CHAPTER-II

MATERIALS AND METHODS EMPLOYED
SECTION : A

EXPERIMENTAL

Materials and Methods Employed:

The sugars, D-Glucose (Loba Chemie), D-Fructose (Alfa Laboratory-Reagents), D-Mannose (Sisco Research Laboratory) D-Galactose (Loba Chemie), L-Sorbose (Sigma) were used. Quinolinium chlorochromate, \( \text{C}_9\text{H}_7\text{N}^+\text{HCrO}_4^-\text{Cl} \), was synthesised as reported in literature\(^{50}\). Acetic acid and perchloric acid used were of A.R. quality. All other materials employed were of the highest purity grade.

Synthesis of QCC Reagent:

Quinolinium chlorochromate \( \text{C}_9\text{H}_7\text{N}^+\text{HCrO}_4^-\text{Cl} \), can be easily and safely prepared from quinoline (Loba Chemie), 6M aqueous hydrochloric acid and chromium(VI) oxide (Sarabhai M. Chemical) in a molar ratio of 1:1:1:1. The procedure followed is as under:

\( \text{CrO}_3 \) (100g, 1 mole) was added to 6M HCl (184 mL, 1.1 mole) rapidly with stirring. After 5 min, the homogeneous solution was obtained. The solution was cooled to 0\(^\circ\)C and quinoline (118 mL, 1 mole) was carefully added with constant stirring over a period of 10 minutes. Recooling to 0\(^\circ\)C gave a yellow orange solid which was collected on a sintered glass funnel and dried for 4 hrs in vacuo. The solid can be stored for extended periods at room temperature without change (decomposition)\(^{50}\).
Conductivity water:

Conductivity water was prepared by the following method: Ordinary water was distilled first with alkaline potassium permanganate. This sample of water was further purified by distillation. The doubly distilled water thus prepared was stored in stoppered bottles and was used to prepare all aqueous solutions used in kinetic measurements.

Preparation of solvent:

Solvent of required composition was prepared by mixing the calculated volume of A.R. acetic acid with distilled water.

Preparation of QCC solution:

Stock solution of quinolinium chlorochromate was prepared by dissolving known weight of the reagent in 50% aqueous acetic acid. The strength of the solution was determined and checked from time to time by titration against a standard solution of ferrous ammonium sulphate using diphenylamine as an indicator. Necessary dilutions were made from the stock solution for the kinetic runs:

Preparation of substrate solutions:

The standard solution of substrates were prepared always fresh by weighing the required amount of the substrate and dissolving in 50% (v/v) aqueous acetic acid in the standard measuring flask. The working solutions were prepared by dilution as and when required.

Perchloric acid:

60% A.R. perchloric acid reagent was used throughout the investigation.
Sodium perchlorate:

Sodium perchlorate was prepared by neutralising 60% A.R. perchloric acid with sodium hydroxide (B.D.H.). The solution was concentrated when crystals of sodium perchlorate were obtained. The crystals were filtered and recrystallised from water. The recrystalised product was dried over silica gel under vacuum. This sample was used for the preparation of stock solution which was employed for maintaining the ionic strength of the medium and also for the study of effect of ionic strength on the rate of reaction.

Preparation of standard solution of $\text{K}_2\text{Cr}_2\text{O}_7$:

Standard solution of $\text{K}_2\text{Cr}_2\text{O}_7$ was prepared by dissolving the requisite amount of A.R. $\text{K}_2\text{Cr}_2\text{O}_7$ in doubly distilled water.

Preparation of ferrous ammonium sulphate solution:

Ferrous ammonium sulphate solution of desired strength was prepared by dissolving requisite amount of A.R. sample of ferrous ammonium sulphate in distilled water. A small quantity of sulphuric acid was added to prevent hydrolysis. The solution was then made up to the mark in the standard measuring flask. This solution was then standardized by a standard $\text{K}_2\text{Cr}_2\text{O}_7$ solution using diphenylamine as an indicator.

Kinetic Method:

All the measuring flasks and reaction vessels were of corning glass with well glass stopper. The flasks, reaction vessels, the pipettes and the burettes used were of standard quality. The glasswares used were
pretreated with chromic acid and rinsed with doubly distilled water before their use.

An electrically operated thermostatic water bath was used. It was provided with sufficient thermal lagging, suitable heaters and stirrers with proper water circulation for continuous work. Contact thermometer (Jumo, D.P.B. German) worked in conjugation with an electric relay maintained at the required temperatures accurately with a precision of ± 0.1°C. The temperatures were recorded by means of an accurate and sensitive thermometer, reading to tenths of a degree celsius.

**Spectrophotometers:**

For absorption measurements, the spectrophotometers used were:

(a) The PE-3B (Perkin-Elmer) double beam UV-Visible spectrophotometer.

(b) Systronics spectrophotometer type No.106.

(a) The PE-3B (Perkin-Elmer) double beam UV-visible spectrophotometer was a single monochromator, having a filter grating of 1200 lines/mm and a wavelength range from 190 to 900 nm. The absorbance value was displayed directly on the digital display and on the recorder. Photometric linearity was checked over the range of concentrations of K₂CrO₄ in KOH solution, as specified in the instruction manual, so as to verify Beer's law at 370nm.

(b) Systronics spectrophotometer model 106 was a single beam 600 lines/mm grating spectrophotometer with a wavelength range of 340 nm to 960 nm. It gave a constant spectral bandwidth of 10 nm over the entire wavelength range. Instrument having 6 filters was selected as
under depending upon the wavelength and provided four digit readout for measurements, as selected by mode switch. The light source was a straight filament tungsten iodide lamp by a regular power supply. The spectrophotometer was connected to the mains through an external voltage stabilizer to minimise the errors in the measurement of absorbance due to fluctuations in voltage and to ensure maximum sensitivity of the instrument. The instrument was calibrated as specified in the instruction manual over the range of concentrations of $\text{K}_2\text{CrO}_4$ in KOH solution so as to verify Beer's law at 370 nm.

**Cuvette:**

Systronics spectrophotometer 106 supplied with 10mm path length rectangular cuvettes. Quartz cuvette of 5 ml capacity was used for spectral determination with the PE-3B double beam UV-visible spectrophotometer (Perkin-Elmer). All the cuvettes used were thoroughly cleaned by aqueous ethanol and acetone and dried before the use for spectral measurements. After the transfer of the solution of cuvette, precaution was taken to see that no solution adhered to the outer surface of the cuvette.

**Location of absorption maxima:**

Solution of quinolinium chlorochromate in acetic acid-water mixture (50% v/v) was prepared. The absorbance of the solution was scanned over the range of wavelength from 400 nm to 600 nm. The maximum absorbance, $\lambda_{\text{max}}$ was located at 440 nm. At this wavelength of
440 nm the maximum absorption due to QCC has been observed. Fig 1 shows the absorption spectrum of QCC.

**FIG.1 : PLOT OF ABSORBANCE AGAINST WAVELENGTH (nm)**

**Verification of Beer’s law:**

At 440 nm, Beer’s law was obeyed over the range of concentrations of solution used. A typical linear graph of the absorbance against concentrations of QCC is shown in Fig 2.

**FIG.2 : PLOT OF [QCC] AGAINST ABSORBANCE**

In this investigation, all the absorbance were measured at a wavelength of 440nm.
Kinetic measurements:

The reactions were carried out under pseudo-first-order conditions by maintaining always a large excess of the substrate concentration over that of the oxidant [QCC]. The solvent used was 50% (v/v) aqueous acetic acid. Reaction mixture containing requisite quantities of substrate, perchloric acid and sodium perchlorate in 50% (v/v) aqueous acetic acid were taken in 100 ml well stoppered flask. In another flask QCC solution was taken. Both the flasks containing reactant solution were half dipped in thermostat maintained at desired temperature. When both the solution attained the required temperature of the bath, the requisite volume of QCC solution was added to the reaction mixture with a standard rapid delivery pipette in order to start the reaction. This was thoroughly mixed and a portion of the solution was quickly transferred to the absorption cell (cuvette). The course of the reaction was followed spectrophotometrically by monitoring the decrease in the absorption of QCC at 440 nm in systronics 106 spectrophotometer, at regular time interval. At this wavelength maximum absorption due to QCC has been observed. No other reactant or product has any significant absorption at this wavelength. The rate constants were evaluated from the equation (1) given in section-C. The reaction mixtures remained homogeneous throughout in the solvent system used.
SECTION : B

OXIDATION OF MONOSACCHARIDES BY QCC:

Prior to the detailed kinetic investigations, preliminary experiments were carried to find out the suitable concentration for the reactants so that rate becomes measurable at the temperature to be investigated. The reactions were studied under the condition, [substrate]>>[oxidant] throughout the investigations. The studies have been carried out at 303K (± 0.1K) unless mentioned otherwise. At this temperature, the rate of oxidation of monosaccharides by QCC is very slow. In the present work, however, it was found that perchloric acid catalyses the oxidation of monosaccharides by QCC.

The oxidation of different substrates by QCC catalysed by HClO₄ at constant ionic strength have been studied at various concentrations of reacting components over the temperature range 293-313K. All the kinetic data of oxidation of substrates by QCC have been listed in subsequent chapters. Only the results of the typical kinetic runs have been given in details. Other kinetic measurements are recorded in summarised form so as to include the value of pseudo-first order rate constant.

Typical kinetic runs:

The main kinetic studies of the oxidation reaction between QCC and monosaccharides were almost similar.
Kinetic runs were repeated several times to check the reproducibility of the results. It was found that the results were reproducible within the limits of experimental error.

The typical kinetic runs of the oxidation of monosaccharides by QCC in acetic acid-water mixture [1:1(v/v)] in the presence of perchloric acid using different concentrations of oxidant, substrate, acid, salt, solvent and at different temperature have been listed in subsequent chapters.

The different kinetic runs for each substrate are as follows:

1. The first typical kinetic run presents the data for the [QCC] dependence. The concentrations of other reactants were kept identical.
2. The second typical kinetic run gives the data for [substrate] dependence, keeping all other conditions constant.
3. In the third typical kinetic run the dependence of [HClO₄] has been presented, keeping the concentrations of other reactants fixed.
4. The fourth typical kinetic run gives the ionic strength dependence.
5. The fifth typical kinetic run shows the dependence on the volume of solvent.
6. The last typical kinetic run presents the data for temperature dependence.
SECTION C

EVALUATION OF KINETIC RESULTS

I. Rate constant:

The pseudo-first order rate constant, \( k_1 \), were evaluated from the plot of log absorbance against time which were linear in each case. The rate constants were checked by substituting values in first order rate equation also,

\[
 k_1 = \frac{2.303}{t} \log \frac{D_0}{D_t}
\] .......... (1)

where, \( D_0 \) was the initial optical density of the reaction mixture and \( D_t \) was the optical density at any time \( t \). The second order rate constant \( k_2 \) were computed by dividing the pseudo-first order rate constant, \( k_1 \), with the concentration of the substrate.

\[
 k_2 = \frac{k_1}{[\text{substrate}]}
\] .......... (2)

II. Activation parameters:

In order to evaluate activation parameters such as temperature coefficient, energy of activation, frequency factor, enthalpy of activation, entropy of activation, free energy of activation from temperature dependent rate data, following method has been applied.

(a) Temperature coefficient:

It is well established that the rate of reaction depends strongly on the temperature. The value of the ratio, called temperature coefficient is given by the expression.\textsuperscript{321}
\[ K_{(\tau+10)}/k_\tau \cong 2 \text{ or } 3 \] ........ (3)

(b) **Arrhenius activation energy**:

Empirically, the activation energy is the parameter which express the temperature dependence of the rate constant.\(^{322}\)

\[ k_{\text{obs}} = A \exp(-E_a/RT) \] ........ (4)

The energy of activation has been calculated numerically as well as by graphical method.\(^{323}\)

(1) **Numerical method**:

The energy of activation can be calculated numerically by appropriately fitting the following equation.

\[ \log \frac{k_2}{k_1} = -\frac{E_a}{2.303R} \left[ \frac{1}{T_2} - \frac{1}{T_1} \right] \] ........... (5)

(2) **Graphical method**:

An alternative form of Arrhenius equation is:

\[ \log k = \log A - \frac{E_a}{2.303RT} \]

The energy of activation can be evaluated from the Arrhenius plot which is linear function of reciprocal temperature.

\[ \text{Slope} = -\frac{E_a}{2.303R} \]

\[ E_a = \text{slope} \times 2.303 \text{ R (kJ mol}^{-1}\) \] ........... (6)

Where, \( R = 8.314 \text{ JK}^{-1}\text{mol}^{-1} \)

(c) **Pre-exponential factor or frequency factor \(A\)**:

The pre-exponential factor "\(A\)" which represents the total frequency of encounters between the two reactant molecules, irrespective of
whether they possess sufficient energy or not is given by the following equation.

\[ \log k_{\text{obs}} = \log A - \frac{E_a}{2.303RT} \] .......... (7)

(d) **Enthalpy of activation (ΔH')**: 

Arrhenius plot has been used to calculate ΔH' from the experimental Ea using the relation.324

\[ \Delta H' = E_a - RT \] .......... (8)

(e) **Entropy of activation (ΔS')**: 

The entropy of activation, ΔS', was computed from the Eyring's equation.325

\[ 2.303 \log \left( \frac{k_{\text{obs}}}{T} \right) = 2.303 \log \left( \frac{k_B}{h} \right) + \frac{\Delta S'}{R} - \frac{\Delta H'}{RT} \] .......... (9)

Where,

- \( k_{\text{obs}} \) = Rate constant
- \( T \) = Absolute temperature
- \( k_B \) = Boltzman's constant = 1.38 x10^{-23} JK^{-1}
- \( h \) = Planck's constant = 6.626x10^{-34}JS
- \( R \) = Universal gas constant = 8.314 JK^{-1}mol^{-1}
- \( \Delta H' \) = Enthalpy of activation.

(F) **Free energy of activation (ΔG')**: 

The free energy of activation, ΔG', was calculated by the following relation.

\[ \Delta G' = \Delta H' - T\Delta S' \] ..... (10)

The computed values of all the thermodynamic parameters have been recorded in Table in subsequent chapters.