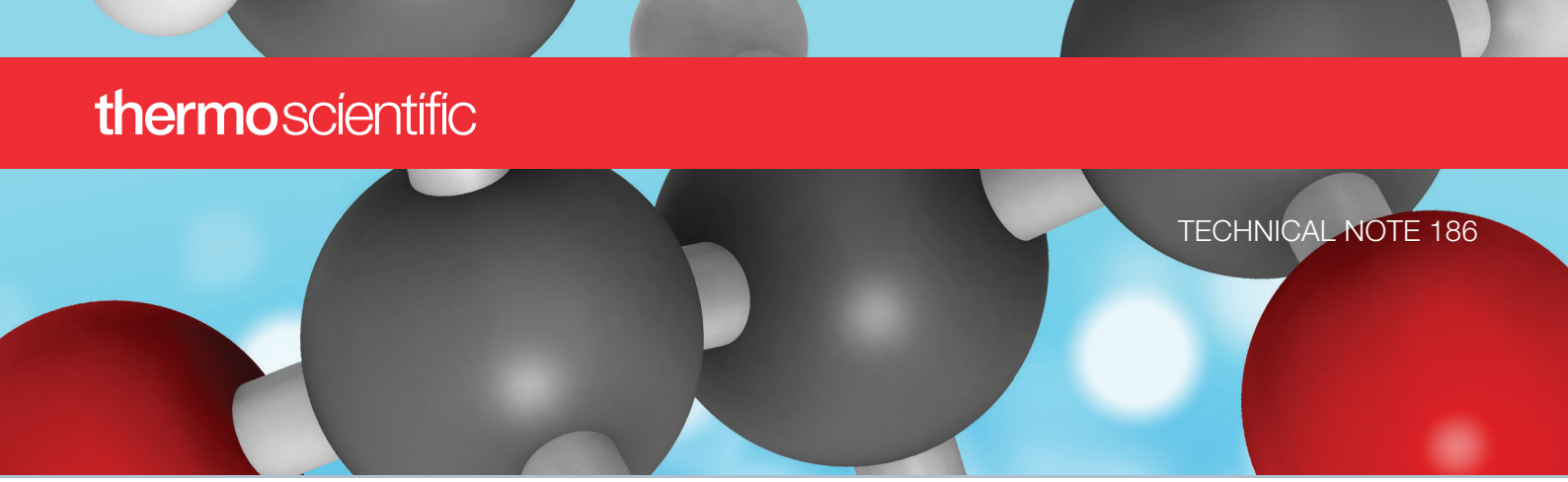

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TECHNICAL NOTE 186

The Effect of Working Electrode Gasket Thickness on the Sensitivity and Linearity of Carbohydrate Response by Pulsed Amperometric Detection

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Keywords
Thermo Scientific Dionex CarboPac PA20 column, Thermo Scientific Dionex CarboPac SA10 column, Glucosamine, Fucose, Glucose, Fructose, Sucrose, Xylose

Goal

To demonstrate the effect of gasket thickness on the sensitivity and linearity of carbohydrate determination using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD).

Introduction

Carbohydrates play vital roles in a variety of biological functions, including cellular communication, gene expression, immunology, organism defense mechanisms, and growth and development. Many samples, ranging from food[1], biological products,[2] biofuel feedstocks,[3] and fermentation media[4], are analyzed for carbohydrate content. They are difficult to analyze using common chromatography methods as they are very polar compounds, exhibit similar structural characteristics, and lack a suitable chromophore.

High-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) is widely used for determination of carbohydrates, including simple monosaccharides, oligosaccharides, sugar nucleotides, sugar alcohols, sugar phosphates, and sugar acids, including sialic acids. HPAE takes advantage of the weakly acidic nature of carbohydrates to give highly selective separations at high pH using a strong anion-exchange stationary phase. Coupled with PAD, it permits direct quantification of native carbohydrates. Carbohydrates are detected by measuring the electrical current generated by their oxidation at the surface of a gold electrode.

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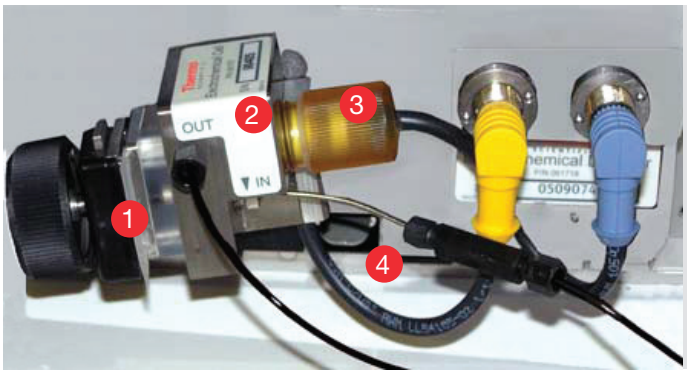
Each complete electrochemical detector (ED) assembly consists of an amperometric detection cell and the detector electronics required to collect data and send it to the computer. The ED cell is a miniature flow-through amperometric detection cell that includes three different electrodes: a titanium cell body (the counter electrode), a working electrode, and either a combination pH-Ag/AgCl reference electrode or a PdH reference electrode (Figure 1).

A 2 mil gasket is recommended for use with disposable working electrodes. Traditionally, a 3 mil gasket has been used for European Pharmacopeia aminoglycoside applications.[5] Thicker gaskets are recommended for high-concentration carbohydrate applications.[6] Increasing the gasket thickness decreases the linear flow rate at the electrode, which extends the linear calibration range to higher concentrations while reducing sensitivity.

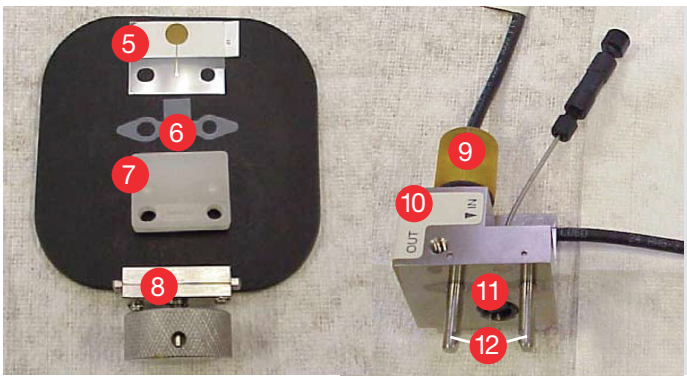


1. Working Electrode
2. Cell Body (counter electrode)
3. pH-Ag/AgCl Reference Electrode
4. Titanium Inlet Tubing

Figure 1. ED cell with pH-Ag/AgCl reference electrode.

Such cells include an amperometric working electrode exposed to a sample flow-through channel, typically enclosed by a plastic gasket held in place by compression (Figure 2). The gasket is typically flexible and is available in different thicknesses. In general, a 1 mil (1 mil = 0.001 inches or 25.4 µm) gasket is recommended for conventional working electrodes and for capillary flow rates.

Here, the sensitivity and linearity of six carbohydrates have been evaluated using 2, 15, and 62 mil gaskets under three chromatographic conditions. Typical conditions were used for the Thermo Scientific™ Dionex™ CarboPac™ PA20 column, and the Thermo Scientific™ Dionex™ CarboPac™ SA10 column with and without postcolumn addition of sodium hydroxide.



5. Disposable Electrode
6. 2 mil PTFE Gasket (with tab)
7. Spacer Block
8. Yoke-Knob Assembly
9. Reference Electrode
10. Cell Body (counter electrode)
11. Working Electrode Contact Pin (Pogo)
12. Alignment Posts

Figure 2. All components of an ED cell.

The six carbohydrates were fucose (deoxyhexose monosaccharide), fructose (hexose monosaccharide – ketose), glucose (hexose monosaccharide – aldose), sucrose (non-reducing disaccharide), glucosamine (aminosugar monosaccharide), and xylose (pentose). Generally, increased gasket thickness reduces response, while increasing the linearity range. The effect of gasket thickness on sensitivity and linearity of carbohydrates was investigated in this technical note.

Equipment

- Thermo Scientific™ Dionex™ ICS-5000⁺ HPIC system, including:
  - Dionex ICS-5000⁺ DP Pump module with the vacuum degas option (P/N 063353) installed
  - Dionex ICS-5000⁺ EG Eluent Generator module with high-pressure degasser module
  - Dionex ICS-5000⁺ DC Detector/Chromatography module
  - 4-port Valve Rebuild Kit (P/N 074699), which includes a 0.4 µL injection loop
- Thermo Scientific™ Dionex™ AS-AP Autosampler with Sample Syringe, 250 µL (P/N 074306) and Buffer line, 1.2 mL (P/N 074989)
- Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator Cartridge (P/N 075778)
- Thermo Scientific™ Dionex™ CR-ATC 500 Continuously Regenerated Anion Trap Column (P/N 075550)
- Thermo Scientific™ Dionex™ ED Electrochemical Detector (P/N 072042)
- Dionex™ ICS-5000⁺ Electrochemical Cell (P/N 072044)
- pH, Ag/AgCl Reference Electrode (P/N 061879)
- Gold on PTFE Disposable Electrode Including four 2 mil gaskets (P/N 066480)
- Gasket, (HDPE) for Disposable Electrodes 0.015" (P/N 057364)
- High Concentration Carbohydrate Analysis Kit (includes 62 mil PTFE gasket and modified spacer block, P/N 085324)
- 10 µL PEEK Sample Loop (P/N 042949)
- Vial Kit, 10 mL Polystyrene with Caps and Blue Septa (P/N 074228)
- Thermo Scientific™ Nalgene™ Syringe Filters, PES, 0.2 µm (Fisher Scientific P/N 09-740-61A)
- AirTite All-Plastic Norm-Ject™ Syringes, 5 mL, Sterile (Fisher Scientific P/N 14-817-28)
- Thermo Scientific Nalgene 1000 mL, 0.2 µm Nylon Filter Units (P/N 09-740-46)
- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2

Reagents and Standards

Reagents

Deionized (DI) water, Type I reagent grade, 18 MΩ-cm resistivity or better filtered through a 0.2 µm filter immediately before use.

Standards

- D(+)-Fucose, 99%, ACROS Organics™ (Fisher Scientific P/N AC225630050)
- Alfa Aesar™ D-Glucosamine hydrochloride, 98+% (Fisher Scientific P/N AAA1553218)
- D-Glucose, Fisher Chemical (Fisher Scientific, P/N D16-1)
- Sucrose, Fisher Chemical (Fisher Scientific P/N S5-500)
- D-Fructose, Fisher Chemical (Fisher Scientific P/N L96-500)
- L(-)-Xylose, 99+%, ACROS Organics™ (Fisher Scientific P/N AC225990050)

Conditions: Method 1	
Columns:	Dionex CarboPac PA20 guard column (3 × 30 mm, P/N 060144) Dionex CarboPac PA20 analytical column (3 × 150 mm, P/N 060142)
Eluent Source:	Dionex EGC 500 KOH Eluent Generator Cartridge with CR-ATC 500
Eluent:	Potassium hydroxide
Isocratic:	100 mM KOH from -15 min to -10.05 min only for column wash, 10 mM KOH from -10.00 min to 0 min for equilibrium, 10 mM KOH from 0 min to 15 min
Flow Rate:	0.5 mL/min
Injection Volume:	10 µL (full loop)
Temperature:	30 °C (column and detector compartments)
Backpressure:	~3110 psi
Detection:	Pulsed amperometric
Background:	~40 nC
Working Electrode:	Disposable Au on PTFE electrode
Electrochemical Cell Gasket:	2, 15, or 62 mil
Reference Electrode:	pH, Ag/AgCl, Ag mode
Noise:	30–60 pC

Carbohydrate 4-Potential Waveform for the ED (used for all methods described here)				
Time (s)	Potential (V)	Gain Region	Ramp	Integration
0.00	+0.1	Off	On	Off
0.20	+0.1	On	On	On
0.40	+0.1	Off	On	Off
0.41	–2.0	Off	On	Off
0.42	–2.0	Off	On	Off
0.43	+0.6	Off	On	Off
0.44	–0.1	Off	On	Off
0.50	–0.1	Off	On	Off

Conditions: Methods 2 and 3	
Columns:	Dionex CarboPac SA10 guard column (4 × 50 mm, P/N 074902) Dionex CarboPac SA10 analytical column (4 × 250 mm, P/N 074641)
Eluent Source:	Dionex EGC 500 KOH Eluent Generator Cartridge with CR-ATC 600
Eluent:	1 mM KOH
Flow Rate:	1.5 mL/min
Injection Volume:	0.4 µL (full loop)
Postcolumn Addition:	200 mM NaOH (Method 3 only)
Postcolumn Flow Rate:	0.4 mL/min (Method 3 only)
Column Temp:	45 °C
Compartment Temp:	30 °C
Backpressure:	~1985 psi
Detection:	Pulsed amperometric
Background:	~40 nC
Working Electrode:	Disposable Au on PTFE electrode
Electrochemical Cell Gasket:	2, 15, 62 mil
Reference Electrode:	pH, Ag/AgCl, Ag mode
Noise:	30–60 pC (Method 2) 200–1000 pC (Method 3)

Preparation of Solutions and Reagents

Eluent Solutions

Generate the potassium hydroxide (KOH) eluent on line by pumping high-quality degassed DI water through the Dionex EGC 500 KOH cartridge. The Chromeleon CDS software tracks the amount of KOH used and calculates the remaining lifetime of the cartridge.

The authors strongly recommend eluents prepared by an eluent generator for concentrations of hydroxide <5 mM, however, eluents can be prepared manually, if needed. If eluents must be prepared manually, use a 50% (w/w) NaOH solution in place of KOH and prepare according to the general instructions for hydroxide eluents in Thermo Scientific Technical Note 71.[7] To prepare the solution for postcolumn addition in Method 3, add 10.4 mL of 50% w/w NaOH to 989.6 mL of degassed DI water to prepare 1 L of 200 mM NaOH. All data in this technical note were collected with eluent-generator-produced eluents.

Stock Standard Solutions

Dissolve solid standards in DI water to prepare a 2 g/L stock solution for fucose, glucosamine, glucose, xylose, and sucrose, as well as a 4 g/L stock solution for fructose. Maintain the stock solution at -20 °C until needed.

Working Standard Solutions

Prepare the mixed carbohydrate working standards by diluting the stock solutions as required. Store working standards at 4 °C. All dilutions are made gravimetrically to ensure high accuracy. The concentrations of each carbohydrate used for Method 1 were in the range of 0.02–200 mg/L. For Methods 2 and 3 the ranges were 2.5–2000 mg/L except for fructose which was 5–4000 mg/L.

Postcolumn Application

Applications requiring the postcolumn addition of NaOH solution require installation of a knitted reaction coil reactor after the column but before the detector. Install a PEEK mixing tee (P/N 048227) after the column and use the second pump of the DP to deliver the postcolumn solution to the tee. Direct the third port on the tee to the reaction coil, followed by the electrochemical detector cell. Increased pump noise (and therefore detector noise) is expected when delivering postcolumn reagents in this manner.

Precautions

System preparation

When using the Dionex ICS-5000$^+$ EG Eluent Generator module for electrochemical applications, install the vacuum degas conversion kit (P/N 063353). This degasser will remove gases generated by the EG and help maintain a stable baseline. This kit is not necessary when preparing eluents manually. Keep the eluent water blanketed under 34–55 kPa (5–8 psi) of nitrogen at all times to reduce carbonate contamination and opportunistic microorganisms.

ED Cell, Reference Electrode and Gasket

While running the ED cell, bubbles may be trapped in the flow through the cell. Air bubbles in the cell can cause pulsations of the baseline, random noise, and low readings. To prevent air from becoming trapped in the cell, increase the backpressure on the cell by connecting backpressure tubing to the cell outlet. The backpressure limit for the ED cell is 690 kPa (100 psi). Do not exceed this limit. One to three feet of black (0.01” i.d.) PEEK tubing at the cell outlet will prevent bubble formation and not exceed the backpressure limit.

Keep a spare, unused pH-Ag/AgCl reference electrode at all times. For best results, replace the reference electrode after six months of use. As a result of exposure to alkaline solutions—such as flowing sodium hydroxide—the 3 M KCl electrolyte inside the reference electrode gradually becomes alkaline and the silver chloride layer on the Ag wire in the electrode dissolves or converts to a mixture of silver oxide and silver hydroxide. When this happens, the reference potential shifts and becomes increasingly unstable.[8]

Reference potential shifting can lead to unusually high background response from the working electrode, reduced signal response, or a combination of both effects. If the reference electrode fails while the electrochemical detection cell is on, replace the disposable working electrode as well as the reference electrode. A large shift in reference potential can damage the disposable working electrode.

Replace the gasket if there is a leak between the gasket and electrode, or between the gasket and cell body.

Other Considerations

Carbohydrates have limited stability unless sterility is maintained. Store solutions and samples at -20 °C or colder. Avoid multiple freeze/thaw cycles to preserve the carbohydrates. Ensure that all carbohydrate solutions are well mixed after thawing stock standards prior to preparing working standards and spiking solutions.

Results and Discussion

Six carbohydrates were chosen to illustrate the effect of working electrode gasket thickness on detection sensitivity and the relationship of concentration to detector response using HPAE-PAD. The six were: fucose, a hexose deoxy sugar; glucosamine, an amino sugar; glucose, a monosaccharide, an aldohexose, and a reducing sugar; fructose, a reducing ketose; sucrose, a non-reducing disaccharide composed of a D-glucose and a D-fructose bonded with a glycosidic linkage; xylose, a monosaccharide of the aldopentose type. For PAD, carbohydrates are detected by integrating the electrical current generated by their oxidation at the surface of a gold electrode over a set period of time. A carbohydrate's electrochemical response is associated with its structure.[9] Carbohydrates that undergo mass transport-controlled oxidations at the electrode surface produce linear calibration plots over larger concentration ranges than oxidations under surface control where electron transfer kinetics at the electrode surface limit the magnitude of the oxidation. Glucose is primarily under mass transport control; however, sucrose is primarily under surface control. Fructose is under mixed control.

Three working electrode gasket thicknesses (2 mil, 15 mil, and 62 mil) were evaluated for their effect on detection sensitivity using Method 1. The same gaskets were used to evaluate high carbohydrate concentrations using Methods 2 and 3.

Separation Using Method 1 on the Dionex CarboPac PA20 Column

The six selected carbohydrates do not resolve on the Dionex CarboPac PA20 column using Method 1 as there is coelution of sucrose and xylose. The Dionex CarboPac PA20 column was designed to give good resolution for carbohydrates common to mammalian glycoproteins and therefore is not appropriate for this set of carbohydrates. Method 1 represents conditions typically used with the Dionex CarboPac PA20 column, thus in our study we divided the six carbohydrates into two sets of three so that we could evaluate the effect of working electrode gasket thickness on each class of carbohydrates under typical conditions.

Figure 3, shows the separation of fucose, glucosamine, glucose, xylose, sucrose, and fructose on a Dionex CarboPac PA20 column with a 2 mil working electrode gasket. The total separation time is 15 min with an additional 5 min wash and 10 min equilibration prior to sample injection.

Column:	Dionex CarboPac PA20, Guard, 3 × 30 mm Dionex CarboPac PA20, Analytical, 3 × 150 mm
Eluent:	100 mM KOH -15 to -10.05 min, 10 mM -10-0 min, 10 mM 0–15 min
Eluent Source:	Dionex EGC 500 KOH cartridge with CR-ATC 500
Temperature:	30 °C
Flow Rate:	0.5 mL/min
Inj. Volume:	10 µL
Detection:	PAD, Au on PTFE (Disposable), 2 mil gasket
Peaks:	1. Fucose, 2. Glucosamine, 3. Glucose, 4. Xylose, 5. Sucrose, 6. Fructose

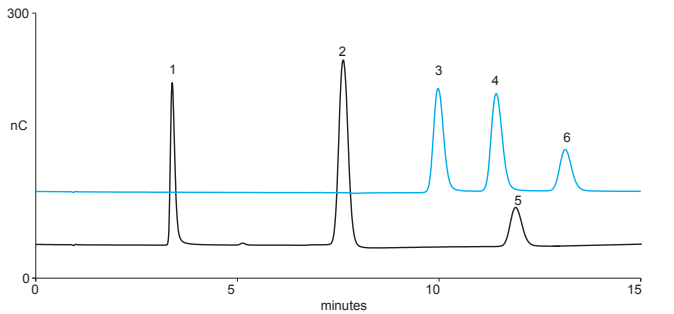


Figure 3. Separation of fucose, glucosamine, glucose, xylose, sucrose, and fructose, each at 10 mg/L, on the Dionex CarboPac PA20 column using Method 1.

Sensitivity of Method 1

Method sensitivity was evaluated by determining limits of detection (LOD). Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably detected. A 3:1 signal-

to-noise ratio is generally considered acceptable for estimating the limit of detection (LOD), and a signal-to-noise ratio 10:1 for limit of quantification (LOQ). The LOD and LOQ were then calculated from the average peak height of five injections of 0.02–0.2 mg/L each of the standards depending on gasket thickness. The results of LOD and LOQ are summarized in Table 1. The results demonstrated that the thinner gasket provides more sensitivity, which is defined as the LOD in this document, under Method 1 conditions and that sensitivity is reduced as the gasket thickness increases. The effect of gasket thickness on sensitivity varied with individual carbohydrates. For instance, the sensitivity of glucose and xylose on the 2 mil gasket is similar, meanwhile the sensitivity of glucose is less than that of xylose on the 15 mil gasket and the opposite on the 62 mil gasket.

Table 1. LOD and LOQ using 2, 15, and 62 mil working electrode

Analyte	2 mil		15 mil		62 mil	
	LOD[a] (mg/L)	LOQ[b]	LOD[a]	LOQ[b]	LOD[a]	LOQ[b]
Fucose	0.007	0.024	0.009	0.031	0.039	0.130
Glucosamine	0.008	0.026	0.016	0.053	0.062	0.205
Glucose	0.013	0.043	0.027	0.090	0.047	0.156
Xylose	0.013	0.042	0.019	0.062	0.084	0.280
Sucrose	0.054	0.180	0.094	0.312	0.233	0.778
Fructose	0.031	0.105	0.032	0.105	0.134	0.448

[a]LOD=3×S/N
[b]LOQ=10×S/N

Response Linearity for Carbohydrates Using Method 1

Linearity of electrochemical response for six sugars was determined by injecting calibration standards in triplicate, ranging from around LOQ concentration to 100 mg/L on 2 and 15 mil gaskets, and from around LOQ concentration to 200 mg/L on the 62 mil gasket. Concentrations above 200 mg/L are not recommended because they overload the Dionex CarboPac PA20 column at the chosen injection volume. The linear ranges of fucose, glucosamine, glucose, xylose, sucrose, and fructose are shown with the coefficients of determination in Table 2. The linearity, as measured by the coefficient of determination (r^2), is generally >0.99. With the thinner gasket, the calibration becomes non-linear at increasing carbohydrate concentration, while for the thicker gasket, the calibration still remained linear up to 200 mg/L concentration. The calibration data for glucose is shown in Figure 4. The thicker gasket extends the linear calibration range to higher concentrations, however, it also decreases the sensitivity. The choice of gasket thickness depends on the sample concentration. Glucosamine showed the largest linear range among six carbohydrates due to the ability of the amine group to adsorb.[9] The plots for glucose and fructose have a greater linear range than shown for sucrose.[10] The differences in linear ranges between different types of sugars are shown in Figure 5 using a 15 mil gasket.

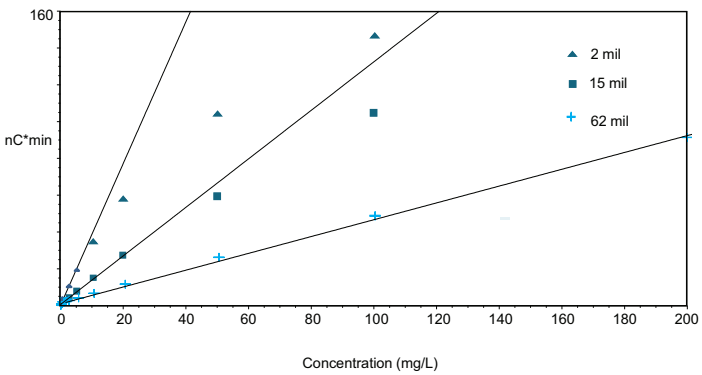


Figure 4. Calibration data for glucose on 2, 15, and 62 mil gaskets in the concentration range of 0.05–200 mg/L using Method 1.

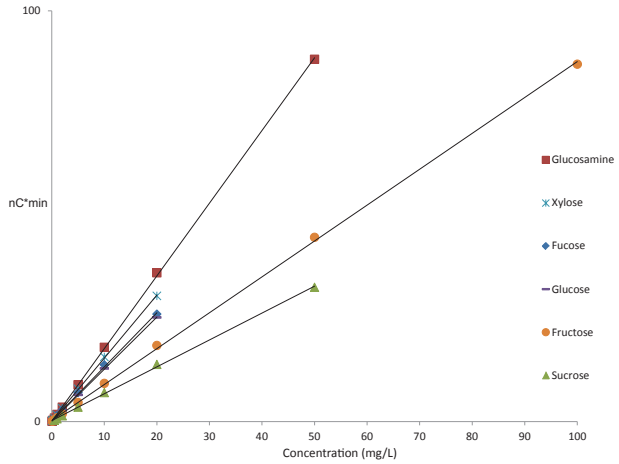


Figure 5. Linear calibration range for each sugar with a 15 mil gasket using Method 1.

analyses, except that the injection volume is 10 µL rather than the 0.4 µL used here. The injection volume was reduced from 10 µL to 0.4 µL to minimize the dilution of high concentration samples. The six carbohydrates were separated with 1 mM KOH at 45 °C within 8 min. To achieve a wider linear range, Method 3 used the postcolumn addition of more concentrated hydroxide (200 mM) to the eluent stream. For comparison, the separation of carbohydrates was run under the same conditions, except working electrode gaskets of different thicknesses were used.

Table 2. Linear range of calibration for the selected carbohydrates using Method 1 and different working electrode gaskets.

Analyte	2 mil		15 mil		62 mil	
	Range (mg/L)	Coefficient of Determination (r^2)	Range (mg/L)	Coefficient of Determination (r^2)	Range (mg/L)	Coefficient of Determination (r^2)
Fucose	0.02–2 0.02–5	0.999 0.998	0.05–20	0.999	0.2–50 0.2–200	1.00 0.993
Glucosamine	0.02–10 0.02–20	0.999 0.991	0.05–50	1.00	0.2–200	0.999
Glucose	0.05–5 0.05–10	0.999 0.997	0.1–20 0.1–50	1.00 0.998	0.2–200	0.999
Xylose	0.05–5 0.05–10	0.999 0.995	0.1–20 0.1–50	1.00 0.998	0.5–200	0.999
Sucrose	0.1–10 0.1–20	0.999 0.996	0.5–50	0.999	1–200	1.00
Fructose	0.1–20 0.1–50	0.999 0.990	0.1–100	1.00	0.5–200	1.00

Separation Using Methods 2 or 3 on the Dionex CarboPac SA10 Column

Figure 6 shows the separation of the sugars in a standard mix. Fucose, sucrose, glucosamine, glucose, xylose, and fructose are easily resolved using Methods 2 or 3 on the Dionex CarboPac SA10 column. The only difference between Methods 2 and 3 is the postcolumn addition of NaOH. Packed with highly porous resin beads, the Dionex CarboPac SA10 column has significantly higher capacity than most Dionex CarboPac columns and is the column of choice for separating high-concentrations of mono- and disaccharides in < 10 min. Method 2 is recommended to evaluate the column performance and for many carbohydrate

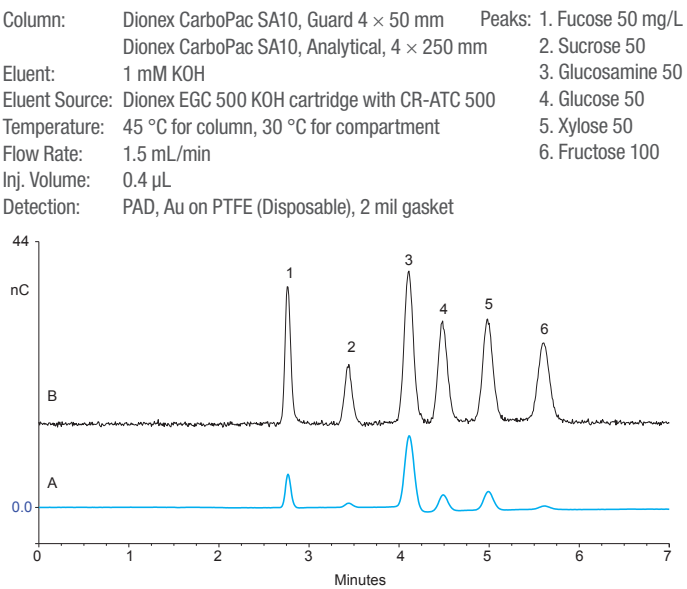


Figure 6. Separation of selected carbohydrates on the Dionex CarboPac SA10 column, (A) Method 2 without postcolumn addition of NaOH and (B) Method 3 with postcolumn addition of 200 mM NaOH at 0.4 mL/min.

Sensitivity of Methods 2 and 3

The LODs and LOQs were calculated from the average peak height of five injections of 2.5–20 mg/L each of the standards for Method 2, and 5–100 mg/L for Method 3, depending on the gasket thickness. Tables 3 and 4 list the LODs and LOQs respectively using Methods 2 and 3. Compared with Method 1, Method 3 demonstrated similar results in that the thinner gasket provides more sensitivity, but the results for Method 2 do not follow that pattern. For fucose, glucose, xylose, and fructose, the 15 mil gasket produced the best sensitivity. For sucrose, the 62 mil gasket is best for sensitivity, and for glucosamine the 2 mil gasket is the most sensitive. These results are probably due to the combination of two variables, the low concentration of hydroxide and a reduction of noise that is observed with the thicker gaskets under these conditions. Postcolumn NaOH addition showed greater noise than chromatograms without this addition. This noise is expected, due to the additional noise that is generated from a second pump delivering the postcolumn sodium hydroxide. Thus, under this method of postcolumn addition of sodium hydroxide, while there is increased signal, the noise also increases so that there is no increase in sensitivity, and, for these experiments, actually a little less sensitivity. Pneumatic addition of sodium hydroxide typically does not increase the baseline noise and therefore there is an increase in sensitivity but it is cumbersome to setup and maintain.

Table 3. LOD and LOQ using Method 2.

Compound	2 mil LOD[a] (mg/L)	LOQ[b]	15 mil LOD[a]	LOQ[b]	62 mil LOD[a]	LOQ[b]
Fucose	1.32	4.40	1.27	4.23	2.67	8.92
Glucosamine	0.534	1.78	1.15	3.85	2.29	7.62
Glucose	3.23	10.8	1.66	5.53	4.02	13.4
Xylose	3.83	12.8	1.70	5.67	3.89	13.0
Sucrose	10.43	34.8	3.49	11.6	2.83	9.45
Fructose	16.0	53.3	3.55	11.8	6.12	20.4

[a]LOD=3×S/N
[b]LOQ=10×S/N

Table 4. LOD and LOQ using Method 3.

Compound	2 mil LOD[a] (mg/L)	LOQ[b]	15 mil LOD[a]	LOQ[b]	62 mil LOD[a]	LOQ[b]
Fucose	6.01	20.0	6.14	20.5	9.92	33.1
Glucosamine	5.78	19.3	7.91	26.4	11.1	36.8
Glucose	8.57	28.6	11.4	37.8	15.2	50.5
Xylose	8.26	27.5	10.1	33.7	14.7	49.0
Sucrose	16.3	54.4	21.2	70.8	26.4	88.0
Fructose	19.8	65.9	19.0	63.2	24.4	81.2

[a]LOD=3×S/N
[b]LOQ=10×S/N

Response Linearity for Carbohydrates Using Methods 2 and 3

Additional hardware modifications (i.e., a thicker gasket, and a reduced injection volume of 0.4 µL using an injection valve with an internal loop) allow for increased linear range, enabling easier handling of samples with high carbohydrate concentrations. Methods 2 and 3 are recommended for such samples when the analyst wishes to minimize sample dilution. The linear ranges for the six selected sugars were determined by injecting calibration standards in triplicate, ranging from around the LOQ concentration to 2000 mg/L, except for fructose which was from around the LOQ concentration to 4000 mg/L. The linear ranges for the carbohydrates varied, but each had a coefficient of determination > 0.99 (Table 5). Two ranges without postcolumn addition are listed in Table 5 and for glucose, plotted in Figure 7. Both ranges produced a good coefficient of determination, > 0.99. However, the smaller range is obviously linear when the data is graphed, and the larger range is obviously non-linear, demonstrating that the coefficient of determination should not be the only measure when evaluating linearity.

The results for Methods 2 and 3 vary somewhat from those for Method 1. The thicker gasket did not always extend the linear calibration range to higher concentrations. In this evaluation, the 15 mil gasket did not extend the linear range of fucose, glucose, and xylose compared with the 2 mil gasket using Method 2. (See the calibration plots for glucose in Figure 8). Different types of sugars showed different linear ranges. Figure 9 shows the linear ranges for each sugar using a 15 mil gasket with Method 2. Second, postcolumn addition with Method 3 greatly improved the linear range of glucosamine on a 2 mil gasket, all carbohydrates on a 15 mil gasket, and the most on a 62 mil gasket. The combination of 15 mil gasket and postcolumn addition of sodium hydroxide showed the best combination of linearity and sensitivity for high concentration samples – up to 2 g/L for fucose, glucosamine, glucose, xylose, sucrose, and 4 g/L for fructose. However, postcolumn base addition requires a second pump to deliver the solution. Without the second pump, 1 mM KOH from eluent generation without postcolumn sodium hydroxide is also good for samples with high concentrations of carbohydrates using a 62 mil gasket. Finally, glucosamine, among all carbohydrates evaluated, was the most sensitive to gasket thickness in both Methods 2 and 3.

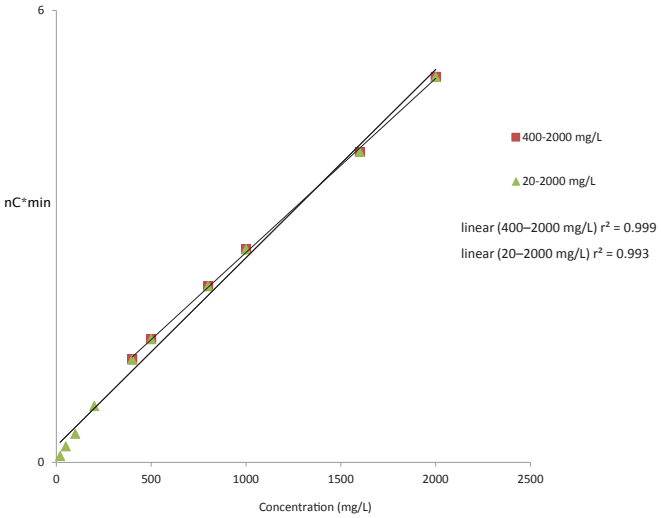


Figure 7. Calibration plots for glucose with a 2 mil gasket using Method 2.

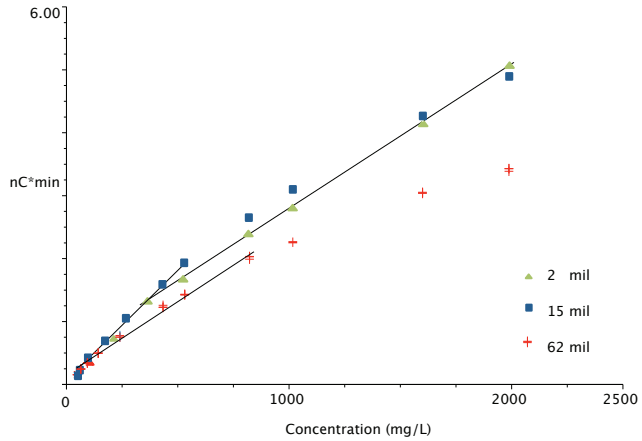


Figure 8. Calibration plots for glucose on 2, 15, and 62 mil gaskets in the concentration range of 10–2000 mg/L using Method 2. The linear ranges fall within the straight lines.

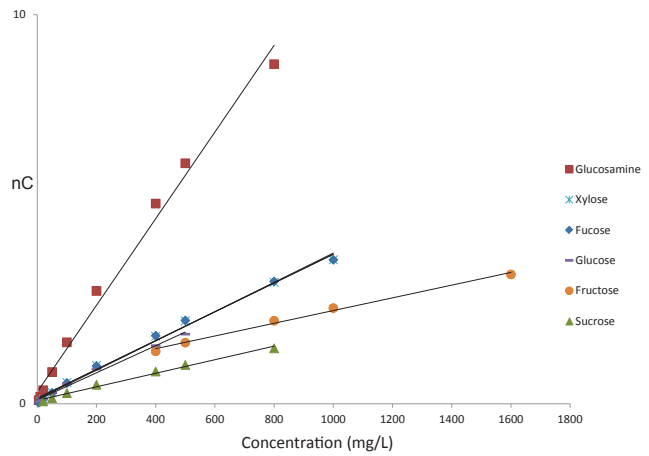


Figure 9. Calibration range for each sugar with a 15 mil gasket using Method 2.

Table 5. Linear calibration ranges using Methods 2 and 3.

Analyte	Method 2 (Without postcolumn addition)				Method 3 (With postcolumn addition)	
	Range (mg/L)	Coefficient of Determination (r^2)	Range (mg/L)	Coefficient of Determination (r^2)	Range (mg/L)	Coefficient of Determination (r^2)
2 mil						
Fucose	10–1000	0.999	10–1600	0.998	20–1000	0.998
Glucosamine	2.5–50	0.999	2.5–100	0.993	20–1600	0.999
Glucose	400–2000	0.999	20–2000	0.993	50–1000 50–2000	0.999 0.995
Xylose	50–500 or 200–2000	0.999	20–2000	0.998	50–1000	0.998
Sucrose	n.a.	n.a.	20–400	0.994	50–1000	0.998
Fructose	n.a.	n.a.	100–1000	0.998	100–4000	0.991
15 mil						
Fucose	n.a.	n.a.	5–1000	0.993	50–2000	0.999
Glucosamine	5–100	0.999	5–800	0.990	50–2000	1.000
Glucose	n.a.	n.a.	10–500	0.991	50–2000	0.999
Xylose	n.a.	n.a.	10–1000	0.994	50–2000	0.999
Sucrose	n.a.	n.a.	20–800	0.990	100–2000	1.000
Fructose	n.a.	n.a.	400–1600	0.994	100–4000	0.999
62 mil						
Fucose	10-400	0.999	10–1600	0.991	50–2000	0.999
Glucosamine	10-1000	0.999	10–2000	0.997	50–2000	0.999
Glucose	n.a.	n.a.	20–800	0.993	100–2000	0.999
Xylose	n.a.	n.a.	20–1000	0.994	50–2000	0.999
Sucrose	n.a.	n.a.	10–1000	0.994	100–2000	0.999
Fructose	n.a.	n.a.	40–1000	0.995	100–4000	0.999

Conclusion

This study evaluated the effect of working electrode gasket thickness on the sensitivity and linearity of carbohydrates using the Dionex CarboPac SA10 column with or without postcolumn addition of sodium hydroxide, and the Dionex CarboPac PA20 column. Under the recommended chromatographic conditions for the Dionex CarboPac PA20 and Dionex CarboPac SA10 columns, the data for fucose, glucosamine, glucose, xylose, sucrose, and fructose were collected, analyzed, and compared. This study provides a basis for choosing the appropriate working electrode gasket based on the carbohydrate concentration in the sample. Using a 2 mil gasket, Method 1 achieved the best sensitivity. A large linear range can be achieved using a 62 mil gasket and either Method 1 or 2. The combination of a 0.4 µL injection valve and the 62 mil gasket might reduce the 10-fold dilution required for high concentration samples using the CarboPac SA10 column, compared to that required when using a 2 mil gasket. The 62 mil gasket helps to reduce or eliminate manual dilutions, thereby minimizing dilution errors, and prevents overloading the working electrode. The combination of a 15 mil gasket and postcolumn addition of sodium hydroxide in Method 3 has good sensitivity and the largest linear range. Choosing the appropriate gasket thickness for a given application will allow measurement of a large concentration range and minimize the amount of sample dilution needed.

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