

Kinetic and mechanistic study of oxidation of lactose by trichloroisocyanuric acid in perchloric acid medium

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Abstract. Kinetics and Mechanism of Trichloroisocyanuric acid to oxidize lactose in perchloric acid solution have been studied. The reaction follows pseudo-first-order kinetics with respect to the oxidant, while exhibiting a fractional order dependence on lactose concentration. The reaction rate increases with increasing acid concentration, indicating that the process is acid-catalyzed. The influence of ionic strength and dielectric constant has an implication of the charged species participating in the rate-limiting step. The evaluation of activation parameters has been performed based on the studies of temperature variation, which shows the formation of an ordered transition state. The plausible mechanism that includes the formation of hypochlorous acid as the reactive species and consequent oxidation through a complex intermediate has been suggested.

1. Introduction

The oxidation of carbohydrates is a major field of study both in organic and biological chemistry since it is an important component in metabolic pathways, food chemistry and industrial applications. Oxidative changes of carbohydrates play a central role in biology, where they are known to participate in energy conversion and biosynthesis. Industrially, the oxidation reaction of sugars are used in the production of value-added products like aldonic acids that are used in pharmaceuticals, food additives and biodegradable materials. [1,2]

Lactose, a naturally occurring disaccharide composed of β -D-galactose and β -D-glucose units linked through a $\beta(1\rightarrow4)$ glycosidic bond, serves as an important model substrate for studying carbohydrate oxidation. Since there are several hydroxyl groups and a reducing end, the reactivity of lactose to oxidizing agents is varied. The oxidation of lactose can be controlled to produce other products like lactobionic acid that finds wide applications in food and pharmaceutical industries because of its antioxidant, chelating and humectant qualities. Thus, a theoretical and practical importance of the kinetics and mechanism of lactose oxidation is important.[3] Over the last few years, there has been a lot of interest in N-halo compounds as a wide-ranging and effective oxidizing agent in organic synthesis. One of them, Trichloroisocyanuric Acid (TCICA), has proven to be a particularly desirable reagent because it possesses high oxidative strength, stability, easy handling and is environmentally friendly. TCICA is reputed as a donor of active chlorine species in aqueous solution and mainly produces hypochlorous acid (HOCl), which is the actual oxidizing species. TCICA reactivity depends on the pH, solvent composition and the structure of the substrate, making it a good choice of kinetic studies.[4]

The literature has reported various kinetic and mechanistic studies of the oxidation of organic substrates by TCICA. The overall trend of these studies is that the reactions are first-order kinetics with respect to the oxidant concentration, but are either first-order or fractional-order with respect to the substrate concentration indicating the formation of an intermediate complex. Moreover, a significant fraction of these reactions are catalyzed by acids, suggesting that protonated species or increased electrophilicity of the oxidant in acidic solvents are involved. Mechanistic pathways tend



to include the production of hypochlorous acid as the reactive species, which then undergoes an electron transfer or hydrogen transfer reaction to produce a product.[5]

In spite of these developments, there is still a conspicuous gap in literature on the oxidation of structurally more complex carbohydrates like lactose in the presence of TCICA. Specifically, there are no systematic kinetic studies on how the rate of the reaction is affected by substrate concentration, oxidant concentration, acidity, ionic strength and temperature. Additionally, the mechanism of oxidation of lactose under acidic conditions has not been studied in detail.

Considering these, the current study will investigate the kinetics and the mechanism of oxidation of lactose by TCICA in perchloric acid medium. The research aims to identify the rate law, assess the impact of different reaction parameters and suggest a feasible reaction mechanism that is in line with the experimental observations.

2. Experimental

2.1 Materials

The chemicals used in the current investigation were of the grade of analytical reagents and were not further purified. Lactose ($\geq 99\%$ purity) was procured and used as the substrate. The oxidant was Trichloroisocyanuric Acid (TCICA) that was obtained at Aldrich. The TCICA stock solution was freshly prepared in a standardized iodometric solution in double distilled water before use to ensure concentration accuracy.

Perchloric acid (HClO_4) of known strength was used to maintain the acidic medium throughout the reaction. Appropriate dilution of the stock solution with the double distilled water was done to arrive at the required acid concentrations. All solutions have been made with freshly distilled water to eliminate interference of impurities.

Iodometric estimation of the amount of oxidant that remained was done using sodium thiosulfate solution, potassium iodide and starch indicator. The glassware used in the experiments was completely cleaned, rinsed with distilled water and dried before using to exclude contamination effects.

2.2 Preparation of Solutions

A stock solution containing lactose was made by dissolving a carefully weighed portion of lactose in a double distilled water. The solution was left to equilibrate so that the solution was completely dissolved.

The solution of the oxidant (TCICA) was freshly made prior to each series of experiments so that there was no decomposition. Appropriate dilution of the stock solution was used to obtain the necessary concentrations of TCICA.

A series of perchloric acid solutions were prepared of different concentrations to investigate the influence of acidity on the rate of reaction. The ionic strength was modified (where necessary) with neutral electrolytes.

2.3 Kinetic Measurements

The kinetics of oxidation of lactose by TCICA were examined in pseudo-first-order conditions by keeping a high concentration of the lactose as compared to the concentration of the oxidant. In such a case, the concentration of lactose essentially does not change as the reaction progresses and the reaction rate is then largely determined by the concentration of TCICA.

The reactions were carried out in a thermostatically controlled water bath maintained at a constant temperature with an accuracy of ± 0.1 °C. Prior to initiation of the reaction, the reaction mixture containing the required concentrations of lactose and perchloric acid was equilibrated at the desired temperature.[6]

The rapid addition of a known volume of TCICA solution to the already equilibrated reaction mixture began the reaction. All experiments had equal total volume of the reaction mixture to maintain consistent concentration calculations.

Aliquots of the reaction mixture were periodically removed and quenched immediately by adding too much potassium iodide solution. The released iodine, equivalent to the unreacted TCICA was titrated with a standard sodium thiosulfate solution in the presence of starch as an indicator towards the endpoint.

The progress of the reaction was thus monitored by determining the decrease in TCICA concentration with time. The pseudo-first-order rate constants (k_{obs}) were calculated from the slope of linear plots of $\log[\text{TCICA}]$ versus time.

Each kinetic run was performed at least in duplicate to ensure reproducibility, and the observed rate constants were found to be consistent within acceptable experimental error limits.

2.4.4 Reaction parameters variation.

The series of controlled kinetic experiments under the pseudo-first-order conditions were conducted to systematically examine the effect of various reaction parameters on the rate of oxidation of lactose by TCICA. In both series of experiments, there was only one parameter which was varied and all other parameters held constant so that the separate effects of each parameter on the reaction rate could be well determined.[7]

Effect of Lactose Concentration:

The concentration of lactose was varied within an appropriate range with constant concentrations of TCICA, perchloric acid, ionic strength, and temperature. The change in rate constants with substrate concentration was used to determine the order of reaction with respect to lactose.[8]

Effect of TCICA Concentration:

The concentration of the oxidant was changed and all other parameters remained constant. To determine the pseudo-first-order behavior and to determine the order in terms of oxidant, the rate dependence on TCICA concentration was examined.

Effect of Perchloric Acid Concentration:

The influence of acidity on the reaction rate was studied by varying the concentration of perchloric acid. This helped in understanding the catalytic role of hydrogen ions and the possible involvement of protonated species in the rate-determining step. [9]

Effect of Ionic Strength:

An inert electrolyte (e.g. sodium perchlorate) was used to adjust the ionic strength of the reaction medium. The ionic strength influence on the rate of reaction gave information on the type of species in the reaction (neutral or ionic) and the presence of charged intermediates.

Effect of Temperature (298–318 K):

The reaction was carried out at different temperatures in the range of 298–318 K. The rate constants obtained at various temperatures were used to calculate activation parameters such as activation energy (E_a), enthalpy (ΔH), and entropy (ΔS) using Arrhenius and Eyring equations.

2.5 Estimation of TCICA

The progress of the reaction was monitored iodometrically by estimating the unreacted TCICA at regular time intervals. The principle of this method is the quantitative liberation of iodine in potassium iodide by TCICA in acidic media, and subsequently titration with standard sodium thiosulfate solution.[10]

The reactions involved are as follows:



A typical procedure involved the removal of a known aliquot of the reaction mixture at a certain time interval and the addition of an excess of potassium iodide solution in acidic conditions. The freed iodine was then titrated with standard sodium thiosulfate solution using starch as an indicator towards the end point.[11]

The amount of sodium thiosulfate that was taken up was directly proportional to the concentration of the unreacted TCICA. Based on these values, the concentration of TCICA that was left in various time intervals were determined and the rate constants were, therefore, established.[12]

3. Results and Discussion

3.1 Stoichiometry

Based on the controlled reaction with excess oxidant, the stoichiometry of the oxidation of lactose by trichloroisocyanuric acid (TCICA) was established by letting the reaction proceed to completion. Iodometrically, the TCICA that was not reacted was estimated.[13]

The results of the experiment indicate that a reaction occurs between one mole of lactose and one mole of TCICA and so the stoichiometric ratio is 1:1. As the major product of the oxidation, lactobionic acid is formed [14]

This stoichiometric equation indicates that the reaction is accompanied by the exchange of an equivalent of active chlorine between TCICA and the substrate, indicating a two-electron oxidation of the aldehydic group of lactose [15]

3.2 Order of Reaction

The kinetic data obtained under pseudo-first-order conditions were analyzed to determine the dependence of reaction rate on various reactants.

Effect of [TCICA]

$$\ln[TCICA] = -kt + \ln[TCICA]_0$$

A linear relationship was observed when $\log[TCICA]$ (or $\ln[TCICA]$) was plotted against time, indicating that the reaction follows first-order kinetics with respect to TCICA. The constancy of slope across different initial concentrations further confirms that the rate depends linearly on oxidant concentration. Figure 1 shows the plot of $\log(TCICA)$ vs time of oxidation of lactose.

Effect of Lactose

The reaction rate is proportional to the concentration of lactose; but the change does not occur in direct proportion. A graph of rate constant vs. lactose concentration plots a non-linear graph, which implies a fractional order dependence.

This behavior indicates that there is an intermediate complex between lactose and TCICA (or its reactive species) before the rate-determining step. Kinetics of this nature are typical of the mechanisms that include the formation of pre-equilibrium complexes. Figure 2 shows the Variation of rate constant (k_{obs}) with lactose concentration.

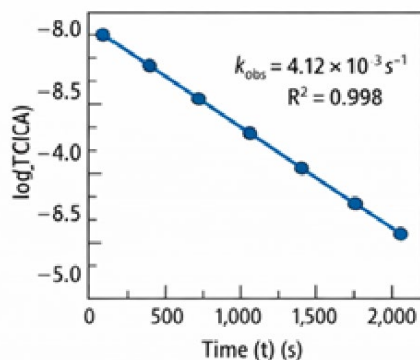


Figure 1. Plot of log[TCICA] vs. time for oxidation of lactose.

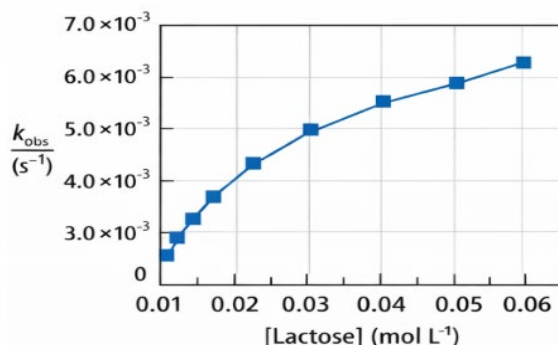


Figure 2. Variation of k_{obs} with lactose concentration.

Effect of $[\text{H}^+]$ (Perchloric Acid)

The reaction rate increases greatly with an increase in the concentration of perchloric acid. The rate dependence on the concentration of hydrogen ions is an indicator that the reaction is catalyzed by acids.

The action of acid could involve:

- TCICA protonation to produce a more active oxidizing species.
- Stabilization of the transition state
- Hydride transfer of lactose.

On the whole, the behavior observed is in line with proton-assisted oxidation mechanisms previously observed with comparable N-halo oxidants.

3.3 Effect of Ionic Strength

It was observed that the rate of the reaction rose with ionic strength of the medium (modulated with an inert electrolyte like sodium perchlorate).

This is a positive salt effect which indicates:

- The participation of charged species in the rate-limiting step.

Possible repulsive force between similarly charged ions, in which the repulsive forces are decreased with increased ionic strength.

The DebyeHuckel theory suggests that such behavior is an indication that the activated complex is probably an ionic reactant complex, which supports the proposed mechanism of protonated oxidant species.

3.4 Effect of Dielectric Constant

Appropriate organic solvents were added to the medium to alter the dielectric constant of the medium. It was observed that:

- Reduction in dielectric constant causes a reduction in the rate of the reaction.

This means that the transition state is more polar than the reactants. The charge or polar species are better stabilized in a medium with a high dielectric constant thus increasing the rate.

Thus, the results suggest:

- Creation of a polar transition state.
- Engagement of the dipole-dipole or an ion-dipole interactions in the process.

3.5 Temperature Effect and Activation Parameters

The effect of temperature on the reaction rate was studied in the range 298–318 K. The rate constants obtained at different temperatures were analyzed using the Arrhenius equation:

$$k = Ae^{-E_a/(RT)}$$

A plot of $\ln k$ versus $1/T$ was found to be linear, confirming the validity of the Arrhenius relationship (Figure 3). From the slope and intercept of the plot, activation parameters were calculated.

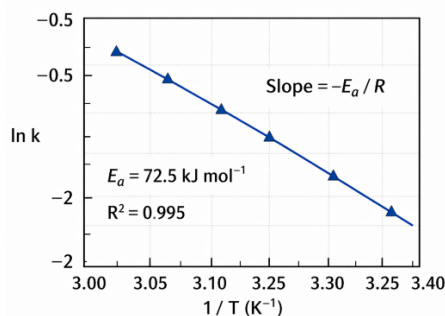


Figure 3. Arrhenius plot for oxidation of lactose.

Activation Parameters

- Activation Energy (E_a):**
The moderate value of activation energy indicates that the reaction proceeds at a reasonable rate without requiring excessive energy input.
- Entropy of Activation (ΔS):**
The negative value of ΔS suggests the formation of a more ordered transition state, possibly due to the association of reactant species during complex formation.
- Gibbs Free Energy of Activation (ΔG):**
The positive ΔG values indicate that the reaction proceeds via an energy barrier, as expected for a non-spontaneous activated complex formation step.

4. Mechanism

4.1 Reactive Species

In aqueous acidic medium, trichloroisocyanuric acid (TCICA) undergoes hydrolysis to generate hypochlorous acid, which acts as the primary oxidizing species:



The formation of HOCl is well documented in N-halo oxidant systems, and it is considered the active electrophilic oxidizing species in the present study. Under acidic conditions, HOCl may further exist in its protonated form (H_2OCl^+), enhancing its oxidizing ability.

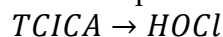
Thus, the observed kinetics and acid dependence strongly support that HOCl (or its protonated form) is responsible for the oxidation of lactose.

4.2 Proposed Mechanism

Based on the kinetic results, stoichiometry, and activation parameters, the following mechanism is proposed:

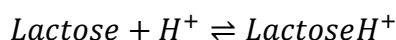
Step 1: Formation of Reactive Species

TCICA rapidly hydrolyzes in aqueous medium to produce HOCl:



Step 2: Protonation of Lactose (Acid Catalysis)

In acidic medium, lactose undergoes protonation, which enhances its susceptibility toward oxidation:



This step increases the electron deficiency at the reactive center, facilitating oxidation.

Step 3: Complex Formation (Pre-equilibrium)

The protonated lactose interacts with HOCl to form an intermediate complex:

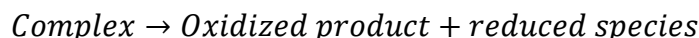


The existence of this intermediate is supported by:

- Fractional order in lactose
- Negative entropy of activation (ΔS)
- Dependence on ionic strength

Step 4: Rate-Determining Step (Slow Step)

The complex undergoes decomposition via hydride transfer or electron transfer, leading to oxidation:



This step is considered the rate-determining step (RDS) and involves the formation of a highly ordered transition state.

Step 5: Product Formation

The final product formed is lactobionic acid, resulting from oxidation of the aldehydic group of lactose.

4.3 Derived Rate Law

Based on the proposed mechanism and assuming a rapid pre-equilibrium followed by a slow rate-determining step, the rate law can be expressed as:

$$Rate = \frac{kK[Lactose][H^+][TCICA]}{1 + K[Lactose]}$$

Interpretation of the Rate Law

The derived rate expression successfully explains all observed kinetic features:

- **Fractional Order in Lactose:**
At low lactose concentration, the denominator term is small, and the rate increases nearly linearly. At higher concentrations, the denominator becomes significant, leading to fractional order behavior.
- **First-Order Dependence on TCICA:**
The rate is directly proportional to TCICA concentration, consistent with experimental observations.
- **Acid Dependence:**
The presence of $[H^+]$ in the numerator confirms the catalytic role of acid, supporting protonation steps in the mechanism.

5. Conclusion

The current study gives a complete kinetic and mechanistic insight into the oxidation of lactose using trichloroisocyanuric acid (TCICA) in acidic solution. It is evident in the results of the experiment that the reaction is governed by the pseudo-first-order kinetics with respect to TCICA, when the latter is in excess. The first-order dependence on the oxidant is confirmed by the linearity of the logarithmic plots.

The rate of the reaction is highly dependent on the concentration of hydrogen ions and this proves that it is an acid catalyzed reaction. The use of perchloric acid is essential in increasing the reactivity of the oxidizing species and also in the formation of the reactive intermediates.

A partial order dependence on lactose concentration has been noted, which is a strong indication that a pre-equilibrium complex between the substrate and the active oxidizing species has occurred. This is typical of reactions that occur via an intermediate complex before the rate-determining step.

The analysis of ionic strength, and dielectric constant also confirms the presence of charged and polar species in the reaction mechanism. The rise in rate as the ionic strength rises and the fall in the rate as dielectric constant decreases points to the fact that the transition state is polar in character.

The temperature dependence and activation parameters analysis indicates a moderate activation energy, and a negative entropy of activation (ΔS). This negative entropy signifies the creation of a highly structured transition state probably by the association of reacting species in the creation of the activated complex. The positive values of Gibbs free energy of activation (ΔG) are an indication that the reaction is through an energy barrier, as is expected of such oxidation reactions.

Mechanistic analysis has affirmed that the active oxidizing species is hypochlorous acid (HOCl), which is produced in the hydrolysis of TCICA. The mechanism is suggested to take place through protonation of lactose, followed by the formation of a complex with HOCl and a rate-limiting step of hydroxide exchange to the production of lactobionic acid as the end product.

In general, the kinetic data, activation parameters, and mechanistic interpretation are consistent with known behavior of N-halo oxidants, thus, offering further understanding of the oxidation chemistry of carbohydrates in acidic conditions.

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