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Kinetics and mechanism of oxidation of chloramphenicol by 1-chlorobenzotriazole in acidic medium

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Abstract. Chloramphenicol (CAP) is an antibiotic drug having a wide spectrum of activity. The kinetics of oxidation of chloramphenicol by 1-chlorobenzotriazole (CBT) in $HClO_4$ medium over the temperature range 293–323 K has been investigated. The reaction exhibits first-order kinetics with respect to $[CBT]_o$ and zero-order with respect to $[CAP]_o$. The fractional-order dependence of rate on $[H^+]$ suggests complex formation between CBT and H^+. It fails to induce polymerization of acrylonitrile under the experimental conditions employed. Activation parameters are evaluated. The observed solvent isotope effect indicates the absence of hydride transfer during oxidation. Effects of dielectric constant and ionic strength of the medium on the reaction rate have been studied. Oxidation products are identified. A suitable reaction scheme is proposed and an appropriate rate law is deduced to account for the observed kinetic data.

Keywords. Chloramphenicol; 1-chlorobenzotriazole; oxidation; kinetics.

1. Introduction

Chloramphenicol (chloromycetin; D-(-)-threo-2-dichloroacetamido-1-*p*-nitrophenyl propane-1,3-diol; CAP) being an antibiotic finds applications in combating a wide range of infections. CAP undergoes hydrolysis in strong acidic and alkaline media at elevated temperature.[1] Oxidative method of assay of CAP in pharmaceutical formulations has been developed[2] and an aldehyde has been identified as the oxidation product of CAP with ozone–air mixture.[3] Review of the literature reveals meagre information about the oxidation kinetics of CAP. 1-chlorobenzotriazole (CBT) is a versatile oxidizing agent and its solution chemistry is reasonably well understood.[4] Kinetics of oxidation of CAP by CBT in alkaline medium has been studied in our laboratory and the reaction is found to be slow[5]. However, no such report is available in acid media. The kinetic aspects of oxidation of dimethylamine by CBT[6] are also reported. There are a few reports on the kinetics of oxidation of medicinal compounds by CBT.[7–9] With this background, we report here results pertaining to the kinetics and mechanism of oxidation of CAP by CBT in $HClO_4$ medium at 323 K for elucidating the mechanism of oxidation of this drug.

2. Experimental

Chloramphenicol (Sigma, USA) was purified before use. CBT was prepared and purified as reported earlier.[10] AnalaR grade chemicals and double distilled water was used throughout the studies. Solvent isotope studies were made with heavy water (D_2O, 99·4% isotopic purity) supplied by the Bhabha Atomic Research Centre, Mumbai.

2.1 *Kinetic measurements*

Kinetic runs were performed under pseudo-first-order conditions with excess of the CAP over the oxidant at 323 K. The reaction was carried out in stoppered Pyrex boiling glass tubes, whose outer surfaces were coated black to eliminate photochemical effects. For each run, requisite amount of solutions of substrate, $HClO_4$, $NaClO_4$ and water (to maintain a constant volume) were measured and thermally equilibrated at 323 K. A measured amount of the CBT solution, also equilibrated at the same temperature, was rapidly added to the reaction mix-

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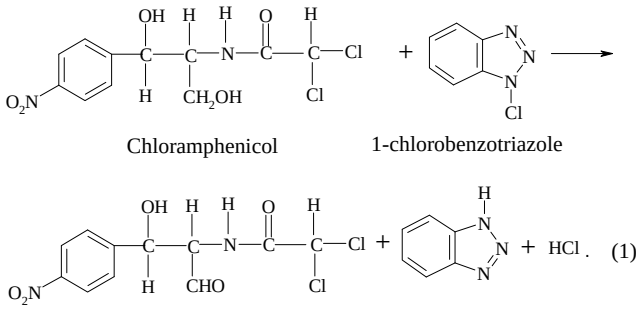
ture in the boiling tube with stirring. The progress of the reaction was monitored by iodometric determination of unreacted oxidant in measured aliquots (5 ml each) of the reaction mixture withdrawn at different intervals of time. The course of the reaction was studied for at least two half-lives. Pseudo-first-order rate constant, *k*, calculated from the linear plots of log [oxidant] vs time were reproducible within ± 3%. Regression analysis of the experimental data were carried out using an fx-100W scientific calculator to obtain the regression coefficient, *r*.

2.2 *Stoichiometry and product analysis*

Different sets of concentrations of the reactants in 0·1 mol dm^{-3} perchloric acid were kept for over 24 h at 323 K. The determination of unconsumed CBT in the reaction mixture showed that one mole of CAP consumed a mole of CBT (table 1). The observed stoichiometry is as shown below,



(1)

The reduction product of CBT, benzotriazole (BTA), was isolated and identified[11] by TLC using butanol–acetic acid–water (4 : 1 : 1 *v*/*v*) as solvent and iodine as the detecting agent (R_f = 0·93). The obtained R_f value was consistent[11] with the literature value. The main product (aldehyde) was the same under different experimental conditions (table 1). The aldehyde was confirmed by IR spectra which show bands at 3440 cm^{-1} and 1720 cm^{-1} for OH stretching and C=O stretching respectively and a band at 2720 cm^{-1} for aldehydic C–H stretching. The aldehyde was also identified as its 2,4-dinitrophenylhydrazone derivative.

Table 1. Stoichiometry of oxidation of CAP by CBT.

10 [$HClO_4$]/M	0·1	0·25	0·50	1·0
Δ [CBT]/Δ [CAP]	0·95	0·98	0·97	1·0
Δ [CBT]/Δ [CAP]	0·97	0·99	0·96	0·99

3. Results

Oxidation of CAP by CBT in aqueous solution is very slow[5] and becomes appreciable only in the presence of H^+ ions and at higher temperature. Hence, detailed kinetic investigations of oxidation of CAP by CBT were made in 0·1 mol dm^{-3} $HClO_4$ solution at 323 K.

With the substrate in excess at constant $[CAP]_o$, $[HClO_4]$ and temperature, plots of log [CBT] vs time were linear (r > 0·9850) indicating first-order dependence of rate on $[CBT]_o$. The pseudo-first-order-rate constant (k) was independent of $[CBT]_o$ (table 2), confirming the first-order dependence on $[CBT]_o$. Values of k did not change with increase in $[CAP]_o$ (table 2), indicating zero-order dependence of the reaction rate on $[CAP]_o$. The rate increased with increase in $[HClO_4]$ and the linear plot of log k vs log $[HClO_4]$ (r = 0·9810) with a slope of 0·62 indicated fractional-order dependence of the reaction rate on $[HClO_4]$ (table 2). Further, a plot of 1/k vs 1/$[H^+]$ (r = 0·9870) gives a straight line, with a y-intercept, confirming fractional-order dependence on $[H^+]$.

Table 2. Effect of varying CBT, CAP and $HClO_4$ concentrations on the reaction rate at 323 K.

10^4 $[CBT]_o$ (mol dm^{-3})	10^3 $[CAP]_o$ (mol dm^{-3})	10 $[HClO_4]$ (mol dm^{-3})	$k \times 10^4$ (s^{-1})
1·0	5·0	1·0	3·05
2·5	5.0	1·0	3·15
5·0	5.0	1·0	3·00
7·5	5.0	1·0	2·98
10·0	5.0	1·0	3·10
1·0	1.0	1·0	3·12
1·0	2.5	1·0	3·08
1·0	5.0	1·0	3·05
1·0	7.5	1·0	3·09
1·0	10·0	1·0	3·14
1·0	5.0	0·10	0·65
1·0	5·0	0·25	1·25
1·0	5·0	0·50	2·15
1·0	5·0	1·0	3·05
1·0[a]	5·0	1·0	3·07
1·0[b]	5·0	1·0	2·90
1·0[c]	5·0	1·0	2·17
1·0[d]	5·0	1·0	5·10

[a][$NaClO_4$] = 0·5 mol dm^{-3}; [b][BTA] = 0·05 mol dm^{-3}; [c]*t*-butanol = 40% *v*/*v*; [d]D_2O = 90%

Addition of the reaction product, benzotriazole ($1{\cdot}0 \times 10^{-3}$ mol dm^{-3}) and variation of ionic strength of the medium (0·1–0·5 mol dm^{-3}) by adding $NaClO_4$ have no significant effect on the rate of reaction. Hence, no attempt was made to keep the ionic strength of the reaction mixture constant for the kinetic runs. Addition of tertiary butanol decreased the rate of reaction. Solvent isotope effect was studied using D_2O. The reaction rate is increased with $k = 5{\cdot}10 \times 10^{-4}$ s^{-1} in D_2O and $k = 3{\cdot}05 \times 10^{-4}$ s^{-1} in H_2O, leading to a solvent isotope effect k (H_2O)/k (D_2O) = 0·60. All these results are reported in table 2.

The reaction was studied at different temperatures (293–323 K). Rate constants at 293, 303, 313 and 323 K were found to be 0·32, 0·65, 1·45, $3{\cdot}05 \times 10^{-4}$ s^{-1} respectively. From the linear Arrhenius plot (r = 0·9993) of log k vs 1/T, the computed activation parameters for the overall reaction were evaluated: E_a = 55·8 kJ mol^{-1}; $\Delta H^{\#}$ = 53·2 kJ mol^{-1}; $\Delta G^{\#}$ = 101 kJ mol^{-1} and $\Delta S^{\#}$ = –149 JK^{-1} mol^{-1}. Addition of acrylonitrile to the reaction mixture had no effect, indicating the absence of free radical species during the reaction.

4. Discussion

The hydrolysis of CAP under the present experimental conditions is negligible.[1] This is further confirmed by testing the absence of free base (–NH_2 group) in the reaction mixture using the reduced form of ninhydrin.[12] CBT being N-haloamine gives several oxidizing species in aqueous solution.[13] The concentration of each species depends on the concentration of CBT, the nature (polar or non-polar) and pH of the medium. Benzotriazole (BTA), the parent compound of CBT, has pK_b 5·8 and hence it might largely exist in protonated form in an aqueous solution,[13]

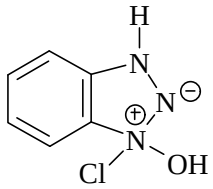
$$CBT + H_3O^+ \rightleftharpoons CBTH^+ + H_2O. \qquad (2)$$

Because of the weakening of the N–Cl bond, it might solvalize further to give H_2OCl^+,

$$CBTH^+ + H_2O \rightleftharpoons BTA + H_2OCl^+. \qquad (3)$$

If H_2OCl^+ is the active oxidant species, a retardation of rate by added BTA is expected. However, no such effect was noticed (table 2) in the present investigation and hence the possibility of H_2OCl^+ as the oxidizing species is ruled out. In order to establish this point, some kinetic experiments were carried out with CBT and HOCl under comparable experimental conditions. The kinetic data with HOCl and CBT were not the same ($k_{HOCl} = 4{\cdot}35 \times 10^{-6}$ s^{-1} and $k_{CBT} = 3{\cdot}15 \times 10^{-4}$ s^{-1}). Hence, the reactive oxidizing species could be $CBTH^+$ which accounts for the observed fractional-order dependence of rate on [H^+]. Such an argument was also proposed earlier[14] for the oxidation of benzyl alcohol by CBT. In view of these facts, the reaction scheme 1 can be proposed to account for the observed kinetics in the oxidation of CAP by CBT in acidic medium:

The probable structure of the intermediate complex (X) is as below:



The total effective concentration of CBT is $[CBT]_t$, then

$$[CBT]_t = [CBT] + [CBTH^+], \qquad (4)$$

From step (i) of scheme 1,

$$[CBT] = \frac{[CBTH^+]}{K_1[H^+]}.$$

By substituting for [CBT] in (4), we get

$$[CBT]_t = \frac{[CBTH^+]}{K_1[H^+]} + [CBTH^+]$$

or

$$[CBTH^+] = \frac{K_1[CBT]_t[H^+]}{1 + K_1[H^+]}. \qquad (5)$$

$$CBT + H^+ \underset{\text{(i)}}{\overset{K_1}{\rightleftharpoons}} CBTH^+, \qquad \text{fast,}$$

$$CBTH^+ + H_2O \xrightarrow[\text{(ii)}]{k_1} X, \qquad \text{slow and rds,}$$

$$X + CAP \xrightarrow[\text{(iii)}]{k_2} \text{products.} \qquad \text{fast.}$$

Scheme 1.

From the slow step of scheme 1,

$$\text{rate} = -d[\text{CBT}]_t/dt = k_1[\text{CBTH}^+][\text{H}_2\text{O}]. \quad (6)$$

By substituting for $[\text{CBTH}^+]$ from (5) into (6), the following rate law is obtained.

$$\text{rate} = \frac{K_1 k_1 [\text{CBT}]_t [\text{H}^+]}{1 + K_1[\text{H}^+]}. \quad (7)$$

The above rate law is in agreement with the observed kinetic results.

Since rate = $k'[\text{CBT}]_t$, (7) can be transformed into,

$$\frac{1}{k'} = \frac{1 + k_1[\text{H}^+]}{K_1 k_1 [\text{H}^+]}, \quad (8)$$

$$\frac{1}{k'} = \frac{1}{K_1 k_1 [\text{H}^+]} + \frac{1}{k_1}. \quad (9)$$

From the linear double reciprocal plot of $1/k$ vs $1/[\text{H}^+]$, (r = 0·9970), values of K_1 and k_1 were found to be 16·4 $\text{dm}^3\ \text{mol}^{-1}$ and $4{\cdot}54 \times 10^{-4}\ \text{dm}^3\ \text{mol}^{-1}\ \text{s}^{-1}$ respectively.

Solvent isotope studies in D_2O medium show an increase in the reaction rate. It is well known that D_3O^+ is a stronger acid[15] than H_3O^+ and hence this observation supports the proposed mechanism. The negligible effect of BTA on the rate of reaction indicates that it was not involved in pre-equilibrium. The change in the ionic strength of the medium did not alter the rate indicating that non-ionic species were involved in the rate determining step. A slight negative dielectric constant effect on the rate supports the fact that the dipole interaction in the rate determining step.[16] The proposed mechanism is supported by the moderate values of energy of activation and other thermodynamic parameters. The fairly high positive values of free energy of activation and enthalpy of activation indicate that the transition state is highly solvated, while the high negative entropy of activation suggests the formation of the compact activated complex with fewer degrees of freedom.

The stoichiometry, products and kinetic results are different in acidic and alkaline[5] media. Energy of activation revealed that the reaction is found to be faster in acid medium compared to alkaline medium (E_a in alkaline medium is 59·0 kJ mol^{-1}).[5] This may be due to the involvement of different reactive species of CBT in acidic (CBTH^+) and alkaline (OCl^-) media of this redox system.

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