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SEPARATION OF MALTO-OLIGOSACCHARIDES BY HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY AT MODERATE TEMPERATURE

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SUMMARY

A procedure for the thin-layer chromatography of malto-oligosaccharides over a temperature range of 22 to 70° is described. Various mixtures of acetone, ethanol, 2-propanol and water were used as solvent. A method is proposed for estimating the temperature at which oligomers of a given degree of polymerization will be resolved.

INTRODUCTION

One of the most powerful methods of modifying the behavior of a chromatographic system is to adjust its temperature. This has been extensively used in most forms of chromatography but has only been used to a limited extent in thin-layer chromatography (TLC)[1–5]. The effect of increasing temperature is to lower the viscosity and raise the vapor pressure of the solvent used. Reports conflict as to whether R_F increases over a temperature range from about 0° up to 60° and whether better resolution is obtained at a higher or lower temperature. The design of the chromatographic tank, the precautions taken to control vapor saturation of the tank and the nature of the solvent and solute are most probably critical to the result obtained.

We have investigated the separation of a series of malto-oligosaccharides on silica gel high-performance TLC plates and have found that oligomers up to a degree of polymerization (D.P.) of about 10 could be separated within 90 min by continuous chromatography. We report here on the advantages of performing continuous chromatography of this series of compounds at elevated temperatures.

EXPERIMENTAL

TLC plates. Precoated silica gel high-performance TLC plates (E. Merck, Darmstadt, G.F.R., Cat. No. 5633). The plates were cut into sections 2.5 × 10 cm.

Solvents. Fisher Scientific (Pittsburgh, Pa., U.S.A.) certified A.C.S. solvents were used.

Oligosaccharides. These were obtained as a gift from Dr. A. W. Wight of the C.S.I.R., Pretoria, South Africa.

Sample application. Twenty milligrams of the oligosaccharide mixture were dissolved in 1 ml of 70% aqueous ethanol. A 200-nl portion of this solution was spotted on the plate using a Pt–Ir capillary (Antech, Bad Dürkheim, G.F.R.) attached to an EVA Chrom-Applicator (W + W Electronic Scientific Instrument, Basle, Switzerland).

Recording of chromatograms. The spots were visualized according to the method of Hansen[6]. The plates were then scanned using a Zeiss KM3 chromatogram spectrophotometer set at 620 nm.

Chromatography. A glass jam jar 8.5 cm in height and 7.5 cm wide, fitted with a screw-on metal cap, was used as the chromatographic tank. A slit of 2 mm × 3 cm was cut in the metal cap. The jar was lined with Whatman chromatography paper. About 15 ml of the solvent were poured into the jar, the slit was covered with a slide and a lead weight was placed on the lid. The jar was then 95% immersed in a large beaker of water equilibrated to the desired temperature on a hot plate. After about 3 min, the slide was removed and the TLC plate was placed in the jar. For chromatography at room temperature the water-bath and hot plate were not used.

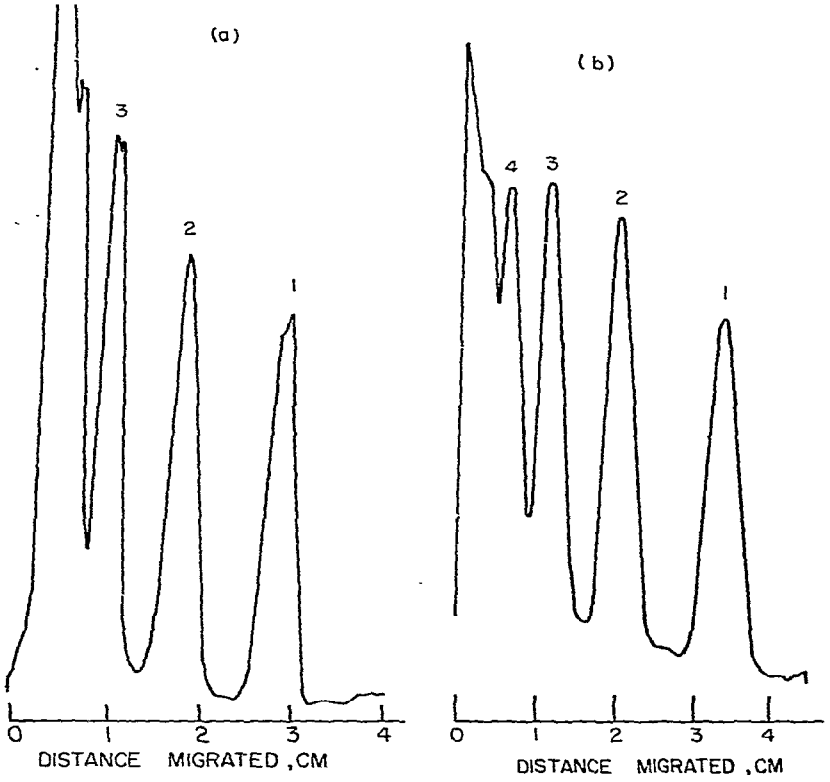

Fig. 1. Chromatograms of oligosaccharide mixture developed with acetone–water (85:15) as solvent. (a) Development for 25 min at 25°; (b) development for 25 min at 53°. Numbers indicate degree of polymerization.

RESULTS AND DISCUSSION

The solvent systems investigated consist of various mixtures of acetone, ethanol, 2-propanol and water. The temperature range studied was from 22 to 60°. With some solvent systems it was necessary to prewarm the plate to prevent excessive condensation on it at the higher temperature whereas with other systems this was not necessary.

It is advisable to remove a strip of silica gel 1.5 mm wide from each side of the plate at the higher temperature. If this precaution is taken, samples spotted in the middle of the plate usually run in a straight line whereas spots placed within about 6 mm from the side tend to migrate to the center of the plate. In some instances, it is possible to extend the temperature range by using a wider plate with a 3-mm strip of silica gel removed on either side.

The sample size spotted corresponds to 4 μg of the oligosaccharide mixture. This corresponds to overloading of the plate for the oligomers of low D.P. which are present at high concentration in the sample used. It allows, however, visualization of the oligomers of high D.P. that are present in low concentration.

The separation achieved at elevated temperature was not in all cases different

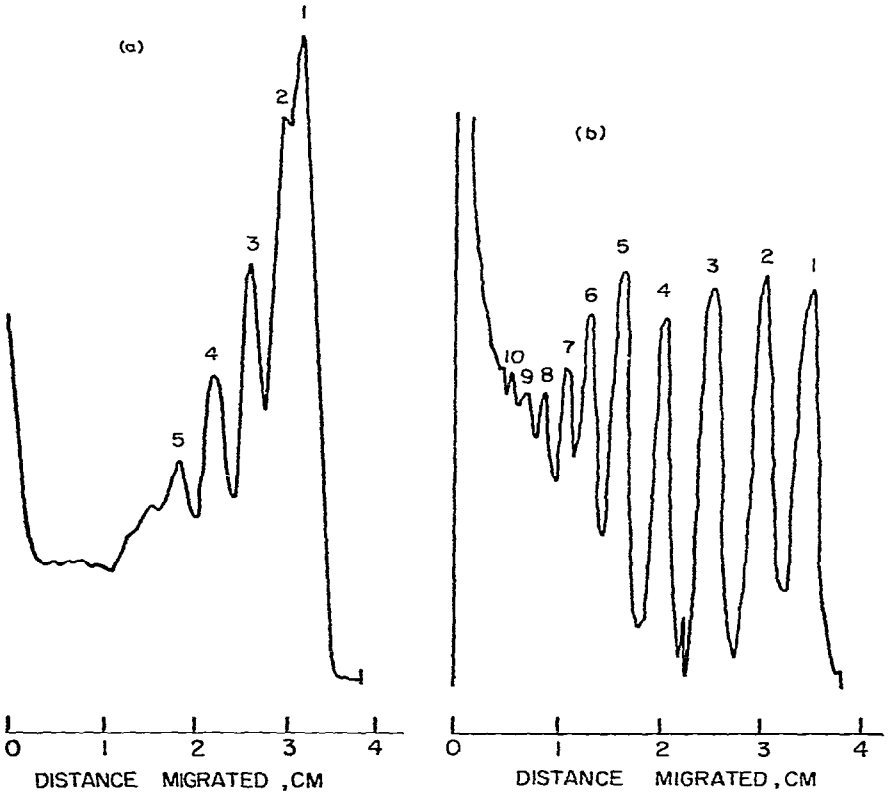

Fig. 2. Chromatograms of oligosaccharide mixture developed with 2-propanol–acetone–water (40:35:25) as solvent. (a) Development for 30 min at 22°; (b) development for 30 min at 60°. Numbers indicate degree of polymerization.

from that found at room temperature. This is illustrated in Figs. 1a and 1b, which show the separation achieved using acetone–water (85:15) as solvent. However, in other cases the increase in temperature results in significant gains in resolution and also significantly extends the range of oligomers separated. Thus, Fig. 2a shows that only a poor resolution of the first five oligomers is achieved at room temperature using a solvent consisting of 2-propanol–acetone–water (40:35:25). Fig. 2b shows a significantly better resolution of the first five oligomers when the separation is performed at 60°. In addition, the range of oligomers that are partially resolved is extended to a D.P. of 10. The distance migrated by the oligomers is not very different

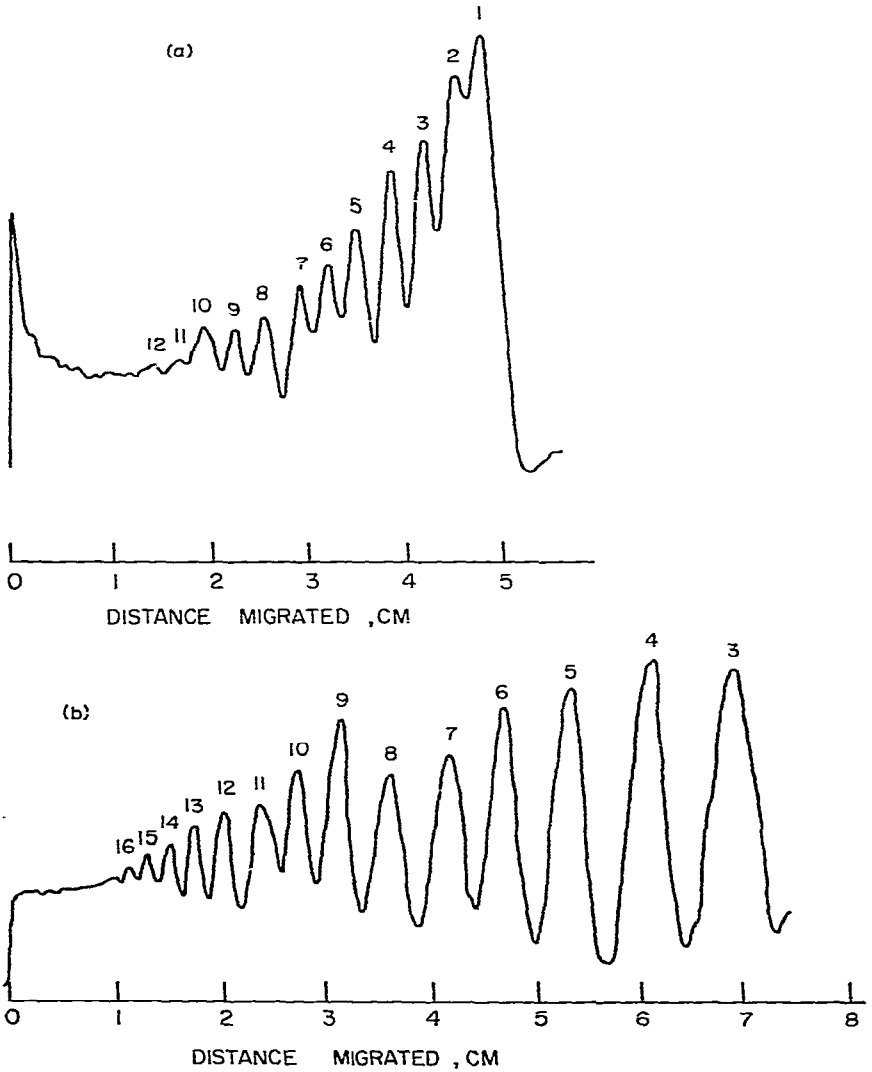

Fig. 3. Chromatograms of oligosaccharide mixture developed with 2-propanol–water (7:3) as solvent. (a) Development for 2 h at 25°; (b) development for 2 h at 60°. Numbers indicate degree of polymerization.

at the two temperatures, indicating that increased resolution is not only due to faster solvent flow but also due to evaporation behind the solvent front owing to non-saturation of the chromatography chamber.

Figs. 3a and 3b show the separation found at room temperature and at 60° using a solvent consisting of 2-propanol–water (7:3). At room temperature the oligomers with a D.P. of up to 12 are partially resolved. At 60° a near baseline separation of the oligomers is achieved with the D.P. resolved extended to 16. Oligomers with a D.P. of over 20 would most probably be resolved in a sample containing more high-molecular-weight material. Glucose and maltose migrate into the solvent front and are not detected.

Fig. 4 shows the plots of log distance migrated *vs.* D.P. for the series of oligomers at three different temperatures using ethanol–acetone–water (45:30:25). Chromatography was performed for 30 min. The shapes of the curves are similar for the three temperatures.

Fig. 5 is a plot of temperature *vs.* D.P. The points are plotted by using Fig. 4 to read the D.P. (even if this be fractional) found at distances of 1, 2 and 3 cm. These points lie on a series of straight lines which may be used to estimate the temperature

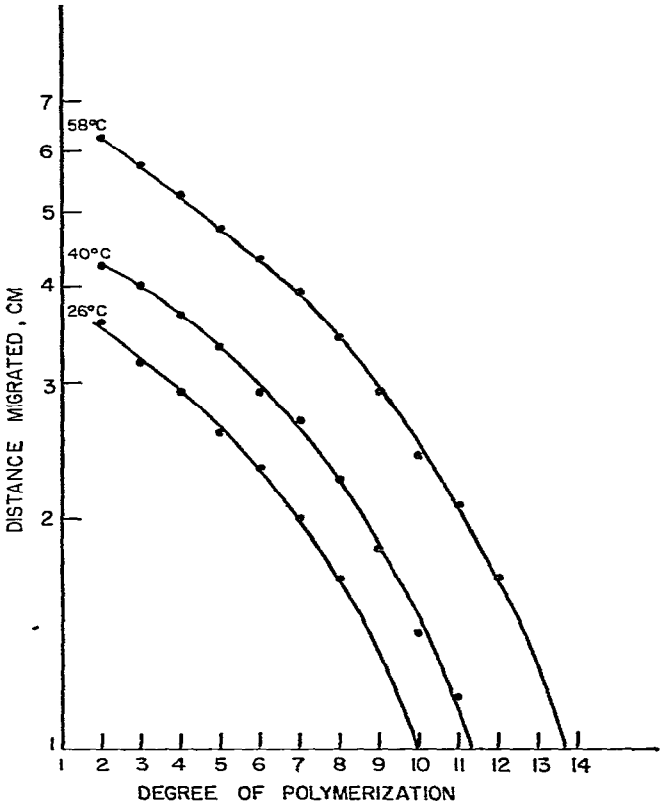

Fig. 4. Plot of distance migrated *vs.* degree of polymerization for the oligosaccharide series at three different temperatures using ethanol–acetone–water (45:30:25) as solvent. Development time, 30 min.

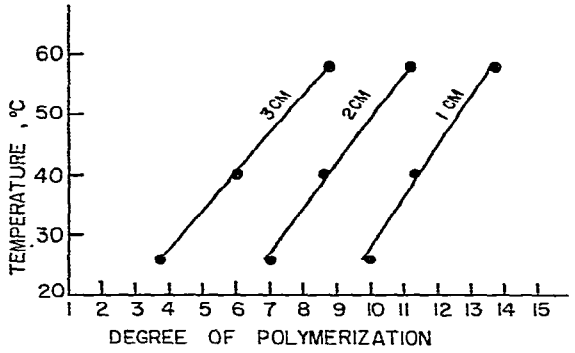

Fig. 5. Plot illustrating relationship between temperature, distance migrated and degree of polymerization for the oligosaccharide series using ethanol–acetone–water (45:30:25) as solvent. Development time, 30 min.

at which an oligomer of a given D.P. would migrate a given distance within 30 min. It is thus possible to estimate the temperature required to resolve oligomers of a given D.P. as partial or complete resolution is generally achieved once oligomers have migrated 1 to 2 cm on a high-performance TLC plate. Thus with this solvent system

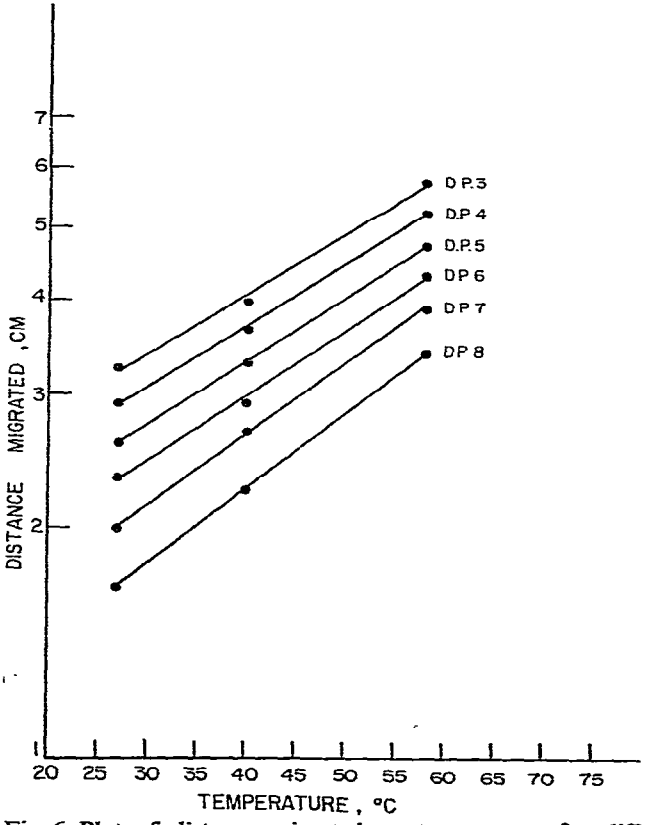

Fig. 6. Plot of distance migrated *vs.* temperature for different degrees of polymerization using ethanol–acetone–water (45:30:25) as solvent. Development time, 30 min.

an oligomer with a D.P. of 20 would be resolved within 30 min at a temperature between 105 and 125°. A diagram of this nature could also be used to estimate the length of plate necessary to avoid loss of low-D.P. oligomers at any given temperature. In practice the system had to be modified to extend the temperature range to 70°. A wider plate with a 3-mm strip of silica gel removed on either side was used and the slit width was increased to about 2.5 mm. This resulted in the oligomers migrating a shorter distance than at 58° but with significantly improved resolution between neighboring compounds. This is undoubtedly due to increased evaporation behind the solvent front owing to the lowered vapor saturation caused by the increased slit width. Fig. 6 shows that there is a linear relationship between the log of distance migrated and temperature for each of the oligomers.

TLC of oligosaccharides at temperatures up to 70° with a non-saturated atmosphere results in improved separations for most of the solvent systems studied. Conversely a given separation may be achieved in a shorter time. These advantages should also be valid for solutes other than the oligosaccharides. It should, in addition, be possible to extend the temperature range by proper experimental design.

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