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EVALUATION OF RATE CONSTANTS AND THERMODYNAMIC PARAMETERS OF OXIDATION OF CHLORAMPHENICOL BY CAS IN MIXED SOLVENT MEDIA

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ABSTRACT

Ceric ammonium sulphate (CAS) is well known to oxidize carbon compounds in various acid media via the formation of 1:1 cerium (IV) – substrate complex which undergoes dissociation unimolecularly in the rate determining step. In the present work, the structure reactivity correlation in oxidation reaction of chloramphenicol, a powerful antibiotic has been ascertained using ceric ammonium sulphate as a versatile oxidizing agent in acetic acid – water 50% (v/v) media. The reaction was found to be first order with respect to the concentration of the oxidant and fractional order with respect to concentration of chloramphenicol. Various thermodynamic parameters have been evaluated. Based on these parameters, the change in microbial activity of Chloramphenicol with respect to oxidation of alcoholic groups has been followed. A probable mechanism has been proposed.

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INTRODUCTION

Chloramphenicol, otherwise commercially known as Chloromycetin is a powerful antibiotic and is mostly used for curing diseases caused by both gram (-) and gram (+) bacteria. Some traditional topics in drug chemistry such as the oxidation or reduction of certain vital functional groups of drugs have continued to attract the attention of several research groups because investigation on the structural modifications and its influence on the activity of the drugs have always revealed new interesting factors. Also the most noticeable trend in current years is to evaluate the kinetic and thermodynamic parameters of the bio active molecules (Gurdeep and Chatwal, 1990). The nitro group in chloramphenicol was shown to be responsible for the antibacterial activity (Alfred Burger, 1970). For instance it has been proved that chloramphenicol loses its antibacterial activity when the nitro group is replaced by a group like -CN, -CONH₂, -NH₂, -OH, -N(CH₃)₂, -Br, -Cl, -F or -C₆H₅.

In general the antibacterial activity is determined by various factors like electro negativity, molecular volume and ability to form quinonoid structure when the benzene ring in chloramphenicol is replaced by another ring like naphthyl or cyclohexyl ring, the antibacterial activity was shown to be greatly reduced. Also it has been proved that the alcoholic group is also a must for microbial activity since its replacement or deletion leads to the decrease of activity (Vogel, 1986). Cerium(IV) salts are considered to be convenient oxidizing agents for the oxidation of large number of carbon compounds and are used for the study of kinetics and mechanism of several organic compounds in general and alcohols in particular. When ceric salts are oxidizing carbon compounds in various acid media they form 1:1 cerium (IV)-substrate complex which then undergo dissociation unimolecularly in the rate determining step. In the oxidation of alcohol by cerium (IV) in nitric acid medium, it was shown that neutral ceric nitrate molecule was active species. But the oxidations of alcohols by cerium (IV) in the sulphuric acid medium, evidences were reported both for the formation of cerium (IV) substrate complex and the nature of active species. These results were found to be conflicting with those of

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earlier works. It is of our interest to carry out experiments to investigate the kinetics and mechanism of oxidation of chloramphenicol in presence of ceric ammonium sulphate as an oxidant in acetic acid – water mixture medium. Chloramphenicol, otherwise commercially known as Chloromycetin is a powerful antibiotic and is mostly used for curing diseases caused by both gram (-) and gram (+) bacteria. Some traditional topics in drug chemistry such as the oxidation or reduction of certain vital functional groups of drugs have continued to attract the attention of several research groups because investigation on the structural modifications and its influence on the activity of the drugs have always revealed new interesting factors.

All chemicals used in the experiments were of the purest available Analar grade. They were used as such without further purification unless otherwise specified. The preparation of chloramphenicol from Chloromycetin capsules and the kinetic experiment was carried out as per the standard procedures available in the literature (Weisberg, 1965).

Preparation of chloramphenicol from Chloromycetin capsules

Known weight of the contents (250 mg) of the chloromycetin capsules (PARAXIN) was dissolved in water (100 ml). The above solution was shaken well and then extracted with petroleum ether (25ml) for four times. The petroleum ether fraction was discarded and the aqueous fraction was extracted four times with ether (50 ml). After discarding the aqueous layer, the ethereal layer was evaporated after drying. The resultant crude sample was recrystallized twice with distilled water. After recrystallisation the chloramphenicol was obtained as a white, needle-like crystals or elongated plates. The melting point (422.8 – 423.3 K) was noted which is found to be in good agreement with the reported value (422 – 423.2 K).

A typical procedure kinetic procedure

A mixture of chloramphenicol solution in glacial acetic acid (4 ml), glacial acetic acid (12 ml) perchloric acid (4 ml) and double distilled water (16 ml) was taken in stoppered conical flask and kept in thermostat at 308 K. In another stoppered conical flask, ceric ammonium sulphate (50 ml) solution was taken and kept in the same thermostat. After about half an hour, the oxidant (4ml) was added to the reaction mixture and shaken well. Immediately a known volume of the reaction mixture (2ml) was pipetted out into a clean conical flask containing ferrous ammonium sulphate solution (2 ml) so as to arrest the oxidation reaction. A piece of ice was also added. To this solution, one drop of ferroin indicator was added and titrated against ceric ammonium sulphate taken in the burette. The end point is the just appearance of pale blue color. The above procedure was

repeated for known intervals of time. The infinite reading was taken by heating the reaction mixture at 50°C for about an hour in a water bath. A graph was drawn by plotting $\log(V^\infty - Vt)$ versus time. A straight line was obtained. The slope of the line was determined and the pseudo – first order rate constant was calculated. The above experiments were repeated with the same substance and the same oxidant and the results were calculated. It was of our interest to carry out experiments to study the effect of changing the concentration of ceric ammonium sulphate and that of chloramphenicol on the rate constant. It was also planned to evaluate thermodynamic parameters. One mole of chloramphenicol and 6 moles of ceric ammonium sulphate were kept under kinetic condition for 72 hours. At the end of the completion of the reaction, the excess /unreacted ceric ammonium sulphate was estimated by pipetting out 2 ml of the reaction mixture into a conical flask containing exactly 2 ml of ferrous ammonium sulphate solution. The excess ferrous ammonium sulphate solution was titrated against standard ceric ammonium sulphate. The stoichiometry was found to be 1:4. The reaction mixture containing chloramphenicol and ceric ammonium sulphate in the molar ratio 10:1 was kept under kinetic condition for 48 hours. The portion of the solution was diluted with sodium acetate solution (4%) and then treated with 2, 4-dinitrophenyl hydrazine solution. After cooling for 24 hours, the separated yellow precipitate was filtered, washed with distilled water and dried. The melting point was determined and it was found to be 453.9 K. The reaction mixture was extracted with ether and the ethereal layer was then extracted with sodium bicarbonate solution. The aqueous layer was then extracted with ether. The ether layer was evaporated. The residue obtained, on qualitative analysis and spectral analysis (FT- IR and NMR) show the presence of carboxylic acid group. The results of kinetic experiments with the change of the concentration of the oxidant on the oxidation of the chloramphenicol in acetic water - medium (50% v/v) are presented in representative table1 and the respective kinetics plots of $\log(V^\infty - Vt)$ are shown in representative figure 1.

Table 1. Effect of variation of concentration of ceric ammonium sulphate

[CMPL] = 2.0×10^{-2} M
HOAc-H₂O = 50 % (v/v)
[CAS] = 1.0×10^{-3} M, Temp. = 308 K
[HClO₄] = 1.0×10^{-2} M

Time, min	V*CAS = V _i ,ml	(V [∞] – Vt) ml	10+log(V [∞] – Vt)
0	4.70	0.50	9.699
3	4.75	0.45	9.653
10	4.85	0.35	9.544
15	4.90	0.30	9.477
20	4.95	0.25	9.398
25	5.00	0.20	9.301
30	5.10	0.10	9.000
∞	5.20	-	-

Correlation coefficient, r = 0.9547

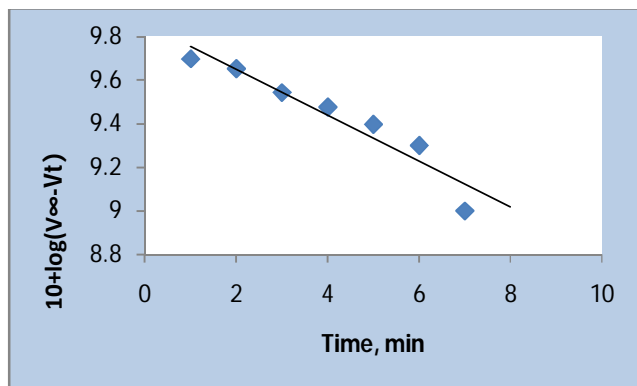


Fig.1. Effect of change of concentration of CAS (1.0×10^{-3} M) on the oxidation of chloramphenicol (Representative plot)

The titre values of the effect of varying the concentration of substrate chloramphenicol are given in representative table 2 and respective representative plot is shown in figure 2. Table 3 represents the effect of varying the concentration of chloramphenicol on rate constant. Figure 3 represents the plot of $\log k'_{\text{obs}}$ vs. $\log_{10} [\text{CMPL}]$. Table 4 represents the effect of variation of $1/[\text{CMPL}]$ on $1/k'_{\text{obs}}$. Fig 4 represents the respective plot. The effect of varying the temperature is given in representative table 5 and the respective representative plot is shown in Figure 5.

The effect of varying the concentration of sodium per chlorate on rate constant is given in representative table 6 and the representative figure 6 represents the respective plot. The effect of varying the concentration of perchloric acid on rate constants are given in representative table 7 and the respective representative plot is shown in figure 7. The effect of change in concentration of ceric ammonium sulphate, sodium per chlorate and perchloric acid on rate constants are given in table-8, table-9 and table -10 respectively. The thermodynamic parameters are given in Table-11. Fig 8 represents the effect of change of concentration of $[\text{HClO}_4]$ on rate constants.

Table 2. Effect of variation of concentration of chloramphenicol

$[\text{CMPL}] = 1.5 \times 10^{-2}$ M
 $\text{HOAc-H}_2\text{O} = 50\%$ (v/v)
 $[\text{CAS}] = 2.0 \times 10^{-3}$ M
 Temp. = 308 K
 $[\text{HClO}_4] = 1.0 \times 10^{-2}$ M

Time, min	$V^* \text{CAS} = V_0, \text{ml}$	$(V_\infty - Vt)$ ml	$\log(V_\infty - Vt) \times 10^2$
0	12.90	9.00	95.40
60	13.65	8.25	91.60
90	15.80	6.10	78.50
120	16.80	5.10	70.80
150	17.90	4.00	60.20
180	19.10	2.80	44.70
∞	21.90	-	-

Correlation coefficient, $r = 0.9617$

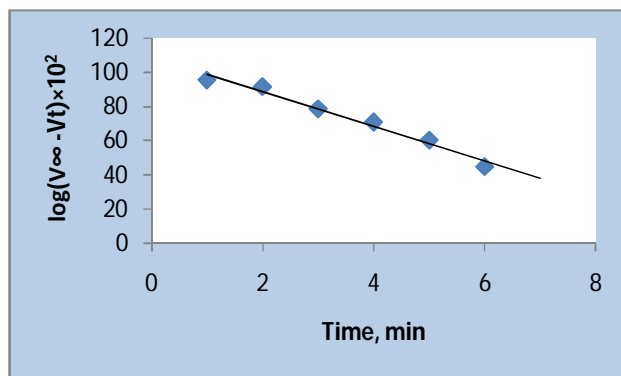


Fig.2. Effect of change of concentration of chloramphenicol

(1.5×10^{-2} M) on the oxidation the oxidation reaction

Table 3. Effect of variation of chloramphenicol on rate constant

$[\text{CAS}] = 2.0 \times 10^{-3}$ M
 $\text{HOAc-H}_2\text{O} = 50\%$ (v/v)
 $[\text{HClO}_4] = 1.0 \times 10^{-2}$ M Temp. = 308K

$[\text{CMPL}] \times 10^2, \text{M}$	$K'_{\text{obs}} \times 10^3, \text{min}^{-1}$	$5 + \log k'_{\text{obs}}$	$5 + \log [\text{CMPL}]$
1.5	8.76	2.943	3.177
2.0	7.32	2.865	3.302
3.0	6.28	2.798	3.478

Correlation coefficient, $r = 0.9900$

Table 4. Effect of variation of $1/[\text{CMPL}]$ on $1/k'_{\text{obs}}$

$K'_{\text{obs}} \times 10^3, \text{min}^{-1}$	$[\text{CMPL}] \times 10^2, \text{M}$	$1/k'_{\text{obs}}$	$1/[\text{CMPL}]$
8.76	1.5	114.15	66.66
7.32	2.0	136.61	50.00
6.28	3.0	159.23	33.33

Correlation coefficient, $r = 0.9999$

Table 5. Effect of variation of Temperature on rate constant

$[\text{CMPL}] = 2.0 \times 10^{-2}$ M
 $\text{HOAc-H}_2\text{O} = 50\%$ (v/v)
 $[\text{CAS}] = 2.0 \times 10^{-3}$ M
 Temp. = 313 K
 $[\text{HClO}_4] = 1.0 \times 10^{-2}$ M

Time, min	$V^* \text{CAS} = V_0, \text{ml}$	$(V_\infty - Vt)$ ml	$\log(V_\infty - Vt) \times 10^2$
0	14.85	9.20	96.4
10	15.60	8.45	92.7
20	16.45	7.60	88.1
30	17.70	6.35	80.3
40	18.40	5.65	75.2
50	19.55	4.50	65.3
70	20.45	3.60	55.6
90	21.30	2.75	43.9
∞	24.05	-	-

Correlation coefficient, $r = 0.9967$

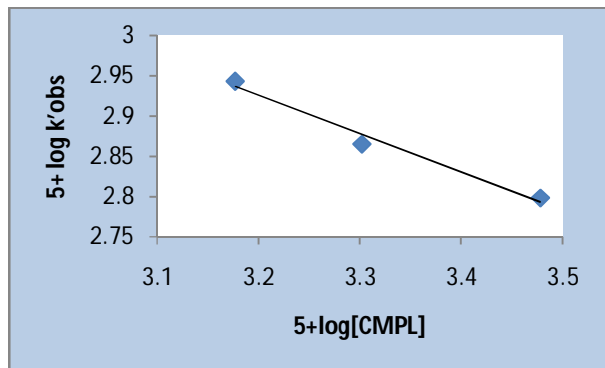


Fig 3. Effect of change of concentration of chloramphenicol on the rate constant

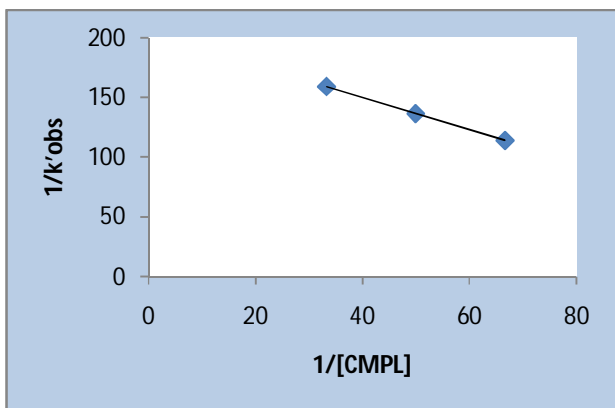


Fig 4. Effect of variation of 1/[chloramphenicol] on 1 / k_obs

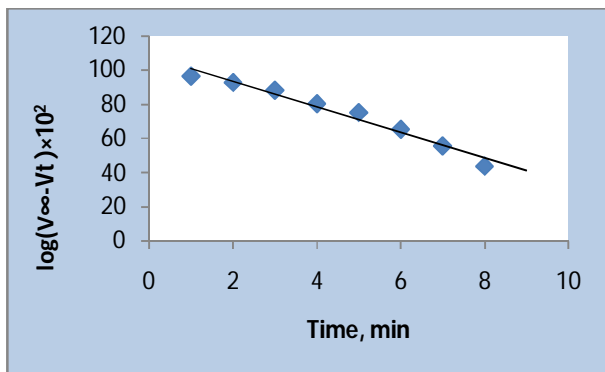


Fig.5 Effect of variation of temperature (313 K) on rate constant

Table 6. Effect of variation of Sodium per chlorate on rate constant

[CMPL] = 2.0 X 10⁻² M
 HOAc-H₂O = 50 % (v/v)
 [CAS] = 2.0 X 10⁻³ M
 Temp. = 308 K [HClO₄] = 1.0 X 10⁻² M [NaClO₄] = 1.0 X 10⁻² M

Time, min	V*CAS = V _v ,ml	(V [∞] - Vt) ml	log(V [∞] - Vt) × 10 ²
0	13.45	7.45	87.2
30	14.50	6.40	80.6
60	15.70	5.20	71.6
90	16.60	4.30	63.3
120	17.55	3.35	52.5
∞	20.90	-	-

Correlation coefficient, r = 0.9968

Table 7. Effect of variation of [HClO₄] on rate constant

[CMPL] = 2.0 X 10⁻² M
 HOAc-H₂O = 50 % (v/v)
 [CAS] = 2.0 X 10⁻³ M Temp. = 308 K
 [HClO₄] = 0.5 X 10⁻² M

Time, min	V*CAS = V _v ,ml	(V [∞] - Vt) ml	log(V [∞] - Vt) × 10 ²
0	17.35	7.45	87.2
30	18.20	6.60	82.0
60	18.85	5.95	77.5
90	19.40	5.40	73.2
120	20.05	4.75	67.6
180	21.05	3.75	57.4
∞	24.80	-	-

Correlation coefficient, r = 0.9994

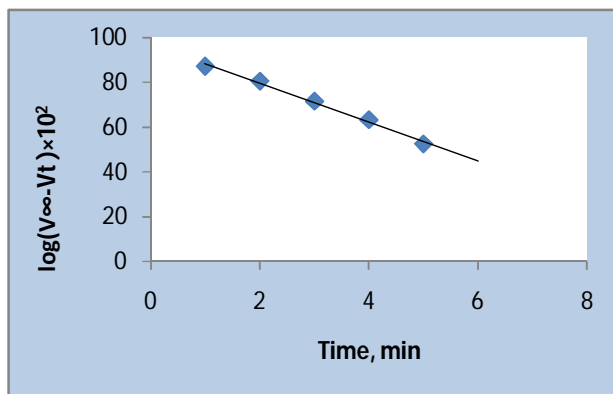


Fig.6. Effect of change of concentration of sodium perchlorate (1.0 × 10⁻² M)

On rate constant

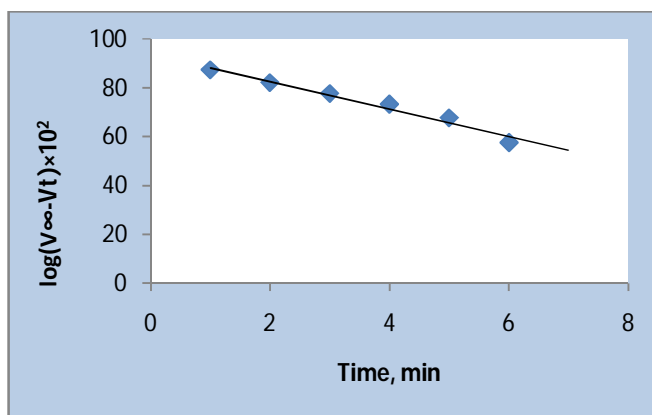


Fig.7. Effect of change of concentration of HClO₄ (0.5 × 10⁻² M) on rate constant

Table 8. Effect of variation of [CAS] on rate constant

[CMPL] = 2.0 X 10⁻² M
 HOAc-H₂O = 50 % (v/v)
 [HClO₄] = 1.0 X 10⁻² M Temp. = 308 K

[CAS] X 10 ³ , M
1.0
1.5
2.0
2.5
3.0
4.0

Table 9. Effect of variation of [NaClO₄] on rate constant

[CMPL] = 2.0 X 10⁻²M
 HOAc-H₂O = 50 % (v/v)
 [CAS] = 2.0 X 10⁻³ M
 Temp. = 308 K
 [HClO₄] = 1.0X 10⁻² M

[NaClO ₄] X 10 ² ,M
1.0

1.5

2.0

Table 10. Effect of variation of [HClO₄] on rate constant

[CMPL] = 2.0 X 10⁻²M
 HOAc-H₂O = 50 % (v/v)
 [CAS] = 2.0 X 10⁻³ M
 Temp. = 308 K

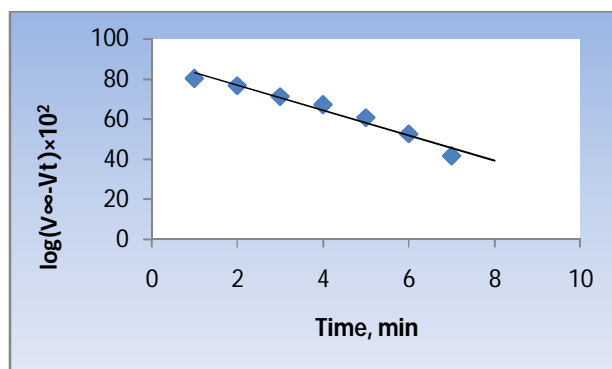
[HClO ₄] X 10 ² ,M
0.5
1.0
1.5
2.0
2.0

Table 11. Thermodynamic parameters for the oxidation of chloramphenicol by CASE = 26125.4 cal.mol⁻¹

Temp., K	K' x 10 ² .min ⁻¹	ΔH [#] , cal mol ⁻¹	ΔS [#] , eu
313	1.381	25503.5	6.2457
318	2.955	25493.5	6.4128
323	5.118	25483.6	6.2111

As a part of our scheme on the structure - reactivity correlation in oxidation reactions, we have carried out investigation on the kinetics and mechanism of oxidation of chloramphenicol by ceric ammonium sulphate. Certain interesting features are presented in this preliminary work. The kinetics of this oxidation reactions were carried out in 50% acetic acid – 50% water (v/v) in the presence of 0.01 M perchloric acid under pseudo-first order conditions. The first order dependency of concentration of ceric ammonium

sulphate on chloramphenicol is evidenced from the linearity of the plots of log (V[∞] – Vt) vs. time.

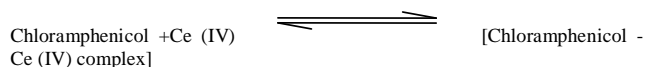
**Fig.8 Effect of change of concentration of HClO₄ (2.0×10⁻² M) on rate constant**

The stoichiometry of chloramphenicol- cerium (IV) carried out by the isolation of dinitrophenyl hydrazones derivative was found to be 1:4. The effect of variation of chloramphenicol on the rate is given in Table-3. For [CAS] = 2.0X10⁻³ M; [HClO₄] = 1.0X10⁻² M; Temp. = 308 K; and medium = 50% acetic acid – 50% water, if 10² x [chloramphenicol] is 1.5, 2.0 and 3.0x10³ k'obs found to be 8.76, 7.32, and 6.28. To deduce order with respect to concentration of chloramphenicol, a graph was drawn using 1/ [chloramphenicol] versus 1/k'obs (Fig. 4). The linear curve without causing any definite intercept reveals that the reaction follows fractional order kinetics to the concentration of chloramphenicol and also the mechanism of this oxidation conforms to the general mechanism in which the formation of a 1:1 complex followed by the slow decomposition of the complex is recognized. We have also carried out spectral (FT-IR, ¹H-NMR and ¹³C-NMR) analysis and product analysis qualitatively and have found that the product was keto acid, since the kinetic reaction mixture at the end of the reaction gives a DNP derivative and also after extraction suitably gives positive results for carboxylic acid. When an alcohol or acetic acid solution of chloramphenicol is mixed with CAS solution, it was found that yellow color indicates the formation of complex. Dainton based on the study of kinetics and mechanism of oxidation of organic compounds by one electron acceptors like cerium (IV) and cobalt (III) has suggested the formation of intermediate free radicals from the former. Duke was the first scientist who proposed that several oxidation-reduction reactions involved the formation of oxidant-substrate coordination complexes. From the effect of variation of concentration of chloramphenicol on rate, it is found that the pseudo - first order rate constant value remains constant with the change in concentration of sodium per chlorate indicating that the reactants in the slow step (rate determining step) of the mechanism are not charged species. The linearity of the plot of 1/k'obs versus 1/ [chloramphenicol] indicates with the slope

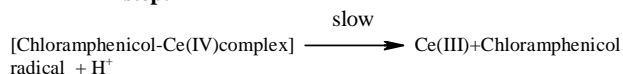
having fractional value indicates that the mechanism involves free radical intermediates. Based on the available data, the following mechanism is proposed.

Mechanism (Scheme 1)

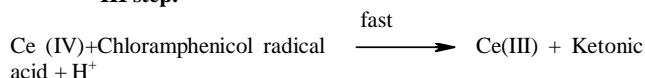
I step:



II step:



III step:



Also it was observed that the rate of the reaction increases with the increase in the concentration of perchloric acid, probably due to the existence of a pre-equilibrium step between chloramphenicol and H^+ ions and the protonated species may be the reactant species rather than chloramphenicol itself. However confirmation of the above mechanism involves the following experimental evidences. The formation of keto acid from chloramphenicol needs confirmation by spectral measurements. It was confirmed by spectral (FT-IR, $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$) analysis and product analysis qualitatively. The nature of complex can be revealed by determining some more thermodynamic parameters. The existence of pre-equilibrium step requires confirmation by carrying out experiments at different pH with constant ionic strength.

Kinetic study of oxidation of chloramphenicol by cerium (IV) ion in aqueous solution of acetic acid 50 % (v/v) shows that the reaction is of first order with respect to the concentration of the oxidant and shows fractional order with respect to concentration of chloramphenicol.

Increase in concentration of sodium per chlorate shows practically no effect on the rate of reaction. Based on the study of effect of variation of concentration of perchloric acid on rate, a pre-equilibrium step has been suggested. Effect of temperature has also been studied and activation parameters were calculated.

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