Kinetic and Mechanistic Studies on the Oxidation of Norfloxacin by Chloramine-B and N-Chlorobenzotriazole in Acidic Medium

N. NANDA, S. M. MAYANNA, N. M. MADE GOWDA

1Department of Post-Graduate Studies in Chemistry, Central College, Bangalore University, Bangalore—560 001, India
2Department of Chemistry, Western Illinois University, 1 University Circle, Macomb, Illinois 61455

Received 8 January 1998; accepted 28 July 1998

ABSTRACT: The kinetics of oxidation of Norfloxacin [1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-\(\text{[l-piperazinyl]}\)-1-quinoline carboxylic acid] by chloramine-B and N-chlorobenzotriazole has been studied in aqueous acetic acid medium (25% v/v) in the presence of perchloric acid at 323 K. For both the oxidants, the reaction follows a first-order dependence on \([\text{oxidant}]\), a fractional-order on \([\text{Norfloxacin}]\), and an inverse-fractional order on \([H^+]\). Dependence of reaction rate on ionic strength, reaction product, dielectric constant, solvent isotope, and temperature is studied. Kinetic parameters are evaluated. The reaction products are identified. The proposed reaction mechanism and the derived rate equation are consistent with the observed kinetic data. Formation and decomposition constants for substrate—oxidant complexes are evaluated.


INTRODUCTION

Chloramine-B (CAB) and N-chlorobenzotriazole (CBT) are positive halogen compounds whose chemistry in aqueous solutions is reasonably understood \[1,2\]. Reports are available in the literature about the mechanisms of oxidation of some medicinal compounds by CAB and CBT \[3±6\]. A review of the literature indicated the absence of kinetics of oxidation of new drugs with CAB and CBT.

Norfloxacin or NRF (C\(_16\)H\(_{18}\)N\(_3\)O\(_3\)F) is a synthetic, broad spectrum, fluoroquinoline antibacterial agent for oral administration. It has \textit{in vitro} activity against Gram-positive and Gram-negative aerobic bacteria. It also inhibits deoxyribonucleic acid synthesis, and is bactericidal \[7,8\]. Hence, it was necessary to perform a systematic kinetic investigation on the oxidation mechanism of NRF using CAB and CBT in acidic medium.

EXPERIMENTAL

Solutions were prepared by using double-distilled water and analytical grade chemicals. Oxidants, CAB and CBT, were prepared and purified using standard pro-
RESULTS

The rate law and other experimental data were obtained for the oxidation of NRF. The kinetic results with CAB and CBT were similar but different in magnitude. Oxidation reactions were carried out with an excess of NRF at a definite concentration of HClO4 (2.5 × 10⁻⁴ M with CAB and 5.0 × 10⁻⁴ M with CBT) with varying concentrations of each oxidant (1.00 ± 1.00 × 10⁻¹ M) and different initial concentrations [2.50 ± 1.00 × 10⁻¹ M] of NRF. The rate constants, kₚ, were evaluated from linear plots of log [oxidant]₀/oxidant vs. time (r > 0.95, 0.97). Here [oxidant]₀ represents the initial concentration while [oxidant] represents the concentration at time t. Duplicate kinetic runs showed that the rate constants were reproducible within ± 3%.

The reactions were studied under pseudofirst-order conditions by keeping an excess of the substrate over the oxidant [6]. The reactions were studied at constant temperature (± 0.1 K), and were followed by monitoring iodometrically the decrease in the oxidant concentrations up to a 75% reaction. Pseudofirst-order rate constants, kₚ, were evaluated from linear plots of log [oxidant]₀/oxidant vs. time (r > 0.95, 0.97). Here [oxidant]₀ represents the initial concentration while [oxidant] represents the concentration at time t. Duplicate kinetic runs showed that the rate constants were reproducible within ± 3%.

The reduction product of oxidants (benzenesulphonamide or BSA and benzotriazole or BTA) were characterized by TLC [11,12]. CO₂ was identified by the lime-water test. The oxidation product of NRF (3-fluoro-4-piperazinyl-6-N-ethylaminophenylglyoxalic acid) was isolated and characterized by IR (Nicolet Impact 400D, FTIR) and NMR (Bruker dmr 500, FT-
NMR, SF = 125.75 MHz) spectral studies.

IR (KBr), υ/cm⁻¹: 1621s (C=O), 1730s (C=O) acid, 3059s (NH), 3500s (OH),
11C-NMR (CDCl₃), δ ppm: 166.1 (C=O), 176.6
(−C−OH), 16.0 (NCH₂CH₃), 51.1 (NCH₃CH₃),
53.6 and 62.0 (−N−), 151.5, 128.9, 128.1,
125.1, 112.4, and 112.2 (benzene ring C atoms).

The reactions were studied under pseudofirst-order conditions by keeping an excess of the substrate over the oxidant [6]. The reactions were studied at constant temperature (± 0.1 K), and were followed by monitoring iodometrically the decrease in the oxidant concentrations up to a 75% reaction. Pseudofirst-order rate constants, kₚ, were evaluated from linear plots of log [oxidant]₀/oxidant vs. time (r > 0.95, 0.97). Here [oxidant]₀ represents the initial concentration while [oxidant] represents the concentration at time t. Duplicate kinetic runs showed that the rate constants were reproducible within ± 3%.
OXIDATION OF NORFLOXACIN

Table I  Effect of [Oxidant] and [NRF] on the Reaction Rate at 323 K in 25% AcOH

<table>
<thead>
<tr>
<th>[NRF]M</th>
<th>[Oxidant]M</th>
<th>k(s⁻¹)</th>
<th>CAB</th>
<th>CBT</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.00</td>
<td>1.00</td>
<td>4.71</td>
<td>7.68</td>
<td></td>
</tr>
<tr>
<td>5.00</td>
<td>2.50</td>
<td>4.75</td>
<td>7.67</td>
<td></td>
</tr>
<tr>
<td>5.00</td>
<td>5.00</td>
<td>4.74</td>
<td>7.70</td>
<td></td>
</tr>
<tr>
<td>5.00</td>
<td>7.50</td>
<td>4.79</td>
<td>7.65</td>
<td></td>
</tr>
<tr>
<td>5.00</td>
<td>10.0</td>
<td>4.73</td>
<td>7.66</td>
<td></td>
</tr>
<tr>
<td>2.50</td>
<td>5.00</td>
<td>2.70</td>
<td>5.55</td>
<td></td>
</tr>
<tr>
<td>5.00</td>
<td>5.00</td>
<td>4.75</td>
<td>7.70</td>
<td></td>
</tr>
<tr>
<td>10.0</td>
<td>5.00</td>
<td>8.70</td>
<td>9.12</td>
<td></td>
</tr>
<tr>
<td>15.0</td>
<td>5.00</td>
<td>10.2</td>
<td>12.0</td>
<td></td>
</tr>
<tr>
<td>25.0</td>
<td>5.00</td>
<td>14.5</td>
<td>12.4</td>
<td></td>
</tr>
<tr>
<td>50.0</td>
<td>5.00</td>
<td>20.0</td>
<td>14.2</td>
<td></td>
</tr>
<tr>
<td>5.00</td>
<td>5.00</td>
<td>4.76</td>
<td>7.71</td>
<td></td>
</tr>
<tr>
<td>5.00</td>
<td>5.00</td>
<td>4.77</td>
<td>7.68</td>
<td></td>
</tr>
<tr>
<td>5.00</td>
<td>5.00</td>
<td>4.74</td>
<td>7.75</td>
<td></td>
</tr>
</tbody>
</table>

(BHClO₄) = 2.5 x 10⁻² M; CAB or CBT = 5.0 x 10⁻³ M.

Table II  Dependence of the Reaction Rate on [HClO₄] in 25% AcOH at 323 K

<table>
<thead>
<tr>
<th>[HClO₄]M</th>
<th>k(s⁻¹)</th>
<th>CAB</th>
<th>CBT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50</td>
<td>12.3</td>
<td>24.0</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>7.77</td>
<td>17.0</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>4.74</td>
<td>10.9</td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>2.91</td>
<td>7.70</td>
<td></td>
</tr>
<tr>
<td>7.5</td>
<td>2.21</td>
<td>6.17</td>
<td></td>
</tr>
<tr>
<td>10.0</td>
<td>1.69</td>
<td>4.70</td>
<td></td>
</tr>
</tbody>
</table>

[NRF] = 5.00 x 10⁻³ M; CAB or CBT = 5.00 x 10⁻³ M.

The fractional-order dependence of the reaction rate on the concentration of NRF indirectly suggests the involvement of the substrate in the fast pre-equilibrium prior to the rate determining step as in the Michaelis-Menten type of kinetics [4].

The reaction was studied in different compositions of the mixture of acetic acid (AcOH) and water. The rate constant decreased with increasing acetic acid content in the reaction medium (Table IV). To know the influence of solvent mixture on the reaction rate and on the reaction mechanisms, plots of log kₒₗ vs. 1/D were obtained. The values of dielectric constant (D) were taken from the literature [15]. These plots were linear for both CAB and CBT (Fig. 3).

DISCUSSION

The oxidants, CAB and CBT, in aqueous solutions exhibit several equilibria [16,17] as in the case of aqueous chloramine-T solution [18].
Table III  The Temperature Dependence and Activation Parameters for the Oxidation of NRF by CAB and CBT in 25% AcOH

<table>
<thead>
<tr>
<th>Temp (K)</th>
<th>293</th>
<th>303</th>
<th>313</th>
<th>323</th>
<th>333</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidant</td>
<td>CAB</td>
<td>CBT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAB*</td>
<td>0.30</td>
<td>1.35</td>
<td>5.59</td>
<td>25.0</td>
<td>84.0</td>
</tr>
<tr>
<td>CBT*</td>
<td>4.0</td>
<td>6.25</td>
<td>9.09</td>
<td>14.2</td>
<td>19.5</td>
</tr>
</tbody>
</table>

* At 293 K.

Table IV  Dependence of the Reaction Rate on Solvent Composition (AcOH–water) at 323 K

<table>
<thead>
<tr>
<th>% ACOH (v/v)</th>
<th>D a</th>
<th>10^4 k obs (s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>73.2</td>
<td>11.4</td>
</tr>
<tr>
<td>15</td>
<td>66.2</td>
<td>9.57</td>
</tr>
<tr>
<td>20</td>
<td>59.2</td>
<td>7.70</td>
</tr>
<tr>
<td>35</td>
<td>52.0</td>
<td>6.10</td>
</tr>
<tr>
<td>50</td>
<td>41.5</td>
<td>3.29</td>
</tr>
</tbody>
</table>

* Dielectric constant values are from ref. 15.

Under the present experimental conditions, the reaction is first-order with respect to [oxidant] and zero-order with respect to [BSA] or [BTA]. Furthermore, the reaction is retarded by the presence of H⁺ ions. On the basis of the observed kinetic data and the available data in the literature on the system, one could expect RNHCl as the most probable oxidizing species involved in the reaction. It is also known that RNHCl undergoes further protonation in slightly higher acidic solution (pH < 2) [19].

RNCl⁻ + H⁺O⁻ → RNHCl + H₂O (5)

2RNCl⁻ → RNH₂⁺ + RNCl₃⁻ (6)

RNHCl + H₂O → RNH₂⁺ + HOCl⁻ (7)

RNHCl + H⁺ → RNH₂Cl⁻ (8)

The following reaction Scheme 1 is proposed for the oxidation of NRF (S) by CAB:

RNHCl as the most probable oxidizing species involved in the reaction. It is also known that RNHCl undergoes further protonation in slightly higher acidic solution (pH < 2) [19].

RNHCl + H⁺ → RNH₂Cl⁻ (8)

The following reaction Scheme 1 is proposed for the oxidation of NRF (S) by CAB:

Scheme 1

RNHCl + S → X (fast) (10)

X → X' (slow/rds) (11)

X' + 3RNHCl + 4H₂O → Products (fast) (12)

(The intermediates X and X' are as shown in Scheme 2.)

Figure 3  Plots of 1/kobs vs. [H⁺] and log kobs vs. 1/D at 323 K. [CAB], or [CBT], = 5.00 × 10⁻³ M; [NRF], = 5.00 × 10⁻³ M; [HClO₄] = 2.50 × 10⁻³ M with CAB and 5.00 × 10⁻³ M with CBT.
Oxidation of Norfloxacin

Scheme 2

---

short
standard
long
A similar scheme can be written for CBT oxidation of NRF.

\[ \text{Rate} = -\frac{d[\text{oxidant}]}{dt} = 4[X] \]  

(13)

Assuming that 

\[ [\text{oxidant}] = [RNH_2\text{Cl}] + [RNHCl] + [X], \]

\[ \text{Rate} = -\frac{d[\text{oxidant}]}{dt} = \frac{K_s K_{eq}[S][\text{oxidant}]}{[H^+] + K_s[1 + K_{eq}[S]]} \]  

(14)

where oxidant is CAB or CBT.

Because rate = \( k_{obs}[\text{oxidant}] \),

\[ \frac{1}{k_{obs}} = \frac{K_s K_{eq}[S]}{[H^+] + K_s[1 + K_{eq}[S]]} \]  

(15)

\[ \frac{1}{k_{obs}} = \frac{1}{K_s[S]} \left( \frac{[H^+]}{K_s} + 1 \right) + \frac{1}{K} \]  

(16)

The linear plots of \( 1/k_{obs} \) vs. \( 1/[S] \) (where \( S = \text{NRF} \)) at constant \( [H^+] \) (Fig. 2) and \( 1/k_{obs} \) vs. \( [H^+] \) at constant \( [S] \) (Fig. 3) gave the values of \( K_s \).

The change in the ionic strength of the medium does not alter the reaction rate, which suggests the involvement of nonionic species at the rate-determining step. The negative dielectric constant effect in the present system supports the rate determining step of the mechanism [20].

The \( E_a \) values show that CBT is more reactive than CAB in oxidizing NRF. Moderate \( E_a \) values support the proposed reaction mechanism, and the low values of \( \Delta \Sigma \) indicate the involvement of rigid transition states during the reaction. The mechanism of NRF oxidation by CAB and CBT in acid medium is similar to that of aspirin oxidation by bromamidine-T, N-bromosuccinimide, and bromophthalimide in aqueous HCOO\text{H} medium [6].

Authors are grateful to Dr. N. R. Krishnaswamy, Retired Professor, Satya Sai Institute of Higher Learning, Prasanthi Nilayam (A.P.), for helpful discussion, and one of the authors (N.N.) thanks the Principal Prof. M. Parvathi, B.M.S. College for Women, Bangalore, for encouragement, and the UGC, New Delhi, for financial assistance.

BIBLIOGRAPHY