diperiodatocuprate(III) in aqueous alkaline medium has been studied spectrophotometrically at 300 K and at constant ionic strength of 0.20 mol dm⁻³. The oxidation products were identified by LC–ESI–MS technique and other spectral studies. The stoichiometry was found to be 1:2 ([NOR]:[DPC]. The active species of DPC is understood to be as monoperiodatocuprate(III). A suitable mechanism was proposed on the basis of experimental results. The reaction constants involved in the different steps of the reaction mechanism were calculated. The activation parameters with respect to the slow step of mechanism were determined and discussed.

Subjects: Engineering & Technology; Health and Social Care; Physical Sciences

Keywords: diperiodatocuprate(III); norfloxacin; oxidation; kinetics; mechanism

1. Introduction

In recent years, the study of highest oxidation state of transition metals has intrigued many researchers. Transition metals in a higher oxidation state can be stabilized by chelation with suitable polydentate ligands. Metal chelates such as diperiodatocuprate(III) (Reddy, Sethuram, & Navneeth Rao, 1984), diperiodatoargentate(III) (Kumar, Kumar, & Ramamurthy, 1999), and periodatonickelate(IV) (Shettar, 1999), and periodatonickelate(IV) (Shettar, 1999) have been extensively studied.

ABOUT THE AUTHORS

The principal investigator and other authors have been actively engaged in the research of the area of mechanisms of uncatalyzed and catalyzed reactions. Kinetic studies provide the most important type of evidence of the reaction mechanism. In the first part of the study, rate, order, stoichiometry, and the final products would be confirmed and estimated by known techniques, while the second part of the study will be made at varying temperatures. From this data, the different thermodynamic parameters will be calculated and the validity of the proposed reaction path will be confirmed. The presence and accumulation of fluoroquinolone antibiotics in aquatic environments, albeit at low concentrations, may pose threat to the ecosystem and human health by inducing increase and spread of bacterial drug resistance due to long-term exposure. This necessitates the development of various advanced oxidation processes for the transformation of fluoroquinolones in water.

PUBLIC INTEREST STATEMENT

This paper reveals the kinetic study of oxidation of an antibacterial agent, norfloxacin, by diperiodatocuprate(III) complex as an oxidant. The extensive usage of fluoroquinolones may enter the environment via wastewater effluent and biosolids from sewage treatment plants and via manure and litters from food-producing animal husbandry. The presence and accumulation of fluoroquinolone antibiotics in aquatic environments, albeit at low concentrations, may pose threats to the ecosystem and human health by inducing increase and spread of bacterial drug resistance due to long-term exposure. This necessitates the development of various advanced oxidation processes for the transformation of fluoroquinolones in water. The present study also demonstrates with the title reaction in order to investigate the redox chemistry of DPC in alkaline media and to compute the thermodynamic quantities of various steps involved in the mechanism to those derived on the basis of kinetic and spectroscopic results.
& Nandibewoor, 2005) are good oxidants in a medium with an appropriate pH value. The oxidation reaction usually involves the copper(II)–copper(I) couple and such aspects are detailed in different reviews (Pierre, 2000; Solomon, Chen, Metz, Lee, & Palmer, 2001). It is used as an analytical reagent and is now well recognized. Copper(III) is involved in many biological electron transfer reactions (Peisach, Alsen, & Bloomberg, 1966). Periodate and tellurate complexes of copper(III) have been used in the estimation of various organic substrates. They have also been used in the differential titration of organic mixtures, in the estimation of chromium, calcium and magnesium from their ores, antimony, and arsenic and tin from their alloys (Sethuram, 2003). When the copper(III) periodate complex is oxidant and when multiple equilibria between different copper(III) species are involved, it would be interesting to know which of the species is the active oxidant.

Norfloxacin[1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid] is a synthetic, broad-spectrum, fluoroquinoline antibacterial agent for oral administration. It has in vitro activity against many Gram-positive and Gram-negative bacteria. It also inhibits deoxyribonucleic acid synthesis, and is bactericidal (Drug information 88, 1988; Kastrup, 1988). Norfloxacin is a fluoroquinolone used in the treatment of several bacterial diseases, including urinary tract infections in humans (Lawrenson & Logie, 2001), enteritis in dogs (Bhaumik, 1997), and chronic respiratory disease in chickens (Sumano, Ocampo, Brumbaugh, & Lizarraga, 1998). A dextran-linked prodrug has been developed from norfloxacin for treating mycobacterium bovis infections (Domurado et al., 2005). Metabolism of norfloxacin via N-acetylation, oxidation, and breakdown of the piperazine ring has been reported for humans (Pauliukonis, Musson, & Bayne, 1984) and fungi (Parshikov et al., 2001). The drug and its formulations are listed in the US Pharmacopoeia (Mack, 1998) and European Pharmacopoeia (European Pharmacopoeia, 1997).

As a result of their extensive usage, fluoroquinolones may enter the environment via wastewater effluent and biosolids from sewage treatment plants and via manure and litters from food-producing animal husbandry. The presence and accumulation of fluoroquinolone antibiotics in aquatic environments, albeit at low concentrations, may pose threats to the ecosystem and human health by inducing increase and spread of bacterial drug resistance due to long-term exposure. This necessitates the development of various advanced oxidation processes for the transformation of fluoroquinolones in water.

The structure of norfloxacin is shown below which consists of piperazine and pyridine moieties.

![Structure of Norfloxacin](image)

In view of potential pharmaceutical importance of norfloxacin and lack of literature on the oxidation of this drug by any oxidant except in one case (Nanda, Mayanna, & Gowda, 1999) and the complexity of the reaction, a detailed study of the reaction becomes important. The present work is aimed at checking the reactivity of norfloxacin toward diperiodatocuprate(III) in an alkaline medium, at determining the redox chemistry of the diperiodatocuprate(III) in such media, and at arriving at a plausible mechanism.

2. Experimental

All chemicals used were of analytical grade. Double-distilled water was used throughout the work. The solution of norfloxacin (Bayer, AG) was prepared by dissolving known amount of compound in 6.0 cm³ of 0.3 mol dm⁻³ NaOH (sd fine Chemicals) and further diluted to 100 cm³ with double-distilled water. KNO₃ and KOH (sd fine Chemicals) were used to maintain the ionic strength and alkalinity of the reaction, respectively. A stock standard solution of periodate was prepared by dissolving a known weight
of KIO₄ (Riedel-de Haen) in hot water and used after keeping for 24 h. Its concentration was ascer-
tained idometrically (Panigrahi & Misro, 1978) at neutral pH, which was maintained using phosphate
buffer. The copper(III) periodate complex was prepared (Murthy, Sethuram, & Rao, 1981) and standard-
ized by a standard procedure (Jeffery, Bassett, Mendham, & Denny, 1996).

2.1. Instruments used
For kinetic measurements: UV–vis spectrophotometer (Varian CARY 50 Bio). For product analysis: LC–ESI–
MS technique: Hewlett Packard 1100 reverse phase high-performance liquid chromatography (HPLC)
system with a phenemene C-18 column, hp 1100 series diode array UV/Visible detector, and hp 1100
MSD Series mass analyzer. FTIR technique: Nicolet-5700 USA. ¹H NMR technique: Bruker 300 MHz.

2.2. Kinetics
The oxidation of norfloxacin by DPC was followed under pseudo–first-order conditions where norfloxa-
cin concentration was excess over DPC at 27.0 ± 0.1°C unless otherwise stated. The reaction was initi-
ated by mixing the required quantities of previously thermostatted solutions of norfloxacin and DPC,
which also contained definite quantities of KOH and KIO₄. The progress of reaction was followed by
measuring the absorbance of unreacted DPC in the reaction mixture present in a 1-cm cell in a ther-
mostatted compartment of a Varian CARY 50 Bio UV–vis spectrophotometer at 415 nm. And extinc-
tion coefficient, ε, was found to be 6213 ± 100 dm³ mol⁻¹ cm⁻¹. The kinetic runs were followed and more
than 75% completion of the reaction and good first kinetics were observed. The pseudo-first-order
rate constants were determined from the slopes of log (absorbance) versus time plots (Figure 1) and
are reproducible within ±5%. The effect of dissolved oxygen on the reaction was studied by preparing
the reaction mixture and following the reaction in an atmosphere of nitrogen. No significant difference
between the results was observed. However, fresh solutions were used during the experiments.

3. Results

3.1. Stoichiometry and product analysis
Different sets of concentrations of reaction mixtures at constant hydroxide ion and periodate ion con-
centrations were kept in a closed container under a nitrogen atmosphere at 27°C. After one hour, the DPC
concentration was assayed by measuring the absorbance at 415 nm. The results indicated that one mole
of norfloxacin reacts with two moles of DPC (1:2) as shown below. The main reaction product was identi-
fied as 1-ethyl-6-fluoro-2-hydroxy-4-oxo-7-piperazin-1-yl-1,4-hydroquinoline-3-carboxylic acid.

The oxidation product of norfloxacin, 1-ethyl-6-fluoro-2-hydroxy-1,4-dihydro-4-oxo-7-(1-piperazinyl)-
3-quinoline carboxylic acid was isolated with the help of TLC and other separation techniques and characte-
rized by LC–ESI–MS, FTIR, and ¹H NMR spectral studies. LC–ESI–MS analysis was carried out
using a reverse phase HPLC system with phenemene’s C-18 column, UV/Visible detector, and series
mass analyzer. Twelve µL of acidified reaction mixture was injected. The mobile phase consisted of
acetonitrile (eluent A) and methanol (containing 0.1% CH₃COOH) at a flow rate of 1 cm³ min⁻¹. Gradient
elution was run to separate the substrate and reaction products. Gradient 0 min/95% A—15 min/35% A—25 min/35% A—30 min/95% A—35 min/35% A. LC–ESI–MS analysis of the reaction indicated the presence of a product with molecular ion of m/z 335 (yield 90%), corresponding to 1-ethyl-6-fluoro-2-hydroxy-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid (Figure 2). The IR spectroscopy shows a peak at 1731 cm$^{-1}$ and is due to acidic C=O stretching; the peak due to ketonic C=O stretching will appear at 1644 cm$^{-1}$; 3056 cm$^{-1}$ is due to NH stretching of the piperazine moiety and the broad peak at 3424 cm$^{-1}$ is due to OH stretching.

$^1$H-NMR (DMSO) shows singlet at 8.9 ppm due to acidic OH, NH of piperazine, and singlet of phenolic OH at 6.6 ppm, which disappears on D$_2$O exchange; this confirms the formation of product 1-ethyl-6-fluoro-2-hydroxy-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid (Figure 3).

3.2. Reaction orders
The reaction orders were determined from the slope of log $k_{obs}$ versus log (concentration) plots by varying the concentrations of norfloxacin and alkali in turn while keeping all other concentrations and conditions constant.

3.3. Effect of [diperiodatocuprate(III)]
At a constant concentration of norfloxacin, $5.0 \times 10^{-4}$ mol dm$^{-3}$, alkali, 0.08 mol dm$^{-3}$, and at a constant ionic strength, 0.20 mol dm$^{-3}$, the DPC concentration was varied in the concentration range of $1.0 \times 10^{-5}$–$1.0 \times 10^{-4}$ mol dm$^{-3}$. The plot of log (absorbance) versus time was linear and almost parallel over three
half-lives of the reaction for different initial concentrations of DPC (Figure 1), indicating the unit order with respect to DPC concentration. This was also confirmed by the constant values of pseudo-first-order rate constants, $k_{\text{obs}}$, for different DPC concentrations.

3.4. Effect of [norfloxacin]
The effect of norfloxacin concentration on the reaction was studied at constant concentrations of alkali and DPC and at a constant ionic strength of 0.20 mol dm$^{-3}$ at 27°C. The substrate, norfloxacin, was varied in the range of $1.0 \times 10^{-4}$–$1.0 \times 10^{-3}$ mol dm$^{-3}$. The $k_{\text{obs}}$ values increased with increase in the concentration of norfloxacin. The order with respect to norfloxacin concentration was found to be less than unity.

3.5. Effect of [alkali]
The effect of increase in concentration of alkali on the reaction was studied at constant concentrations of norfloxacin and DPC at a constant ionic strength of 0.2 mol dm$^{-3}$ at 27°C. The pseudo-first-order rate constant, $k_{\text{obs}}$, was found to be increase with increase in alkali concentration (Table 1). The order with respect to OH$^-$ ion concentration was found to be less than unity.

3.6. Effect of [periodate]
The effect of increase in concentration of periodate was studied by varying the periodate concentration from $1.0 \times 10^{-5}$ to $1.0 \times 10^{-4}$ mol dm$^{-3}$, keeping all other reactant concentrations constant. It was found that the added periodate had a retarding effect on the rate of reaction (Table 1). The order with respect to periodate concentration was found to be negative and less than unity.
3.7. Effect of ionic strength and dielectric constant of the medium
The effect of ionic strength was studied by varying the KNO₃ concentration from 0.40 to 0.20 mol dm⁻³ at constant concentrations of DPC, norfloxacin, and alkali. It was found that increase in ionic strength had no effect on the rate of reaction. The effect of 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 decrease in dielectric constant of the reaction medium has no effect on the rate of reaction.

3.8. Effect of initially added product
The Cu(II) ion concentration was varied from 1.0 × 10⁻⁵ to 1.0 × 10⁻⁴ mol dm⁻³ at constant concentrations of DPC, norfloxacin, alkali, and ionic strength. It was found that initially added Cu(II) ion had no effect on the rate of reaction.

3.9. Polymerization study
Under the reaction conditions used for kinetic measurements, a 100-cm³ solution of norfloxacin contained 8% acrylonitrile mixed with a 100-cm³ DPC in a three-neck flask; both solutions were flushed for 30 min with nitrogen gas before mixing. By stirring the reaction mixture for 3 h, under the protection of nitrogen gas, precipitation of polyacrylonitrile could be noticed. This observation implies the involvement of free radical in the reaction mixture.

3.10. Effect of temperature
The influence of temperature on the rate of reaction was studied at 17, 27, 37, and 47°C, under varying concentrations of norfloxacin, alkali, and periodate, keeping other conditions constant. The rate constant (k) of the slow step of Scheme 1 was obtained from the slopes and intercepts of 1/kₖₜₚₑₚₑₚₑₒₚₑₚₑₛₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑège亨
values are given in Table 2. The activation parameters for the rate-determining step were obtained by the least square method of plot of log $k_1$ versus $1/T$ and are presented in Table 2.

4. Discussion

The water-soluble copper(III) periodate complex is reported (Reddy, Sethuram, & Navneeth, 1987) as $[\text{Cu(HIO}_6^2\text{O}_2\text{OH}^2]^-$. However, in an aqueous alkaline medium and at a high pH range, as employed in the study, periodate is unlikely to exist as $\text{HIO}_4^-\text{O}_6$ (as present in the complex), as is evident from its involvement in the multiple equilibria (Bailar, Emeleus, Nyholm, & Trotman Dikenson, 1975) (1)–(3) depending on the pH of the solution.

$$\text{H}_5\text{IO}_6 \rightleftharpoons \text{H}_4\text{IO}_6^- + \text{H}^+ \quad (1)$$

$$\text{H}_4\text{IO}_6^- \rightleftharpoons \text{H}_3\text{IO}_6^{2-} + \text{H}^+ \quad (2)$$

$$\text{H}_3\text{IO}_6^{2-} \rightleftharpoons \text{H}_2\text{IO}_6^{3-} + \text{H}^+ \quad (3)$$

Periodic acid exists as $\text{H}_5\text{IO}_6$ in an acid medium and as $\text{H}_4\text{IO}_6$ around pH 7. Thus, under the conditions employed in alkaline medium, the main species are expected to be $\text{H}_3\text{IO}_6^{2-}$ and $\text{H}_2\text{IO}_6^{3-}$. At higher concentrations, periodate also tends to dimerise (Sethuram, 2003). However, formation of this species is
negligible under conditions employed for kinetic study. Hence, at the pH employed in this study, the soluble copper(III) periodate complex exists as diperiodatocuprate (III), \([\text{Cu(OH)}_2(H_3\text{IO}_6)_2]^{3-}\), a conclusion also supported by the literature (Bal Reddy, Sethuram, & Navaneeth Rao, 1981).

The reaction between the diperiodatocuprate(III) complex and norfloxacin in an alkaline medium has the stoichiometry 1:2 (norfloxacin:DPC), with a first-order dependence on [DPC] and an apparent order of less than unity in [substrate] and [alkali], and negative fractional order dependence on the periodate. No effect of added products was observed. Based on the experimental results, a mechanism is proposed for which all the observed orders in each constituent such as [oxidant], [reductant], [OH\(^{-}\)], and \([H_3\text{IO}_6]^{2-}\) may be well accommodated.

The result of increase in rate with increase in alkali concentration can be explained in terms of prevailing equilibrium of formation of \([\text{Cu(OH)}_2(H_3\text{IO}_6)(H_2\text{IO}_6)]^{4-}\) from \([\text{Cu(OH)}_2(H_3\text{IO}_6)_2]^{3-}\) as

\[
[\text{Cu(OH)}_2(H_3\text{IO}_6)_2]^{3-} + \text{OH}^{-} \rightleftharpoons [\text{Cu(OH)}_2(H_3\text{IO}_6)(H_2\text{IO}_6)]^{4-} + \text{H}_2\text{O}
\]

Also, decrease in rate with increase in \([H_3\text{IO}_6]^{2-}\) suggests the equilibrium of Cu(I) periodate complex to form monoperiodatocuprate(III) (MPC)

\[
[\text{Cu(OH)}_2(H_3\text{IO}_6)(H_2\text{IO}_6)]^{4-} \rightleftharpoons [\text{Cu(OH)}_2(H_3\text{IO}_6)]^{-} + (H_2\text{IO}_6)^{3-}
\]

Such type of equilibria has been noticed in the literature (Shetti & Nandibewoor, 2009). It may be expected that a lower periodate complex such as monoperiodatocuprate(III) (MPC) is more important in the reaction than the DPC. The inverse fractional order in \([H_3\text{IO}_6]^{2-}\) concentration might also be due to this reason. Therefore, MPC is the reactive form of the oxidant in the present study.

The fractional order with respect to norfloxacin concentration indicates the formation of a complex between the norfloxacin and the DPC species. Such a complex formation between the oxidant and norfloxacin has also been observed in the literature (Hiremath, Kiran, & Nandibewoor, 2007). Then, this complex (C) breaks up in the slow step resulting in the formation of an intermediate free radical species of norfloxacin. This intermediate species further reacts with another molecule of MPC species in a fast step to yield the products. On this basis, a general mechanism involving MPC is as follows:

\[
\begin{align*}
\text{Cu(HL)}_2 + [\text{OH}^{-}] & \rightleftharpoons \text{CuL(HL)} + \text{H}_2\text{O} \\
\text{CuL(HL)} & \rightleftharpoons \text{Cu(HL)} + \text{L} \\
\text{Cu(HL)} + \text{S} & \rightleftharpoons \text{Complex (C)} \\
\text{Complex (C)} & \rightarrow \text{S}^{\cdot} + \text{Cu(II)} \\
\text{S}^{\cdot} + \text{Cu(HL)} & \rightarrow \text{Products} + \text{Cu(II)}
\end{align*}
\]

So, the detailed mechanistic scheme for the oxidation of norfloxacin by diperiodatocuprate(III) is as follows:

\[
[\text{Cu(OH)}_2(H_3\text{IO}_6)_2]^{3-} + \text{OH}^{-} \rightleftharpoons [\text{Cu(OH)}_2(H_3\text{IO}_6)]^{-} + (H_2\text{IO}_6)^{3-} + \text{H}_2\text{O}
\]

\[
[\text{Cu(OH)}_2(H_3\text{IO}_6)(H_2\text{IO}_6)]^{4-} \rightleftharpoons [\text{Cu(OH)}_2(H_3\text{IO}_6)]^{-} + (H_2\text{IO}_6)^{3-}
\]
The probable structure of complex(C) is given below

Since Scheme 1 is in accordance with the generally well-accepted principle of non-complementary oxidations taking place in sequence of one-electron steps, the reaction between the substrate and oxidant would afford a radical intermediate. A free radical scavenging experiment revealed such a possibility (see infra). This type of radical intermediate has also been observed in the literature (Chougale, Hiremath, & Nandibewoor, 1997).

Spectroscopic evidence for the complex formation between the oxidant and substrate was obtained from UV–vis spectra of norfloxacin (5.0 × 10⁻⁴), DPC (5.0 × 10⁻⁵) and [OH⁻] (0.06 mol dm⁻³), and mixture of both. A bathochromic shift of about 6 nm from 342 to 348 nm in the spectra of DPC was observed (Bal Reddy et al., 1981; Hiremath et al., 2007; Murthy et al., 1981; Shetti & Nandibewoor, 2009). However, the Michaelis–Menten plot also proved the complex formation between DPC and norfloxacin, which explains the less than unit order dependence on [norfloxacin]. From Scheme 1, the rate law (8) may be derived as follows:

\[
\text{Rate} = -\frac{d[DPC]}{dt} = k[C] = \frac{kK_1K_2K_3[Cu(OH)\_2(H\_3IO\_6)]^-[\text{NOR}][OH^-]}{[H\_3IO\_6^{2-}]}
\]  

The total concentration of DPC, i.e. \([DPC]_t\), is given by,

\[
[DPC]_t = [DPC]_r + [Cu(OH)\_2(H\_3IO\_6)]^{4-} + [Cu(OH)\_2(H\_3IO\_6)]^- + [\text{Complex(C)}]
\]
where “t” and “f” refer to the total and free concentrations. Similarly, the total concentration of norfloxacain is given by

\[ [\text{NOR}]_t = [\text{NOR}]_f + [C] \]

Similarly,

\[ [\text{OH}^-]_t = [\text{OH}^-]_f + [\text{Cu(OH)}_2(\text{H}_3\text{IO}_6^2)]^{2-} + [\text{Cu(OH)}_2(\text{H}_3\text{IO}_6)]^{-} + [\text{Complex(C)}] \]

\[ [\text{OH}^-]_f = [\text{OH}^-]_t \]

In view of lower concentrations of DPC, OH\(^-\), and H\(_2\)IO\(_6\)\(^-\) used,

\[ [\text{NOR}]_t = [\text{NOR}]_f \]  

Similarly,

\[ [\text{OH}^-]_t = [\text{OH}^-]_f + [\text{Cu(OH)}_2(\text{H}_3\text{IO}_6)(\text{H}_2\text{IO}_6)]^{4-} + [\text{Cu(OH)}_2(\text{H}_3\text{IO}_6)]^{-} + [\text{Complex(C)}] \]

\[ [\text{OH}^-]_f = [\text{OH}^-]_t \]

Substituting Equations 5, 6 and 7 in Equation 4 and omitting subscripts “t” and “f”, we get,

\[ \text{Rate} = -\frac{d[DPC]}{dt} = \frac{kK_1 K_2 K_3 [\text{DPC}][\text{NOR}][OH^-]}{[H_2\text{IO}_6^{2-}] + K_1 [OH^-][H_2\text{IO}_6^{2-}] + K_2 [OH^-] + kK_1 K_2 [OH^-][\text{NOR}] \]  

or

\[ k_{obs} = \frac{\text{Rate}}{[\text{DPC}]} = \frac{kK_1 K_2 K_3 [\text{NOR}][OH^-]}{[H_2\text{IO}_6^{2-}] + K_1 [OH^-][H_2\text{IO}_6^{2-}] + K_2 [OH^-] + kK_1 K_2 [OH^-][\text{NOR}] \]  

The rate law (8) can be rearranged to Equation 9, which is suitable for verification.

\[ \frac{1}{k_{obs}} = \frac{[H_2\text{IO}_6^{2-}]}{kk_c K_2 K_3 [\text{NOR}][OH^-]} + \frac{[H_2\text{IO}_6^{2-}]}{kk_c K_3 [\text{NOR}]} + \frac{1}{k} + \frac{1}{k} \]

According to Equation 9, the plots of 1/k\(_{obs}\) versus 1/[OH\(^-\)], 1/k\(_{obs}\) versus 1/[NOR], and 1/k\(_{obs}\) versus [H\(_2\)IO\(_6\)\(^-\)] should be linear and are found to be so (Figure 4). The slopes and intercepts of such plots
lead to the values of $K_1$, $K_2$, $K_3$, and $k$ as $(1.3 \pm 0.4) \times 10^{-2}$ mol dm$^{-3}$, $(1.90 \pm 0.06) \times 10^{-2}$ mol dm$^{-3}$, $(5.6 \pm 0.3) \times 10^3$ dm$^3$ mol$^{-1}$, and $(3.9 \pm 0.2) \times 10^{-3}$ s$^{-1}$, respectively. The equilibrium constant $K_1$ is far greater than $K_2$. This may be attributed to the greater tendency of DPC to undergo hydrolysis compared to the dissociation of the hydrolyzed species in an alkaline medium. The negligible small effect of ionic strength and dielectric constant of medium on the rate explains qualitatively the reaction between neutral and negatively charged ions, as seen in Scheme 1.

The thermodynamic quantities for the first, second, and third equilibrium steps of Scheme 1 can be evaluated as follows. The $[\text{H}_2\text{IO}_6^-]$, [NOR], and $[\text{OH}^-]$ (as in Table 2) were varied at four different temperatures. The plots of $1/k_{\text{obs}}$ versus $1/[\text{OH}^-]$, $1/k_{\text{obs}}$ versus $1/[\text{NOR}]$, and $1/k_{\text{obs}}$ versus $[\text{H}_2\text{IO}_6^-]$ should be linear (Figure 4). From the slopes and intercepts, the values of $K_1$ were calculated at different temperatures (Table 2). A van’t Hoff plot was made for variation of $K_1$ with temperature ($\log K_1$ versus $1/T$) and the values of enthalpy of reaction, $\Delta H$, entropy of reaction, $\Delta S$, and free energy of reaction, $\Delta G$, were calculated for the first equilibrium step. These values are given in Table 2. A comparison of the latter values with those obtained for the slow step of the reaction shows that these values mainly refer to the rate-limiting step, supporting the fact that the reaction before rate-determining step is fairly fast and involves low activation energy (Weissberger & Lewis, 1974). In the same manner, $K_2$ and $K_3$ values were calculated at different temperatures and their corresponding values of the thermodynamic quantities are given in the Table 2.

The values of $\Delta H^\circ$ and $\Delta S^\circ$ were both favorable for electron transfer processes. The negative value of $\Delta S^\circ$ indicates that the complex(C) is more ordered than the reactants (Weissberger & Lewis, 1974). The value of $\Delta S^\circ$ within the range for radical reaction has been ascribed (Walling, 1957) to the nature of electron pairing and unpairing processes and to the loss of degrees of freedom formerly available to the reactants upon the formation of rigid transition state. 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. This conclusion is supported by the literature (Hiremath, Sirsalmath, & Nandibewoor, 2008).

5. Conclusion
Among various species of DPC in an alkaline medium, monoperiodatocuprate(III) (MPC) is considered as an active species for the title reaction. The results demonstrate that in carrying out this
reaction, the role of pH in the reaction medium is crucial. Rate constant of slow step and other equilibrium constants involved in the mechanism are evaluated and activation parameters with respect to slow step of reaction were computed.

**Nomenclature and abbreviations**

| Abbreviation | Definition |
|--------------|------------|
| DPC | diperiodatocuprate(III) |
| NF | norfloxacin |
| ε | molar absorption coefficient |
| kobs | observed rate constant |
| k | rate constant with respect to slow step of the mechanism |
| K1 and K2 | equilibrium constants |
| ΔH | change in enthalpy of reaction |
| ΔS | change in entropy of reaction |
| ΔG | change in free energy of reaction |
| ΔH° | enthalpy of activation |
| ΔS° | entropy of activation |
| ΔG° | free energy of activation |
| D | dielectric constant of the medium |
| I | ionic strength of the medium |
| FT-IR | Fourier transform infrared spectra |
| 1H NMR | proton nuclear magnetic resonance |
| UV | ultraviolet spectra |
| TLC | thin layer chromatography |
| LC–ESI–MS | liquid chromatography–electrospray ionization–mass spectrometry |

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