Oxidation of amoxicillin by hexacyanoferrate(III) in aqueous alkaline medium—A kinetic and mechanistic approach

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The kinetics and mechanism of oxidation of amoxicillin by hexacyanoferrate(III) in aqueous alkaline medium at constant ionic strength of 0.10 mol dm\(^{-3}\) is studied spectrophotometrically at 25 °C. The reaction exhibits 2:1 ([Fe(CN)\(_6\)]\(^{3-}\): amoxicillin) stoichiometry. The reaction products have been identified with the help of TLC and characterized by FT-IR, GCMS and LCMS. The reaction is first order in hexacyanoferrate(III) concentration but fractional order in both amoxicillin and alkali concentrations. The effects of ionic strength and dielectric constant have been investigated. In a composite equilibrium step, amoxicillin binds to hexacyanoferrate(III) to form a complex that subsequently decomposes to the products. Based on investigation of the reaction at different temperatures, computation of the activation parameters with respect to the slow step of the proposed mechanism was evaluated.

Keywords: Kinetics, Reaction mechanism, Oxidation, Amoxicillin, Hexacyanoferrate(III)

Amoxicillin (AMX), D-\(\alpha\)-amino-p-hydroxybenzylpenicillin trihydrate, is one of the most frequently used \(\beta\)-lactam antibiotics\(^1,2\). It is usually the drug of choice within the class because it is better absorbed following oral administration, than other \(\beta\)-lactam antibiotics. Amoxicillin is susceptible to degradation by \(\beta\)-lactamase-producing bacteria, which are resistant to a broad spectrum of \(\beta\)-lactam antibiotics, such as penicillin\(^3\).

Hexacyanoferrate(III) has been widely used to oxidize numerous organic and inorganic compounds in basic, acidic and neutral media\(^4,5\). Hexacyanoferrate(III) is a one-electron oxidant with a redox potential of +0.36 V in acidic medium and +0.45 V in basic medium\(^6\). In most of its oxidations, hexacyanoferrate(III) is mainly used as a hydrogen atom abstractor\(^7\) and/or free radical generator\(^8\). Hexacyanoferrate(III), due to its strong oxidizing properties, has also been extensively employed as a reagent in analytical investigations of compounds like hydrazine hydrate, atropine sulfate and arginine\(^9,10\), esters\(^11\) etc. The title reaction was investigated using different oxidants like Cr(VI), Ce(IV), and DPC in both alkaline and acidic medium, but the rate of reaction was not significant. However, the reaction is facile only when hexacyanoferrate(III) is used as an oxidant in alkaline medium. Thus, to explore the mechanism of oxidation by hexacyanoferrate(III) in aqueous alkaline medium and to check the selectivity of amoxicillin towards hexacyanoferrate(III) during oxidation, we have undertaken a detailed study of the title reaction to arrive at a plausible mechanism.

Materials and Methods

All chemicals employed in the present work were of analytical reagent grade. All stock solutions were prepared in millipore water. The stock solution of amoxicillin (Sigma-Aldrich) was prepared by dissolving a known amount in millipore water. The stock solution of the oxidant, hexacyanoferrate(III), was prepared by dissolving K\(_3\)Fe(CN)\(_6\) (SISCO CHEM) in millipore water and the solution was standardized iodometrically\(^12\). Hexacyanoferrate(II) solution was prepared by dissolving a known amount of K\(_4\)Fe(CN)\(_6\) (SD Fine Chem) in millipore water. In the reaction solutions, the required alkalinity and ionic strength were maintained with KOH (Fisher Scientific) and KNO\(_3\) (Fisher Scientific), respectively. \(t\)-Butyl alcohol (Spectrochem) was used to vary the dielectric constant of the medium. All solutions were prepared freshly.

For kinetic measurements, a Peltier Accessory (temperature control) attached Varian Cary 50 Bio
UV-vis spectrophotometer (Varian, Victoria-3170, Australia) was used. For product analysis, a QP-2010S Shimadzu gas chromatograph mass spectrometer and Nicolet 5700-FT-IR spectrometer were used.

**Kinetic measurements**

The oxidation of amoxicillin(AMX) by hexacyanoferrate(III) was followed under pseudo first-order condition where concentration of [AMX] was 10-fold in excess over concentration of hexacyanoferrate(III) at a constant ionic strength of 0.10 mol dm$^{-3}$ in alkaline medium and at a constant temperature, 25 ± 0.1 °C. The reaction was initiated by mixing thermally equilibrated solutions of hexacyanoferrate(III) and amoxicillin, which also contained the required quantities of KOH (0.06 mol dm$^{-3}$) and KNO$_3$ to maintain alkalinity and ionic strength, respectively. The reaction was monitored by the decrease in absorbance of hexacyanoferrate(III) at its maximum absorption of 420 nm. It was verified that there are no interference from other reagents at this wavelength. Beer’s law was verified under the present experimental conditions, and $\varepsilon$ was found to be 1050±10 dm$^3$ mol$^{-1}$ cm$^{-1}$. The pseudo first-order rate constants $k_{obs}$ were evaluated from the slopes of the plots of log [Fe(CN)$_6$]$^{3-}$ versus time. The plots in all cases were linear over 70% completion of the reaction (Fig. 1). The $k_{obs}$ values were reproducible within ±5% and are the average of minimum three sets of kinetic run (Table 1).

The reaction mixture containing excess concentration of [Fe(CN)$_6$]$^{3-}$ over [AMX] was allowed to react for 4 h at 25 °C. One part of the reaction mixture was used to analyse the remaining hexacyanoferrate(III) spectrophotometrically and the remaining reaction mixture was acidified, concentrated and extracted with ether. The ether layer was basified using aqueous sodium bicarbonate and the aqueous layer was separated and neutralized carefully to obtain 2-amino-2-formylacetic acid. The ether layer was further basified using aqueous sodium hydroxide and the aqueous layer was neutralized with dilute HCl to get the 4-hydroxybenzonitrile. The ether layer was finally evaporated to get 1-amino-2-methylpropane-2-thiol. These products are characterized by FT-IR, GCMS and LCMS.

**Results and Discussion**

The kinetic studies indicate that one mole of amoxicillin requires two moles of hexacyanoferrate(III) according to Eq. (1).

The FT-IR spectra of 4-hydroxybenzonitrile shows (CN) band at 2075 cm$^{-1}$, because of presence of hydroxyl group which is evident through broad peak at 3424 cm$^{-1}$ (Supplementary Data, Fig. S1). The product 2-amino-2-formylacetic acid shows a peak at 104 m/z in LCMS (Supplementary Data, Fig. S2), while 1-amino-2-methylpropane-2-thiol is evident in the GCMS showing a peak at 105 m/z (Supplementary Data, Fig. S3). The product, [Fe(CN)$_6$]$^{4-}$, was isolated and analysed by titrating it against Ce(IV) solution$^{13}$. The CO$_2$ was qualitatively detected by bubbling nitrogen gas

<table>
<thead>
<tr>
<th>[Fe(CN)$_6$]$^{3-}$ (mol dm$^{-3}$)</th>
<th>[AMX]×10$^3$ (mol dm$^{-3}$)</th>
<th>[OH$^-$]×10$^2$ (mol dm$^{-3}$)</th>
<th>$k_{obs}$×10$^3$ (s$^{-1}$)</th>
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<tbody>
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<td>1.90</td>
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<td>2.0</td>
<td>5.0</td>
<td>8.0</td>
<td>2.11</td>
</tr>
<tr>
<td>2.0</td>
<td>5.0</td>
<td>10.0</td>
<td>2.55</td>
</tr>
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</table>

Fig. 1—First order plots of oxidation of amoxicillin by hexacyanoferrate(III) in alkaline medium at 25 °C. [AMX] = 5.0×10$^{-3}$ mol dm$^{-3}$; [OH$^-$] = 0.06 mol dm$^{-3}$; $I$ = 0.10 mol dm$^{-3}$, [Fe(CN)$_6$]$^{3-}$×10$^{-3}$ mol dm$^{-3}$ = (1) 0.50; (2) 1.0; (3) 2.0; (4) 3.0; (5) 4.0; (6) 5.0.
through the acidified reaction mixture and passing the liberated gas through tube containing limewater.

The reaction orders were determined from the slope of log $k_{obs}$ versus log concentration plots, by varying the concentration of the reductant, and alkali in turn while keeping the others constant.

**Effect of reaction parameters on the rate of oxidation**

With constant concentrations of $[\text{AMX}] = 5.0 \times 10^{-3}$ mol dm$^{-3}$, $[\text{OH}^-] = 0.06$ mol dm$^{-3}$ and at constant ionic strength $= 0.10$ mol dm$^{-3}$, the oxidant, hexacyanoferate(III), concentration was varied in the range of $0.50 \times 10^{-4}$ – $5.0 \times 10^{-4}$ mol dm$^{-3}$.

The observed rate constants, $k_{obs}$, were almost constant (Table 1) and the linearity of the plot of log $[\text{Fe(CN)}_6^{3-}]$ versus time (Fig. 1) over 70% completion of the reaction, indicates the unit order with respect to hexacyanoferate(III) concentration.

The substrate, amoxicillin, concentration was varied in the range of $0.50 \times 10^{-3}$ – $5.0 \times 10^{-3}$ mol dm$^{-3}$ at 25 °C keeping all other reactant concentration and conditions constant. As the concentration of amoxicillin increases the $k_{obs}$ also increases (Table 1). The apparent order in $[\text{AMX}]$ was found to be less than unity.

The concentration of OH$^-$ was varied in the range $0.01$–$0.10$ mol dm$^{-3}$ at constant concentrations of $[\text{Fe(CN)}_6^{3-}]$, amoxicillin, and at constant ionic strength and temperature. The rate of reaction increased with increase in [alkali] (Table 1) and the order was found to be less than unity.

The initially added product, hexacyanoferate(II), did not have any significant effect on the rate of reaction.

The reaction was studied by varying the ionic strength from 0.1 mol dm$^{-3}$ to 1.0 mol dm$^{-3}$ by varying the concentration of potassium nitrate at constant concentrations of hexacyanoferate(III), amoxicillin and alkali. The values of $k_{obs}$ were found to increase with increasing the ionic strength. The plot of log $k_{obs}$ versus $\sqrt{I}$ was found to be linear with positive slope (Fig. 2) indicating reaction between two ions of similar charges. The effect of dielectric constant was studied by varying the $\alpha$-butyl alcohol-water ($v/v$) composition from 0 – 20%. It was found that as the composition of $\alpha$-butyl alcohol increased in the reaction medium, the rate of reaction decreased and the plot of log $k_{obs}$ versus $1/D$ is linear with negative slope (Fig. 2).

**Test for free radicals**

The possible occurrence of free radicals in this reaction was tested by the addition of known quantity of acrylonitrile (scavenger) to the following reaction solutions: (1) $0.01$ mol dm$^{-3}$ hexacyanoferate(III), (2) $0.01$ mol dm$^{-3}$ amoxicillin, and, (3) $0.01$ mol dm$^{-3}$ hexacyanoferate(III) + $0.01$ mol dm$^{-3}$ amoxicillin; all in $1.0$ mol dm$^{-3}$ KOH + $0.2$ mol dm$^{-3}$ KNO$_3$. To all three solutions known quantities of acrylonitrile (scavenger) was added.
initially, and kept in an inert atmosphere for 1 h at room temperature. These solutions were diluted with methanol. It was observed that none of the solutions became turbid, suggesting that there was no participation of free radicals in the reaction.

**Effect of temperature on rate of oxidation**

The reaction was studied at four different temperatures, 15, 25, 35 and 45 °C and varying the concentration of amoxicillin and alkali, keeping other conditions constant. The rate of reaction increased with the increase in temperature. The rate constant, $k$, of the slow step of the Scheme 1 was obtained from the slopes and intercepts of $1/k_{obs}$ versus $1/[AMX]$ and $1/k_{obs}$ versus $1/[OH^-]$ plots at four different temperatures (Table 2). The activation energy corresponding to these rate constants was evaluated from the Arrhenius plot of log $k$ versus $1/T$ from which other activation parameters were also obtained (Table 2).

**Table 2—Activation parameters and thermodynamic quantities for the oxidation of amoxicillin by alkaline hexacyanoferrate(III)**

(A) Effect of temperature with respect to slow step of the Scheme 1 and activation parameters

<table>
<thead>
<tr>
<th>Temp. (K)</th>
<th>$k \times 10^3$ (s$^{-1}$)</th>
<th>Parameter</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>288</td>
<td>5.13</td>
<td>$E_a$ (kJ mol$^{-1}$)</td>
<td>44 ± 3</td>
</tr>
<tr>
<td>298</td>
<td>9.30</td>
<td>$\Delta H^o$ (kJ mol$^{-1}$)</td>
<td>42 ± 3</td>
</tr>
<tr>
<td>308</td>
<td>16.8</td>
<td>$\Delta S^o$ (J K$^{-1}$mol$^{-1}$)</td>
<td>-141 ± 5</td>
</tr>
<tr>
<td>318</td>
<td>30.0</td>
<td>$\Delta G^o$ (kJ mol$^{-1}$)</td>
<td>84 ± 3</td>
</tr>
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</table>

(B) Equilibrium constants $K_1$ and $K_2$ at different temperatures

<table>
<thead>
<tr>
<th>Temp. (K)</th>
<th>$K_1$ (dm$^3$ mol$^{-1}$)</th>
<th>$K_2$ (dm$^3$ mol$^{-1}$)</th>
</tr>
</thead>
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<td>288</td>
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<td>298</td>
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<td>308</td>
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<td>318</td>
<td>2.7</td>
<td>143</td>
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</table>

(C) Thermodynamic quantities with respect to $K_1$ and $K_2$

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<th>Using $K_1$ values</th>
<th>Using $K_2$ values</th>
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<tbody>
<tr>
<td>$\Delta H$ (kJ mol$^{-1}$)</td>
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</tr>
<tr>
<td>$\Delta S$ (J K$^{-1}$mol$^{-1}$)</td>
<td>-11</td>
</tr>
<tr>
<td>$\Delta G$ (kJ mol$^{-1}$)</td>
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**Scheme 1**
DURGANNAVAR et al.: OXIDATION OF AMOXICILLIN BY HEXACYANOFERRATE(III) IN ALKALINE MEDIUM

1089

The variation in the concentrations of the oxidant, substrate, and alkali, while keeping the others constant, showed that the reaction is first order in oxidant and less than unit order in substrate and alkali concentrations. The reaction between amoxicillin and $[\text{Fe(CN)}_6]^{3-}$ has a stoichiometry of 1:2. Based on the experimental results, a mechanism is proposed in which all the observed orders in each constituent such as [oxidant], [reductant] and [OH$^-$] has been well accommodated. On oxidation of amoxicillin by hexacyanoferrate(III) in KOH media, the oxidation state of the oxidant and reductant are changed by different number of units, hence this oxidation is a non-complementary reaction with oxidant undergoing one equivalent change.

**Reaction mechanism**

In the present study, alkali combines first with amoxicillin to give the anionic form of amoxicillin in a prior equilibrium step, which is also supported by the observed fractional order in [OH$^-$] and [AMX]. The hexacyanoferrate(III) species reacts with the anionic form of amoxicillin resulting in the formation of a complex (C), which further decomposes in a slow step to form one of the product, 4-hydroxybenzonitrile, an intermediate species (i) and $[\text{Fe(CN)}_6]^{4-}$. This intermediate species containing β-lactum undergoes rapid hydrolysis in the fast step to form intermediate species (ii) which is highly unstable as compared to the penicilloic acids.$^{14}$ Hence, it undergoes subsequent fission of C-S bond to yield the final products 1-amino-2-methylpropane-2-thiol and 2-amino-2-formylacetic acid with the elimination of $2\text{CO}_2$. All these results may be interpreted in the detailed mechanism given in Scheme 1.

In most of the oxidation reactions, hexacyanoferrate(III) resembles Cu(II), which involves free radical formation and rapidly oxidises it.$^{15,16}$ However, our experimental results are contrary to that expected. The hexacyanoferrate(III)–hexacyanoferrate(II) system, which has higher redox potential than Cu(II)–Cu(I) couple, has a better possibility for the rapid oxidation of the free radical with hexacyanoferrate(III) in the alkaline medium. Hence, there may be a rapid oxidation of the free radical that could mask the polymerization of acrylonitrile. As found experimentally, sometimes the vinyl compounds themselves are oxidized under the experimental conditions used, resulting in the failure of free radicals test.$^{17}$

The probable structure of the complex is given in (I).

**Fig. 3**—Verification of rate law (8) in the form of Eq. (9) for the oxidation of amoxicillin by hexacyanoferrate(III) in alkaline medium. Plot of $1/k_{obs}$ versus $1/\text{[AMX]}$ (1) and $1/k_{obs}$ versus $1/\text{[OH}^-\text{]}$ (2) at 25 °C.

Such a complex formation between substrate and hexacyanoferrate(III) has been reported earlier. The formation of such complex is proven kinetically by the non-zero intercept of $1/k_{obs}$ versus $1/\text{[AMX]}$ (Fig. 3).

From Scheme 1 the rate law can be derived as follows:

\[
\text{Rate} = -\frac{d[\text{Fe(CN)}_6]^{3-}}{dt} = k[\text{Complex}]
\]

\[
= kK_2[\text{Fe(CN)}_6]^{3-}[\text{AMX}]
\]

\[
= kK_1K_2[\text{Fe(CN)}_6]^{3-}[\text{OH}^-]_f [\text{AMX}]_f \quad (2)
\]

Total hexacyanoferrate(III) concentration can be written as,

\[
[\text{Fe(CN)}_6]^{3-} = [\text{Fe(CN)}_6]_f^{3-} + [\text{Complex}]
\]

\[
= [\text{Fe(CN)}_6]_f^{3-} + K_1K_2[\text{Fe(CN)}_6]^{3-}[\text{OH}^-]_f [\text{AMX}]_f
\]

\[
= [\text{Fe(CN)}_6]_f^{3-}
\]

\[
+ \left[ 1 + K_1K_2[\text{OH}^-]_f [\text{AMX}]_f \right]
\]

\[
\]
Therefore,
\[
[\text{Fe(CN)}_6^{3-}]_f = \frac{[\text{Fe(CN)}_6]^{3-}}{1 + K_1 K_2 [\text{OH}^-]_f [\text{AMX}]_f}
\]  
\tag{3}

where subscripts ‘t’ and ‘f’ stands for total and free hexacyanoferrate(III) concentrations respectively.

Similarly,
\[
[\text{AMX}]_t = [\text{AMX}]_f + [\text{AMX}]_t + \text{[Complex]}
\]
\[
= [\text{AMX}]_f + K_1 [\text{OH}^-]_f [\text{AMX}]_f
\]
\[
+ K_1 K_2 [\text{Fe(CN)}_6]^{3-} [\text{OH}^-]_f [\text{AMX}]_f
\]
\[
= [\text{AMX}]_f [1 + K_1 [\text{OH}^-]_f + K_1 K_2 [\text{Fe(CN)}_6]^{3-} [\text{OH}^-]_f]
\]

Therefore,
\[
[\text{AMX}]_f = \frac{[\text{AMX}]_t}{1 + K_1 [\text{OH}^-]_f + K_1 K_2 [\text{Fe(CN)}_6]^{3-} [\text{OH}^-]_f}
\]
\tag{4}

In view of the low concentration of $[\text{Fe(CN)}_6]^{3-}$ used herein the term $K_1 K_2 [\text{OH}^-]_f [\text{AMX}]_f$ is neglected.

\[
[\text{AMX}]_f = \frac{[\text{AMX}]_t}{1 + K_1 [\text{OH}^-]_f}
\]
\tag{5}

Substituting Eqs (3), (4) and (5) in Eq. (2) and omitting the subscripts, we have

\[
\text{Rate} = \frac{-d[\text{Fe(CN)}_6]^{3-}}{dt}
\]
\[
= \frac{k K_1 K_2 [\text{Fe(CN)}_6]^{3-} [\text{AMX}]}{1 + K_1 K_2 [\text{OH}^-] [\text{AMX}] + K_1 [\text{OH}^-]}
\]
\[
+ K_1 K_2 [\text{Fe(CN)}_6]^{3-} [\text{AMX}]_f
\]
\tag{6}

Rearranging Eq. (6) we have:

\[
\text{Rate} = \frac{k K_1 K_2 [\text{Fe(CN)}_6]^{3-} [\text{AMX}]}{1 + K_1 K_2 [\text{OH}^-][\text{AMX}] + K_1 [\text{OH}^-] + K_1 K_2 [\text{OH}^-]^2 [\text{AMX}]}
\]
\tag{7}

The term, $K_1^2 K_2 [\text{AMX}][\text{OH}^-]^2$ in Eq. (7) can be omitted due to the low concentrations of amoxicillin and OH$^-$ used. Thus Eq. (7) becomes,

\[
\frac{[\text{Fe(CN)}_6]^{3-}}{[\text{AMX}]} = \frac{k K_1 K_2 [\text{OH}^-] [\text{AMX}]}{1 + K_1 K_2 [\text{OH}^-][\text{AMX}] + K_1 [\text{OH}^-]} + k K_2 [\text{AMX}] + \frac{1}{k}
\]
\tag{8}

Equation (8) is verified in the following form (Eq. 9).

\[
1 = \frac{1}{k K_1 K_2 [\text{OH}^-][\text{AMX}]} + \frac{1}{k K_2 [\text{AMX}]} + \frac{1}{k}
\]
\tag{9}

The plots of $1/k_{obs}$ versus $1/[\text{OH}^-]$ and $1/k_{obs}$ versus $1/[\text{AMX}]$ (Fig. 3) should be linear and are found to be so. From the slopes and intercept, the constants $k$, $K_1$, and $K_2$ were calculated at four different temperatures (Table 2).

The van't Hoff plot were drawn for the variation of $K_j$ and $K_2$ with temperature and the values of enthalpy of reaction $(\Delta H)$ entropy of reaction $(\Delta S)$ and free energy of reaction $(\Delta G)$ are calculated (Table 2). The moderate values of $\Delta H^0$ and $\Delta S^0$ are both favorable for electron transfer processes, while, that of negative value of $\Delta S^0$ supports the proposed mechanism and indicates the formation of a transition state fairly rapidly with a lower degree of freedom$^{19}$. The smaller value of $k$ also points towards the formation of activated complex.

The effect of ionic strength on the rate of the reaction is also in the expected direction as similar charged species, $[\text{Fe(CN)}_6]^{3-}$ and the anionic form of amoxicillin are involved in the reaction. Similarly, decrease in the dielectric constant of the medium results in a decrease in the rate of reaction, supporting the involvement of the same charged species (Scheme 1). The activated complex may be more polar than the reactants, $[\text{Fe(CN)}_6]^{3-}$ and the amoxicillin anion, which may be more solvated in water than in the low dielectric medium$^{20}$ as compared to its reactants.

\section*{Conclusions}

The oxidation of amoxicillin by hexacyanoferrate(III) in aqueous alkaline medium was investigated. Based on the experimental observations, a mechanism is proposed via the formation of an intermediate complex between amoxicillin and hexacyanoferrate(III). The rate constant of the slow step and other
equilibrium constants involved in the mechanism were evaluated and activation parameters with respect to the slowest step of the reaction were computed. The overall sequence described herein is consistent with experimental findings, including the mechanistic and kinetic studies.

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References