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Capillary zone electrophoresis of reducing mono- and oligo-saccharides as the borate complexes of their 3-methyl-1-phenyl-2-pyrazolin-5-one derivatives

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ABSTRACT

Various aldoses were derivatized to their 3-methyl-1-phenyl-2-pyrazolin-5-one derivatives and analyzed by capillary zone electrophoresis as their borate complexes. All derivatives of aldopentoses and aldohexoses were completely separated from each other in 100mM borate buffer (pH 9.5) and detected by u.v. absorption in the on-column mode. This system also provided good separation of derivatives of homologous oligoglucans having various types of interglycosidic linkages.

INTRODUCTION

Gas-liquid chromatography (g.l.c.) has played an important role for carbohydrate analysis in the preceding decade, and it is still in use in some areas of the field; however, it has a shortcoming in that samples need to be converted to volatile derivatives prior to analysis. During the last decade g.l.c. has given way, in part, to high-performance liquid chromatography (h.p.l.c.), because various separation and detection modes have been introduced to allow direct, sensitive analysis of carbohydrates. However, this method is still not entirely satisfactory in covering the analysis of a wide range of homologues and isomers of carbohydrates.

Under such circumstances, recent advances in high-performance capillary electrophoresis are quite attractive to carbohydrate researchers because of the high-resolution separations and the reproducible on-column detection; however, there is a fundamental problem in that the analytes must be ionic in order for the direct zone electrophoresis method to be employed. While the micellar electrokinetic chromatography mode can separate neutral compounds, the sample components must possess moderate hydrophobicity. Thus, carbohydrate compounds that have quite weak hydrophobicity cannot be analyzed by this separation mode.

It has been well established that carbohydrates react readily with the borate ion to form anionic complexes, and we have utilized this reaction for analysis of monosaccharides as their *N*-2-pyridylglycamine derivatives. The result was satisfactory, as almost all naturally occurring monosaccharides were well separated from each other

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under simple conditions[1]. We have further extended this method to the analysis of 3-methyl-1-phenyl-2-pyrazolin-5-one (MPP) derivatives, which were initially developed for precolumn labeling in h.p.l.c.[2], since the derivatives are more easily derivable from reducing carbohydrates. In this paper are presented data on capillary zone electrophoresis (c.z.e) of MPP derivatives of reducing mono- and oligo-saccharides in the indirect mode.

EXPERIMENTAL

Chemicals. — 3-Methyl-1-phenyl-2-pyrazolin-5-one (MPP) was purchased from Kishida Chemical Co. (Dosho-machi, Higashi-ku, Osaka, Japan) and used without further purification. This reagent was marketed with the name 1-phenyl-3-methyl-5-pyrazolone, but ^{1}H-n.m.r. spectroscopy in deuterochloroform indicated that its structure is 3-methyl-1-phenyl-2-pyrazolin-5-one having the double bond between C-2 and C-3 of the pyrazoline ring. A mixture of α-(1→3)-linked (laminara-) oligoglucans was obtained from Seikagaku Kogyo Co. (Nihonbashi, Chuo-ku, Tokyo). Mixtures of α-(1→6)-(isomalto-) and β-(1→4)- (cello-) oligoglucans were obtained by partial acid hydrolysis of dextran[3], and by acetolysis, followed by deacetylation, of cellulose[4], respectively. All other chemicals and carbohydrate samples were of the highest grade available.

Precolumn derivatization with 3-methyl-1-phenyl-2-pyrazolin-5-one (MPP). — A 0.5M methanolic solution (50 μL) of MPP and 0.3M sodium hydroxide (50 μL) were added to a dried sample of a reducing carbohydrate or a mixture of reducing carbohydrates. The mixture was kept for 30 min at 70°, then cooled to room temperature. Hydrochloric acid (50 μL, 0.3M) was added to the reaction mixture, and the mixture was evaporated to dryness under reduced pressure. Water (200 μL) and chloroform (200 μL) were added to the residue, and the mixture was vigorously shaken. The aqueous layer was evaporated to dryness, the residue was dissolved in a small volume of methanol, and the solution was introduced to the capillary tube for c.z.e.

Capillary zone electrophoresis (c.z.e.). — The apparatus used for c.z.e. was set up from a Matsusada Precision Devices HER-30P1 high-voltage power supply, a JASCO UVIDEC 100-V u.v. monitor, and two hand-made PTFE blocks, each having a 1-mL cavity, in which a platinum wire was fixed. A fused silica capillary tube (Scientific Glass Engineering, Melbourne, Australia, 50 μm i.d., 78 cm) was cut from a roll of fused silica tubing, and a part of the polyimide coating was removed by burning at a distance of 15 cm from one end. The transparent portion was fixed to a slit (50 × 700 μm), which was screwed to a plastic holder positioned in the center of the u.v. source. The tube was rinsed with 0.1M sodium hydroxide and conditioned with carrier before each run. After every 10 runs, the tube was rinsed with methanol. Samples were introduced into the tube by siphoning, *i.e.*, by raising the level of the sample solution 5 cm higher than that of the cathode solution for 5 s. Detection was carried out at 245 nm.

RESULTS AND DISCUSSION

Separation of aldopentose and aldohexose MPP derivatives. — The mechanism of separation of MPP-aldoses is the same as that described previously for *N*-2-pyridylglycamines[1]. When a sample was introduced into the tube from the anodic end, the component MPP-aldoses were converted *in situ* to anionic borate complexes and retarded by electrophoresis. At the same time there was a constant flow of carrier from anode to cathode, induced by electro-osmosis, which drove the MPP-aldoses to the detector. Separation was achieved by the degree of complexation and by the molecular size of the resulting complex. The column efficiency was quite high (*e.g.*, theoretical plate height for MPP–xylose, 4.1 μm) because the velocity of electro-osmotic flow was uniform across the tube, *i.e.*, the electro-osmotic flow was a plug flow, and also because the heat evolved during analysis was efficiently dissipated from the capillary wall.

Since the apparent electrophoretic mobility (μ_{ep}) of every MPP-aldose as the borate complex is proportional to the mole fraction of the complexed aldose, and since the mole fraction increases with the pH and borate concentration (C) of carrier, μ_{ep} gave ascending curves with pH and C in a similar fashion as that observed for *N*-2-pyridylglycamines[1]. The μ_{ep}–pH as well as μ_{ep}–C curves of MPP-aldopentoses were parallel and did not cross each other. Although both *N*-2-pyridylglycamines and MPP-aldoses have structures similar to 1-substituted alditols, there was a slight difference in ease of complexation between these two types of derivatives, presumably due to the participation of the substituent (MPP) group in complexation. Thus the optimum pH for the separation of MPP-aldoses (9.5) was slightly different from that for *N*-2-pyridylglycamines (10.5). Using 100mM borate buffer (pH 9.5) as carrier, all MPP-aldopentoses in the D-series were completely separated from each other as shown in Fig. 1. Amobarbital was used as an internal standard, since it migrated ahead of these aldopentose derivatives, well separated from the peaks of these derivatives. The μ_{ep} value can be calculated from the equation, $l \cdot L(1/t_0 - 1/t)/V$ (ref. 5), where l, L, t_0, t and V are respectively the effective length of the tube between the inlet and the detection slit, the whole length of the tube, the migration time of a neutral marker (methanol), the migration time of a MPP-aldose, and the applied voltage. The values obtained ($\times 10^4$, $cm^2 \cdot s^{-1} \cdot V^{-1}$) for the authentic samples of xylose, arabinose, ribose and lyxose derivatives were 2.56, 2.62, 2.68, and 2.72, respectively. The peaks in Fig. 1 were assigned by comparing their μ_{ep} values with those of the authentic samples.

Separation of MPP-aldohexoses was also excellent, as all MPP-aldohexoses in the D-series were almost completely separated from each other under the same conditions as those for MPP-aldopentoses (Fig. 2). The migration order was glucose (2.62) > allose (2.67) > altrose (2.75) > mannose (2.78) > idose (2.86) > gulose (2.88) > talose (2.96) > galactose (2.98). The numbers ($\times 10^4$, $cm^2 \cdot s^{-1} \cdot V^{-1}$) in parentheses are μ_{ep} values. Comparison of the μ_{ep} values of MPP-aldohexoses with those of MPP-aldopentoses can predict that the peaks of a few species of pentose and hexose derivatives will overlap. Therefore, the present system should be further improved, based on optimization studies, if complete separation of all these derivatives is required.

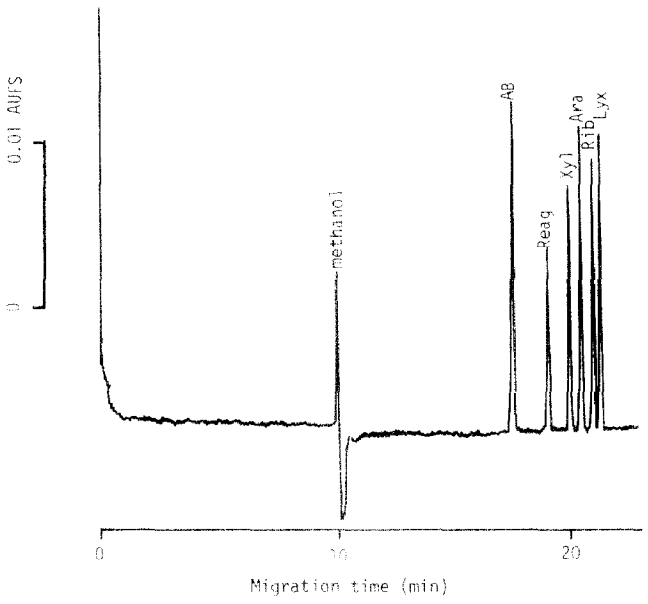

Fig. 1. Separation of MPP-aldopentoses by c.z.e. Capillary: fused silica (50 μm i.d., 78 cm, effective length 63 cm); carrier: 200mM borate buffer (pH 9.5); applied voltage: 15 kV; detection: u.v. at 245 nm. AB, amobarbital (internal standard); Reag, excess reagent (MPP); Xyl, xylose; Ara, arabinose; Rib, ribose; Lyx, lyxose.

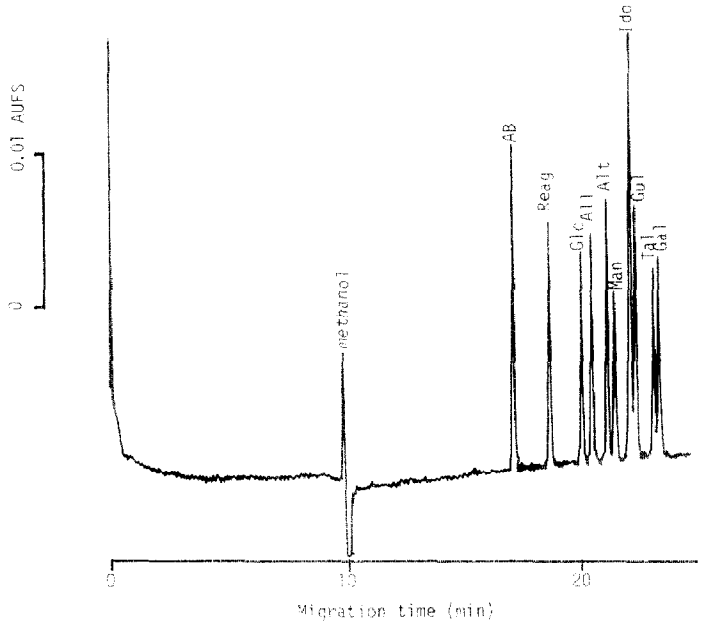

Fig. 2. Separation of MPP-aldohexoses by c.z.e. The analytical conditions were the same as those in Fig. 1. Glc, glucose; All, allose; Alt, altrose; Man, mannose; Ido, idose; Gul, gulose; Tal, talose; Gal, galactose. The other abbreviations are the same as those in Fig. 1.

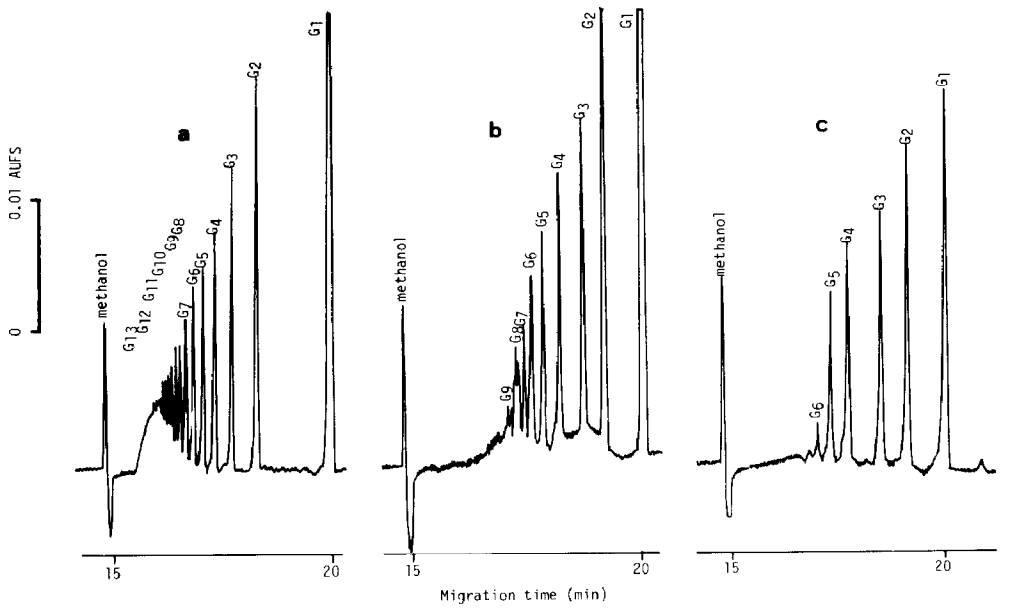

Fig. 3. Analysis of MPP-derivatives of homologous oligoglucans by c.z.e. (a) Isomalto series; (b) laminara series; (C) cello series. The analytical conditions were the same as those in Fig. 1.

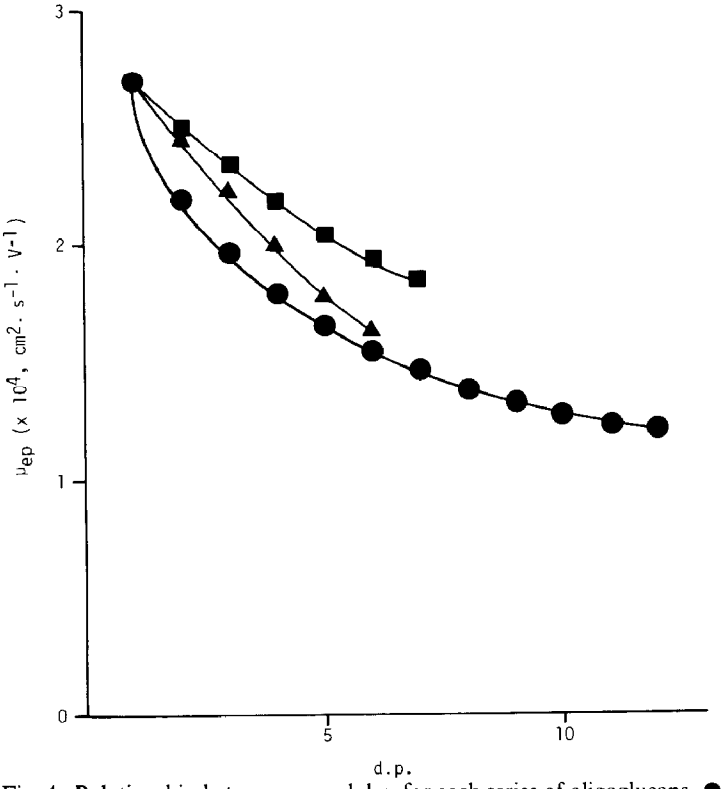

Fig. 4. Relationship between μ_{ep} and d.p. for each series of oligoglucans. ●, isomalto series; ■, laminara series; ▲, cello series.

Separation of homologous oligoglucans. — Fig. 3 shows the separation of various series of homologous oligoglucans. Separation among the MPP-oligoglucans was good for each in these series, but it should be taken into consideration that the methods of preparation of samples varied among samples; hence, the range of degree of polymerization (d.p.) differs from sample to sample. The μ_{ep}–d.p. plots of MPP-oligoglucans in every series gave a descending curve, and the rate of decrease varied among series, as shown in Fig. 4. All curves were slightly concave and logarithmic plots also gave no straight lines.

Since μ_{ep} in c.z.e. of borate complexes depends on the ease of complexation, the μ_{ep} is affected by the disposition of hydroxyl groups, *i.e.*, by the type of interglycosidic linkage of these oligosaccharides. For this reason the pattern of electrophoretogram was also considerably varied among the series. Thus this separation mode has a quite different feature from either size-exclusion or partition in liquid chromatography, in which oligomers are separated by the differences in molecular size and hydrophobicity, respectively. Therefore, comparison of analysis of the partial acid hydrolyzates by this method with those by other methods will undoubtedly provide useful information on the structure of polysaccharides.

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