Tetrahedron, 1967, Vol. 23, pp. 1189 to 1196. Pergamon Press Ltd. Printed in Northern Ireland

KINETICS AND MECHANISM OF OXIDATION OF SOME DISACCHARIDES—MELIBIOSE, CELLOBIOSE, LACTOSE AND MALTOSE—BY HEXACYANOFERRATE (III) IN ALKALINE MEDIUM

R. K. SRIVASTAVA, N. NATH and M. P. SINGH
Chemistry Department, University of Allahabad, Allahabad
India

(*Received 24 November 1965; accepted for publication 3 August 1966*)

Abstract—The kinetics of oxidation of four disaccharides, namely melibiose, cellobiose, lactose and maltose, have been carried out in alkaline ferricyanide. The order of reaction is zero with respect to ferricyanide and first with respect to reducing sugar. The reaction is base catalysed. The rate expression proposed is

$$-\frac{d[Fe(CN)_6]^{-3}}{dt} = k[OH^-]\,[\text{Reducing sugar}]$$

The rates of oxidation of disaccharides follow the order: melibiose > maltose > cellobiose > lactose. The compensating effect between heat and entropy of activation has also been observed.

THE kinetics of the oxidation of reducing sugars have been studied using halogens[1] in alkaline and acidic media. In the kinetics of oxidation of simple monosaccharides as well as disaccharides[2] by alkaline hypoiodide, the order of the reaction with respect to each reactant was found to be unity, that is, the total order of the reaction is two.[2] In the oxidation of different anomeric pairs of aldoses with aqueous bromine,[4] it was found in general that β-forms react faster. It has also been reported[3] that under certain conditions (pH about 9·2), the β-D-form is oxidized approximately 28 times faster than α-D-anomer. However, Reeve[5] has reported that the rate of mutarotation of the free sugars is greatly enhanced in alkaline solutions, and, beyond the pH range of 11·8, becomes much faster than oxidation, the result being that under these circumstances, both forms are oxidized at identical rates. Prior to the present work, the kinetics of oxidation of some monosaccharides[6] (pentoses and hexoses) was studied with bivalent copper complexed with tartrate and citrate in presence of sodium hydroxide. The reaction being heterogeneous created some difficulty in the measurement of velocity constants. Recently, the kinetics of some aldo and keto

---

[1] *The halogen oxidation of simple carbohydrates* by John W. Green in *Advances in Carbohydrate Chemistry* Vol. 3. Academic Press, New York (1948).

[2] O. G. Ingels and E. C. Israel, *J. Chem. Soc.* 810 (1948).

[3] H. S. Isbell and W. W. Pigman, *J. Research Natl. Bur. Standards* **10**, 337 (1933); *Ibid.* **18**, 141 (1937).

[4] *Formation and Cleavage of the oxygen rings in sugars* by F. Shafizadeh in *Advances in Carbohydrate Chemistry* Vol. 13, Academic Press, New York (1958).

[5] K. D. Reeve, *J. Chem. Soc.* 172 (1951).

[6] *a* M. P. Singh, B. Krishna and S. Ghosh, *Proc. Natl. Acad. Sci. India* **A28**, Part I, pp. 21–29 (1959), *b*. M. P. Singh, B. Krishna and S. Ghosh, *Z. Physik. Chem.* **204**, 1 (1955); *Ibid.* **208**, 265 (1958).

hexoses have been studied with alkaline ferricyanide.[7] The reaction in this case was homogeneous and thus the results obtained were more accurate. In both cases where bivalent copper and ferricyanide were taken as oxidants, the order of the reaction was found to be zero with respect to oxidant and unity with respect to the reducing sugar. Further, the reactions were base-catalysed.

The present paper deals with the kinetics of oxidation of melibiose, cellobiose, lactose and maltose with potassium ferricyanide in alkaline medium. The effect of previous treatment of reducing sugars with alkali on the reaction rate has also been studied in case of lactose and maltose.

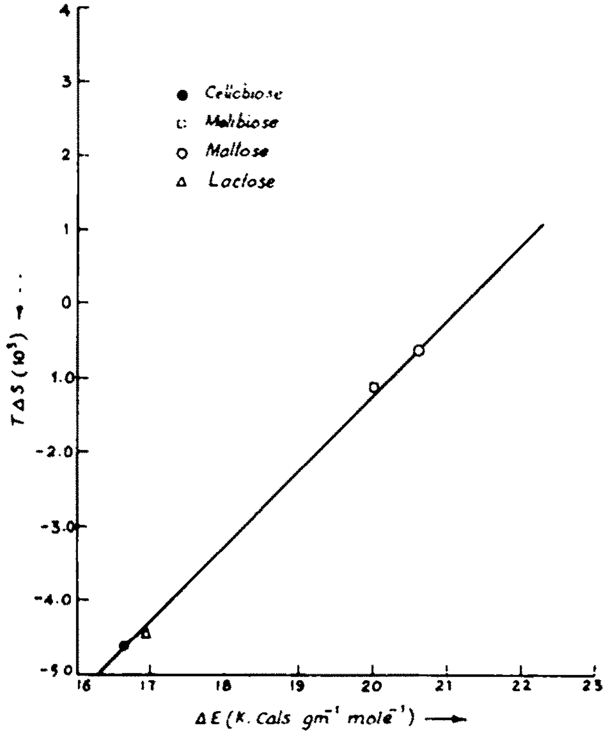


FIG. 1.

EXPERIMENTAL

The mixture of $K_3Fe(CN)_6$ and alkali was kept in a black coated Jena bottle maintained at constant temp. within ±0·1° by an electrically operated thermostat, and the fresh soln of reducing sugar was also kept separately in the same bath. The reaction was started by adding the sugar and the time was measured with a stop watch. Immediately, 5 ml of the aliquot was taken out and poured into a beaker containing 10 ml fresh soln of KI and 10 ml 4N H_2SO_4 (Analar). Then the remaining $K_3Fe(CN)_6$ was titrated iodometrically. The function of H_2SO_4 was to check the reaction by neutralizing the alkali, as well as to help in the liberation of I_2. All the samples used were of E. Merck grade except lactose, $K_3Fe(CN)_6$ and alkali which were of B.D.H. (Analar) grade.

Determination of the order of reaction with respect to ferricyanide

In order to determine the order of reaction with respect to ferricyanide ion, the concentration of the reducing sugar was kept high. If the concentration of the reducing sugar is in excess in respect

---

[7] *a* N. Nath and M. P. Singh, *Z. Physik. Chem.* **221**, 204 (1962); *Ibid.* **224**, 419 (1963), *b* N. Nath and M. P. Singh, *J. Phys. Chem.* **69**, 2038 (1965).

of the concentration of ferricyanide ion, the variation in the velocity of the reaction is determined by the concentration of the latter.

TABLE 1

Temp 30°; total vol
100 ml; overall concn;
$K_3Fe(CN)_6$ 2·5 × 10^{-3}M
NaOH 6·6 × 10^{-2}N
Melibiose 6·6 × 10^{-3}M

Time (min)	Vol of N/980 hypo (ml)	$k_0 \times 10^6 = \frac{\Delta x}{\Delta t} \times 10^6$
0	12·00	—
10	11·00	10·0
20	9·90	11·0
31	8·30	14·5
40	7·30	12·0
50	6·10	12·0
60	4·86	12·5
70	3·70	11·6
80	2·54	11·6
∞	0·00	—

Mean k_0 11·88 × 10^{-6}
k_2 2·42 × 10^{-4}
(mole l^{-1} min^{-1})

TABLE 2

Temp 35°; total vol
100 ml; overall concn;
$K_3Fe(CN)_6$ 2·5 × 10^{-3}M
NaOH 2·02 × 10^{-2}N
Cellobiose 2·5 × 10^{-3}M

Time (min)	Vol of N/960 hypo (ml)	$k_0 \times 10^6 = \frac{\Delta x}{\Delta t} \times 10^6$
0	11·10	—
5	10·40	14·0
10	9·70	14·0
20	8·20	15·0
30	6·75	14·5
40	5·30	14·5
50	3·90	14·0
60	2·55	13·5
∞	0·00	—

Mean k_0 14·2 × 10^{-6}
k_2 2·95 × 10^{-4}
(mole l^{-1} min^{-1})

TABLE 3

Temp 35°; total vol
100 ml; overall concn;
$K_3Fe(CN)_6$ 3·3 × 10^{-3}M
NaOH 2·0 × 10^{-2}N
lactose 1·0 × 10^{-3}M

Time (min)	Vol of N/950 hypo (ml)	$k_0 \times 10^6 = \frac{\Delta x}{\Delta t} \times 10^6$
0	14·6	--
5	12·6	40·0
10	10·5	42·0
15	8·2	46·0
20	5·9	46·0
251	3·6	46·0
30	1·3	46·0
∞	0·0	—

Mean k_0 44·3 × 10^{-6}

k_2 93·03 × 10^{-4}
(mole l^{-1} min^{-1})

TABLE 4

Temp 30°; total vol
100 ml; overall concn;
$K_3Fe(CN)_6$ 2·0 × 10^{-3}M
NaOH 6·6 × 10^{-2}N
maltose 6·6 × 10^{-3}M

Time (min)	Vol of N/870 hypo (ml)	$k_0 \times 10^6 = \frac{\Delta x}{\Delta t} \times 10^6$
0	8·50	—
10	7·90	6·0
20	7.00	9·0
30	6·00	10·0
40	5·06	9·4
50	4·14	9·2
60	3·20	9·4
70	2·24	9·6
∝	0·00	—

Mean k_0 9·43 × 10^{-6}
(Neglecting first constant)
k_2 21·60 × 10^{-4}
(mole l^{-1} min^{-1})

It was observed that one mole reducing sugar required six moles ferricyanide. Therefore, from Tables 1–4 it is obvious that the minimum ratio of the concentration of reducing sugar to ferricyanide is 6 (Table 2). In other Tables, the molar concentration of reducing sugar is more than 6 times the concentration of ferricyanide. So, the rate of the reaction is determined by the decrease in the concentration of ferricyanide ion. The Tables show that the zero order velocity constants are practically uniform having a slight induction period in some cases. Below each Table k_0 values have been calculated by multiplying k_0 by X/V, where X is the strength of the hypo and V the volume of aliquot taken. Further, order of the reaction with respect to ferricyanide can be confirmed from Table 5.

TABLE 5. EFFECT OF VARYING THE FERRICYANIDE ION CONCENTRATION
Total volume of reaction mixture 10 ml

Temp	Overall conc of sugar and alkali		Overall conc of Ferricyanide $\times 10^4$, M	Mean zero-order $k_0 \times 10^4$ (Mole l^{-1} min^{-1})
30°	Melibiose	$6{\cdot}6 \times 10^{-3}$M	20·00	2·33
	NaOH	$6{\cdot}6 \times 10^{-3}$N	25·00	2·42
			33·00	2·38
			50·00	2·45
35°	Cellobiose	$2{\cdot}5 \times 10^{-3}$M	20·00	2·89
	NaOH	$20{\cdot}20 \times 10^{-3}$N	25·00	2·95
			33·00	2·97
			50·00	2·91
			50·00	2·91
35°	Lactose	$1{\cdot}0 \times 10^{-3}$M	20·00	9·68
	NaOH	$2{\cdot}0 \times 10^{-3}$N	25·00	9·81
			33·00	9·68
			50·00	9·60
30°	Maltose	$6{\cdot}6 \times 10^{-3}$M	20·00	2·16
	NaOH	$6{\cdot}6 \times 10^{-3}$N	25·00	2·11
			33·00	2·12

Table 5 shows that the reaction rates are independent of ferricyanide ion concentration. This further confirms that the reaction is of zero order with respect to ferricyanide under the experimental conditions used.

From Table 6 it is obvious that the rate constant in case of each sugar, increases in direct proportion to the increase in concentration of reducing sugar. In other words the reaction is of first order with respect to reducing sugar.

From Table 7 it is clear that on dividing k_0 by the sodium hydroxide concentration, practically constant values have been obtained. This leads to the conclusion that the reaction rates are also directly proportional to hydroxyl ion concentration.

Effect of the previous treatment of lactose and maltose with sodium hydroxide on the reaction rate

The reactants (50 ml) containing alkali and lactose were kept in the thermostat for the period mentioned in Table 8 and then from another bottle 50 ml of $K_3Fe(CN)_6$, previously kept in the thermostat, were added to study the course of reaction. It is clear that during the treatment the conc. of alkali and sugar are twice those of the final concentration.

TABLE 6. EFFECT OF VARYING THE SUGAR CONCENTRATION
Total volume of the reaction mixture 100 ml; overall conc of
$K_3Fe(CN)_6$ 2·5 × 10^{-3}M

Temp	Overall conc of NaOH, N × 10^3	Overall conc of sugar × 10^4, M	$k_s \times 10^6$ (mole l^{-1} min^{-1})	$\frac{k_s}{[sugar]} \times 10^3$
	Melibiose			
30°	6·60	100·00	3·63	3·63
		50·00	1·80	3·60
		40·00	2·45	3·62
		33·00	1·22	3·66
	Cellobiose			
35°	20·00	66·00	7·62	11·43
		50·00	5·83	11·66
		33·00	3·76	11·28
		25·00	2·95	11·80
	Lactose			
35°	20·00	66·00	7·01	10·51
		50·00	5·11	10·22
		33·00	3·63	10·08
		25·00	2·52	10·08
	Maltose			
30°	6·60	200·00	6·07	3·03
		125·00	3·70	2·96
		66·00	2·11	3·16
		50·00	1·52	3·04

TABLE 7. EFFECT OF VARIATION OF HYDROXYL ION CONCENTRATION
Total volume of reaction mixture 100 ml; overall conc of
$K_3Fe(CN)_6$ 2·5 × 10^{-3}M

Temp	Overall conc of sugar, M × 10^3	Overall conc of NaOH × 10^4, N	$k_s \times 10^6$ (mole l^{-1} min^{-1})	$\frac{k_s}{[NaOH]} \times 10^3$
	Melibiose			
30°	6·60	100·00	3·53	3·53
		66·00	2·40	3·60
		50·00	1·81	3·62
		40·00	1·46	3·65
	Cellobiose			
35°	6·60	200·00	7·62	3·81
		125·00	5·37	4·21
		100·00	4·18	4·18
		66·00	2·90	4·35
	Lactose			
35°	6·60	200·00	7·01	3·50
		125·00	4·42	3·53
		100·00	3·51	3·51
		66·00	2·31	3·46
	Maltose			
30°	10·00	200·00	8·80	4·40
		125·00	5·52	4·41
		100·00	4·44	4·44
		66·00	3·07	4·60

TABLE 8
Temp 35°; total vol 100 ml; overall conc of
$K_3Fe(CN)_6$ 2·5 × 10^{-3}M

Overall conc of sugar and alkali		Time of treatment hr	Mean zero order $k_0 \times 10^6$ (mole l^{-1} min^{-1})
Lactose	5·0 × 10^{-3}M	0	5·58
		1	7·65
NaOH	20·0 × 10^{-3}N	2	10·71
		3	11·86
Maltose	5·0 × 10^{-3}M	0	7·34
		1	14·40
NaOH	20·0 × 10^{-3}N	2	17·90
		3	20·23

Table 8 shows that there is gradual increase in k_0 values with the time of treatment. This is due to the formation of keto sugars, lactulose[6a,b] and maltulose,[6c] during alkali treatment of lactose and maltose respectively. Since the rates of oxidation of these keto sugars are higher than aldehydo sugars, the enhancements in the reaction rates with time of treatment have been observed. The formation of these keto sugars during alkali treatment is due to Lobry de-Bruyn transformation,[7] according to which, when an aldehydo sugar is treated with alkali, an equilibrium between the keto sugar produced and aldehydo one is established. On the other hand in case of keto sugar after treatment with alkali, an equilibrium is established between the aldehydo sugar produced and the keto one taken.

Energy of activation, entropy and comparison in the rates of oxidation

Energy of activation has been calculated from the slope of Arrhenius linear plot of log k_0 vs $1/T$ and also the entropy of activation has been calculated by employing the equation.

$$k = e \cdot \frac{KT}{h} \times \exp(\Delta S/R) \exp(-\Delta E/RT)$$

where k is the reaction rate constant, K is Boltzmann constant and h is the Planck's constant. The results obtained are shown in Table 9.

TABLE 9

Sugars	k at 30° × 10^4 (l $mole^{-1}$ sec^{-1})	ΔS (E.U.)	Energy of activation (cal/mole)
Melibiose	84·76	−3·917	20,040
Cellobiose	70·35	−15·32	16,690
Lactose	61·5	−14·71	16,950
Maltose	79·125	−2·087	20,630

The values of T × ΔS have been plotted against ΔE and it gives a straight line of unit slope shown in Fig. 1. This indicates that there is a tendency for heat and entropy to compensate each other, so that change in free energy is much smaller. Such observations are not common in the literature.

[6a] E. M. Montgomery and C. S. Hudson, *J. Amer. Chem. Soc.* **52**, 2101 (1930);
[b] J. P. L. Bots, *Rect. Trav. Chem.* **76**, 515 (1957);
[c] S. Peat, P. J. P. Roberts and W. J. Whelan, *Biochem. J.*, 51, XVII (1952).

[7] *The Lobry de- Bruyn-Alberda van Ekenstein transformation* by John C. Speck. Jr. in *Advances in Carbohydrate Chemistry* Vol. 13; p. 63. Academic Press, New York (1958).

This is frequently a case for the given reaction investigated in a series of solvents, and also for homologous reaction in which substituents are introduced in the reactant.[10] Fairclough and Hinshelwood[11] have reported such compensated hydrolysis of ethyl benzoate in alcohol-water mixture. In the present work also the substituents are changing since in the case of disaccharides the reducing units are attached to non-reducing units of hexoses.

Further, in comparing the rates of oxidation of these disaccharides it has been observed that the rates of oxidation of melibiose, maltose and cellobiose are 1·40, 1·28 and 1·14 times the rate of oxidation of lactose. In other words the rates of oxidation of these disaccharides follow the order

melibiose > maltose > cellobiose > lactose

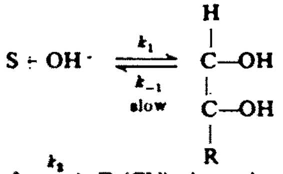
DISCUSSION

It is interesting to note that in the halogen oxidations of disaccharides in alkaline media, the total order of the reaction was found to be two, one with respect to each reactant.[8] But in the present oxidations of disaccharides by ferricyanide in alkaline medium, it is evident that the total order of the reaction is one, zero with respect to ferricyanide and one with respect to the reducing sugar. This difference arises since in the halogen oxidations, the sugars are oxidized directly to their corresponding acids as shown

$$\mathrm{RCHO + I_2 + 3NaOH \rightarrow RCOONa + 2NaI + 2H_2O}$$

while the present oxidation takes place through 1,2-enediol which is an intermediate product.

On the basis of the experimental results, it appears that the first step involves the reaction between the hydroxyl ion and the reducing sugar leading to the formation of an intermediate enediol which is subsequently oxidized by ferricyanide, the latter being a faster process, so that the reaction becomes zero order with respect to ferricyanide ion. Accordingly, the first step is the slow transformation of the sugar into the intermediate enediol.



$$\mathrm{S + OH^-} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \begin{array}{c}\mathrm{H}\\|\\\mathrm{C{-}OH}\\|\\\mathrm{C{-}OH}\\|\\\mathrm{R}\end{array} \quad \text{slow} \qquad (1)$$

$$\mathrm{en + Fe(CN)_6^{-3}} \xrightarrow[\text{fast}]{k_2} \mathrm{Fe(CN)_6^{-4}} + \text{other reaction products}$$

where S and en represent the reducing sugar (disaccharides) and intermediate enediol. Considering steady state, the rate expression is

$$-\frac{d[\mathrm{Fe(CN)_6^{-3}}]}{dt} = \frac{k_1k_2[\mathrm{S}][\mathrm{OH^-}][\mathrm{Fe(CN)_6^{-3}}]}{k_{-1} + k_2[\mathrm{Fe(CN)_6^{-3}}]}$$

Since the value of k_{-1} is very small in comparison to $k_2[\mathrm{Fe(CN)_6}]^{-3}$ it can be neglected. Therefore the final rate expression is

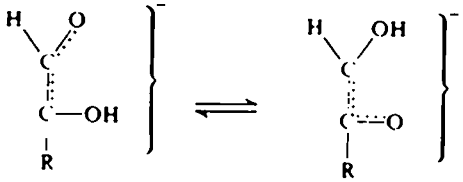
$$-\frac{d[\mathrm{Fe(CN)_6}]^{-3}}{dt} = k_1[\mathrm{S}][\mathrm{OH^-}]$$

---

[10] K. J. Laidler, *Reaction Kinetics* Vol. 2; p. 46, Pergamon Press, Oxford.

[11] R. A. Fairclough and C. N. Hinshelwood, *J. Chem. Soc.* 1573 (1937).

This shows that the reaction is of first order both with respect to reducing sugar and hydroxyl ion and independent of ferricyanide ion concentration. Bamford and Collins[12] have studied the kinetics of interconversion of glucose and fructose in strongly alkaline solution and have suggested the existence of two enolate ions in tautomeric equilibrium, instead of intermediate enediol as



It may also be noted that reaction rate is not affected by the initial presence of ferrocyanide ion.

---

[12] C. H. Bamford and J. R. Collins. *Proc. Roy. Soc.* **A204**, 85 (1950); **A228**, 100 (1955).