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KINETICS OF OXIDATION OF SOME DISACCHARIDES IN AMMONIACAL MEDIUM

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Abstract—Kinetics of oxidation of lactose, maltose, cellobiose and melibiose has been studied by hexacyanoferrate(III) in the presence of ammonia. A general mechanism involving the formation of intermediate enodiol anion has been suggested. The effects of dielectric constant and salts have been studied in detail. The oxidation products have been characterised by descending paper chromatography. Compensation effects have been observed.

INTRODUCTION

The kinetics of oxidation of reducing saccharides has been studied previously in strong alkaline medium by Conant[1] and Singh *et al.*[2-5] by using hexacyanoferrate(III) as an oxidant. A single scheme governing the oxidation as well as the interconversion of reducing sugars through a comon intermediate 1,2-enediol has been suggested. The results have been later confirmed by Lambert[6] and Wiberg.[7] However, resin formation, degradation, polymerisation, lack of product analysis and influence of solvent are some limitations in such systems. The present paper deals with the kinetics of oxidation of some disaccharides, i.e. lactose, maltose, melibiose and cellobiose by hexacyanoferrate(III) in the presence of ammonia.

RESULTS AND DISCUSSION

The reactions have been studied with respect to $K_3Fe(CN)_6$. The standard zero-order velocity constant K_s has been calculated as:

$$K_s = \frac{K_0 XS}{V}; \quad K_0 = \frac{\Delta x}{\Delta t}.$$

Where S is the strength of hyposolution and V is the volume of aliquot taken. The collected data (Tables 1-3) indicate that the rate of reaction is independent of $Fe(CN)_6^{3-}$ ions and directly proportional to the concentrations of disaccharides and the square root of ammonia concentration. The initial presence of $K_4Fe(CN)_6$ has no effect on the rate of oxidation. However, on addition of NH_4Cl the reaction rate retards due to the common ion effect, showing thereby that reaction is generally base-catalysed in nature. Further the reactions have been studied by varying NH_4Cl at fixed pH or ratio of $[NH_3]/[NH_4cl]$. An increase in the rate constants due to increase in the concentration of undissociated ammonia has been observed.[8] The total rate is governed by the equation

$$K_s = K_{OH^-}\cdot[OH^-] + K_{NH_3}\cdot[NH_3].$$

Where K_{OH^-} and K_{NH_3} are the catalytic constants of $[OH^-]$ and $[NH_3]$ and have been calculated from the intercepts and slopes of the linear plots between K_s vs $[NH_3]$.

Further, reactions have been studied at different ionic strength (by adding KCl) and varying dielectric constant of the medium which has been maintained by varying the volume percentage of methanol in methanol-water mixture. The data has been summarized in Tables 4 and 5.

To predict the effect of dielectric constant and ionic strength on ion-dipolar molecule reactions, the two different equations have been derived by Laidler and Eyring[9] and Amis and Jaffé.[10] In the reactions between dipolar molecules (disaccharides) and negative ion, $[Fe(CN)_6^{3-}]$, the reaction rate decreases (Table 4) with the decrease in dielectric constant and increases with the increase (Table 5) in ionic strength. The observed linear plots (Fig. 1) between $\log K_s$ vs μ with deviation at low ionic strength due to complication of secondary salt effect (Fig. 2) are in agreement with the results predicted by Laidler and Eyring. However, the linear plots (Fig. 3) between $\log K_s$ vs $1/D$ with negative slopes in contrast to

Table 1. Effect of $[K_3Fe(CN)_6]$ on the reaction rate

[Lactose] = 2.50×10^{-2}M; $[NH_3] = 20.00 \times 10^2$M; Temp = 35°C

$[K_3\,Fe(CN)_6]$ $M \times 10^3$	μ M	$K_s \times 10^5$ mol lit^{-1} min^{-1}
12.50	.08	3.153
5.00	.08	3.000
3.33	.08	2.974
2.50	.08	2.974

Table 2. Effect of [substrate] on the reaction rate $[K_3Fe(CN)_6] = 2.50 \times 10^{-3}$ M; $[NH_3] = 20.00 \times 10^{-2}$ M

[Lactose] M$\times 10^2$	Ks x 10^5 mol lt^{-1}min^{-1}	$\frac{Ks \times 10^4}{[Lactose]}$	Temperature
1.00	0.641	6.41	30°C
1.25	0.800	6.40	
1.66	1.080	6.48	
2.50	1.609	6.43	
5.00	3.244	6.48	
1.00	2.068	20.68	40°C
1.25	2.592	20.73	
1.66	3.460	20.76	
2.50	5.172	20.68	
5.00	10.369	20.73	
1.00	3.853	38.53	45°C
1.25	4.796	38.32	
1.66	6.360	38.16	
2.50	9.512	38.04	
5.00	12.024	38.04	

Table 3. Effect of $[NH_3]$ on the reaction rate $[K_3Fe(CN)_6] = 2.50 \times 10^{-3}$ M; [Melibiose] = 1.66×10^{-2} M, Temp. 35°C

$[NH_3]$ M x 10^2	Ks x 10^5 mol lt^{-1} min^{-1}	$\frac{Ks \times 10^5}{[NH_3]^{1/2}}$
33.33	4.940	8.543
25.00	4.240	8.480
20.00	3.820	8.547
10.00	2.692	8.512
8.33	2.430	8.420
6.66	2.180	8.447

Table 4. Effect of dielectric constant on the rate constant $[K_3Fe(CN)_6] = 2.50 \times 10^{-3}$ M; $[NH_3] = 20.00 \times 10^{-2}$ M; Temp. 40°C [Lactose] = 2.50×10^{-2} M; $\mu = 0.015$ M

Percentage of Methanol by Vol.	D	$\frac{1}{D}$ x 10^2	K_s x 10^5 mol lt^{-1} min^{-1}
0	73.38	1.3627	5.172
5	72.77	1.3741	4.365
10	70.83	1.4118	3.515
15	69.21	1.4448	2.804
20	67.82	1.4744	2.429

Eyring's observation are due to the fact that the ionisation constant[11] of ammonia varies as 1/D.

Compensation effect

The reactions have been studied at four different temperatures. The activation parameters have been calculated from the expression:

$$K_r = \exp\frac{KT}{h}\exp(\Delta S/R)\exp(-\Delta E/RT)$$

where K_r is the specific rate constant, ΔS is the entropy

Table 5. Effect of dielectric constant and ionic strength on the rate constant $[K_3Fe(CN)_6] = 2.50 \times 10^{-3}$ M; $[NH_3] = 20.00 \times 10^{-2}$ M; Temp. 35°C [Lactose] = [Maltose] = 2.50×10^{-2} M

Percentage of methanol by vol.	D	μ M	$K_s \times 10^5$ mol $lit^{-1} min^{-1}$ for	
			Lactose	Maltose
0	76.03	0.015	2.916	3.037
5	73.93	0.015	2.473	3.093
I		0.100	2.631	3.363
		0,500	3.078	4.204
		0.800	3.157	4.681
		1.000	3.263	5.090
		1.500	3.578	5.977
10	72.33	0.015	1.973	2.606
II		0.100	2.078	3.181
		0.500	2.657	3.909
		0.800	2.763	4.272
		1.000	2.973	4.659
		1.500	3.315	5.590
15	71.23	0.015	1.763	2.426
III		0.100	1.815	2.750
		0.500	2.421	3.502
		0.800	2.605	4.045
		1.000	2.736	4.295
		1.500	3.131	5.227
20	69.59	0.015	1.394	2.089
IV		0.100	1.526	2.431
		0.500	2.026	3.136
		0.800	2.289	3.659
		1.000	2.473	3.909
		1.500	2.789	4.863

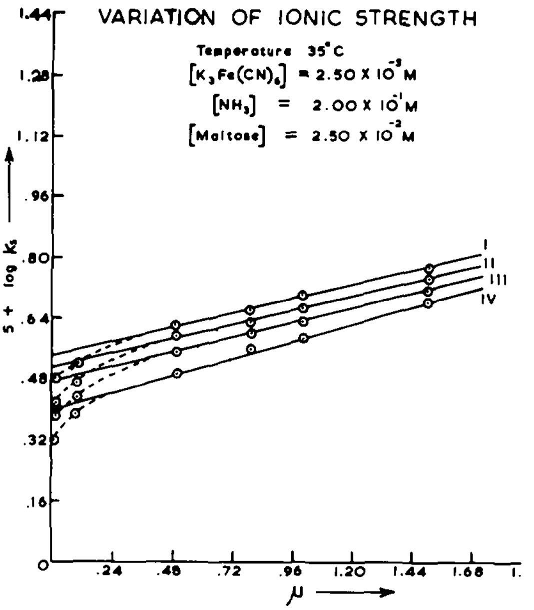


Fig. 1.

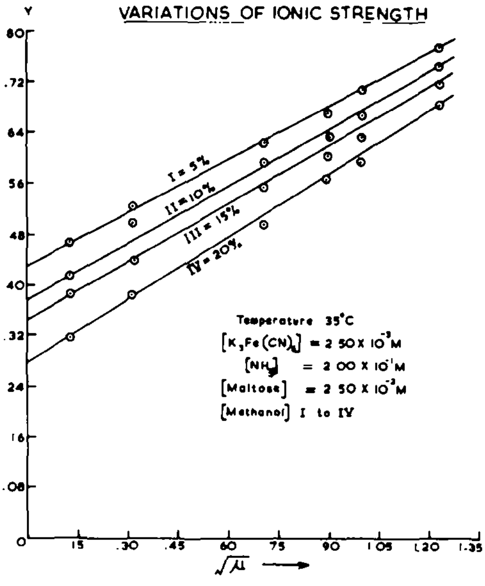


Fig. 2.

of activation and ΔE is the energy of activation calculated from $\log K_s$ vs 1/T plots. The results have been summarized in Table 6. The negative entropy of activation indicates that the activation complex is more polar and solvated. The free energy of activation changes are much smaller in comparison to their velocity constants. The linear plots between T · ΔS vs ΔE of different disaccharides (Fig. 4) with unit slopes indicate the presence of a compensation effect.

A possible reason is that there is a general tendency in

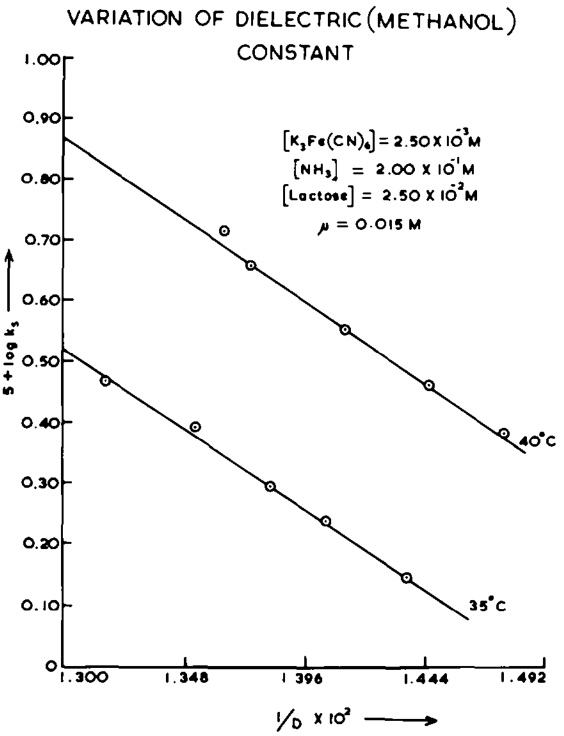


Fig. 3.

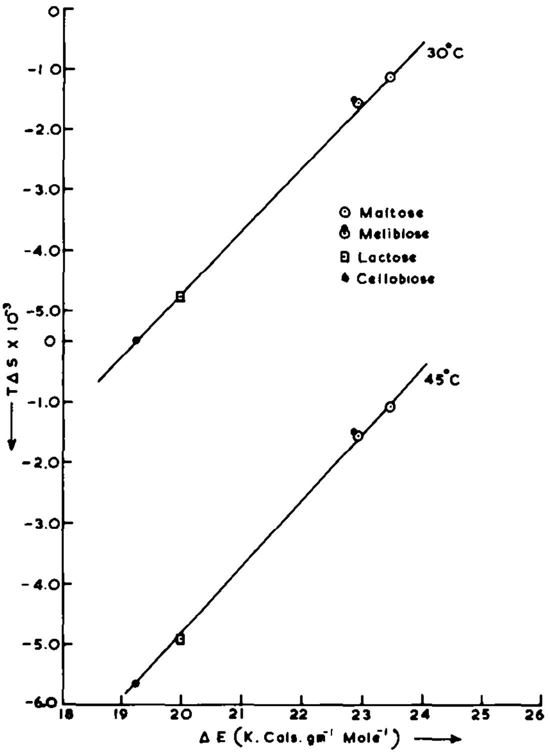


Fig. 4.

Table 6. Table showing activation parameters $[K_3Fe(CN)_6] = 2.50 \times 10^{-3}$ M; $[NH_3] = 20.00 \times 10^{-2}$ M; [Disaccharide] $= 1.66 \times 10^{-2}$ M

Parameters	[Lactose]	[Maltose]	[Melibiose]	[Cellobiose]	Temperature
Kr (lit mol^{-1}sec^{-1})	2.415×10^{-5}	3.236×10^{-5}	4.180×10^{-5}	2.973×10^{-5}	30°C
	14.221×10^{-5}	22.817×10^{-5}	29.338×10^{-5}	14.645×10^{-5}	45°C
ΔE (Cals./gm mol)	19980	23450	22890	19240	
A (C.C. mol^{-1}sec^{-1})	6.312×10^{9}	2.698×10^{12}	1.374×10^{12}	2.271×10^{9}	30°C
	7.766×10^{9}	3.029×10^{12}	1.605×10^{12}	2.478×10^{9}	45°C
ΔS (E. U.)	-15.708	-3.673	-5.013	-18.054	30°C
	-15.392	-3.451	-4.802	-17.661	45°C
ΔF Cals/gm mol	24739	24563	24409	24710	30°C
	24874	24547	24417	24856	45°C

$$K_r = K_1K_b^1; \quad K_s = K_1K_b^1[DS]\,[NH_3]^{\frac{1}{2}}$$

solution for heats and entropies to compensate one other.[12] Observance of the compensation effect in these disaccharides is due to substitution (linking) of D-glucose or D-galactose by α- or β-glycosidic linkage to D-glucose unit. As a result of this linkage only one reducing group is free (D-glucose) in each case.[13] The comparison of the oxidation rates indicates that rate of oxidation are in the order

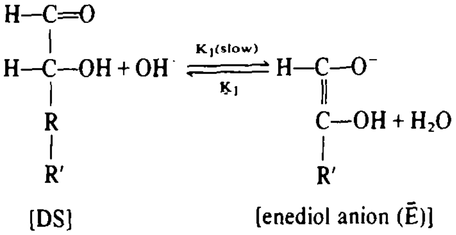
melibiose > maltose > cellobiose > lactose.

Further, there occurs no resin formation and rapid transformation of reducing disaccharide during the process of oxidation.

Mechanism of oxidation. From the discussion it appears that the first step involves action between a disaccharide molecule and OH^- ions furnished by ammonia and forms an active intermediate enediol anion which is subsequently oxidised by $Fe(CN)_6^{3-}$. The latter being the faster process so the reaction becomes zero-order with respect to $Fe(CN)_6^{3-}$ ions.

$$NH_3 + H_2O \overset{K_b}{\rightleftharpoons} OH^- + NH_4^+$$

$$[OH^-] = K_b^1[NH_3]^{1/2}$$



$$\begin{array}{c}H-C=O\\ |\\ H-C-OH\\ |\\ R\\ |\\ R'\end{array} + OH^- \underset{K_1}{\overset{K_1(slow)}{\rightleftharpoons}} \begin{array}{c}H-C-O^-\\ \|\\ C-OH\\ |\\ R'\end{array} + H_2O$$

[DS] [enediol anion ($\bar{E}$)]

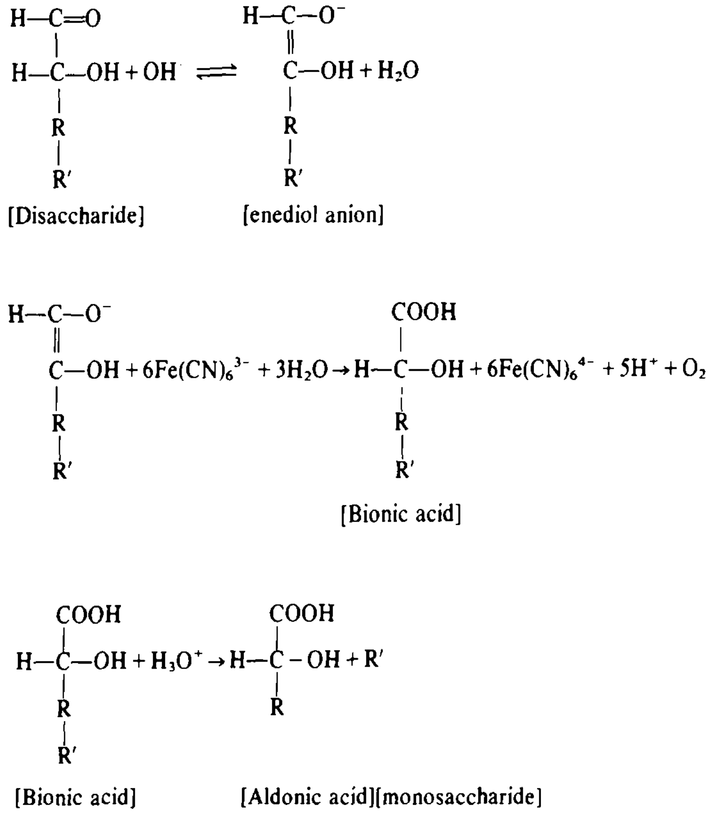
$$[\bar{E}] + Fe(CN)_6^{3-} \xrightarrow[(fast)]{K_2} Fe(CN)_6^{4-} + \text{other reaction products.}$$

Applying steady state condition for $\bar{E}$ and in equality of $K_1[H_2O] \ll K_2$ the final rate expression comes out to be:

$$-\frac{d[Fe(CN)_6^{3-}]}{dt} = K_1[DS][OH^-] = K_1K_b^1[DS][NH_3]^{1/2}.$$

The derived rate expression fully satisfies our experimental findings.

Product analysis. The reaction mixtures in which $[Fe(CN)_6^{3-}]$ and ammonia were present in large excess as compared to disaccharides were boiled for 5–10 min on water bath and the number of equivalents were determined by estimating produced $[Fe(CN)_6^{4-}]$ equivalent to disaccharides. It was observed that one equivalent of disaccharides requires 4–6 moles of $[Fe(CN)_6^{3-}]$ for its complete oxidation. The stoichiometric equation can be shown as:



$$\begin{array}{c}H-C=O\\ |\\ H-C-OH\\ |\\ R\\ |\\ R'\end{array} + OH^- \rightleftharpoons \begin{array}{c}H-C-O^-\\ \|\\ C-OH\\ |\\ R\\ |\\ R'\end{array} + H_2O \qquad (1)$$

[Disaccharide] [enediol anion]

$$\begin{array}{c}H-C-O^-\\ \|\\ C-OH\\ |\\ R\\ |\\ R'\end{array} + 6Fe(CN)_6^{3-} + 3H_2O \rightarrow \begin{array}{c}COOH\\ |\\ H-C-OH\\ |\\ R\\ |\\ R'\end{array} + 6Fe(CN)_6^{4-} + 5H^+ + O_2$$

[Bionic acid]

$$\begin{array}{c}COOH\\ |\\ H-C-OH\\ |\\ R\\ |\\ R'\end{array} + H_3O^+ \rightarrow \begin{array}{c}COOH\\ |\\ H-C-OH\\ |\\ R\end{array} + R'$$

[Bionic acid] [Aldonic acid][monosaccharide]

Table 7. R_f values of sugar acids at raom temperature

Solvents	(1)	(2)	(3)
Development time in hrs.(descending)	22 (Rfx10 cm)	72 (Rf x 10 cm.)	120 (Rf x 10 cm)
R. Mix. of Lactose	3.64	3.05	2.70
R. mix. of Maltose	3.64	3.05	—
R. mix. of Melibiose	3.64	3.05	—
R. mix. of Cellobiose	3.64	3.05	2.70
Glucuronic acid	4.05	—	—
Gluconic acid	3.64	3.05	2.70
Galacturonic acid	4.46	—	—
Formic acid	—	2.29	—
Acetic Acid	—	2.54	—

Solvents (1) Methyl-Ethyl Ketone saturated with H_2O
(2) n - Butanol saturated with (1.4N)NH_3
(3) n - Butanol : Acetic acid : H_2O (4:1:5)

Table 8. R_f values of monosaccharides at room temperature

Solvents	(1)	(2)
Development time in hrs (descending)	24 (Rf x 10 cm.)	65 (Rf. x 10 cm)
R. mix of Lactose	2.30	3.39
R. mix. of Maltose	2.02	3.05
R. mix. of Melibiose	2.30	3.39
R. mix. of Cellobiose	2.02	3.05
D-glucose	2.02	3.05
D-galctose	2.30	3.39

Solvents (1) Methyl-Ethyl Ketone saturated with H_2O
(2) n-Butanol : Toluene(2:1)

The produced bionic acids are unstable and are hydrolysed soon after the oxidation process and are converted into aldonic acid (gluconic acid) and the attached non reducing unit into a monosaccharide. The liberated O_2 goes into the atmosphere. However, the possibility of subsequent breaking of the glycoside linkage and oxidation of R′ should not be ruled out.

Lactobionic acid + $H_3O^+ \rightarrow$ Gluconic acid + galactose
Maltobionic acid + $H_3O^+ \rightarrow$ Gluconic acid + glucose
Melibionic acid + $H_3O^+ \rightarrow$ Gluconic acid + galactose
Cellobionic acid + $H_3O^+ \rightarrow$ Gluconic acid + glucose

The existence of the gluconic acid as one of the oxidation products has been confirmed by descending paper chromatography. The same reaction mixtures as taken for kinetic studies were made acidic and $Fe(CN)_6^{4-}$ was removed by adding ZnO at 70–80°. The filtrate was again made alkaline by adding aqueous NH_3 such as to maintain pH between 7–7.5 and evaporated to a small volume was used for the spot tests. The identification of the spots were made from their positions on the chromatogram by spraying bromo cresol-green[14] or bromophenol-blue.[14] The comparison of the R_f values of acids in different solvents at a different time (Table 7) confirms that in each case gluconic acid is formed.

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