

CHAPTER - II
EXPERIMENTAL TECHNIQUES

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Oxidation of D-ribose and D-arabinose by alkaline potassium bromate solution has been studied here. The sugar (substrate), potassium bromate (oxidant) and potassium hydroxide in the requisite amounts were mixed in a reaction flask at constant temperature and an aliquot of the reaction mixture was analysed for the unused oxidant by the Iodometric method at definite time intervals. Various experiments were designed to cover the following studies.—

- (i) effect of variation of oxidant, sugar and KOH concentration on the rate of reaction and determination of order of the reaction with respect to each reactant and overall order of reaction,
- (ii) effect of variation in ionic strength of the solution on reaction rate,
- (iii) effect of temperature variation and to evaluate temperature coefficient, frequency factor and other thermodynamic parameters for the reaction,
- (iv) determination of mole ratio for oxidant and reductant,
- (v) Identification of end products of oxidation of sugars.

2.1 MATERIALS AND PURITY :

The double-distilled water was used throughout the work which was obtained by redistillation of distilled water in the presence of a few

crystals of KMnO_4 and a few pellets of KOH using corning glass distillation assembly.

All the chemicals used for the experiments were of AR grade. Pure samples of D-ribose and D-arabinose (Hi-media) were used without further purification after drying *in vacuo* for atleast four hours. Potassium bromate, potassium iodide, sodium thio sulphate, potassium chloride and potassium sulphate were from B.D.H. (A.R.) whereas potassium hydroxide and sulphuric acid were from E. Merck. Potassium chloride, potassium sulphate, potassium iodide, potassium hydroxide, sodium thiosulphate and sulphuric acid were used directly as received but potassium iodide was tested for absence of iodate impurity by adding dilute sulphuric acid and starch. Finely powdered potassium bromate was dried for 1-2 hours at 120°C and kept in a desiccator for cooling before use.

2.2 PREPARATION OF SOLUTIONS AND STANDARDISATION :

The double-distilled conductivity water was boiled for atleast 15 minutes and cooled in air-tight container and then immediately used for preparation of stock solutions. The stock solutions were further stored in air-tight polythene bottles. This was found to be very necessary to avoid contact of solutions with air.

The stock solutions used were of the following concentrations :

1. Sugar solution	1 M
2. Potassium bromate	7×10^{-3} M
3. Potassium hydroxide	1 M
4. Potassium chloride	1 M
5. Potassium sulphate	5×10^{-1} M
6. Potassium iodide	10 %
7. Sodium thiosulphate	7.5×10^{-2} N
8. Sulphuric acid	2 N

The 1 M stock solutions of KOH and KCl and 0.5 M solution of K_2SO_4 were prepared by exactly weighing the calculated quantities and dissolving them in double - distilled water. KOH was standardised against standard (1 M) oxalic acid solution using phenolphthalein as an indicator.

Standard (0.075 N) stock solution of sodium thiosulphate was prepared by dissolving calculated quantity of sodium thiosulphate pentahydrate in conductivity water. Few drops of chloroform were added to improve the stability of solution. Solution of requisite concentration (0.0015 N) was prepared as and when required.

Solutions of sugar (1 M), $KBrO_3$ ($7 \times 10^{-3}M$) and KI (10 %) were freshly prepared everyday by dissolving calculated amounts of these in double distilled water. The potassium bromate solution was standardized iodometrically⁶⁹. The 5 ml of the stock solution was diluted to 50 ml with double distilled water in a standard volumetric flask. From this diluted potassium bromate solution 5 ml solution was pipetted out in an iodine flask containing 10 ml of 2 N H_2SO_4 and 10 ml of 10 % KI solutions (Before adding KI solution to H_2SO_4 , nearly 0.2 gm $NaHCO_3$ was added to avoid air oxidation of KI). After two minutes the liberated iodine was titrated against 0.0015 N $Na_2S_2O_3$ solution using freshly prepared starch as an indicator. The stock solutions of sugars were always used for the experiments within 12 hours.

The stock solutions were kept in dark to prevent any photo-chemical deterioration.



2.3 METHOD OF FOLLOWING KINETICS :

The main features of the experimental method are as follows :

- i) The standard pyrex glasswares were used throughout the experimental work.
- ii) The temperature of the reaction mixture was adjusted and maintained by using auto-thermostated water-bath ($\pm 0.1^{\circ}\text{C}$).
- iii) The reaction was carried out in a stoppered conical flask (250 ml.) blackened from outside.
- iv) The reaction was carried out under the pseudo order condition where -
 $[\text{Sugar}] \gg [\text{KBrO}_3]$ and $[\text{KOH}] \gg [\text{KBrO}_3]$.
- v) The standard KOH solution was used to get the alkaline medium and the ionic strength of reaction medium was adjusted with standard solution of neutral salt (KCl).
- vi) The calculated quantity of standard solution of sugar was taken in one (250 ml) stoppered conical flask.

The calculated quantity of standard potassium hydroxide solution was taken in another stoppered conical flask.

The calculated quantities of standard KBrO_3 solution, KCl solution and double distilled water were taken in a third stoppered conical flask.

- vii) All the three conical flasks were thermostated for 15-20 minutes.

- viii) The reaction was initiated by adding the thermostated mixture of KOH, KBrO_3 and KCl to the thermostated sugar solution. The time of initiation of reaction, i.e., time of mixing the solutions was recorded as zero time.
- ix) Aliquots (5 ml) of the reaction mixture were pipetted out at definite time intervals (5 min.) in iodine flask containing 10 ml of 2N H_2SO_4 , ~ 0.2 gm NaHCO_3 and 10 ml of 10% KI solutions. A period of 2 minutes was found to be sufficient to decompose all the bromate left in solution unused. Hence, after two minutes, the liberated iodine was titrated against standard (0.0015 N) $\text{Na}_2\text{S}_2\text{O}_3$ solution using freshly prepared starch as an indicator.
- x) The reaction was usually followed upto 70% completion or more.
- xi) The titre values (Tt) so obtained were then subjected to various methods to determine the order and the specific reaction rate.

2.3.1 TEMPERATURE VARIATION :

In the preliminary work, experiments were carried out in order to decide the range of temperature, concentrations of potassium bromate, sugar and potassium hydroxide.

It was observed that the reaction was very slow at ordinary temperatures (25 to 35°C) and hence a higher temperature range (45 to 65°C) was employed. The temperature was kept constant during kinetic study of the reaction by means of a thermostat (Ultra - Thermostat , German) with $\pm 0.1^\circ\text{C}$ accuracy.

2.3.2 VARIATION IN CONCENTRATIONS :

It was observed that satisfactory results were not obtained at higher concentrations ($C > 0.001$ M) of potassium bromate solution, and rate of oxidation reaction was found to vary inversely as the potassium bromate concentration but at lower concentrations of potassium bromate solution the reaction rate was found to be nearly constant. Hence very low concentration of potassium bromate in the range 3×10^{-4} M to 11×10^{-4} M was employed. In case of sugar and alkali, however, the rate of reaction was found to be very low at low concentrations. Hence higher concentrations of sugar and alkali were employed to get consistent results. As such it was decided to elucidate the order of the reaction with respect to potassium bromate by Ostwald's isolation method by using concentrations of sugar and alkali at least 5-10 times greater than that of bromate. Hence sugar in the concentration range 0.005 M to 0.1 M and alkali in the range 0.025 M to 0.125 M were used. To maintain ionic strength (μ) constant at 0.2, requisite amounts of KCl solution were used.

2.3.3 VARIATION IN IONIC STRENGTH (μ) AND SALT EFFECT :

The effect of ionic strength on reaction rate was studied by varying concentration of potassium chloride at fixed concentrations of sugar, potassium bromate and alkali. The ionic strength was varied between 0.15 to 0.3. The experiments were repeated by replacing potassium chloride by potassium sulphate to study the specific ion effect.

The effect of variation in concentrations, variation in ionic strength and salt effect were studied at 60°C .

2.4.1 DETECTION OF REACTION INTERMEDIATES :

Tests were carried out to decide whether free radicals are formed during the course of the reaction. The reaction mixture was mixed with 1 ml of acrylonitrile and the mixture was kept at room temperature for one hour and tested for polymerization. Also, the reaction mixture was treated with allyl acetate to see if the reaction could be arrested by its presence.

2.4.2 IDENTIFICATION OF END PRODUCTS :

The final product of the oxidation of monosaccharide by potassium bromate was identified by paper-chromatographic technique as described by Iyengar, Puttaswamy, and Mahadevappa^{60(a,b)} for aldonic acids. Whatmann Paper No.1 was used for the purpose. A mixture of n-butanol, acetic acid and water (4:1:5) was used as a developing solvent and p-anisidine phosphate in ethanol⁷⁰ was used as the spotting reagent. In the reaction mixture, excess potassium bromate was used to get large proportion of the product and was concentrated to 30% before use. As a reference, the corresponding aldonic acid was prepared by the action of bromine water on the sugar at 25°C and the solution was used for chromatography.

The reaction mixture and the aldonic acid solution (prepared as above) were spotted very carefully side by side on a Whatmann Paper No.1 with the help of a micropipette. Pure sugar solution was also spotted for comparison. The spots were dried in air.

The solvent was prepared and placed in the developing jar about an hour before use. The walls of the jar were thoroughly wetted. The

spotted paper was suspended in the chamber by taking care that the paper was dipped only in the organic phase. The development was allowed to proceed until the solvent-front had advanced about 20 cm. The paper was then removed and dried in sun-light after marking the solvent-front boundary. The paper was sprayed with p-anisidine phosphate reagent and heated carefully in an oven at 95^o-100^oC. A violet spot for aldonic acid and a brown spot for pure sugar were developed within five minutes.

2.4.3 DETERMINATION OF MOLE RATIO :

To study the stoichiometry of the reaction, reaction mixtures containing excess of potassium bromate over sugar were allowed to equilibrate at 60^oC in potassium hydroxide solution of suitable concentration. However, in the present case it was difficult to arrive at some definite conclusion because of the following factors.

It was observed that :

- i) The reaction rate is adversely affected by higher concentration of oxidant to the extent that the reaction seems to be practically stopped.
- ii) Even at moderate concentration of oxidant, the titration reading in experimental run decreased initially, followed by an increase to regain almost the initial titration reading.

The bromate ion is reduced to bromide ion which in presence of H⁺ ions reacts with bromate ion to liberate bromine, which is also an oxidising agent. Further, it has been reported^{71,32(b)} that the redox reaction between an aldose and bromate ion in acidic medium is an Oscillation reaction.

Hence, it was not possible to determine the mole-ratio (Oxidant-reductant ratio) or the Stoichiometry of the reactions studied. Such a difficulty in establishing stoichiometry of the oxidation reaction has been reported earlier in acidic^{41(a)} as well as alkaline⁶⁶ medium.

The results obtained on the Kinetic studies of D-ribose and D-arabinose and the relevant discussion on these results is included in Chapter-III.