CHAPTER - I

INTRODUCTION

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1. INTRODUCTION

1:1 : HISTORICAL INTRODUCTION :

A review of the earlier work shows that the estimation of different Sugars has been done mainly by the following methods.

1. Optical rotation,
2. Colorimetric,
3. Chromatographic,
4. Fermentation,
5. Enzymic,
6. Isotopic,
7. Visual,
8. Polarographic,
9. Potentiometric, and
10. Coulometric

The aim of the present study is to estimate some sugars with electrogenerated bromine coulometrically, and to compare the results with those obtained with visual and Potentiometric methods. Hence, historical survey of these three analytical methods is presented in this Chapter.

1:1:1 : VISUAL METHODS :

From the survey of literature, it seems that the principal chemical methods for quantitative determination of sugars make use of the reducing action of sugars on alkaline solutions of certain metallic salts like Copper, Silver, Mercury, and Bismuth. However, it seems that Copper has been employed most extensively in sugar analysis.

In this method an aldehyde or ketone is oxidized by withdrawal of oxygen from the base formed by the action of the alkali upon the salt. The reduced base is precipitated either as the free metal or as the suboxide :

$$RCHO + Ag_2O \longrightarrow 2\,Ag + RCOOH$$

$$RCHO + 2\,CuO \longrightarrow Cu_2O + RCOOH$$

The reaction does not proceed stoichiometrically, products are many and variable. But it has been found that, it is possible to standardize the conditions so that the amount of cuprous oxide may be used as a measure of the quantity of sugar.

Trommer (1841) used alkaline copper sulphate to distinguish between grape sugar (glucose) and cane sugar (sucrose). In 1844 Barreswil reported that the addition of potassium tartrate to alkaline copper sulphate solution greatly increases the stability due to the formation of complex salts. Citrates, Oxalates, Salicylates, glycerol, and Cane Sugar also stabilize alkaline solution of cupric salts. Some of these, citrates in particular, have been used in the preparation of copper solution for sugar analysis.

The copper method was further improved in 1848 by Fehling, who worked out analytical details of the alkaline copper method. He gave stoichiometrical equivalents as : 5 molecules of copper to one molecule of glucose. But apparently he did not realize that the amount of copper which was reduced varies with experimental conditions and is quantitative only within a narrow range of concentrations and of reaction times. The ratio 1:5 was employed subsequently until Soxhlet in 1878 showed that the ratio varies with the degree of excess of copper present during the reaction.

Since the copper reduction method was used so generally for sugar analysis, number of modifications have been suggested which were based on the same fundamental principles but which differed in analytical details. Fehling solution was rather unstable. Hence, efforts have been made to improve its stability. Consequently, other copper solutions were frequently employed, especially in biological analysis. But with all the numerous modifications the Fehling-Soxhlet solution was the most widely used of the copper solutions. No other has been found to equal it for general usefulness in sugar analysis although others may be more suitable under specific circumstances.

The amount of copper which was reduced by various sugars has been found to vary according to the alkalinity, the temperature, the time of heating, the sugar concentration, the nature of the sugar, the type of the tartrate, the amount of contact with air, etc. Therefore, it was necessary to standardize the conditions so that the amount of cuprous oxide may be used as a measure of the quantity of sugar present initially. The early tendency was to devise a particular method for each sugar under examination. This procedure required different reagents and procedures for each sugar and as such not useful for estimation of mixtures of sugars. This difficulty led to the establishment of unified procedures for which the same reagents and procedure were used regardless of the nature of the sugar. The unified methods were then developed by Munson and Walker, Quisumbing and Thomas, Bertrand, Brown, Morris, and Millar, Lane and Eynon, and Scales. After the establishment of standard conditions for the reaction, the cuprous oxide may be estimated by gravimetric or volumetric method, and related to the amount of sugar.

Instead of measuring the copper reduced by a given amount of sugar, the copper solution may be titrated directly by the addition of sugar to the boiling copper solution. The end point is distinguished by the discharge of the blue color (methods of violette and Pavy), by spot tests with ferrocyanide(Soxhlet), or by the internal indicator methylene blue (Lane and Eynon).

Among the many methods available for sugar analysis, the most widely used is the Lane and Eynon[1,2] method, for which some modifications better suited to routine analysis and process control have been suggested[3-7]. The method is the official method of analysis for sugar determination in food and invert sugar in honey[8].

Ohashi, Kazuji and others[9] in 1971 analysed mixtures containing sucrose, invert sugar and maltose using modified Lane-Eynon method. The quantities of these found in known mixtures agreed well with those taken.

Kurokawa and co-workers[10] (1971) estimated reducing sugars using modified Fehling's solution. In this method excess Cu(II) was determined iodometrically and milligram amounts of sugars were estimated.

Rebelein and Hans[11] successfully determined, the sugar content of wine by oxidation of sugar with $CuSO_4.5H_2O$ and back titrating unreacted $CuSO_4$ iodometrically. For the first time in 1950, Beck[12] used Cu(III) for the titrimetric determination of some sugars. The direct titrimetric method at room temperature as recommended by Beck[13] suffered from serious limitation. The end point indicated by the appearance of the transient green colour was stable only for 30 sec. He used glucose as a primary standard. Keyworth and Stone[14] also found Beck's method to be unsuitable for detection of the end point and hence adopted the potentiometric dead stop end point detection method. Beck reported that 8 equivalents of oxidant were required per mole of gulcose, whereas Chandra and Yadava[15] have shown that a maximum of 24 equivalents may be needed for the oxidation of glucose, depending on experimental conditions.

The oxidising capacity of periodato complex of Cu(III) has been utilized by Jaiswal and Yadava[16], (1973) in determining 2 to 36 moles of sugars and some organic acids. They observed that the oxidation remained incomplete at room temperature. Periodate did not interfere as an oxidant because Cu(III), being a stronger oxidant, reoxidized any iodate that may be formed from the reaction between periodate and the substrate.

In 1974, Bose and Co-workers[17] used Cu(III) as IO_4^- or tellurate complexes as oxidant for the determination of Carboxylic acids and sugars, IO_4^- complexes being prefered owing to higher thermal stability. Sugars were determined by oxidation at 30-100 K° in alkaline solution with an excess of the Cu(III) oxidant, the unconsumed portion of which was titrated with standard arsenite solution. The results were reproducibile within ± 0.5 % .

Soliman[18,19] and Belal developed argentimetric titration method for semi micro estimation of some reducing sugars. Ammoniacal $AgNO_3$ was used to estimate sugars. The ppt of Ag was dissolved in hot HNO_3 (1:1) and estimated by titration with NH_4SCN. The results were comparable with those obtained by the hypoiodite oxidation method.

Kaushik, and Prasad[20] (1973) have described use of periodate as an analytical reagent for estimation of Hg(II) and its application in the indirect determination of sugars.

The mercurimetric titration method was applied by Belal and Soliman[21,22] (1974) to both monosaccharides and disaccharides using the Nessler's reagent. The Hg produced was treated with a standard iodine solution, and the excess was back titrated with a standard $Na_2S_2O_3$ solution.

Cerium (IV) sulphate has been used indirectly by a number of workers for the determination of reducing sugars. Birstein and Blumental[23] observed that both glucose and fructose consumed 13 moles of Cerium (IV) per mole of sugar while Sharma[24] observed that these sugars consumed 12 and 14 moles respectively. Grant[25] (1961) and Pais[26] (1963) observed that Ce(IV) decomposed noticeably at high temperatures in sulphuric acid medium. In view of the previous observations, Rao and Rao[27], in 1973, carried out the oxidation reactions at 60-65° in the presence of manganese (II) sulphate as catalyst and developed an improved method for determination of glucose, fructose, sucrose and lactose having an accuracy of ± 0.5 %. They observed that under the mild oxidation conditions employed, formaldehyde was stable whereas it under went further oxidation to formic acid under more drastic conditions employed by Sharma or with the more powerful oxidising agent, Cerium (IV) perchlorate in perchloric acid medium, employed by Smith[28] and Getz or by Michalskii and co-workers[29].

In 1979, Shukla and Beg[30] estimated mono and disaccharides by oxidation with excess Ce(IV) (as ammonium hexanitrato cerate) at 25° and the unreacted Ce(IV) reagent was titrated against Fe(II) using ferroin indicator. The oxidising reagent was fairly stable and gave clear and sharp end point within the accuracy of ± 1 %.

The oxidation of sugars by ferricyanide ion in alkaline solution was also used frequently for the sugar analysis. The method was open to the same objections as the copper reduction method, namely, the lack of a stoichiometric reaction and the dependence of the method on arbitrarily chosen conditions. The ferricyanide may be used to titrate the sugar solution directly by the use of picric acid or of methylene blue as an indicator. Or, the reduced ferrocyanide may be precipitated as the zinc salt, and the excess ferricyanide determined iodometrically. The ferricyanide is stable in alkaline solution, the reaction is less complex, the reduction product ferrocyanide is not reoxidized as readily by atmospheric or dissolved oxygen, hence experimental conditions are less demanding than those for the copper reduction methods. These advantages were taken into consideration in the procedures being employed in many automated reducing sugar methods[31,32].

Alkaline hypoiodite has been proposed as a reagent for the quantitative determination of aldehyde groups by Romijn[33]. With careful control of conditions, aldoses are converted practically quantitatively to aldonic acids. Measurement of the iodine consumed gives the amount of aldose originally present. Under well defined conditions and in the absence of interfering materials, the method is stoichiometric. In 1975-76, Bose[34] and co-workers used a modified hypoiodite oxidation method for the determination of glucose in honey, the oxidation was carried in Na_2CO_3 solution instead of the usual NaOH, thus avoiding complications of sugar isomerization that occur during the standard determination.

Grover and Mehrotra[35] in 1958 estimated some sugars using alkaline solution of bromine and observed that the sugars were oxidised to the stage of gluconic acid. Rathor and Grover[36] attempted to estimate sugars by using alkaline bromine (pH-8) solution by visual and potentiometric methods. In the case of estimation of these sugars it has been found that they involved a two electron change and the aldehydic group present in these sugars was oxidised to carboxylic group.

Kiliani[37] reaction has been made the basis of a stoichiometric method for determination of reducing aldose sugars[38]. The sugar was reacted with cyanide, ammonia from the hydrolysis of the nitrile was steam-distilled into alkali. One ammonia is equal to one aldose reducing group.

In addition to above, a number of different methods are also described in the literature, but only important methods are mentioned in this Chapter.

1:1:2 : POTENTIOMETRIC METHODS :

The routine visual methods of analysis to determine reducing sugars are mostly based on the reducing action of sugars on alkaline Copper Sulphate solution. These procedures, involving various techniques, like gravimetric or volumetric, are generally complicated and time consuming.

The potentiometric methods of analysis being rapid and accurate were applied successfully for the determination of sugars. In general, in these methods, the reducing sugars were oxidised by a proper oxidising agent and changes in oxidation-reduction potentials were studied potentiometrically using a suitable indicator reference electrode system.

In 1970, Rathor and Grover[36] studied oxidation of glucose, galactose, xylose and arabinose by bromine in buffer solution of borax and disodium hydrogen phosphate (pH=8), potentiometrically using platinum as an oxidation reduction electrode and saturated calomel electrode as reference electrode. These potentiometric titrations were found to be smooth and reproducible only in case of indirect titrations (when the bromine solution was in the cell). In the case of estimation of these sugars, it has been found that they involved a two electron change and the aldehydic group in these sugars was oxidised to carboxylic acid.

Reducing sugars were determined by measuring the changes in oxidation reduction potential as the sugar solution was added to an alkaline solution of Hg(II) EDTA complex by Abou-El-Kheir and Co-workers[39] (1974). They carried out the titrations at 70-80°C and found that 1N NaOH provided the best alkaline medium for the purpose. The responses of the carbohydrates upon titrations with this reagent were studied and their reduction values were ascertained. The average error for the determination of 1.5 - 6 % sugar solution was 0.5 - 1.5 %. An another method was described by the same workers[40], in which they heated a sugar with 0.1M $HgCl_2$ and estimated the unconsumed Hg(II) without filtration by adding an excess amount of 0.1M Na_2S solution and back titrating excess sulfide with 0.1M EDTA disodium salt solution. The end point was detected potentiometrically using a sulfide selective electrode.

Mannose and galactose were oxidised with excess $NaIO_4$ at room temperature in the dark by Dusic, Zirka[41] in 1976.

The excess $NaIO_4$ was back titrated potentiometrially with $N_2.H_4.H_2SO_4$ at a platinum electrode. They observed that one mole of mannose in acid, neutral and alkaline solutions and one mole of galactose in acid medium reacted quantitatively with 5 moles of IO_4^- within 10 minutes. The consumption of IO_4^- in the oxidation of galactose at pH 8-10 was smaller, and quantitative oxidation was not achieved even after 90 minutes. A potentiometric method for the determination of reducing sugars was described by D.S. Papastathopoulos and coworkers[42] in 1970. In this method the sample was treated with Stanley Benedict reagent and the amount of unreacted Copper (II) was determined with a copper-selective electrode by use of the standard addition method[43], it was then related directly to the amount of reducing sugar. Amounts of glucose in the range 0.25 - 2 mg have been determined with an average error of about 2 % .

In a similar way Palanivel and Riyazuddin[44] (1984) have described the fabrication of a simple Cu-selective electrode and its application to determine reducing Sugars in natural products, employing standard addition technique[43].

Bhatnagar and others[45] (1978) determined different sugars with trivalent copper by visual and potentiometric methods. They obtained comparable results with maximum error of ± 1.0 % .

In 1980, Grobler and Van Whyk[46] developed a potentiometric sensor based on the selective fermentation of carbohydrates by streptococcus mutans, for the estimation of glucose, mannose and fructose in a mixture of hexoses and pentoses. The response was based on the measurement of hydronium ion produced during the bacterial fermentation. The lactic acid produced during the fermentation caused a change in pH which was sensed by the glass electrode and a potentiometric steady state response,proportional to the sugar concentration was obtained.

The construction of immobilized enzyme electrodes have been reported for the measurement of glucose concentrations by Wingard, Lemnel and coworkers[47] in 1982. An immobilized glucose oxidase/catalase electrode when placed in buffered glucose,gave a potentiometric response to glucose with respect to a Ag/AgCl reference electrode. This response was found to be reproducible in the physiological range of glucose concentrations. In this paper they have described the immobilization technique, several factors affecting the sensitivity of these electrodes, and possible applications. Several immobilized enzyme electrodes for the determination of carbohydrates have been suggested by Martin[48], Bowers[49], and Diamandis[50]. However, these electrodes are difficult to fabricate and not commercially available. Potentiometric flow injection technique for the determination of sugars using a metallic copper electrode was developed by Alexander, Haddad and Trojanowicz[51] (1985). Various sugars including arabinose, maltose, Sorbose, lactose and xylose, gave an electrode response when injected into different carrier streams composed of cupric ions in weakly complexing ligand solutions comprised of either NH_3 or tartrate. Optimisation of response found to be dependent on the flow rate, temperature, pH, and concentration of carrier component.

Lopatina[52] and Ruvinskii (1985) and Stupakova and Ruvinskii[53] (1987), reported a pH metric method for determination of invert sugars in wines and related compounds. The method was based on the interaction of sugar components (glucose and fructose) with Na-tetraborate in 50 % water - alcohol solution followed by measuring the pH of the solution. The method was found to be simple, rapid and reliable.

1:1:3 : COULOMETRIC METHODS :

The electrochemistry of organic compounds has played an important role in the field of carbohydrates and their derivatives and degradation products. In the early stage of development, much attention has been given to design classical electrochemical experiments and to study their utility, chiefly for the electrosynthesis of carbohydrates and their derivatives.

The first systematic work on the electrolytic oxidation of D-glucose was due to Loeb[54-55], Neuberg and coworkers[56-59], and others[60-62]. For the electro-oxidation of D-glucose in solution in 5 % aqueous sulphuric acid, Loeb used lead as anode, and platinum cathode. He obtained, on the one hand, oxidation products (D-arabinonic acid and D-glucaric acid), and on the other hand, fragmentation products (D-arabinose and formaldehyde) which were further oxidized to D-arabinonic acid and D-arabinaric acid , and formic acid, respectively.

Neuberg and coworkers[56-59] carried out the electrolysis of many aldoses, ketoses, alditols, and aldonic acids in aqueous solution, using platinum electrodes. The most noteworthy result of this work was the discovery that carbondioxide can be electrolytically split from aldonic acids with the formation of the corresponding, lower aldoses; this observation was later verified by Carabinos[60].

A successful series of studies dealing with the indirect, electro oxidative preparation of aldonic acids from the corresponding aldoses was started in 1931 by Isbell and Frush[63]. Their method consisted of the oxidation of an aldose with hypobromite (formed by the electrooxidation of bromide) in the presence of calcium carbonate, with formation of an aldonic acid anion and bromide ion. In this way, aldonic acids were obtained in the form of calcium salts, and the only side products of the reaction were hydrogen and carbon dioxide.

Following the work of Isbell and Frush, a number of papers[64-83] have been published from time to time suggesting some modifications to the previous procedures for optimal and economical production of ca-salts of aldonic acids.

Since then, there has been a rapid progress in the field of electro-chemistry of carbohydrates, so as to take advantage of the electrochemical methods to develope various procedures for the electro synthesis, and indirect methods for the quantitative determination of carbohydrates, based on the consumption of an oxidant in their oxidation. We have however, confined our attention, to study the coulometric estimation of different reducing sugars by electrogenerated bromine, and hence a brief review of coulometric methods is given in this Chapter.

Coulometry, as a separate electrochemical method, has contributed significantly to our knowledge of the chemical and electro chemical properties of carbohydrates and their derivatives. It constitutes a great enrichment of electro-chemistry from the methodological point of view, as it can resolved practical and theoretical problems that could not be solved by the classical electrochemical method. Furthermore, it is a fast, sensitive and more precise analytical method that can be used successfully in quantitative determination of carbohydrates. In this technique a suitable reagent is electrogenerated and made to react directly or indirectly and quantitatively with the substance under investigation. By timing the electrolysis at a constant current the amount of the substance can be determined using Faraday's first law of electrolysis. Variety of reagents can be electrogenerated and number of substances can be estimated using this electro chemical method.The versatility and wide range of its applicability will be more clear from the review of the work in this field. A review of the literature shows that electrogenerated bromine has proved to be particularly useful among the oxidising agents and the development of the most of interesting coulometric titration methods has been based upon this substance.

Both the controlled potential as well as controlled current coulometry find extensive applications in all the branches of Chemistry, such as for Inorganic,Organic,Biochemical, Clinical, Industrial pollusion and Environmental etc.analysis, Hence,it is impossible to list all the applications in a systematic manner.Some applications of the coulometric titrations involving redox reactions are summarized in table below :

Reagent	Generator Electrode Reaction	Substance determined
Br_2	$2\ Br^- \longrightarrow Br_2 + 2e^-$	As(III),Sb(III), U(IV) Tl(I), I^-, SCN^-, NH_3, N_2H_4, NH_2OH C_6H_5OH $C_6H_5NH_2$
Cl_2	$2\ Cl^- \longrightarrow Cl_2 + 2e^-$	As (III), I^-

Reagent	Generator Electrode Reaction	Substance determined
I_2	$2I^- \longrightarrow I_2 + 2e^-$	Ascorbic acid[84], dihydroxy alkyl benzene[85], Urea[87], Halides[88] As(III), Sb(III)[86], $S_2O_3^{2-}$[89], H_2S
Ce^{4+}	$Ce^{3+} \longrightarrow Ce^{4+} + e^-$	Fe(II), Ti(III), U(IV), As(III) I^-
U^{4+}	$UO_2^{2+} + 4H^+ + 2e^- \longrightarrow U^{4+} + 2H_2O$	Cr (VI), Ce(IV)

In addition to above work a number of papers have been published recently, to estimate different substances coulometrically using electrogenerated bromine.

Thus, oxine was estimated by electrogenerated bromine with 100 percent current efficiency by the oxidation of bromide ion at a platinum anode. Bromination of oxine proceeded according to the equation.

$$C_9H_7ON + 2Br_2 \longrightarrow C_9H_5ON\ Br_2 + 2H^+ + 2Br^-$$

and thus four faraday constants are required per mole of oxine. The end point was detected with two small platinum plates connecting to apparatus for the amperometric detection of the end point.

In 1972, Brooks and Purdy[90] determined acid and alkaline phosphates with electrogenerated bromine. They titrated, enzymatically released phenols with Bromine. In this method they used two simple platinum indicator electrodes for detection of the end point biamperometrically.

The current efficiencies of iodine and bromine electrogenerated with an internal current source and platinum and graphite anodes and a platinum cathode, immersed in 8 M H_2SO_4 solution, Saturated with $KMnO_4$ were calculated by using polarization curves by Basov and coworkers[91] (1973). Electrogenerated bromine was used for the coulometric titration of hydrazine and iodine for the $Na_2S_2O_3$ and Na_2SO_3 titrations. J.E.O'Reilly[92] (1975) studied successfully bromination and estimation of compounds of some nucleotides such as cytosene, thiamine and Uracil by electrogenerated bromine using biamperometric end point detection technique. In this method first he generated a definite amount of bromine in the coulometric cell corresponding to indicator current of 20 µA. Next, an aliquot of an analyte was added, the current decreased due to consumption of bromine. Then he generated the bromine by passing a constant current until indicator current became 20 µA , noting the time and current he calculated the amount of substance under investigation. In addition, he studied the effects of pH of the solutions and current magnitudes etc and compared the results with those obtained with spectrophotometric method.

Shunichi Uchiyana and Giichi Muto[93] (1984) determined bromination number by controlled potential coulometry with controlled current coulometric generation of bromine. In this technique, the concentration of Br_2 in the electrolytic solution was maintained at a constant steady state value when the rates of controlled potential reduction of Br_2 and controlled current coulometric oxidation of Br^- were equal. This technique was applied to study the oxidation of L-ascorbate and the bromination of adenine.

An excellent review on the direct or indirect determination of Inorganic and Organic compounds by titration with coulometrically generated bromine and utilization of the Br-Cu(I) system and kinetics of bromination have recently been given by Barek, Jiri and coworkers[94].

In 1985, Nikolic, Kosta I; and Velasevic[95], K.R. have described an accurate and reproducible method for coulometric determination of Paracetamole with electrogenerated bromine. Similarly Liu, Haikun and others[96] estimated ascorbic acid in food by coulometric titration with electrogenerated bromine from KBr solution.

A new method based on potentiostatic coulometry to study the redox reaction rates is described by Ramette, R.W. and coworkers[97] (1987).

The quantitative determination of carbohydrates, especially sugars, has been studied by number of workers using different electrogenerated reagents. Thus, McGlothlin and Purdy[98](1977) have described a coulometric determination of serum galactose. This method is based on the determination of H_2O_2 produced in the oxidation of galactose with galactose oxidase. The peroxide reacts with iodide in the presence of a molybdenum catalyst to produce iodine which is determined with thiosulphate. The method was found to be applicable to serum samples containing 5-100 mg of galactose per 10 ml and results were comparable with a colorimetric method based on peroxidase and o-dianisidine.

Mitev, S. and others[99] (1981) have described a coulometric method for the estimation of milligram amounts of sugars (such as sucrose). In this method analyte was oxidised to CO_2 with an excess of 0.167 M $K_2Cr_2O_7$ in hot 20-25 percent H_2SO_4, and the unconsumed $Cr_2O_7^{--}$ was titrated by electrogenerated Fe(II) by using an automatic titrator. The same authors[100] (1985) have developed a new coulometric method for determination of glucose, fructose and Sucrose. They have established optimum conditions for the oxidation of these sugars with electrogenerated C_e^{4+} in H_2SO_4 medium by complete factorial experiment method. The unreacted C_e^{+4} was determined by titration with Fe^{2+} in the presence of ferroin indicator or by using potentiometric indicator. This method had better repeatability, was more rapid and more accurate. The proposed coulometric method gave better results due to the formation of fresh and active forms of reagents on the electrode at the moment of reaction, and due to the elimination of the solutions volume measurements.

Markova and coworkers[101] in 1982, determined glucose by constant current coulometry by oxidation with iodine generated at a platinum anode in 0.2M KI + 0.1 N NaOH (current efficiency = 100 % at 20 mA). Addition of $Na_2S_2O_3$ + H_2SO_4 and back titration of excess $S_2O_3^{--}$ by electrogenerated Iodine. The end point was determined potentiometrically by using two platinum electrodes. The errors were comparable to those obtained with conventional iodometry. Later on, same workers[102] have described a coulometric method for determination of invert sugars in wine and grape juice, based on combining electrochemical generation of reagent and automated iodometric titration.

Turyan, Ya I. et.al[103] (1983) described a coulometric method for the determination of glucose. In this method glucose was oxidised by coulometrically generated iodine, and the excess of iodine was back titrated with thiosulphate solution and glucose was estimated. In order to increase the accuracy and speed of the detection; iodine was generated coulometrically at pH 11-13 at 10-20 mA.

Appleby, A.J. and Van Drunen, C[104] studied the catalytic effects of different electrode materials such as Au, Pt, Ir, Ru, Rh, and Pt/Ru alloy on the anodic oxidation of carbohydrates and related compounds in saline solutions.

In addition to above, few excellent review articles have been published on Coulometric analysis notably by Burangey and Dhaneshwar (1979)[105], Stuzka, Vaclav[106] (1981) and Barek, Jiri and coauthors[107] (1984).

1:2 THEORY

1:2:1 : THEORY OF POTENTIOMETRIC METHOD :

A) OXIDATION REDUCTION REACTIONS :

Under this heading are included all reactions involving a change in oxidation number or transfer of electrons among the reacting substances.

In quantitative analysis we are chiefly concerned with reactions which take place in solution, i.e. ionic reactions. The oxidation of ferrous chloride by Cl_2 in aqueous solutions may be written :

$$2\ FeCl_2 + Cl_2 = 2\ FeCl_3$$

or may be expressed ionically.

$$2\ Fe^{++} + Cl_2 = 2\ Fe^{+++} + 2\ Cl^-$$

The ferrous ion Fe^{++} is converted into the ferric ion Fe^{+++} (oxidation) and the neutral chlorine molecule into negatively charged chloride ions, Cl^- (reduction). According to the electronic conception of the constitution of matter the conversion of Fe^{++} into Fe^{+++} requires a loss of one electron, and the transformation of the neutral chlorine molecule into chloride ion necessitates the gain of two electrons. This leads to the view that, for reaction in solution oxidation is a process involving a loss of electrons, as in

$$Fe^{++} - e = Fe^{+++}$$

and reduction is the process resulting in a gain of electrons as in

$$Cl_2 + 2e = 2\ Cl^-$$

In the actual oxidation reduction process electrons are transferred from the reducing agent to the oxidising agent. Thus oxidation is the process which results in the loss of one or more electrons by atoms or ions and reduction is the process which results in the gain of one or more electrons by atoms or ions. An oxidising agent is one that gains electrons and is reduced to a lower valency condition and a reducing agent is one that loses electrons and is oxidised to a higher valency condition.

In all oxidation reduction processes (or redox processes) there will be a reactant undergoing oxidation and one undergoing reduction, since the two reactions are complementary to one another and occur simultaneously one cannot take place without the other. The reagent suffering oxidation is termed the reducing agent or reductant, and the reagent undergoing reduction is called the oxidising agent or oxidant.

B) ELECTRODE POLENTIALS :

When a metal is immersed in a solution containing its own ions, say, zinc in Zinc sulphate solution, a potential difference is established between the metal and the solution. The potential difference E for an electrode reaction.

$$M^{n+} + ne \longrightarrow M$$

at 25°C is given by the Nerst equation as

$$E = E^{o} + \frac{0.0591}{n} \text{ Log } C_{M}n^{+}$$

where,

E = Potential difference developed

E^{o} =Standard (or reduction) potential of the metal

n = Number of electrons involved in electrode reaction

$C_{M}n^{+}$ = Concentration of the ions in solution.

In order to determine the potential difference between an electrode and a solution, it is necessary to have another electrode and a solution of accurately known potential difference. The two electrodes can then be combined to form a voltaic cell, the em.f. of which can be directly measured. The e.m.f. of the cell is the arithmetical sum or difference of the electrode potentials (depending upon the sign of these two potentials);the value of the unknown potential can then be calculated.

The primary reference electrode is the normal or standard hydrogen electrode. But the standard hydrogen electrode is rather difficult to manipulate. Hence in practice electrode potentials on the hydrogen scale are usually determined indirectly by measuring the e.m.f. of the cell formed from the electrode in question and a convenient reference electrode whose potential with respect to the hydrogen electrode is accurately known. The reference electrodes generally used are the calomel electrode and the Silver-silver chloride electrode.

C) OXIDATION REDUCTION CELLS :

Oxidation is accompanied by a loss of electrons and a reduction by a gain of electrons. In a system containing both an oxidising agent and its reduction product,there will be an equilibrium between them and electrons. If an inert electrode, such as platinum, is placed in a redox system, for example,one containing ferric and ferrous ions, it will assume a definite potential indicative of the position of equilibrium. If the system tends to act as an oxidising agent, then $Fe^{+++} \longrightarrow Fe^{++}$ and it will take electrons from the platinum, leaving the latter positively charged; if, however, the system has reducing properties ($Fe^{++} \longrightarrow Fe^{+++}$), electrons will be given up to the metal, which will then aquire a negative charge. The magnitude of the potential will thus be a measure of the oxidising or reducing properties of the system.

To obtain comparative values of strengths of oxidising agent, it is necessary, as in the case of the electrode potentials of metals, to measure under standard experimental conditions the potential difference between the platinum and the solution relative to a standard of reference. The standard experimental conditions for the redox system are those in which the ratio of the activity of the oxidant to that of the reductant is unity. Thus for the ferric - ferrous Fe^{+++} , Fe^{++} electrode, the redox cell would be :

$$\text{Pt, } H_2 \;\Big|\; H\ (a=1) \;\Big\|\; \begin{matrix} Fe^{+++} & (a=1) \\ Fe^{++} & (a=1) \end{matrix} \;\Big|\; \text{Pt}$$

The potential measured in this way is called the standard reduction potential.

The reversible oxidation reduction system may be written in the form

$$\text{Oxidant} + ne \longrightarrow \text{Reductant}$$

$$\text{or} \quad OX + ne \longrightarrow Red$$

The electrode potential which is established when an inert or unattackable electrode is immersed in a solution containing both oxidant and reductant at 25°C is given by the expression :

$$E_T = E^o + \frac{0.0591}{n} \log \frac{[OX]}{[Red]}$$

Where, $[OX]$ = Concentration of oxidant and

$[Red]$ = Concentration of reductant

It follows from the above expression that potential of oxidation reduction cell is mainly governed by the ratio of concentration of oxidant and concentration of reductant.

D) POTENTIOMETRIC TITRATIONS :

According to the theory of oxidation reduction reaction such as

$$\text{Oxidised form} + n \text{ electrons} \longrightarrow \text{Reduced form},$$

The potential of a metal electrode immersed in it at 25°C is given by the expression.

$$E_{25} = E^{o} + \frac{0.0591}{n} \log \frac{[ox]}{[Red]}$$

The potential of the immersed electrode is thus controlled by the ratio of these concentrations. During the oxidation of a reducing agent or the reduction of an oxidising agent the ratio, and therefore the potential, changes more rapidly in the vicinity of the end point of the reaction. Thus titration involving such reactions (eg. ferrous iron with potassium permanganate or potassium dichromate or cerric sulphate) may be followed potentiometrically and afford titration curves characterised by a sudden change of potential at the equivalence point. The indicator electrode is usually a bright platinum wire or foil, and the oxidising agent is generally placed in the burette.

When a potentiometric titration is being performed, interest is focused upon changes in the emf of an electrolytic cell as a titrant of precisely known concentration is added to a solution of the analyte. The method can be applied to all titrimetric reactions provided that the activity of atleast one of the substances involved can be followed by means of a suitable indicator electrode. Reproducible equilibrium cell emf is of no concern here. Requirements for reference electrodes are greatly relaxed, and it is only necessary that the response of one of the two electrodes of a pair be substantially greater or faster than that of the other. The cell emf can be measured at zero current (null balance).

Chief advantages of the potentiometric titration method are applicability to turbid, fluorescent, Opaque, or colored solutions, or when suitable visual indicators are unavailable or inapplicable. The method presents the possibility of a succession of end points in the titration of a mixture. As compared with color indicators, the end point can be located precisely, even with dilute solutions.

1:2:2 : THEORY OF COULOMETRIC METHOD :

A) INTRODUCTION :

Coulometry is a primary or absolute method of analysis. It is an application of Faraday's first law of electrolysis which may be expressed in the form that the extent of chemical reaction at an electrode is directly proportional to the quantity of electricity passing through the electrode. For each equivalent of chemical change at an electrode 96487 coulombs of electricity (the Faraday constant) are required, a coulomb is that quantity of electricity represented by the flow of one ampere for one second.

The fundamental requirement of a coulometric analysis is that the electrode reaction used for the determination proceeds with 100 percent efficiency so that the quantity of substance reacted can be expressed by means of Faraday's law from the measured quantity of electricity (coulombs) passed. In other words, the current obtained due to the electrolysis must be entirely due to the electrode reaction, and must not be due to the side reaction or any other simultaneous reaction. The substance being determined may directly undergo reaction at one of the electrodes (Primary coulometric analysis), or it may react in solution with another substance generated by an electrode reaction (Secondary coulometric analysis).

The weight W of the substance produced or consumed in an electrolysis involving Q coulombs is given by the expression

$$W = \frac{MXQ}{96487\ n}$$

Where M is the atomic or the molecular weight of the substance being electrolysed, and n is the number of electrons involved in the electrode reaction. Analytical methods based upon the measurement of a quantity of electricity and the application of the above equation are termed " Coulometric titrations."

Coulometric technique is further subdivided into two subtechniques namely as ,

i) Coulometric analysis with controlled potential of the working electrode (CPC), and

ii) Coulometric analysis with constant current (CCC).

B) CONTROLLED POTENTIAL COULOMETRY :

In this method the substance being determined reacts with 100 percent current efficiency at a working electrode the potential of which is controlled. The completion of the reaction is indicated by the current decreasing to practically zero, and the quantity of the substance reacted is computed from the reading of a coulometer in series with the cell or by means of a current time integrating device.

In this technique, current is noted with time, as soon as the controlled potential is applied to the electrode system. With time, the current goes on decreasing in an exponential manner according to the equation,

$I_t = I_o \; e^{-Kt}$, where I_t is the current at time t, I_o is the initial current, t is the time in seconds and $K = \frac{DA}{SV}$; D being the diffusion constant of the reducible or oxidisable species ($Cm^2 \; S^{-1}$), A the electrode area (Cm^2), S the thickness of the diffusion layer (Cm), and V the same solution volume (Cm^3). In electrolysis at controlled potential, the quantity of electricity Q (coulombs)passed from the begining of the determination to time t is given by

$$Q = \int_o^t I_t \, dt$$ where It is the current at time t.

Experimental curve obtained is plot of current Vs time and the number of coulombs are obtained by the integration of the area under the curve. The integration can be accomplished by means of mechanical, electrical or electronic integrators, Now a days digital accumulators are in use.

The necessary components of a controlled potential coulometric set up are :

i) the coulometer or other method for determining the quantity of electricity.
ii) the controlled source of current.
iii) the electrolysis vessel.

C) CONTROLLED CURRENT COULOMETRY OR COULOMETRIC TITRATIONS :

When the current efficiency of the electrode falls below 100 percent, it is not possible to employ the controlled potential coulometry. This may happen when the concentration of the substance becomes very low or electrode surface gets adsorbed by the species present in the solution. Constant current coulometry consists of generating a titrant with a current of 100 % efficiency and this titrant then undergoes a stoichiometric reaction with the species being determined. The quantity of substance reacted is calculated with the aid of Faraday's law, and the quantity of electricity passed can be evaluated simply by timing the electrolysis at constant current. Since the current can be varied from 0.1 to 100 mA., amounts of material corresponding to 1×10^{-9} to 1×10^{-6} equivalent per second of electrolysis time can be determined. In titrimetric analysis the reagent is added from a burette; in Coulometric titrations the reagent is generated electrically, either directly within the test solution or, less frequently, it may be generated in an external solution which is allowed to run continuously into the test solution. Then, its amount is evaluted from a knowledge of the current and the generating time. The electron becomes the standard reagent.

The coulometric titrimetry has several advantages over classical titrimetry.

I) ADVANTAGES OF COULOMETRIC TITRATION :

1) Standard solutions are not required, hence no problem of their preparation, standardisation and storage. In their place the coulomb becomes the primary standard.

2) Unstable reagents, such as bromine, chlorine, argentic ion (Ag^{+2}) and titanous ion, can be utilised successfully, since they are generated and consumed immediately.

3) The method is practically useful in the range from milligram to microgram quantities.

4) The same solution is not diluted in the internal generation procedure.

5) By pre-titration of the generating solution before the addition of the sample, more accurate results can be obtained. The end point indicator corrections are thus automatically cancelled and the effect of impurities in the generating solution is minimised.

6) Only fundamental quantities such as current and time are involved which can be measured with high precision and hence high accuracy can be achieved.

7) The method, being largely electrical in nature, is readily adapted to remote control. This is significant in the titration of radioactive or dangerous materials.

II) DETECTION OF END POINT :

Several methods are used for the detection of end points in coulometric titrations. These are :

1) Use of Chemical Indicators :

The end point can be found by means of chemical indicators, Provided the indicator itself is not electro-active. Examples include methyl orange for bromine, starch for iodide, dichlorofluoroscein for chloride, and eosin for bromide and iodide.

2) By Potentiometric Observations :

In this method the electrolytic generation is continued until the emf of the indicator reference electrode assembly placed in the test solution attains a predetermined value corresponding to the equivalence point.

3) By Amperometric Procedures :

These are based upon the establishment of conditions such that the substance being determined, or, more usually, titrant undergoes reaction at an indicator electrode to produce a current which is proportional to the concentration of the electroactive substance, maintaining the potential of the indicator electrode constant, the end point can be established from the course of the current change during the titration.

4) By Application of the Dead-Stop Procedure(Biamperometric):

In this method the titration is performed by using two small but similar platinum electrodes to which a small emf (1-100 milli volts) is applied, the end point is usually shown by either the disappearance or the appearance of the current flowing between the two electrodes. The essential requirement of this procedure is that a reversible oxidation reduction system be present either before or after the end point.

5) By Spectrophotometric Observations :

In this method the end point is determined by measuring the optical density of the test solution during the course of titration at an appropriate wavelength. Before the end point the optical density increases slowly, but rapid and linear response occurs beyond the equivalence point.

III) INSTRUMENTATION FOR COULOMETRY :

The necessary components for a Coulometric analysis are :

1) Constant Current Sources :

The currents used in coulometric titrations are usually in the range 1-50 milliamperes. Fairly constant currents are conveniently obtained from batteries with a series regulating resistance : Seven 6 Volt car (or storage batteries in series yielding the equivalent of a 42 volt) battery will be found satisfactory. More rigorous control of the current may be achieved by the use of commercially available stabilised power units, designed to replace batteries for voltages of 0-100 volts and currents upto 50 or 100 milliampere

2) Current Measuring Devices :

The obvious means for measuring the current is a carefully calibrated milliammeter. A more precise method is to determine with a good potentiometer the voltage drop E, across a precision resistance (50-100 Ohms, R_1) in series with the electrolysis cell. The electrolysis current Ie is calculated from the equation $I_e = \frac{E_1}{R_1}$

3) Time Measurement :

An ordinary electric stop clock operated by closing and opening the electrolysis circuit is not very satisfactory because of the appreciable lag and 'Coast'of the motor. The best type of electric stop clock is that fitted with magnetic breaks. Sometimes ordinary stop-watch is satisfactory.Current time integrating motor is also used for measuring quantity of electricity directly.

4) Electrolysis Cell for Coulometry :

The electrolysis cell contains the working or generator electrode, at which the reagent is electrogenerated, and the auxiliary electrode. Generator electrode may be of platinum, Silver, or mercury in the form of mercury pool; auxiliary electrode is usually of platinum.

The auxiliary electrode is generally placed in a separate glass tube closed at its lower end by a fine porosity glass disc, the level of the solution in this compartment must be maintained at a higher level and greater ionic strength than the solution in the titration cell, so as to prevent diffusion of the latter into the isolated compartment. The electrolyte in which auxiliary electrode is immersed may be either the same as the supporting electrolyte in the test solution or some other innocuous electrolyte appropriate to the particular case.

In addition there are two indicator electrodes. These may consist of a tungsten pair for a dead-stop end point;for an amperometric end-point the indicator electrodes may both be of platinum foil or one can be platinum and the other a saturated calomel reference electrode.

The coulometric cell consists of a tall form beaker (without lip) of about 150 or 200 ml capacity. Provision is made for magnetic stirring and for passing a stream of inert gas through the solution. The main generator electrode may consist of a platinum foil (1 x 1 cm or 4 x 2.5 cm)and the auxiliary electrode may consist of platinum foil (1 x 1 cm or 4 x 2.5 cm) bent into a half cylinder so as to fit into a wide glass tube. The isolation of the auxiliary generator electrode within the glass cylinder (closed by a sintered glass disc) from the bulk of the solution avoids any effects arising from undesirable reactions at this electrode. The nature of the indicator electrodes will depend upon the procedure adopted for the detection of the end point. In general, the indicator electrodes should be positioned outside the electric field (current path) between the generator electrodes, otherwise spurious indicator currents may be produced, particularly in the amperometric detection of the end point.

1.3: THE AIM OF THE PRESENT WORK :

It is evident from the survey of literature that considerable work has been done on estimation of different reducing sugars by a number of methods. For example, estimation of sugars by bromine has been reported by visual and potentiometric methods. 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, estimations 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 explanation for the observed results. The present study would be of value in sugar industry, biochemistry and medicinal chemistry.