EFFECT OF CHAIN LENGTH ON CERTAIN PROPERTIES OF OLIGOSACCHARIDES

by

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INTRODUCTION

Maltooligosaccharides are dextrose (glucose) polymers with α-D-1,4-glycosidic linkages which occur repeatedly in the homologous glucose series. Although these compounds rarely occur in nature, they are often obtained as hydrolytic products from polysaccharides, such as starch, which on hydrolysis yield fragments of various molecular weights. Corn syrup is a well-known example of a hydrolyzate resulting from acid or acid-enzyme cleavage of corn starch.

The use and application of corn syrup as well as hydrolyzed cereal starch solids has increased widely in industry, especially where products of low hygroscopicity and high water-solubility are desired. These products are used in many food products to modify food flavors and sweetness. They also modify crumb and texture properties of bread and baked products.

At present, the technology of glucose production from starch is quite advanced. After production of a glucose syrup, the glucose can be partially enzymically converted to fructose which increases the sweetening power of the syrup. The increase in demand for corn syrup can be attributed to the wide range of properties that may be imparted to syrups through various modifications.

For many years, maltooligosaccharides have been isolated because of their potential biological significance. Chromatographic methods of isolation and identification of oligosaccharides from starch hydrolyzates are the most powerful tools available at present to the carbohydrate chemist. Among these techniques, adsorption chromatography using charcoal (carbon) columns and gradient elution of the oligosaccharides with aqueous alcohol has been most extensively used for isolation of individual oligosaccharides from

starch hydrolyzates. Preparative paper chromatography may be superior to column techniques for isolation of individual oligosaccharides from corn syrup.

It is evident that differences in dextrose equivalents of commercial syrups are due to variations in the amount of each oligosaccharide present. These combinations also exhibit different physical properties.

The physical and chemical properties of glucose and maltose have been studied extensively. However, there is a limited amount of data reported in the literature for the higher oligosaccharides and there is some disagreement among this limited data. The lack of information seems to be due to the difficulty associated with isolation and purification of higher oligosaccharides. Because of the importance of this problem to industry, considerable effort has been expended by scientists but the results have been of limited value.

The purpose of this investigation was to isolate and purify oligosaccharides from hydrolyzed cereal starch and to determine certain chemical and physical properties for each of the purified oligosaccharides.

REVIEW OF LITERATURE

Research has been conducted by numerous scientists on the isolation, purification, and identification of linear maltooligosaccharides from starch hydrolyzates. The main trend of research on the chemistry of the oligosaccharides has been to improve techniques for isolation and purification. A few studies have been conducted to establish the chemical and physical properties. An excellent review of procedures for isolation used has been made by Bailey and Pridham (5).

The separation of oligosaccharides on columns of charcoal was first described by Whistler and Durso (67). They separated a mixture of mono-, di- and trisaccharides by chromatographic adsorption on charcoal using water, 5 and 15% ethanol for displacement. They followed the desorption process by polarimetry. They found that the presence of salts such as sodium chloride, sodium bicarbonate and acetate would not affect either the adsorption or the desorption of sugar from the charcoal column. Bailey, Whelan and Peat (4) applied the method devised by Whistler and Durso (67) for the chromatographic separation of mono-, di-, trisaccharides, and dextrins derived from amylose by acid hydrolysis. They obtained pure samples of glucose, maltose, maltotriose, maltotetraose, maltopentaose and maltohexaose by using ethanol concentrations of 0, 7.5, 15, 20, 20-23 and 25% respectively. Glucose, maltose and maltotriose were obtained directly by using 0, 7.5 and 15% ethanol solutions. The other three oligosaccharides, obtained with higher concentrations of ethanol, were hygroscopic, amorphous and contained a small amount of inorganic acid.

Whelan, Bailey and Roberts (63) described the use of carbon-Celite columns for separation of maltodextrins. The carbon was pretreated with 0.1 N citrate

buffer, pH 7.0, until the inorganic material was eluted. A mixture of carbon and Celite, 1:1 ratio, was washed with distilled water and thoroughly dried at 80°C. The column, with pads of glass wool and cotton at the bottom, was packed with a thick slurry in water. The powder was allowed to settle under gravity.

Alm, William and Tiselius (2) introduced the gradient elution technique. The method consisted of the use of a continuously changing concentration of an eluting solution which was produced outside the column in a mixing chamber. They also studied the effect of pretreatment of the carbon column with stearic acid. Alm (1) applied the above method to the separation of malto-oligosaccharides. He gave the idea that application of gradient elution analysis in combination with pretreatment of the carbon adsorbent had the possibility of providing for the quantitative resolution of sugar mixtures.

Lindberg and Wickberg (41) treated a carbon-Celite mixture (1:1) in concentrated hydrochloric acid to remove inorganic material and deactivate the carbon. The mixture was washed with large quantities of water and ethanol and then poured into the column in a thick slurry. They succeeded in separating the isomeric pairs 2,3,6- and 2,3,4-tri-O-methyl glucose and di-, tri- and tetra-methyl ethers of the sugars by using an ethanol gradient of increasing concentration or ethylmethylketone as eluents. This method is of value in structural carbohydrate chemistry.

Whistler and Hickson (68) isolated tetrasaccharide from corn syrup on a carbon-Celite (1:1) column. They washed the column successively with water, 3.5 and 7.5% aqueous ethanol. A fraction containing most of the tetra-saccharide was then eluted with 15% aqueous ethanol. The eluate was evaporated to dryness at 50°C under reduced pressure. The sugars thus obtained were

further refined chromatographically by elution from a column of Whatman cellulose powder with a solution of ethyl acetate-pyridine-water (10:4:3 v/v).

Whistler and Duffy (66) resolved maltopentaose by the method described by Whistler and Hickson (69). A crude pentasaccharide was concentrated in the fraction eluted by 20% aqueous ethanol. This fraction was evaporated and refined first on a cellulose column (29) using 85 and 80% aqueous ethanol as eluting agents. The final purification was carried out on a cellulose column eluting with butanol-ethanol-water (3:2:1 v/v).

Isolation of maltohexaose was accomplished by Whistler and Moy (70) in a carbon column. Four liters of 16% aqueous ethanol were used to remove glucose and maltooligosaccharides below a degree of polymerization (D.P.) of 6. Subsequent elution with 4 liters of 20% aqueous ethanol removed maltohexaose contaminated with neighboring homologs. After evaporation of this fraction it was purified on a cellulose column eluted with decreasing concentrations of ethanol (85, 82 and 80.5%). Maltohexaose was found in the 80.5% ethanol eluate and was confirmed by paper chromatography.

Miller (44) successfully resolved both malto- and cellodextrins on a micro-column using Darco G 60 (40 g) and Celite 545 (40 g) which was pretreated for half an hour in 320 ml of 2.5% stearic acid in absolute ethanol.

Barker, Bourne and Theander (7) complexed isomeric compounds with borate and separated them on a hydrochloric acid-pretreated carbon column. In this way, they could separate mixtures of melibiose (1-6 linkage), maltose (1-4 linkage) and dextran (1-6 linkage) which were eluted with lower than normal ethanol concentration. Sato, Ito and Ono (51) used borate buffer in ethanol (pH 10.0) to separate isomaltose and panose from hydrol.

Salem and Johnson (50) isolated gram quantities of the oligosaccharides from corn syrup in order to study the influence of various oligosaccharides on bread staling. They used equal parts of carbon (Darco G-60) and Celite 545 in a 60 x 750 mm column. Twenty per cent solution of corn syrup [dextrose equivalent (D.E.) = 42] was applied, and elution with stepwise increases in ethanol concentration was performed. Oligosaccharides G_1 to G_7 and dextrins were obtained as white amorphous solids with a tinge of yellow. The separated saccharides were confirmed to be homologous maltooligosaccharides by the linear relationship between log R_M [$R_M = \log_{10}(1/Rf-1)$] value and average chain length (32). The linear relationship obtained in a plot of A/n versus $\frac{n-1}{n}$; where A is the molecular rotation and n is the number of glucose residues per molecule, indicated that maltose is the lowest member of the maltooligosaccharides.

Hoover, Nelson, Milner and Wei (27) isolated a series of maltooligosaccharides of 3-10 glucose units from commercial acid-converted 42 Dextrose Equivalent corn syrup with large scale carbon column chromatography. They used a 1:1 mixture of Darco G 60 and Darco S 51 charcoal packed firmly in the large column. Water was pulled through the packed charcoal with vacuum. The column was treated with concentrated hydrochloric acid and then washed with distilled water to a pH of 3.5. The chloride ions in the saccharides were removed by the use of ion-exchange columns. The samples, thus isolated, were white or sometimes yellowish amorphous powders.

Lately, French, Robyt, Weintraub and Knock (20) succeeded in resolution of branched as well as linear oligosaccharides up to 15 D-glucose units on a pretreated carbon-Celite column. They found that pretreatment with 0.01 N hydrochloric acid was equally effective as pretreatment with 0.01 N stearic

acid when using ethanol as eluant. However, stearic acid pretreatment of the charcoal gave superior results when tertiary butanol was used as eluant. Carbohydrate in the eluate was monitored by a continuous recording polarimeter or by automatically analyzing fractions colorimetrically with a Technicon Autoanalyzer.

Fractionation of oligosaccharides on charcoal could be readily accomplished using the modification described by Andrews, Hough and Powell (3) in which the charcoal is packed as a short, wide column in a sintered glass Buchner funnel. This method was particularly useful for purifying individual oligosaccharides or for removing a very high concentration of a mono- or a disaccharide from a solution containing trace amounts of other sugars. Lammers (39) found a reproducible separation of α- and β-cyclodextrin on a charcoal column. He eliminated very small carbon particles by flotation in water.

Partition on cellulose was first used by Hough, Jones and Wadman (29) for the resolution of monosaccharide mixtures. It was applied to the separation of mixtures of homologous saccharides by Thoma, Wright and French (58). At elevated temperatures they could separate oligo- and megaoligosaccharides (D.P.$>$10) up to D.P. 18. The isolated saccharides were purified by paper chromatography using a butanol-pyridine-water ternary solvent system. They failed to isolate cellodextrin by partition chromatography on a Celite column.

Several chromatographic techniques led to separation of oligosaccharides in other media. Stefanovic (54) used a carbon-aluminum oxide column. Gel filtration on polyacrylamide gel had been used by John, Trenel and Dellweg (36) and by Luby and Kuniak (42) on cross-linked starch and cellulose to separate oligosaccharides.

Beadle (9) determined the composition of starch hydrolyzates by gas-liquid chromatography. Maltooligosaccharides were converted to trimethylsilyl ether derivatives. The columns were packed with 3% JXR on 80-100 mesh Chromosorb W (AW-DMCS). They were conditioned at 350°C for 48 hours with a carrier gas flow rate of 10 to 20 ml per minute. The accuracy of the procedure was claimed to be equal to or better than that obtained by paper chromatography.

Partridge (48) was the first to introduce partition chromatography on paper to the resolution of mixtures of monosaccharides. Hough, Jones and Wadman (29) had improved methods for separation and detection of sugars and their methylated derivatives on paper chromatograms. Jeanes, Wise and Dimler (32) improved the technique to resolve disaccharides and oligosaccharides. They also studied the interrelationships between the degree of resolution of the sugars, their R_f values, and solvent composition.

French and Wild (19) investigated the paper chromatogram mobilities of homologous oligosaccharide series. Straight line relationships were obtained when the logarithm of a partition function ($R_f/1-R_f$) was plotted against molecular size. This relationship also held true for the maltooligosaccharide separated by thin-layer chromatography on silica gel G and Kieselguhr G (24). A procedure for selecting solvent proportions and components for paper chromatography of homologous saccharides was devised by Thoma and French (57). Solvent systems containing very basic nitrogenous carrier solvents destroyed reducing sugars during one or two developments (57).

Commerford, VanDuzee and Scallet (15) modified macro-paper chromatography to isolate maltooligosaccharides from corn syrup. They used an n-propanol-ethylacetate-water (14:3:7) ternary system to develop the paper chromatogram.

Because of the chemical and biochemical importance of the maltooligosaccharides, the isolation and characterization of individual members were undertaken. Whelan, Bailey and Roberts (63) studied carbohydrase action and suggested that the lowest member of oligosaccharides is not the monosaccharide glucose but the disaccharide maltose which is the smallest molecule containing one glycosidic linkage.

Whelan and Robert (64) found that only maltose and maltotriose were end-products of α-amylase action on maltooligosaccharides or amylose. Thompson and Wolfrom (55) determined the structure of maltotriose by resolving the acetylated partial hydrolysate of the alditol in silica column chromatography.

The structures of maltotetraose and maltopentaose were determined by Whistler and Hickson (69) and Whistler and Duffy (66), respectively. The saccharides were oxidized with hypoidite and with periodate or hydrolyzed with β-amylase and the results used to define the structures. Reduction of the saccharides to maltotetraitol and maltopentaitol followed by acetylation yielded a crystalline pentadeca-O-acetyl and octadeca-O-acetyl derivative, respectively. Attempts have been made to determine the structure of maltohexaose by this procedure, but reduction of maltohexaose followed by acetylation failed to yield a crystalline glucitol acetate.

Thoma and French (56) examined the starch-iodine-iodid complex of maltodextrins (G-4-G-15) spectrophotometrically. They suggested that a chain length of 8 glucose units could form an iodide complex.

Hoover, Nelson, Milner and Wei (28) evaluated the oligosaccharides for density, refractive index, viscosity, optical rotation, reducing powder and infrared absorption characteristics. Statistical treatment of data was used to predict certain values. Reducing powers were unusually high,

especially for maltopentaose. Solubility in water was different for each oligosaccharide. They concluded that density, refractive index, viscosity, optical rotation and reducing power were related to molecular weight.

Specific optical rotations of maltooligosaccharides were reported by Whistler (65), and Whistler and associates (66, 68). The oligosaccharides were obtained by desorption from a carbon column. Specific optical rotations have been reported elsewhere (49). Hoover and associates reported the specific rotation of oligosaccharides form maltotriose through maltodecaose.

Commerford and Scallet (16) used the Schoch and Jensen procedure (25) to determine the ferricyanide number of the oligosaccharides prepared by macro-paper chromatography. They also investigated the effect of different bases on the ferricyanide reaction. A saturated calcium hydroxide reagent gave a stoichiometric reaction that was relatively constant for all of the oligosaccharides studied. Commerford and Scallet (17) also have determined dextrose equivalents for a homologous series of maltooligosaccharides from G-1 through G-6 by modification of the Lane and Eynon (40) procedure. They found that observed D.E. is always higher than the theoretical D.E. One millimole of maltooligosaccharide consumed about 5 milliequivalents of copper.

Birch, Kheiri and Hufton (10) used Commerford and Scallet's (17) results to calculate a $D.E._{n+1}/D.E._{n}$ ratio (n = number of glucose units). They found a close relationship of the calculated ratio of D.E. value to the ratio of $M.W._{n}/M.W._{n+1}$ with the exception of glucose. From the oxidation of oligosaccharides by copper, it was suspected that oxidation of the sugar occurred beyond the terminal reducing group. Thus maltose gave 0.88 equivalents of glucose and maltotriose gave 1.60 equivalents of glucose after oxidation.

Johnson, Ruliffson and Cooks (33) established the mass spectra for cellobiose, lactose, gentibiose, isomaltose, melibiose and maltooligosaccharides (G-2 to G-5). They prepared the acetylated 1-phenylflavazole derivative and established the structure and sequence of the oligosaccharides.

Chizhov, Kochetkov and Malysheva (13, 14) also examined the mass spectra of oligosaccharides. They prepared N-phenylosotriazole acetate and N-arylglycosylamine acetate derivatives of glucose, maltose, cellodextrins (G-2 to G-5). By mass spectrometry they determined the molecular weight, the location of the glycosidic linkage and the sequence of the sugar units.

Vasko, Blackwell and Koenig (62) investigated infrared and Raman spectra related to the C-H and C-H variation modes of D-glucose, maltose, cellobiose and dextran by a deuterium-substitution method. The Raman spectra at approximately 1349, 1071, 1021 and 913 cm^{-1} could be assigned to be characteristic mode related to the C-O-H group. Bands at 1457, 1337, 1219 and 1011 cm^{-1} for α-D-glucose were assigned to CH_2-related modes, and those at 1404, 1360, 1250, 1076, 1047, 911 and 836 cm^{-1} to C-H-related modes.

The preceding review has illustrated the remarkable value of various chromatographic techniques which are available for separation of oligosaccharides, whose chemical and chemical properties were then determined. It is not yet possible to reach an absolute conclusion relating to critical properties of oligosaccharides. Different experimental systems have been employed. The results often cannot be compared in a meaningful way. There still appears a need for confirming critical data on the properties of pure oligosaccharides.

MATERIALS AND METHODS

Isolation of Oligosaccharides

A partially-hydrolyzed corn starch, Morrex T.E. Code No. 1918[1], was used as a crude source of oligosaccharides. Morrex is a dry, hygroscopic powder consisting of glucose polymers from G_1 to G_{14} and higher dextrins. The major proportion consists of G_6 and G_7 polymers.

The saccharides in Morrex were crudely isolated on a carbon-Celite column. This was made by filling the column with a mixture of Darco G-60, granulated carbon (20 mesh) and Celite 535 (4:4:2). The mixture was made into a thick slurry with water and packed 10' deep in a 6" by 12' Pyrex column. After packing, the column was washed first with one gallon of 40% hydrochloric acid, followed with distilled water until the eluate reached a pH of 3.5. The carbon column was never allowed to drain dry but was kept covered with a layer of liquid.

After preparation of the carbon column, 450 g of Morrex dissolved in 1 liter of water was added and allowed to perculate down the column. The column was further washed with approximately 20 gallons of distilled water before addition of the 5% ethanol eluant. Attempts were made to use a regulated vacuum to accelerate the flow rate. This was found to create channels which would have led to uneven elution bands in the column. Instead of vacuum, a head of pressure was developed by closing the column system with appropriate cap and elevating the eluant reservoir 6' above the carbon column. The flow rate of the column did not remain constant but decreased toward the end of elution with higher concentrations of ethanol. Initially, with 10% ethanol, the flow rate was approximately

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[1]Corn Products International, Argo, Illinois.

5 gallons/24 hour period. Later the flow rate was 2 gallons/24 hour period.

A homologous series of maltooligosaccharides was subsequently eluted with increasing concentrations of ethanol including 10, 15, 17, 20, 25, 30, 35 and 50% ethanol. It was found that glucose and maltose was removed with 10% ethanol. Maltotriose was eluted by 15% ethanol. Seventeen per cent ethanol eluted the last traces of maltotriose and maltotetraose was subsequently eluted mainly with 20% ethanol. Higher oligosaccharides usually were eluted as mixtures of the neighboring sugars. The degree of separation was found to be fair with the lower members of the homologous series of maltooligosaccharides, but with the higher members, although requiring a higher ethanol concentration, separation was increasingly less distinct.

Detection of Oligosaccharides in Eluant

Each gallon of eluant was analyzed for total carbohydrate by the Dubois et al. method (18, 45). One-half ml of each gallon of eluate was mixed with 1.5 ml of distilled water and 1 ml of 8% phenol was added. Five ml of concentrated sulfuric acid was added rapidly and solution thoroughly mixed with a Vortex mixer. After 30 minutes the absorbance was measured at 489 nm with a Bausch and Lomb Spectronic 20 spectrophotometer.

Oligosaccharides in the eluate fractions were identified by paper chromatography. Two ml of eluate were concentrated under reduced pressure in a vacuum desiccator containing phosphorus pentaoxide. The concentrated sugar solution was spotted on Whatman No. 4 chromatographic paper. The chromatogram was developed with n-propanol-ethyl acetate-water (6:1:3) for 18 or more hours. The chromatogram was dipped successively in silver nitrate, sodium hydroxide and thiosulfate solutions to make the sugars on the paper

visible and to fix the color (60).

After the eluants had been collected and analyzed for type and concentration of oligosaccharides, they were combined according to type of oligosaccharide present and concentrated in a vacuum evaporator (a dry-milk evaporator) to approximately 3/4 of a gallon for each oligosaccharide fraction. The dry-milk evaporator was operated at 26 inches of mercury vacuum and 110°F. The concentrated solution never represented a single oligosaccharide, which was usually admixed with neighboring sugars. Furthermore, some chloride ion was inevitably present.

The concentrated solution of sugar was passed through a column of cation-exchange resin, Amberite IR-100 (H^+ form), which was generated with 500 ml of 10% sulfuric acid and washed with water to a pH of 5.5 to 6.0. The concentrated solution was next passed through column of anion-exchange resin, Dowex 1-X 8 (Cl^- form), which was generated by treatment with 2,000 ml of 10% ammonium carbonate and washed with water to a pH of 5.6 to 6.0. The removal of the chloride ion was checked with silver nitrate.

The purified, concentrated sugar solutions were then further concentrated in a rotary evaporator and finally dried to a white powder by freeze-drying. The freeze-drier was operated at 0.2 mm Hg of pressure and 80°F.

Further Purification of Oligosaccharides

During the isolation of the oligosaccharides on the carbon column it became obvious that each oligosaccharide fraction was not a single sugar but rather consisted of several neighboring sugars in each fraction. Attempts were made to further purify the fractions by applying them to a smaller (2" x 23") carbon column and eluting with appropriate ethanol solutions. The flow rate was controlled by application of vacuum regulated with a monostat. Fair

resolution was obtained with the lower members of the homologous series of oligosaccharides but, even with applied vacuum, purification of a single fraction consumed as long as 3 months.

A decision was made to use macro-paper chromatography as described by Commerford, VanDuzee and Scallet (15) for further purification of the fractions. The individual freeze-dried fractions were dissolved in distilled water and streaked on prewashed Whatman 3MM chromatography paper (18" x 22"). The papers were washed by soaking for 20-30 minutes in a tray containing distilled water followed by rinsing before drying. The chromatograms were developed in a descending system using n-propanol-ethyl acetate-water (14:3:7). Irrigation of the chromatogram required from 3 to 5 days for separation of polymers G_3 to G_7 and 8 to 14 days for G_7 to G_{12} oligosaccharides. One inch strips were cut from each edge and the center of the air-dried chromatogram. These strips were treated alkaline silver reagent (60) to establish the location of each oligosaccharide. The large pieces of paper were then cut into strips corresponding to the location of the oligosaccharides on the strips. The individual oligosaccharides were then eluted with 5 to 10 ml of distilled water. The sugar solutions were placed in 50 ml beakers and freeze-dried to obtain the individual oligosaccharide in pure form. The freeze-dried oligosaccharides were stored in vials placed in a desiccator containing phosphorous pentaoxide.

Many individual oligosaccharide samples were obtained and later combined by dissolving them in water, filtering and freeze-drying. The samples were further dried in a vacuum oven at 0.2 mm Hg pressure and 100°F and stored in a desiccator until used for analysis of physical and chemical properties.

Measurement of Physical and Chemical Properties of Oligosaccharides

The following physical and chemical properties of the purified oligosaccharides were determined:

Reducing power

Specific gravity

Refractive index

Viscosity

Hygroscopicity

Infrared spectra

Raman spectra

Reducing power. The reducing power of the maltooligosaccharides was determined by the Somogyi method using the 1952 alkaline copper sulfate reagent (52). The reagents — alkaline copper sulfate, potassium iodide, sodium thiosulfate, starch and phenol red indicators — were prepared as described by Hodge and Hofreiter (26).

Five ml of alkaline copper-reagent was added to a series of test tubes containing not more than 0.5 mg of glucose equivalent in 5 ml of distilled water. The tubes were flushed with nitrogen gas as they were filled. Blanks were included using 5 ml of distilled water. The tubes were stoppered with aluminum foil-covered stoppers and placed in a boiling water bath for 15 minutes. The tubes were then cooled to 25°C and 1.5 ml of 2N sulfuric acid was added with shaking to liberate the iodine which reacted with the reduced copper. The excess of iodine was titrated with 0.005 N thiosulfate.

Calculations:

$$\text{Dextrose Equivalent (D.E.)} = \frac{\text{mg of reducing sugars expressed as glucose} \times 100}{\text{mg of polymer}}$$

$$\text{Stoichiometry} = \frac{\text{Milliequivalent copper}}{\text{Millimole of polymer}}$$

$$= \frac{(\text{ml for blank-ml for sample}) \times \text{N thiosulfate}}{\text{mg sugar} \times \text{molecular weight}}$$

Specific Gravity. Maltooligosaccharides were dissolved in distilled water to form a 10% (w/v) solution. These solutions were subsequently diluted to provide oligosaccharide solutions of 8, 4, 2 and 1% concentration, respectively.

Specific gravity of the solutions was determined at 20°C over the entire range of concentrations using a 2 ml pycnometer. Deionized, distilled water was used as the reference liquid. Before each determination, the pycnometer was placed in a bath of water maintained at 20°C. The sample solution, which was previously equilibrated to 20°C, was placed in the pycnometer, and the cap fitted. The excess liquid was removed from the stem and the pycnometer was dried with Kimwipe paper. It was then weighed on a Mettler analytical balance, which was accurate to 0.00001 gm. Prior to weighing the next sample solution, the pycnometer was washed with distilled water, rinsed with acetone and dried in the hot air oven. While filling the pycnometer and inserting the cap, care was taken to prevent the introduction of air bubbles.

The apparent specific gravity (S') of maltooligosaccharides was calculated as follows:

$$S' = \frac{vs^{20^\circ}}{vw^{20^\circ}} \quad \text{(weight in air)}$$

where:

s = weight of sample
w = weight of water
v = volume

The true specific gravity (S) was calculated by consideration of the buoyancy factor according to Hann (23).

$$B = P\left(w - \frac{w}{8.4}\right)$$

where:

B = buoyancy factor
P = air density at 20°C = 0.0012
w = weight of water in air at 20°C

$$B = 0.0012 \left(w - \frac{w}{8.4}\right)$$

$$= 0.00105\ w$$

This value was added to both the numerator and denominator of the specific gravity equation to give the true value.

Thus:
$$S = \frac{s + B}{(1.00177 \times w) + B}$$

$$= \frac{s + 0.00105w}{1.00177w + 0.00105w}$$

$$= \frac{w + 0.00105w}{1.00282w}$$

8.4 = the density of brass weight

1.00177 = the volume of water at 20°C

Refractive Index. Maltooligosaccharide solutions prepared for specific gravity determinations were used to measure refractive indices. A Bausch and Lomb refractometer controlled at 20°C was used. Two drops of the sugar solutions were placed on the prism using a disposable pipette. Between each reading, the prisms were cleaned with 95% ethanol and wiped dry with tissue paper.

Calculations:

$$\text{Specific refraction} = \frac{(n^2-1)}{(n^2+2)} \times 1/d$$

$$\text{Molecular refractivity} = \text{Mol. wt.} \times \frac{(n^2 - 1)}{(n^2 + 2)} \times 1/d$$

where:

n = refractive index n_D^{20}
d = density ($d_{4o}^{20°C}$)

Viscosity. The same oligosaccharide solutions used for determination of specific gravity were used for determination of the effect of polymer size on viscosity of solutions. Five ml each solution were placed in an Oswald capillary viscosimeter (type 100) which was held at a constant temperature of 20 ± 0.05°C. The flow times were determined in triplicate by electric timer. Distilled water served as a reference.

Calculations:

Relative viscosity,

$$\eta_r = \frac{\eta_s}{\eta_o} = \frac{S_s T_s}{S_o T_o}$$

Specific viscosity,

$$\eta_{sp} = \frac{\eta_s - \eta_o}{\eta_o} = \eta_r - 1$$

Intrinsic viscosity

$$[\eta] = \lim_{c \to 0} \frac{\eta_{sp}}{c}$$

where:

η = viscosity
S = specific gravity
t = time in seconds
c = concentration (w/v)

Hygroscopicity. Maltooligosaccharides tend to absorb moisture from the atmosphere. Oligosaccharides will absorb or desorb moisture until reaching an equilibrium with the atmospheric moisture. This property of the oligosaccharides is basically due to residue valence forces and hydrogen bonding, which varies with the chain length and the hydroxyl groups exposed.

The hygroscopicity of oligosaccharides was estimated by placing a weighed quantity of each sugar in aluminum dishes which were placed in desiccators containing sulfuric acid of known density (22). The relative humidities

inside the desiccator were recorded with an Abbeon relative humidiscope and temperature indicator. The change in weight with time of storage under $60\pm5\%$ relative humidity was determined by weighing the samples periodically. The moisture content is expressed as:

$$\% \text{ dry basis} = \frac{\text{wt. of water}}{\text{wt. of dry material}} \times 100$$

Infrared spectra. Infrared spectra were measured with a Perkin-Elmer infrared spectrophotometer, Model 421. The oligosaccharide samples, G_1 to G_{12}, were ground to a fine, uniform powder in a stainless steel capsule in a Cresent Dental Company Wig-L-Bug. Two methods were used to prepare samples for scanning in order to study infrared absorption throughout the spectrum. To measure absorption in the region of 4000-2000 cm^{-1}, the samples were ground and approximately 10 mg was deposited on the face of a 25 mm calcium fluoride (CaF_2) crystal window. Several drops of hexachloro-1,3-butadiene was applied to the window sample. Several more drops were applied to a second window and the two sandwiched together, pressing to spread the sample uniformly between windows. To measure absorption in the 2000-500 cm^{-1} region, the ground sample was mixed with petroleum jelly in an agate mortar. The "mull" was placed on the face of a 25 mm potassium bromide (KBr) crystal window. A second window was pressed on the first window containing the sample to provide a uniform film.

Raman spectra. Raman spectra of the samples G_1 to G_{12} were obtained with the Raman spectrometer in the Department of Physics at Kansas State University. The spectrometer used was a Spex model 1401 double beam Spectrometer with an associated photodetector and counting circuit. The laser beam source was

a coherent radiation model 52 argon ion laser. The data were recorded at a scan rate of 50 $cm^{-1}min^{-1}$ with a time constant of two seconds and a slit width of 160 microns. This allowed a resolution of $\pm$ 4 cm^{-1}. The data were plotted by a Mosely Autograph model 2D-2 X-Y recorder.

All sugar samples were prepared identically by forcing small amounts into 1 mm ID Kimax capillary tubes with the aid of a small stiff wire. The samples were loosely tamped but not packed. The ends of the tubes were sealed with wax after packing.

The 514-5 line of the argon ion laser was chosen for use in this study. The laser had a maximum output of 750 mW at this wavelength. There was more than enough power and, in most cases, the beam was attenuated by neutral density filters before it hit the sample.

RESULTS AND DISCUSSIONS

Oligosaccharides from partially-hydrolyzed corn starch (Morrex) were separated on a carbon-Celite column. Glucose and maltose which were present in low concentration in Morrex were eluted with water and 10% aqueous ethanol, respectively. These two fractions were not collected since they are commercially available in a relatively pure form. Maltotriose and other higher maltooligosaccharides were eluted with increasing concentrations of aqueous ethanol. Fifteen per cent ethanol was used to elute maltotriose. The first fraction eluted with 15% aqueous ethanol was pure maltotriose. Seventeen per cent ethanol was used in an attempt to eliminate tailing, but no carbohydrates were detected in the eluate using the phenol-sulfuric acid procedure (45) for detection. When the concentration of aqueous ethanol was increased to 20%, maltotriose and maltotetraose were eluted with the latter predominating. Later fractions, using 20% aqueous ethanol, contained pure maltotetraose, maltotetraose mixed with maltopentaose, a mixture of maltotetraose through maltohexaose, and a mixture of maltopentaose through maltoheptaose. These were obtained in the sequence listed using 20% ethanol as the eluting solvent. Twenty-five per cent ethanol eluted no additional carbohydrates.

Attempts were made to elute higher oligosaccharides (G_8-G_{10}) with 30, 35 and 50% aqueous ethanol. Maltotriose and maltotetraose were found chromatographically pure in dilute solution but trace quantities of homologous oligosaccharides were found after concentration and freeze-drying. They were further purified in a small carbon column by elution with increasing concentrations of ethanol. The yields obtained from this chromatographic purification were only a few milligrams which were not sufficient to determine properties such as viscosity. Presumably, the carbon had a high sugar

adsorption capacity and this resulted in the low yields obtained.

The chloride-free (as detected by silver nitrate), partially separated oligosaccharides were further purified by chromatography on washed Whatman 3MM chromatographic paper. The sugars separated by this method were amorphous and white. Table 1 gives the amounts of each pure oligosaccharide obtained by this method. Purity and identity were established by the appearance of only one spot for each oligosaccharide on paper chromatograms and by comparing the relative rates of mobility of the saccharides with the reference corn starch hydrolysate (Morrex), respectively. Figure 1 illustrates the paper chromatographic purity of maltooligosaccharides obtained by preparative paper chromatography.

Reducing Power

The Somogyi micro-copper method (52) was selected for measuring reducing power because this method has been widely and successfully applied on both milligram and microgram samples. The 1952 alkaline copper reagent is especially useful for 0.015 to 0.5 mg of glucose or equivalent (26). Oligosaccharide solutions were prepared such that a 5.0 ml aliquot contained saccharides equivalent to approximately 2.0×10^{-3} milliequivalent (0.36 mg) of dextrose. Figure 2 shows the standard curve obtained with glucose. The standard curve failed to pass through the origin although precaution was taken to prevent air oxidation of the alkaline copper solution.

The dextrose equivalent (D.E.) values obtained for the oligosaccharides are shown in Table 2 along with theoretical and previously reported values. The D.E. values found for G_1-G_6 tend to be about 5-10% higher than the theoretical value. These values were in agreement with those reported by Commerford and Scallet (17) using the Lane and Eynon procedure (42) which

Table 1

Pure Oligosaccharides

Obtained by Preparative Paper Chromatography

D.P.	Yield (gm)
G_3	1.4
G_4	1.3
G_5	1.1
G_6	2.3
G_7	1.9
G_8	0.9
G_9	0.6
G_{10}	0.5
G_{11}	0.1
G_{12}	0.1

D.P. = Degree of Polymerization

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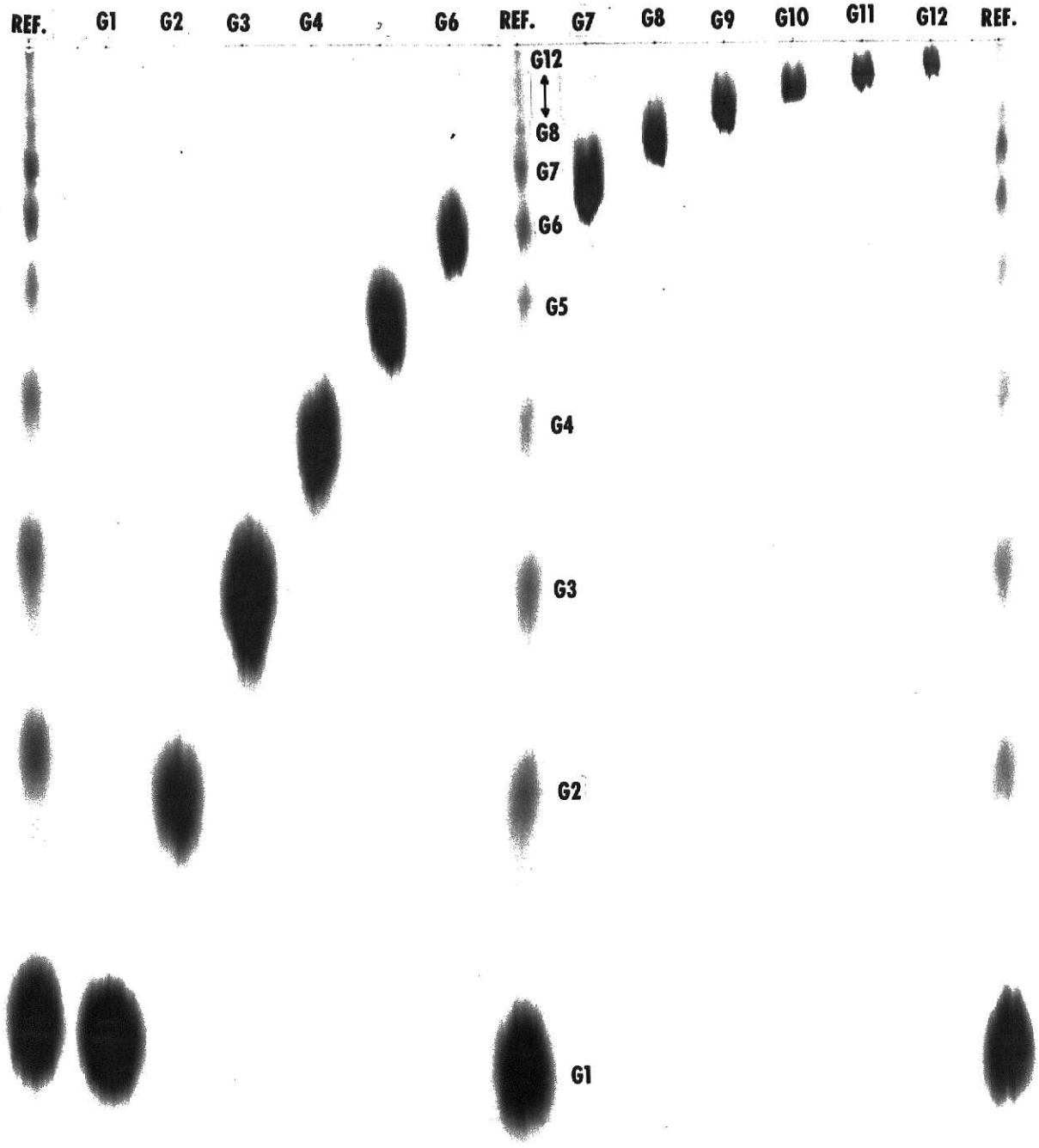

Figure 1. Paper chromatogram of purified maltooligosaccharides separated by preparative paper chromatography using n-propanol-ethyl acetate-water (14:3:7)

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Figure 2. Standard curve for reducing power by the micro-Somogyi alkaline copper method using a glucose standard.

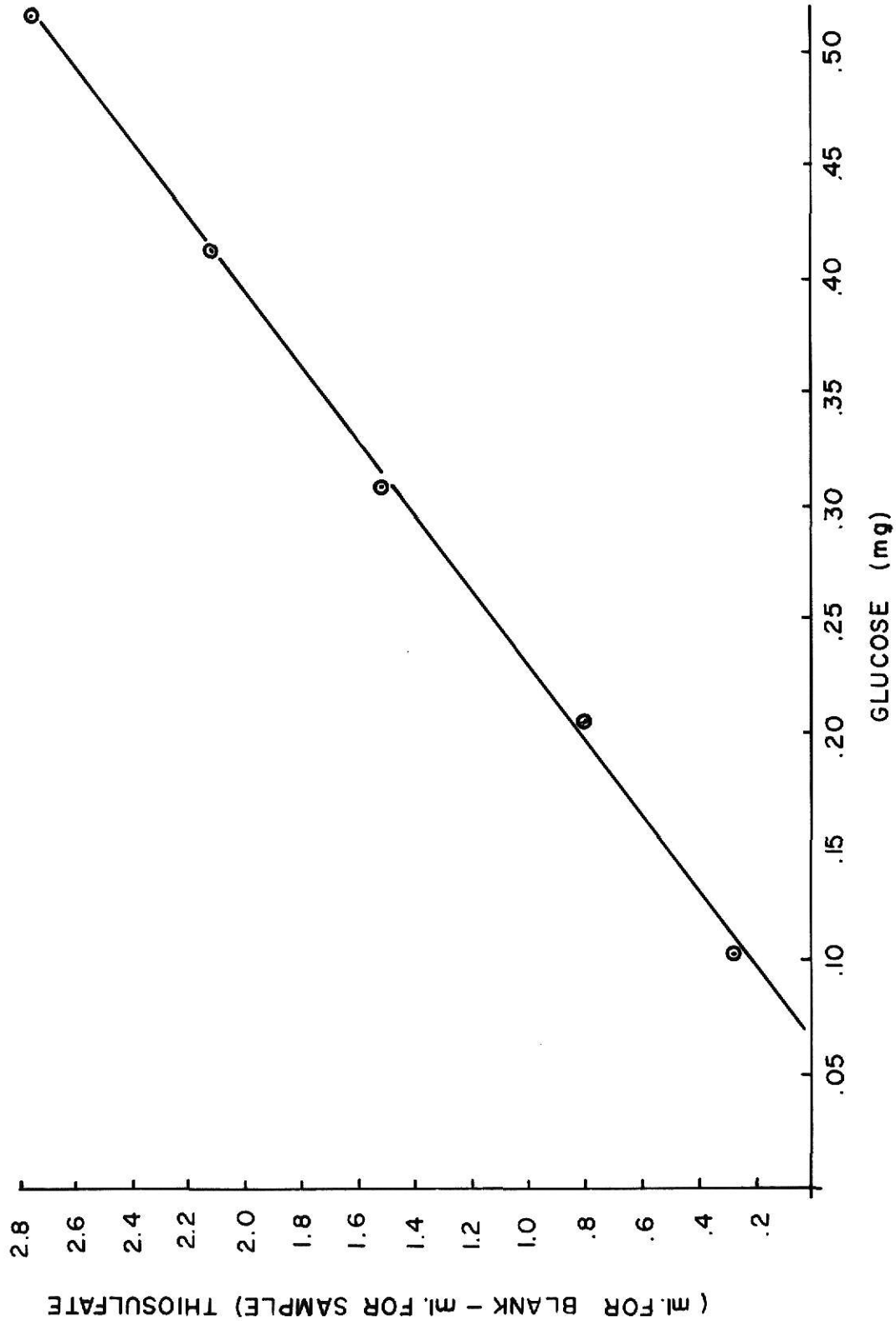
GLUCOSE (mg)
.05
.10
.15
.20
.25
.30
.35
.40
.45
.50
(ml. FOR BLANK – ml. FOR SAMPLE) THIOSULFATE
.2
.4
.6
.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8

Figure 2.

Table 2

Dextrose Equivalent for Maltooligosaccharides

D.P.	Hoover et al[a]	Commerford and Scallet[b]	Present Investigation	Theoretical Values
G_1	100.08	100.00	106.39	100.00
G_2	71.95	58.10	54.34	52.63
G_3	61.63	39.50	37.83	35.71
G_4	52.27	29.80	29.58	27.03
G_5	53.97	24.20	23.19	21.74
G_6	41.14	20.80	20.36	18.18
G_7	38.04	-	15.67	15.63
G_8	33.84	-	13.65	13.70
G_9	31.83	-	12.57	12.20
G_{10}	28.67	-	10.91	10.99
G_{11}	-	-	9.63	10.00
G_{12}	-	-	8.42	9.17

a. Ferricyanide Procedure

b. Lane and Eynon Procedure

Table 3

Stoichiometry of the Copper-Sugar Reaction

D.P.	Polymer meq.	meq. copper / meq. polymer	Stoichiometry of Polymer / Stoichiometry of Glucose
G_1	2.000×10^{-3}	5.51	1.00
G_2	2.158×10^{-3}	5.39	0.98
G_3	1.972×10^{-3}	5.46	0.99
G_4	2.000×10^{-3}	5.67	1.03
G_5	2.000×10^{-3}	5.53	1.01
G_6	1.994×10^{-3}	5.86	1.06
G_7	2.000×10^{-3}	5.13	0.93
G_8	2.024×10^{-3}	5.14	0.93
G_9	1.864×10^{-3}	5.23	0.95
G_{10}	2.109×10^{-3}	5.11	0.93
G_{11}	2.030×10^{-3}	4.90	0.89
G_{12}	2.071×10^{-3}	4.63	0.84

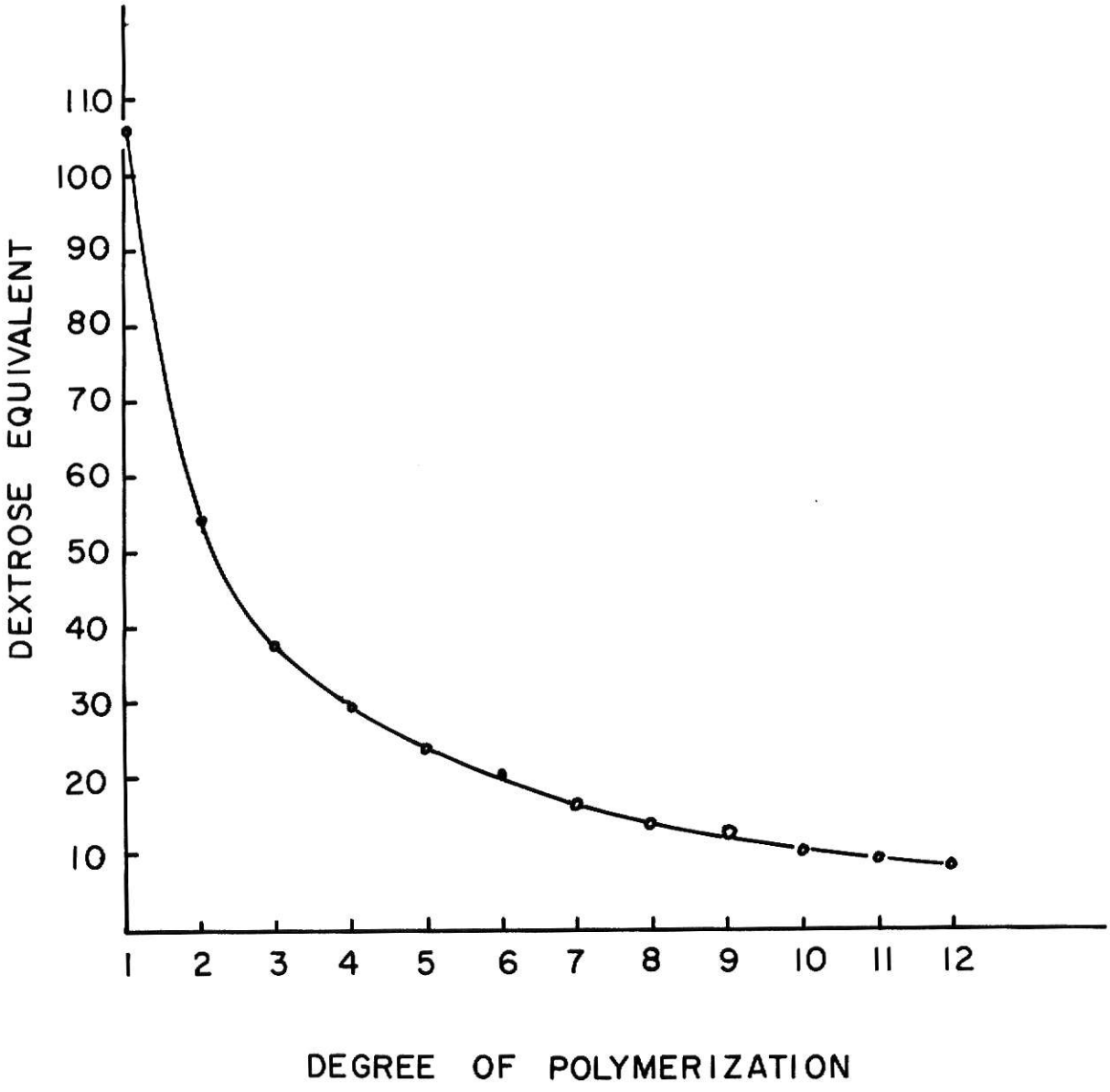

Figure 3. Reducing power expressed as dextrose equivalent of maltooligosaccharides ranging from G_1 to G_{12}.

is also based on an alkaline copper reagent. The D.E. values found for G_7-G_{10} are in close agreement with the theoretical values. G_{11}-G_{12} give values approximately 10% lower than the theoretical values. Figure 3 shows a plot of reducing power v.s. degree of polymerization of oligosaccharides.

Preparation of sugar samples with the same equivalent weights has advantages in comparing the stoichiometry of each oligosaccharide in the reaction. The stoichiometries of oligosaccharides found in this investigation are shown in Table 3. One millimole of polymer reacted with approximately 5 milliequivalents of copper. This stoichiometry was in agreement with values reported by Commerford and Scallet (17). G_1-G_6 gave approximately equal relative responses (app. 1.0) while G_7-G_{12} tended to be 10-15% less than an equivalent amount of glucose. G_7-G_{12} might need longer time to react with copper than G_1-G_6.

Reducing powers determined by the Somogyi micro-method are in quite close agreement with the theoretical values, and only small amounts of sample are required. It is an excellent method for determining reducing power on a limited quantity of sample.

Specific Gravity

Specific gravity is a characteristic physical property which serves as a criterion of identity. Specific gravity measurements are best applied to the testing of solutions consisting of one component dissolved in water. When the change in specific gravity with unit change in concentration is large, densimetric methods yield an accurate, as well as rapid, means of analysis.

Bates and associates (8) reported the specific gravity for glucose, fructose and sucrose and McDonald and Turcotte (43) reported the specific

gravity of lactose at 20°C. Using a statistical procedure Hoover and associates (28) predicted values for the apparent density (density in air) of maltooligosaccharides from G_3 to G_{10}.

The true specific gravity (specific gravity in vacuo as calculated by considering buoyancy factor) of maltooligosaccharides (G_1-G_{10}), determined with a 2 ml-pycnometer using concentrations (w/v) ranging from 1-10%, are listed in Table 4. These data indicate that solutions of different sugars at equal concentration have slightly higher specific gravity than the next lower homolog except glucose. The specific gravity of G_9 and G_{10} are varied. This might be due to the fact that these saccharides did not completely dissolve in aqueous solution, thus reducing the accuracy of the values.

The sugar solutions when first eluted from paper were dilute, clear and colorless. The small amount of these sugars obtained after freeze-drying was light, puffy, amorphous and white. A light yellow color occurred when the saccharides were concentrated. The light yellow color of the oligosaccharide solutions probably came from the chromatographic papers which were used to separate and purify these maltooligosaccharides. Solutions of G_1 and G_2 were clear and colorless. These two saccharides used in this investigation were repurified commercial samples. Solutions of G_3 to G_6 were clear and slightly yellow. Solution of G_7-G_{10} contained small amounts of floccuent material. These precipitates were completely dissolved at the following saccharide concentration: G_7, 8%; G_8, 4%; G_9, 1%. One per cent G_{10} still contained some precipitate. The occurrence of precipitation in solutions of higher oligosaccharides (G_7-G_{10}) was also noted by Hoover and associates (28). The relationship between specific gravity and degree of polymerization is shown in Figure 4. A linear relationship between specific

Table 4

True Specific Gravity* of Solutions of Maltooligosaccharides at 20°C

Degree of Polymerization	True Specific Gravity				
	1%	2%	4%	8%	10%
G_1	1.00177	1.00496	1.01314	1.02821	1.03477
G_2	1.00115	1.00471	1.01263	1.02660	1.03371
G_3	1.00127	1.00486	1.01291	1.02871	1.03640
G_4	1.00143	1.00510	1.01297	1.02895	1.03687
G_5	1.00149	1.00528	1.01301	1.02904	1.03691
G_6	1.00162	1.00563	1.01356	1.02855	1.03861
G_7	1.00190	1.00583	1.01383	1.02974	1.03860
G_8	1.00199	1.00615	1.01396	1.02961	-
G_9	1.00208	1.00565	1.01401	-	-
G_{10}	1.00202	1.00556	1.01390	-	-

* Specific gravity in Vacuo

Figure 4. Relationship between true specific gravity and degree of polymerization for purified maltooligosaccharides

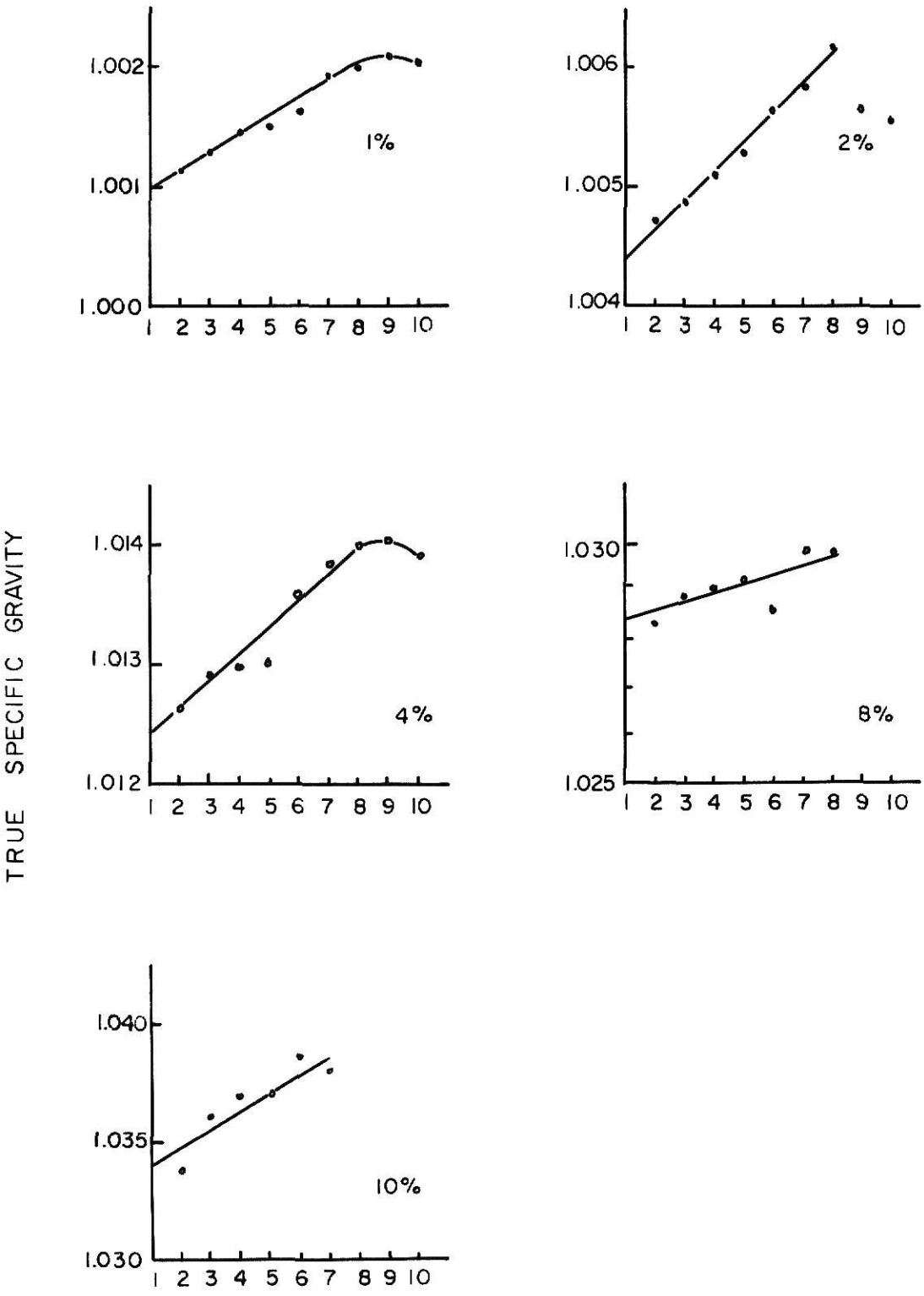
1.002
1.001
1.000
1%
1.006
1.005
1.004
2%
1.014
1.013
1.012
4%
1.030
1.025
8%
1.040
1.035
1.030
10%
1 2 3 4 5 6 7 8 9 10
TRUE SPECIFIC GRAVITY
DEGREE OF POLYMERIZATION

Figure 4.

gravity and D.P. was observed when a true solution of the saccharide was obtained.

Refractive Index

The index of refraction of a pure substance is a characteristic physical constant. It may be used as effectively as specific gravity to determine the concentration of sugar solutions. The refractive index of a solution increases linearly with concentration only when the concentration is expressed in terms of weight of solute per unit volume of solution (21). Grover and Goulden (21) deduced an equation relating refractive index, n, to concentration, c, and showed that a linear relationship exists between the slope of (n_2-n_1/ρ_2), where ρ = density, when refractive increments are plotted against concentration in w/v units, whereas a curvilinear relationship exists when w/w units are used.

The refractive indices obtained for maltooligosaccharides, using concentrations varying from 1 to 10 per cent are shown in Table 5. At lower concentration, oligosaccharides were observed to have the same refractive index. The reason for this may be due to the slightly yellow color of the saccharide making it difficult to measure accurately the closely similar refractive indices at these low concentrations. At higher concentrations of oligosaccharide (8% and 10%), slight differences in the index of refraction occur with the oligosaccharides of higher chain length giving higher value. A ten per cent solution of maltoheptaose gives a lower value than maltohexaose but this may be due to the presence of a precipitate. Specific refraction and molecular refractivity are reported in Table 6. The specific refraction is also termed "the Lorentz-Lorenz" constant, and is nearly independent of temperature, pressure, and phase. Specific refraction is used

in checking abnormalities in observed values of refractive index. Tolman and Smith (59) calculated the average value of the Lorentz-Lorenz constant for sucrose solution as 0.20614. The Lorentz-Lorenz constants of malto-oligosaccharides were found to decrease as chain length increased. The values for G_9 and G_{10} should probably be less than those reported here because insufficient data was obtained to allow an accurate calculation. The Lorentz-Lorenz constant for glucose is an exception to the apparent decrease, probably because it is a monomer rather than containing the glycosidic linkage of the oligosaccharides. The density of a sugar solution can be calculated from the refractive index. Table 7 lists the density of oligosaccharides calculated from the refractive indices observed in this investigation. This will render an accurate prediction of the density of a sugar solution. Molecular refractivity reflects the refraction of the molecule as a whole. The contribution of each hexose residue to the molecule decreases as the chain length increases. Each additional glycosidic bond decreases the contribution of the hexose unit.

Viscosity

Measurements of viscosities of high-polymer solutions offer an indirect method for determination of molecular weight. The unit of viscosity is usually denoted by the Greek letter eta (η) and is defined as the ratio of shearing stress per square centimeter to the velocity gradient produced by it (37). Water at 20°C has a viscosity of almost exactly 1.00 centipoise.

The general method for measuring viscosity of sugar solutions was discussed by Browne and Zerban (12). Viscosities of pure glucose, fructose, galactose, lactose, maltose and raffinose, and also mixtures of sucrose and glucose are reported in the International Critical Table (31). At a

Table 5

Refractive Indices of Maltooligosaccharide Solutions at 20°C

Degree of Polymerization	Refractive Index				
	Oligosaccharide Concentration of Solution				
	1%	2%	4%	8%	10%
G_1	1.3350	1.3367	1.3392	1.3451	1.3480
G_2	1.3349	1.3362	1.3390	1.3448	1.3480
G_3	1.3348	1.3360	1.3390	1.3448	1.3480
G_4	1.3348	1.3361	1.3390	1.3449	1.3482
G_5	1.3348	1.3361	1.3390	1.3450	1.3483
G_6	1.3348	1.3360	1.3390	1.3450	1.3483
G_7	1.3348	1.3360	1.3390	1.3450	1.3482
G_8	1.3348	1.3360	1.3390	1.3450	-
G_9	1.3348	1.3360	1.3390	-	-
G_{10}	1.3348	1.3360	1.3390	-	-

Table 6

Specific Refraction and Molecular Refractivity of Maltooligosaccharides

Degree of Polymerization	Specific Refraction	Molecular Refractivity (Mr)	$\frac{Mr}{D.P.}$
G_1	0.20652	37.17360	37.17360
G_2	0.20665	70.67430	35.33715
G_3	0.20642	104.05568	34.68523
G_4	0.20641	137.46906	34.36727
G_5	0.20642	170.91576	34.18315
G_6	0.20632	240.25680	34.04280
G_7	0.20626	237.60000	33.94286
G_8	0.20622	270.97308	33.87164
G_9	0.20622	304.38072	33.82008
G_{10}	0.20623	337.80474	33.78047

Table 7

Density ($20^{\circ}/4^{\circ}$) calculated from Refractive Index

Degree of Polymerization	Density ($20^{\circ}/4^{\circ}$)				
	Solution Concentration				
	1%	2%	4%	8%	10%
G_1	1.00145	1.00605	1.01283	1.02877	1.03651
G_2	1.00116	1.00665	1.01161	1.02729	1.03586
G_3	1.00136	1.00518	1.01274	1.02844	1.03701
G_4	1.00141	1.00470	1.01279	1.02873	1.03760
G_5	1.00136	1.00489	1.01274	1.02897	1.03784
G_6	1.00184	1.00514	1.01323	1.02947	1.03834
G_7	1.00213	1.00543	1.01353	1.02977	1.03835
G_8	1.00233	1.00563	1.01372	1.02997	-
G_9	1.00233	1.00563	1.01372	-	-
G_{10}	1.00228	1.00558	1.01367	-	-

fixed temperature, all sugar solutions of the same concentration gave almost the same viscosity. Browne and Zerban (12) reported the viscosity of pure sucrose solutions for a number of temperatures and concentrations ranging from 0 to 100%.

Ihnat and Goring (30) measured the intrinsic viscosity of cellodextrins (cellobiose through cellohexaose) in aqueous solution at 25°C along with diffusion coefficients and other factors to study the shape of these oligomers in aqueous solution. The cellodextrins appeared to be rodlike and gave no indication of pronounced flexibility or folding in aqueous solution at 25°C. Intrinsic viscosities were reported as follow (30): cellobiose, 0.0274; cellotriose, 0.0303; cellotetraose, 0.0357; cellopentaose, 0.037; and cellohexaose, 0.047 at 25°C. By the method of least squares, Hoover et al. (28) predicted values of viscosities and intrinsic viscosities at 20°C for several maltooligosaccharides.

In this study, the relative viscosity of 1%, 2%, 4%, 8% and 10% (w/v) maltooligosaccharide solutions are reported in Table 8. As expected, the maltooligosaccharide of higher D.P. exhibited slightly higher viscosity at the same concentrations. This property of oligosaccharides was significantly observed at concentrations higher than 2%. The relationship between relative viscosity and D.P. at various concentrations are shown in Figure 5. The relationship between relative viscosity and chain length of oligosaccharides was found to be linear so long as the true solution could be obtained. For threadlike high polymer molecules, which consist of chains of monomer units, the specific viscosity, η_{sp}, is often calculated since the specific viscosity is independent of the size of the polymer and is dependent on the volume fraction that they occupy (35). The specific viscosities of

Table 8

Relative Viscosity at 20°C of Solution of

Maltooligosaccharides

D.P.	Relative Viscosity (centipoise)				
	Concentration				
	1%	2%	4%	8%	10%
G_1	1.02213	1.04755	1.10848	1.22869	1.29722
G_2	1.02401	1.05046	1.10984	1.23001	1.31284
G_3	1.02540	1.05442	1.11780	1.27276	1.35286
G_4	1.02556	1.05745	1.12809	1.28733	1.38879
G_5	1.03131	1.06753	1.13580	1.31470	1.42415
G_6	1.02765	1.07044	1.14409	1.33223	1.45922
G_7	1.03363	1.07066	1.15718	1.34937	1.47624
G_8	1.03246	1.07608	1.16820	1.40893	-
G_9	1.04013	1.08315	1.17848	-	-
G_{10}	1.04260	1.08686	1.20390	-	-

Table 9

Specific Viscosity at 20°C of Solutions of Maltooligosaccharides

D.P.	Specific Viscosity				
	Solution Concentration				
	1%	2%	4%	8%	10%
G_1	0.02213	0.04755	0.10848	0.22869	0.29722
G_2	0.02401	0.05046	0.10984	0.23001	0.31284
G_3	0.02540	0.05442	0.11780	0.27276	0.35286
G_4	0.02556	0.05745	0.12809	0.28733	0.38879
G_5	0.03131	0.06753	0.13580	0.31470	0.42415
G_6	0.02765	0.07044	0.14409	0.33223	0.45922
G_7	0.03363	0.07066	0.15718	0.34937	0.47624
G_8	0.03246	0.07608	0.16820	0.40893	-
G_9	0.04013	0.08315	0.17848	-	-
G_{10}	0.04260	0.08686	0.20390	-	-

Figure 5. Relationship between relative viscosity and degree of polymerization for purified maltooligosaccharides.

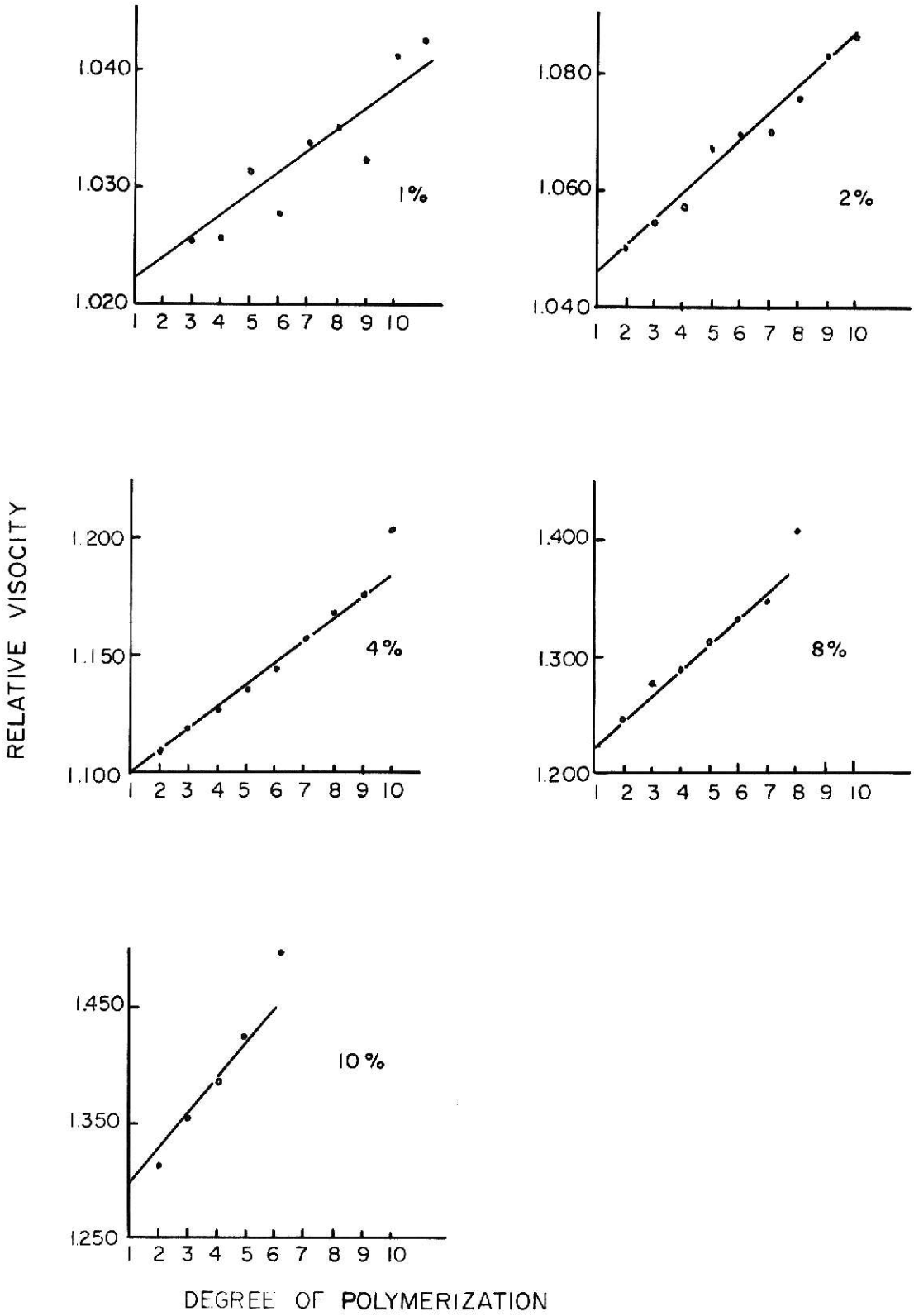
1.040
1.030
1.020
1%
1 2 3 4 5 6 7 8 9 10
1.080
1.060
1.040
2%
1 2 3 4 5 6 7 8 9 10
RELATIVE VISOCITY
1.200
1.150
1.100
4%
1 2 3 4 5 6 7 8 9 10
1.400
1.300
1.200
8%
1 2 3 4 5 6 7 8 9 10
1.450
1.350
1.250
10%
1 2 3 4 5 6 7 8 9 10
DEGREE OF POLYMERIZATION

Figure 5.

Table 10

Intrinsic Viscosity of Maltooligosaccharides

D.P.	Intrinsic Viscosity
G_1	0.0223
G_2	0.0235
G_3	0.0253
G_4	0.0285
G_5	0.0310
G_6	0.0324
G_7	0.0344
G_8	0.0353
G_9	0.0393
G_{10}	0.0430

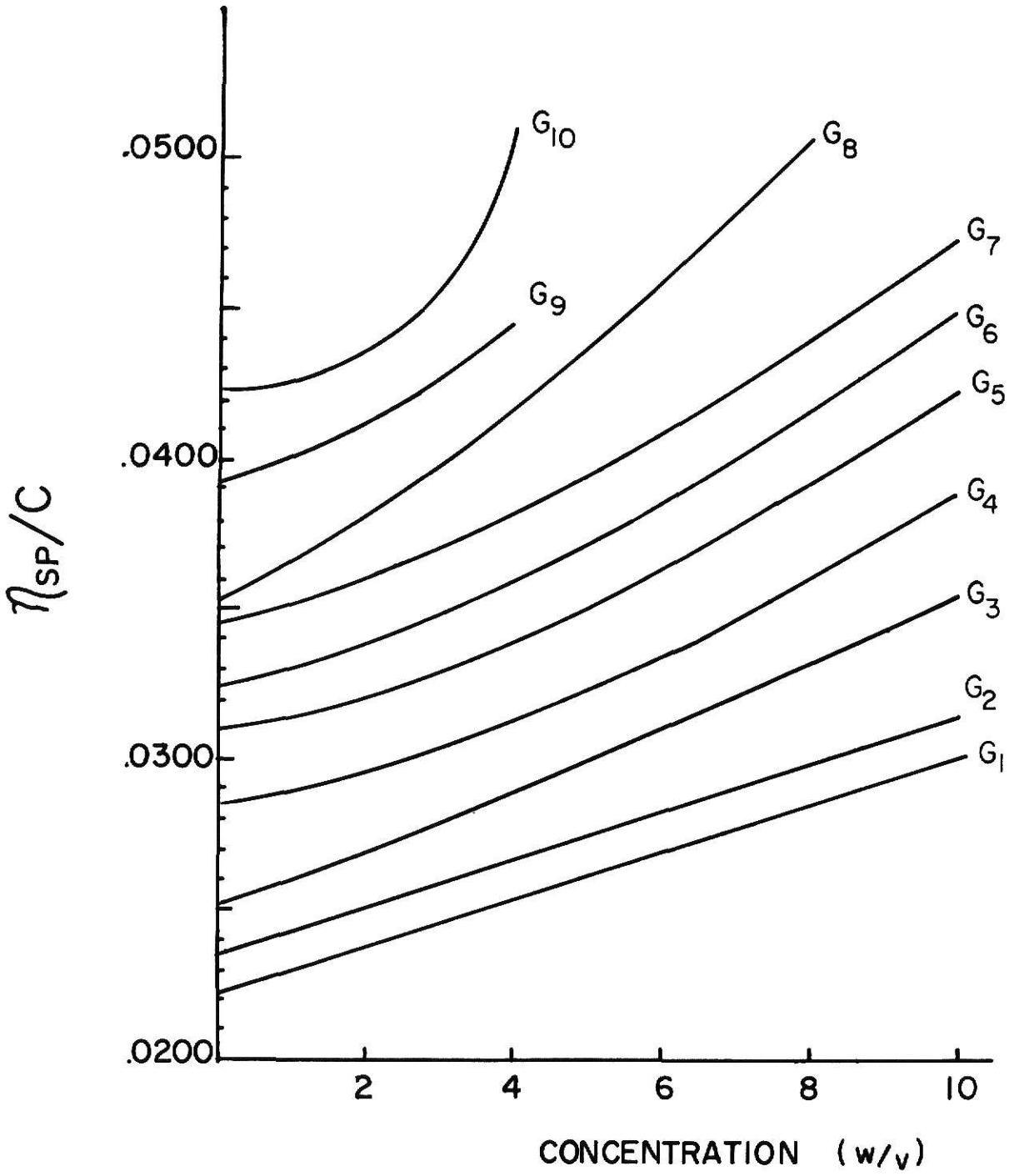

Figure 6. The ratio of specific viscosity to concentration v.s. concentration of maltooligosaccharides.

maltooligosaccharides are listed in Table 9. This shows more pronounced differences of the effect of chain length on viscosity of oligosaccharides since the solvent effect, in this case water, is eliminated.

The intrinsic viscosity or the limit of η_{sp}/c as $c \rightarrow 0$ depends on the properties (shape and specific volume) of the molecule in the solution, since the effects of interaction are eliminated by extrapolation. The relationship between the ratio of specific viscosity to concentration and concentration for a series of saccharide solutions with extrapolation to zero concentrations is shown in Figure 6. Intrinsic viscosities are listed in Table 10. The values of G_1 to G_3 are lower and G_4 to G_{10} are higher when compared to the intrinsic viscosities reported by Hoover and associates (28). The intrinsic viscosity of cellodextrin is higher than maltodextrin at equal chain lengths (30).

Hygroscopicity

Maltooligosaccharides are known to be hygroscopic but it is not known how chain length influences this property. To measure hygroscopicity of the maltooligosaccharides, the individual samples were placed in aluminum dishes stored in desiccators with $60 \pm 5\%$ relative humidities. The hygroscopicity was expressed in terms of percent moisture absorbed by the oligosaccharide. Data illustrating moisture absorption as a function of storage time are summarized in Table 11. It is evident that as chain length increases, the hygroscopicity also increased.

Infrared Spectra

Among the physical properties of a carbohydrate, the infrared spectrum is usually much more specific and characteristic than the melting point, density, refractive index, or ultraviolet spectrum. Changing the orientation

Table 11

Hygroscopicity of Maltooligosaccharides at 60±5 Relative Humidity at Room Temperature

Time (min)	D.P.									
	G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_8	G_9	G_{10}
	% Moisture									
15	1.72	1.45	3.82	4.21	6.20	7.76	4.01	7.48	9.62	8.90
45	2.10	-0.05	5.00	6.47	8.21	8.05	8.14	8.45	8.55	8.19
90	0.52	0.33	7.93	9.22	9.94	9.14	9.67	11.53	12.73	12.30
150	0.60	0.33	8.20	9.83	10.38	9.01	9.12	10.54	11.88	12.30
210	0.49	0.27	9.89	10.12	9.66	9.60	9.83	11.72	13.15	13.26
270	0.43	0.34	11.27	10.82	10.10	10.96	11.44	13.23	14.32	13.92
330	0.51	0.38	11.41	10.38	10.12	9.61	10.01	11.49	12.75	12.12

at only one carbon atom in anomers of a sugar or glycoside changes the spectrum markedly (47). Kuhn (38) recorded the infrared spectra of a number of sugars and their derivatives. He found that glucose is typical of the sugar spectrum with a very strong band about 3300 cm^{-1} due to the OH stretching frequency of the hydrogen-bonded OH group, and several closely spaced bands between 1100-1000 cm^{-1} which are due to carbon-carbon and carbon-oxygen vibration.

Katon and Miller (38) investigated infrared transmission spectroscopy in the 2,000-200 cm^{-1} range for α-αtrehalose, glucose admixed with fructose (1:1), and lactose at room temperature and at lower temperature [-196°C (liquid nitrogen) and -253°C (liquid hydrogen)]. They found spectra of improved quality at both temperatures. This technique is useful for identification and differentiation of complex compounds of biological interest.

Hoover and associates (28) determined the infrared spectra of maltooligosaccharides in potassium bromide pellets. They found that all the saccharides gave spectra so similar that identifying and distinguishing between the various maltooligosaccharides were not possible.

Using the "mull" technique, the spectra of maltooligosaccharides (G_1-G_{12}) were determined (Figure 8 to 10). Figure 7 shows the absorption band of petroleum jelly and hexachloro-1,3-butadiene used to prepare the samples for IR analysis. Principally these oligosaccharides absorb infrared radiation at approximately the same frequency. A broad and deep absorption band centered at about 3600-3100 cm^{-1} can be identified as O-H stretching modes. A smaller band at 2900 cm^{-1} is C-H stretching vibration. The absorption at 1640 cm^{-1} along with the band at 3600-3100 cm^{-1} are characteristic of the molecular vibration in water (61). All samples except G_1 show strong

absorption at 1640 cm^{-1}. This is an indication that maltooligosaccharides are hygroscopic.

The region between 1320-1220 cm^{-1} contains O-H in-plane and C-H, CH_2 bending modes. Absorption in this region decreased in intensity as chain length increased. The bands between 1150 cm^{-1} and 980 cm^{-1} are broad, intense and close together. The peak at 1147 cm^{-1} could be assigned to C-C in-plane bending and the peaks at 1105 and 1150 cm^{-1} are C-O-C bending (53).

The region of adsorption by the anomeric carbon atom of pyranose structures at 960-730 cm^{-1} indicates that these oligosaccharides are α-D-glucopyranosides. The absorption spectra increases from 907 to 930 cm^{-1} for type 1, and from 778 to 758 ± 2 cm^{-1} for type 3, from maltose to maltodecaose, and type 2 absorption is at 844 ± 8. Type 1 has been assigned to the antisymmetrical ring C-O-C stretching, type 3 to the symmetrical ring breathing vibration and type 2 is assigned to be vibration involved in an anomeric C-H deformation (6, 46), which showed very small deviation.

Although there is some shifting of the peacks from 960-730 region, the infrared spectra of G_1, G_2 and G_3 are appreciably different in the intensities of these bands and this may be used to distinguish between these three. Spectra for the other oligosaccharides (G_4-G_{12}) are very much like G_3. The infrared spectra could not be used to identify the higher molecular weight sugars. The reason for this is because all maltooligosaccharides contain the same functional group and the same type of glycosidic linkages.

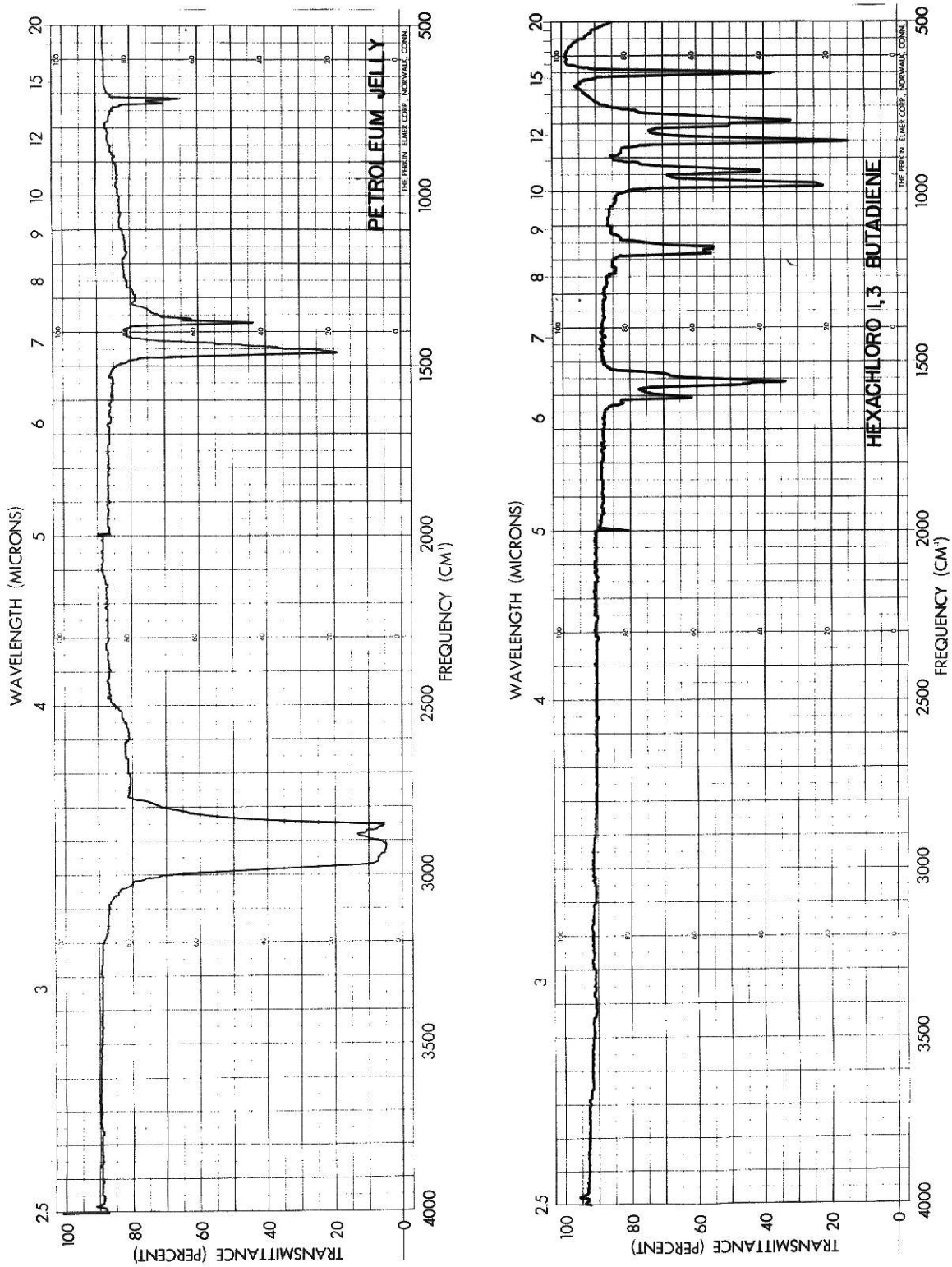

Figure 7. Infrared spectra of petroleum jelly and hexachloro-1,3-butadiene

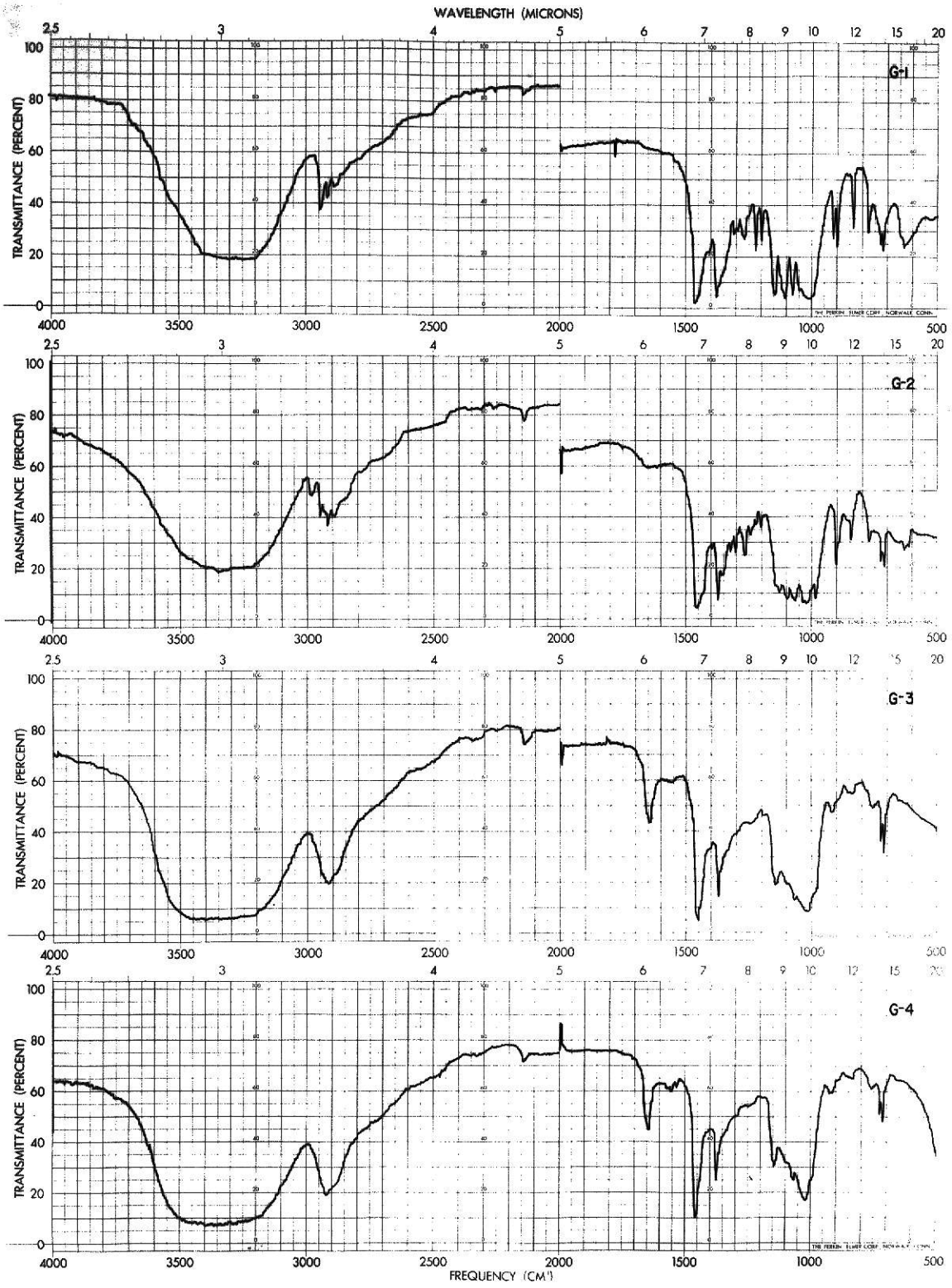

Figure 8. Infrared spectra of glucose, maltose, maltotriose and maltotetrose

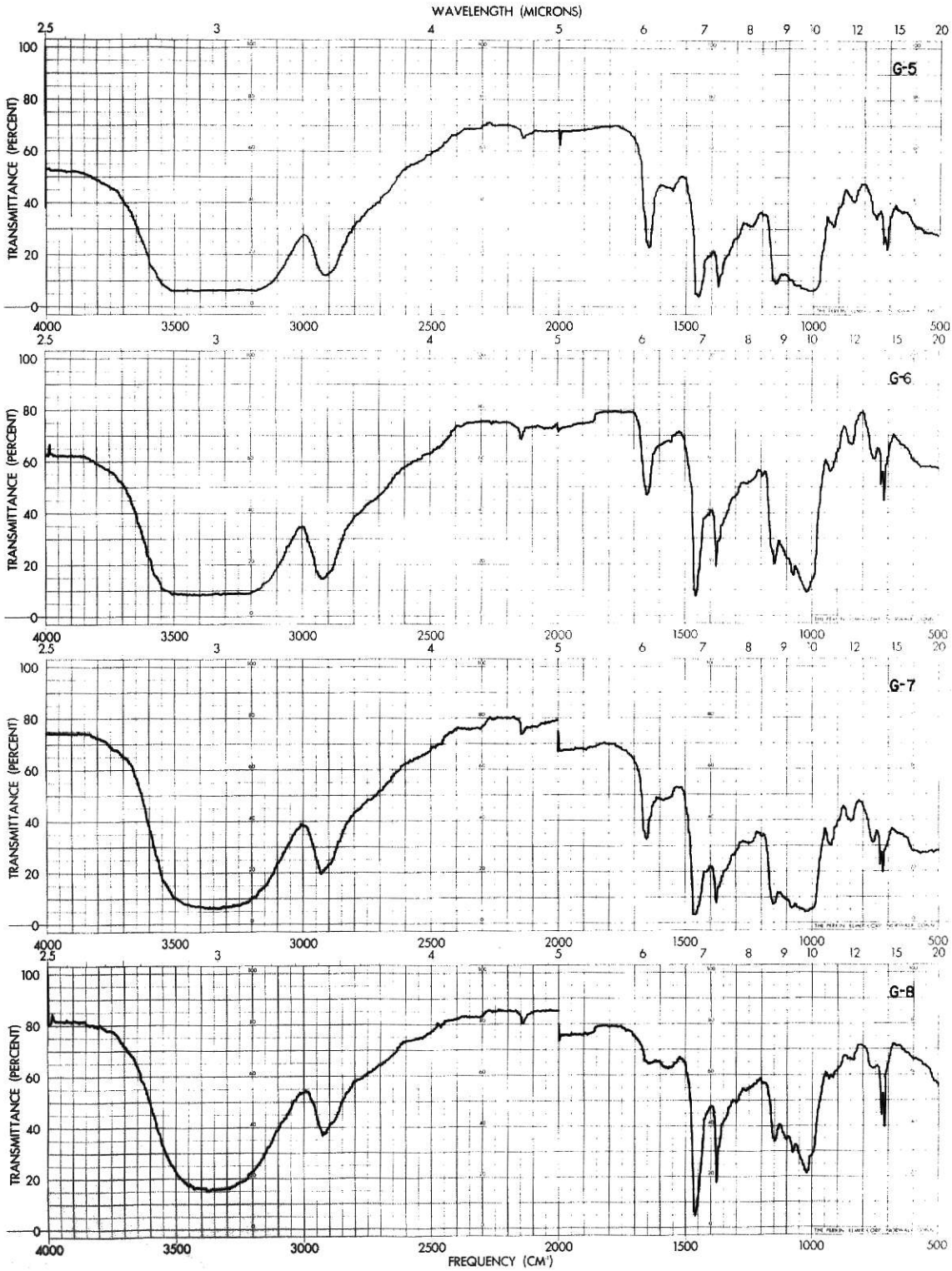

Figure 9. Infrared spectra of maltopentaose, maltohexaose, maltoheptaose, and maltooctaose.

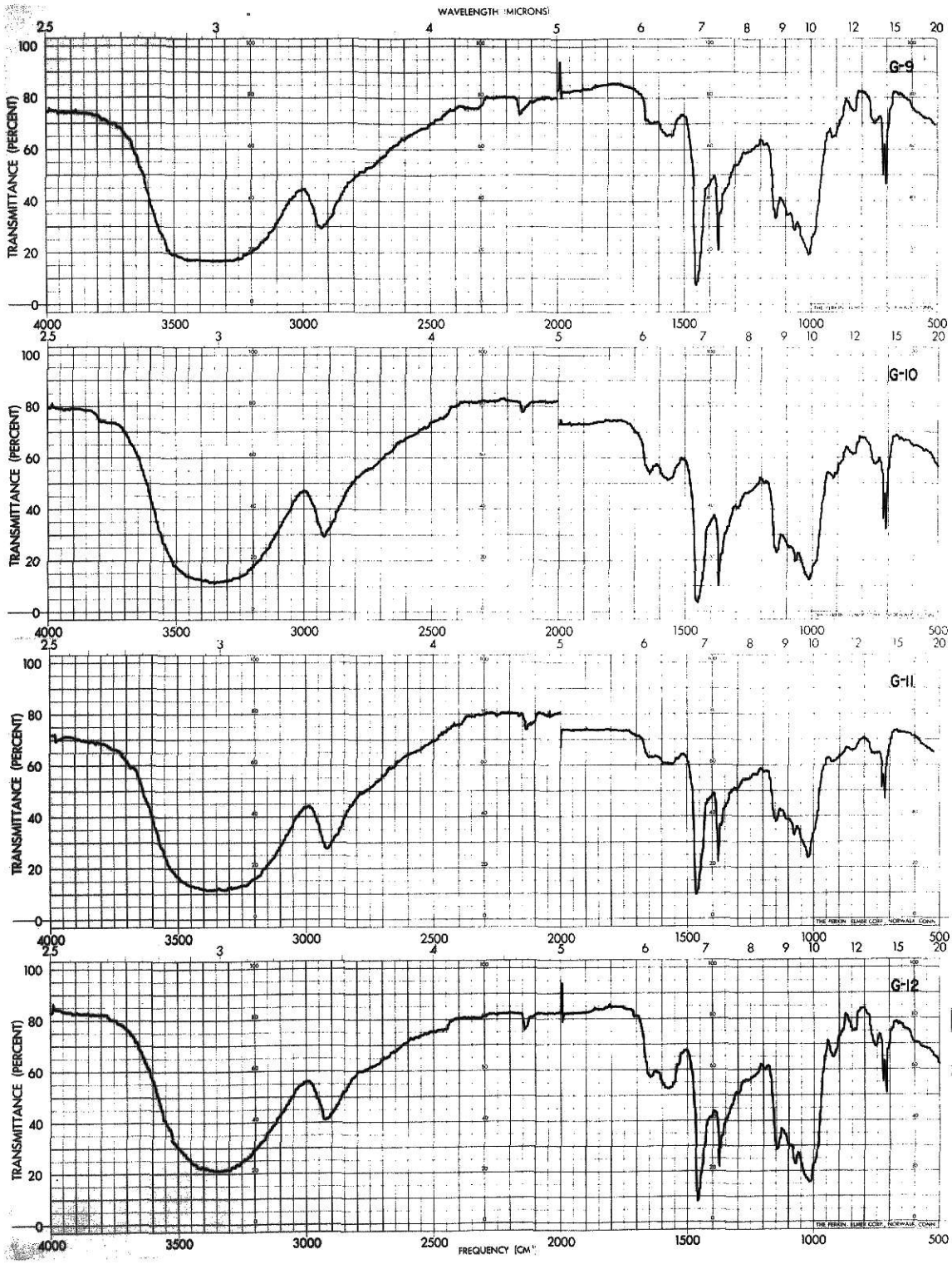

Figure 10. Infrared spectra of maltononaose, maltodecaose, maltoundecaose and maltododecaose

Raman Spectra

Since the infrared spectra did not appear promising for identifying these maltooligosaccharides, attempts have been made to use Raman spectra to identify these homologs. The Raman spectra of glucose, maltose and maltooligosaccharides were studied in the region 100 to 3600 cm^{-1}. Glucose and maltose showed interesting spectra, but maltotriose and higher oligosaccharides showed fluorescence over the available spectrum. Vasko and associates (62) studied Raman spectra of glucose, maltose, cellobiose and dextran in water and deurterium oxide solution. They could assign C-O-H, CH_2-, and C-H related vibrational modes.

The major peaks in the Raman spectra of crystalline glucose and maltose obtained in this investigation are listed in Table 12. Solid samples seem to give more absorption bands than those in solution. Raman spectra of crystalline glucose and maltose are shown in Figure 12.

Scanning of maltooligosaccharides at the anti-stoke side (frequency higher than the laser exciting line) has also been tried to prevent fluorescence. Glucose and maltose showed good structure and were distinguishable. G_3 through G_{12} were superficially different in appearance but close examination reveals that they are almost the same, except that the intensities of the bands vary from sample to sample.

Thus, it is concluded that both stokes and anti-stokes spectra yield no clearcut way to differentiate the maltooligosaccharides.

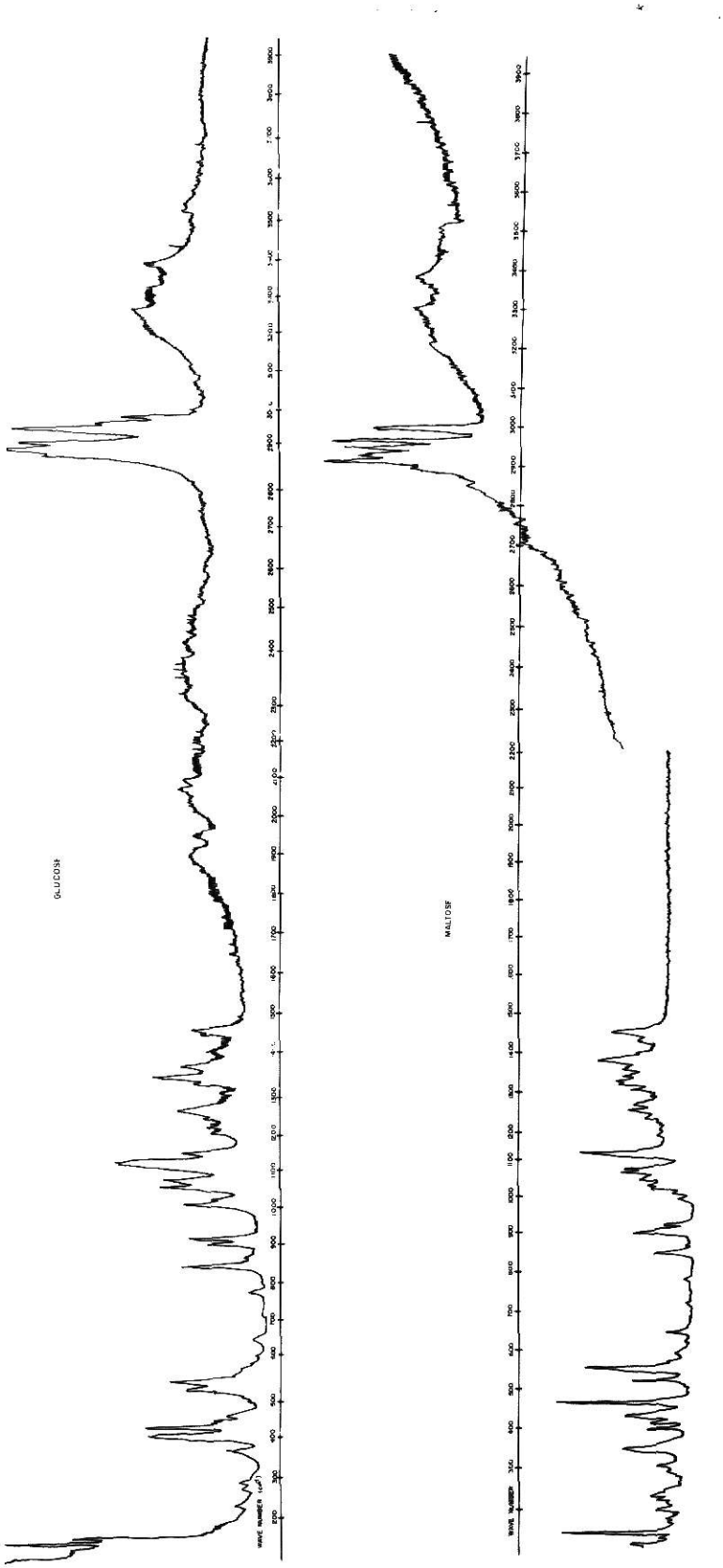

Figure 11. Raman spectra of glucose and maltose

Table 12

Observed Frequencies (cm^{-1}) and Intensity of Glucose and Maltose

Glucose	Maltose	Glucose (cont.)	Maltose (cont.)
3400s		1257s	1254m
3354w(sh)		1240m	1238m
3320sh		1223m	1226w
3263m		1205m	1208w
3110w(sh)			1192w
3070sh		1150m	
3000w		1120vs	1125vs
2977m	2978s(sh)	1075s	1070s
	2973s(sh)		1063s(sh)
2948m(sh)	2950vs	1052s	
2930vs	2930vs		1046m(sh)
	2913m(sh)		1025vs
	2910s	1020sh	1020sh
2900vs	2897vs	1007s	1007w
2880vs	2883sh		990w
2867s(sh)		918s	920m
	2845m	900s	900s
1460s		866w	868w
1450sh	1453s		847m
	1432w	840s	
1407m	1400w(sh)	800w	
	1380s		780w

Table 12 (cont.)

Glucose	Maltose	Glucose (cont.)	Maltose (cont.)
1370s		770m	
	1360m	713w	720w
1343vs	1344m	650sh	640m
1330sh	1328m	610w	620w
	1320sh		590w
1310w	1308m		568w(sh)
	1292w	553vs	550vs
	1270m	532s	
	519m	290m	290w
500w		273m	
465m	463vs	230m	234m
450m			2223m
	432sh		200m
425vs	427s		176w
406vs	412m		167w
	395m		
367m			
	346s(sh)		
	305m		

s = strong
vs = very strong
sh = shoulder
m = medium
w = weak

SUMMARY AND CONCLUSIONS

A study was made of certain chemical and physical properties of a series of oligosaccharides, G_1 to G_{12}, isolated from a partially-hydrolyzed corn starch.

The oligosaccharide fractions were isolated by carbon-Celite and preparative paper chromatography. After isolation and purification, the following chemical and physical properties were established: reducing power, specific gravity, refractive index, viscosity, hygroscopicity, infrared and Raman spectra.

The reducing power was found in close agreement with theoretical values for each of the oligosaccharides. The true specific gravity, corrected for buoyancy, increased with chain length. The refractive index of low concentration solutions, except glucose and maltose, were identical for all oligosaccharides, but with high concentration, the refractive index increased with chain length. Specific refraction decreased with chain length. Likewise, relative, specific and intrinsic viscosity increased with chain length. The relationship between relative viscosity and degree of polymerization was linear. The hygroscopicity increased with higher molecular weight oligosaccharides. Infrared absorption bands were similar for all sugars and it appeared impossible to distinguish different oligosaccharides by this procedure. Raman spectra had distinct absorption bands for glucose and maltose but higher oligosaccharides exhibited such a high fluorescent background that absorption bands were obliterated.

SUGGESTIONS FOR FUTURE WORK

1 Maltooligosaccharide can be separated directly using preparative paper chromatography. With this method more attention is required, but the cost of operation is cheap and the sugar separation is reliable.

2 The maltooligosaccharide solutions used in determination of specific gravity, refractive index and viscosity were slightly yellow. This yellow color which was suspected to have been derived from the chromatographic paper could be eliminated (a) by passing the oligosaccharide solutions through ion exchange columns after separating these with unwashed chromatographic paper; (b) by irrigating the chromatographic paper with water in the separatory chamber before use.

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APPENDIX

Table 1

Data collected to calculate the reducing power included: sample weight, volume of sample, volume of sodium thiosulfate used for titration.

Degree of polymerization	Volume Prepared ml.	Wt. of Dry Sample (mg) in 5 ml	Blank-titer	Dextrose read from Std. curve
G_1	100	0.360	1.935	0.383
G_2	100	0.738	2.040	0.401
G_3	25	0.994	1.935	0.376
G_4	25	1.332	1.990	0.394
G_5	25	1.652	1.940	0.384
G_6	25	1.972	2.050	0.402
G_7	25	2.304	1.800	0.361
G_8	25	2.660	1.825	0.363
G_9	25	2.752	1.710	0.346
G_{10}	25	3.454	1.890	0.377
G_{11}	25	3.654	1.745	0.352
G_{12}	25	4.064	1.680	0.342

Table 2

Average Flow Time of Maltooligosaccharides

in Oswald Viscometer

D.P.	Time (second)				
	Solution Concentration				
	1%	2%	4%	8%	10%
G_1	80.90	82.65	86.75	94.75	99.40
G_2	81.10	82.90	86.90	95.00	100.70
G_3	81.20	83.20	87.50	98.10	103.50
G_4	81.20	83.65	88.30	99.20	106.20
G_5	81.65	84.20	88.90	101.30	108.90
G_6	81.35	84.40	89.50	102.70	111.40
G_7	81.80	84.40	90.50	103.90	112.70
G_8	81.70	84.80	91.35	108.5	-
G_9	82.30	85.40	92.15	-	-
G_{10}	82.50	85.70	94.10	-	-

Flow time for water = 79.43 seconds

Table 3

Average Weight of Maltooligosaccharides

Solution in Pycnometer

D.P.	Weight (gm)				
	Concentration				
	1%	2%	4%	8%	10%
G_1	2.00965	2.01605	2.03249	2.06275	2.07593
G_2	2.00840	2.01556	2.03146	2.05952	2.07379
G_3	2.00864	2.01585	2.03202	2.06376	2.07920
G_4	2.00897	2.01634	2.03215	2.06423	2.08014
G_5	2.00909	2.01671	2.03223	2.06442	2.08022
G_6	2.00935	2.01740	2.03333	2.06344	2.08363
G_7	2.00992	2.01880	2.03387	2.06582	2.08254
G_8	2.01010	2.01845	2.03414	2.06557	-
G_9	2.01028	2.01744	2.03424	-	-
G_{10}	2.01016	2.01726	2.03402		

Weight of water = 2.00255 gm

EFFECT OF CHAIN LENGTH ON CERTAIN PROPERTIES OF OLIGOSACCHARIDES

by

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B.S., Kasetsart University, 1967
Bangkok, Thailand

AN ABSTRACT OF A MASTER'S THESIS

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Manhattan, Kansas

1972

The present investigation was conducted to study the effect of chain length on some physical and chemical properties of oligosaccharides.

Maltooligosaccharides (G_3-G_{12}) from corn starch hydrolyzate were separated on a charcoal-Celite column (6"x12') and eluted with increasing concentrations of aqueous ethanol. The presence of sugars in the eluate was estimated by the phenol-sulfuric acid method and confirmed by paper chromatography. Maltotriose and maltotetraose, which were eluted with 15 and 20% aqueous ethanol, respectively, were found to be relatively pure. Saccharides ranging from maltopentaose to maltoheptaose were found admixed with each other in fractions eluted with 20% aqueous ethanol. Maltooctaose to maltododecaose were eluted from the column using 25, 30, 35 and 50% aqueous ethanol. Eluates of lower fractions of maltooligosaccharides (G_3-G_4) and fractions of closely admixed homologous members were evaporated under vacuum. Chloride ions in the concentrated solutions were eliminated by use of ion-exchange columns.

Preparative paper chromatography using prewashed Whatman 3MM paper, with n-propanol-ethyl-acetate-water (14:3:7) solvent was used to isolate and purify the partially-separated sugars; the chromatographically pure, white, amorphous saccharides were collected for chemical and physical analysis.

The reducing power of the maltooligosaccharides measured by the Somogyi micro-copper method was found in close agreement with the theoretical values. Stoichiometry of the copper-maltooligosaccharide reaction was also in close agreement with reported values. The specific gravity, refractive index and viscosity were determined for each oligosaccharide. True specific gravity of oligosaccharides, except glucose, at various concentrations increased as

the chain length increased. A linear relationship between the specific gravity and chain length was obtained so long as the sugars remained in true solution. At low concentration, the refractive indices of maltooligosaccharides, except glucose and maltose, had the same value. At high concentration, saccharides of higher D.P. showed a higher index of refraction. The specific refraction decreased as chain length increased. The molecular refractivity of maltooligosaccharides increased with increasing chain length, but each glucose unit in the polymer contributed less refraction as the chain length increased. Relative viscosity, specific viscosity, and intrinsic viscosity of maltooligosaccharides of various concentrations were found to increase as the chain length increased. Relationship between relative viscosity and D.P. was linear so long as the sugars remained in true solution. Except glucose and maltose, the hygroscopicity of maltooligosaccharides at $60\pm 5\%$ relative humidity increased as the chain length increased. Infrared spectra of all maltooligosaccharides were similar and although there was some shift in the 930-760 cm^{-1} region, that shift was not significant enough for use in identification and characterization. The Raman spectra of glucose and maltose showed absorption bands. However, maltotriose and all higher oligosaccharides exhibited such great fluorescence that the absorption bands were obliterated. Thus, maltooligosaccharides could not be identified and characterized by Raman spectra.

The results indicated that the reducing power of maltooligosaccharides decreases, whereas the specific gravity, refractive index, viscosity and hygroscopicity increase with increasing chain length. Changes observed in the infrared and Raman spectra were not significant enough for identification and characterization.