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KINETIC AND MECHANISTIC STUDY OF OXIDATION OF CHOLESTEROL BY CHLORAMINE-T IN ALCOHOL MEDIUM

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ABSRACT: Kinetics of oxidation of cholesterol by N- chloro- p-toulene sulfonamide or chloramine-T (CAT) in alcohol medium has been investigated at 303K. The reaction rate shows first-order each on $[CAT]_0$ and $[cholesterol]_0$. Stoichiometry and oxidation products are identified by spectroscopic and chemical technique. The reaction has been studied at different temperatures and thermodynamic parameters have been calculated, mechanism consistent with the kinetic preceding data has been proposed.
Keywords: Cholesterol, Oxidation, Kinetics, Mechanism, Chloramine-T

INTRODUCTION

Aromatic N-chloro-p-toulenesulphonamide is mild oxidants containing a strongly polarized N-linked halogen in its +1 oxidation state. The prominent member of this group chloroamine-T (CAT) is a well known analytical reagent and the mechanistic aspects of many of its reactions have been documented [1, 2].

Cholesterol is white Fainty yellow almost odourless, pearly leaflets powder or granules a prolonged exposure to light and air acquires a yellow to tan colour. Cholesterol is used in cosmetics and topical pharmaceutical formulations at concentration between 0.3 -5.0% as emulsifying agent. It requires water- absorbing power to an ointment and has emollant activity. This is soluble in organic solvent but insoluble in water, cholesterol generally regarded as an essentially non toxic, non irritant material at the levels employed as an excipient [3].

Cholesterol has been exploited with great advantage to detect any oxidation processes in cell membranes, in contrast with unsaturated fatty acids, cholesterol excites as a single molecular species, its oxidation products are thus much less complicated to isolate and characterize. Sterol cholesterol (cholest–5 en–p–ol) is an essential metabolite required for major biological functions, such as the cell membrane structure where the steroid forms, together with phosphor lipid molecules, cholesterol is inserted into membrane bilayers with its long axis perpendicular to the plane of the membrane, preventing the crystallization of fatty acyl chains by fitting between them (yeagle 1985) and modifying the activity of membrane bounded enzymes. Cholesterol synthesis is highly-regulated process that occurs in almost all animal tissues, but in higher mammals the liver, the adrenal gland, the ovaries and testis show the most significant biosynthetic activity.

In adult human approximately 400 mg of cholesterol per day are converted to bile acids and only approximately 50 mg are converted to harmones (Ishibashi et.at.1996). Cholesterol is a molecule with an unsaturated bond at position Δ 5-6 of the sterol nucleus. Therefore it is prone to oxidation (Maerker, 1987). The substrate undergoes oxidation by a free - radical mechanism leading to the formation of hydro peroxides and then to a number of oxidation products, the so-called oxysterols. These oxidation products are a group of sterols similar in structure to cholesterol. But that contain an additional oxygen function such as a hydroxyl group, ketone group or an epoxide group at the sterol nucleus or at the side chain of the molecule.

It has been estimated that approximately 1% of the cholesterol consumed in a mixed western diet is oxidized cholesterol (Van de Boven Kamp et al 1988). Cholesterol containing foods, when subjected to high temperatures during manufacture or processing may form variable amounts of oxysterols, depending on the analytical method applied for their identification.

The most commonly detected oxysterols in foods are the major products of cholesterol oxidation. Cholestrol is a waxy steroid of fat that is produced in the liver or intestines. It is used to produce harmones and cell membranes and is transported in the blood plasma of all mammals [4]. Cholestrol is an important component for the manufacture of bile, acids, steroid harmones and vitamin D. Small quantities can be synthesized in other eukaryotes such as plants and fungi. It is almost completely absent among prokaryotes including bacteria [5]. High levels of cholesterol in the blood can damage arteries and are cardiovascular system [6]. A very few kinetic studies of cholesterol oxidation, ozonolysis are reported in literature [7- 10].

MATERALS AND METHODS

Experimental

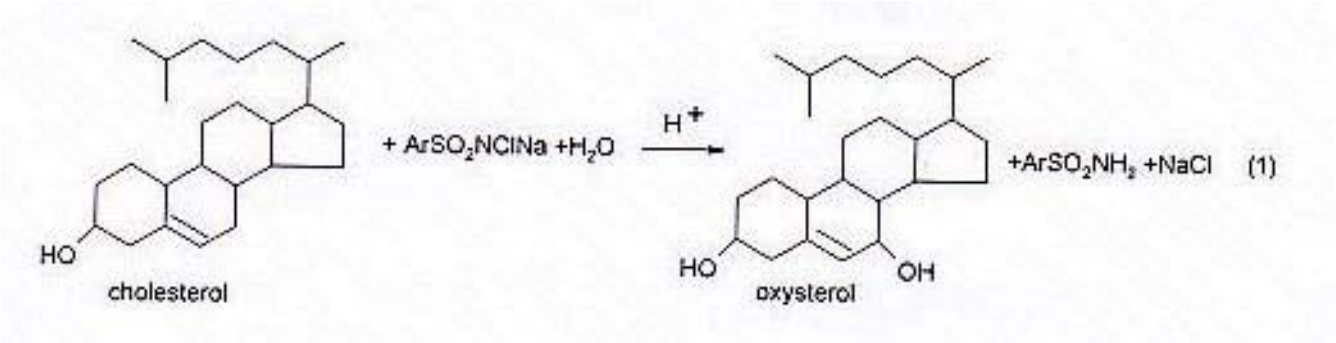
Chloramine-T (CAT) (E merck) was purified by the method of Morris etal [11] an aqueous solution of CAT was prepared, standardized, perodically by the iodometric method. Analar grade of cholesterol (spectrochem) was used and the solution was prepared in ethyl alcohol. All other chemicals used were acceptable grades of purity.

Kinetic Measurements

Mixtures containing requisite amounts of the substrate (cholesterol), alcohol were taken in stoppard pyrex glass tubes, whose outer surfaces were coated black. Required amount of alcohol was added to maintain a constant volume. The tube was thermo stated in a water bath to a given temperature (303K for most of the runs). To this solution was added a measured amount of pre-equilibrated CAT solution to give a known concentration. The reaction mixture was shaken well for uniform concentration. The progress of the reaction was monitored iodometrically for two-half lives by withdrawing aliquots of the reaction mixture at a regular time intervals. Under pseudo first-order conditions, rate constants, k' were reproducible with in ±3% regression coefficient of experimental data was carried out on an origin 5.0 by HP computer.

Reaction Stoichiometry

Varying ratios of oxidant to cholesterol in the presence of alcohol were equilibrated at 303K for 24 h. The unreacted CAT in the reaction mixture was determined iodometrically, indicated that one mole of cholesterol consumed one mole of CAT to give the corresponding oxysterol which is stoichiometrically represented as in equation.(1).



Product analysis:

The reaction mixture in the stoichiometric ratio in the presence of alcohol medium was allowed to progress for 24 h at 303K. After completion of the reaction (monitored by TLC), the reaction mixture was neutralized and the products were extracted with ether. The organic products were subjected to spot tests and chromatographic analysis (TLC method). However the substrate undergoes oxidation leading to the formation of hydro peroxides and then to a number of oxidation products, the so-called oxysterols.

The products corresponded to oxysterol of cholesterol, were confirmed by GC-MS analysis and IR spectra. GC-MS data were obtained on a 17A Shimadzu gas chromatograph with LCMS-2010A Shimadzu mass spectrometer. The mass spectra showed a molecular ion peak at (M = 404 amu) (Fig. 1) and IR spectra confirms a presence of strong band observed at ≈ 3433cm^{-1} (Fig. 2) indicates the presence of hydroxyl groups of oxysterol, which was not observed for pure cholesterol. The reaction product toulenesulfonamide ($ArSO_2NClNa$) was detected by TLC [12] using light petroleum-chloroform-butan-1-ol (2: 2: 1; v/v/v) as the mobile phase and iodine as the detection agent (R_f = 0.88).

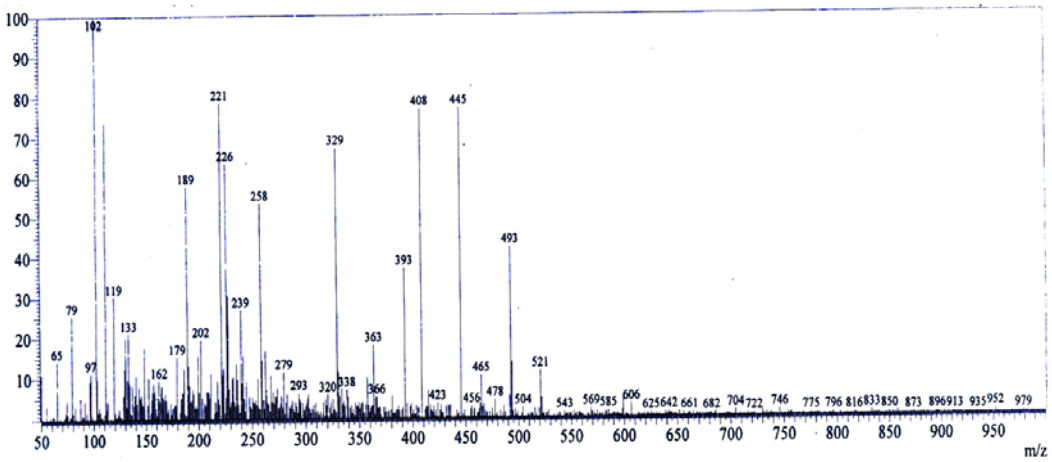


Fig.1. GC-Mass spectrum of oxysterol with molecular peak at 404 amu

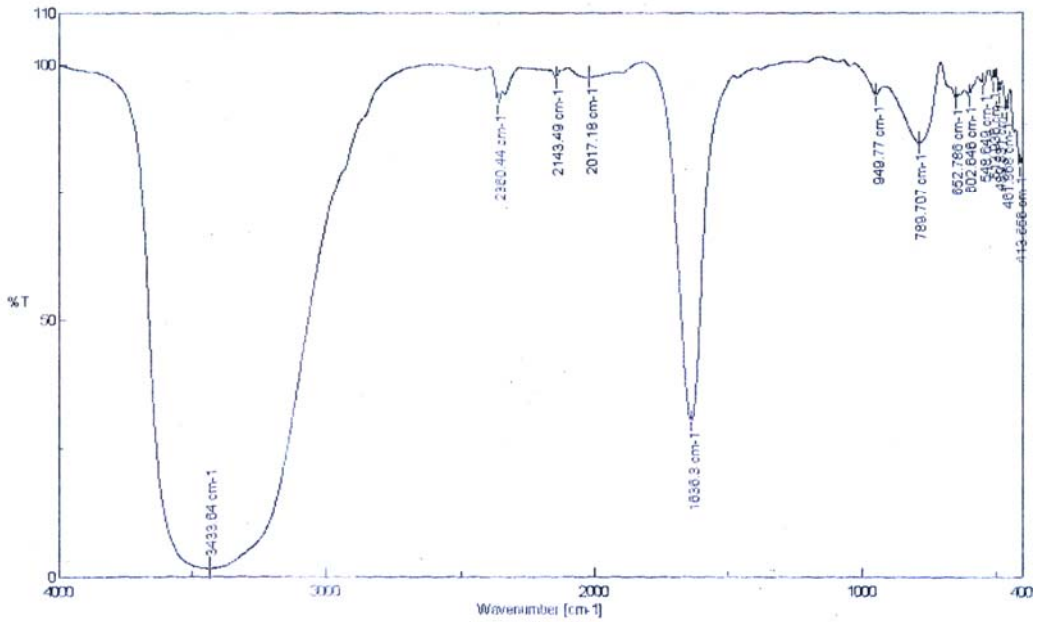


Fig.2. IR Spectra confirms a presence of strong band observed at ≈ 3433cm^{-1} indicates the presence of hydroxyl groups of oxysterol

RESUTS AND DISCUSSION

Results

The kinetics of oxidation of cholesterol by CAT was investigated at several initial concentrations of the reactants in alcohol medium. Under comparable experimental conditions, the oxidation kinetic behavior was observed for the cholesterol.

Effect of reactant concentrations on the rate:

Under pseudo-first-order conditions of $[cholesterol]_0 >> [CAT]_0$ at constant $[cholesterol]_0$, and temperature, plots of log [CAT] *vs.* time were linear ($r > 0.9972$) showing a first-order dependence of the rate on $[CAT]_0$. The pseudo-first order rate constant k′ obtained of 303K are listed in Table1. The values of k′ remain unaffected with a change in $[CAT]_0$ confirming the first -order dependence on $[CAT]_0$. The rate increased with increase in $[cholesterol]_0$ (Table 1) and plots of log k′*vs* log [cholesterol] were linear with unit slopes, indicating the first-order dependence of rate on [cholesterol].

Effect of HCl or NaOH concentration on the rate

Addition of HCl or NaOH to the reaction mixture enhances the oxidation of alcohol itself and hence the effect of HCl or NaOH is not studied.

Effect of Temperature on the reaction rate:

The reaction was studied at different temperatures, 298 K to 313 K, while keeping the $[CAT]_0$ and other experimental conditions constant. The rate constants are presented in Table 2. The thermodynamic parameters (Table 3) were calculated from the slopes of Arrhenius plots of log k′*vs* 1/T ($r > -0.9915$) in fig.3.

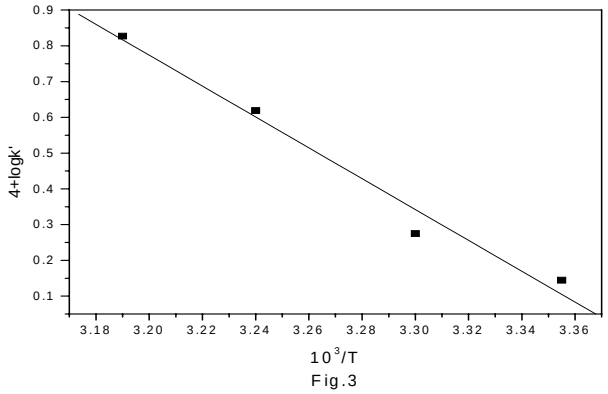


Fig.3

Table 1: Effects of varying CAT, Cholesterol concentrations on the rate of reaction

T = 303 K

10^5 [CAT] mol dm^{-3}	10^4 [Cholesterol] mol dm^{-3}	10^5 k′ (s^{-1})
2.00	3.33	2.787
4.10	3.33	2.810
8.30	3.33	2.900
16.6	3.33	2.878
25.0	3.33	2.787
8.33	1.66	0.332
8.33	3.33	2.787
8.33	4.16	4.138
8.33	5.00	4.792
8.33	6.66	5.182

Table-2 Effect of Temperature on the rate of reaction

[CAT] = 8.33 x 10^{-5} mol dm^{-3}: [Cholesterol] = 3.33 x 10^{-4} mol dm^{-3}:

Temperature(K)	1/T x 10^3	k′ x 10^3 s^{-1}
298	3.35	1.395
303	3.30	1.882
308	3.25	4.158
313	3.19	6.717

Table-3 Thermodynamic parameters for the oxidation of cholesterol by CAT in alcohol medium

Ea (KJ mol $^{-1}$)	$\Delta H^{\neq}$ (kJ mol $^{-1}$)	$\Delta S^{\neq}$ (J K^{-1} mol $^{-1}$)	$\Delta G^{\neq}$ (kJ mol $^{-1}$)
85.20	82.65	- 42.49	95.63

DISCUSSION

Pryde and Sopper [13] Morris et al and Bishop and Jennings [14] have shown the existence of similar equilibria in acid of N-metallo-N-haloarylsulfonamides. Chloramine-T ($ArSO_2NClNa$) or p-$CH_3C_6H_4SO_2NClNa.3H_2O$) behaves as a strong electrolyte in aqueous solution forming different species as shown in equation (2) to (6)

$$ArSO_2NClNa \rightleftharpoons ArSO_2NCl^- + Na^+ \quad (2)$$

$$ArSO_2NCl^- + H^+ \rightleftharpoons ArSO_2NHCl \quad (3)$$

$$2ArSO_2NHCl \overset{K^1d}{\rightleftharpoons} ArSO_2NCl_2 + ArSO_2NH_2 \quad (4)$$

$K^1d = 1.13 \times 10^{-2}$ at 25°c

$$ArSO_2NHCl + H_2O \rightleftharpoons ArSO_2NH_2 + HOCl \quad (5)$$

$$HOCl + H^+ \rightleftharpoons H_2OCl \quad (6)$$

The pH of CAT [8.33×10^{-5}], cholesterol [3.33×10^{-4}] and alcohol are 6.89, 2.70 and 2.60 respectively. The pH of reaction mixture shows 3.0. Therefore the reaction between CAT and cholesterol takes place in acidic medium. Since addition of HCl or NaOH enhance the oxidation of alcohol itself, the effect of HCl or NaOH was not studied.

In acidic solutions, the probable oxidizing species are the free acid ($ArSO_2NHCl$), dichloramine-T ($ArSO_2NCl_2$), HOCl and H_2OCl^+, as equation (4) & (5) indicates a slow hydrolysis. If HOCl were the primary oxidizing species, a first order retardation of the rate by the added $ArSO_2NH_2$ would be expected which was not observed and $ArSO_2Cl_2$ was not formed. Therefore it is likely that the $ArSO_2NHCl$ itself acts as the reactive oxidant species interacting with the cholesterol, based on the preceding discussion, scheme-1 is proposed.

$$ArSO_2NHCl + Cholesterol \overset{K_1}{\underset{k_{-1}}{\rightleftharpoons}} X \quad \text{r.d.s} \quad (I)$$

$$X \xrightarrow{k_2} Product \quad \text{slow} \quad (ii)$$

Scheme-1

$$Rate = k_2 [X] \quad (7)$$

$$K_1 = \frac{[X]}{[ArSO_2NHCl]\,[Cholesterol]} \quad (8)$$

$$X = K_1 X$$

$$\frac{d[X]}{dt} = k_1[ArSO_2NHCl]\,[Cholesterol] - k_{-1}[X] = 0$$

$$[X] = K_1\,[ArSO_2NHCl]\,[Cholesterol]$$

$$Rate = k_2 K_1\,[ArSO_2NHCl]\,[Cholesterol] \quad (9)$$

∴The equation (9) is a good agreement with experimental result showing first order w.r.t both [CAT] and [Cholesterol].

Clinical study of cholesterol oxidation by Erba Analiser method.

The concentration of cholesterol before oxidation and after oxidation was estimated by clinical method

Cholesterol	198 mg% [Before oxidation]
CAT + Cholesterol (1: 5)	131 mg % [After oxidation]

The above table indicates 67mg of cholesterol decrease after oxidation. Thus the kinetic reaction up to half life indicates 35% cholesterol is oxidized by CAT in to its product.

CONCLUSION

The oxidation of cholesterol was carried out by chloramine-T as oxidant. The kinetic study of this reaction helpful for decrease of cholesterol concentration. Hence chloramine-T may act as a drug for the decrease of concentration of cholesterol, which plays important role in pharmaceutical chemistry.

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