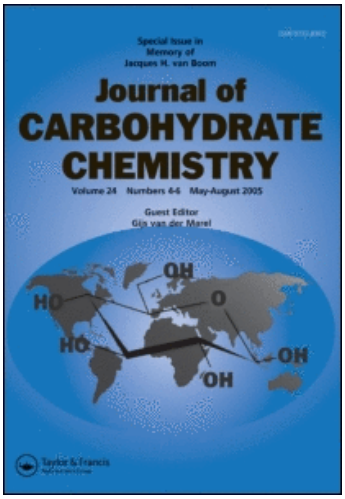

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REVIEW

CAPILLARY-ELECTROCHROMATOGRAPHIC METHODS FOR THE SEPARATION OF *p*-NITROPHENYL AND 1-PHENYL-3-METHYL-5-PYRAZOLONE DERIVATIZED MONO- AND OLIGOSACCHARIDES

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INTRODUCTION

In this review the basic principles and recent developments in instrumentation, stationary phases, buffer systems and derivatization techniques for the capillary electrochromatography of *p*-nitrophenyl and 1-phenyl-3-methyl-5-pyrazolone derivatized carbohydrates and their anomers is presented and new developments are discussed. Since 1974 Pretorius *et al.*[1] demonstrated the potentials of capillary electrochromatography (CEC), a hybrid of high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE), this technique has advanced steadily toward becoming an efficient microcolumn separation method.[2-4] In CEC, transport of the eluent is accomplished by applying an electric field across the

length of the column. The hybrid nature of CEC provides unique selectivity and high resolution[5] that can not be achieved by either HPLC or CE alone, and offers higher loading capacity than CE. The theoretical basis for CEC was established by Knox and Grant[6] in 1987. In CEC the electroosmotic flow (EOF), generated on the surfaces of capillary inner walls as well as packing materials, drives separated analytes as in free-solution CE.[7] Since EOF is a plug flow with uniform velocity everywhere in the capillary, better separation than with pressure-driven laminar flow LC, can be achieved. A major advantage of CEC results from the possibility of attaining relatively high flow rate in capillary columns packed with small particles that would require ultra high column inlet pressures in micro-HPLC because of their low permeabilities. Analytes introduced into the capillary are separated based on the difference of the magnitude of distribution between mobile and stationary phases. If the analytes have ionic properties, they may also be separated by zone electrophoresis. Although some investigations have been reported concerning approaches for gradient elution,[8,9] frits and stationary phase making,[10, 11] no investigations on the CEC separation of crude samples, such as biological samples have been published, because these samples often contain large proportions of impurities, which may strongly affect the performance of CEC in these miniaturized separation systems.

There are many investigations which show that the unique selectivity of CEC is suitable for the separation of closely related carbohydrates, namely derivatives of mono- and oligosaccharides. As in other separation techniques, a prerequisite for achieving the separation and detection of carbohydrates by CEC is to derivatize the sugar analytes with fluorophores or chromophores to yield preferably neutral derivatives. Neutral sugar derivatives can be electrochromatographed on ODS capillary columns exhibiting moderate electroosmotic flow velocity for which kind and concentration of the buffer system used plays an important role. Negatively charged sugar derivatives may not be transported through the capillary column within a reasonable analysis time under moderate velocity in packed ODS capillary columns. Positively charged derivatives may undergo silanophilic interaction with unreacted silanols on the surface of the ODS stationary phase, a phenomenon that may lead to band broadening and loss of resolution between the separated analytes. The advantage of carbohydrate derivatization is twofold: 1) the detection sensitivity is increased, and 2) the hydrophobicity necessary for reversed-phase CEC is ensured.

BASIS OF CAPILLARY ELECTROCHROMATOGRAPHY OF DERIVATIZED CARBOHYDRATES

Instrumentation

Most capillary electrochromatographic separations are carried out using capillary electrophoresis systems that allow slight alteration to facilitate pressurization of both the inlet and outlet end of the packed capillary column and applying isocratic elution at voltages up to 30 kV. A schematic of a home built capillary elec-

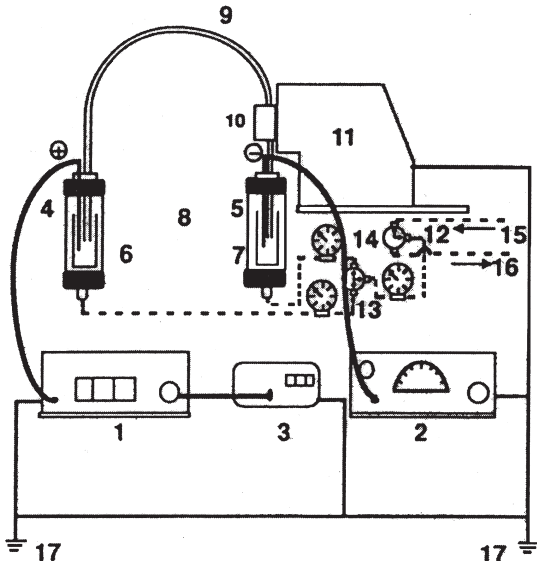

Figure 1. Schematic of the modular capillary electrochromatograph with a 90 kV dual power supply and pressurizable chambers for the column inlet and outlet. (1) 60 kV power supply, (2) 30 kV power supply, (3) digital electrometer, (4,5) electrodes, (6,7) reservoir for mobile phase or the sample, (8) pressurizable chambers, (9) packed capillary column, (10) cell for on-column detection, (11) detector, (12) four-port three-way valve, (13) four-port three-way valves, (14) pressure gauges, (15) from nitrogen cylinder, (16) vent, (17) ground.[12]

trochromatograph by Horváth *et al.*[12] equipped with a 90 kV dual power supply and pressurizable vials at both the column inlet and outlet is shown in Figure 1.

Using a fused silica capillary column packed with 6 μm ODS particles EOF velocities as high as 6-7 mm/sec can be realized at an applied voltage of 60 kV, whereas it was found that the EOF velocity is not a linear function of voltage when the field strength exceeded 100 kV/m. In order to separate all sample components without compromising analysis time, resolution and peak sensitivity, gradient elution is employed in order to increase gradually and in a controlled way the eluent strength during the chromatographic run. In order to exploit the full potential of CEC for the analysis of complex mixtures it is advisable to employ instrumentation having gradient elution capabilities.

Stationary Phase

Most commonly used stationary phases in reversed-phase HPLC are alkyl-silica bonded phases. Stationary phases used in CEC should ensure the required selectivity for the components and should allow rapid mass transfer in order to minimize band spreading at high plate efficiencies. Furthermore, the stationary phase should have fixed charges at the surface (about 75%) to generate EOF in the high electric field. As a result even charged and uncharged analytes show dif-

ferent retention behaviour when they are separated both in the micro-HPLC and CEC modes under otherwise identical conditions.[13] Reversed-phase CEC can be used for the analysis of neutral species having widely different molecular structures. The employment of RP-CEC with alkyl silica stationary phases and neutral or alkaline hydroorganic mobile phases is possible because the dissociated silanol groups generate a high EOF while the alkyl chains provide the hydrophobic surface for retention by solvophobic interactions.[14] Generally, two types of columns are currently used in CEC. In one type of packed capillaries, the column is composed of a segment that is packed with a given stationary phase and a segment that is empty. This kind of column is a partially packed capillary column. One of the retaining frits (the outlet frit) is fabricated at the interface of the two segments and a detection window is opened on the empty side close to the interface.[15-17] In this configuration the packed segment is less conductive than the open one. Thus, for the same current across the whole capillary, the electric field strength in the packed segment is higher. Also, Joule heating in the open segment is higher than in the packed segment. Furthermore, the intrinsic EOF in the open segment is higher than in the packed segment because of column porosity, reduced number of free surface silanols of the packing, and the orientation between the capillary axis and the interconnected channels. However, the mass conservation law dictates the volumetric flow rate of the mobile phase to be the same in the open and packed segments. Thus, an average flow across the capillary will be established. Under these conditions, there will be an acceleration of the intrinsic flow-rate in the packed segment exerted by the higher intrinsic EOF in the open segment, and the EOF may partially degenerate to viscous flow. This degeneration of EOF to a viscous flow and the difference in Joule heating between the open and packed segments are believed to be principal contributors to the formation of air bubbles in partially packed columns.

The other type of column is fully packed with stationary phase.[3,10] Fully packed capillary columns are more homogenous in terms of conductance, Joule heating and EOF. These properties reduce air bubble formation when compared to partially packed columns. The drawback of fully packed columns is that detection sensitivity is reduced due to light scattering by the packing material at the detection window.[18,19]

Preparation of Frits

The retaining frits of a CEC column must ensure good column permeability to achieve high separation efficiency. Also, the frits should be strong enough to withstand the packing pressure and allow reproducible column performance. There are at least three approaches for frit fabrication.[19] In one type, the frit is formed by *in situ* polymerization of potassium silicate solution with formamide according to the method of Cortes et al..[20] Usually, the material is completely polymerized after 1 h at 120 °C. In a second approach, the frit is made by tapping the capillary tip into silica wetted with potassium silicate solution, and the frit is sintered using an

electrically heated hot iron.[19] In a third type, the frit is formed by sintering water-wetted silica.

NEW DEVELOPMENTS IN CAPILLARY ELECTROCHROMATOGRAPHY OF DERIVATIZED CARBOHYDRATES

Separation of *p*-Nitrophenyl α-D-Derivatives of Monosaccharides and *p*-Nitrophenyl α-D-Oligosaccharides

p-Nitrophenyl modified monosaccharides and oligosaccharides (Figure 2) can be purchased from Sigma (St. Louis, MO, USA). The electroosmotic flow depends on the nature of both the stationary and the mobile phases. The influence of the organic modifier and its concentration in the mobile phase on the electroosmotic flow velocity, retention and selectivity has been systematically studied.[21,12] The most widely used organic modifier in CEC has been acetonitrile because of its UV-transparency and the highest ratio of dielectric constant to viscosity (ε/η).

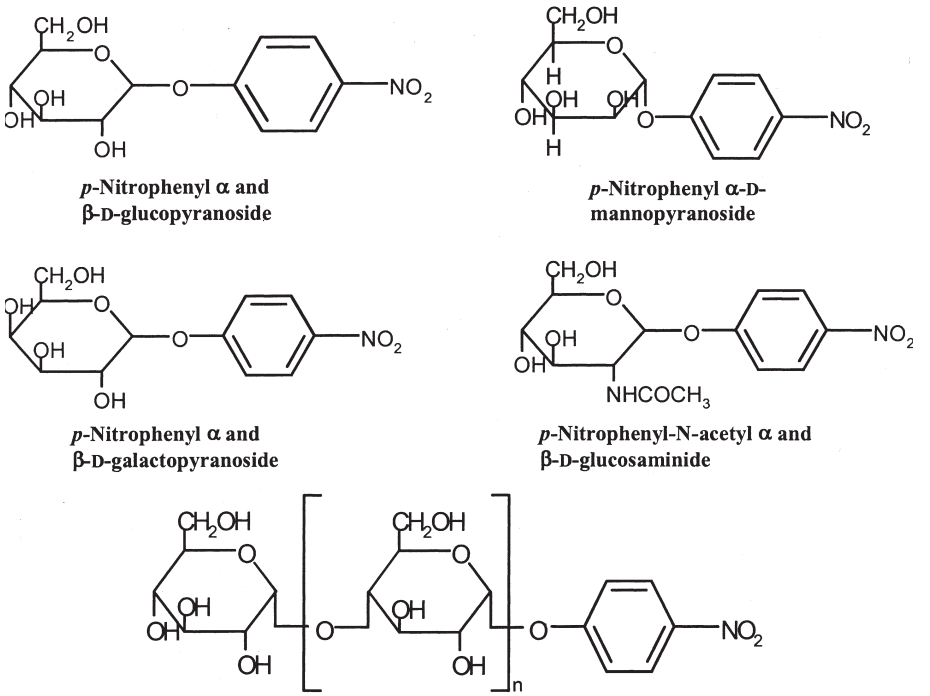

Figure 2. Structures of *p*-nitrophenyl derivatives of some mono- and oligosaccharides.

For the separation of carbohydrates the percentage of acetonitrile (v/v) in the mobile phase was changed in order to determine the optimum mobile phase composition for rapid elution time and high separation efficiency. Therefore, *p*-nitrophenyl α-D-maltooligosaccharides and *p*-nitrophenyl α-D-glucopyranosides were used as standards. Figure 3 shows the CEC electrochromatograms of *p*-nitrophenyl α-D-glucopyranoside and *p*-nitrophenyl α-D-maltooligosaccharides at different portions of acetonitrile. By optimizing the content of acetonitrile the retention times of the investigated solutes can be halved.[22]

Satisfactory separation was obtained with a mobile phase at relatively low acetonitrile content (20 % v/v) and low electric field strength (370 V/cm). Under these circumstances, the reproducibility of the electrochromatographic system in terms of retention time was shown to be good, with % RSD < 0.55.[22] The column separation efficiency was relatively high and varied between 72,000 and 152,000

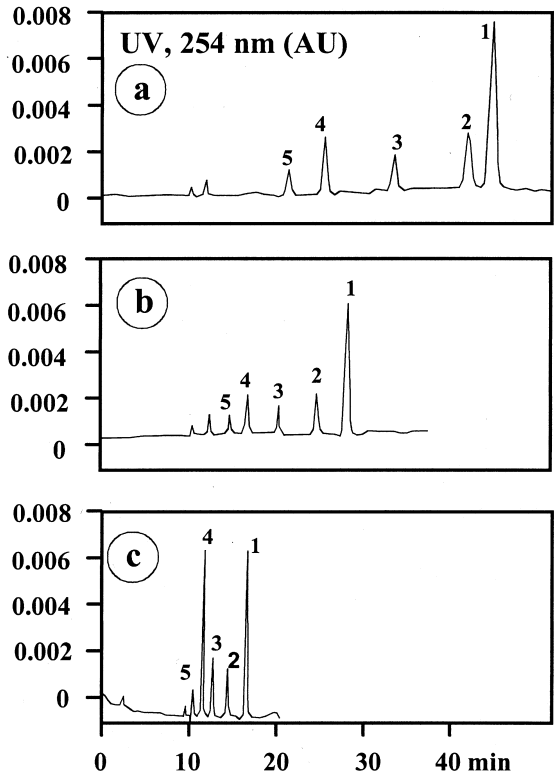

Figure 3. Electrochromatograms of *p*-nitrophenyl α-D-glucopyranosides and maltooligosaccharides. Mobile phases: (c), 40% v/v of 5 mM NaH_2PO_4 (pH 6.0), 40% v/v H_2O and 20% v/v acetonitrile; (b) 42.5% v/v of 5 mM NaH_2PO_4 (pH 6.0), 42.5% v/v H_2O and 15% v/v acetonitrile; (a) 45% v/v of 5 mM NaH_2PO_4 (pH 6.0), 45% H_2O and 10% v/v acetonitrile; voltage, 10 kV; detection window at 20 cm from column inlet; detection wavelength, 254 nm; column temperature, 15 °C. Solutes: *p*-nitrophenyl α-D-glucopyranoside; 2, *p*-nitrophenyl α-D-maltoside; 3, *p*-nitrophenyl α-D-maltotrioside; 4, *p*-nitrophenyl α-D-maltotetraoside; 5, *p*-nitrophenyl α-D-maltopentaoside.[22]

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Table 1. Column Efficiency (plates/m) and Retention Time Reproducibility (% RSD)

p-Nitrophenyl derivative of	Retention time (% RSD)	Column efficiency (plates/m)
Galactose	0.54	95000
Glucose	0.47	92000
N-Acetylglucosamine	0.21	83000
Mannose	0.31	84000
Maltose	0.55	95000
Maltotrioside	0.23	85000
Maltotetraoside	0.49	152000
Maltopentaoside	0.26	72000

plates/m (Table 1). The elution order of the glycosides under these conditions is *p*-nitrophenyl α-D-maltopentaoside, -maltotetraoside, -maltotrioside, -maltoside, and -glucoside. This elution order is the same as that observed in RPC,[23] and is believed to be determined by the hydrophobicity of the glycosides as well as by organic induced conformational change of the glycosides. It has been shown that the homologs of degree of polymerization (d.p.) of 2-5 are increasingly more soluble in water due to the decreasing effect of the *p*-nitrophenyl residue. This means that the polarity of the solute increases when going from a d.p. of 2 to a d.p. of 5. Linear plots for the dependence of log k on percent acetonitrile in the range of 10-30% v/v can be deduced from Figure 4. By extrapolating to 0% acetonitrile in the mobile phase, the curve of the *p*-nitrophenyl α-D-maltopentaoside intersects the y-axis at a log k value higher than that of glucose, and the order of elution becomes maltotetraoside, maltotrioside, maltoside, glucose and maltopentaoside. These irregularities in the elution order when changing the organic content of the mobile phase have been observed previously in RPC of carbohydrates.[24]

The change in the elution order of the *p*-nitrophenyl α-D-maltopentaoside may be attributed to organic solvent induced conformational change.[25] Figure 5 shows the electrochromatograms of *p*-nitrophenyl α-D-glycoside derivatives of monosaccharides, e.g., galactose, *N*-acetylglucosamine, glucose and mannose at different portions of acetonitrile in the mobile phase. Optimum conditions are found at 25% acetonitrile in the sodium hydrogenphosphate buffer system which allows for a fast separation of the investigated solutes within five minutes.

As can be seen in Figure 2 glucose and galactose differing only by the orientation of the OH on C4 (equatorial vs axial) separated very well on the ODS capillary column. The axial OH in galactose at C4 confers more hydrophilic character to the molecule than the equatorial OH on the C4 of glucose because the OH group on C4 of galactose may not engage in a significant interaction with other OH groups in the galactose molecule. This reasoning may explain why galactose is less retained than glucose. For mannose, the axial OH at C2 can interact with the axial glycosidic linkage.

Under these conditions the hydroxyl group at C2 in mannose will confer less polar character to the molecule than the OH at C2 in glucose. This may explain the

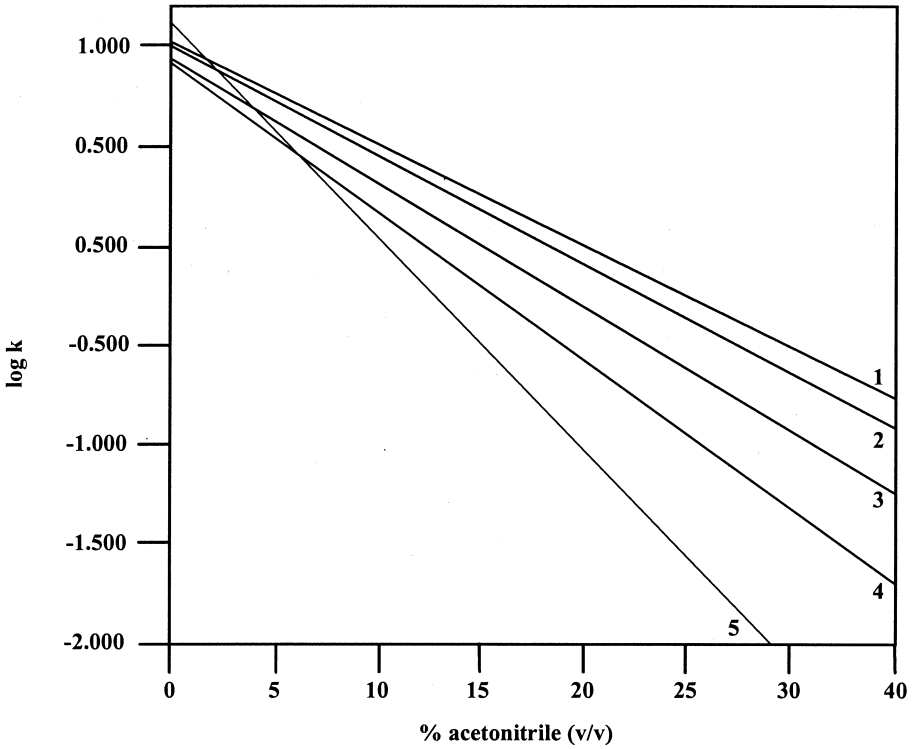

Figure 4. Plots of logarithmic retention factor (log k) of *p*-nitrophenyl α-D-glucopyranosides and maltooligosaccharides vs percent acetonitrile in the mobile phase. Mobile phases: diff. % v/v of 5 mM NaH_2PO_4 (pH 6.0), water and acetonitrile; voltage, 10 kV; Other conditions as in Fig. 3.

higher retention time of mannose. Finally, because of the presence of the *N*-acetyl group (slightly hydrophobic), *N*-acetylglucosamine is more retarded than glucose.

Furthermore, Yang and Rassi[22] investigated the retention behaviour of α– and β–anomers of *p*-nitrophenyl *N*-acetyl-glucosaminide, *p*-nitrophenyl galactopyranoside, and *p*-nitrophenyl glucopyranoside. By adding borate to the hydroorganic mobile phase, baseline resolution was obtained after optimization of the mobile phase composition for α– and β–anomers of *p*-nitrophenyl glucopyranoside as shown in Figure 6. Figure 7 shows the separation of *p*-nitrophenyl galactopyranoside anomers. Figure 7a shows that a separation of the α- and β-anomer is not possible running a phosphate buffer.

Change of sodium dihydrogenphosphate with boric acid and slight decrease of the pH allows the baseline separation of both p-nitrophenyl galactopyranoside anomers. Under these conditions *p*-nitrophenyl galactopyranoside anomers show shorter retention times than those of *p*-nitrophenyl glucopyranoside. β-Anomers eluted earlier than α-anomers because β-anomers complexed stronger than α-anomers with borate.[25]

The reason for this is that under these conditions, the borate complex of the β-anomer is more negatively charged than the borate complex of the α-anomer,

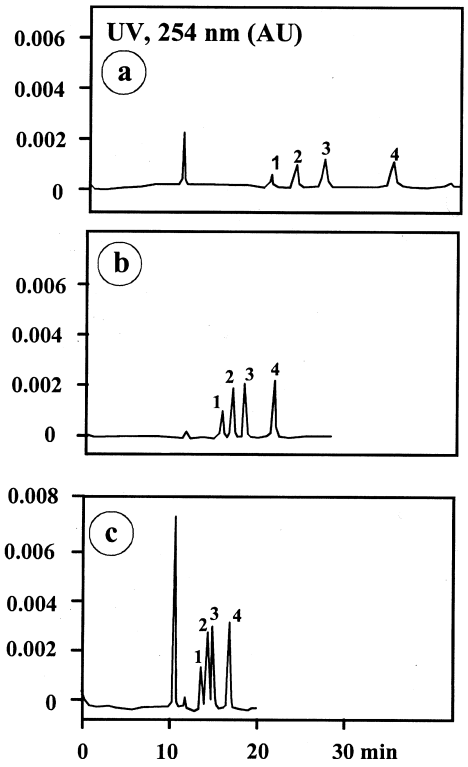

Figure 5. Electrochromatograms of *p*-nitrophyl α-D-glycoside derivatives of monosaccharides. Mobile phases: (a) 42.5 % v/v of 5 mM NaH_2PO_4 (pH 6.0), 42,5% v/v H_2O and 15% v/v acetonitrile; (b) 40% v/v of 5 mM NaH_2PO_4 (pH 6.0), 40% v/v H_2O and 20% v/v acetonitrile; (c) 37.5% v/v of 5 mM NaH_2PO_4 (pH 6.0), 37.5% v/v H_2O and 25% v/v acetonitrile. Solutes: 1, *p*-nitrophenyl α-D-galactopyranoside; 2, *p*-nitrophenyl α-D-glucopyranoside; 3, *p*-nitrophenyl-*N*-acetyl α-D-glucosaminide; 4, *p*-nitrophenyl α-D-manopyranoside. Other conditions as in Fig. 3[22].

and consequently the former is less retained than the latter. In the α-anomer, the glycosidic *p*-nitrophenyl group occupies an axial position and interacts strongly with the hydrogen atoms on C3 and C5, thus destabilizing the borate complex. This is not the case for the β-anomer where the *p*-nitrophenyl substituent occupies an equatorial position, and is free from strong nonbonding interaction. Separation of α- and β-anomers of *p*-nitrophenyl *N*-acetylglucosaminide was not possible under these conditions.

Separation of 1-Phenyl-3-methyl-5-pyrazolone (PMP) Derivatives of Monosaccharides

Derivatization

The procedure for the derivatization of monosaccharides with 1-phenyl-3-methyl-5-pyrazolone (PMP) (Figure 8) was described by Honda *et al.* .[26] Suzuki

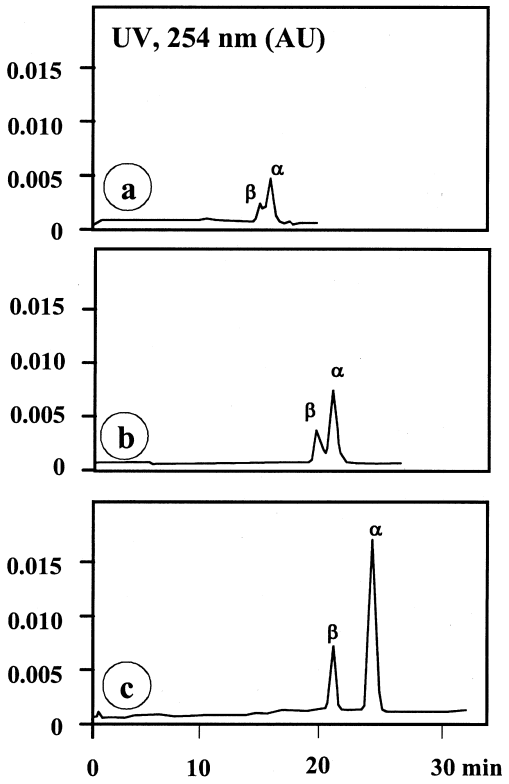

Figure 6. Electrochromatograms of β-anomers and α-anomers of *p*-nitrophenyl glucopyranoside. Mobile phase: (a) 42.5% v/v of 30 mM H_3BO_3 (pH 7.0), 42.5% v/v H_2O and 15% v/v acetonitrile; (b) 42.5% v/v 50 mM H_3BO_3 (pH 7.5), 42.5% v/v H_2O, and 15% v/v acetonitrile; (c) 45% v/v of 50 mM H_3BO_3 (pH 7.5), 45% v/v H_2O and 10% v/v acetonitrile; detection wavelength, 280 nm; other conditions as in Fig. 3.[22] Elution order : β-anomer first and α-anomer second.

et al.[27] dissolved a mixture of monosaccharides (10-50 nmol each) in 50 μL of 0.3 M sodium hydroxide. The solution was then mixed with 50 μL of methanolic 0.5 M PMP. The mixed solution was heated for 30 min at 70 °C, the reaction mixture neutralized with 50 μL of 0.3 M hydrochloric acid and the solution concentrated to dryness. The residue was dissolved in 200 μL of water, and the excess reagent removed three times by extraction with 200 μL of chloroform. The aqueous layer finally obtained was concentrated to dryness, the residue dissolved in 50 μL of acetonitrile-water (1:2 v/v), and the solution subjected to CEC.

Influence of Buffer, pH and Eluent Composition

In a preliminary experiment phosphate buffer and borate buffer were tested as eluent at low concentrations (lower than a few millimoles per liter) by Suzuki

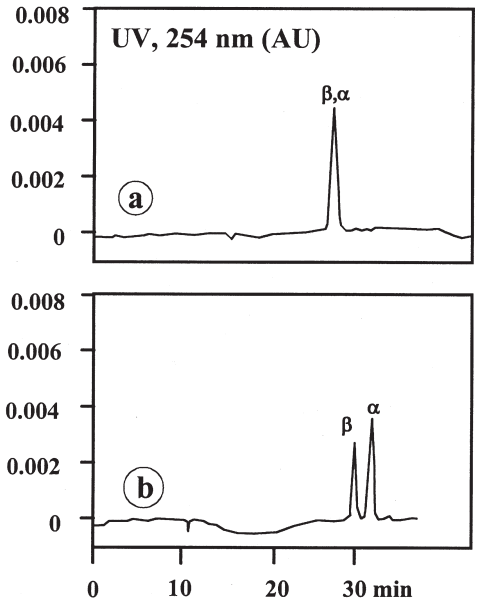

Figure 7. Electrochromatograms of β- and α-anomers of *p*-nitrophenyl galactopyranoside. Mobile phases: (a) 42.5% v/v of 5 mM NaH_2PO_4 (pH 6.0), 42.5% v/v H_2O and 15% acetonitrile; (b) 45% v/v of 50 mM H_3BO_3 (pH 7.5), 45% v/v H_2O and 10% v/v acetonitrile; detection wavelength, 280 nm; other conditions as in Fig. 3. Elution order: β-anomer first and α-anomer second.[22]

et al..[26,27] The results were broad peaks with fluctuating retention times. Higher concentrations induced a noisy baseline and caused damage of the ODS bed. Further investigations showed that the use of HEPES (*N*-(2-hydroxyethyl)-piperazine-2′-(2-ethanesulfonic acid)) buffer resulted in better separations because of its much lower conductivity. Suzuki *et al.*[27] investigated the influence of the pH of HEPES buffer, the concentration of HEPES (mM) in the buffer and the HEPES buffer/CH_3CN volume ratio (Figure 9). PMP derivatives of the common

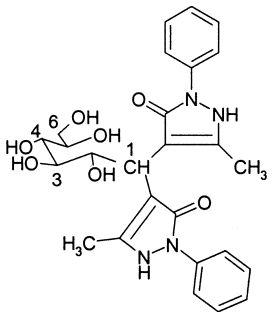

Figure 8. Structure of glucose-PMP.

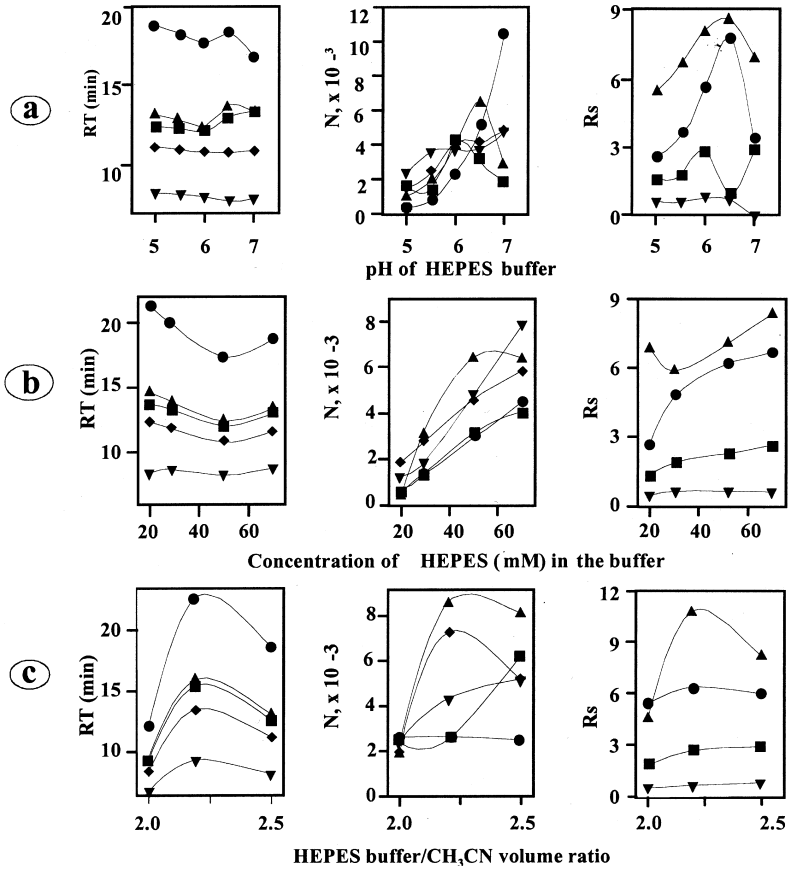

Figure 9. Dependence of the separation of PMP derivatives of monosaccharides found in glycoproteins on: (a) the pH and (b) the HEPES concentration of HEPES buffer in eluent; and (c) the volume ratio of the HEPES buffer to acetonitrile. Capillary, fused silica (100 μm ID, 25 cm) packed with Hypersil ODS (particle size, 3 μm); capillary temperature, 25 °C; applied voltage, 25 kV; detection, absorption at 245 nm; eluent: HEPES buffer-acetonitrile mixtures; RT, retention time; N, theoretical plate number; R_s, resolution. ▼, mannose; ◆,*N*-acetylglucosamine;∝, *N*-acetylgalactosamine; ▲, galactose; ●, fucose; △, mannose/*N*-acetylglucosamine; □,*N*-acetylglucosamine/*N*-acetylgalactosamine; ▽, *N*-acetylgalactosamine/galactose; ○, galactose/fucose.

monosaccharides in glycoproteins (fucose, galactose, mannose, *N*-acetylgalactosamine and *N*-acetylglucosamine) were relatively well separated from each other within 30 min, when the volume ratio of HEPES buffer to acetonitrile ranged from 2.0 to 2.5 in acidic eluents with a pH above 5 (Figure 9c). At pH 7, a few PMP monosaccharides were coeluted (Figure 9a). Complete resolution was achieved again above pH 8 but the separation was not reproducible under these conditions. The reason for this can be attributed to the zwitterionic property of HEPES. Thus, the imino group in the piperazine ring will be protonated more

strongly as the pH decreases, and the resultant ammonium ion will associate with dissociated silanol groups resulting in a loss of local charge. Suzuki and coworkers found that the elution order of PMP monosaccharides was mannose $<$ *N*-acetylglucosamine, *N*-acetylgalactosamine, galactose $<$ fucose. The elution order is identical with that in HPLC using an ODS column and phosphate buffer-acetonitrile mixture as the stationary and mobile phases.[26] The changes in retention time with pH and HEPES concentration of the buffer were relatively small over the ranges examined. A pH dependence was observed, the mechanism including distribution to the stationary phase which caused the decrease of RT with pH increase. A PMP derivative of monosaccharide exists in an equilibrium state between the keto and the enol forms and the enol form becomes predominant as pH increases.[28] When the enol group is dissociated to the less hydrophobic enolate ion, conversion to the enol form by pH increase will reduce hydrophobicity, resulting in lower distribution. Furthermore, Suzuki *et al.*[27] found that the relationship between retention time and HEPES concentration was similar to that of the pH dependence of retention time. This can be explained by a similar mechanism. The increased HEPES concentration facilitated ionization of the solutes to reduce hydrophobicity, and therefore caused low distribution. The relationship between retention time and buffer/acetonitrile volume ratio is conflicting and could not be interpreted clearly. The peaks of the PMP monosaccharides became sharp with increasing HEPES concentrations, but the baseline became noisy as the HEPES concentration exceeded 50 mM. The volume ratio of the HEPES buffer to acetonitrile greatly affected peak broadening, and the resolution was gradually increased with increasing volume ratios. Furthermore, the conditions for the separation of PMP derivative of epimeric aldopentoses (arabinose, lyxose, ribose, and xylose) were also optimized in the same way.

Therefore, increasing pH and buffer/acetonitrile volume ratios generally enhanced the resolution of aldopentoses within the examined ranges. The effect of the pH and the HEPES concentration in the buffer on retention time resembled those for PMP derivatives of glycoprotein monosaccharides, but the effect of buffer/acetonitrile volume ratio was different.

The retention time continued to increase and there were no maximum points in the range of 2.0-2.5. Finally 50 mM HEPES buffer (pH 6.0 $\sim$ 6.3) - acetonitrile (2.2:1 v/v) was chosen for separation as the best eluent. Figure 10 shows the electrochromatograms of the above two kinds of mixtures under optimized conditions. The PMP monosaccharides in each mixture were well separated from each other, although the *N*-acetylgalactosamine/galactose pair (Figure 10a) and the arabinose/xylose pair (Figure 10b) were not resolved to the baseline. Knox and Grant[6] reported that the theoretical plate numbers in CEC were on the order of 10^4 and CEC gave better column efficiency than pressure-driven HPLC by a factor of two. However, the theoretical plate numbers obtained for the PMP monosaccharides were one order of magnitude lower than this reported standard value.

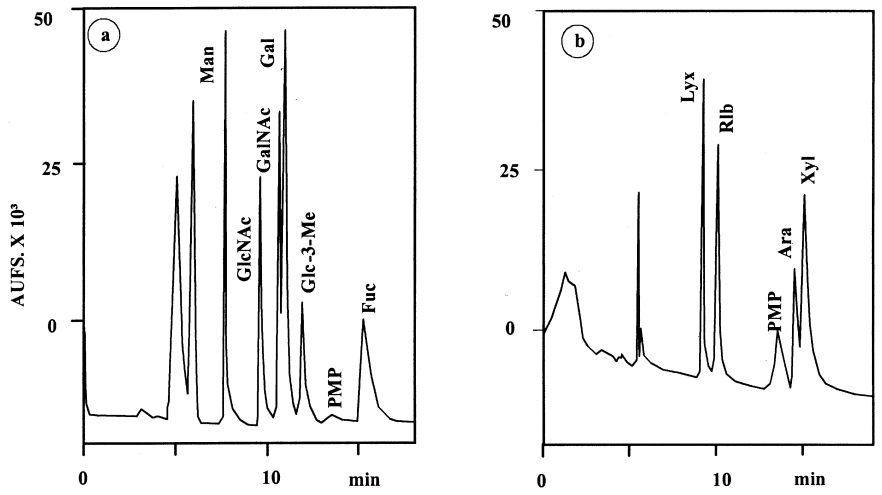

Figure 10. Separation of PMP derivatives of the (a) common monosaccharides in glycoproteins and (b) epimeric aldopentoses by CEC under optimized conditions. Eluent: 50 mM HEPES/sodium hydroxide buffer, (a) pH 6.0 or (b) pH 6.3 - acetonitrile (2.2:1, by volume). Other analytical conditions as in Fig. 9. Man, mannose; GalNAc, *N*-acetylgalactosamine; GlcNAc, *N*-acetylglucosamine; Gal, galactose; Fuc, fucose; Glc-3-Me, 3-O-methylglucose.

CONCLUSIONS

This review has shown the recent advances in CEC analysis of derivatized carbohydrates, thus enlarging that scope of applications of the newly emerging microseparation technique. It is reported that the ODS stationary phase produces relatively high EOF velocities which allows the rapid separation of the derivatized carbohydrates. Reversed-phase CEC offers high selectivity toward the *p*-nitrophenyl derivatives of monosaccharides, thus permitting the separation of monosaccharides differing by only the orientation of an OH-group. By adding small amounts of borate to the hydroorganic eluent, the separation of the α- and β-anomers of the *p*-nitrophenyl derivatives of some monosaccharides was possible. The use of HEPES (*N*-(2-hydroxyethyl)-piperazine-2′-(2-ethanesulfonic acid)) and optimization of the pH and the acetonitrile content allows 1-phenyl-3-methyl-5-pyrazolone (PMP) derivatives of glycoproteins and epimeric monosaccharides to be separated.

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