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CARBOHYDRATE ANALYSIS AND KINETICS

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ABSTRACT

As products of natural origin, carbohydrates are among the most abundant, and are widely distributed in both the plant and animal kingdoms. We eat them directly in such foods as bread, potatoes, corn and peas and indirectly in meat, eggs and fats from animals which feed on carbohydrates in the form of grains and grasses. Cotton and linen, the traditional clothing fabrics, are both almost pure carbohydrates. Wood consists largely of cellulose and hence a good portion of houses in which we live, as well as much of our furniture, is constructed of carbohydrates. The paper is mostly carbohydrate. Fructose is the sweetest of all the sugars. It occurs widely and abundantly in nature. Fructose has been determined by various authors using various techniques like Liquid Chromatography, HPLC, Fused Silica Gas Chromatography, Mass Spectroscopy, High Performance Union Exchange Chromatography with Pulse Amperometry, Reversed Phase HPLC etc. The author has used polarographic methods of analysis for the analysis of fructose in different samples. In the present study Fructose has been determined polarographically in honey and in various fruits. Sugars such as the hexoses, give polarographic curve but the disaccharides such as sucrose and lactose, do not. Analytical use of reduction of fructose has been made in the study of inversion of sucrose.

Key words: Carbohydrate, Fructose, Glucose, Polarography, Direct current polarography, Differential pulse polarography.

INTRODUCTION

A carbohydrate is an organic compound that consists only of carbon, hydrogen, and oxygen, Structurally they are polyhydroxy aldehydes and ketones. Carbohydrates perform numerous roles in living organisms. Polysaccharides serve for the storage of energy (e.g., starch and glycogen), and as structural components (e.g., cellulose in plants and chitin in arthropods). The 5-carbon monosaccharide ribose is an important component of coenzymes (e.g., ATP, FAD, and NAD) and the backbone of the genetic molecule known as RNA. The related deoxyribose is a component of DNA. Saccharides and their derivatives include many other important biomolecules that play key roles in the immune system, fertilization, preventing pathogenesis, blood clotting, and development[1].

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In food science and in many informal contexts, the term carbohydrate often means any food that is particularly rich in the complex carbohydrate starch (such as cereals, bread, and pasta) or simple carbohydrates, such as sugar (found in candy, jams, and desserts).

Carbohydrates are often found in many energy bars and isotonics due to the energy that is contained. The production of carbohydrate in nature occurs in green plants by process called photosynthesis. Plants contain the green pigment chlorophyll, which catalyses the conversion of carbon dioxide and water into sugar. The necessary energy for the reaction is supplied by the sun in the form of sunlight. Carbohydrates are usually classified as monosaccharides, oligosaccharides and polysaccharides. D-fructose which occurs naturally is laevorotatory and is therefore also known as levulose or fruit sugar. In a free state, it is found in fruits, honey[2], and as sole sugar in bull[3] and human[4] semen. In combination, invariable as a D-fructofuranose, it is present in sucrose[5]. In trisaccharides such as melezitose, gentianose and raffinose, in the tetrasaccharide slachyose, the pentasaccharide verbascose and in many polysaccharides notably inulin, phlein, triticin and irisin. L-fructose does not occur naturally[6]. Carbohydrates have been studied by various analytical methods during the past. These include chromatographic and electrophoretic methods, chemical methods, titration methods, gravimetric methods, colorimetric methods, enzymatic methods, Polarimetry, Infrared[7] chromatographic purification techniques are followed by mass spectrometry and/or NMR spectroscopy. Absolute molecular weights of larger molecules are determined by light scattering[8]. Lee has used high-performance anion-exchange chromatography for carbohydrate analysis[9]. Carbohydrate analysis by a phenol-sulfuric acid method in microplate format are also reported[10].

Polarography is a very simple and accurate method for analysis of carbohydrates. Polarographic method have been used for determination of 3-keto sugars[11]. The polarographic determination of periodate and lead (IV) ions in the analysis of carbohydrate oxidations have been reported in the research work of Corlett[12]. Differential pulse polarographic determination of Cd(II) and Pb(II) in milk samples after solid phase extraction using amberlite XAD-2 rasin modified with 2,2'-$DPED_3P$ is done by Shreedhar *et al.*[13] The hydrolysis of sucrose by boiling with a mineral acid, or by the enzyme invertase, produce a mixture of equal molecules of D-glucose and D-fructose.

$$C_{12}H_{22}O_{11} + H_2 \xrightarrow[\text{or invertase}]{H^+} \underset{\text{D-glucose}}{C_6H_{12}O_6} + \underset{\text{D-fructose}}{C_6H_{12}O_6}$$

Sucrose solution is dextro rotatory but during hydrolysis it becomes laevorotatory.

The specific rotation of sucrose is + 66.5°. D-glucose has specific rotation +52° and D-fructose-92°. Therefore the net specific rotation of an equimolar mixture of D-fructose is –

$$\frac{+52-92^{o}}{2} = -20^{o}$$

In the process of hydrolysis, the specific rotation changes from +66.5° to -20°. The sign of the specific rotation changes from (+) to (-), or is said to 'Invert'. Hence the hydrolysis of sucrose to D-glucose and D-fructose is termed 'Inversion' and the hydrolysis mixture is called 'Invert-sugar'.

$$\underset{(\text{Specific rotation} + 66.5)}{\text{Sucrose}} \xrightarrow{\text{Inversion}} \underbrace{\text{D Glucose} + \text{D Fructose}}_{\text{Invert sugar (Specific rotation} - 20^{o})}$$

The enzyme that brings about inversion is named as invertase. The rate of inversion can be studied from the increase in concentration of fructose.

In the present paper, the polarographic behaviour of fructose has been reported. It's qualitative and quantitative determination in various fruits like grapefruit (*Vitis vinifera*) and apple (*Malus domestica*) has been done.

The author has also determined fructose, qualitiatively and quantitatively, in honey. The rate of inversion of sugar, has been studied polarogrpahically from the increase in the wave of fructose.

EXPERIMENTAL

Solutions

1 M solution of fructose, LiCl and calcium chloride were prepared by dissolving a requisite quantity of the compound in distilled water. 0.1% gelatin was prepared by dissolving a weighed amount in warm distilled water. pH adjustments were made using dilute solutions of HCl/NaOH. Sugar, honey and fruit juice solutions were prepared by dissolving a requisite quantity of sample in distilled water.

Determination of fructose

1 mL of 1 M solution of fructose was transferred to a polarographic cell containing 10 mL of 1 M $CaCl_2$. 5 mL of 0.1% gelatin was added to the solution as a maximum suppressor. The volume of the analyte was made up to 100 mL with distilled water. The pH

of the solution was adjusted to 6.7. Polarogram was recorded keeping the initial EMF set to -1.5 V in the presence of air[14].

Calibration curve was obtained by taking various concentrations of fructose under the identical experimental conditions.

Fructose was also polarographed using LiCl as supporting electrolyte. 1 mL of 1 M fructose was transferred to a polarographic cell containing 10 mL of 1 M LiCl. The volume of the test solution was made up to 100 mL with distilled water. pH of the solution was adjusted to 7.0 with dilute solutions of NaOH/HCl. The solution was polarographed keeping the initial emf set to -1.6V in the presence of air.

Calibration curve was obtained under the identical experimental conditions with varying concentrations of fructose.

Determination of fructose in samples

(i) Determination in fruit juices

2.5 mL of fruit juice (freshly extracted) was transferred to the polarographic cell. 10 mL of 1 M $CaCl_2$ and 5 mL of 0.1% gelatin was added to it and the solution was diluted to 100 mL with distilled water. The polarogram was then recorded in the presence of air, under the above mentioned experimental conditions. The concentration of fructose was determined by standard addition method and calibration curve.

(ii) Determination in honey

The determination of fructose in honey was carried out by preparing a 1% solution of honey in water and adding 3 mL of this solution to 10 mL of 1 M LiCl. The volume was made up to 100 mL with distilled water. The polarogram was then recorded under the above mentioned experimental conditions. The concentration of fructose was determined by standard addition method and calibration curve.

(iii) Study of inversion of sugar

Polarographically the study of the rate of inversion of cane sugar was carried out with a 6% solution of sucrose in 0.1 N HCl in a thermostat at 25^{o}C. At fixed intervals of time, a 10 mL sample was taken out, made slightly alkaline with 10 mL of 1 M LiCl and NaOH, diluted 10 times with distilled water and polarographed in air under the indentical conditions as discussed earlier.

RESULTS AND DISCUSSION

Sugars such as the hexoses, give polarographic curve but the disaccharides such as sucrose and lactose, do not[15]. Normal waves comparable with diffusion currents are given only by ketoses where as glucose gives a reduction wave with a height which is much smaller than what would be expected for its given analytical concentration[16].

Fructose produces a polarographic wave/peak in 0.1 M solution of $CaCl_2$ at pH 6.7 with half wave potential of - 1.65 V and peak potential (DPP mode) of -1.74 V *vs* SCE (Fig. 2 and Fig. 3). The log plot slope of fructose in 0.1 M $CaCl_2$ and 0.1 M LiCl are shown in Fig 5 and 6. The log plot slope suggest a 2 electron reduction of fructose. There is no change in the $E_{1/2}$ value with change in pH. However, in an acid medium the hydrogen wave starts before the ketone wave[17]. The effect of pH on DC and DP polarograms of fructose in 0.1 M $CaCl_2$ is shown in Fig. 7 and Fig. 8.

Disaccharides (sucrose, maltose, lactose) are reported not to be reduced at the DME[18]. Analytical use of reduction of fructose can be made in determination of this substance in the presence of sucrose and glucose[19].

The height of the wave/peak of fructose is directly proportional to the concentration of fructose in the solution. The DC and DP polarograms of fructose in 0.1 M $CaCl_2$ at pH 6.7 are depicted in Fig. 2 and Fig. 3. The effect of concentration on DC and DP polarograms of fructose in 0.1 M LiCl at pH 7.0 is depicted in Fig. 1 and Fig. 4. The DC and DP polarograms of grape fruit juice, apple juice and honey are shown in Fig. 9 and Fig. 10. By this technique, the results that are found under the above mentioned experimental conditions are shown in Table 1.

Table 1: Results* on various plant products for their fructose content (mg/mL)

Sample	Parameter	DCP		DPP	
		Added	Found	Added	Found
Grape fruit juice	Amount	-	20.16	-	20.8
		18.0	38.10	18.0	38.78
	%R	99.8		99.9	
	RMD	0.001		0.001	
	SD	0.03		0.03	
	CV	0.14		0.14	

Cont…

Sample	Parameter	DCP		DPP	
		Added	Found	Added	Found
Apple juice	Amount	-	66.24	-	66.30
		72.0	138.15	72.0	138.22
	%R	99.9		99.9	
	RMD	0.001		0.001	
	SD	0.15		0.15	
	CV	0.22		0.22	
Honey	Amount	-	173.5	-	178.0
		180.0	352.8	180.0	353.8
	%R	99.8		99.9	
	RMD	0.006		0.003	
	SD	1.5		0.7	
	CV	0.8		0.4	

[*]Average of four determinations

The concentration of fructose in grape fruits, apple and honey was found to be 2.0%, 6.6% and 17.4%, respectively. The results are in good agreement with those reported in the literature[14].

The statistical analysis of the results is shown in Table 2 percentage recovery in all the cases is found to be more than 99.8%. The values of RMD, SD and CV, which were less than 0.006, less than 0.7 and less than 0.8, respectively, speak the reliability of the data.

Table 2: Final analyses results on plant products for their fructose content (Percentage)

Sample	DCP	DPP	Reported in literature[*]
Grapefruit juice	2.05	2.08	2.15
Apple juice	6.65	6.68	6.66
Honey	17.5	17.8	20.0

[*]M. Brezina and P. Zuman, Polarography in Biochemistry, Pharmacy and Medicine, Interscience Publishers, INC, New York (1958) p. 284

Figs. 11 and 12 show the polarographic investigation of the inversion of sucrose from the increase in the wave of fructose. The various curves shown in the figure are obtained by taking 10 mL of solution from 6% solution of sucrose in 0.1 N HCl at 25°C, after a definite time (given in hours). The fructose wave increases at -1.7 V proportionally to its concentration. The reaction -

$$\underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} + H_2O \longrightarrow \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Fructose}}{C_6H_{12}O_6}$$

Obeys the first order rate law as the amount of water present is in such an excess that its concentration may be considered to remain constant. The reaction is therefore a pseudounimolecular reaction. It has been studied by observing the increase in the polarographic wave of fructose at regular time intervals.

The data has been depicted in Tables 3 and 4. The constant value of k shows that the reaction is of first order. On the basis above data and ongoing discussion it could be concluded that the polarographic method is an accurate and reliable method for the study of kinetics of inversion of sucrose.

Table 3: Change in concentration of fructose with time

Time (hrs.)	Concentration of fructose (mM)
1	0.031
2	0.051
3	0.054
24	0.057
48	0.059

Table 4: Rate constant for inversion of sucrose

t	a	a - x	k_1
1	1.75	1.719	0.0177/hr
2	1.75	1.699	0.0176/hr
3	1.75	1.696	0.0175/hr
24	1.75	1.693	0.0174/hr
48	1.75	1.691	0.0174/hr

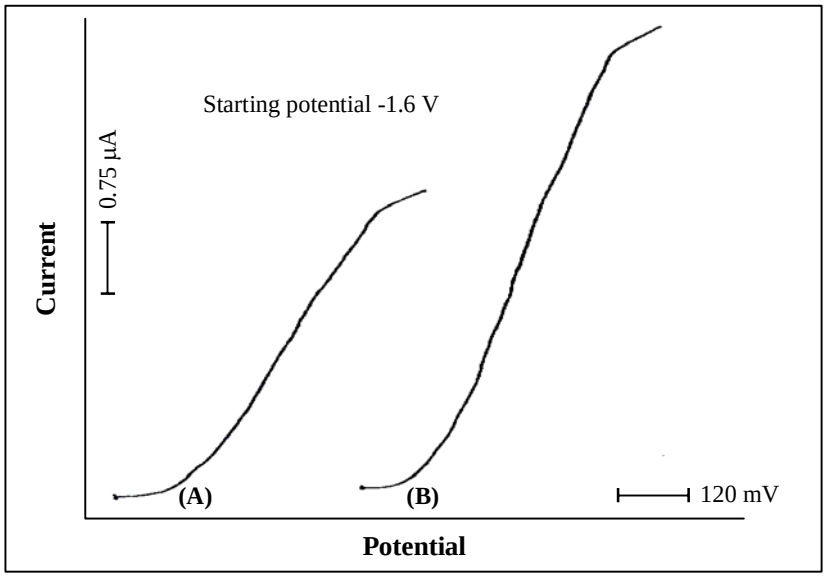

Fig. 1: DC Polarograms of fructose in 0.1 M LiCl at pH 7.0 showing effect of concentration (A) 18.0 mg, (B) 36.0 mg (per 100 mL of analyte)

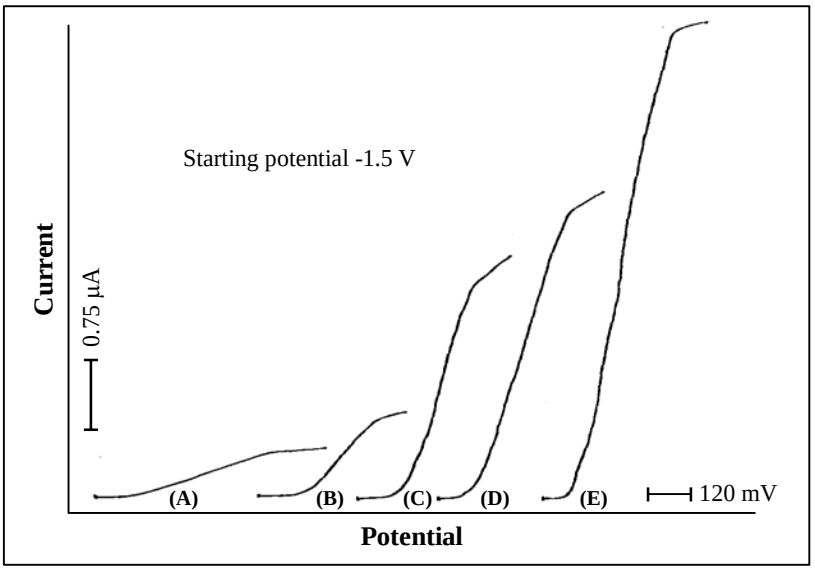

Fig. 2: DC Polarograms of fructose in 0.1 M $CaCl_2$ at pH 6.7 showing effect of concentration (A) 18.0 mg, (B) 36.0 mg, (C) 72.0 mg, (D) 90.0 mg, (E) 270 mg (per 100 mL of analyte)

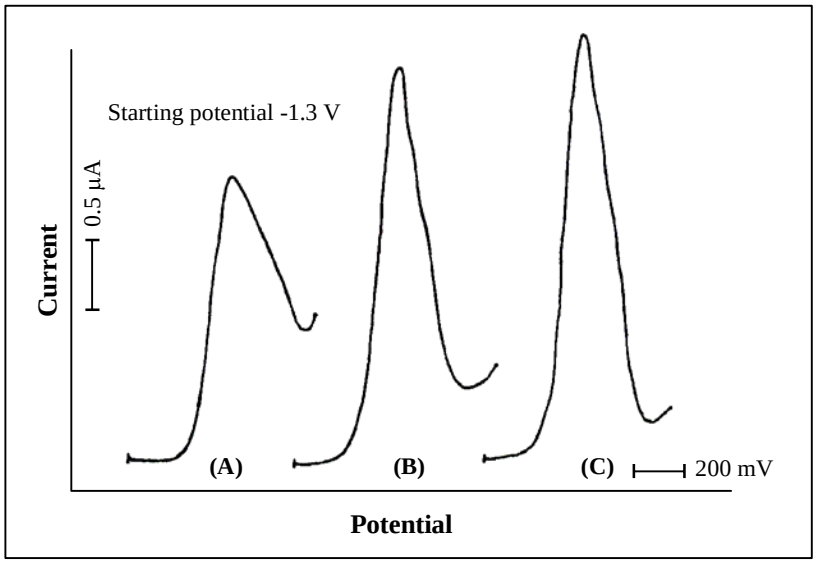

Fig. 3: DC Polarograms of fructose in 0.1 M $LaCl_2$ at pH 6.7 showing effect of concentration (A) 57.0 mg, (B) 72.0 mg, (C) 90.0 mg (per 100 mL of analyte)

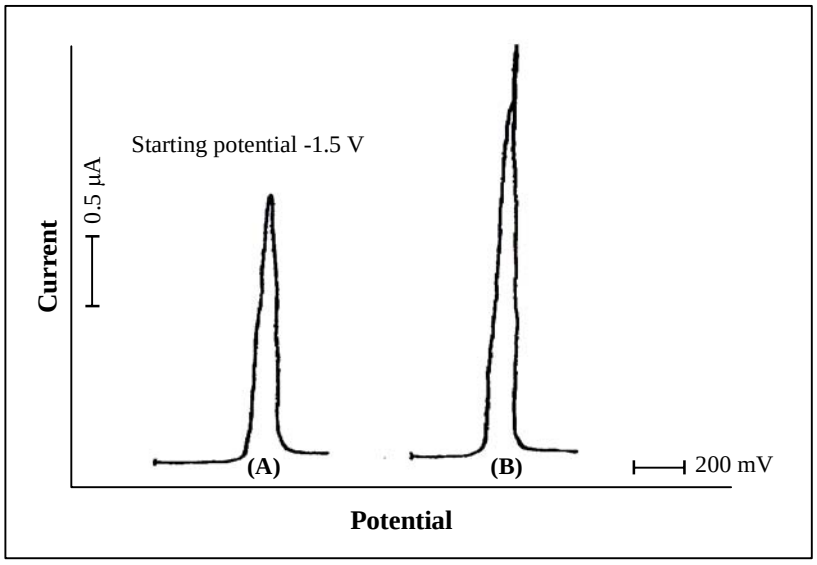

Fig. 4: DP Polarograms of fructose in 0.1 M LiCl at pH 7.0 showing effect of concentration (A) 18.0 mg, (B) 36.0 mg (per 100 mL of analyte)

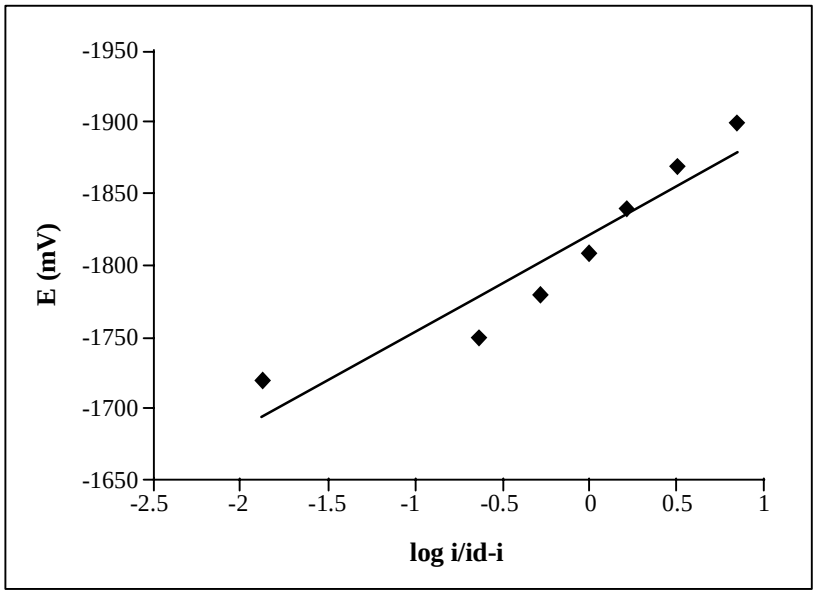

Fig. 5: Plot of E vs log i/(id-i) for fructose in 0.1 M LiCl at pH 7.0

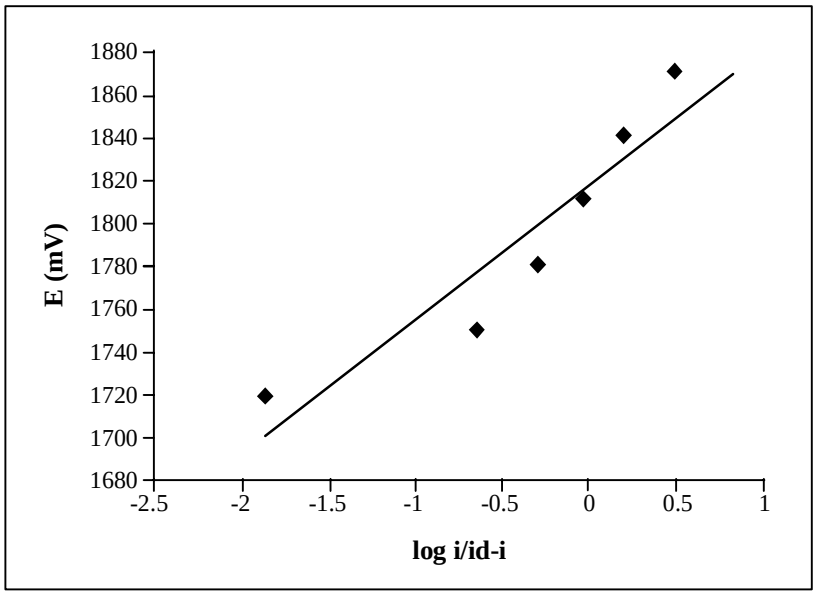

Fig. 6: Plot of E vs log i/(id-i) for fructose in 0.1 M $CaCl_2$ at pH 6.7

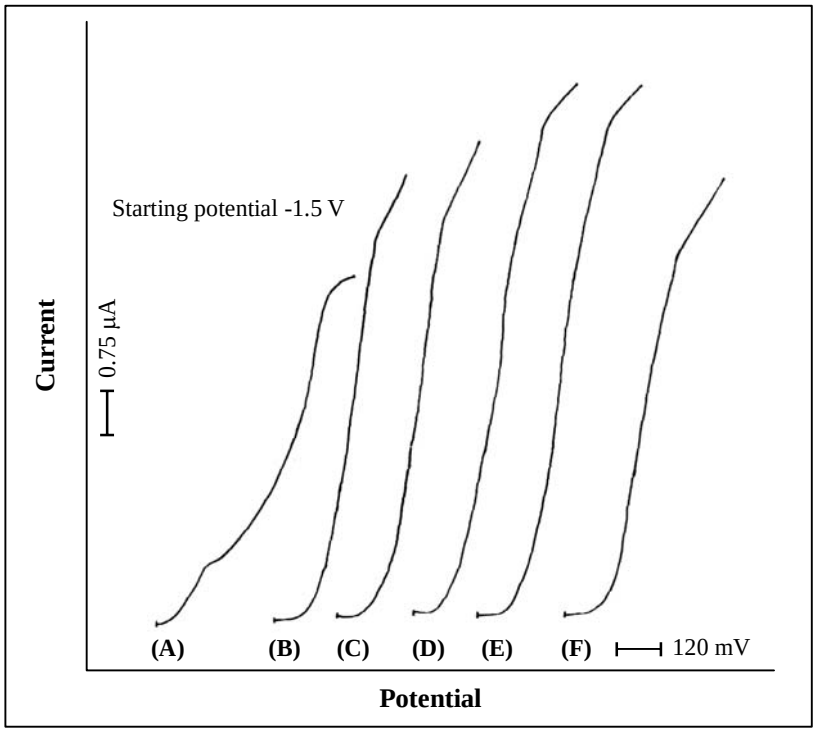

Fig. 7: DC Polarograms of 90.0 mg fructose in 0.1 M $CaCl_2$ at various pH. Total volume of analyte was 100 mL (A) pH 2.5, (B) pH 3.4, (C) pH 5.0, (D) pH 6.7, (E) pH 8.0, (F) pH 9.5

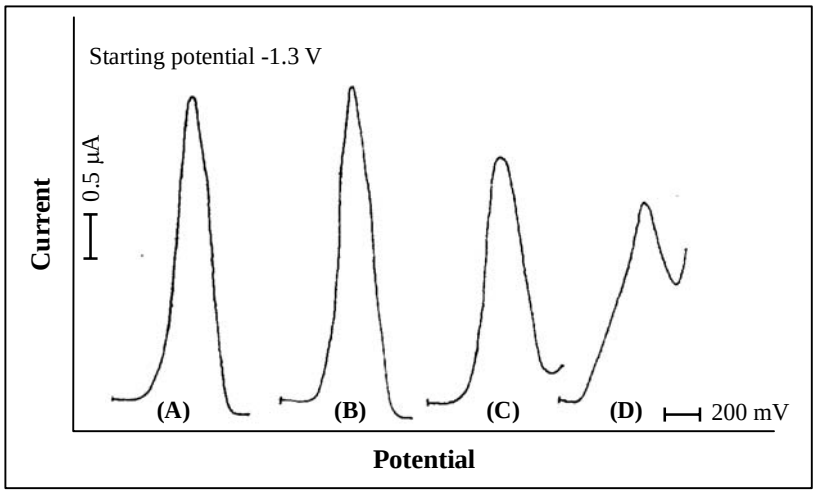

Fig. 8: DP Polarograms of 90.0 mg fructose in 0.1 M $CaCl_2$ at various pH. Total volume of analyte was 100 mL (A) pH 3.2, (B) pH 5.1, (C) pH 7.8, (D) pH 8.1

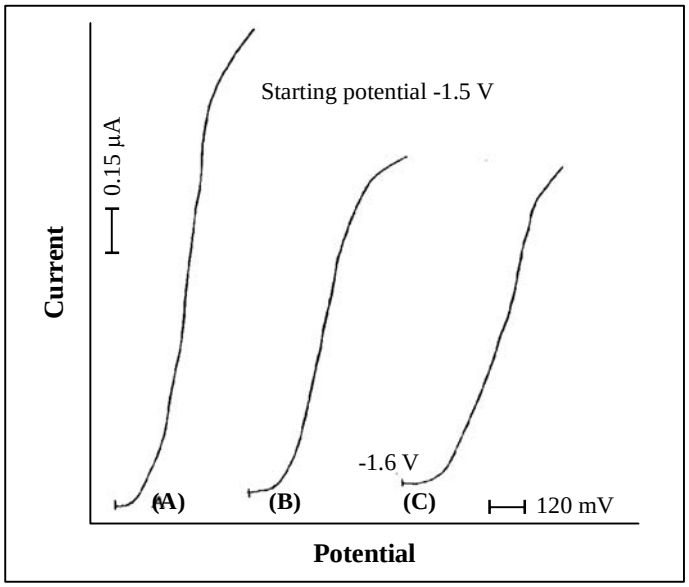

Fig. 9: DC Polarograms of plant products (A) Apple juice in 0.1 M $CaCl_2$ at pH 6.7, (B) Grape fruit juice in 0.1 M $CaCl_2$ at pH 6.7 (2.5 mL of fruit juice per 100 mL of analyte) (C) Honey in 0.1 M LiCl at pH 7.0 (3 mL of 1% honey per 100 mL of analyte)

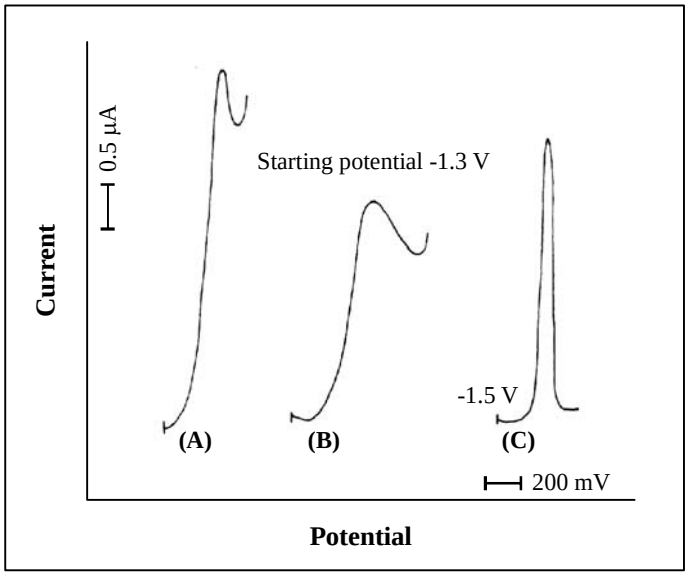

Fig. 10: DP Polarograms of plant products (A) Apple juice in 0.1 M $CaCl_2$ at pH 6.7, (B) Grape fruit juice in 0.1 M $CaCl_2$ at pH 6.7 (2.5 mL of fruit juice per 100 mL of analyte) (C) Honey in 0.1 M LiCl at pH 7.0 (3 mL of 1% honey per 100 mL of analyte)

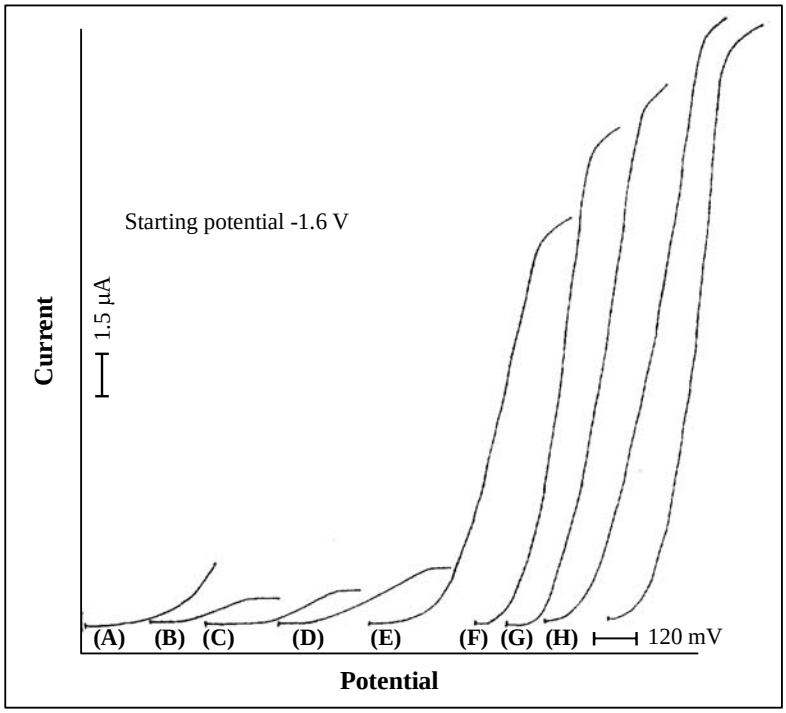

Fig. 11: DC Polarographic investigation of the inversion of sucrose from the increase in the wave of fructose. From a 6% solution of sucrose in 0.1 N HCl, after a definite time (given in hrs), 10 mL solution was taken, neutralized with 5 mL of 1 M LiCl and 5 mL of 0.5 M NaOH and diluted ten fold before polarographic analysis (A) 0 hrs, (B) 2 hrs, (C) 3 hrs, (D) 4 hrs, (E) 24 hrs, (F) 48 hrs, (G) 72 hrs, (H) 120 hrs, (I) 144 hrs

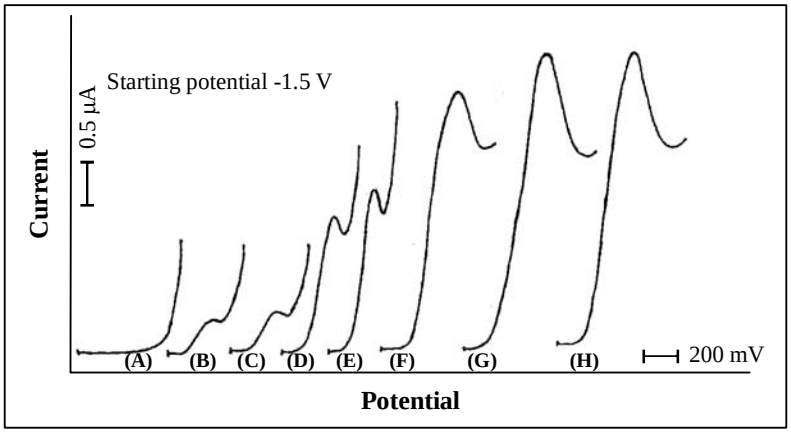

Fig. 12: DP Polarographic investigation of the inversion of sucrose (A) 0 hrs, (B) 1 hrs, (C) 2 hrs, (D) 3 hrs, (E) 4 hrs, (F) 48 hrs, (G) 72 hrs, (H) 120 hrs

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