Improved Mono-, Di-, and Oligosaccharide Separation on CarboPac Columns with Lower Eluting Hydroxide Concentration

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INTRODUCTION

High-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) has been widely used for separation and detection of carbohydrates ranging from simple monosaccharides to branched oligosaccharides, and large linear polysaccharides.[1,2] CarboPac® columns are a family of high-resolution, strong anion-exchange columns developed for carbohydrate separations with HPAE-PAD under alkaline conditions. They have been proven to be capable of separating monosaccharides; positional, linkage, and branch isomers of oligosaccharides; and homopolymer oligosaccharides that differ only in length.[3,4] Sodium hydroxide or potassium hydroxide is commonly used to elute carbohydrates on these columns. Sodium acetate is sometimes added to elute multiply-charged carbohydrate species that strongly bind to these columns. Here we demonstrate that the separation of carbohydrates on the CarboPac PA20 and CarboPac PA200 columns can be improved by adjusting the concentration of the eluting hydroxide solution.

EXPERIMENTAL

Instrumentation

Analyses were performed on a Dionex ICS-3000 chromatography system, which includes an ICS-3000 DP gradient pump, EG Eluent Generator with EluGen® EGC-KOH cartridge and continuously-regenerated anion trap column (CR-ATC), AS Autosampler, and DC Detector/Chromatography compartment with an electrochemical cell. The carbohydrates were detected by pulsed amperometry with a gold electrode and an Ag/AgCl reference electrode using the standard quadruple waveform developed at Dionex.[5]

Chromatography was controlled by Chromeleon® chromatography management software (Dionex Corporation).

Columns

CarboPac PA20 analytical column (3 × 150 mm, Dionex, P/N 060142)
CarboPac PA20 guard column (3 × 30 mm, Dionex, P/N 060144)
CarboPac PA200 analytical column (3 × 250 mm, Dionex, P/N 062896)
CarboPac PA200 guard column (3 × 50 mm, Dionex, P/N 062895)

Reagents and Standards

Mono- and disaccharide standards (Sigma)
Oligosaccharide standards (Dextra labs)
PNGase F (Bio-rad)
Deionized water, Type 1 reagent grade, 18.2 MΩ-cm resistivity
Sodium acetate, HPLC grade (Dionex, P/N 053926)
Sodium hydroxide, 50% (w/w) (Fisher Chemicals, P/N SS254-500)

RESULTS

Separation of Mono- and Disaccharides on the CarboPac PA20 Column

The CarboPac PA20 column was developed to provide fast, efficient separations for mono- and disaccharides. Rapid separation of common monosaccharides is usually achieved by isocratic elution with a 12–20 mM NaOH or KOH solution on this column. Here we demonstrate that the separation of a mix of eight mono- and disaccharides, commonly found in plant hydrolysates, can be improved by using lower hydroxide concentrations than commonly employed (Figure 1). At 30 °C with a flow rate of 0.5 mL/min, optimal separation was achieved by isocratic elution with 3.5 mM KOH produced by the EG. At 25 °C and 0.4 mL/min, the eight sugars can be baseline resolved by isocratic elution with 2 mM KOH (Figure 2). The EG eluent regenerator produces carbonate-free hydroxide solution, and therefore column washing with high concentration hydroxide and reconditioning are not needed. This can reduce the analysis time for each cycle from ~45 min to ~25 min, which greatly improves the throughput.

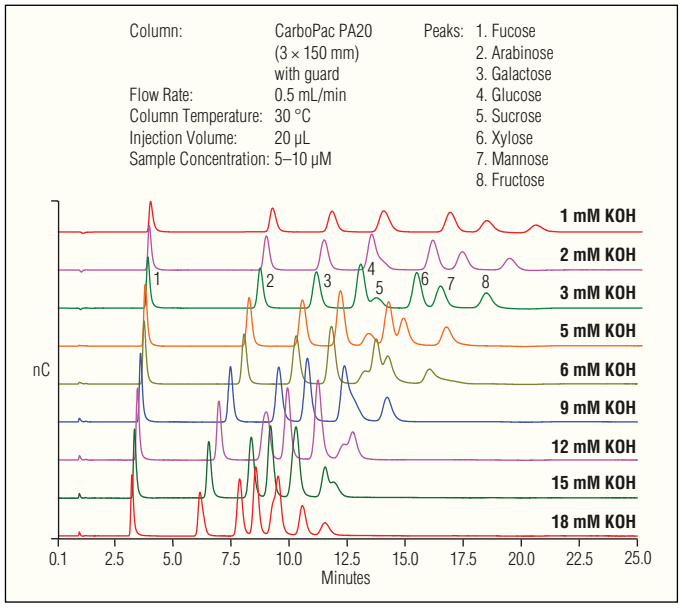

Figure 1. Separation of eight mono- and disaccharides on the CarboPac PA20 column by isocratic elution with different concentrations of KOH.

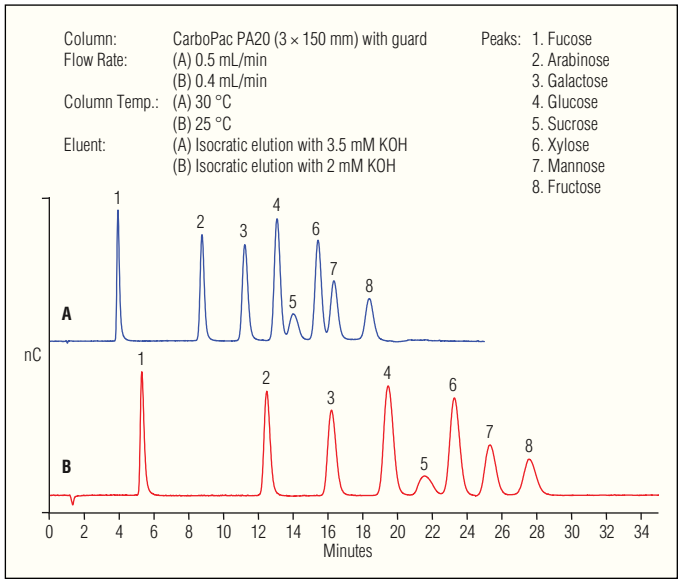

Figure 2. Optimized separation of eight mono- and disaccharides on the CarboPac PA 20 column. Optimizing KOH concentration, column temperature, and flow rate allows baseline separation of all eight saccharides.

Separation of Neutral Oligosaccharides on the CarboPac PA200 Column

The CarboPac PA200 column is designed to provide high-resolution separations for oligosaccharides. In many applications the CarboPac PA200 column produces better resolution than the CarboPac PA100 column.[6] A common gradient used for the PA200 and PA100 columns is 0–200 mM sodium acetate in 100 mM sodium hydroxide. However, this gradient has proven incapable of resolving many neutral oligosaccharides, especially neutral *N*-linked glycans. Improved separation of neutral *N*-glycan oligosaccharides was achieved by decreasing the NaOH concentration. Figure 3 shows the effect of NaOH concentration on the separation of a mixture of seven neutral *N*-glycan oligosaccharides.

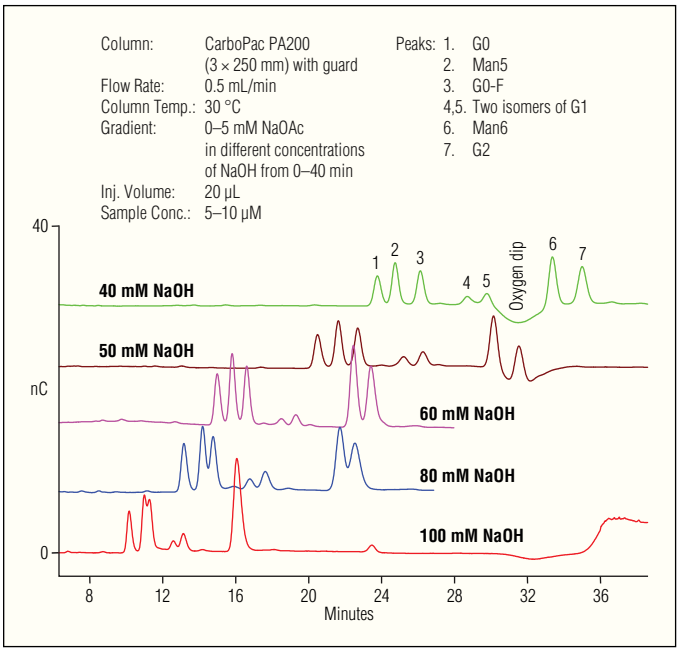

Figure 3. Separation of a mix of neutral oligosaccharides on the CarboPac PA200 column.

Decreasing NaOH concentration resulted in longer retention time and under certain conditions sample peaks eluted in the "oxygen dip" (~32 min, Figure 3). The oxygen dip is due to oxygen dissolved in the sample being introduced into the column upon injection. In certain cases, this can be minimized by using a full-loop injection. By selection of appropriate adjustments to the flow rate and hydroxide concentration, we moved the peaks away from the oxygen dip. Figure 4 shows the separation of the mixed oligosaccharides with the optimized gradient, which is overlaid with individual oligosaccharide standards. The two fucosylated $Man_3GlcNAc_4Gal$ linkage isomers (G1 isomers, Table 1) were well resolved with this gradient.

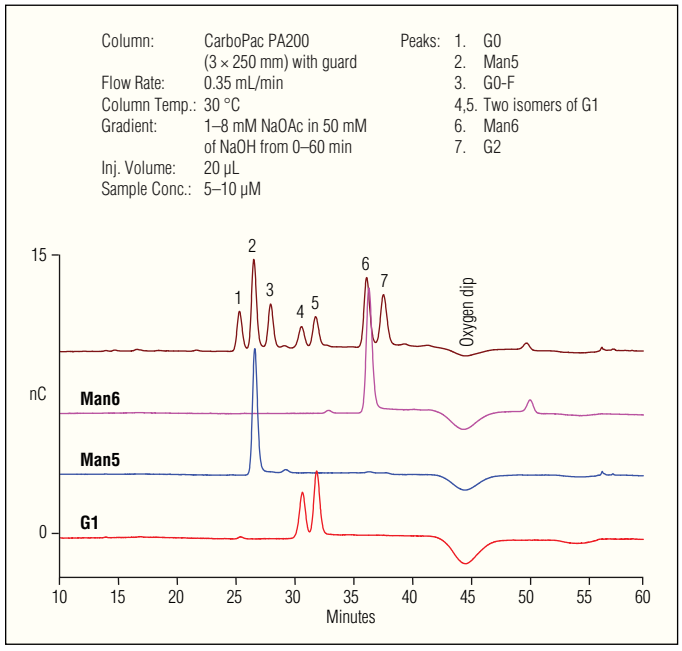

Figure 4. Optimized separation for the mixed neutral oligosaccharides on the CarboPac PA200 column.

Standard	Structure M Mannnose G Galactose GlcN *N*-acetyl-glucosamine F Fucose
Man 5	M α1–6 M α1–6 M α1–3 M α1–4 GlcN β1–4 GlcN M α1–3
Man 6	M α1–6 M α1–6 M α1–3 M α1–4 GlcN β1–4 GlcN M α1–3 M α1–2
G0-F	GlcN β1–2 M α1–6 M β1–4 GlcN β1–4 GlcN GlcN β1–2 M α1–3
G2	G β1–4 GlcN β1–2 M α1–6 F α1–6 M β1–4 GlcN β1–4 GlcN G β1–4 GlcN β1–2 M α1–3
G1	G β1–4 GlcN β1–2 M α1–6 F α1–6 M β1–4 GlcN β1–4 GlcN GlcN β1–2 M α1–3
	GlcN β1–2 M α1–6 F α1–6 M β1–4 GlcN β1–4 GlcN G β1–4 GlcN β1–2 M α1–3
G0	GlcN β1–2 M α1–6 F α1–6 M β1–4 GlcN β1–4 GlcN GlcN β1–2 M α1–3

Table 1. Structures of the oligosaccharide standards.

Profiling of *N*-glycans Cleaved from Two Glycoproteins on CarboPac PA200

N-glycans were cleaved from two glycoproteins with PNGase F. After digestion, proteins were removed by ethanol precipitation. The released *N*-glycans were vacuum dried and then resuspended in deionized water before separation on a CarboPac PA200 column with the optimized gradient. The major species were well resolved and correlated well with the standards (Figure 5).

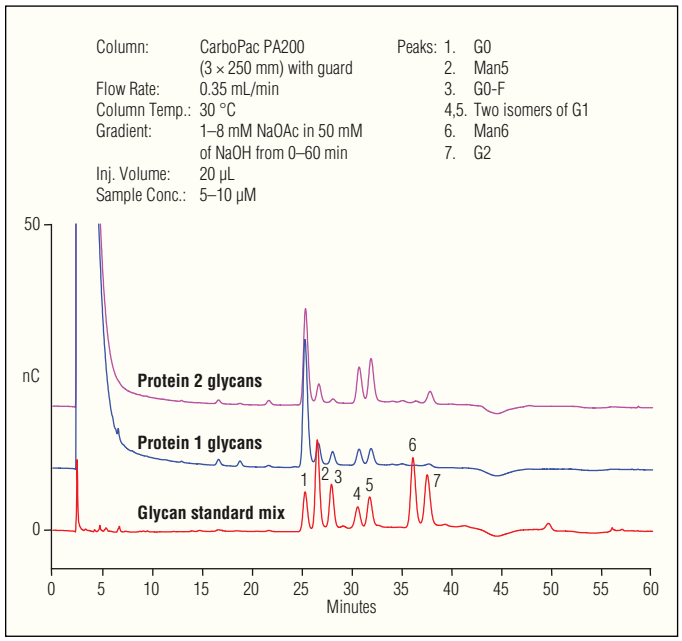

Figure 5. Profiling of the glycans cleaved from two glycoproteins, separated on a CarboPac PA200 column using the optimized gradient.

CONCLUSIONS

- Separation of a mixture of eight mono- and disaccharides on the CarboPac PA20 column was improved by reducing KOH concentration from the commonly used range of 12–20 mM down to 3.5 mM. The mixture was baseline resolved with 2 mM KOH isocratic elution at 25 °C. This method may be useful for biofuel research and food industries that are interested in mono- and disaccharide analysis.
- We developed a method optimized for separation of a mixture of seven neutral *N*-glycan type oligosaccharides on the CarboPac PA200 column. The method achieves near baseline resolution by reducing the NaOH concentration to 50 mM. We also adjusted the flow rate and NaOAc concentration gradient to move the *N*-glycan elution positions away from the oxygen dip. This method is useful in the biotechnology and pharmaceutical industries for analysis of glycans released from glycoproteins during development and quality control of glycoprotein therapeutics.
- Selectivity of carbohydrate retention on CarboPac columns is controlled by adjusting the eluting NaOH concentration. The concentration of the eluting NaOH is an important factor to consider when optimizing carbohydrate separations on these columns.

References

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6. Dionex Corporation. *CarboPac PA200 Column Solutions for Oligosaccharide Analysis Data Sheet.* LPN 1618-03, Sunnyvale, CA, 2006.

Note: All Dionex product manuals, data sheets, and technical notes are available at www.dionex.com.

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