DIONEX

Application Update 150

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Determination of Plant-Derived Neutral Oligo- and Polysaccharides Using the CarboPac™ PA200

INTRODUCTION

High-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) is a well-established technique for determining plant-derived oligo- and polysaccharides. (An oligosaccharide is sometimes defined as a sugar composed of two to eight monosaccharides linked by glycosidic bonds.) The success of this technique is due to the high-resolving power of HPAE and its ability to determine higher degrees of polymerization (DPs) than other techniques. The larger DP polysaccharides are determined by HPAE-PAD because the high-performance anion-exchange column (CarboPac) has the capacity necessary to resolve low concentrations of the large DP polysaccharides in the presence of high concentrations of smaller saccharides and PAD has the sensitivity to detect low concentrations of the larger polysaccharides. The larger polysaccharides also have greater solubility in the typical eluents used for HPAE-PAD—sodium hydroxide and sodium acetate—than the eluents used for other liquid chromatography techniques. The application of HPAE-PAD to the determination of many types of oligo- and polysaccharides in foods and agricultural products was recently reviewed.[1]

In Application Note 67, "Determination of Plant-Derived Neutral Oligo- and Polysaccharides", we showed how to develop HPAE-PAD methods to separate the titled compounds.[2] That application note demonstrated how the CarboPac PA100 achieved slightly higher resolution separations compared to the CarboPac PA1. Since the publication of Application Note 67, Dionex introduced the CarboPac PA200, a column designed for even higher-resolution separations than the PA1 or PA100 columns. The improved resolution is due in part to the PA200's significantly smaller substrate (5.5 µm) and latex (43 nm) beads. These and other changes produce a column that has greater resolution and requires a lower sodium acetate concentration to elute a given oligosaccharide than the PA100. The PA200 is produced in a 3 × 250 mm format that has an optimal flow rate of 0.5 mL/min. Therefore, in addition to requiring a lower sodium acetate concentration to achieve a given separation, the PA200 also requires a lower volume of eluent, reducing the need and expense of preparing eluents, and reducing the generation of aqueous waste.

EQUIPMENT

Dionex BioLC® chromatography system consisting of:

- GP50 Gradient Pump with vacuum degas option
- ED50 Electrochemical Detector
- E01 Eluent Organizer
- AS50 Autosampler with Thermal Compartment
- Chromeleon® Chromatography Management Software

REAGENTS AND STANDARDS

Deionized water, 17.8 MΩ-cm resistivity or better (used for all eluent and standard preparations)

Sodium Hydroxide, 50% (w/w) (Fisher Scientific)

Sodium Acetate, anhydrous (Fluka, Microselect)

Inulin, Chicory Root (Sigma Chemical)

Maltrin® M040 (Grain Processing Corporation)

OTHER CONSUMABLES

Syringe Filters (0.45 µm PVDF) (Acrodisc® LC, Gelman Sciences)

Nylon Filter disks (0.2 µm, 47 mm) (P/N 66602, Gelman Sciences)

Autosampler vials

CONDITIONS

Column: CarboPac PA200 Analytical (3 × 250 mm, P/N 062896)
CarboPac PA200 Guard (3 × 50 mm, P/N 062895)

Tubing for Post Injector Plumbing: 0.005 inch PEEK

Expected Operating Pressure: 18.6 MPa (2700 psi)

Degas: 30 s every 10 min

Injection Volume: 5 µL

Injection Loop: 10 µL

Eluents: (A) 100 mM NaOH
(B) 100 mM NaOH/ 1 M sodium acetate

Flow Rate: 0.5 mL/min

Detection: Pulsed amperometry, Carbohydrate Certified Disposable Gold Working Electrode (P/N 060139 or 060216), Ag/AgCl reference

Waveform (3):

Time(s)	*Potential (V)*	*Integration*
0.00	+0.1	
0.20	+0.1	Begin
0.40	+0.1	End
0.41	–2.0	
0.42	–2.0	
0.43	+0.6	
0.44	–0.1	
0.50	–0.1	

Note: This waveform should be used only with a disposable gold working electrode (all the work in this application update with the CarboPac PA200 used disposable gold working electrodes), a new gold working electrode, or a gold working electrode used previously only with this waveform.

Collection Rate: 2 Hz

Expected Background: 20–37 nC

Temperature: 30 °C

Needle Height: 2 mm

Cut Segment Volume: 0 µL

Flush Volume: 200 µL

Example* Gradient:

Eluent A: 100 mM sodium hydroxide

Eluent B: 100 mM sodium hydroxide/ 1.0 M sodium acetate

Flow Rate: 0.5 mL/min

Separation of Maltodextrins in Beer

Time (min)	*%A*	*%B*	*Curve*
0.0	93	7	5
30.0	70	30	4
31.0	93	7	5
40.0	93	7	5

*Other gradients in this application note are shown in the figures or described in the text.

PREPARATION OF SOLUTIONS AND REAGENTS

Sample Preparation

Maltrin M040

Weigh out 25 mg of M040 and add water to prepare a 5.0-mg/mL solution.

Inulin

Weigh out 25 mg of inulin and add 100 mM sodium hydroxide, prepared in the same manner as the eluent, to prepare a 5.0-mg/mL solution.

General Advice On Preparing Samples Containing Oligo- And Polysaccharides for HPAE-PAD

Samples can be dissolved in water or 100 mM sodium hydroxide (reconstitute just prior to analysis). Higher concentrations of sodium hydroxide may be used, but the effect on the chromatography for a given sample, sample concentration, and injection volume must be evaluated. Cloudy samples should be filtered using a 0.45 µm syringe filter. If samples that are cloudy—even after filtration—are injected, they will damage the guard column and clog the injector, injection valve, injection loop, or tubing leading to the guard column. For additional advice on sample considerations for CarboPac columns, see the CarboPac column manual (Document Number 031824-02).

Eluent Preparation

100 mM Sodium Hydroxide

It is essential to use high-quality water of high resistivity (17.8 MΩ-cm or better) that contains as little dissolved carbon dioxide as possible. Biological contamination should be absent. Sodium hydroxide eluent should be prepared with 50% (w/w) sodium hydroxide. Sodium hydroxide pellets are covered with a thin layer of sodium carbonate and should not be used under any circumstances. To prepare 2 L of 100 mM NaOH, use a 10-mL graduated plastic pipet to deliver 10.4 mL of 50% (w/w) sodium hydroxide into 1 L of water and then add water to reach 2 L. Stir this solution only one to two minutes. Excessive mixing will increase the carbonate ion in the solution by trapping carbon dioxide from the air. After preparation, keep the eluent blanketed under helium at 34–55 kPa (5–8 psi) at all times. If maintained under helium, this eluent is viable for approximately one week.

100 mM Sodium Hydroxide/1.0 M Sodium Acetate

Measure approximately 800 mL of water into a 1-L graduated cylinder. Add a stir bar and begin stirring. Weigh out 82.0 g of anhydrous crystalline sodium acetate. Steadily add the sodium acetate to the briskly stirring water. After the salt dissolves, remove the stir bar with a magnetic retriever and use a 10-mL graduated plastic pipet to add 5.2 mL of 50% (w/w) sodium hydroxide to the sodium acetate solution. Add water to the solution to reach a final volume of 1 L, replace the stir bar, and stir briefly to mix. Vacuum filter this solution through a 0.2 µm nylon filter. This vacuuming may be slow as the filter may clog with insoluble impurities from the sodium acetate. After preparation, keep the eluent blanketed under helium at 34–55 kPa (5–8 psi) at all times. If maintained under helium, this eluent is viable for approximately one week.

RESULTS AND DISCUSSION

Figure 1 compares the separation of chicory inulin, a polyfructan, separated on a CarboPac PA100 column to the same sample separated on the CarboPac PA200 column. While these separations were not performed under exactly the same conditions, they reveal the major differences between the two columns. Though the starting sodium acetate concentration in the PA200 separation was 30 mM higher than the PA100 separation, the final concentration was the same (Note: A difference in the gradient delivery for the PA100 separation will slightly delay the delivery of the high sodium acetate concentration compared with the linear gradient used for the PA200 column). Under these conditions, the final sample peaks elute from the PA200 column ten to twenty minutes before the same peaks elute from the PA100 column. This difference reflects the lower capacity of the PA200 compared to the PA100 (35 meq vs 90 meq) and demonstrates that less acetate is required for eluting an oligosaccharide from the PA200 compared to the PA100. If oligo- or polysaccharides must be purified for subsequent experiments, the presence of less acetate and the lower flow rate of the

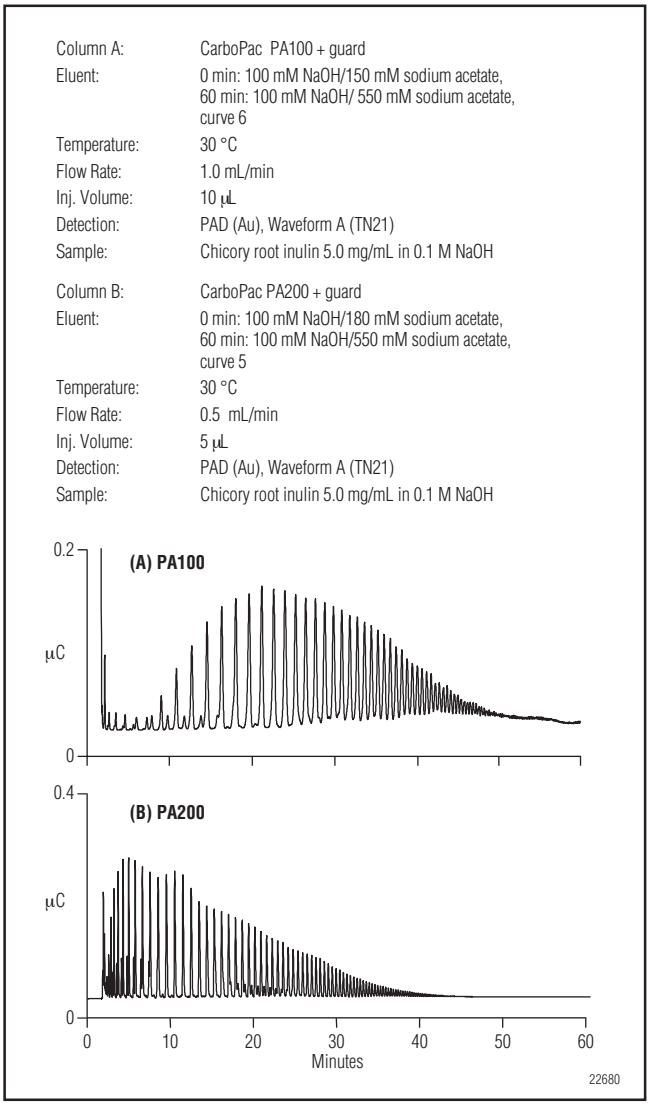

Figure 1. Comparison of the CarboPac PA100 and PA200 columns for the separation of chicory inulin.

separation improves sodium removal by the Carbohydrate Membrane Desalter (CMD™), and possibly increases the number of large polysaccharides that can be successfully treated with the CMD after a HPAE-PAD separation.[4] The narrower diameter of the PA200 compared to the PA100 column (3 mm vs 4 mm) requires only half the sample used for the PA100 separation to achieve the PA200 separation. Despite using half the sample, the peak heights are larger due to the higher efficiency of oligosaccharide separations with the PA200 column. The higher efficiency due in part to the smaller substrate and latex beads, yields higher resolution and that is especially evident for a series of polysaccharide peaks between approximately 17 and 23 min where a small peak elutes just after the main peak. The lower capacity and higher efficiency of the PA200 column should allow optimization of the separation in Panel B so that it is significantly shorter than the PA100 separation.

Figure 2 compares the separation of Maltrin M040, a maltodextrin sample, separated on a CarboPac PA100 column to the same sample separated on the CarboPac PA200 column. Maltodextrins are α 1,4 –linked polyglucans. The same small difference in the gradient delivery applies as in Figure 1. We observe, as we did with the chicory inulin sample, higher peak efficiencies and earlier elution of the larger DP polysaccharides (i.e. these polysaccharides are eluting at lower sodium acetate concentrations) compared to the PA100 column. The higher peak efficiencies result in the identification of four additional higher DP polysaccharides.

Figure 3 shows a quick attempt to optimize the separation of M040 to take advantage of the higher resolution and lower capacity of the PA200 column. The separation in Figure 3 required 29 min less time from injection to injection and identified four additional peaks compared to the separation in Figure 2, Panel B. The same method was applied to the analysis of maltodextrins in beer. Figure 4 compares the separation on the PA100 column (Figure 2 in Application Note 67) to that using the PA200 column with the shorter separation time. Once again we observe higher efficiency peaks and some resolution of smaller peaks near the baseline that are not observed using the PA100 column. The resolution of these minor peaks may facilitate their identification.

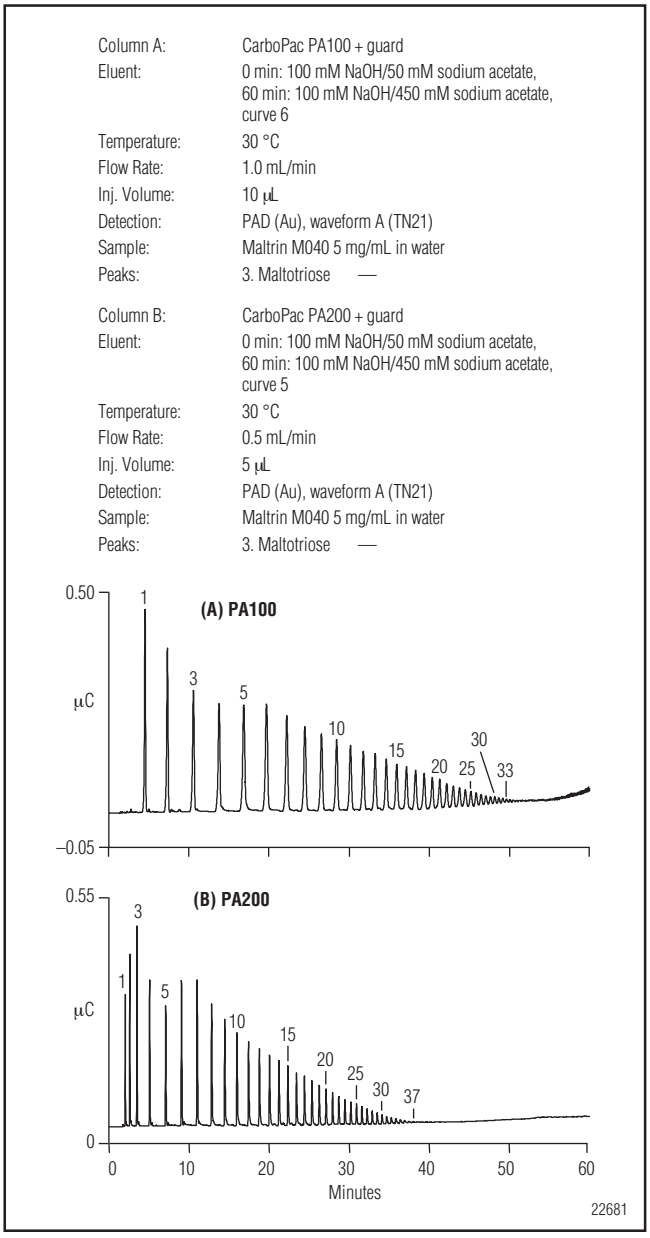

Figure 2. Comparison of the CarboPac PA100 and PA200 columns for the separation of maltodextrins.

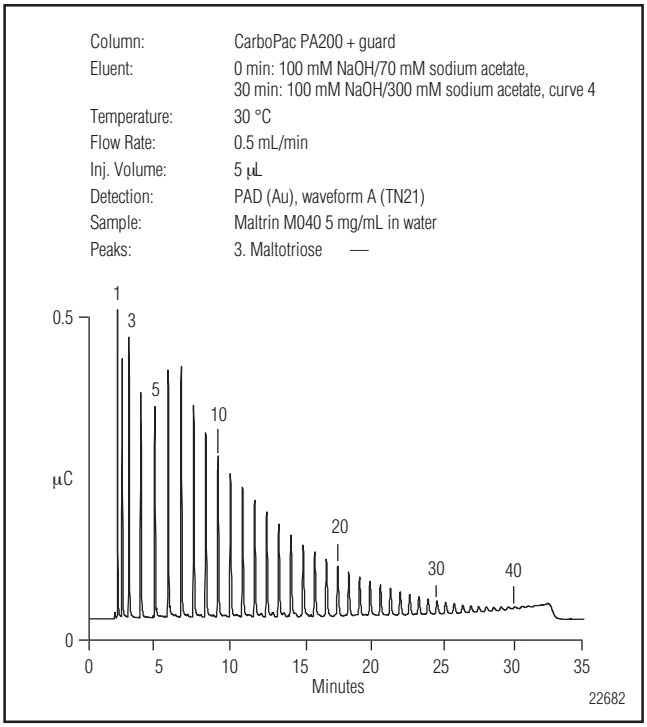

Figure 3. Faster separation of maltodextrins with the CarboPac PA200 column.

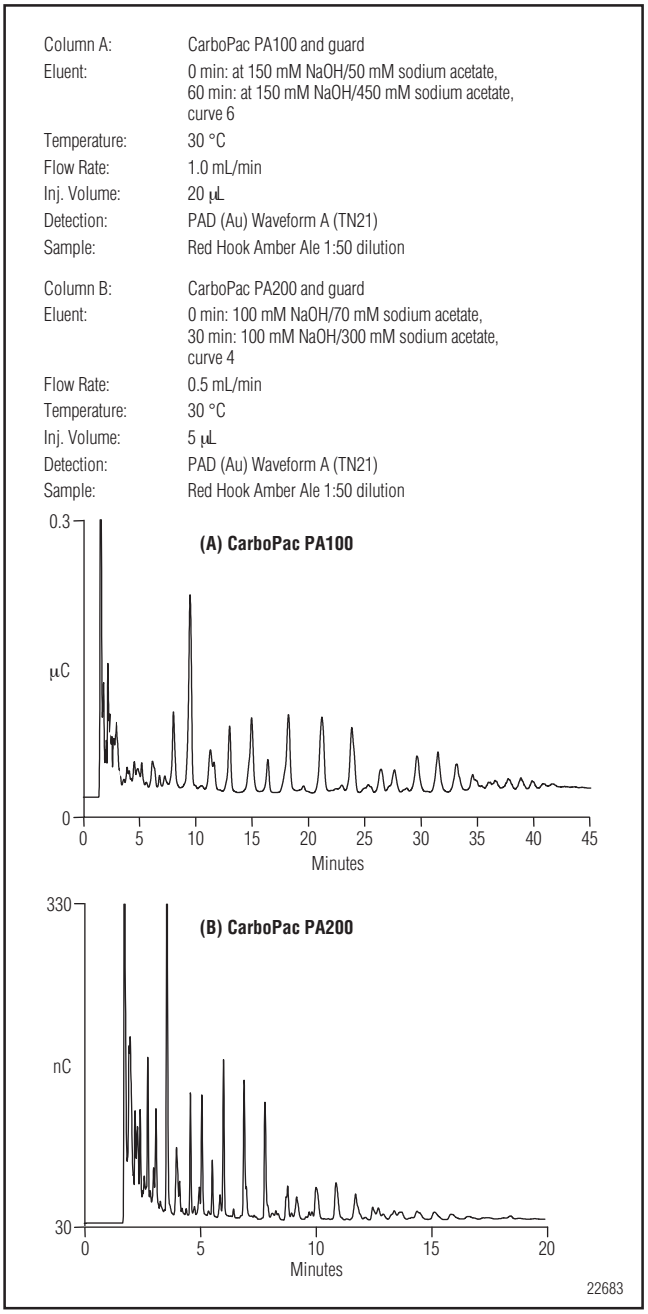

Figure 4. Comparison of the CarboPac PA100 and PA200 columns for the separation of maltodextrins in beer.

CONCLUSION

The CarboPac PA200 yields higher-resolution oligosaccharide and polysaccharide separations than existing CarboPac columns used for HPAE-PAD. Less sample, less eluent, less time, and lower sodium acetate concentrations are required to achieve these higher-resolution separations.

PRECAUTIONS AND RECOMMENDATIONS

For best results, the Ag/AgCl reference electrode should be replaced every three to six months.

REFERENCES

1. Rohrer, J. S. "High-Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD) for the Determination of Oligosaccharides in Food and Agricultural Products". *In ACS Symposium Series 849: Oligosaccharides in Food and Agriculture;* Chap. 2; Eggleston, G. and Côté, G. L., Eds.; Oxford University Press, 2003; pp 16–31.
2. Dionex Corporation. Application Note 67; LPN 1474; Sunnyvale, CA.
3. Dionex Corporation. Technical Note 21; LPN 0032025-02; Sunnyvale, CA.
4. Thayer, J.; Rohrer, J. S.; Avdalovic, N.; Gearing, R. P. *Anal. Biochem.* **1998**, *256*, 207–216.

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