

MACRO PAPER CHROMATOGRAPHY OF CORN STARCH HYDROLYSATES¹

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ABSTRACT

In order to study the physical and chemical properties of the constituents of corn starch hydrolysates, large quantities of individual oligosaccharides were required. Glucose polymers up to a D.P. of 11 were separated by application of the techniques of quantitative paper chromatography. Whatman 3-mm. chromatography paper was loaded with 2-3 g. of substrate in a band about 1½ in. wide. The polymers were separated using a solvent made up of n-propyl alcohol, ethyl acetate, and water.

During the course of investigating various physical and chemical properties of corn starch hydrolysates such as dextrans and syrups, the need arose for gram quantities of the component oligosaccharides. Thus, for example in the development of new corn syrups for candy-makers, knowledge of the hygroscopicity of the individual sugars is needed to predict the behavior of the finished product. Glucose polymers higher than maltose were, for all practical purposes, unavailable for studies such as these; therefore, a program was initiated to obtain them.

There are several methods of separation, but most of them have some limitations. Solvent precipitation and dialysis methods give mixtures of oligosaccharides which in most instances are little better than the starting substrate. Many workers have employed column chromatography using charcoal, charcoal-Celite, cellulose, etc. In most cases a few grams of substrate were used; the packing and operation of the column were difficult and time-consuming; many fractions had to be taken, and each fraction had to be analyzed for the presence of sugars. The most successful workers were Whelan *et al.* (6), Thoma *et al.* (5), and Hoover (4). Whelan separated fractions up to maltoheptaose on a charcoal-Celite column using various alcohol-water solvents. Thoma, using a cellulose column at elevated temperatures, was able to separate 50- to 100-mg. quantities of sugars up to a D.P. of 18. Hoover scaled up a charcoal column chromatography apparatus to pilot-plant size and separated 10- to 50-g. quantities of the sugars up to a degree of polymerization of 10. Hoover used an 8-ft. column made up of 6-in.-diameter Plexiglas tubing. He collected over 400 1-liter fractions in the preliminary separation. He combined selected fractions, evapo-

¹Manuscript received May 29, 1963.

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rated them to syrups, and refractionated them on a smaller column to obtain his pure materials. One device, the LKB Chro Max Column, is commercially available. This consists of a metal cylinder in which rolls of cellulose (paper) are inserted. In many respects it behaves like a paper chromatogram; however, fractions have to be removed periodically and analyzed. Furthermore, it is necessary to wash all of the desired components off the paper in order to collect them.

The most rapid and easiest method of separating corn starch hydrolysates is paper chromatography. Various workers have used both ascending and descending procedures to separate corn syrup, amylose hydrolysates, etc. (3,7). Analytical procedures for the quantitative determination of the components of syrups have been developed using this technique. However, most workers have stressed the importance of using microgram quantities and the necessity of keeping the spotting area at a minimum. Thus, although the method is simple and rapid, gives excellent separation and requires little personal attention, it is limited by the amount of material which can be obtained.

Each of the above-mentioned methods had been employed at one time or another in our laboratory with some success. Several hundred mg. of the lower sugars (G_3 and G_4) were obtained by a combination of dialysis and charcoal-Celite column chromatography from a corn starch dextrin. The crude polymers were purified by paper chromatography. Later it was found that by applying a narrow band of corn syrup in a continuous line on No. 1 Whatman chromatography paper, good resolution of the polymers was obtained using a solvent made up of n-propanol, ethyl acetate, and water. The next step was to use Whatman 3-mm. paper on which up to 3 g. of solids could be applied in bands ranging in width from $1/2$ to $1 1/2$ in.

Materials and Methods

To obtain the lower polymers such as maltotriose, maltotetraose, and maltopentaose, 42 D.E. acid-hydrolyzed corn syrup was used as the substrate. This material has appreciable quantities of the reversion disaccharides and trisaccharides. In order to cut down on the possibility of contamination from this source, special all-enzyme-converted syrups were prepared ranging in D.E. from 18 to 35. The 18-D.E. syrup proved to be the best source of polymers higher than maltohexaose.

The chromatography paper was laid out in the usual manner by drawing a line 3 in. from the top of the paper to form a crease, from which the paper was hung on rods. Three-fourths inch below this line a 1-in. band was drawn. Guide lines were drawn in 1 in. from either side. The substrate was applied by painting a 25% solids solution or

suspension in the 1-in. band. When the area was dry, another layer of substrate was applied. This was repeated until 2 to 3 g. of solids were deposited on the sheet. At this stage a glossy layer of carbohydrates coated the surface of the 1-in. band. It was found that by brushing on water this glossy layer could be soaked into the paper. If this procedure was not followed, poor resolution resulted, owing to excessive streaking at various points along the sheet. Spots of known sugars were placed on the guide strips.

The paper was then suspended in a cabinet. The developing solvent contained *n*-propanol, ethyl acetate, and water in a ratio of 14:2:7 by volume. After 2 or 3 days the lower sugars, glucose, maltose, and maltotriose, had run off the sheet. The guide strips were cut off, together with a small margin of the spotted carbohydrates. These strips were sprayed with the Bacon and Edelman (1) detecting reagent made up of benzidine (0.5 g.), acetic acid (10 ml.), trichloroacetic acid (40% w/v, 10 ml.), and ethanol (80 ml.) and heated for several minutes in a forced-air oven. From the location of the known sugars the positions of the polymers were determined. The sheet was then divided into three sections. The lower portion contained mostly maltotetraose, maltopentaose, maltohexaose, and traces of maltoheptaose. The center section contained traces of maltohexaose but was predominantly the polymers up to nine glucose units. The upper section contained the higher homologs. A very small band which did not react with the spray reagent remained along the top of the sheet just below the area which was spotted.

It was possible in some instances, particularly in the case of the lower polymers, to separate the individual sugars at this point. In general, however, the entire section was cut up into small pieces and the sugars eluted with water. The water extracts were evaporated to near dryness *in vacuo* at 55°C., decolorized with charcoal, and respotted on Whatman 3-mm. paper as before. After development, individual sugars were located and eluted from the paper. At this point they could be rechromatographed to ensure complete separation or dried in a laboratory-size freeze-dryer.

The lower sugars required about 4 days to separate, the intermediate sugars 8 to 10 days, and the higher polymers up to 20 days under typical conditions.

Results and Discussion

From 40 sheets of paper, each impregnated with 2 to 3 g. of solids, were obtained about 30 g. of pure sugars G_4 to G_{11} . They ranged in

quantity from 350 mg. for the 11-glucose-unit polymer to nearly 5 g. of the maltotetraose. No attempt was made to recover the sugars lower than maltotetraose. To ensure the chromatographic purity of the sugars, only the centers of the bands were recovered; hence, substantial quantities of "heads" and "tails" of fractions were discarded.

The pure fractions, together with known samples of glucose, maltose, and maltotriose, were spotted on Whatman No. 1 chromatography paper and developed with a solvent containing slightly less water (14:2:6) for 25 hr. The ratio of the movement of the polymers to that of glucose (R-G values) is shown below:

Solvent: Propanol:ethyl acetate:water (by volume)

14 : 2 : 6

G_1 (glucose) = 1

$G_2 = 0.835$ $G_7 = 0.223$

$G_3 = 0.655$ $G_8 = 0.168$

$G_4 = 0.505$ $G_9 = 0.127$

$G_5 = 0.390$ $G_{10} = 0.097$

$G_6 = 0.292$ $G_{11} = 0.081$

To substantiate the purity and identity of the polymers, they were compared with the sugars obtained by the acid hydrolysis of corn

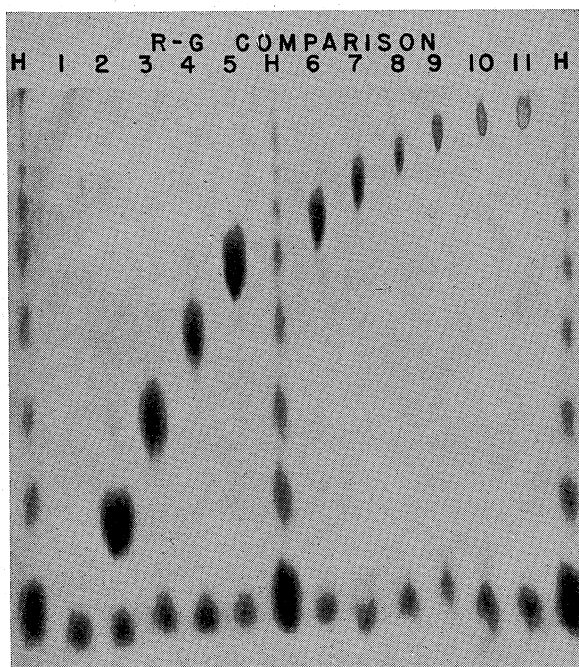


Fig. 1. Comparison of the relative rates of movement of glucose polymers, G_1 to G_{11} . H-spots are from an acid-hydrolyzed corn amylose.

amylose (Fig. 1). Further analytical work has also been initiated which likewise indicates the purity and identity of each member of the series and will be reported later.

The solvent made up of n-propanol, ethyl acetate, and water in a ratio of 14:2:7 is satisfactory for this type of separation. The resolution is not as good as others employed, but by decreasing the water this can be slightly altered. Using higher levels of water causes some streaking and running of the substrate. The early separations were made at ambient temperatures; later a new stainless-steel cabinet insulated with styrofoam was employed. By heating the cabinet to 90°F. with a heating tape, the time required for the separations could be cut nearly in half.

The 1-in. bands usually tend to spread slightly during the application procedure, and in the subsequent rewetting process frequently the bands may be nearly 1½ in. wide in places. If too much substrate is applied in one spot it is advisable to scrape it off; otherwise the solvent will not penetrate that area and bad streaks will result.

Summary

Large quantities of glucose polymers ranging up to a D.P. of 11 were separated from starch hydrolysates by paper chromatography. The substrate was spotted in a wide band on Whatman 3-mm. paper and developed with a solvent made up of n-propanol, ethyl acetate, and water in a ratio of 14:2:7.

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