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GEL CHROMATOGRAPHIC SEPARATION OF OLIGOSACCHARIDES

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1. INTRODUCTION

The methods most commonly used to achieve separation of oligosaccharides are chromatography on paper and on thin layers, gas liquid chromatography and ion exchange chromatography. Chromatography on cellulose and charcoal-celite columns has also been used with success.

Gel chromatography is a column chromatographic method, which separates solute molecules according to differences in their size. If a solution containing a mixture of solutes of different molecular sizes is percolated through a column packed with porous gel particles, the smaller molecules penetrate farther into the gel pores than do the larger, and they are therefore retained for a longer time on the column. The solute molecules are thus eluted from the column in order of decreasing molecular size. Immediately after elution of all solutes, another sample can be applied to the column and the process may be repeated. Regeneration is thus unnecessary.

In recent years this technique has been improved for a more effective separation of carbohydrates occuring in biological materials. Fractionations of dextrins[1], dextran[2], dextrins from glycogen and amylopectin[3], xylodextrins[4], cellodextrins[5], mannodextrins[6], and the isolation of cell wall components from yeast[7,8,9], and from bacteria[10] have been achieved by means of dextran gels (Sephadex). Oligosaccharides of enzymatically degraded heparin[11], hyaluronic acid and chondroitin-4-sulfate[12] have also been fractioned on Sephadex columns.

Polyacrylamide gel, first introduced in 1962 by HJERTEN and MOSBACH[13,14]

and simultaneously by LEA and SEHON[15], is a more useful support for the separation of carbohydrates because in contrast to dextran gels it does not split off carbohydrates and it is not attacked by bacteria. This gel has been used for the fractionation of fructosans with a chain length of 10 fructose units[16], chitin oligomers[17], xylodextrins[4], cellodextrins[5] and mannodextrins[6].

This contribution describes the separation of mono- and oligosaccharides on a column of Bio-Gel P-2, minus 400 mesh, and their detection by an automated system based on the orcinol colorimetric method. With this technique we succeeded in fractionating oligomers containing two to twenty-one glucose units, as well as separating a mixture of isomeric compounds with similar molecular weights. It is the purpose of this chapter to give some of the experimental details on the use of this method.

2. EXPERIMENTAL

2.1. Preparation of the gel

Gel chromatographic separation of carbohydrates was carried out with Bio-Gel P-2, minus 400 mesh, with a fractionation range for molecular weights from 100 to 3.400. The gel was purchased from Bio-Rad Laboratories.
In order to obtain a high column efficiency it is necessary to use gel fractions with a narrower particle size distribution than are commercially available.
In our laboratory a small batch of dry gel was shaken over a set of two sieves (60 μ and 28 μ, Monodur, Verseidag, Krefeld, Germany). A sufficient amount of the 28-60 μ gel fraction was allowed to swell in excess water overnight. After hydration the gel was fractionated by repeated settling and decanting the fines until a sharp zone of settling gel particles was attained. This operation was carried out in a large glass cylinder (14 x 80 cm). Before packing the column, the gel slurry should be degassed by aspiration in order to prevent bubble formation within the gel bed.

2.2. Column

For most analytical and preparative purposes a glass-jacketed column (1,5 x

200 cm), screwed at both ends, was used. The upper end of the column was sealed by inserting a flow adaptor which permits automatic sample application. A screw cap containing a fine nylon net (10μ) and a coarse support screen was attached to the end of the Duran-glass tube. The cap is sealed with an inserted washer against the end of the tube. In order to avoid loss of resolution and dilution of effluent fractions, a capillary PTFE outlet tubing was used. Wall effects were minimized by coating the column with a solution of 1 % dichlorodimethylsilane in benzene[18].

2.3. Packing the column

The column was packed in the following way: It was filled with degassed water and air bubbles were removed carefully. An extension tube (150 cm) was attached to the top of the column by means of a screw coupling. Sufficient gel suspension, previously degassed in a vacuum flask was poured into the lengthened column and the gel was allowed to settle. When a layer of 2 - 4 cm has formed the outlet of the column was opened and a slow stream of water allowed to flow out until a horizontal zone of densely packed material became visible in the extension tube. Tight and close packings were obtained by pumping a solution of 0,1 % NaCl or Brij-35 by means of a Milton-Roy Minipump at a flow rate of about 50 ml/h through the lengthened gel bed till the settling of the gel was complete. The flow rate should be reduced when the pressure exceeds 175 psi.

After packing, the extension tube is removed and the flow adaptor is installed and screwed on top of the column. Care must be taken to avoid entrapment of air between bed surface and flow adaptor. The NaCl- or Brij-35 solution was replaced by pumping water at a flow rate of 20 - 25 ml/h through the bed. The column is now heated to 65 °C by means of a Haake constant temperature circulator. During this operation no eluant should be pumped through the gel bed. The column is then connected to a reservoir containing the eluant (distilled water) and stabilized by allowing at least 2 - 4 bed volumes of water to pass through the bed.

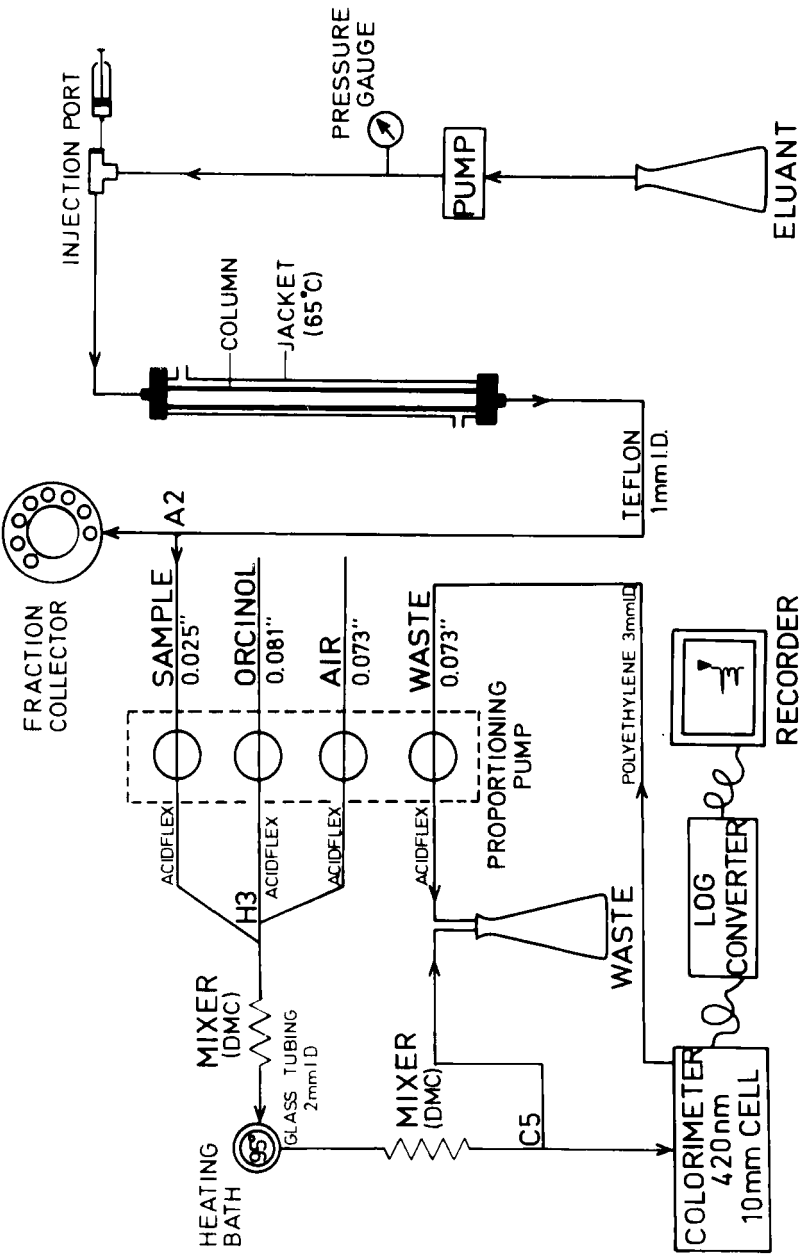

FIGURE 1

Flow scheme for the separation of carbohydrates and analyzing system.

Since Bio-Gel P-2 is relatively rigid due to a low water regain, rather high pressures can be used to achieve an optimal flow rate. In our experiments all separations were carried out at a column temperature of 65 °C and a flow rate of 25 ml per hour. Usually the pressure rises to 35 - 100 psi ; but if the pressure exceeds 145 psi, the screw cap at the bottom of the column was opened and the dehydrated and compressed gel layer was removed carefully. Then the cap was filled with water and air bubbles were removed before screwing it to the end of the column again. Thus, repacking the column can be avoided.

2.4. Analytical procedure

Figure 1 shows the flow scheme for the separation and analyzing system which was essentially an Auto Analyzer using orcinol-sulfuric acid for the detection of the carbohydrates in the effluent [19]. The eluant (twice-distilled water) was degassed by boiling and the eluant reservoir was kept at a temperature of about 80 °C. A Milton-Roy Minipump was used to pump the eluant through the column at a fixed rate of 25 ml per hour. For the automatic application of the sample a Swagelok Union Tee (1/8"), covered with a rubber septum, is connected by a Teflon tubing to the column and is used as an injection port. This device allows the application of the sample through a rubber septum by means of a micro-syringe without interrupting the process of elution. Thus, as regeneration of the column is unnecessary, the next sample may be applied before the slowest compound of the foregoing sample has been eluted. In a typical run the sample was dissolved in water and was applied to the column by means of a 100 µl Hamilton syringe. For preparative purposes 500 to 1000 µl of sample solution may be applied to the column at reduced column pressure and the effluent flows to a fraction collector. For analytical purposes the best results were obtained when 20µl of a 10 % solution were injected.

A constant part of the effluent (about 18 ml/h) is withdrawn by means of a peristaltic pump and is mixed with the orcinol-reagent. In order to avoid mutual influence of the fractions, the reagent stream is segmented by air.

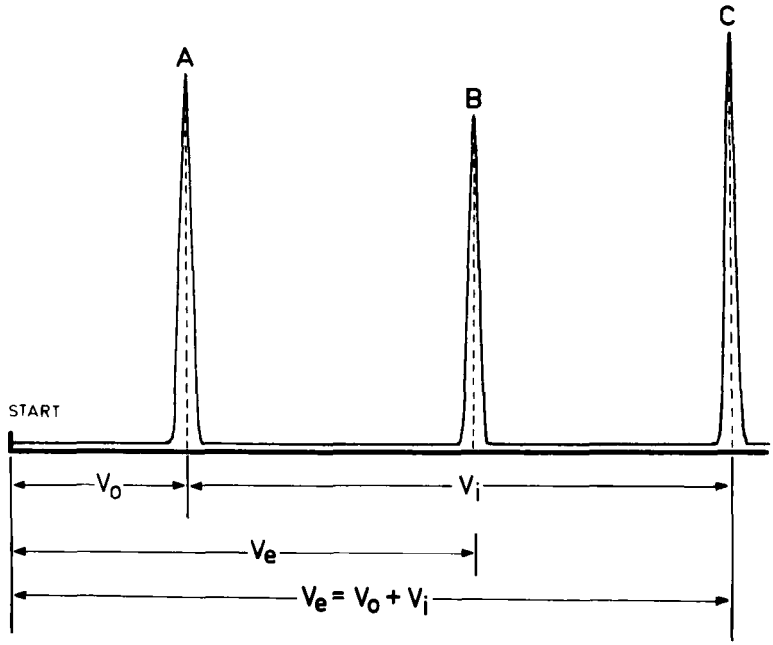

FIG. 2. Elution diagram of three solutes: A is totally excluded from gel pores (K_d=0), B is excluded only from the smaller pores ($K_d \approx 0,5$) and C is not excluded at all (K_d=1). Reproduced from ref. 22.

After passing a reaction coil of 12 m length in a 95 °C heating bath, the developed color is measured at 420 nm by means of a colorimeter (Zeiss, Elko 3 or PL 4), equipped with a 10 mm flow cell (Hellma, Müllheim, Germany) and a logarithmic converter. The absorbance was recorded by a Moseley/Hewlett-Packard strip-chart recorder (7101 BM). Reaction coils, glass fittings and acid-flex tubings were obtained from Technicon.

Orcinol reagent was prepared by dissolving 8,6 g orcinol in 8,6 l cold 70 % sulfuric acid in a brown glass bottle.

2.5. Exclusion limit and determination of distribution coefficients

The theory of gel chromatographic separation has been comprehensively reviewed by DETERMANN[20] and ALTGELT[21].

One possible interpretation of the separation process on gel columns is as follows (Fig.2).

Large solute molecules (substance A) that cannot penetrate the gel pores are eluted in a volume corresponding to the volume of liquid between the gel particles which is called the void volume V_o. At the other extreme small solutes (substance C), which can penetrate the gel pores freely, will be retarded and emerge at a volume $V_o + V_i$, where V_i is the volume of liquid inside the gel particles. With decreasing molecular size the accessible volume increases and the elution volume of solutes with intermediate sizes (substance B) lies in the volume range between V_o and $V_o + V_i$. Separation efficiency obtainable in gel chromatography is therefore greatly restricted by the limited volume in which the separation occurs.

Usually in gel chromatography the solute behavior is given by the distribution coefficient K_d, which is the ratio between the concentrations of a solute in the mobile and stationary phase. According to this interpretation, the stationary phase or gel phase is the water imbibed in the gel particles and the mobile phase the water moving in the void space between the particles. A solute will be distributed between the two phases to an extend measured by the distribution coefficient K_d. This constant is defined as the fraction of the internal volume V_i of a column accessible to the solute molecules[23].

The elution volume of a solute therefore is

$$V_e = V_o + K_d \cdot V_i \qquad (1)$$

The distribution coefficient becomes

$$K_d = \frac{V_e - V_o}{V_i} \qquad (2)$$

For large solutes which are totally excluded from the gel pores, $K_d = 0$, whereas small solutes that can freely penetrate the pores of the gel will have K_d - values of 1. Between these two extremes lie all solutes that can enter the gel pores to various limited extents ($0 < K_d \leqslant 1$).

The distribution coefficient is a valuable constant for studying solute behavior under different conditions, because K_d is independent of column geometry, bed size and packing density of the gel bed.

For calculating K_d of maltodextrins, the inner volume V_i was estimated by separate runs with ribose and erythrose. Furthermore, using equation (3), V_i was calculated from the water regain of the gel

$$V_i = a \cdot W_r \qquad (3)$$

In equation (3), a is the mass of xerogel used in preparation of the bed and W_r is the water regain of the gel (gram/gram of dry gel) given by the manufacturer. The void volume V_o was determined by measuring the elution volume of excluded dextran 2000. For the determination of the elution volume of maltodextrins the effluent was measured from start of sample application until the sugar emerges in maximum concentration.

3. FRACTIONATION OF CARBOHYDRATES

In the following chapter the high resolution of oligosaccharides by chromatography on a polyacrylamide gel column will be demonstrated. The column has been used repeatedly during a period of several months and no deterioration of the column properties was observed.

3.1. Separation of homologous oligosaccharides

Maltooligosaccharides, synthesized by the action of amylomaltase on maltose, were chosen as one of the first sugar mixtures to study the efficiency of the Bio-Gel column. It was found that elevated temperatures and the use of a very fine particle size gel increases the resolution of a polyacrylamide gel column[24].

Fig.3 illustrates the excellent properties of Bio-Gel P-2 for the separation of glucose (G1), maltose (G2) and higher oligosaccharides up to ten glucose units. The sugars were eluted from the gel bed in an order of decreasing molecular size. The maltodextrins were synthesized by amylomaltase, an enzyme which is induced in E.coli ML 30 by maltose[25]. According to WIESMEYER and COHN[26], amylomaltase releases the reducing end of the maltose molecule as free glucose, whereas the non-reducing end is contributed toward the

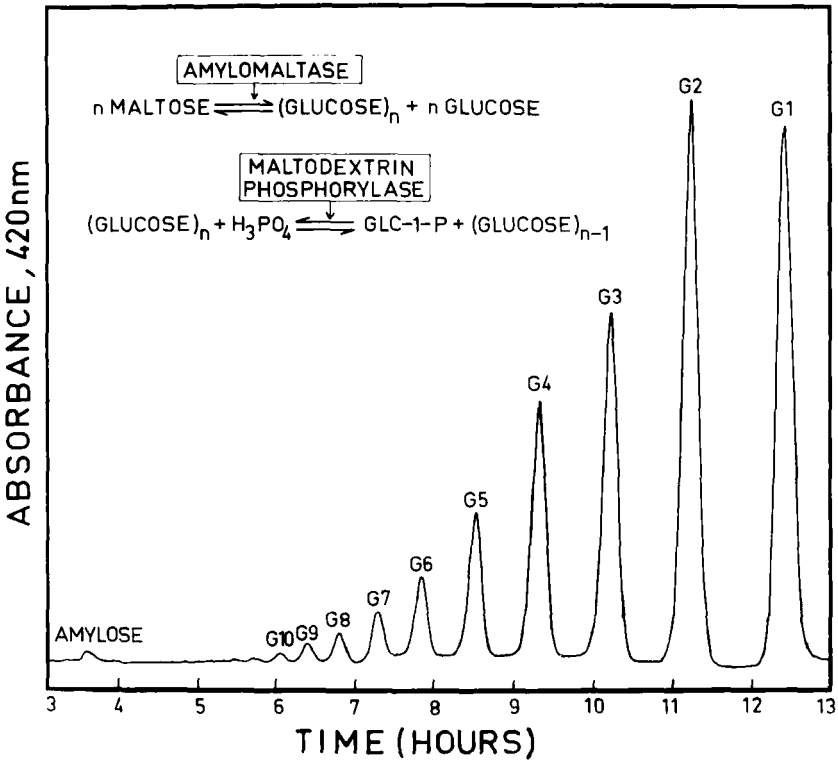

FIGURE 3

Separation of glucose and maltodextrins synthesized by the action of E.coli ML 30 on maltose. Bio-Gel P-2, *minus 400 mesh; column temperature*, 65°C; column, 1,5 x 200 cm ; eluant, water ; flow rate, 25 ml/h ; sample, 20 µl of a 10 % solution; peaks G1, G2, G3 etc. denotes glucose, maltose, maltotriose etc. For the preparation of maltodextrins, 0,5 g freeze-dried E.coli ML 30 cells were incubated at pH 6,8 for 4 hours with 1,5 g maltose contaminated with traces of maltotriose. During incubation at 28 °C the pH was kept constant by means of an autotitrator. The reaction was stopped with TCA and the solution was centrifuged (36.000 x g, 15 min.) and the supernatant was freeze-dried.

formation of the polymer in which all glucose units are exclusively bound by α-1,4-glucosidic linkages[27].

In the equilibrium state of this reaction, successive transglucosylation leads to the formation of α-1,4-linked glucans (amylose) that stain blue with iodine.

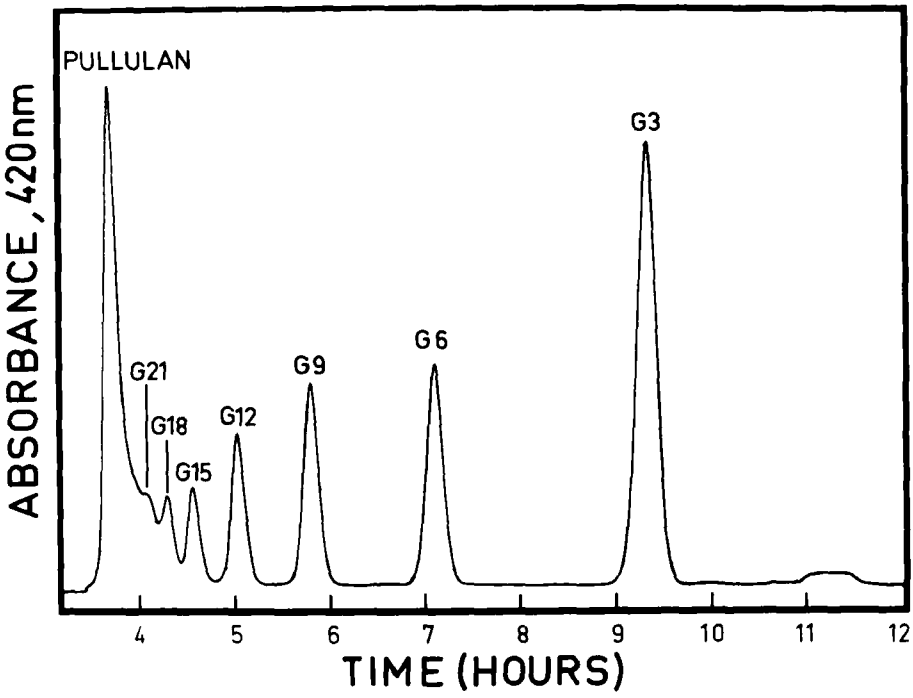

FIGURE 4

Enzymatic hydrolysis of pullulan and gel chromatography of the degradation products. Sample, 70 µl of a 1 % solution ; flow rate, 22,8 ml/h ; conditions as in Fig.3.

The induction of amylomaltase is accompanied by the formation of a maltodextrin phosphorylase[28], so that the elution profile shown in Fig.3 does not describe the typical action pattern of amylomaltase, because the maltodextrin phosphorylase catalyses predominantly the phosphorolysis of short-chained maltodextrins.

In order to resolve higher glucose homologs on a Bio-Gel P-2 column, pullulan was degraded by an Aerobacter aerogenes pullulanase (Fig.4).

Pullulan, an extracellular α-glucan produced by the yeast-like fungus Pullularia pullulans, is composed of repeating maltotriose units joined through

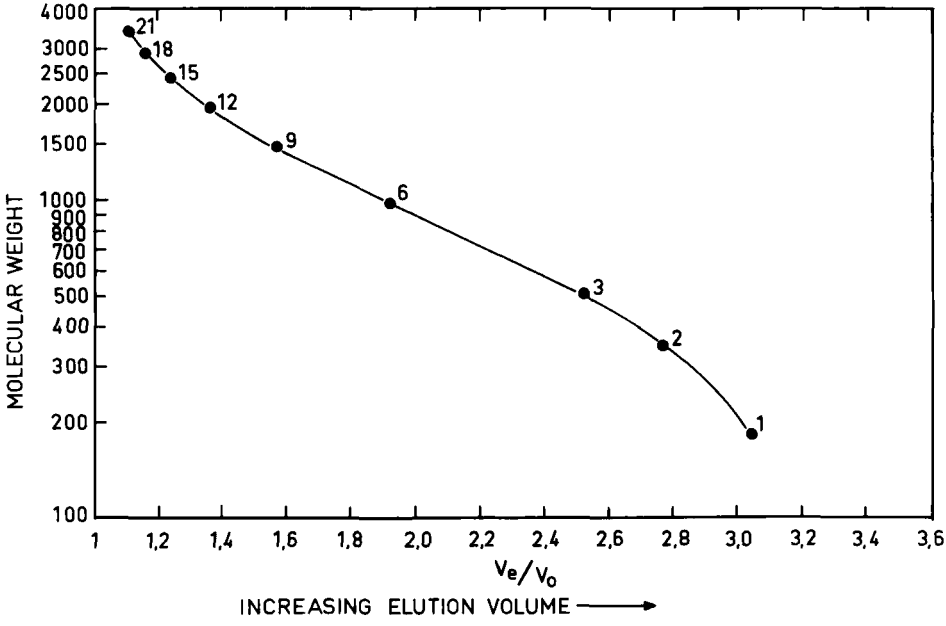

FIGURE 5

Semi-logarithmic plot of molecular weight against elution volume (V_e / V_o) for gel chromatography of glucose, maltose, maltotriose and polymaltotrioses.

α-1,6-glucosidic linkages and is hydrolysed by the enzyme pullulanase (bacterial R-enzyme, EC 3.2.1.9) by an endomechanism, yielding a mixture of polymaltotrioses with different chain lengths[29]. Because of great differences in molecular weights, the polymaltotriose molecules can be fractionated up to 21 glucose units (Fig.4).

3.2. Relation between elution volume and molecular weight

As separation of compounds by gel chromatography occurs according to their molecular sizes, this method has often been used to infer the molecular weight of an unknown substance from its elution volume. In most practical cases the logarithmic relation between elution volume and molecular weight is applied as reported by DETERMANN and MICHEL[30]. This can be expressed as

$$V_e = k \cdot \log M \qquad (4)$$

where M is the molecular weight.

The plot in Fig. 5 shows the relationship between the logarithm of the molecular weight of α-1,6-linked polymaltotrioses (from Fig. 4) and the elution volume. A linear function between elution volume and the logarithm of the molecular weight was obtained for oligosaccharides with more than three glucose units up to a degree of polymerization of 9. The curve deviates markedly from the linear form for the sugars glucose (1) and maltose (2), which were added to the mixture, and for the higher polymers. Therefore, it may be concluded that in these regions separation effects other than pure exclusion may exist, such as adsorption or partition effects.

3.3. Fractionation of isomeric compounds

These interactions of the solute molecules with the gel matrix are probably the reason for some unexpected separation properties of the gel column.

In Fig. 6 the separation of a synthetic mixture of mono-, oligo- and polysaccharides on Bio-Gel P-2 is shown. Although there is no difference in molecular weight, maltotriose and raffinose are separated, as well as maltose and melibiose. Furthermore, 2-deoxyglucose is eluted prior to glucose, although its molecular weight is about 10% lower than that of glucose. In the fractionation range of high molecular weights the separation of glycogen and amylose is remarkable, since both polysaccharides should be excluded from the gel pores because of their high molecular weights , which are far beyond the fractionation range of Bio-Gel P-2.

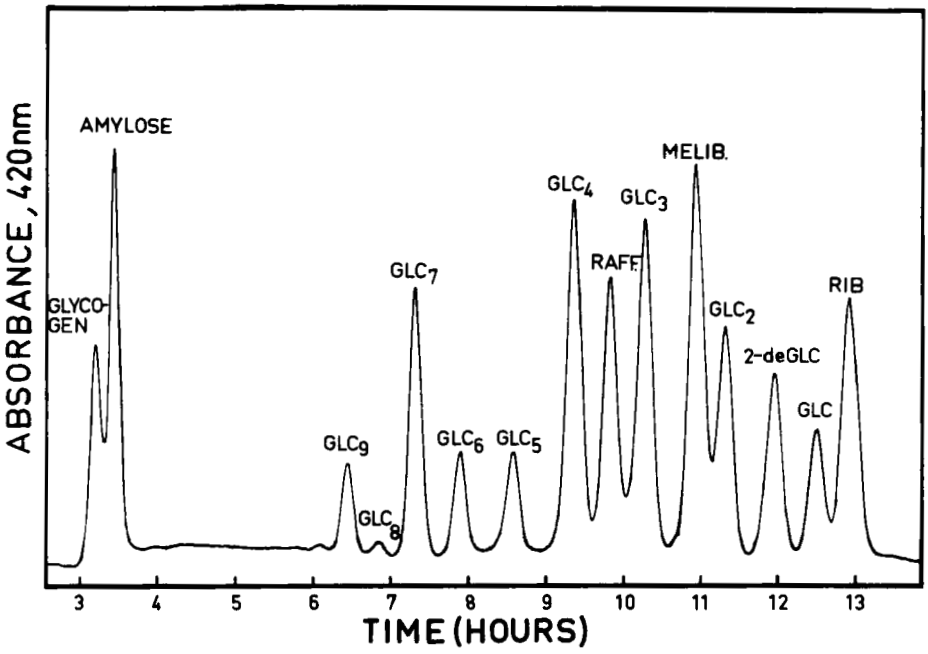

FIGURE 6

Gel chromatography of a synthetic mixture of mono-, oligo-and polysaccharides. Compounds from right to left are: Ribose, glucose, 2-deoxyglucose, maltose, melibiose, maltotriose, raffinose, maltotetraose, maltopentaose etc.

These interacting effects between solute and gel matrix could be observed clearly by fractionating mononucleotides on polyacrylamide gel. As previously reported[31], 3′-AMP , 5′-AMP and cyclic 3′,5′-AMP were separated using a column of Bio-Gel P-2.

3.4. Separation of maltodextrins at different temperatures

In an earlier publication[24] we observed the influence of temperature on the elution volume, leading to an improvement of the separation effect at elevated temperatures.

According to the exclusion concept, the elution volume of a given solute would be expected to be independent of temperature. This has been established by

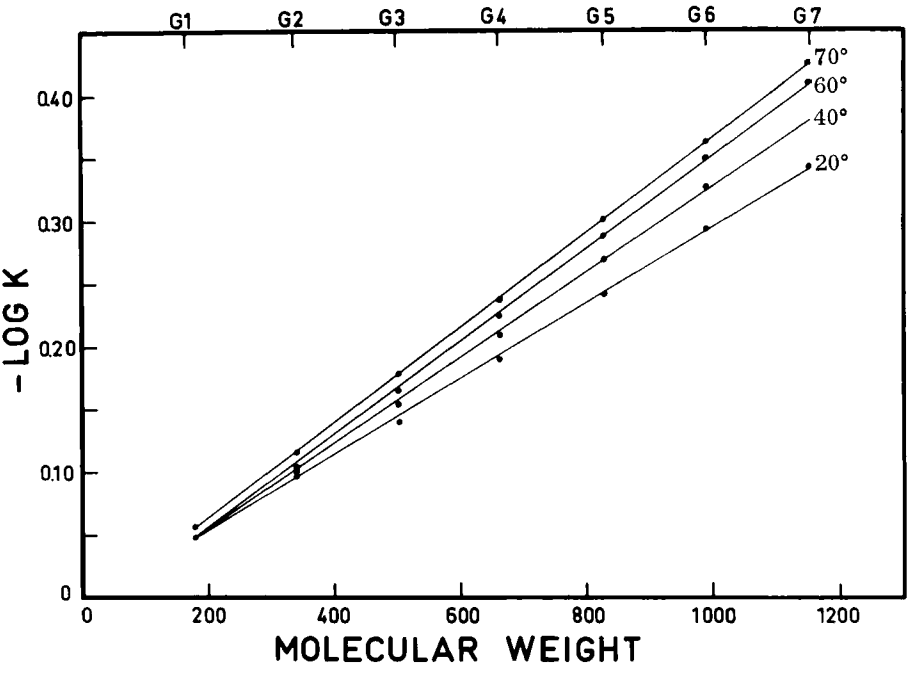

FIGURE 7

Plots of - log K against the molecular weight of maltodextrins at various temperatures.

MOORE and HENDRICKSON[32], who studied the separation of polystyrols and polyethyleneglycols in a polystyrol gel.

Taking into consideration some kind of solute-gel interactions one should expect a temperature dependence of the distribution coefficient. Therefore we studied the separation of maltodextrins (glucose to maltoheptaose) on polyacrylamide gel at various temperatures[33]. The results indicated that the elution volumes and the distribution coefficients decrease with increasing temperature. As may be seen from Fig.7 the values of -log K versus molecular weight are in quite good agreement with a linear function in the temperature range of 20 to 70 °C.

Using the form of equation proposed by HJERTEN [34]

$$-\log K = C_1 \cdot M + C_o \qquad (5)$$

where M is the molecular weight and K the distribution coefficient, one can estimate the constant C_1 as the slope of the straight line and C_o as the ordinate at the origin. These values are given in Table 1.

TABLE I

Values of constants C_1 and C_o from eqn.5 for different temperatures as calculated by the method of least squares

Temperature	T	C_1	C_o	C_1/T
20 °	293	$3{,}01 \cdot 10^{-4}$	-0,006	$1{,}026 \cdot 10^{-6}$
30 °	303	$3{,}26 \cdot 10^{-4}$	-0,010	$1{,}077 \cdot 10^{-6}$
40 °	313	$3{,}31 \cdot 10^{-4}$	-0,008	$1{,}057 \cdot 10^{-6}$
50 °	323	$3{,}53 \cdot 10^{-4}$	-0,011	$1{,}091 \cdot 10^{-6}$
60 °	333	$3{,}72 \cdot 10^{-4}$	-0,021	$1{,}117 \cdot 10^{-6}$
70 °	343	$3{,}78 \cdot 10^{-4}$	-0,013	$1{,}100 \cdot 10^{-6}$

Table 1 shows that the slope C_1 increases nearly proportional with the absolute temperature.

3.5. Isolation of the mono- to tetrasaccharides from wort and trehalose from bakers yeast

The application of polyacrylamide gel chromatography to the isolation of large amounts of mono- and oligosaccharides from wort is shown in Fig.8. An amount of 200 mg of freeze-dried wort was applied to the column (1,5 x 200 cm) and the effluent containing the mono-to tetrasaccharides was collected in 70 fractions. After the fractions corresponding to each peak in the resulting elution diagram had been pooled and freeze-dried, gas chromato-

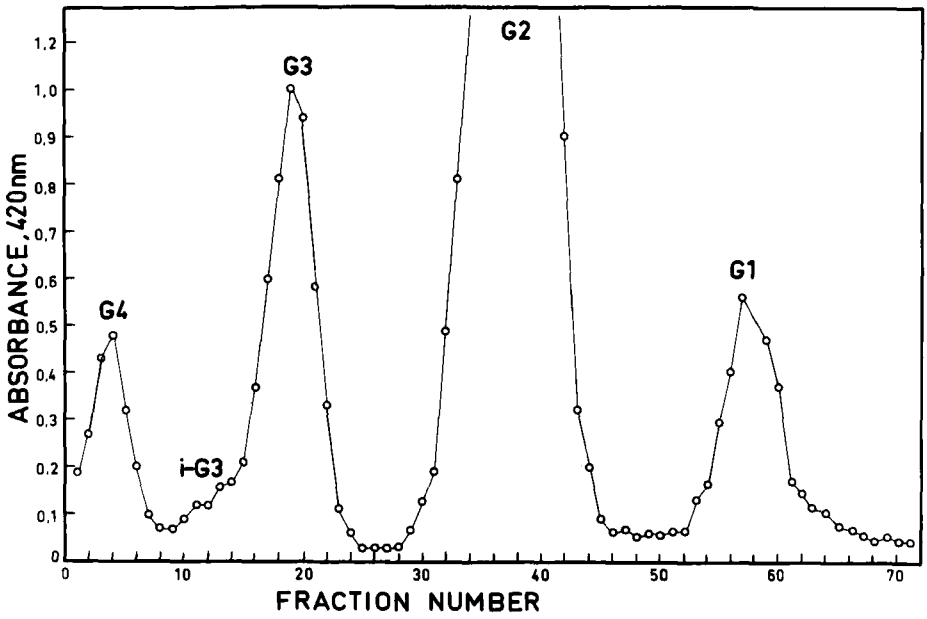

FIGURE 8

Elution diagram of the mono- to tetrasaccharides of 200 mg freeze-dried wort, fractionated on a 200 x 1,5 cm Bio-Gel P-2 column. Sample size, 700 µl ; conditions as in Fig.3. Peak G1 (monosaccharides, arabinose, ribose, glucose and fructose) ; peak G2 (disaccharides, sucrose and maltose) ; peak G3 (trisaccharide, maltotriose) ; peak i-G3 (trisaccharides, raffinose and panose) ; peak G4 (tetrasaccharide, maltotetraose). Samples of eluted fractions were analyzed by the orcinol-sulfuric acid method.

graphic analysis of the sugars as volatile polytrimethylsilyl ethers showed each peak to be composed of different sugars.

Fraction G1 contained the hexoses glucose and fructose and traces of the pentoses arabinose and xylose. The disaccharides maltose and sucrose have been detected in the peak G2 while in the fractions G3 and G4 maltotriose

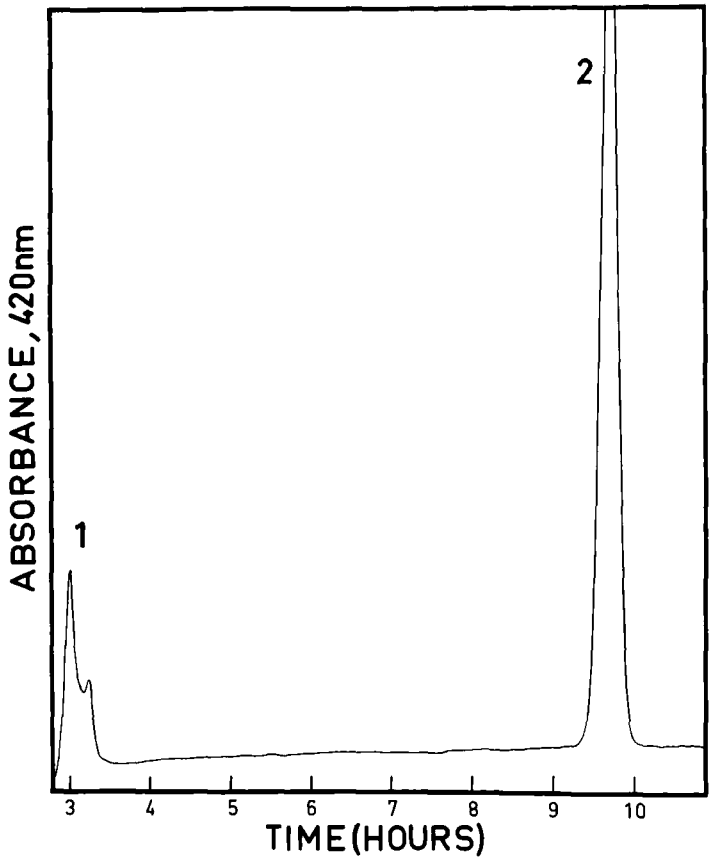

FIGURE 9

Gel chromatography of a cell extract from Saccharomyces cerevisiae on Bio-Gel P-2. Column, 200 x 1,7 cm ; flow rate, 30 ml/h. The other chromatographic conditions were the same as described for Fig.3. Polysaccharides (1) and trehalose (2). Extraction of carbohydrates was carried out by boiling 20 g of pressed yeast in 20 ml of water for 10 min. After centrifugation (20.000 x g, 5 min.) the supernatant was freeze-dried and dissolved in water (1 ml). An appropriate sample (about 100 µl) was applied to the column.

and maltotetraose were found by gas liquid chromatography. In fraction i-G3 the two trisaccharides panose and raffinose have been identified.

The preparative chromatogram in Fig.8 demonstrates that gel chromatography of carbohydrates is a simple and efficient way to isolate large amounts of various sugars from biological materials with relatively high purity. Furthermore, combined use of gas liquid chromatography and gel chromatography allows the complete quantitative determination of polysaccharides, dextrins and fermentable sugars occuring in wort and beer [35].

Another example of the usefulness of gel chromatography in the purification of sugars from biological material is shown in Fig.9.
Fig.9 illustrates an experiment in which the carbohydrates of a yeast extract were fractionated in a Bio-Gel P-2 column. Two major peaks were obtained. The compounds which were eluted in the void volume of the column (peak 1) are polysaccharides, e.g. glycogen and cell wall components, while the second peak contained pure trehalose as identified by molecular weight determination and gas liquid chromatography [36].

3.6. Characterization of different polysaccharide degrading enzymes by typical elution patterns

Using gel chromatography, it is possible to define the action patterns of polysaccharide degrading enzymes much more precisely than had been possible with other chromatographic methods before. In particular, three types of enzymes were examined : α-amylase from B.subtilis and from pig pancreas, dextranase from a Lipomyces yeast and cellulase from Trichoderma viride.
A more detailed study concerning the action patterns of various amylases was published recently [37].

Fig.10 shows the action of two α-amylases from different origin on amylose. Alpha-amylases hydrolyse α-glucans by cleaving the internal α-1,4-glucosidic linkages to yield a mixture of oligosaccharides. Although early investiga-

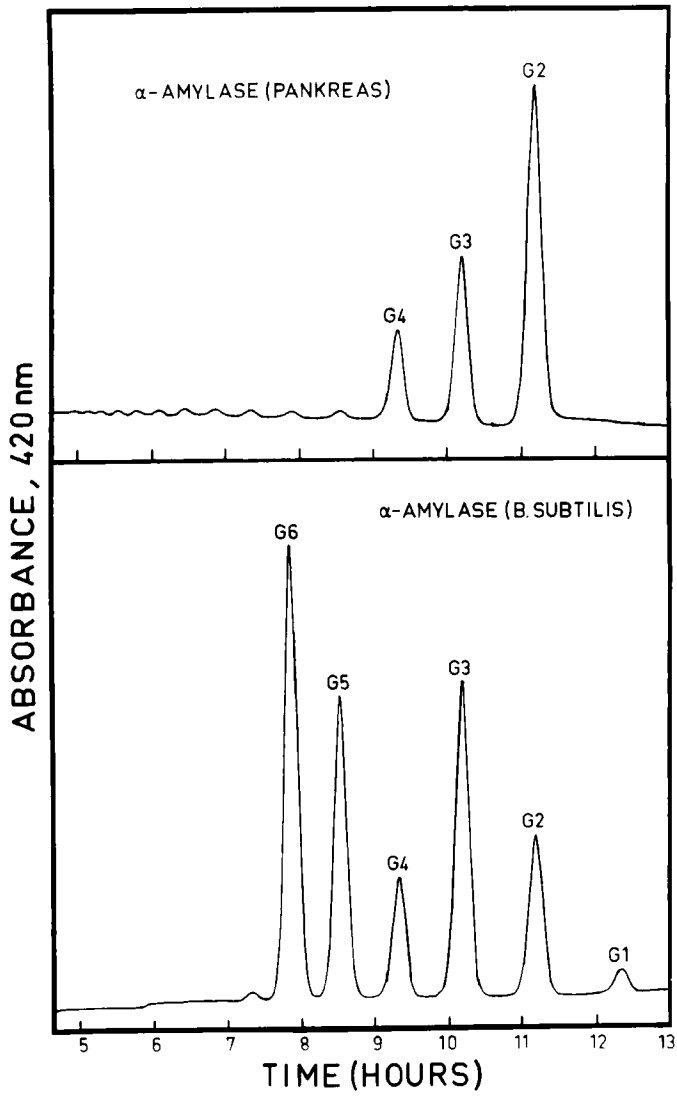

FIGURE 10

Hydrolysis of amylose by two different α-amylases and gel chromatography of the degradation products.

tions indicated that α-amylolytic attack on amylose is random and that this mode of attack is independent of the source of the enzyme, it is now known that the detailed action pattern of an α-amylase depends on the source of the enzyme. A comparison of the action patterns of α-amylase from B.subtilis and from hog pancreas shows that they are very different (Fig.10).
At the achroic point of amylose hydrolysis by B.subtilis α-amylase, oligomers as the maltopentaose (G5) and maltohexaose (G6) tend to accumulate as shown in the lower part of Fig.10. This is due to a relatively low affinity of B.subtilis α-amylase for maltodextrins of about seven glucose units or less. A further difference is that B.subtilis α-amylase can liberate glucose (G1) directly from relatively large maltodextrins.

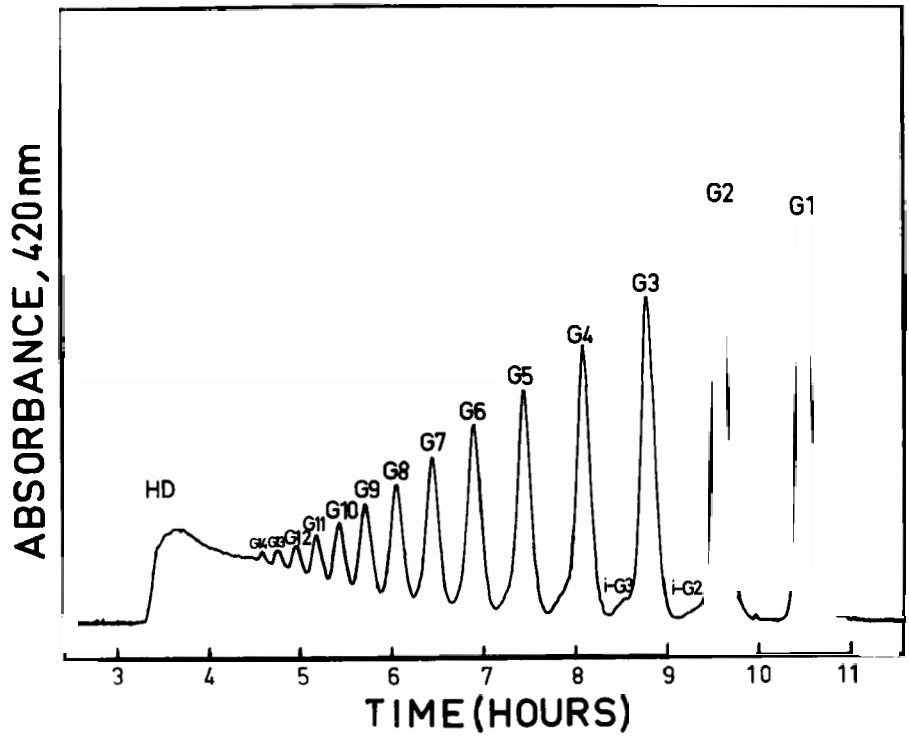

FIGURE 11

Chromatography of a glucose sirup manufactured by acid hydrolysis of starch.

The spectrum of oligosaccharides produced by the action of pig-pancreatic amylase is shown in the upper part of Fig.10. During the hydrolysis of amylose by this enzyme , there is no accumulation of such sugars as maltohexaose and maltopentaose, for these are rapidly degraded to the biose, triose and tetraose. At the achroic point, the main products of mammalian amylase action are maltose (G2) , maltotriose (G3) and maltotetraose (G4) as can be seen in Fig.10. At this stage of hydrolysis no glucose is formed.

In contrast to the enzymatic degradation of starch components the chromat-

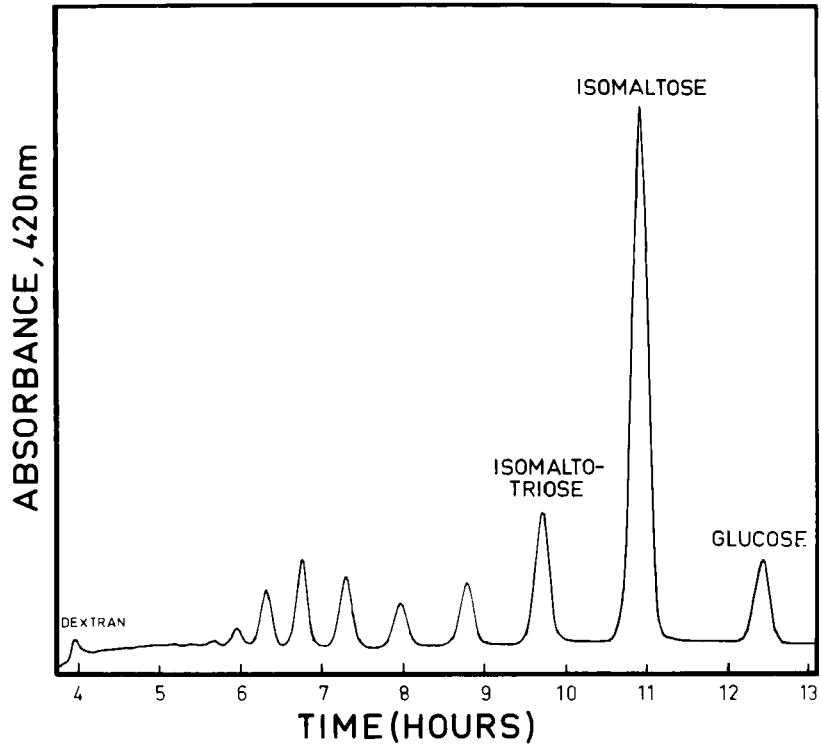

FIGURE 12

Gel chromatography of the mono- and oligosaccharides produced by the enzymatic hydrolysis of dextran. The dextranase used was a preparation from a Lipomyces yeast.

ographic separation of the products obtained by acid hydrolysis of starch shows a quite different elution profile (Fig.11). The chromatogram in Fig.11 illustrates that the concentration of the degradation products decreases

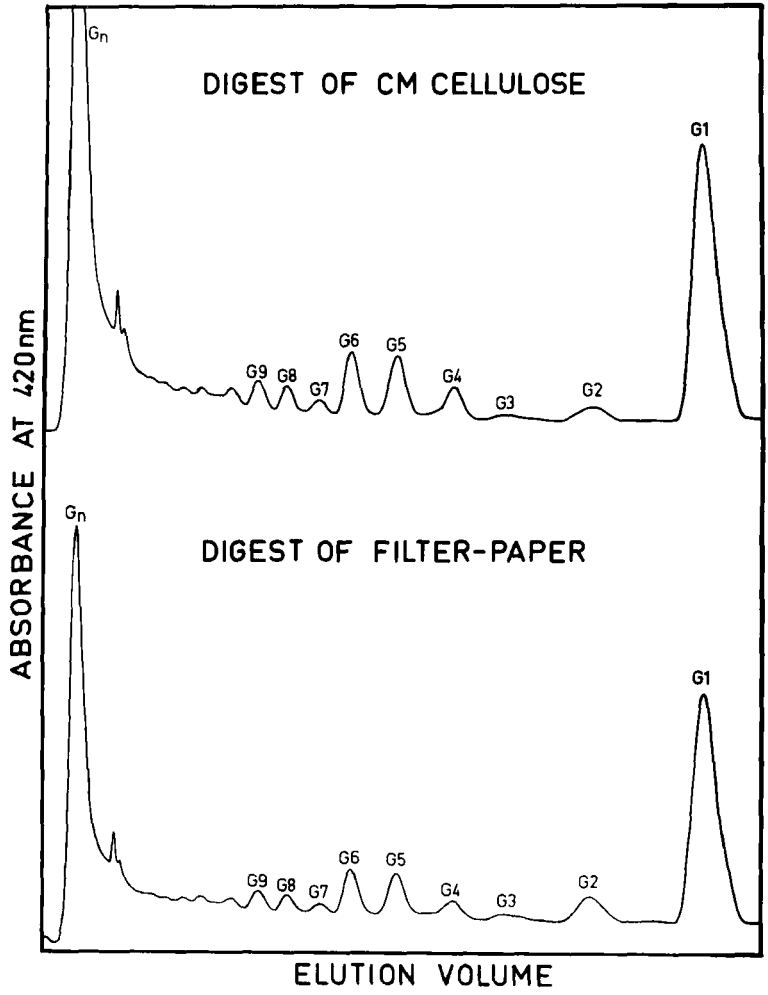

FIGURE 13

Action of cellulase from Trichoderma viride on two different substrates and gel chromatography of the degradation products. Top: Hydrolysis of carboxymethylated cellulose; bottom: Hydrolysis of filter paper. The cellulase was a gift of Worthington Biochemical Corporation, Freehold, New Jersey.

logarithmically with increasing degree of polymerisation[38].

Fig. 12 demonstrates the action pattern of a dextranase from a Lipomyces yeast. As can be seen from this figure the main product of dextran hydrolysis is isomaltose. Moreover, larger oligosaccharides have been formed which have not been identified.

The chromatograms in Fig.13 illustrate the separation of mono- and oligosaccharides produced by the action of a partially purified cellulase prepara-

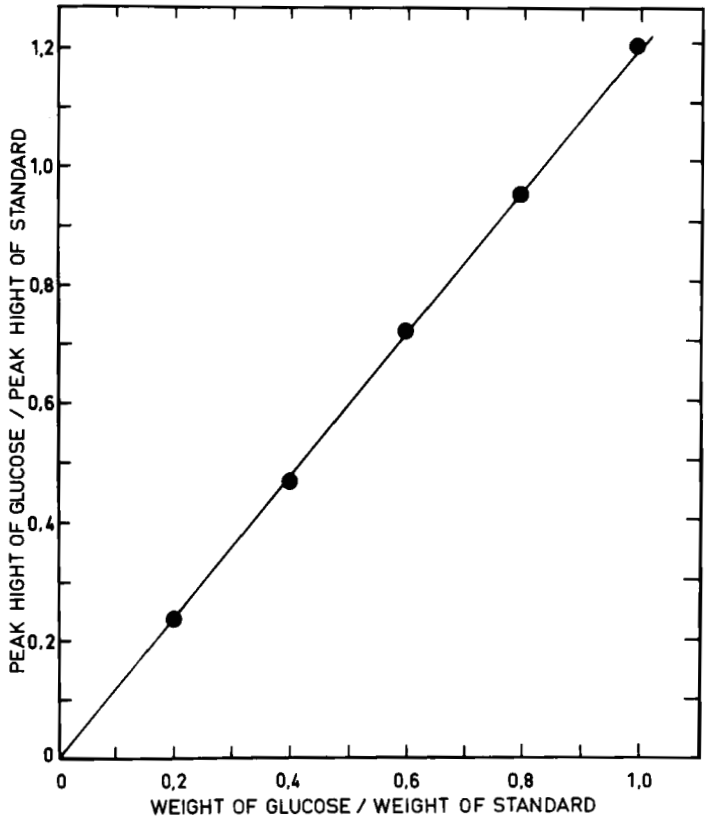

FIGURE 14

Calibration curve for glucose with 2-deoxyglucose as an internal standard.

tion from Trichoderma viride on two different celluloses. In both cases nearly identical elution patterns were obtained. The occurence of oligomers indicate that the polymer is degraded by random attack. The peak G1 contained glucose as evidenced by testing aliquots of this fraction with glucose test-paper. Minor amounts of pentoses are supposed to be present in peak G1. Other peaks have not been identified.

3.7. Calibration and calculation

The internal standard technique as described by SAWARDEKER and SLONEKER[39] was used for the quantitative determination of carbohydrates. Because of its desirable retention time 2-deoxyglucose was chosen as an internal standard (compare Fig.6).

A linear calibration curve was obtained for glucose by chromatographing varying amounts of glucose with a constant amount of internal standard and plotting the ratio of peak hight of glucose to that of the standard against the ratio of the weight of glucose to that of the standard (Fig.14). The calibration factor for glucose was then determined by the slope of the curve.

$$F_{glc} = \frac{\text{Peak hight glucose / peak hight internal standard}}{\text{Weight of glucose / weight of internal standard}} = 1,2$$

For the determination of carbohydrates with more than one glucose unit the calibration factor for glucose was used. The amount of sugar in the sample was calculated by the expression

$$\text{Weight of unknown} = \frac{\text{Weight of standard} \times \text{peak hight of unknown sugar}}{1,2 \times \text{peak hight of internal standard}}$$

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SUMMARY

1. A method for the separation and quantitative determination of mono- and oligosaccharides is described. The procedure is based on the use of Bio-Gel P-2 , minus 400 mesh, in a properly designed column with water as eluant at 65 °C.

2. For the colorimetric estimation of carbohydrates the effluent is monitored by an automated analyzing system using the orcinol-sulfuric acid reagent.

3. Maltooligosaccharides containing up to 15 glucose units and polymaltotrioses with a chain length of 21 units are easily fractionated within twelve hours for analytical and preparative purposes. Furthermore, separation of isomeric compounds is possible.

4. The separation of maltodextrins (glucose to maltoheptaose) on polyacrylamide gel has been studied at various temperatures. Increasing temperature leads to a decrease of the elution volumes and partition coefficients.

5. Chromatography on polyacrylamide gel turns out to be an excellent method for characterizing different polysaccharide degrading enzymes by typical elution patterns. Several applications are demonstrated.

REFERENCES

1. P.Nordin , Arch.Biochem.Biophys., 99 , 101 (1962).
2. T.C.Laurent and K.A.Granath , Biochim.Biophys.Acta, 136 , 191 (1967).
3. J.Heller and M.Schramm , Biochim.Biophys.Acta, 81 , 96 (1964).
4. W.Brown and Ö.Andersson , J.Chromatogr., 57 , 255 (1971).
5. W.Brown , J.Chromatogr., 52 , 273 (1970).
6. W.Brown and K.Chitumbo , J.Chromatogr., 66 , 370 (1972).
7. Y.C.Lee and C.E.Ballou , Biochemistry, 4 , 257 (1965).
8. T.S.Stewart, P.B.Mendershausen and C.E.Ballou, Biochemistry, 7 , 1843 (1968).

9. G.H.Jones and C.E.Ballou , J.Biol.Chem., 244 , 1043 (1969).
10. J.C.Speck and D.J.Rynbrandt, Anal.Biochem.,19 , 426 (1967).
11. C.P.Dietrich, Biochem.J.,108 ,647 (1968).
12. P.Flodin,J.D.Gregory and L.Rodén , Anal.Biochem.,8 , 424 (1964).
13. S.Hjertén and R.Mosbach, Anal.Biochem.,3 , 109 (1962).
14. S.Hjertén , Arch.Biochem.Biophys.,Suppl.1 , 147 (1962).
15. D.J.Lea and A.H.Sehon , Can.J.Chem.,40 , 159 (1962).
16. H.G.Pontis , Anal.Biochem.,23 , 331 (1968).
17. M.A.Raftery,T.Rand-Meir,F.W.Dahlquist,S.M.Parsons,C.L.Borders,Jr., R.G.Wolcott,W.Beranek,Jr.,and L.Jao, Anal.Biochem.,30,427 (1969).
18. A.Schwartz and B.A.Zabin, Anal.Biochem.,14 , 321 (1966).
19. R.B.Kesler , Anal.Chem.,39 , 1416 (1967).
20. H.Determann, "Gel Chromatography" , Springer-Verlag,New York,1968.
21. K.H.Altgelt, Advan.Chromatogr.,7 ,3 (1968).
22. P.Andrews,*in* "Methods of Biochemical Analysis ",Vol. 18, D.Glick,ed., Interscience,New York , 1970 , p.4.
23. B.Gelotte, J.Chromatogr.,3 ,330 (1960).
24. G.Trênel ,M.John and H.Dellweg, FEBS Letters,2 ,74 (1968).
25. M.John,G.Trênel and H.Dellweg, J.Chromatogr.,42 , 476 (1969).
26. H.Wiesmeyer and M.Cohn , Biochim.Biophys.Acta,39 , 427 (1960).
27. S.A.Barker and E.J.Bourne, J.Chem.Soc.,209 (1952).
28. M.Schwartz and M.Hofnung, European J.Biochem.,2 , 132 (1967).
29. G.S.Drummond,E.E.Smith,W.J.Whelan and H.Tai, FEBS Letters, 5 , 85 (1969).
30. H.Determann and W.Michel, J.Chromatogr.,25 , 303 (1966).
31. M.John , H.Skrabei and H.Dellweg, FEBS Letters, 5 ,185 (1969).
32. J.C.Moore and J.G.Hendrickson, J.Polym.Sci.,8C ,233 (1965).
33. H.Dellweg,M.John and G.Trênel, J.Chromatogr.,57 , 89 (1971).
34. S.Hjertén, J.Chromatogr., 50 ,189 (1970).
35. M.John , Thesis , Technische Universität Berlin,1972.

36. G.Richter, M.John and H.Dellweg, Mschr.Brauerei, 25 , 123 (1972).

37. H.Dellweg and M.John , Mschr.Brauerei, 25 ,271 (1972).

38. G.Trênel and C.C.Emeis, Stärke, 22 ,188 (1970).

39. J.S.Sawardeker and J.H.Sloneker, Anal.Chem., 37 , 945 (1965).