# CHAPTER - II

## EXPERIMENTAL

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Our intension in the present work was mainly to study the estimations of sugars such as D-arabinose, D-xylose, D-glucose, maltose, lactose, and raffinose coulometrically with electrogenerated bromine and to compare the results with those obtained by visual and potentiometric methods.

The experimental work done for this study can be divided into three parts, namely, estimation of sugars by ,

1. Coulometric method,
2. Visual method and
3. Potentiometric method.

### 2.1 : ESTIMATION OF SUGARS BY COULOMETRIC METHOD :

#### 2.1.1 : Instrumentation :

a) Coulometric Cell :

The constant current coulometric estimations were carried out with a cell ( cf Fig.1 Page No.36)constructed in our laboratory. The electrolysis cell contained a tall form beaker (without lip) of 250 $cm^3$ capacity. The main working or generating electrode A was of platinum plate ( 4 x 2.5 cm),at which the reagent was electrogenerated, and the auxiliary electrode C was of platimum plate ( 1 x 1 cm ). The isolation of the auxiliary generating electrode C, was achieved by placing it in a separate glass tube closed at its lower end by a fine porosity glass disc; over which agar-agar gel was poured. This tube was filled with KBr solution, the level and ionic strength of which was maintained at a higher level than the solution in

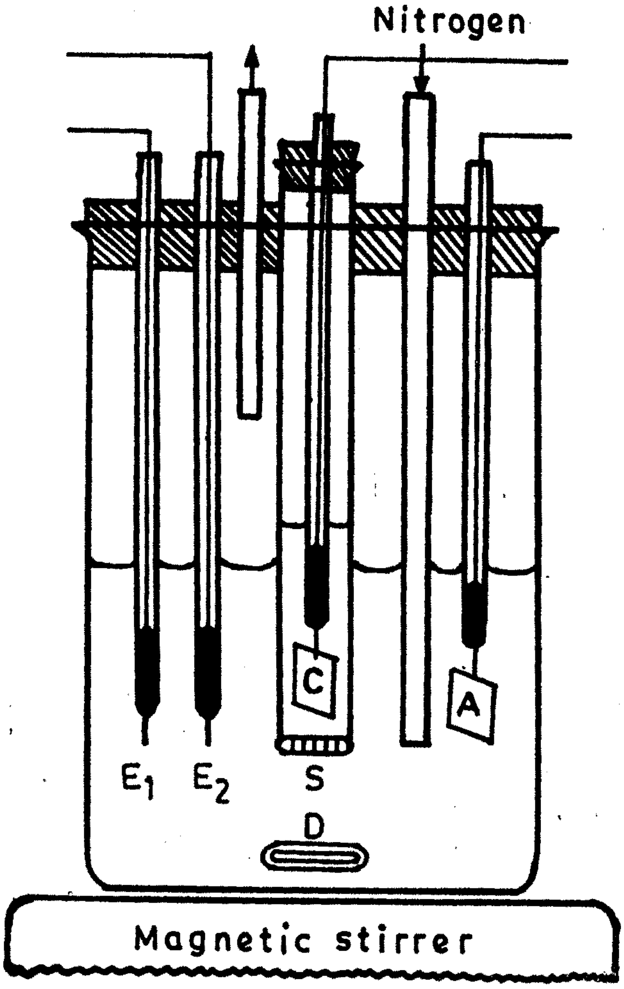


FIG. 1 – A CELL FOR COULOMETRIC ESTIMATION.

NOTATIONS :—

A — Platinum plate generating electrode.

C — Platinum plate generating electrode.

$E_1$ — Platinum Wire, Micro indicator electrode.

$E_2$ — Platinum Wire, Micro indicator electrode.

S — Sintered glass disc.

D — Stirring bar.

the electrolysis cell, so as to prevent the diffusion of solution from one compartment to another.

Two similar, micro indicator electrodes $E_1$ and $E_2$ were constructed from 20 gauze platinum wire. They were located approximately 5.0 mm apart and positioned outside the electric field (current path) between the generator electrodes to avoid the spurious indicator currents. All the electrodes were fixed in a rubber stopper which was tightly fitted on the top of the beaker. Provision was made for magnetic stirring and for passing a stream of nitrogen gas through the solution.

### b) Current Measuring Devices :

Two generating electrodes, A and C were connected to the stabilized D.C. Power Unit ( APLAB regulated D.C. Power supply model 7113 ) through a calibrated multimeter (Simpson multimeter 260M), which enabled to maintain constant current and exact measurement respectively of the current passing through the cell.

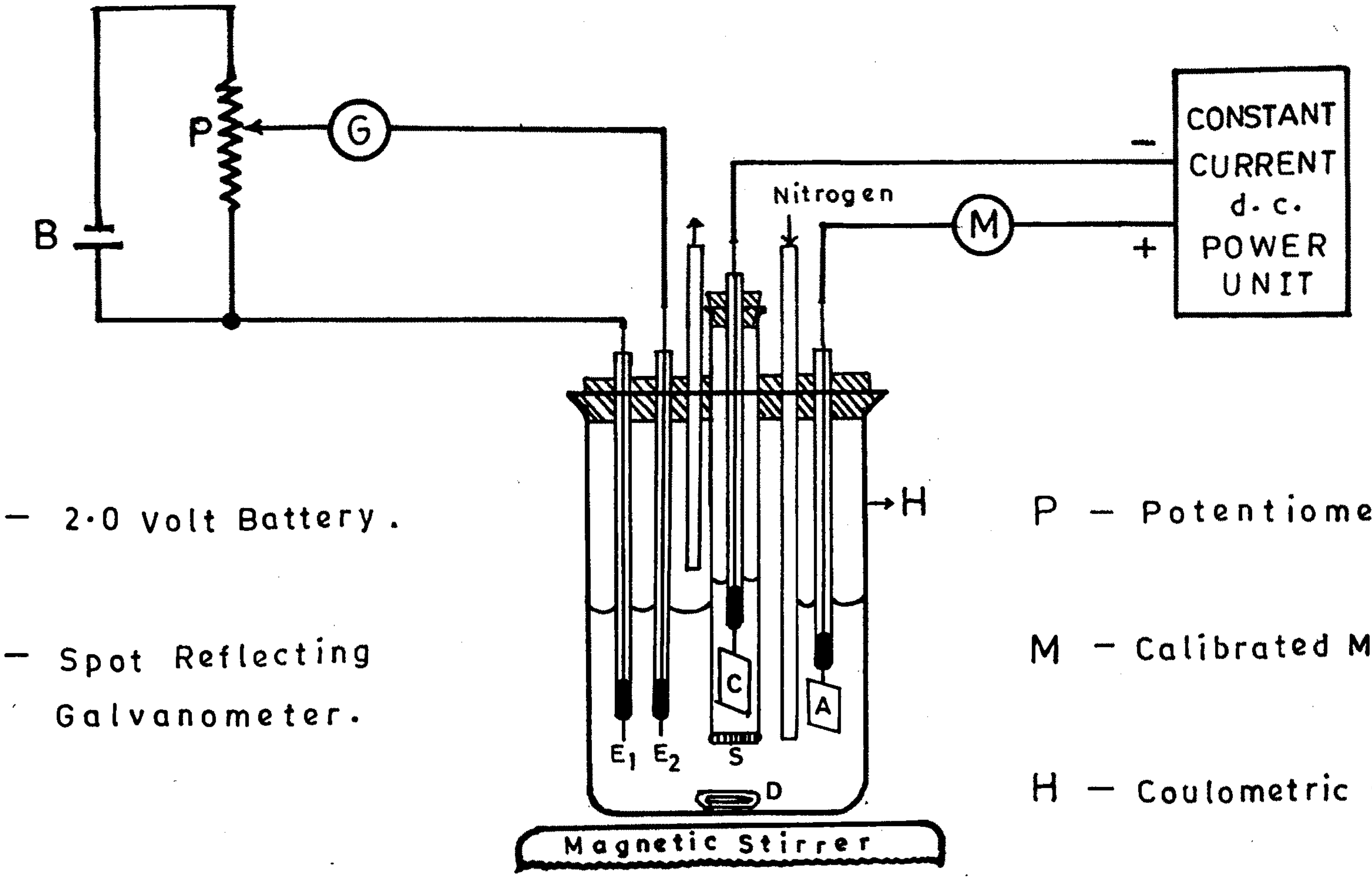
Two micro indicator electrodes $E_1$ and $E_2$ connected to a potentiometer, P (500 Ohm, 0.50 watt potentiometer) through a sensitive spot reflecting galvanometer (OSAW, sensitivity : 0.04 to 0.005 µA per mm scale division), fitted with a variable shunt. The voltage impressed across these electrodes was supplied by battery B (2.0 volts) Via a potentiometer, P and the indicator current was recorded by galvonometer G, in terms of deflection.

### c) Stirring Device :

The magnetic stirrer (Remy 2-LH) was used for proper stirring.

### d) Time Measurement :

Results of moderate accuracy were obtained by measuring the time by the use of a good stop watch (RICAR 1/10).



B — 2·0 Volt Battery.

G — Spot Reflecting Galvanometer.

P — Potentiometer.

M — Calibrated Multimeter.

H — Coulometric Cell.

FIG. 2 — BLOCK DIAGRAM OF EXPERIMENTAL SET UP FOR COULOMETRIC ESTIMATION.

This assembly was found to have the following advantages.

1. The large area generating electrode A , permits the electrogeneration of bromine with 100 percent current efficiency by the oxidation of the bromide ion at this platinum anode.
2. The perfect isolation of the auxiliary generator electrode, C, within the isolated compartment from the bulk of the solution, prevented the diffusion of the solution from one compartment to another and avoided any effects arising from undesirable reactions at this electrode.
3. Maintainance of a reasonable constant current and its exact measurement can be achieved.
4. The disturbing fluctuations in the measurements of indicator currents can be minimised by using two similar micro indicator electrodes $E_1$ and $E_2$. The spurious indicator currents which can be produced during electrolysis, are completely avoided by locating these indicator electrodes outside the electric field (current path) between the generator electrodes.
5. The losses of electrogenerated bromine were completely supressed by tightly fixing the electrode assembly on the top of the electrolysis cell.
6. The necessity to stir the solution effectively at a constant rate, throughout the time required for the experiment to be completed and also to keep the temperature constant, is achieved by using magnetic stirring.

e) pH Measurement :

pH of all the solutions were measured with the help of digital pH meter (Electronic Corporation of India Ltd.,Model pH 5651, S.NO. 372).

## 2.1.2: Chemicals, reagents and Preparation of Solutions :

Analytical grade chemicals were used to prepare all stock solutions. The solutions were prepared fresh for each set of experiment. Water, double distilled in glass was used for preparation of various solutions and throughout the procedure. All the glass wares were cleaned with chromic acid, washed repeatedly with distilled water and rinsed with double distilled water before being used for preparing the solutions.

### 2.1.2 : a) Supporting Electrolyte :

5.0 g of Potassium bromide (Merck product) previously dried at 110°C were dissolved in 100 ml buffer solutions of known pH.

### b) Buffer Solutions :

#### i) 0.1 M Disodium hydrogen phosphate solution :

35.816 g of $Na_2HPO4 \; 12 \; H_2O$ (BDH product) were dissolved in distilled water and diluted to 1000 ml in a volumetric flask.

#### ii) 0.1 M Borax solution :

38.138 g of $Na_2B4 \; 07 \; 10 \; H_2O$ (Riedel De Haen, AG) were dissolved in distilled water and diluted to 1000 ml in volumetric flask.

#### iii) Buffer of pH = 8 :

This buffer solution was prepared by mixing 40 ml of 0.1M $Na_2HPO4$ solution and 10 ml of 0.1M borax solution.

#### iv) Buffers of pH = 8.4 and 8.8 :

These buffer solutions were prepared by adding requisite amount of orthophosphoric acid in 0.1M $Na_2HPO4$ solution.

The pH of all the above solutions were measured with the help of digital pH meter. The pH meter was standardised by using 0.05M Potassium hydrogen phthalate solution, prepared by dissolving 10.2115 g in 1000 ml of distilled water, before measurement of pH of the solution.

v) Buffer of pH = 9.1 :

The 0.1M Disodium hydrogen phosphate solution as prepared above had a pH = 9.1

c) Sugar Solutions ( 2 mg per ml).

The solutions of D-Arabinose, Maltose, Raffinose (Loba products), Lactose (Sisco product), D-glucose (E.Merck product) and D(+) xylose (Fluka product), were prepared by dissolving 200 mg of each sugar in 100 ml distilled water in volumetric flask.

### 2.1.3 : Procedure :

All the Pt-electrodes were cleaned by dipping in concentrated nitric acid and thereafter washing them repeatedly with double distilled water before their use for electrolysis. The coulometric cell was charged with 50 ml KBr supporting electrolyte (Prepared in buffer of known pH). The electrode assembly was properly positioned. The isolated compartment was filled with Potassium bromide solution ( ~ 1M), the level and ionic strength of which was higher than the solution in the cell. All electrical connections were made as described previously. A 0.1V potential was impressed across the two indicator electrodes and the galvonometer shunt was set to give maximum deflection on the galvonometer. The nitrogen gas was passed through the solution to remove dissolved oxygen. The solution was continuously stirred by means of magnetic stirrer.

Bromine was generated by passing a constant current. The concentration of bromine was measured in terms of galvanometer deflection. A definite excess of bromine corresponding to definite deflection was generated and current was stopped.

An aliquot of sugar solution under investigation was added into the cell by means of a calibrated microburette. Galvanometer deflection decreased due to consumption of bromine in the oxidation of sugar. Then constant corrent was passed and bromine regeneration was continued until galvanometer deflection attained original value. As soon as deflection attained original value, current was stopped immediately. The time required for this regeneration of bromine was measured by means of a stop watch. Each reading was repeated three times to confirm reproducibility. Thus knowing the current and time the amount of sugar reacted was evaluated by using the following expression.

$$W = \frac{M \times C \times t}{96500 \times n}$$

Generally, about 5 to 6 aliquots of each sugar were analysed before changing the electrolyte solution.

In order to establish the optimum experimental conditions for the estimation of each sugar, the effects of various factors such as pH of the solutions, current magnitudes, and temperatures were studied.

The results obtained in all these cases are given in table Nos. 1 to 13 on Page Nos. 49-75.

## 2.2 : ESTIMATION OF SUGARS BY VISUAL METHOD :

### 2.2.1 : Chemicals, Reagents and Preparation of Solutions :

Analytical grade chemicals were used to prepare all stock solutions. The solutions were prepared fresh for each set of experiment. Water, double distilled in glass was used for preparation of the various solutions and throughout the procedure. All the glass wares were cleaned with chromic acid, washed repeatedly with distilled water and rinsed with double distilled water before being used for preparing the solutions.

### 2.2.1 : a) Preparation of Reagents :

#### i) Sugar Solutions : (2 mg per ml.)

The different sugar solutions were prepared as given in coulometric method.

#### ii) Sodium Thiosulphate solution: (0.05 M)

12.409 g of $Na_2S_2O_3\ 5H_2O$ (BDH product) were dissolved in distilled water and diluted to 1000 ml in a volumetric flask. The flask was ~~cool~~ closed tightly and kept away from the light. The solution was standardised against 0.05 M Potassium dichromate solution.

#### iii) Bromine Solution : ( 0.025 M)

Approximate 0.025 M bromine solution was prepared by dissolving 1.5 ml of liquid bromine (S.D. Fine Chem.Pvt.Ltd.) in distilled water containing 16 g per litre of KBr (Merck Product) and diluted to 1000 ml in volumetric flask. The flask was tightly closed. The solution was standardised against 0.05N sodium thiosulphate solution.

iv) Buffer Solution of pH = 8.8

The solution was prepared as described in coulometric method.

v) Potassium Iodide Solution : (0.2 N)

33.2 g of Potassium bromide (BDH product) were dissolved in distilled water and diluted to 1000 ml in a volumetric flask. The flask was closed tightly.

vi) Sulphuric Acid :( 2 N)

56 ml of concentrated sulphuric acid (Merck product) were added to distilled water very slowly with constant shaking and volume was made upto 1000 ml in a volumetric flask.

### 2.2.2 : Procedure :

50.0 ml of buffer solution of pH = 8.8 were taken in a glass stoppered bottle. 20 ml of 0.025 M bromine solution and a definite quantity of the sugar solution (2 mg per ml) say, 10 ml were added to it. The stoppered bottle was kept aside with occasional stirring. After sufficient time interval (30 minutes) at room temperature, 20 ml of 0.2N KI solution and 15.0 ml of 2N sulphuric acid were added and bottle was kept aside for, atleast, 20 minutes after which the liberated iodine was titrated against 0.05N Sodium thiosulphate solution.

Blanks were simultaneously run and the difference between the two titration readings gave a measure of the bromine used up in the oxidation of the sugar under investigation. From this difference the quantity of sugar was calculated, using standard relations.

It was found in coulometric method that the optimum pH for oxidation of sugars was 8.8 and hence this pH was maintained in these cases. The results obtained in these cases are tabulated in Table Nos. 14 to 19 on Page Nos. 76-81.

## 2.3 : ESTIMATION OF SUGARS BY POTENTIOMETRIC METHOD :

### 2.3.1 : Chemicals, Reagents and Preparation of Solutions :

Analytical grade chemicals were used to prepare stock solutions. The solutions were prepared fresh for each set of experiment. Water, double distilled in glass was used for preparation of the various solutions and throughout the procedure. All the glass wares were cleaned with chromic acid, washed repeatedly with distilled water and rinsed with double distilled water before being used for preparing the solutions.

### 2.3.1 : a) Preparation of Reagents :

i) Sugar Solutions :(0.025 M)

All the sugar solutions were prepared by dissolving the calculated weights of these sugars in the required volumes of double distilled water.

ii) Sugar Solutions :(5 mg per ml).

Sugar solutions were prepared by dissolving 500 mg of each sugar in double distilled water and diluting to 100 ml in a volumetric flask.

iii) Bromine solution : (0.025 M)

Bromine solution was prepared and standardised as described in visual method.

iv) Buffer Solution of pH = 8

Buffer solution of pH = 8 was prepared as described in coulometric method.

### 2.3.1 : b) Pt-Electrode :

A bright platinum electrode was cleaned by dipping in concentrated nitric acid and thereafter washing it repeatedly with double distilled water. The platinum electrode was kept under distilled water when it was not in use.

### 2.3.2 : Procedure :

A known amount of bromine solution ( 10 ml, 0.025 M)was taken in a beaker and requisite quantity of buffer (pH= 8) (40.0 ml 0.1M $Na_2HPO_4$ + 10 ml 0.1M Borax) was added to it. The mixture was titrated potentiometrically with 0.025 M solutions of different sugars at room temperature.

A bright platinum wire electrode, immersed in the solution to be estimated, was used as an oxidation reduction electrode and this was coupled with a saturated calomel electrode. The mixture was kept stirred with a magnetic stirrer during the titration. The progress of the reaction was studied by measuring the potential of the cell with direct reading potentiometer (OSAW) in conjunction with a spot reflecting galvanometer (OSAW sensitivity : 0.04 to 0.005 $\mu A$ per mm. scale division). At the equivalence point, a sharp jump in potential was observed in each titration.

Preliminary experiments showed that these potentiometric titrations were smooth only in case of indirect titrations (when the bromine solution was in the cell). In the direct (reductant in the cell)titrations,repeatable results could not be obtained due to a drift in potentiometric readings. A series of potentiometric titrations were performed in each case by taking different amounts of bromine in a cell.

Sufficient time was allowed (30 min) after each addition for attainment of steady potential and reproducible results were obtained in these cases. A graph of $\Delta E / \Delta V$ against the mean volume of reducing agent was plotted and the maximum inflection of the curve gave the end point.

It was found, however, that the potentiometric method (being successful only by indirect procedure with the oxidant in the cell) was useful only in conjuction with visual procedure. This is because out of bromine solution only the hypobromite reacts with the reductant and its amount must be known separately by visual titration method.

It was found in coulometric method that the optimum pH for estimation of sugars was 8.8. However, it was found on the basis of some preliminary experiments that the potentiometric method gives reproducible results at pH = 8. And hence this pH was maintained in these cases.

The results obtained by this method in these cases are recorded in Table Nos. 20 to 29 on Page Nos. 82-96.