Carbohydrate Research, 65 (1978) 265–270

Note

The separation of malto-oligosaccharides by high-performance thin-layer chromatography

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(Received July 5th, 1977; accepted for publication, September 26th, 1977)

The separation of oligosaccharides by thin-layer chromatography has been reported for plates coated with cellulose[1–3], kieselguhr[4–7], silica gel[7–10], or mixtures of silica gel and kieselguhr[11,12]. For a given solvent system, the rate of migration on kieselguhr is higher than on silica gel[7] and to date the oligomers of highest degree of polymerization (D.P.) have been separated on the former support or on combinations of the two supports. Oligomers with a D.P. up to 6 have been separated by Hansen[10] on silica gel within 3 h, and oligomers with a D.P. up to ~9 have been separated by de Stefanis and Ponte[9] on silica gel within 4 h.

Recently, high-performance t.l.c. plates coated with silica gel have become commercially available. These plates are made of silica gel having a very narrow particle-size distribution compacted to a layer density comparable to that of a well packed h.p.l.c. column. The capacity is smaller than that of conventional t.l.c. plates with the result that smaller samples must be applied. Owing to the hard layer surface, a very fine capillary can be employed for sample application. The small spot size coupled with the high efficiency of the plates results in resolution of spots within a smaller distance than that found in conventional t.l.c. with the result that analysis times are significantly reduced.

Individual sugars are generally considered separated on the basis of a visual inspection of the t.l.c. plate after formation of a colored derivative. However, the actual diameter of a spot is considerably larger than its visual size, because of the relative insensitivity of the eye to low concentration of sugar derivative. Thus, not all of the reported separations of oligomers should be considered complete separations. The only satisfactory method of recording the true separation of oligomers is to measure the spot concentration with a suitable densitometer connected to a recorder[11,12].

Figures 1–4 illustrate the use of high-performance t.l.c. plates for the separation of malto-oligosaccharides. The size of the sample varied from 0.2 μg to 4 μg. The smallest spot size in general gives better resolution for the oligomers of low D.P. but results in poor detection of the oligomers of high D.P., which were present in low concentration in the sample used. The plates may be scanned from the front of the

plate or through the back of the plate, giving traces of somewhat different appearance. As a rough generalization a marginally better resolution is obtained from the front whereas a less "noisy" scan is obtained from the back. The spectrophotometer response is generally higher when scanning is made through the back of the plate.

Figure 1a shows 3 oligomers separated in 17:3 (v/v) acetone–water and Fig. 1b shows 5 oligomers partially or fully separated with 4:1 (v/v) acetone–water. Development was for 30 min in the large tank. When the water content was increased to 30%, the range was extended to 7 oligomers, but the quality of the separation decreased to such an extent that baseline resolution was not obtained for any of the oligomers.

Continuous development, where one end of the plate is exposed to the atmosphere, is a useful technique for extending the range of oligomers separated. Figure 2 shows a separation in 8:7:5 (v/v) 2-propanol–acetone–water with developing for 80 min. Oligomers up to a D.P. of 10 were separated with a near baseline separation of several members of the series. Figure 3 shows a separation in 9:6:5 (v/v) ethanol–acetone–water with developing for 85 min. Oligomers up to a D.P. of 12 were separated but the first 2 members of the series merged.

The solvent system 2:2:1 (v/v) butanol–ethanol–water gave a good separation of the peaks corresponding to oligosaccharides of low D.P., as well as a partial

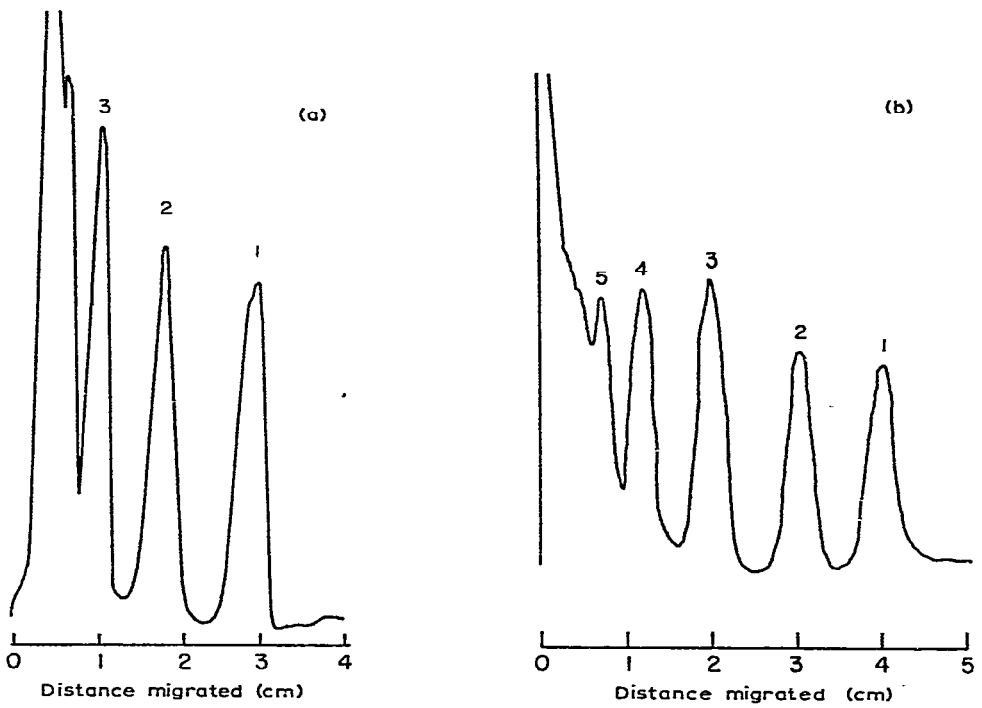

Fig. 1. Chromatogram of oligosaccharide mixture developed for 30 min in (a) 17:3 (v/v) acetone–water and (b) 4:1 (v/v) acetone–water. Numbers indicate degree of polymerization.

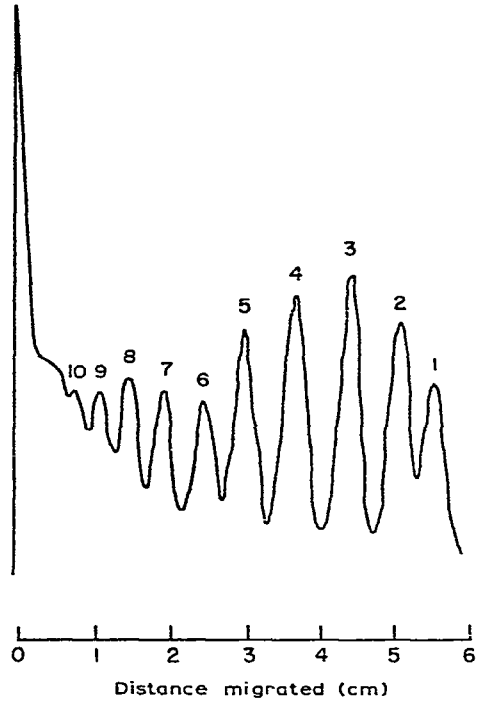

Fig. 2. Chromatogram of oligosaccharide mixture developed for 80 min in 8:7:5 (v/v) 2-propanol–acetone–water solvent. Numbers indicate degree of polymerization.

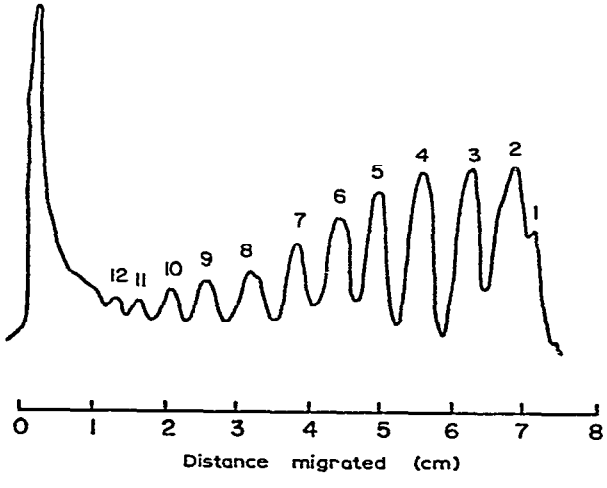

Fig. 3. Chromatogram of oligosaccharide mixture developed for 85 min in 9:6:5 (v/v) ethanol–acetone–water. Numbers indicate degree of polymerization.

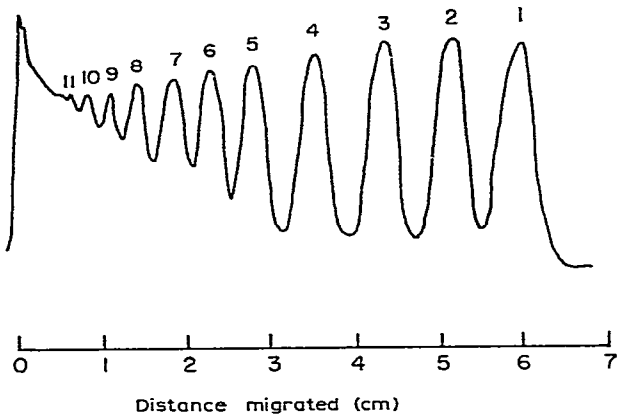

Fig. 4. Chromatogram of oligosaccharide mixture developed for 3 h in 2:2:1 (v/v) butanol–ethanol–water. Numbers indicate degree of polymerization.

separation of oligosaccharides up to a D.P. of 11 (see Fig. 4). However the development time of 3 h is longer than for the other solvent systems discussed here.

Figure 5 shows 9 consecutive scans of a mixture of D-glucose and D-maltose in a 4-μg sample run under the conditions of Fig. 1a, but scanned at a lower chart speed. The relative standard deviation for the peak ratio determined from these scans is 0.4%, which compares favorably to a previous densitometry report[11] and in fact, with a few exceptions, is better than the reported precision of the separation of sugars by gas–liquid chromatography.

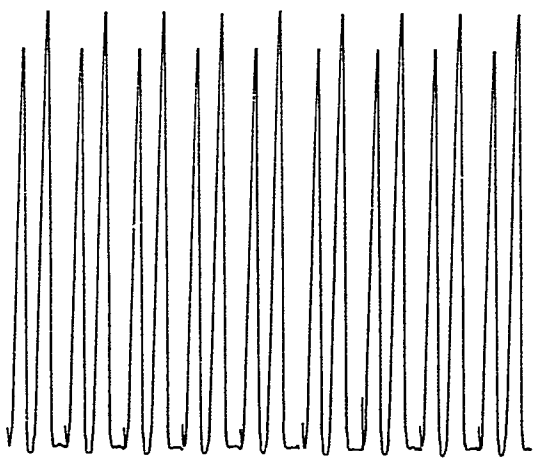

Fig. 5. Repetitive scans of D-glucose and D-maltose separated under the same conditions as given for Fig. 1a.

In conclusion, malto-oligosaccharides of low D.P. may be separated within 30 min, with a single development, on high-performance thin-layer plates coated with silica gel. The range of oligomers may be extended to a D.P. of 12 by use of continuous development for 85 min. In many cases, the separation is virtually baseline. In addition, these separations are more rapid than those reported using conventional thin-layer plates coated with silica gel. By careful selection of solvents and by performing multiple developments, it should be possible to separate oligomers with a D.P. of 20 or higher.

EXPERIMENTAL

Materials. — The precoated Silica Gel high-performance TLC plates (E. Merck, Darmstadt, Germany, cat. no. 5633) were cut into sections 2.5 cm × 10 cm. The solvents were Fisher-certified A.C.S. solvents. The oligosaccharides were gifts from Dr. A. W. Wight of the C.S.I.R., Pretoria, South Africa.

Sample application. — Solutions of 0.01%, 0.05%, and 0.2% of the oligosaccharide mixture in 70% aqueous ethanol were prepared. A sample of 200 nl of solution, corresponding to 200 ng, 1 μg, or 4 μg, respectively, of the oligosaccharide mixture, was deposited on the plates with a Pt–Ir capillary (Antech GmbH, Bad Dürkheim, Germany) attached to an EVA Chrom-Applicator (W+W Electronic Scientific Instrument Co., Basel, Switzerland).

Recording of chromatograms. — The spots were detected according to the method of Hansen[10]. The plates were dipped into a solution of aniline (4 ml), diphenylamine (4 g), acetone (200 ml), and 85% phosphoric acid (30 ml), and then placed for 20 min in an oven set at 110°. The plates were allowed to cool and then scanned with a Zeiss KM3 chromatogram spectrophotometer set at 620 nm. A Spectra-Physics Minigrator was used for the quantitative analysis.

Chromatography. — For single irrigation, a glass tank (26 × 22 × 7 cm), lined with Whatman Chromatography paper, was used. For continuous chromatography, a glass bottle 8.5 cm in height and 7.5 cm wide, lined with Whatman Chromatography paper and fitted with a screw-on metal cap was used as the chromatography tank. The t.l.c. plate was inserted into the tank through a slit (2 mm × 3 cm) cut in the metal cap.

ACKNOWLEDGMENT

The authors gratefully acknowledge the support of the National Aeronautics and Space Administration, Life Sciences Directorate, Johnson Space Center, Houston, Texas.

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