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Research Article

Chemistry for New drug discovery

1,2-Bis(4-Pyridyl) Ethane Catalysed Oxidative Conversion of D-Lactose to Lactobionic Acid by Cr(VI) in Aqueous Acetic Acid Medium: A Kinetic and Mechanistic Study

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Abstract: The kinetics of oxidation reactions and the investigations of the reaction mechanisms from the kinetic data have been always the more interesting in the chemical research and industrial applications. The kinetics of oxidation of sugars has been a subject of extensive research in recent years. The biological as well as the economic importance of carbohydrates is responsible for the great interest in the study of their bio- and physio -chemical properties. The kinetics of oxidative degradation of different sugars by metal ions including Cr (VI) has been studied under different conditions. Chomium (VI) compounds are powerful oxidants at low or neutral pH as well as Cr (VI) is a well-known oxidant in acetic media. Aim of our present investigation deals with oxidation of D-Lactose using Cr (VI) Oxidizing agent, Trimethylammonium fluorochromate [TriMAFC] which was carried out in protic solvent as a medium at 308 K in the presence of 1,2-bis(4-pyridyl)ethane as a catalyst . The oxidation of D-Lactose yields the corresponding aldonic acid and Cr^{3+} as final products. Lactobionic Acid (LBA) has been used for food and pharmaceutical applications. Thus the kinetics and mechanism of carbohydrates using Cr (VI) as oxidant, 1,2-bis(4-pyridyl)ethane as a catalyst is very important from biochemical and kinetic viewpoints. Our experimental studies showed that reaction progressed with first order kinetics with respect to [TriMAFC], [1,2-bis(4-pyridyl)ethane] and $[H^+]$ with the fractional Order dependence on [D-Lactose]. It was found that introduction of sodium perchlorate results in decrement of the reaction rate substantially. The rate constant increased with increase in the concentration of perchloric acid. Also, the rate of reaction decreases with increasing the concentration of acetic acid. For acrylonitrile, no polymerization occurs. The reaction was carried out at four different temperatures to determine the activation parameters. An appropriate mechanism was suggested based on the observed kinetic effects.

Keywords [D-Lactose], [LBA], [TriMAFC], [1,2-Bis(4-pyridyl)ethane], Oxidation, Kinetics and mechanism.

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1. INTRODUCTION

Carbohydrates are the fuels of life, being the main energy source for living organisms and supply for most cells. The study of the carbohydrates and their derivatives has greatly enriched chemistry, particularly with respect to the role of molecular shape and conformations in chemical reactions[1]. Influences of oxygen on reaction rate depends on the concentration of the reactants that increases in the pseudo first order conditions during the oxidation process [Reactants] comes decreasing category. Hence [Products] increases during the oxidation process. The biological as well as the economic importance of carbohydrates is responsible for the great interest in the study of their bio- and physio -chemical properties[2]. Sugars are very attractive natural ligands for both toxic and essential metal ions. Besides acting simply as effective chelators[3], in many cases they are also reducing agents, (e.g) for metal ions such as Ce (IV), V (V), Co (III), Fe III[4-7], depending on the acidity of the medium. There is also an indication that some sugars, or their derivatives, may play an important role in the chemistry of chromium, especially in the environment[8-10], where Cr (VI) may present a serious hazard became of its mutagenic and carcinogenic activity. Due to the carcinogenic and toxic effects of Cr (VI) [11-13], studies on the kinetics and mechanism of C (VI) oxidation of biologically relevant reducing agents is of interest to both biochemists and inorganic chemistry[13,14]. During the reduction of Cr (VI) to Cr (III), the intermediate oxidation states of chromium may interact with biologically active molecules to induce toxicity[15]. Thus in chromate toxicity, it is reasonable to assume that the reducing agents may have an important role. Purified lactose can also act as high calorie diet additive[16]. These biological and economic importance of the carbohydrates and especially the monosaccharides and disaccharides have been largely responsible for the interest in the study of bio and physiochemical properties and relativities[17]

1.1. TriMAFC as an oxidant

In synthetic organic chemistry, halochromates have been used as mild and specific oxidizing reagents[18-31]. Trimethylammonium fluorochromates has been used as mild selective oxidant in synthetic organic chemistry[32-34]. TriMAFC $C_3H10CrFNO_3$, has certain advantages over similar oxidizing agents in terms of amount of oxidant and solvent required, easier working up and high yield. Further trimethylammounium fluorochromate does not react with acetonitrile, which is a suitable medium for studying kinetics and mechanism[35]. TriMAFC & TriMACC on alumina have also been used for oxidation of carbohydrates such as 1,2:5,6- Di-O- isopropylidine –α-D-G lucofuranose to its relative ketosugar as other chromium reagents by use of the equimolar ratio of the reagents. Kinetics of oxidation of D-Lactose by some other oxidizing reagents has been well documented[36].The literature survey revealed that the kinetics and mechanism of oxidation of D-Lactose by TriMAFC has not been reported. Hence, in the current investigation, the oxidant Trimethylammonium fluorochromate [TriMAFC] is used in aqueous acetic acid medium.

1.2. Oxidation of D-Lactose

Lactose is an important secondary product of the cheese and certain manufacture, whose make up is expected to about 1.2 million tons per year. Because of its low solubility and sweetness, as well as a certain intolerance of some population segments, the usage of lactose is limited in many applications[37]. Lactose is composed of β-D-galactose and β-D-glucose. The linkage is between C1 of galactose and C4 of glucose. Hence it is also a reducing sugar. A method for Cr (VI) oxidation of lactose to lactobionic acid in aqueous medium at room temperature by combination of best promoter and micellar catalyst has been deduced[38]. The kinetics of oxidative degradation of different sugars by metal ions including Cr(VI) has been studied under different conditions. The kinetics and oxidation of D-Lactose by [TriMAFC] has not been documented in the literature survey. As a result in the present study, we have used TriMAFC to oxidize D-Lactose in the presence of 1,2-bis(4-pridyl)ethane, which act as a catalyst . The corresponding mechanisms are explained in this research paper.

1.3.-bis (4-pyridy1) ethane as an catalyst

The 1,2-bis (4-pyridy1) ethane catalyst is a versatile building block for the purpose of crystal engineering. 1,2 bis (4-pyridyl) ethane (bpa) which is a well-known ligand in supramolecular chemistry[39]. It is extensively employed as organic building block for construction of 1-D,2-D and 3-D networks. It can be used in Co-ordination chemistry as a bidentate bridging ligand, and act as a terminal ligand[40] or as a host molecule[41].

2. MATERIALS AND METHODS

2.1. Materials

All stock solutions for kinetic investigations were prepared in double distilled water. TriMAFC were synthesized with the belief that the reagents could be used for the organic substrates. Further TriMAFC does not react with acetonitrile, which is a suitable medium for studying kinetics and mechanism**.** D-Lactose and all other chemicals without further purification utilized in this research are all of Analar grade (AR).A chemical reaction occurs faster in the presence of catalyst because, the catalyst provides an alternative reaction pathway or mechanism with lower activation energy than the non-catalysed mechanism.

- Catalyst alters the reaction path and enhances the flexibility of entire reactions.
- An enzyme is a biological catalyst and also important for controlling reactions in cells.
- Selective oxidation of D-Lactose in presence of 1,2-bis (4-pyridy1) ethane, were satisfactory fitted by applying pseudo-homogeneous models[42]

Characterisation of Catalyst

1. 1,2-bis (4-pyridy1) ethane catalyst is a white to light yellow crystals and also having $P_{ka}(P_{kb})$/Solubility-5.9(8.1)[19]/5.3g/L[20]M.F.$C_{12}H_{12}N_2$M.Wt.is184.24.MP:110-112°C(lit.),BP:310.3±22.0°C at760mmHg FP:117.2±14.0°C Density:1.1±0.1g/cm3 Purity:97%

2. Structure of 1,2-bis (4-pyridy1) ethane catalyst[43]

3. **IR** and **UV** spectrum of free 1,2-bis (4-pyridy1) ethane.[39],[44,45,46]

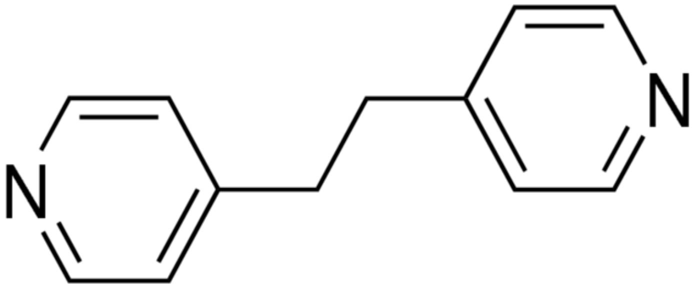


Fig.1. 1,2-bis (4-pyridy1) ethane (bpa)

Kinetic Modelling

Only one step (slow step) of the proposed mechanism is considered as the controlled one, but the reaction occurs in reversible (or) equilibrium (or) fast step in non-controlling step

2.2. Kinetic Procedure and measurement

All the kinetic measurements were carried out in pseudo first order conditions maintained throughout the study. Homogeneous medium of reaction combination was conserved throughout the reaction unless otherwise mentioned, the process performed out in a solvent system of 50%(v/v) acetic acid-water medium at 308K, and the reaction was controlled through using iodometric approach. The order of the reaction was found to be first with respect to [TriMAFC] as evidenced by the plot of [TriMAFC] Vs time and $[H^+]$ Vs log K_{obs} yielded a straight line, its slope , the first order rate constant were calculated.

2.3. Product Analysis and Stoichiometric studies

For the completion of the reaction, the reaction mixture was allowed to stand for 48 hrs. The product was extracted with chloroform and the organic layer was washed with water, dried over anhydrous sodium sulfate. The chloroform layer was evaporated. After the completion of kinetic experiment, a portion of the oxidized reaction mixture was treated with alkaline hydroxylamine solution. To prepare a neutral fraction, certain amount of barium carbonate was added to rest of the reaction mixture. A probing of the resulting mixture with a phenol-colored violet solution of $FeCl_3$ gave bright-yellow coloration, thereby indicating the presence of aldonic acid. This was a sign that lactone, which was formed in the rate determining step, was rapidly hydrolysed to lactonic acid in neutral medium. We required 0.1 mole of [D-Lactose] and 0.1 mole of [TriMAFC] for the stoichiometric studies of the reaction. The reaction mixture were prepared by mixing of above reagents with one another in perchloric acid medium and 50% (v/v) aqueous acetic acid medium (total volume 100ml).

3. RESULT AND DISCUSSION

The oxidation of [D-Lactose] by [TriMAFC][38] in the presence of a 1,2-bis(4-pyridyl) ethane catalyst was conducted in a 50-50% (v/v) acetic acid – water medium at 308K under pseudo-first order condition, and the findings were addressed on the following pages,

3.1. Influence of changing [TriMAFC]

The Oxidation reactions were conducted with varying concentrations of TriMAFC[18-31] in the range 0.50 to 2.25 x 10^{-3} mol dm^{-3} and maintaining all other reactant concentrations constant and rate constant were calculated (Shown in Table 1). It is 1,2-bis(4-pyridyl)ethane catalysed reactions. The lack of difference in the first order rate constant at different TriMAFC concentrations showed that the order is unity with respect to [TriMAFC].The linearity of the log titre versus time plot (r=0.999) up to 80% completion of the reaction validated these observations.[47]

Table.1 Effect of variation of oxidant concentration

10^3 [TriMAFC](mol dm^{-3})	10^4 K_{obs} (S^{-1})
0.50	2.77
1.00	2.78
1.25	2.80
1.50	2.81
1.75	2.82
2.00	2.87
2.25	2.88

[D-Lactose] = 1.50×10^{-2} mol dm^{-3}; [1,2-bis(4-pyridyl)ethane] = 1.25×10^{-3} mol dm^{-3}
[$HClO_4$] = 10.00×10^{-3} mol dm^{-3}; AcOH-H_2O = 50-50 (v/v); Temperature = 308K

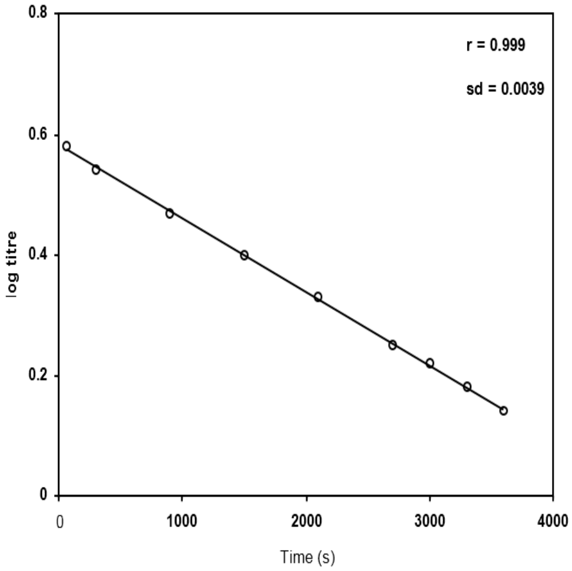


Fig.2. Plot of log titre *versus* time (s)

3.2 Influence of changing [D-Lactose]

At 308K, the Substrate [**D-Lactose**] was varied from 0.50 to 3.00 X10^{-3} mol dm^{-3} , while all other reactant concentrations were kept stable, and the corresponding rate constant was calculated (Table 2).With the increase in the concentration of substrate, the rate of reactions also increased[48]. A plot of log K_{obs} versus log [**S**] is straight line[47] and r=0.999 in the case of 1,2-bis(4-pridyl)ethane catalysed reaction.

Michelis constant for D-Lactose

The Michaelis constant for D-Lactose was determined by an Iodometric method

$$r = \text{Rate law} = \frac{K_1K_2k_3[\mathrm{S}][\mathrm{H}^+][\mathrm{Cr(VI)}][1{,}2\text{ - bis(4 - pyridyl)ethane}]}{1 + k_3\,[\text{substrate}]}$$

Table.2 Effect of variation of Substrate concentration

10^3 [D-Lactose](mol dm^{-3})	10^4 K_{obs} (S^{-1})
0.50	1.77
1.00	2.31
1.50	2.80
2.00	3.14
2.50	3.46
3.00	3.72

[TriMAFC]] = 1.25x10^{-3} mol dm^{-3}; [1,2-bis(4-pyridyl)ethane] = 1.25x10^{-3} mol dm^{-3}
[$HClO_4$] = 10.00 x10^{-3} mol dm^{-3}; AcOH-H_2O = 50-50 (v/v); Temperature = 308K

3.3 Influence of varying [$HClO_4$]

The processes were carried out with perchloic acid concentrations ranging from 5.00 to 15.00 mol dm^{-3} while retaining all other reactant concentrations unchanged. The rates were calculated (Table.3) The plot of log K_{obs} versus log[H^+] shows that first order dependence is observed (slope= 0.8136, r=0.9997, sd=0.0032) in this reaction[49]

Table.3 Effect of variation of Perchloric acid concentration

10^3 [$HClO_4$](mol dm^{-3})	10^4 K_{obs} (S^{-1})
5.00	1.61
7.50	2.21
10.00	2.80
12.50	3.38
15.00	3.94

[TriMAFC]] = 1.25x10^{-3} mol dm^{-3}; [1,2-bis(4-pyridyl)ethane] = 1.25x10^{-3} mol dm^{-3}
[D-Lactose] = 1.50x10^{-2} mol dm^{-3}; AcOH-H_2O = 50-50 (v/v); Temperature = 308K

3.4. Influence of varying [1,2-bis(4-pridyl)ethane]

The 1,2-bis(4-pridyl)ethane catalytic process was performed out with differing concentrations of 1,2-bis(4-pridyl)ethane with range 0.50 to 1.75 mol dm^{-3} while maintaining all other reactant concentrations unchanged and the rate constant values are calculated.(Table.4). The rate constant increased with increasing the concentrations of catalyst[50]. The straight line plot of log K_{obs} versus log [Catalyst] with r=0.999 and sd=0.0029 implies that there is first order dependency with respect to [1,2-bis(4-pridyl)ethane].

Table.4 Effect of variation of Catalyst concentration

10^3 [1,2-bis(4-pridyl)ethane] (mol dm^{-3})	10^4 K_{obs} (S^{-1})
0.50	1.54
1.00	2.38
1.25	2.80
1.50	3.13
1.75	3.44

[TriMAFC] = 1.25×10^{-3} mol dm^{-3}; [$HClO_4$] = 10.00×10^{-3} mol dm^{-3};
[D-Lactose] = 1.50×10^{-2} mol dm^{-3}; AcOH-H_2O = 50-50 (v/v); Temperature = 308K

3.5. Influence of changing solvent composition

The reaction was conducted with different solvent composition of hydrophilic solvent mixture and catalysed by [1,2-bis(4-pridyl)ethane] keeping all other reactant concentrations constant. The corresponding rate constants were given in Table.5. The kinetic result demonstrates that as the percentage of acetic acid in the reaction mixture increases, the rate constant decreased. This suggests that an ion-dipole interaction may be involved in the mechanistic pathway. A plot of log K_{obs} Vs 1/D is linear with a positive slope in this reactions. [49]

Table.5 Effect of Solvent Composition

ACOH-H_2O (%v/v)	10^4 K_{obs} (S^{-1})
30-70	6.89
40-60	3.94
50-50	2.80
60-40	2.21
70-30	1.81

[TriMAFC]] = 1.25×10^{-3} mol dm^{-3}; [$HClO_4$] = 10.00×10^{-3} mol dm^{-3}; Temperature = 308K
[D-Lactose] = 1.50×10^{-2} mol dm^{-3}; [1,2-bis(4-pyridyl)ethane] = 1.25×10^{-3} mol dm^{-3}

3.6.1 Influence of added [$NaClO_4$],[Acrylonitrile] and [MnO_4] on the Rate of Reaction

The rate data was measured with different concentrations of $NaClO_4$ and keeping all others reactant concentration constant with increase in the concentration of $NaClO_4$, the rate constant decreased slightly. By adding Mn^{2+} to 1,2-bis(4-pridyl) ethane catalysed reaction, the reaction rate slightly increased, indicating a catalytic effect. Added acrylonitrile rules out the possibility of radical pathway mechanism[49,50]

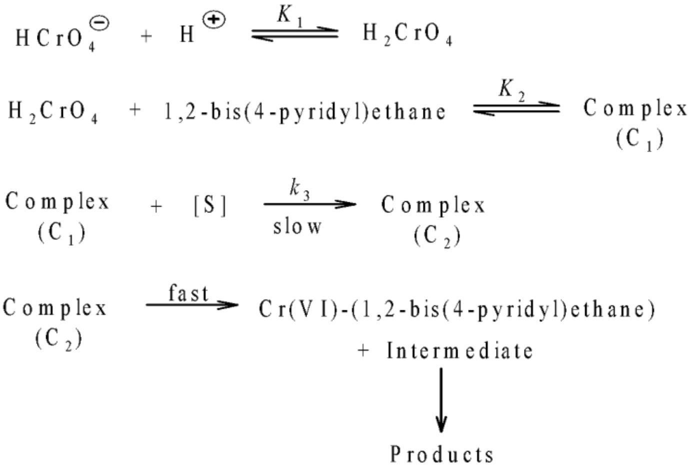
Table 6. Rate data for the effect of added [$NaClO_4$], [Acrylonitrile] and [$MnSO_4$]

10^4[$NaClO_4$] (mol dm^{-3})	10^4 kohs (S^{-1})	10^4[Acrylonitrile] (mol dm^{-3})	10^4 kohs	10^4[$MnSO_4$] (mol dm^{-3})	10^4 kohs (S^{-1})
0.00	2.80	0.00	2.80	0.00	2.80
5.00	2.46	5.00	2.81	5.00	3.09
10.00	2.26	10.00	2.86	10.00	3.17
15.00	2.15	15.00	3.08	15.00	3.23
20.00	1.21	20.00	3.18	20.00	3.38

[TriMAFC]] = 1.25×10^{-3} mol dm^{-3}; [$HClO_4$] = 10.00×10^{-3} mol dm^{-3}; Temperature = 308K
[D-Lactose] = 1.50×10^{-2} mol dm^{-3}; ; [1,2-bis(4-pyridyl)ethane] = 1.25×10^{-3} mol dm^{-3}

3.7. Mechanism in rate of law

The oxidative conversion of organic substrate using Trimethylammonium fluorochromate [32-34] **were** carried out in protic solvent as a medium at 308 K in presence of 1,2-bis(4-pyridyl)ethane catalyst, and the kinetic results were summarized as follows. The unit order dependence of the reaction was with respect to [TriMAFC], [1,2-bis(4-pyridyl)ethane] and [H^+]. The fractional order dependence of the reaction was with respect to [S]. The reaction rate increased with increasing concentration of 1,2-bis(4-pyridyl)ethane and first order dependence is observed. Slight catalyst behaviour of Mn^{2+} is observed by the addition of $MnSO_4$[47]. For acrylonitrile, no polymerization occurs[35] and the reaction was carried out at four different temperatures to determine the activation parameters. Reaction product has been recognized as analogous aldonic acid, which is confirmed by the formation of bright yellow colour with corresponding reagents. From the observed kinetic results the following suitable mechanism has been proposed[49,51].



$$HCrO_4^{\ominus} + H^{\oplus} \underset{}{\overset{K_1}{\rightleftharpoons}} H_2CrO_4$$

$$H_2CrO_4 + \text{1,2-bis(4-pyridyl)ethane} \overset{K_2}{\rightleftharpoons} \underset{(C_1)}{\text{Complex}}$$

$$\underset{(C_1)}{\text{Complex}} + [S] \xrightarrow[\text{slow}]{k_3} \underset{(C_2)}{\text{Complex}}$$

$$\underset{(C_2)}{\text{Complex}} \xrightarrow{\text{fast}} \text{Cr(VI)-(1,2-bis(4-pyridyl)ethane)} + \text{Intermediate} \longrightarrow \text{Products}$$

The rate law is as follows,

$$\boxed{\text{r} = \text{Rate law} = \frac{K_1K_2k_3[\text{S}][\text{H}^+][\text{Cr(VI)}][\text{1,2 - bis(4 - pyridyl)ethane}]}{1 + k_3\,[\text{substrate}]}}$$

In the first step, $HCrO_4^-$ protonates to given H_2CrO_4 in an equilibrium step[49,51]. In the second step complex (C_1) formed from the association of H_2CrO_4 and 1,2-bis(4-pyridyl)ethane. Further, the complex (C_1) reacts with substrate molecule to yield a complex (C_2), which is disproportionate to given the product in the final step[49, 51]

4. CONCLUSION

In conclusion, after D-Lactose was oxidised by TriMAFC in an aqueous acetic acid medium at 308k.The reaction is 1,2-bis(4-pridyl)ethane catalysed and exhibited first order dependence with respect to the oxidant, H^+ and catalyst then followed fractional order respect to substrate in concentration. The product of D-lactose is Lactobionic acid (LBA), is a high-value added chemical and the Acrylonitrile is not induced by the polymerization reaction, which showed that there is no free radical route. Added Mn^{2+} increased with slightly increase of reaction rate .In the case of 1,2-bis(4-pridyl)ethane, the oxidation reaction has been conducted at four different temperature and the activation parameters calculated from the Eyring's plot .

5. APPLICATIONS OF PRODUCT

- Kinetic of oxidation reaction is much more interest in the chemical research and industrial application.
- The main commercial use of LBA is a constituent of the solutions employed for stabilizing organs earlier to Transplanation.
- LBA is also known as a chelating agent, it can be useful in the fortification of food products with respect to the mineral content.
- The food industry has great interest or attention to the use of Lactobionic Acid as a food additive in the last years as calcium carrier, efficient acidifier in dairy products that need a gelation step.
- LBA was proved to be a reliable water retention agent (WRA) in meat based products and it is considered an antioxidant chelator..
- LBA is used as an ingredient in an organ preservative in solution.
- LBA and its salts are mainly used in high-quality speciality products.
- Mineral salts formed by LBA

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7. AUTHOR'S CONTRIBUTION STATEMENT

All Authors equally worked to the manuscript, collection of references as well as interpretation of results. All authors contributed equally in whole investigation processes

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