CHAPTER - IV

SUMMARY

SUMMARY

Large amount of work seems to have been done on the estimation of reducing sugars by various methods such as, Optical rotation, Colorimetric, Chromatographic fermentation, enzymic, Visual, Potentiometric, coulometric etc. The quantitative determination of carbohydrates, especially sugars, has been studied by number of workers using different electrogenerated reagents. However, no work seems to have been done to estimate sugars by electrogenerated bromine using coulometric methods.

Hence, it is the general object of the present work to estimate some sugars like D-arabinose, D-xylose, D-glucose, maltose, lactose and raffinose by using electrogenerated bromine coulometrically. In order to establish the optimum experimental conditions for the estimation of these sugars, the effects of various factors, such as pH of the solutions, current magnitudes and temperatures are studied.

Furthermore, it seems that very little work has been done, and no systematic efforts have been made in past for estimation of these sugars by bromine using potentiometric methods. Therefore, at this stage, we think that a fresh experimentation is necessary. Keeping this view in mind an attempt has also been made to estimate these sugars potentiometrically using bromine.

In addition, estimation of these sugars is also studied by visual method with bromine, and results of all the three methods are compared with a view to give merits and demerits of these methods. An attempt has been made to give a suitable theoretical explaination for the observed results. The present study would be of value in sugar industry, biochemistry and medicinal chemistry.

The experimental work done for this study was divided into the following parts namely,

I) Coulometric method,

II) Visual method and

III) Potentiometric method.

1) Estimation of Sugars by Coulometric method :

a) Coulometric Cell :

The coulometric cell contained a tall form beaker (without lip) of 250 ml capacity. There were two generator electrodes (main and auxiliary). The main generator electrode was of a platinum plate (4 x 2.5 cm). The auxiliary generator electrode was also of platinum plate (1 x 1 cm), which was isolated by placing it in a separate glass tube fitted with a porous glass disc overwhich agar-agar gel was poured. This tube was fitted with potassium bromide solution, the level and ionic strength of which was higher than the solution in the cell. Two micro indicator electrodes were constructed from 20 gauze platinum wire. They were located approximately 5 mm apart and positioned outside the current path between the generator electrodes, 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 also for passing a stream of nitrogen gas through the solution.

b) Current Measuring devices :

Two generator electrodes were connected to the stabilised D.C. power unit (APLAB regulated D.C. power supply model 7113) through a Calibrated multimeter (Simpson multimeter 260M), which enabled to maintain a constant current and its measurement respectively.

Two micro indicator electrodes were connected to a potentiometer (500 ohm, 0.5 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. A voltage impressed across these electrodes was supplied by 2 volt battery via potentiometer and indicator current was measured in terms of galvanometer deflection.

c) Stirring devices :

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

d) Time Measurement :

A good stop watch (ROCAR 1/10) having sensitivity 1/10 sec, was used for the measurement of time.

e) pH Measurement :

A digital pH-meter (Electronic Corporation of India Ltd. Model 5651 S.NO. 372) was used to measure the pH of the solution.

The coulometric cell was charged with 50 ml potassium bromide supporting electrolyte (Prepared in a buffer of known pH). All the necessary electric connections were made and 0.1V potential was impressed across the indicator electrodes. The solution was continuously stirred.

A definite excess of bromine was generated by passing a constant current, the concentration of which was measured in terms of galvanometer deflection. A definite amount of sugar solution under investigation was added into the cell by means of a Calibrated microburette. Galvanometer deflection decreased due to the consumption of bromine (hypobromite) in the oxidation of sugar. Then a constant current was passed to regenerate bromine until the galvanometer deflection attained the original value. The time required for this regeneration of bromine was measured. Knowing the time and current, the amount of sugar reacted was evaluated by using the following expression,

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

In order to confirm the reproducibility each reading was repeated for several times.

Effect of pH :

In the first phase of our study attempts have been made to estimate sugars in acidic medium. The experimental results in this case indicate the under-estimation of sugars. This under-estimation in acidic medium may be due to the accumulation of hydrobromic acid during the oxidation of sugar with bromine, which slows up the oxidation resulting in under-estimation.

Similarly estimations were carried out in highly alkaline medium. We observed that there was no generation of bromine (hypobromite) and it was practically impossible to estimate sugars in this medium. In highly alkaline solution, an active oxidant, hypobromite, being rapidly converted into inactive bromate, may not be available for the oxidation of sugars.

These observations suggest that the acidic as well as highly alkaline media are not suitable for coulometric estimation of these sugars. Therefore we have confined our attention to estimate these sugars in the pH range from 8 to 9.1 only.

In the second phase our study we have estimated these sugars coulometrically with electrogenerated bromine at several different pH values of 8, 8.4, 8.8 and 9.1 with current magnitude of 40 mA at 25°O. These observations indicate that the pH of 8.8 is suitable for these estimations.Therefore we think that this is a pH at which the rate of generation of hypobromite and the rate of its consumption in the oxidation of the sugars may be identical and no undesired side reactions occur. The hypobromite continuously regenerates and oxidises sugars as represented in the following scheme

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

$$BrO^- + RCHO \longrightarrow RCOO^- + Br^-$$

Effect of Current:

The effect of the current on the estimation of sugars has been studied by estimating sugars at different current values of 35, 40,45 and 50 mA at pH 8.8 and temperature 25°C. From the results obtained in this case, it seems that the effect of current is rather complicated due to the simultaneuous occurance of number of side reactions such as :

i) generation of hypobromite;

ii) Consumption of hypobromite in the oxidation of sugars;

iii) decomposition of hypobromite to bromate etc.

In addition, because of the narrow range of the current magnitudes selected by us and difficulty in precise measurement of time, at this stage we are not in position to predict the exact role played by the current in these estimations. Therefore, we think that the fresh experimentation is necessary in this case by using wider range of current magnitudes and a sophisticated time measuring device.

Effect of Temperature :

To study the effect of temperature, we have attempted to estimate the sugars at various temperatures such as 25°, 30°, 35° and 40°C at current magnitude of 45 mA at pH 8.8. The results obtained show that the average percentage errors are low at 25°C and 30°C. The underestimation is observed at 35°C and the overestimation at 40°C. The underestimation at 35°C may be due to the formation of active species such as, peroxide ion ($HO-O^-$) in the solution simultaneously, which oxidises sugar as represented in the scheme given below.

$$2\,OH^- - 2e^- \longrightarrow HO-O^- + H^+$$

$$HO^- + BrO^- \longrightarrow 2HO-O^- + Br^- + H_2O$$

$$RCHO + HO-O^- \longrightarrow RCOOH + HO^-$$

Most of the sugar may be oxidised by these active species. Thus original concentration of hypobromite is, less affected due to the lowering of its consumption in the oxidation of sugars. Therefore less time must have been required to regenerate original amount of hypobromite in the solution leading to the underestimation of these sugars at this temperature.

The overestimation of sugars at 40°C may be due to the more losses of hypobromite by its decomposition to inactive bromate as reported by Palmen. The average percentage errors at 30°C are relatively small and hence this temperature seems to be optimum for these estimations.

The results obtained by this method show that the method gives accurate and reproducible results in lower concentration range (below 10 mg) of the sugar.

Estimation of sugars by visual method :

The selected sugars have been estimated by visual method at pH 8.8 and temperature 25°C. In this method 50 ml of the buffer solution (pH = 8.8) were taken in a glass stoppered bottle. A definite amount of 0.025 M bromine solution and sugar solution was added to it. The stoppered bottle was kept aside with occasional stirring. After 30 minutes 20 ml of 0.2N potassium iodide solution and 15 ml of 2N sulphuric acid were added to the stoppered bottle and bottle was kept aside again for 20 minutes, after which the liberated iodine was titrated against 0.05N sodium thiosulphate solution.

Blanks were run simultaneously and from the difference of these two readings the quantity of sugar reacted was calculated using standard relations.

The results obtained in this case except raffinose show that this method gives reproducible results only in higher concentration range (above 10 mg) of the sugar. The underestimation is observed in case of raffinose. This underestimation may be due to the slow rate of oxidation of this sugar which agrees with the results reported by Isbell and Pigman.

Estimation of Sugars by Potentiometric method :

The estimation of all these sugars except raffinose has been studied by potentiometric method at pH 8.0 and temperature 25°C.

In this method a known amount of bromine solution was taken in a cell and requisite quantity (50 ml) of buffer (pH=8.0)was added to it. The mixture was titrated potentiometrically against 0.025M solution of different sugars.

A bright platinum wire electrode was used as an oxidation reduction electrode. It was coupled with a saturated calomel electrode.The progress of the reaction was studied by measuring the potential of the cell with direct reading potentiometer(OSAW) in conjunction with spot reflecting galvonometer(OSAW, Sensitivity: 0.04 to 0.005 µA per mm Scale division).At equivalence point,a sharp jump in potential was observed.

In general,in potentiometric study,the underestimation of all the sugars is noted which may be due to incomplete oxidation. This method seems to be suitable for the estimation of higher amounts of sugars in solution.The attempt has not been made to estimate raffinose by this method because the rate of oxidation of raffinose being very slow,the method becomes lengthy and time consuming.

Comparison of results of Coulometric,Visual & Potentiometric methods:

The results obtained by these three methods have been compared with a view to give merits and demerits of these methods. From this comparison it is evident that the coulometric method is most suitable for estimation of sugars in low concentration range (below 10 mg).However,for higher concentrations (above 10 mg)this method seems to be not suitable, because of the losses of oxidant, hypobromite by its decomposition.

The visual and potentiometric methods are suitable only for estimation of sugars in higher concentration range (above 10 mg) Though these methods are suitable for estimation of higher amounts of sugars, the procedures are complicated and time consuming. In addition, the potentiometric method is effective only by indirect procedure and useful only in conjunction with the visual method.

On the other hand the proposed coulometric method is more convenient and rapid. It gives accurate and reproducible results quickly due to the formation of fresh and active form of reagent, hypobromite, at the moment of the reaction. It is an absolute method of analysis and involves the fundamental quantities such as current and time which can be measured precisely and hence high accuracy can be achieved.