REACTIONS OF OLIGOSACCHARIDES

I. Ferricyanide Numbers[1]

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ABSTRACT

The ferricyanide number of a carbohydrate (the number of ml. of 0.1000*N* ferricyanide reacting with 1 g. of substance) is used in determining the reducing power of starches, dextrins, and syrups. The Schoch and Jensen procedure for determining the ferricyanide number was applied to glucose and to a series of glucose polymers prepared by macro paper chromatogaphy and ranging in size from 2 to 10 glucose units. When the results were calculated in terms of equivalents of ferricyanide reagent consumed per equivalent of reducing sugar, a constant stoichiometry was observed for the members of the series higher than maltotriose. G_1 to G_3 had lower but progressively increasing molecular reducing power. Changing the base employed in the ferricyanide reagent altered the stoichiometry. Use of a saturated calcium hydroxide reagent gave a reaction stoichiometry that was relatively constant for glucose and for all of the glucose polymers. Variation of sugar concentration during the determination had little effect on the results when sodium carbonate was the alkali used, but was important when barium or calcium hydroxide was used.

For many years it has been evident that the properties of naturally occurring or man-made carbohydrate materials containing mixtures of oligosaccharides are at least partially dependent upon the properties of the individual sugars. However, study of the isolated components of such mixtures has been limited because of the difficulty of obtaining quantities sufficient for analytical work. Recent development of macro paper chromatography of amylose hydrolysates (1) has led to isolation of the whole series of chromatographically pure linear glucose[3] polymers, up to a degree of polymerization of 11 in gram quantities.

A property of great analytical interest in this field is reducing power. Unfortunately, there are nearly as many procedures for determining reducing power of cereal products as there are investigators, and each is essentially an empirical method. These procedures can be divided into four main groups: copper, ferricyanide, hypohalite, and colorimetric (2,3,4,5,6,7). Others which do not fall into these general classifications are applicable in special situations. The present work is concerned with potassium ferricyanide oxidation. This method

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[3] The term "glucose" in this article represents alpha-D-glucose. Use and repetition of the longer term would be cumbersome.

was originally chosen for investigation because of its use in dextrin analysis; however, as more information has become available, its utility in the analysis of other starch hydrolysis products has become evident.

In the analysis of dextrins the term "ferricyanide number" has been used for some time (8). In more recent years it has been defined as the number of ml. of 0.1*N* ferricyanide reagent which will react with 1 g. of substance (9). The opinion of most investigators has been that the ferricyanide oxidation of reducing sugars is variable, depending on pH, time, quantity of reagent, temperature, etc. (3).

In this study the Schoch and Jensen procedure was applied to glucose and to a series of glucose polymers ranging from maltose to maltodecaose in order to determine the stoichiometry of the reaction under those conditions. When it was found that ferricyanide number was not directly proportional to the number of mols of sugar present for this series, an attempt was made to establish reaction conditions under which each mol of any of the sugars would reduce the same amount of ferricyanide.

The procedure was modified by employing calcium hydroxide or barium hydroxide as the alkaline ingredient of the ferricyanide reagent, and by varying the concentration of sugar in the samples analyzed.

Materials and Methods

Reagents. The reagents used in the ferricyanide procedure were prepared according to the method given by Hodge and Davis (9).

1. Potassium ferricyanide solutions. (a) Dissolve 16.5 g. of potassium ferricyanide and 22 g. of sodium carbonate in water and make up to 1 liter (0.2*M* carbonate). (b) Dissolve 16.5 g. of potassium ferricyanide and 3.8 g. of barium hydroxide in water and make up to 1 liter (0.0446*N* hydroxide). (c) Dissolve 16.5 g. of potassium ferricyanide and 1.65 g. of calcium hydroxide in water and make up to 1 liter (0.0446*N* hydroxide). Age these solutions 3 days, filter, and store in brown bottles away from sunlight.

2. Zinc sulfate-potassium chloride-acetic acid solution. Dissolve 20 g. of zinc sulfate heptahydrate, 70 g. of potassium chloride, and 200 ml. of glacial acetic acid in water and make up to 1 liter.

3. Potassium iodide solution. Dissolve 200 g. of potassium iodide in water and make up to 1 liter.

4. Sodium thiosulfate solution, 0.05*N*. Dissolve 12.4 g. of sodium thiosulfate pentahydrate in boiled water and make up to 1 liter.

Standardize the thiosulfate against reagent grade potassium dichromate. Borax may be added to prevent spoilage.

5. Starch indicator. Pour 5 g. of soluble starch slurried in 20 ml. of water slowly into a stirred boiling salt solution containing 150 g. of sodium chloride in 480 ml. of water. The cooled starch suspension should remain stable for several months.

Oligosaccharides. Reagent grade glucose and maltose were recrystallized and dried *in vacuo* to give the anhydrous sugars. The glucose polymers, maltotriose through maltodecaose, were separated from corn starch hydrolysates by macro paper chromatography (1) and isolated as amorphous powders by freeze-drying aqueous solutions of the eluted sugars. All of the polymers were rechromatographed twice and showed only one spot on a chromatogram. The solvent used in these separations contained 1-propanol, ethyl acetate, and water in the ratio of 7:1:3 by volume (Fig. 1).

Exhaustive efforts were made to assure both the purity of the individual sugars and their freedom from water or chromatographic solvents.

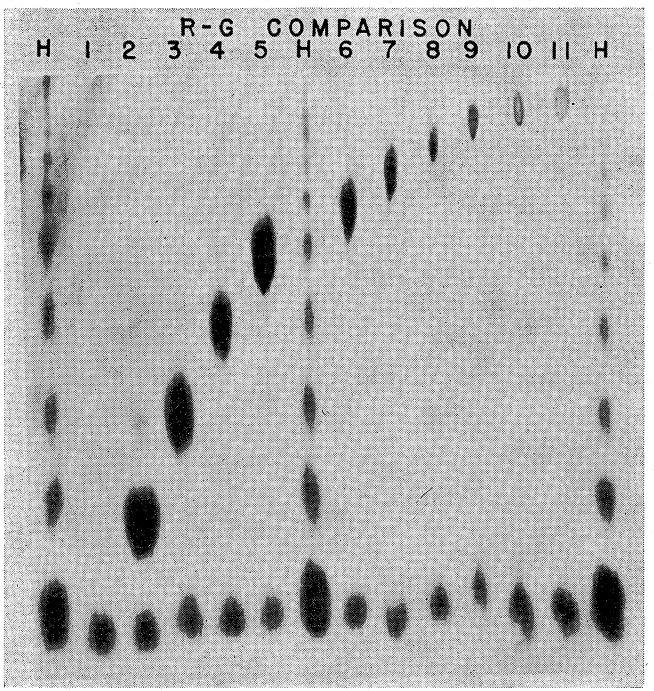

Fig. 1. Comparison of the relative rates of movement of glucose polymers, G_1 to G_{11}. H spots are from an acid-hydrolyzed corn amylose.

The heads and tails of the spots were cut off and discarded in each of three chromatographic purifications. Samples of the purified oligosaccharides were also chromatographed, using a solvent consisting of ethyl acetate, pyridine, and water (8:2:1), in an effort to separate them further. The materials used showed only one spot for each oligomer in each solvent system.

All oligosaccharides were freeze-dried at room temperature under high vacuum. They were then transferred immediately to an Abderhalden apparatus, where they were dried at 87°C. for 4 hr. at a pressure less than 0.1 mm. Hg. They were then stored in glass bottles in a desiccator over Drierite until required for analysis. Immediately prior to use they were dried again for 16 hr. in an oven at 75°C. under a vacuum of 27 in. of mercury. Air bled into the oven was passed through a drying train containing concentrated sulfuric acid.

Considering that the sugars were eluted from air-dried paper with a large quantity of water and that prior to freeze-drying the aqueous solutions were evaporated under reduced pressure in a rotating flash evaporator to about 15% solids, it is unlikely that any traces of 1-propanol from the solvent remained associated with the oligosaccharides.

Procedure. Transfer exactly 25 ml. of the alkaline ferricyanide reagent to the reaction flask containing about 0.05 meq. of oligosaccharide dissolved in 25 ml. of water. Cover the flask with a vented cap and heat the solution in a boiling-water bath for exactly 15 min. Quench the reaction by cooling in tap water and add 60 ml. of zinc sulfate solution to complex the ferrocyanide and to acidify the reaction mixture. Add 20 ml. of the potassium iodide solution to reduce the excess ferricyanide and titrate the liberated iodine with the standard thiosulfate, using the starch indicator. Run a blank on 25 ml. of water.

Calculation. The ferricyanide number is obtained from the expression:

$$\frac{(\text{ml. for blank} - \text{ml. for sample}) \times (\text{normality of thiosulfate})}{(\text{sample weight in g.}) \times (0.1)}$$

The divisor (0.1) is used to conform to the definition of ferricyanide number which is based on 0.1*N* thiosulfate.

To determine the equivalents of ferricyanide consumed per equivalent of oligosaccharide, the following expression is used:

$$\frac{(\text{ferricyanide number}) \times (0.1) \times (\text{polymer molecular weight})}{1{,}000}$$

Results and Discussion

The data obtained in these experiments are summarized in Tables I, II, III, and IV and in Figs. 2 and 3.

TABLE I
FERRICYANIDE NUMBERS OF GLUCOSE POLYMERS

	Na_2CO_3, 0.2*M*	$Ba(OH)_2$, 0.0446*N*	$Ca(OH)_2$, 0.0446*N*		Na_2CO_3, 0.2*M*	$Ba(OH)_2$, 0.0446*N*	$Ca(OH)_2$, 0.0446*N*
Glucose (G_1)	305	307	255	G_6	105.5	78	67
Maltose (G_2)	243	202	125	G_7	87	61	44.5
G_3	176	128	102	G_8	75	55.5	39
G_4	147	99	70	G_9	68	..	..
G_5	123	81	56	G_{10}	66	46	33

In Table I the ferricyanide numbers of the various members of the series are shown. There appears to be a regularly decreasing order for the sodium carbonate figures; however, closer examination shows that the change from glucose to maltose and from maltose to maltotriose is different. In the barium and calcium hydroxide data the change is regular except for the sixth member of the series. This anomalous behavior was investigated thoroughly and was found to be reproducible. The chromatogram in Fig. 2 shows that the G_6 member is located between the G_5 and the G_7 and that the polymer is chromatographically pure. This information on the ferricyanide numbers is also shown graphically in Fig. 3. The displacement of the G_6 data

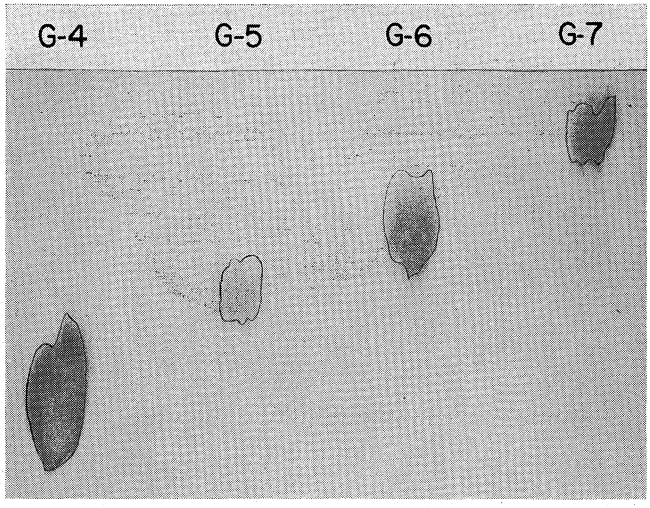

Fig. 2. Comparison of the chromatographic purity of maltotetraose, maltopentaose, maltohexoase, and maltoheptaose. Solvent composed of 1-propanol, ethyl acetate, and water. Polymer separation, 72 hr. (7:1:3).

is more evident in this representation and shows up even in the sodium carbonate curve. The G_9 data for the barium and calcium hydroxides were not obtained, owing to scarcity of materials.

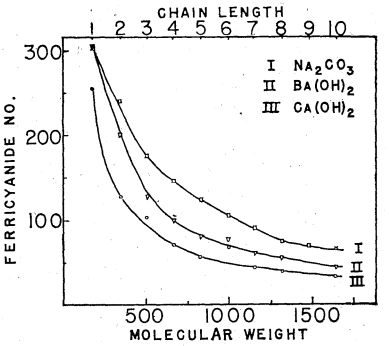

Fig. 3. The effect of 0.2*M* sodium carbonate, 0.0446*N* barium hydroxide, and 0.0446*N* calcium hydroxide on the ferricyanide numbers of the glucose polymers ranging from G_1 to G_{10}.

Table II shows the effect of reducing sugar concentration on the ferricyanide number for various members of the series with the sodium carbonate procedure.

The effect of glucose concentration on the ferricyanide numbers for sodium carbonate-, barium hydroxide-, and calcium hydroxide-catalyzed oxidation is shown in Table III. Variations of the results for sodium carbonate are considered to be within experimental error, but with barium and calcium hydroxides the ferricyanide number drops significantly with increasing sugar concentration. Currently, investigations are being carried out using the higher members of the polymer series with the alkaline earth hydroxides; preliminary results indicate that the concentration effect is less pronounced or negligible for the sugars above glucose. For example, the ferricyanide

TABLE II

EFFECT OF SUGAR CONCENTRATION ON THE FERRICYANIDE NUMBER (Na_2CO_3)

Glucose	$Fe(CN)_6$ No.	Maltotriose	$Fe(CN)_6$ No.	Maltopentaose	$Fe(CN)_6$ No.	Maltoheptaose	$Fe(CN)_6$ No.
mg.		*mg.*		*mg.*		*mg.*	
5	300	9.2	174	12.1	124	14.6	89
10	305	18.4	174	24.3	125	51.2	87
15	303	27.7	178	36.4	125		
20	307	36.9	176	48.5	125		
		46.1	175	60.6	124		

number of maltotriose, using 0.0446*N* calcium hydroxide reagent, is 101 for 10 mg. of sugar and 103 for 20 mg.

TABLE III

EFFECT OF GLUCOSE CONCENTRATION ON THE FERRICYANIDE NUMBERS

GLUCOSE	FERRICYANIDE NUMBER		
	0.2*M* Na_2CO_3	0.0446*N* $Ba(OH)_2$	0.0446*N* $Ca(OH)_2$
mg.			
5	300	330	270
10	305	310	265
15	303	300	253
20	307	290	235

The ferricyanide numbers obtained for glucose and maltose with sodium carbonate as the catalyst (305 and 243) compare favorably with figures given in the literature. Usually these figures are presented as mg. of glucose or maltose per ml. of 0.1*N* thiosulfate, or in some similar form. Various factors and tables have been developed for converting the number of ml. of thiosulfate consumed by an unknown reducing sugar mixture to mg. of glucose or maltose. However, unless the unknown sample contains predominantly glucose or maltose, the use of such factors will give misleading results. To demonstrate this more strikingly, the data for the ferricyanide numbers have been tabulated by the number of equivalents of ferricyanide reagent consumed per equivalent of reducing sugar. This gives the stoichiometric factors shown in Table IV. They indicate that the reaction stoichiometry for glucose is about 5 to 1 for all three alkaline reagents studied. However, the carbonate stoichiometry approaches 10 to 1 for the higher members of the series, the barium hydroxide 7 to 1, and the saturated calcium hydroxide 5 to 1, with the outstanding exception of G_6, which gives high results with all alkalis. Other deviations which will require further investigation are the low maltose and high maltotriose values in the calcium hydroxide series.

TABLE IV

STOICHIOMETRIC FACTORS[a]

POLYMER	Na_2CO_3, 0.2*M*	$Ba(OH)_2$, 0.0446*N*	$Ca(OH)_2$, 0.0446*N*	POLYMER	Na_2CO_3, 0.2*M*	$Ba(OH)_2$, 0.0446*N*	$Ca(OH)_2$, 0.0446*N*
G_1	5.5	5.5	4.5	G_6	10.5	7.7	6.6
G_2	8.3	6.9	4.3	G_7	10.0	7.0	5.1
G_3	8.9	6.5	5.0	G_8	9.9	7.3	5.1
G_4	9.8	6.6	4.7	G_9	10.0	..	..
G_5	10.2	6.7	4.6	G_{10}	10.8	7.5	5.4

[a] (Molecular weight × ferricyanide number)/1,000.

These studies indicate that it may be possible to develop an analytical system in which the stoichiometry will be the same for all members of the series. Such a system, if developed, could eliminate a large number of the empirical reducing-power methods and provide a rational method for sugar determination.

Fortunately, the analysis of dextrins, which contain little glucose or maltose, is not seriously affected by the differences in stoichiometry observed with sodium carbonate, the reagent normally used in ferricyanide oxidations. Use of this reagent for mixtures of low-molecular-weight glucose polymers becomes of doubtful value, particularly if an attempt is made to calculate the sugar content as being equivalent to a certain amount of a single sugar such as glucose or maltose. A better relationship would be obtained with saturated calcium hydroxide (0.0446*N*), in which the stoichiometry of glucose and the entire range of polymers is more nearly the same.

Some of the applications of this procedure range from the determination of degree of polymerization of dextrin fractions and starch enzyme conversion products to determination of dextrose equivalent (ferricyanide) of corn syrups.

Summary

Glucose and a series of glucose polymers ranging in degree of polymerization from 2 to 10 were oxidized with potassium ferricyanide reagents containing different alkalis. For glucose the number of equivalents of ferricyanide consumed per mole of reducing sugar was about 5 for each of the alkaline catalysts; however, for the higher members of the series the factor approached 10 when sodium carbonate (0.2*M*) was employed and was of the order of 7 when barium hydroxide (0.0446*N*) was used. With saturated calcium hydroxide (0.0446*N*) the value remained at approximately 5 for the entire series. The G_6 polymer gave anomalously higher results in each case.

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