*Journal of Chromatography*, 86 (1973) 184–189
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 6920

## Note

# Gel permeation chromatography of glucose oligomers using polyacrylamide gels

NABIH K. SABBAGH and IRVING S. FAGERSON
*Chenoweth Laboratory, University of Massachusetts, Amherst, Mass. 01002 (U.S.A.)*
(Received June 29th, 1973)

Separation of glucose polymers, as encountered in corn syrups, by use of gel chromatography has been described by Trenel and Emeis[1]. John *et al.*[2] reported quantitative chromatography of glucose oligomers and other saccharides with low exclusion limit polyacrylamide gels (Bio-Gel P-2, 200–400 mesh and −400 mesh). The effect of different temperatures on the separation of malto-oligosaccharides with such systems has been investigated by Trenel *et al.*[3] and Dellweg *et al.*[4]. In studying the nature of the separation of cellodextrin series, glucose-cellohexaose, Brown[5] found that the interaction between gel and solute increased with molecular weight. This effect was more pronounced with the dextran gels when compared with polyacrylamide gels. Brown and Anderson[6,7], who studied the separation of xylodextrins on polyacrylamide and dextran gels, concluded that the solubility-determined partitioning is an important factor in separations in tightly cross-linked dextran gels. Brown[8] has emphasized the importance of solubility-determined partitioning in a study of the influence of solvent and temperature in dextran gel chromatography. Low exclusion limit gels (Bio-Gel P-2 and Sephadex G-15) have been usually used as materials for the separation of glucose by gel permeation chromatography. We wish to report here the use of an acrylamide gel of higher exclusion limit (Bio-Gel P-4) which we believe offers an improved resolution for the higher polymers of glucose.

## EXPERIMENTAL

Polyacrylamide gels Bio-Gel P-2, −400 mesh and P-4, −400 mesh (Bio-Rad Lab., Richmond, Calif., U.S.A.) were hydrated overnight with an excess of deionized distilled water. Removal of fine particles was achieved by decantation. Once the fine particles were removed, the slurry was heated in a water-bath to about 60° for 10 min, then degassed using a water aspirator. Two K16/100 analytical columns (Pharmacia, Piscataway, N. J., U.S.A.) were used in this study. Uniform packing was achieved by use of a reservoir Sephadex R 15/16 (Pharmacia). After filling, deionized, distilled and previously degassed water was pumped through columns making certain that the system was free of entrapped air. A Milton Roy Minipump (Milton Roy, Philadelphia, Pa., U.S.A.) was used to provide flow-rates of 20.0 and 18.0 ml/h for P-2 and P-4 columns, respectively. Constant temperature

was maintained in the column jackets by circulating water at 45°. Eluant was pumped through the columns for 10 h before any sample injection was made to allow equilibration to take place. Samples were prepared by making a solution of 8% (w/v) of a commercial, acid-hydrolyzed corn syrup of 42 dextrose equivalents. 200-$\mu$l samples were injected via a Chromatronix off-column septum injector, Model 164A11 (Chromatronix, Berkeley, Calif., U.S.A.) which was in series with the column inlet. The column effluent was monitored with a differential refractive index monitor (Pharmacia) equipped with a recorder. The temperature of the detector cells was maintained constant by circulating water from a bath held at 40°.

## RESULTS AND DISCUSSION

Typical chromatograms obtained are shown in Figs. 1 and 2. The successive oligomers are identified as $G_1$, $G_2$, $G_3$, ... etc. corresponding to the number of glucose units, *i.e.*, glucose, maltose, maltotriose, etc. The identity of the emergent fractions was verified by comparisons of elution volumes with known standards

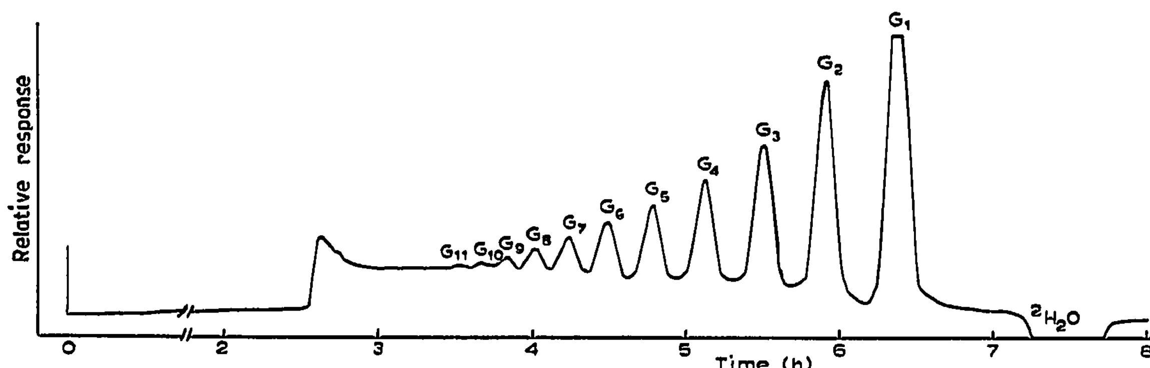


Fig. 1. Chromatogram of corn syrup (42 dextrose equivalents). Column, 1 m × 1.6 cm, packed with Bio-Gel P-2 (−400 mesh); flow-rate, 20.0 ml/h; temperature, 45°.

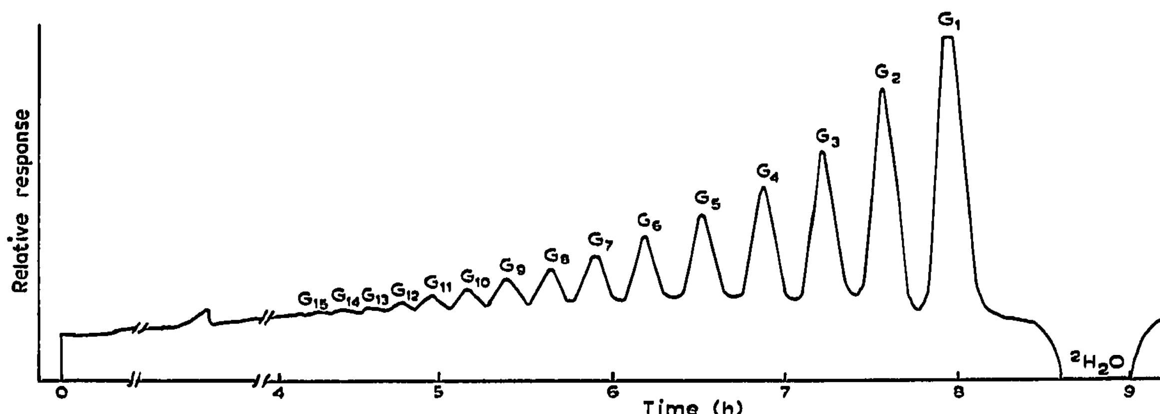


Fig. 2. Chromatogram of corn syrup (42 dextrose equivalents). Column, 1 m × 1.6 cm, packed with Bio-Gel P-4 (−400 mesh); flow-rate, 18.0 ml/h; temperature, 45°.

TABLE I

BIO-GEL COLUMN OPERATING PARAMETERS

| | Bio-Gel | |
|---|---|---|
| | *P-2* | *P-4* |
| Total volume (ml): | 188.0 | 188.0 |
| Void volume (ml): | 52.9 | 45.6 |
| Internal volume (ml): | 97.6 | 113.5 |
| Polymer volume (ml): | 37.5 | 28.9 |
| Elution volume ($^2H_2O$) (ml): | 150.5 | 159.1 |
| Sample volume | 200 μl. | 200 μl. |
| Flow-rate | 20.0 ml/h. | 18.0 ml/h. |
| Temperature | 45° | 45° |
| Detector attenuation | 4× | 4× |
| Column | 1.6×100 cm | 1.6×100 cm |

TABLE II

COMPARISON OF THEORETICAL PLATE DATA AND RESOLUTION FOR BIO-GEL P-2 AND P-4

| *Compound* | $n_{req}$ | | $n_{eff}$ | | $N_{req}$ | | $N$ | | $R$ | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *P-2* | *P-4* | *P-2* | *P-4* | *P-2* | *P-4* | *P-2* | *P-4* | *P-2* | *P-4* |
| $G_1$ | | | 3408 | 6208 | | | 9840 | 13360 | | |
| | 960 | 3294 | | | 2800 | 7123 | | | 1.96 | 1.39 |
| $G_2$ | | | 3040 | 5776 | | | 9920 | 12992 | | |
| | 992 | 3222 | | | 3248 | 7259 | | | 1.74 | 1.36 |
| $G_3$ | | | 2304 | 5360 | | | 8544 | 12720 | | |
| | 928 | 2960 | | | 3456 | 7056 | | | 1.61 | 1.37 |
| $G_4$ | | | 1904 | 4960 | | | 8096 | 12448 | | |
| | 928 | 2342 | | | 3984 | 5776 | | | 1.42 | 1.48 |
| $G_5$ | | | 1440 | 4464 | | | 7120 | 11920 | | |
| | 736 | 2342 | | | 3600 | 6336 | | | 1.43 | 1.40 |
| $G_6$ | | | 1135 | 4192 | | | 6784 | 12016 | | |
| | 976 | 2541 | | | 7600 | 7328 | | | 1.13 | 1.30 |
| $G_7$ | | | 1056 | 3840 | | | 7328 | 11840 | | |
| | 928 | 2662 | | | 6528 | 8171 | | | 1.08 | 1.23 |
| $G_8$ | | | 880 | 3600 | | | 7328 | 11920 | | |
| | 912 | 2830 | | | 7600 | 9293 | | | 0.98 | 1.10 |
| $G_9$ | | | 656 | 2832 | | | 6656 | 9920 | | |
| | 816 | 2662 | | | 8240 | 9448 | | | 0.95 | 1.07 |
| $G_{10}$ | | | 608 | 2912 | | | 7680 | 11152 | | |
| | 1024 | 2499 | | | 12816 | 9683 | | | 0.80 | 1.09 |
| $G_{11}$ | | | 512 | 2704 | | | 8032 | 11328 | | |
| | – | 2830 | | | – | 11750 | | | – | 1.00 |
| $G_{12}$ | | | – | 2576 | | | – | 11664 | | |
| | – | 2746 | | | – | 12365 | | | – | 1.02 |
| $G_{13}$ | | | – | 2784 | | | – | 13728 | | |
| | – | 2662 | | | – | 13088 | | | – | 1.06 |
| $G_{14}$ | | | – | 2704 | | | – | 14496 | | |
| | – | 2461 | | | – | 13088 | | | – | 1.10 |
| $G_{15}$ | | | – | 3040 | | | – | 14400 | | |

TABLE III

COMPARISON OF DISTRIBUTION COEFFICIENTS AND CAPACITY FACTORS FOR BIO-GEL P-2 AND P-4

| *Compound* | *Mol. wt.* | $V_e(ml)$ | | $K_D$ | | $W$ (ml) | | $K'$ | |
|---|---|---|---|---|---|---|---|---|---|
| | | *P-2* | *P-4* | *P-2* | *P-4* | *P-2* | *P-4* | *P-2* | *P-4* |
| $G_1$ | 180 | 128.1 | 143.2 | 0.770 | 0.860 | 5.16 | 4.96 | 1.422 | 2.140 |
| $G_2$ | 342 | 118.4 | 136.4 | 0.671 | 0.800 | 4.76 | 4.78 | 1.238 | 1.991 |
| $G_3$ | 504 | 110.1 | 130.0 | 0.586 | 0.745 | 4.76 | 4.61 | 1.081 | 1.851 |
| $G_4$ | 666 | 102.6 | 123.8 | 0.510 | 0.689 | 4.56 | 4.43 | 0.940 | 1.715 |
| $G_5$ | 828 | 96.1 | 117.3 | 0.443 | 0.643 | 4.56 | 4.30 | 0.817 | 1.572 |
| $G_6$ | 990 | 89.7 | 111.4 | 0.377 | 0.580 | 4.36 | 4.07 | 0.696 | 1.443 |
| $G_7$ | 1152 | 85.0 | 106.2 | 0.329 | 0.534 | 3.97 | 3.90 | 0.607 | 1.329 |
| $G_8$ | 1314 | 80.8 | 101.5 | 0.286 | 0.492 | 3.77 | 3.72 | 0.527 | 1.226 |
| $G_9$ | 1476 | 77.1 | 97.3 | 0.248 | 0.456 | 3.77 | 3.90 | 0.458 | 1.134 |
| $G_{10}$ | 1638 | 73.7 | 93.3 | 0.213 | 0.420 | 3.37 | 3.54 | 0.393 | 1.046 |
| $G_{11}$ | 1800 | 71.1 | 89.5 | 0.186 | 0.387 | 3.17 | 3.37 | 0.344 | 0.963 |
| $G_{12}$ | 1962 | – | 86.2 | – | 0.358 | – | 3.19 | – | 0.890 |
| $G_{13}$ | 2124 | – | 83.1 | – | 0.330 | – | 2.83 | – | 0.822 |
| $G_{14}$ | 2286 | – | 80.2 | – | 0.305 | – | 2.66 | – | 0.758 |
| $G_{15}$ | 2448 | – | 77.4 | – | 0.280 | – | 2.50 | – | 0.697 |

from glucose through maltoheptaose. Standards were not available for verification of higher polymers.

Table I summarizes the column parameters. The total volume ($V_t$) was calculated from the column geometry. The void volume ($V_0$) was taken as the elution volume of the excluded dextrans in the syrup samples and co-incides with the $V_0$ of Blue Dextran as previously determined. The internal volume ($V_i$), which is the total volume occupied by the solvent contained within the gel particles, was determined by measuring the elution volume of deuterated water [$V_e(^2H_2O)$] and from that value subtracting $V_0$. The polymer volume ($V_p$), which represents the volume occupied by the gel matrix *per se*, was calculated as $V_t - V_e(^2H_2O)$.

The different relationships used in calculating the data for both columns and the symbols used are shown on p. 188. Tables II and III summarize the data and compare them for both P-2 and P-4 columns.

Throughout the whole series the P-4 column shows higher values for elution volumes, partition coefficients, capacity factor and number of theoretical plates than those for the P-2 column. This can be explained by the higher internal volume of the P-4 column (Table I). On the other hand, the higher value of $V_i$ for the P-4 column results from the smaller values of $V_0$ and $V_p$ for this column compared to the P-2 column. The resolution in the P-2 column decreases more rapidly than in the P-4 column, although the former shows higher values for the first 4 polymers in the series. A resolution of at least 1.00 is obtained for all components up to $G_{15}$ in the P-4 column indicating a separation of about 98% of the individual components which is satisfactory for practical purposes. The number of theoretical and effective theoretical plates required are higher for the P-4 column than the one for P-2, but this is more than compensated for by the theoretical and effective theo-

retical number of plates in the P-4 column. More peaks are separated and detected with the P-4 gel, as expected, due to its higher exclusion limit (4000 daltons), nevertheless, the resolution obtained is much better than that obtained on the P-2 material. Water regain for the P-4 gel (2.4) is higher than that for P-2 (1.5) permitting the use of smaller amounts of the dry material in order to fill columns with the same total volume. There are some disadvantages, however, in using the P-4 gel. The main one is the longer analysis time. This is due to the limitations of using higher flow-rates since the gel is soft and tends to collapse at flow-rates higher than 18 ml/h. If higher resolution is to be achieved, one has to sacrifice time.

## RELATIONSHIPS AND DEFINITIONS USED IN CALCULATION OF COLUMN DATA

$$K_D = \frac{V_e - V_0}{V_i} \qquad K_{av.} = \frac{V_e - V_0}{V_t - V_0} = \frac{V_e - V_0}{V_i + V_p}$$

$$N = 16\left(\frac{V_e}{W}\right)^2 \qquad N_{req} = 16\left(\frac{\alpha'}{\alpha' - 1}\right)^2$$

$$n_{eff} = 16\left(\frac{V_e - V_0}{W}\right)^2 \qquad n_{req} = 16\left(\frac{\alpha}{\alpha - 1}\right)^2$$

$$\alpha = \frac{V_{e_1} - V_0}{V_{e_2} - V_0} \qquad \alpha' = \frac{V_{e_1}}{V_{e_2}}$$

$$R = \frac{2(V_{e_1} - V_{e_2})}{(W_1 + W_2)} \qquad K' = \frac{V_e - V_0}{V_0}$$

$K_D$ = Distribution coefficient
$K_{av}$ = Distribution coefficient (when $V_i$ is unknown)
$N$ = Number of theoretical plates in the column
$N_{req}$ = Number of theoretical plates required
$n_{eff}$ = Number of theoretical effective plates in the column
$n_{req}$ = Number of theoretical effective plates required
$\alpha$ = Relative permeation value
$\alpha'$ = Relative retention value
$K'$ = Capacity of column for a given solute
$R$ = Resolution
$V_e$ = Elution volume
$W$ = Peak width.

## ACKNOWLEDGEMENT

Acknowledgement is made to the National Research Council of Brazil for its financial support to N. Sabbagh during this investigation.

## REFERENCES


1 G. Trenel and C. C. Emeis, *Die starke-Starch*, 22 (1970) 41.
2 M. John, G. Trenel and H. Dellweg, *J. Chromatogr.*, 42 (1969) 476.
3 G. Trenel, M. John and H. Dellweg, *FEBS Lett.*, 2 (1968) 74.
4 H. Dellweg, M. John and G. Trenel, *J. Chromatogr.*, 57 (1971) 89.
5 W. Brown, *J. Chromatogr.*, 52 (1970) 273.
6 W. Brown and O. Anderson, *J. Chromatogr.*, 67 (1972) 163.
7 W. Brown and O. Anderson, *J. Chromatogr.*, 57 (1971) 255.
8 W. Brown, *J. Chromatogr.*, 59 (1971) 335.