## 3.2 DISCUSSION

3.2.1: Estimation of Sugars by Coulometric method :

3.2.1 a): Effect of pH

In the first phase of our study attempts have been made to estimate coulometrically, D-arabinose, D-xylose, D-glucose, maltose, Lactose and raffinose, with electrogenerated bromine in acidic medium at pH 3,4,5 and 6.5 etc. The experimental results (not tabulated) indicate that the consumption of generated bromine in the oxidation of sugars was very less and the generation time measured was short resulting in the under-estimation of these sugars. In fact, this may be due to the slow rate of oxidation of sugars in acidic medium. Similar results have been reported by Ling and Nanji[108], Bunzel and Matthews[109] and Kiliani et.al.[110-112].

Bunzel and Matthews[109] studied the rate of reaction of bromine with D-glucose quite extensively and concluded that the free bromine, rather than hypobromous acid, was the oxidant. This is because bromine in water undergoes hydrolysis giving hypobromous acid as

$$Br_2 + H_2O \rightleftharpoons HBrO + HBr$$
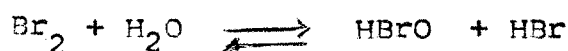
since the equilibrium for the above reaction is far to the left in acidic medium, the equilibrium concentration of free bromine is very high as compared with that of hypobromous acid. Hence free bromine is an active oxidant in acidic medium.

The oxidation of aldose sugars with bromine soon leads to the formation of strongly acidic solution, regardless of whether the oxidant be considered as hypobromous acid or a free bromine, due to the formation of hydrobromic acid as in the following reactions :

$$Br_2 + RCHO \rightleftharpoons RCOOH + 2HBr$$

$$HBrO + RCHO \rightleftharpoons RCOOH + HBr$$
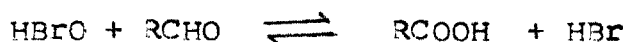
The accumulation of the hydrobromic acid results in slowing up the oxidation. In addition Bunzel and Matthews[109] pointed out that the decrease in the rate of oxidation was not only due to the increased acidity but also due to the hydrobromic and hydrochloric acid which have stronger inhibiting effects than other acids.

Thus, the results of these workers support to justify our experimental observations of underestimation of these sugars in acidic medium.

Similarly a series of experiments have been performed to estimate these sugars coulometrically in strongly alkaline medium at pH 10, 11, 12 etc. In these experiments, we observed that there was no generation of bromine (hypobromite) and it was practically impossible to estimate sugars in strongly alkaline solution. This may be due to the absence of active oxidant in the solution.

Bromine, in general gives hypobromite in alkaline solution, which can be expressed by the following equation.

$$Br_2 + 2NaOH \rightleftharpoons NaBrO + NaBr + H_2O$$
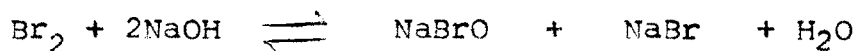
Since, the equilibrium is far to the right, the concentration of hypobromite is greater than that of the free bromine. Therefore, hypobromite, being active oxidant oxidises the aldose sugars to aldonic acids:

$$RCHO + BrO^- \rightleftharpoons RCOO^- + Br^-$$
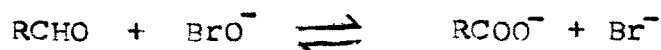
However, the hypobromite is unstable and decomposes
readily. The rate of decomposition of hypobromite solutions in
presence of excess alkali has been determined at various concent-
rations and at different temperatures by Palmen[113,114]. He pointed
out that under these conditions the hypobromite gets converted
into bromate which is quite inactive

$$3\ BrO^- \longrightarrow BrO_3^- + 2Br^-$$

Thus, the hypobromite, being converted into bromate in
highly alkaline solution, is not available for the oxidation of
sugars. Similar observations have been reported by Hönig and
Tempus[116]. Therefore, the higher pH range and elevated temperatures
are not suitable for the estimation of aldose sugars coulometri-
cally with electrogenerated bromine.

Hönig and Ruzicka[115] studied the oxidation of various
aldose sugars with bromine to obtain aldonic acids and noted that
the formation of aldonic acids by oxidation with bromine was
favoured by low alkali and sugar concentrations. Recently, Rathor
and Grover[36] attempted to estimate some aldose sugars by
oxidation with bromine and obtained comparable results at pH of
about 8 because these reactions were fast at this pH.

Our experimental observations for the estimation of these
sugars in acidic and in highly alkaline medium suggest that the
coulometric estimations can conveniently be studied in the pH
range 8 to 9.1. Hence we have confined our attention to estimate
these sugars in this pH range only.

In the second phase of our study, we have attempted to
estimate these sugars coulometrically with electrogenerated bromine

at several different pH values of 8, 8.4, 8.8 and 9.1 with
the current magnitude of 40 mA at 25°C. The results obtained
are presented in table Nos. 1,2,3 and 4 and Summarised results
are shown in table No.5. These observations indicate that the
average percentage errors are least, for all sugars, at the pH
of 8.8. Therefore, we think that this is a pH at which the rate
of generation of hypobromite and the rate of its consumption
in oxidation of sugars may be identical and no undesired side
reactions occur. The hypobromite is continuously regenerated
during the reaction so that a small quantity of it can oxidise
a large amount of sugar as represented in the following scheme.

$$Br^- + H_2O - 2e^- \longrightarrow BrO^- + 2H^+$$

$$BrO^- + RCHO \longrightarrow RCOO^- + Br^-$$
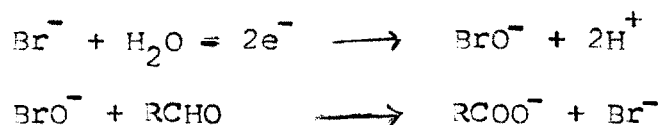
3.2.1 b) Effect of Current :

The effect of current on the estimation of sugars has
been studied by using different current values of 35, 40, 45
and 50 mA at pH 8.8 and temperature 25°C. The results are
recorded in table Nos. 6,3,7 and 8 respectively and summarised
results are shown in table No.9. Our observations seem to be
in accordance with the following theoretical explination based
on Faraday's first law of electrolysis which is mathematically
expressed as

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

Where W, M, C, t and n have usual meanings. It is obvious
from the above relation that at constant W, M and n, the
generation time, 't' and current, C are inversely related with
each other. At lower current values the generation times are more
and vice versa.

From the table No.9 it is clear that the average percentage errors are maximum at the current magnitude of 35 mA and seem to decrease with increase in current magnitude for all sugars except D-arabinose, D-xylose, and D-glucose. In D-arabinose, D-xylose and D-glucose ,the average percentage errors decrease upto 45 mA and on further increase in current a curious behaviour is observed at current of 50 mA. In these sugars the average percentage errors increase at 50 mA rather to decrease. This increase in average percentage errors at 50 mA may be due to a shifting of anodic potential to more positive values leading to undesirable oxidation of hypobromite to bromate. Similar observations are reported by Avrutskaya and coworkers[117,118] in the electrooxidative preparation of D-gluconic acid from D-glucose.

In case of maltose, lactose and raffinose it is observed that there is a decrease in average percentage errors at 50 mA current. This may be due to the slow oxidation rates of these high molecular weight sugars. This observation is in accordance with the results of Isbell and Pigman[119] who found that a buffered bromine solution attacked pentoses most rapidly; hexoses were oxidised more slowly and disaccharides still more slowly.

The effect of current on the estimation of these sugars is rather complicated due to the simultaneous, occurance of number of side reactions in the electrolytic cell; such as

1   generation of hypobromite;

2   consumption of hypobromite in the oxidation of sugars;
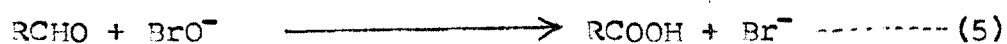
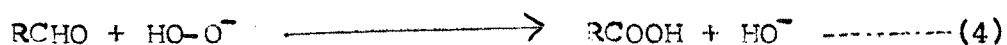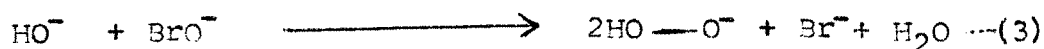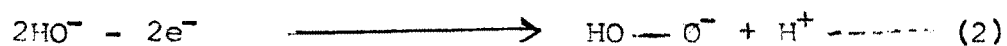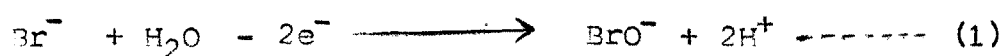3   decomposition of hypobromite to bromate etc.

In addition , in our study we have selected a very narrow range of current values and it is evident from the results that the time plays a key role in the estimation. A slight error of even one second in measurement affects the percentage error enormously. Hence, at this stage, it is very difficult to predict the exact role played by the current in the estimation of these sugars.

We think that the fresh experimentation is necessary in order to decide the exact role of current in the estimation by using a wider range of current magnitudes and a sophisticated time measuring device.

3.2.1 c): Effect of temperature :

An attempt has also been made to study the effect of temperature on the estimation of sugars and for the shake of convenience the effect was studied by using a current magnitude of 45mA at pH 8.8. The results of experiments performed at the temperatures 25°, 30°, 35° , and $40^\circ C$ are presented in table Nos.7,10, ... 11 and 12 respectively and summarised results are shown in table No.13. From these results it is evident that the average percentage errors are low at 25° and $30^\circ C$. The average percentage errors become negative indicating thereby the under estimation of sugars at $35^\circ C$. On the other hand the average percentage errors become positive indicating the overestimation of these sugars at $40^\circ C$. At 25° and $30^\circ C$ it seems that the rate of generation of hypobromite and the rate of its consumption in the oxidation of sugars are identical.This may be due to the lower losses of hypobromite.

The underestimation at $35^{\circ}C$ may be due to the formation of some active species, such as, peroxide ion ($HO-O^-$) in the solution which also oxidises sugar as represented in the scheme given below.

$$Br^- + H_2O - 2e^- \longrightarrow BrO^- + 2H^+ \quad \text{------ (1)}$$

$$2HO^- - 2e^- \longrightarrow HO-O^- + H^+ \quad \text{------ (2)}$$

$$HO^- + BrO^- \longrightarrow 2HO-O^- + Br^- + H_2O \quad \text{---(3)}$$

$$RCHO + HO-O^- \longrightarrow RCOOH + HO^- \quad \text{------(4)}$$

$$RCHO + BrO^- \longrightarrow RCOOH + Br^- \quad \text{------(5)}$$

The active species, peroxide ion, is formed in the solution as shown by the reactions (2) and (3) in the above scheme, which oxidises sugar as represented by the reaction (4). From reaction (3) it is evident that the single hypobromite ion produces two peroxide ions by the combination with hydroxyl ion (HO^-). Hence, the concentration of peroxide ion in the solution increases rapidly, oxidising more and more amount of sugar. The consumption of hypobromite in the oxidation of sugar as represented by the reaction (5) is lowered. Thus the original concentration of hypobromite ion is less affected. Therefore the time required to regenerate the original amount of hypobromite in the solution must have been decreased leading to the underestimation of these sugars.

At $40^{\circ}C$, the decomposition of hypobromite to bromate may be predominant as reported by Palmen[113,114] leading to more losses of hypobromite which ultimately results in the overestimation of sugars. The average percentage errors at $30^{\circ}C$ are relatively small and hence this temperature seems to be optimum for the coulometric estimation of these sugars with electrogenerated bromine.

3.2.2: <u>Estimation of Sugars by Visual method</u> :

In addition, an attempt has also been made to estimate these sugars by visual method at pH 8.8 and temperature $25^{\circ}C$. The results obtained are presented in table Nos 14-19. From these results it is observed that the percentage errors are low for higher concentrations (above 10 mg) and high for lower concentrations (below 10 mg) of these sugars except raffinose. Hence, this method seems to be suitable only for higher concentration range of these sugars.

In case of raffinose, (results shown in table No.19), the underestimation is observed, in general for all concentrations. This may be due to the slow rate of oxidation of this high molecular weight sugar, leading to the underestimation. This observation is in accordance with the results reported by Isbell and Pigman[119].

3.2.3: <u>Estimation of sugars by potentiometric method</u> :

The estimation of all these sugars except raffinose has been studied by potentiometric method at pH 8.0 and temperature $25^{\circ}C$. The results obtained are shown in table Nos. 20-29 and graphically represented in fig. Nos. 3-7. In general, in potentiometric study, the underestimation for all sugars is observed. This may be due to the incomplete oxidation of sugars. However, it seems that this method is suitable only for the estimation of higher amounts of sugar as visual method. The oxidation of raffinose, being very slow, as indicated by visual method, the attempt has not been made to estimate this sugar by method potentiometric method.

3.2.4 : Comparison of results of Coulometric, Visual and

Potentiometric methods :

The results obtained by these three methods are compared in table No.30 with a view to give merits and demerits of these three methods. From this comparison it is obvious that the proposed coulometric method gives more accurate and reproducible results than the other two methods particularly in the lower concentration range (below 10 mg). However, the errors in the estimation are likely to be incorporated in higher concentration range (above 10 mg) due to the losses of hypobromite during the longer generation times involved. Thus we think that the proposed coulometric method is most suitable for estimation of sugars in low concentration range (below 10 mg).

The visual and potentiometric methods give reproducible results only in higher concentration range (above 10 mg).However, these conventional methods involve complicated procedures and are rather time consuming. In addition the potentiometric method is (being successful only by indirect procedure with bromine in the cell) useful only in conjunction with the visual method.

This is because out of bromine solution only hypobromite reacts with the sugar and its concentration must be separately known by visual titration method.

On the other hand the proposed coulometric method is made more convenient and rapid. It gives accurate and reproducible results quickly due to the formation of fresh and active form of reagent (hypobromite)in the solution at the moment of reaction. It is an absolute method of analysis involving only the fundamental quantities such as current and time,which can be measured with high precision and hence high accuracy can be obtained.