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Capillary zone electrophoresis of pyridylamino derivatives of maltooligosaccharides

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ABSTRACT

Maltooligosaccharides derivatized with 2-aminopyridine were separated by capillary zone electrophoresis in the pH range 3.0–4.5 using 0.1 *M* phosphate solutions as the running electrolyte. The inclusion of small amounts of tetrabutylammonium bromide in the electrolyte solution facilitated the separation at pH 5.0 and yielded high separation efficiency. The separated zones of pyridylamino derivatives of maltooligosaccharides migrated across the fused-silica capillary and passed the detection point in the order of increasing size. The "overall mobility" was a linear function of the number of glucose residues in the homologous series.

INTRODUCTION

Capillary zone electrophoresis (CZE) is increasingly employed for the separation and analysis of a wide variety of compounds ranging in size from small ions and molecules[1–7] to high-molecular-weight substances such as proteins[8–10] and nucleic acid fragments[11,12]. However, the potential of the technique has not been yet exploited in many areas including carbohydrates. This may be due to the lack of charges and chromophores in the carbohydrate molecules.

Indeed, neutral carbohydrates are not directly amenable to electrophoresis. However, they may become charged as the result of complex formation with other ions. The complex formation equilibria, *e.g.* borate complexes, have been exploited in traditional paper electrophoresis[13,14]. Very recently, the sugar-borate complexes at pH 10 have been utilized in CZE for the separation of reducing monosaccharides[15] tagged with 2-aminopyridine.

On the other hand, pre-column derivatizations to produce a chromophore or fluorophore have been extensively used in high-performance liquid chromatography (HPLC) of carbohydrates to allow their sensitive detection[16,17] (for review see refs. 18 and 19). It is expected that many of these detection schemes will also be applied to carbohydrate detection in CZE.

In the present report, we extend the potential of CZE to the separation of pyridylamino (PA) derivatives of maltooligosaccharides. The derivatization of mono-

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and oligosaccharides with 2-aminopyridine is well documented[15,20] and was used for HPLC analysis of carbohydrates by UV[21] or fluorescence detection[17]. The tagging of maltooligosaccharides with 2-aminopyridine provides the homologues with a positive charge, which allowed their analysis by paper electrophoresis[20]. Indeed, the pK_a value of the derivatives has been found to be 6.7 (ref. 20), a value equal to that found for free 2-aminopyridine[22].

This paper presents the results of a study of the electrophoretic conditions for the separation of PA derivatives of maltooligosaccharides by CZE. High separation efficiencies were obtained in the pH range 3.0–4.5, which is ideal for the operation of fused-silica capillary columns as far as the stability of the column and the reproducibility of the separations are concerned. In addition, the inclusion of small amounts of tetrabutylammonium bromide in the running electrolyte was a useful adjunct to affect full separation of the homologous series at pH 5.0. The PA derivatives of the maltooligosaccharides eluted in the order of increasing size. The "overall mobility" was a linear function of the number of glucose residues in the homologous series. The slope of the lines, which we refer to as "overall mobility" decrement, is expected to facilitate the identification of oligosaccharides at large.

EXPERIMENTAL

Electropherograph

The instrument for capillary electrophoresis used in this study resembled that reported by others[3,4]. It was constructed from a Glassman High Voltage (Whitehouse Station, NJ, U.S.A.) Model EH30P3 high-voltage power supply and a Linear (Reno, NV, U.S.A.) Model 200 UV–VIS variable-wavelength detector equipped with a cell for on-column capillary detection. The detection wavelength was set at 240 nm. The electropherograms were recorded with a Kipp and Zonen Model BD 40 strip chart recorder. The high-voltage output and the ground were connected to platinum electrodes, which were placed in 2.0 ml electrolyte reservoirs. The ends of the fused-silica capillary were dipped in both reservoirs and that completed the circuit. The separation and sample introduction were carried out in the positive polarity mode, *i.e.* the anodic reservoir served as the high-voltage output (injection end) and the cathodic reservoir was at the ground potential (detection end).

Capillary columns

Fused-silica capillary columns of 50 μm I.D. and 365 μm O.D. having polyimide-clad were obtained from Polymicro Technology (Phoenix, AZ, U.S.A.). The total column length was 80 cm while the separation distance was 50 cm, *i.e.* from the injection end to the detection point. The polyimide-clad of the capillary was burned off at the detection point and the exposed quartz tubing was placed in the detector cell.

Reagents and materials

The following PA derivatives of maltooligosaccharides: PA-maltotetraose (PA-G_4), PA-maltopentaose (PA-G_5), PA-maltohexaose (PA-G_6), and PA-maltoheptaose (PA-G_7) were a gift by Dr. A. Mort from the Biochemistry Department at Oklahoma State University. The covalent attachment of 2-aminopyridine to the reducing end of the maltooligosaccharides was carried out using the procedure of

reductive amination[20]. Reagent-grade sodium phosphate monobasic and dibasic, phosphoric acid, hydrochloric acid, sodium hydroxide, phenol and tetrabutylammonium bromide were obtained from Fisher Scientific (Pittsburgh, PA, U.S.A.). Distilled water was used to prepare the running electrolyte as well as the solutions used in column cleaning and pretreatment. All solutions were filtered with 0.2-μm UniPrep syringeless filters obtained from Genex (Gaithersburg, MD, U.S.A.) to avoid column plugging.

Procedures

In all experiments (except where indicated) the voltage drop across the capillary during electrophoresis was fixed at 20 kV, while injection was made by electromigration at 18 kV for 15 s.

All experiments were carried out with uncoated fused-silica capillaries. The untreated silica capillary column was flushed with 1 *M* sodium hydroxide followed by water and the running electrolyte. The running electrolyte was renewed after 5–6 runs, and the capillary column was flushed with fresh buffer before each injection in order to ensure reproducible separations[23].

The electroosmotic flow-rate was measured by the ratio of the volume of the capillary (volume of a cylinder in 50 cm length and 50 μm I.D.) and the time required for phenol, an inert tracer[9], to migrate the 50 cm distance between the injection end and the detection point.

The "overall mobility" of the solute's zone is the sum of the electrophoretic mobility and the electroosmotic mobility; in this study both are directed toward the negative electrode. The "overall mobility," $\mu_{overall}$, is given by the equation[4], $\mu_{overall} = L^2/Vt$, where L, V and t are the total length of the capillary tube, the applied voltage, and the time for the zone to migrate the entire length of the tube, respectively. In CZE and using on column detection, the time t is given by the equation, $t = t_d L/l$, where l = distance from injection to the detector, and t_d = time for species to migrate the distance, which is readily obtained from the electropherogram. Substituting t in the above equation yields $\mu_{overall} = Ll/Vt_d$, which was used to measure the overall mobility.

RESULTS AND DISCUSSION

The electrophoretic behavior of each PA-maltooligosaccharide was examined using 0.1 *M* aqueous phosphate solutions at different pH values. The results are depicted in Fig. 1 in terms of migration time *versus* pH of the electrolyte solution. As expected, the migration time of the different homologues from the injection to the detection point decreased with increasing pH. This behavior is primarily due to the increase in electroosmotic flow[24], because higher electrolyte pH produces an increase in the ionization of silanol groups on the surface of fused-silica capillaries. Indeed, as can be seen in Figs. 1 and 2 phenol which is believed to migrate with the electroosmotic or bulk flow[9] clearly demonstrated this trend. In the pH range from 3.0 to 5.5 the positively charged PA-maltooligosaccharides migrated ahead of phenol and were separated according to their size. At pH 6.0 and 7.0, the derivatives practically coeluted at approximately the same migration time as phenol (see Fig. 1). Obviously in this pH range the PA-maltooligosaccharides become less positively charged. Under these

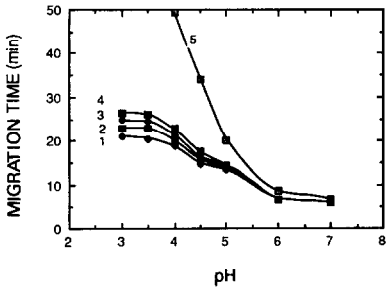


Fig. 1. Migration times of PA-maltooligosaccharides and phenol as a function of electrolyte pH. Capillary, fused-silica tube, 50 cm (to the detection point) × 50 μm I.D.; electrolytes, 0.1 *M* phosphate solutions at different pH values; voltage, 20 kV; current was *ca.* 60 μA; injection by electromigration for 15 s at 18 kV; temperature, 25°C. 1 = PA-G_4; 2 = PA-G_5; 3 = PA-G_6; 4 = PA-G_7; 5 = phenol.

circumstances neutral and quasi-neutral solutes are swept with the electroosmotic flow (bulk flow).

Fig. 3 illustrates the high separation efficiency obtained with a 50-cm (separation distance) untreated fused-silica capillary tube at 20 kV using 0.1 *M* phosphate, pH 4.0, as the running electrolyte. As can be seen in Fig. 3, base line resolution is obtained in less than 25 min.

Fig. 4 illustrates the "overall mobility" of PA-maltooligosaccharides at different pH values as a function of the number of glucose residues in the homologous series. The slope of the lines, which is the "overall mobility" decrement, δ, was relatively constant over the pH range 3.0 to 4.5 (see Table I). On the other hand, δ dropped sharply at pH 5.0, and reached almost zero at pH 6.0 and 7.0 whereby the PA-maltooligosaccharides moved virtually together with the bulk flow.

The effect of electrolyte concentration on the electrophoretic behavior of the homologues was studied at pH 4.0 using 0.025, 0.05, 0.075 and 0.1 *M* phosphate solutions. The results are depicted in Fig. 5 in terms of "overall mobility" *versus* the molar concentration of the running electrolyte. As expected, the "overall mobility" decreased with increasing phosphate concentration in the running electrolyte solution. This is due to the reduction in electroosmotic flow[25], as a consequence of an increase in

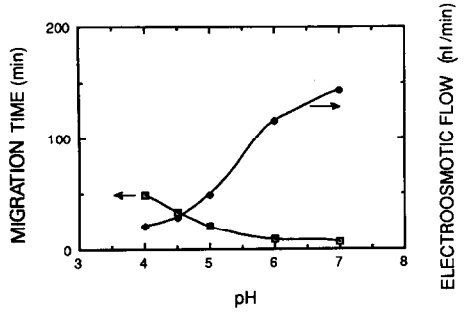
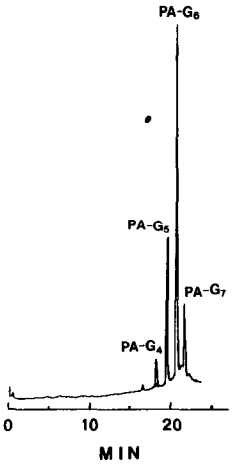


Fig. 2. Migration times for phenol and electroosmotic flow as a function of electrolyte pH. Other conditions as in Fig. 1.



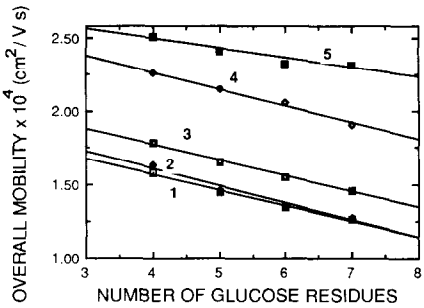


Fig. 3. Separation of PA-maltooligosaccharides. Electrolyte: 0.1 *M* phosphate, pH 4.0. UV detection at 240 nm. Other conditions as in Fig. 1.

Fig. 4. "Overall mobility" as a function of the number of glucose residues in the homologous series at different pH values. Other conditions as in Fig. 1. 1 = pH 3.0; 2 = pH 3.5; 3 = pH 4.0; 4 = pH 4.5; 5 = pH 5.0.

the viscosity of the electrolyte solution and a decrease in both the thickness of the double layer and the ζ potential of the capillary wall[26]. It has to be noted that a concentration of 0.075 to 0.1 *M* phosphate in the running electrolyte yielded sharper peaks and consequently higher resolution than a lower phosphate content. This may be due to the salt shielding effect of silanol groups on the capillary walls, which minimizes wall interaction with the positively charged PA-maltooligosaccharides.

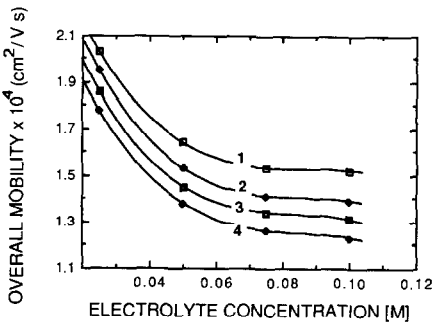
For each electrolyte concentration, the "overall mobility" data from the preceding experiments are plotted in Fig. 6 *versus* the number of glucose residues in the homologous series. Straight lines are obtained with a correlation coefficient equal or greater than 0.99 (see Table II). The "overall mobility" decrement, δ, for the homologues increased slightly with the electrolyte concentration by a factor of 1.13 when going from 0.025 to 0.1 *M* phosphate.

TABLE I

OVERALL MOBILITY DECREMENT, δ, AS ESTIMATED FROM THE SLOPE OF THE PLOTS OF OVERALL MOBILITY *VERSUS* THE NUMBER OF GLUCOSE RESIDUES IN THE HOMOLOGOUS SERIES

Electrolytes: 0.1 *M* phosphate solutions at different pH values.

pH	$\delta \cdot 10^6$ *cm²/Vs*	*Correlation coefficient*
3.0	−10.6	0.98
3.5	−11.5	0.97
4.0	−10.6	0.99
4.5	−11.4	0.99
5.0	− 6.5	0.92



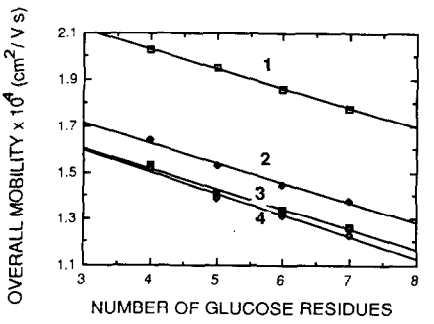


Fig. 5. "Overall mobility" as a function of electrolyte concentration. Electrolytes: phosphate solutions at different concentrations, pH 4.0. Currents: 15 μA, 25 μA, 40 μA and 60 μA at 0.025, 0.05, 0.075 and 0.1 *M*, respectively. Other conditions as in Fig. 1. 1 = PA-G_4; 2 = PA-G_5; 3 = PA-G_6; 4 = PA-G_7.

Fig. 6. "Overall mobility" as a function of glucose residues in the homologous series at different phosphate concentrations in the electrolyte. 1 = 0.025 *M*; 2 = 0.05 *M*; 3 = 0.075 *M*; 4 = 0.1 *M*. Other conditions as in Fig. 5.

As indicated above the resolution between the homologues decreased with increasing pH. Fig. 7 shows the separation of PA-maltooligosaccharides at pH 5.0 with and without tetrabutylammonium bromide in the running electrolyte solution. The inclusion of 50 m*M* tetrabutylammonium bromide in the electrolyte solution yielded higher separation efficiency and concomitantly higher resolution. A further increase in resolution was achieved by using 15 kV instead of 20 kV. This may be due to a lesser heat overload of the system and decreased electroosmotic flow at the lower voltage.

To study the effect of tetrabutylammonium bromide on separation, 50 m*M* of this organic salt were added to 0.1 *M* phosphate solutions at different pH values. The results are summarized in Table III in terms of migration modulus, η, which is the ratio of the migration time of the solute in the presence to that in the absence of tetrabutylammonium bromide. At pH 5.0 and above, η is almost equal to unity, whereas at pH 4.5 and 3.0 η is greater than 1 and 2, respectively. In the pH range 5.0–7.0, the small changes in the migration of PA-maltooligosaccharides upon adding tetrabutylammonium bromide to the electrolyte solution may be viewed as the result of

TABLE II

OVERALL MOBILITY DECREMENT, δ, AS ESTIMATED FROM THE SLOPE OF THE PLOTS OF OVERALL MOBILITY *VERSUS* THE NUMBER OF GLUCOSE RESIDUES IN THE HOMOLOGOUS SERIES

Electrolytes: solutions at different phosphate concentrations, pH 4.0.

Phosphate concentration (M)	$\delta \cdot 10^6$ *cm²/Vs*	*Correlation coefficient*
0.025	−8.4	0.99
0.05	−8.6	0.99
0.075	−8.8	0.99
0.1	−9.5	0.99

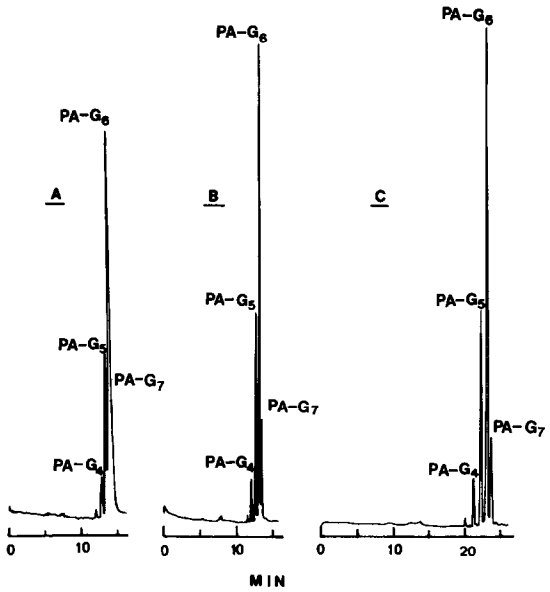


Fig. 7. Separation of PA-maltooligosaccharides. Electrolytes, 0.1 *M* phosphate, pH 5.0, without and with 50 m*M* tetrabutylammonium bromide in A and B, respectively. In both cases the voltage was 20 kV and the corresponding currents were 60 and 125 μA. In C as in B except voltage was 15 kV and current was 60 μA. Other conditions as in Fig. 1.

two opposing effects: increased overall mobility due to attenuation in wall interaction of the positively charged derivatives *versus* a reduction in the electroosmotic flow as a consequence of increasing the ionic strength of the electrolyte solution. At pH 3.0 and 4.5, where wall interaction is less pronounced, the enhancement in migration time upon adding the organic salt (see Table III) may be explained by the decrease in the electroosmotic flow resulting from an increase in the ionic strength of the running electrolyte.

TABLE III

MIGRATION MODULUS, η, OF PYRIDYLAMINO DERIVATIVES OF MALTOOLIGOSACCHARIDES AT DIFFERENT pH

Electrolytes: 0.1 *M* phosphate solutions with or without 50 m*M* tetrabutylammonium bromide.

PA-maltooligosaccharide	*Migration modulus, η*		
	pH 3.0	*pH 4.5*	*pH 5.0*
PA-G_4	1.8	1.5	0.96
PA-G_5	2.0	1.5	0.96
PA-G_6	2.3	1.5	0.96
PA-G_7	2.5	1.5	0.96

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