Errata

Product Manual for Dionex CarboPac™ SA10 Columns
065384-02

For new orders of the following parts discussed in this manual, please use the updated part numbers listed below.

Part	Old Part Number in this manual	Updated Part Number to use for new orders
PROD,ELCTD,AU,GSKT,ICS3/5	*061749*	*079850*

PRODUCT MANUAL

for

CarboPac® SA10

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IC | HPLC | MS | EXTRACTION | PROCESS | AUTOMATION

PRODUCT MANUAL

FOR

CARBOPAC® SA10

4 x 250 mm (P/N 074641)
4 x 50 mm (P/N 074902)

Document No. 065384
Revision 02

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Guide to Important Information

 **SAFETY**	Safety information can help prevent bodily harm.
 **WARNING**	Warning information can help prevent equipment harm.
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 **NOTE**	Note information can help with tips for improved use.

SECTION 1 - INTRODUCTION

1.1. CarboPac SA10 column

The CarboPac® SA10 column has been developed to provide fast, high-resolution separations for most mono- and disaccharides in biofuel and food & beverage research. The eight common biofuel sugars, fucose, sucrose, arabinose, galactose, glucose, xylose, mannose and fructose, can be separated on this column within 10 minutes. The six common sugars in food & beverage industry, sucrose, glucose, fructose, lactose, cellobiose, and maltose, can be separated within 10 minutes on this column.

The CarboPac SA10 columns are packed with a hydrophobic, polymeric, macroporous anion exchange resin stable over the range of pH 0-14. This unique pH-stability of the packing material allows the use of eluent compositions that are conducive to anodic oxidation of carbohydrates at gold electrodes.

1.1.1. Resin Characteristics:

Particle Size:	6 µm
Pore Size:	macroporous (2000 Å)
Cross-linking:	55%
Ion exchange capacity:	290 µeq per 4.0 x 250 mm column

1.1.2. Latex Characteristics:

Functional Group:	quaternary ammonium ion
Latex Diameter:	55 nm
Latex Cross-linking:	4.5 %

1.1.3. Typical Operating Parameters:

pH range:	0-14
Temperature Limit:	4-55°C
Pressure Limit:	3500 psi
Organic Solvent Limit:	100% compatible
Typical eluents:	potassium hydroxide or sodium hydroxide

SECTION 2 - SYSTEM REQUIREMENTS

2.1. System Configuration

The Dionex Carbohydrate system should be configured to comply with the following key requirements:

- Mobile phase eluents are kept under helium or nitrogen at all times
- On-line degassing of eluents
- Ag/AgCl reference electrode
- Programmable PAD waveforms with frequencies of 1 Hz or higher
- Minimized contribution to the background signal by contaminants from the system and reagents
- Constant temperature control of the guard column, separation column and detection cell.
- Use of KOH/NaOH eluent from a compatible eluent generator is highly recommended.

2.2. Carbohydrate System

2.2.1. System Components and Description

The Dionex ICS-5000 system is recommended for carbohydrate analysis with the CarboPac SA10 column.

- Dual pump (DP) or single pump (SP) module
- Eluent Generation module (ICS-5000) with EGC, and CR-ATC
- Autosampler (AS) module
- Dual Column (DC) module (ICS-5000)
- Electrochemical cell with a gold electrode, PdH or Ag/AgCl reference electrode
- PTFE gasket
- Eluent organizers (EO1)

2.3. System Requirements

The carbohydrate separations using the CarboPac SA10 column are optimized for use with DIONEX ICS3000 system. All of the surfaces in contact with eluent and samples are metal-free.

Tubing anywhere between the injection valve and detector should be < 0.005 in I.D. PEEK tubing. Minimize the length of all liquid lines, but especially that of the tubing between the column and the detector cell. The use of larger diameter and/or longer tubing will decrease peak resolution.

The AS module offers multiple sampling options; however, a consistently reproducible quantitation and an absence of disturbing artifacts are achieved best using the “full loop” mode. Good reproducibility of retention times requires the use of temperature control. For high concentration samples, a 0.4 µL internal injection valve (P/N 072050), special 62 mil thick ED gasket (P/N 075499), and a compatible ED block, (P/N 075501), may be needed to minimize requirement of dilution.

2.4. Disposable Gold Working Electrodes

Carbohydrates separated by anion exchange chromatography at high pH and are detected by pulsed amperometric detection. The signal is reported in coulombs (C). Electrochemical detection (ED) is used to measure the current or charge resulting from oxidation or reduction of analyte molecules at the surface of a working electrode. During oxidation reactions electrons are transferred from molecules of electroactive analytes, such as carbohydrates, to the working electrode in the amperometry cell. Detection is sensitive and highly selective for electroactive species, since many potentially interfering species cannot be oxidized or reduced, and are not detected. When a single potential is applied to the working electrode, the detection method is DC amperometry. Pulsed amperometry and integrated amperometry employ a repeating sequence of potentials. Pulsed electrochemical detection (PAD) at a gold working electrode is a reproducible and sensitive method for the detection of all carbohydrates of molecular weight up to ten-thousand.

Although carbohydrates can be oxidized at a gold working electrode, some products of the oxidation reaction poison the surface of the electrode, inhibiting further analyte oxidation. By repeatedly pulsing between high positive and negative potentials, a stable and active electrode surface can be maintained. However, the gold working electrode is very slowly consumed during this process and will eventually need to be replaced. Occasionally the electrode may be 'poisoned' by other contaminants, resulting in a significantly reduced response. When this occurs, the active surface can be renewed by polishing the electrode. However, this can be a tedious and time-consuming process.

The Dionex disposable gold electrodes (P/N 060139 for 6, P/N 060216 for 4 packages of 6) make electrode reconditioning by polishing and other methods unnecessary. They are less expensive and can thus be replaced more often than the conventional electrodes. The more frequent replacement of working electrodes renders electrochemical detection more predictable and reproducible. The disposable electrodes also make easier any troubleshooting of electrochemical detection problems. The gold hydroxide (AuOH) catalyzed mode of oxidation of carbohydrates differs from the gold oxide catalyzed oxidation of amino acids at higher potentials. Although both gold electrodes can be mounted in the same electrochemical detection cell, and thus in principle it is feasible to convert a gold electrode from one mode of detection to another, in practice this may require an extensive period of time and is thus not recommended. The Au electrodes for carbohydrate analysis have been tested for and are guaranteed to work for carbohydrate analyses.

WARNING	***Dionex Technical Note 21*** ***You MUST USE the quadruple waveform (Waveform A) with disposable electrodes. Waveform B and Waveform C CANNOT BE USED with Disposable Electrodes. Waveforms B and C will strip the gold surface of the disposable electrode within 24 hours***.

2.5. System Start-up

Configure the system with the AS autosampler on the left, the DC module in the middle and the pump on the right. EG Module should be placed on top of the pump. Nitrogen or helium should be delivered to the eluent organizer with about 5-6 psi at each bottle. Make all fluidic and electrical connections, but do not install the column yet. Instead install some backpressure tubing, such as a length of 0.005" I.D tubing between the injector and detector cell inlet. Minimize the number of unions and the length of all the liquid lines. Tubing between the injection valve and the detector, on either side of the column, should be 0.005" ID PEEK tubing. The use of larger tubing will decrease peak resolution. Verify that the modules are communicating.

2.5.1. System Rinse

1. Rinse a new system with 2M NaOH prior to use DO NOT install the CarboPac SA10 column before confirming that the background < 30nC.
2. Prepare a solution of 2M NaOH to rinse each bottle, by diluting 104 mL of 50% sodium hydroxide to 1 L with deionized water
3. Place the 2 M NaOH in a pre-rinsed bottle and place all the four eluent lines in it. Withdraw at least 40 mL of sodium hydroxide from the line into waste, using a priming flow rate.
4. Close the solvent draw-off valve and leave the pump running at 1.0 ml/min for 15 minutes, with equal proportioning from all four eluent lines.
5. Make sure that all surfaces come into contact with the sodium hydroxide; rotate the injector valve.
6. Repeat the process with 18 megohm-cm water.

2.5.2. Column Installment

1. Install the column according to the direction indicated by the arrow.
2. Flush the column with 100mM KOH or NaOH at 1ml/min for 30min or until a stable baseline is achieved.
3. Flush the column with the starting eluent with at least 10 column volumes.
4. Start your analysis.

SECTION 3 - OPERATION

3.1. CarboPac SA10 Column Operational Parameters

pH range:	pH = 0 - 14
Temperature limit:	55°C
Pressure limit:	3,500 psi
Organic Solvent Limit:	100% Acetonitrile, methanol, acetone, if required for cleaning
Typical Eluents:	potassium hydroxide from the Eluent Generator

3.1.1. The Most Important Rules

3.1.1.1. ALWAYS…

- use dedicated glassware and disposable glass or plastic ware for volume adjustments.
- use high purity water (18.2 MΩ-cm resistivity).
- keep your water blanketed with helium or nitrogen. Use new filtered water if left unblanketed for more than 30 minutes.

3.1.1.2. NEVER…

- go to the next step of the installation if the previous step has failed.
- start an installation with any of the check list items below missing.
- use 'communal' filtration units or filters made of unknown or unsuitable (cellulose derivatives, polysulfone) materials.
- use MeOH or other organic solvents as rinse fluid in the autosampler. Use only water, replaced daily. NEVER run above 60 °C or 3,200 psi.

3.1.2. Initial Check List

The following items MUST be available in your lab. The absence of any of these may compromise your analysis.

- Laboratory water unit delivering 18.2 megohm-cm water at the installation site.
- Vacuum pump available for use with the vacuum filtration units
- Inert gas cylinder (helium or nitrogen) with a regulator valve (for example, a 0-200 psi gauge on the low pressure side) and the appropriate size adaptors plus tubing
- Plastic eluent bottles

3.2. Purity Requirements for Chemicals

Obtaining reliable, reproducible and accurate results requires eluents that are free from impurities and prepared only from the chemicals recommended below. DIONEX cannot guarantee proper column performance when alternate suppliers of chemicals or lower purity water are utilized.

3.2.1. Deionized Water

The deionized water used to feed the Eluent Generator should be Type I reagent grade water with a specific resistance of 18 megohm-cm. The water should be free from ionized impurities, organics, microorganisms and particulate matter larger than 0.2 µm. The availability of UV treatment as a part of the water purification unit is recommended. Follow the manufacturer's instructions regarding the replacement of ion exchange and adsorbent cartridges. All filters used for water purification must be free from electrochemically active surfactants. Expanding their period of use beyond the recommended time may lead to bacterial contamination and as a result, a laborious cleanup may be required. Use of contaminated water for eluents can lead to high background signals and gradient artifacts.

3.2.2. Potassium Hydroxide

Use Dionex KOH Eluent Generator Cartridge installed with CR-ATC in the EG module.

3.3. Preparation of Eluents and Standards

NOTE	***Always sanitize the entire analyzer with 2M NaOH prior to initial start-up (see Section 2.5.2) and after idle periods.***

Obtaining reliable, consistent and accurate results requires eluents that are free of ionic and electrochemically active impurities. Chemicals and deionized water used to prepare eluents must be of the highest purity available. Maintaining low trace impurities and low particle levels in eluents also helps to protect your ion exchange columns and system components. DIONEX cannot guarantee proper column performance when the quality of the chemicals, solvents and water used to prepare eluents is substandard.

3.3.1. Deionized Water

Vacuum degas the water by placing the eluent reservoir in a sonicator and drawing a vacuum on the filled reservoir with a vacuum pump. Vacuum degas the reservoir for 5-10 minutes while sonicating. Cap each bottle and minimize the length of time the bottle is opened to the atmosphere. Vacuum filtration through 0.2 µm Nylon filters is a good alternative to vacuum degassing under sonication and is sufficient in the majority of cases. On-line degassing is supported through the use of the DP gradient pumping systems.

3.3.2. Eluent: Potassium Hydroxide

The first step in the preparation of potassium hydroxide eluent is to degas an aliquot (typically 1000 mL) of the deionized water, as described above. In the second step, start the pump flow and verify that the water is exiting from the Eluent Generator exit tubing. In the third step, select an appropriate KOH concentration (usually 10 mM) in the EG panel and verify that the eluent is exiting from the CR-ATC outlet tubing, then turn on the CR-ATC in the eluent generator panel.

3.4. Sample Preparation

The CarboPac columns are strong anion exchangers. Thus, the sample matrix precautions applicable to ion exchange chromatography apply to these columns. High salt concentrations in the samples should be avoided where possible. Special care should be taken with samples containing high concentrations of anions, which are strong eluents for the CarboPac columns (e.g. chloride, carbonate, phosphate, etc.). Avoid extremes of sample pH (especially extremely acid samples). The presence of anionic detergents (e.g. SDS) in samples should be avoided entirely. Nonionic or cationic detergents may be acceptable in low concentrations.

When using PED for detection, beware of high concentrations of electrochemically-active components (e.g. TRIS buffer, alcohols, and other hydroxylated compounds). Small amounts of organic solvents in the sample will not harm the column, although the organics may interfere with the chromatography or detection of the analytes of interest.

3.5. Introduction to the Detection Methods

The carbohydrate oxidation at gold electrodes is made possible by a rapid sequence of potentials (waveform) adjusted between the working electrode (gold) and the reference electrode (Ag/AgCl). Resulting currents are measured by integration during a short time interval of the detection waveform. The standard, recommended carbohydrate waveform is shown in Table 1.

Table 1
Carbohydrate Quadruple Waveform

Time (s)	Ag/AgCl reference potential (mV)	PdH potential for pH 11.5 (mV)	Integration
0	100	950	
0.2	100	950	Start
0.4	100	950	End
0.41	-2000	-1150	
0.42	-2000	-1150	
0.43	600	1450	
0.44	-100	750	
0.5	-100	750	

CAUTION	***Do not polish a new gold electrode prior to use. NEVER POLISH the disposable gold electrodes***.

Refer to "Section 5 – Troubleshooting Guide" of this manual for an overview of reconditioning techniques for conventional gold working electrodes.

The reference electrode for the ED is either a combination pH-Ag/AgCl electrode or a PdH electrode. For carbohydrate analysis, the former electrode is used in the Ag mode. Always verify the correct selection of reference electrode is made in the program file and on the ED panel prior to turning the cell voltage on.

Always have available at least one unused "known good" pH-Ag/AgCl reference electrode. If stored in saturated KCl, a reference electrode can be kept for years with its reference potential virtually unchanged. In contrast, the reference electrodes mounted inside the electrochemical cell and exposed to flowing sodium hydroxide have only a limited lifetime of approximately 3 to 6 months. As a result of prolonged exposure to alkaline solutions, the 0.1 M KCl solution inside the reference electrode gradually becomes alkaline and the silver chloride layer on the Ag wire immersed into that solution either dissolves or converts to a mixture of silver oxide and silver hydroxide. As that happens, the reference potential shifts and becomes increasingly unstable. Shifting reference potential is experienced by the user either as an unusually high background or as a decrease in signal response. A combination of both effects is also possible.

The PdH electrode does not undergo any change during a prolonged exposure to alkaline conditioned. However, its potential can change slightly during a longer storage outside of the detection cell.

CAUTION	***Never leave pH-Ag/AgCl electrode reference electrode inside a disconnected electrochemical cell***

A pH-Ag/AgCl electrode reference electrode can be irreversibly damaged by drying out. This happens most frequently by leaving the reference electrode inside a disconnected electrochemical cell. Always remove the pH-Ag/AgCl electrode reference cell from the electrochemical cell, when the system is not in proper use (i.e. cell inlet and outlet are not plugged or connected to a flowing eluent). After removal from the electrochemical cell, keep the reference electrode immersed in 3M KCl solution (224 g KCl/L) at all times.

SECTION 4 - APPLICATIONS

4.1. Biofuel mono- and disaccharides

The CarboPac SA10 column has been designed to provide fast speed separations for mono- and disaccharides. Particularly, the eight biofuel sugars can be separated within 7 min on this column.

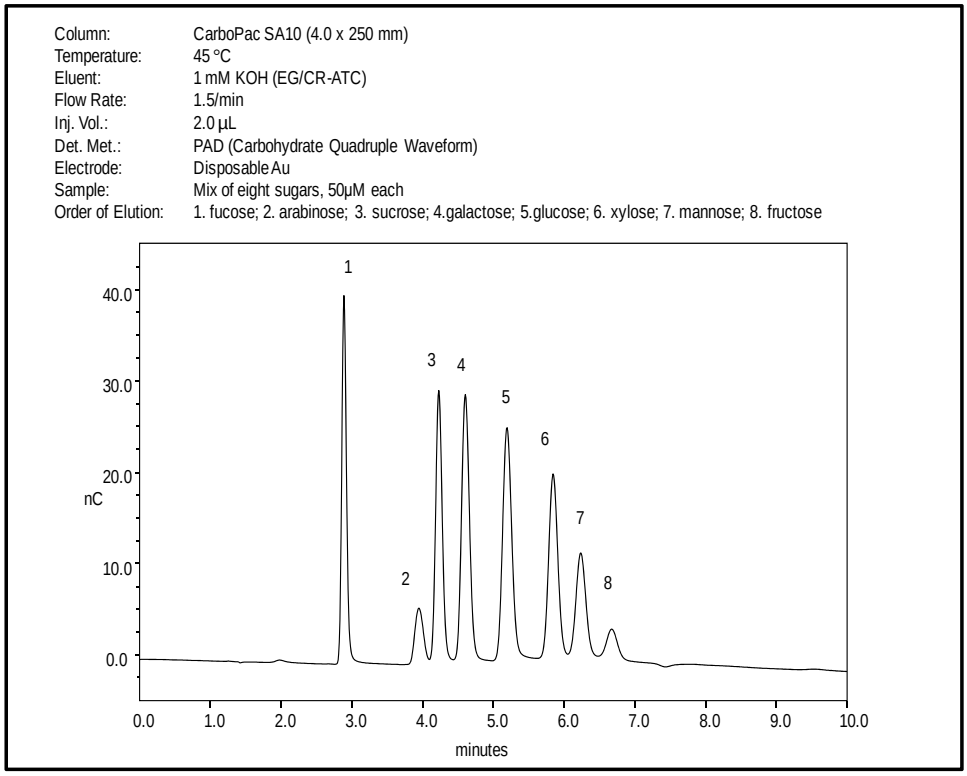

Figure 1

Separation of mono- and disaccharides

4.2. Corn Stover Hydralysate

The CarboPac SA10 column can be used for high concentration sample loading and analysis. Figure 2 demonstrates the high resolution separation of a high concentration corn stover hydrolysate sample on the CarboPac SA10 column. The corn stover hydrolysate sample was analyzed with only a 1:200 dilution using a 0.4µl injection valve and a 15 mil ED gasket. The use of a 15 mil gasket reduces the sensitivity of the cell so that the detector is not overloaded.

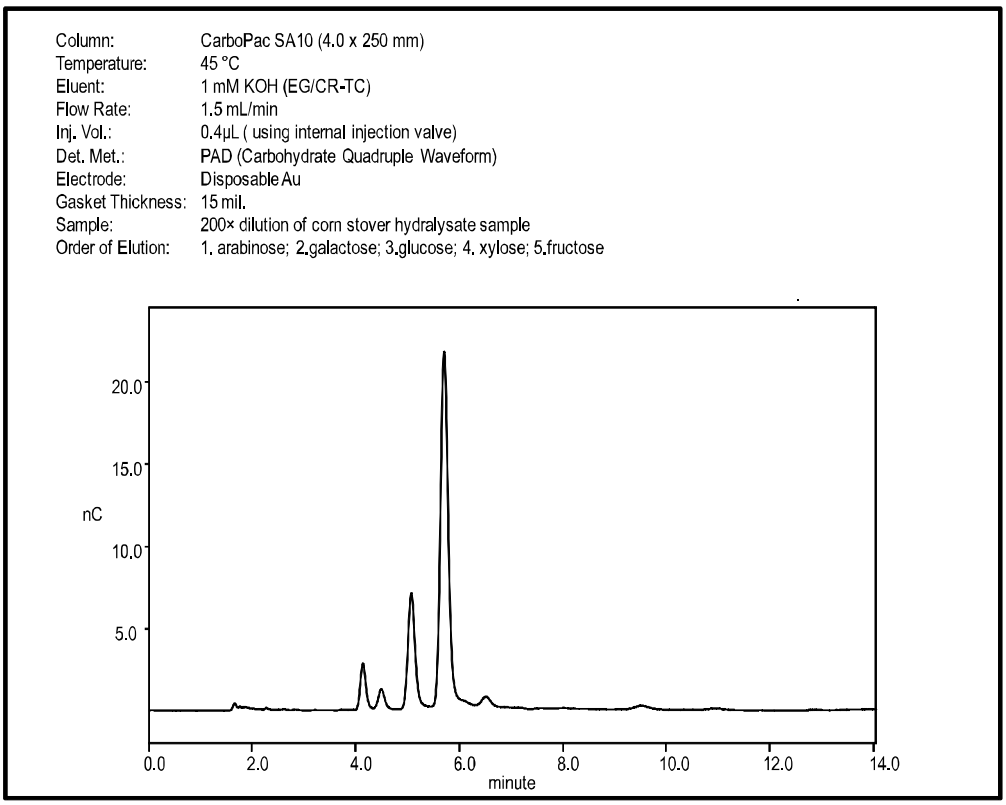

Figure 2

Separation of corn stover hydralysate

NOTE	***Note: Alternate approaches for reducing required dilutions is to use a 0.4µl injection valve with a 62 mil gasket, P/N 075499, and modified spacer block, P/N 075501.***

4.3. High concentration sample loading and analysis of Corn Stover Hydrolysate mono- and disaccharides

Pulsed amperometric detection (PAD) is well known for high sensitivity. Biomass samples often contain high concentration (over 100 g/L) sugar contents, which usually require a dilution factor of 1000 to avoid saturating the column or the detector. By reducing the injection volume to 0.4 µL with an internal injection valve, and reducing the detector sensitivity with 62 mil thick ED gasket, corn stover hydrolysate sample with ~150 g/L total sugar concentration can be analyzed quantitatively on the column after 100× dilution (Figures 3 & 4).

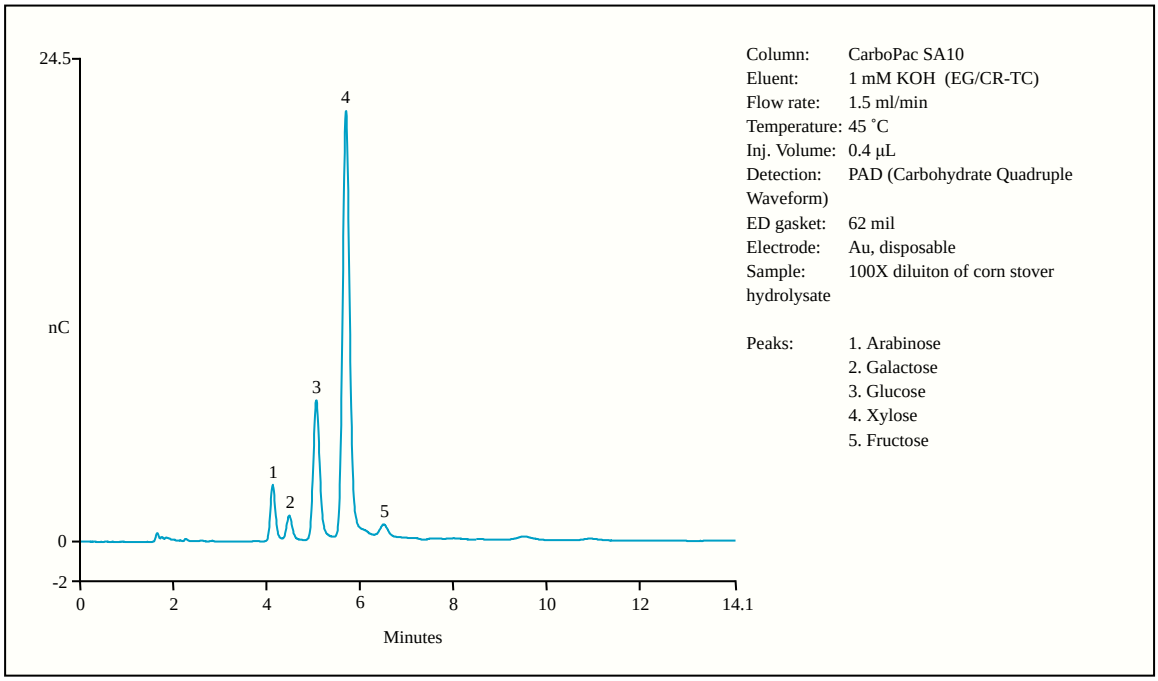

Figure 3

Analysis of corn stover hydrolysate

NOTE	***Note: 62 mil gasket, P/N 075499, was used with a modified Spacer Block, P/N 075501***

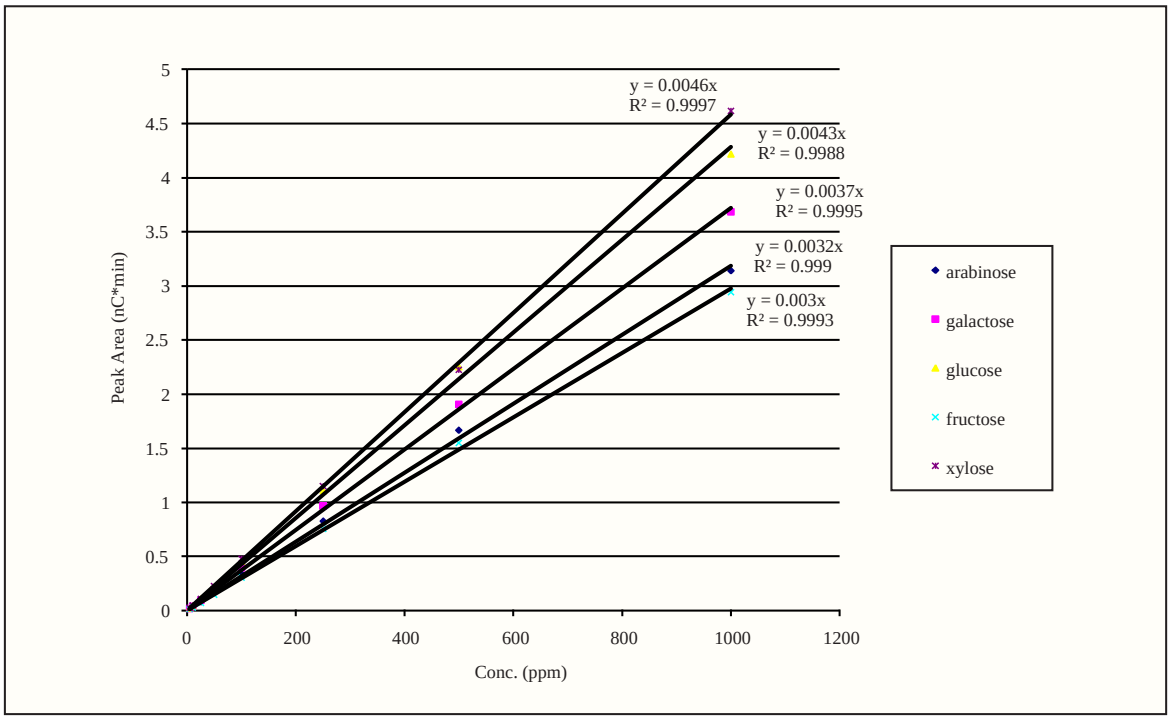

Figure 4

Linearity on the CarboPac SA10 Column

4.4. Food and Beverage

The CarboPac SA10 column has been designed to provide fast speed separations for mono- and disaccharides. Particularly, the sugars found in food and beverages. The six below can be separated within 10 min on this column.

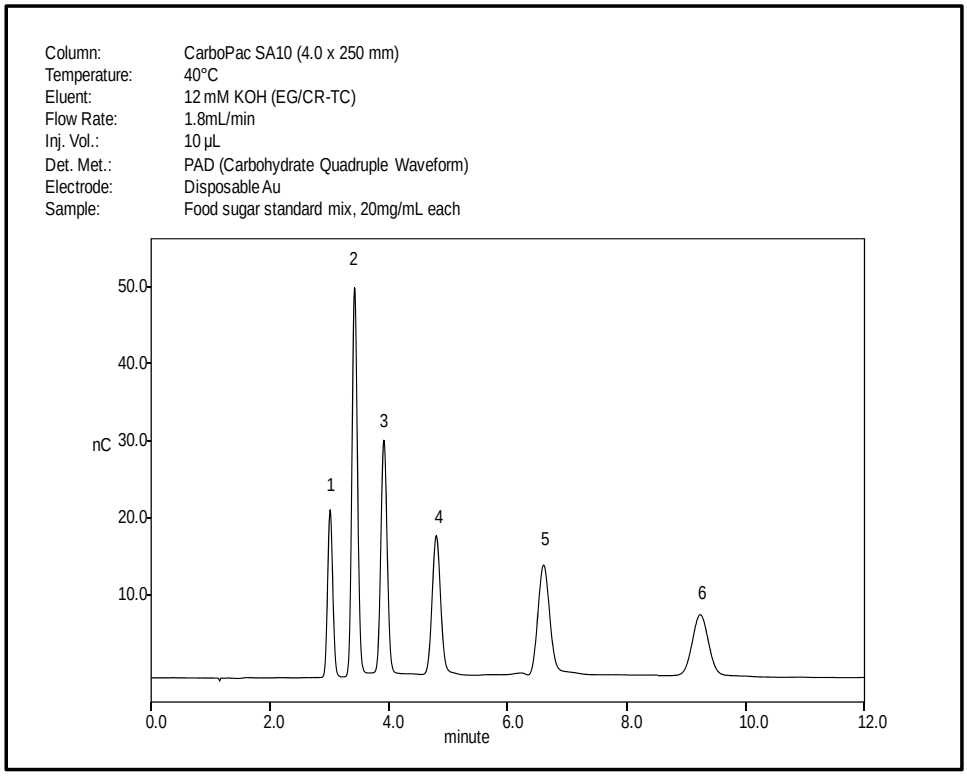

Figure 5

Separation of food & beverage sugars

4.4 Sialic acid

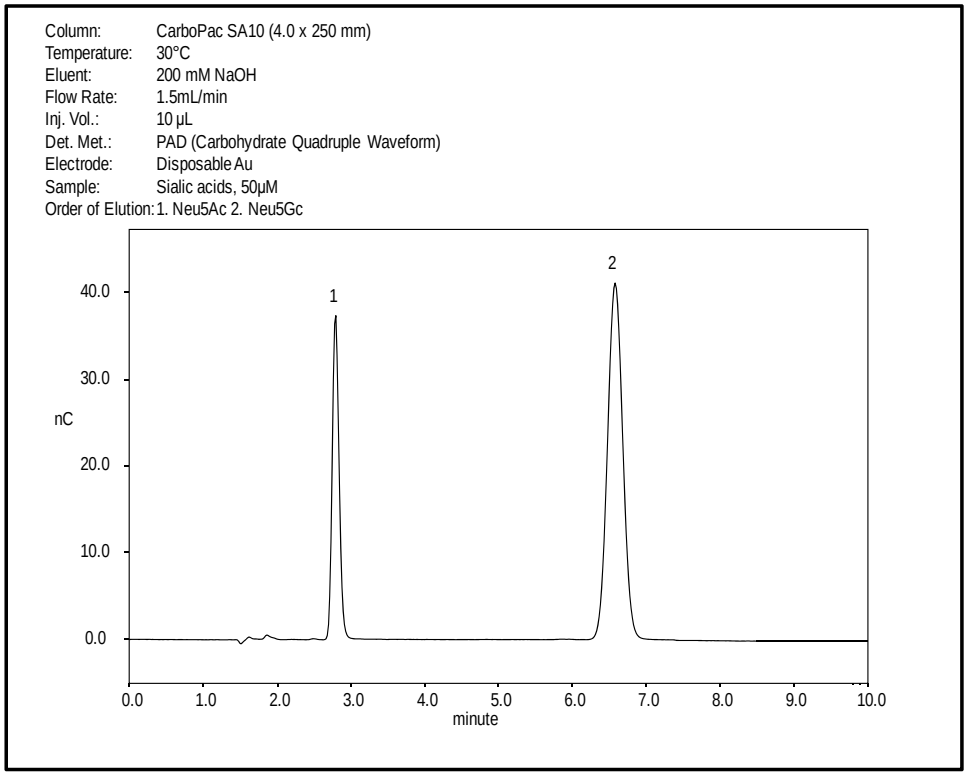

Figure 6

Separation of Sialic Acids

SECTION 5 - TROUBLESHOOTING GUIDE

Problems such as sample contamination, imprecision during sample transfer, problems during peptide or protein hydrolysis, may be related to specific experimental protocols.
Make sure to follow the rules from 3.1.1 “The Most Important Rules”, and to recheck all of the items from 3.1.2, “Initial Check List.”

5.1. High Background

While it may be possible to obtain reasonable performance even with elevated levels of detection background according to some requirements, high background frequently brings about an increased size of gradient artifacts and can be accompanied by a presence of ghost peaks. Detection sensitivity may also change suddenly when the detection background is too high.
A background >30 nC with 10 mM potassium hydroxide at 1.0 mL/min and 30°C using the quadruple waveform shown in Table 1 indicates one of the following possibilities:

5.1.1. Incorrect detection parameters

Verify that “Ag” or “PdH” is specified in detector panel. Check all values of waveform in the program against those in Table 1, “Carbohydrate Quadruple Waveform,” in Section 3.6. Make sure the correct waveform for the selected reference mode is being applied. If the pH reading at 10 mM KOH) is above 13.2 replace the Ph/Ag/AgCl reference electrode.

5.1.2. Compromised working electrode surface

Briefly install a new working electrode and check the background as above, If the reading remains > 30 nC, remove the new electrode within 30 minutes and continue testing for column or system contamination. Otherwise continue with your work with the new electrode installed.

5.1.3. Column contamination

Remove the column set from the system first and replace it with a length of 0.010” ID PEEK tubing, generating a pressure drop between 1000 and 2000 psi. If the background reading improves after the column is removed from the system, go to section 5.3.

5.1.4. Water contamination

Prepare eluents using a freshly filtered aliquot of water, investigate the source of contamination in the original aliqout of water.

5.1.5. System contamination

If the background remains high even with fresh water and without the column, carry out the 2 M sodium hydroxide rinse described in section 5.5.

5.2. Decreased Detection Sensitivity

5.2.1. Working Electrode

CAUTION	***Never install a new electrode without an aggressive system cleanup (Section 5.6, “EDTA Cleanup”) Exceptions to this rule are described below.***

Any decrease in detection sensitivity means that the working electrode surface has been affected. The operator should install a new working electrode. Spare gold working electrodes should always be available in order to avoid unnecessary delays.

5.2.2. Reference Electrode

If pH/Ag/AgCl reference electrode is installed, check the pH reading. If the value is out of range of >13.2, install a new pH/Ag/AgCl electrode and then install a new gold working electrode (P/N 061749 or P/N 060139 disposable Au electrode). The system cleanup is not necessary. The decrease in sensitivity was caused by a gold-oxide-buildup on the electrode surface. This was because the reference potential was too high. The non-disposable gold working electrode can be reconditioned by the repair polishing described in Section 5.7.1, "Mechanical Polishing."

After installing a new working electrode (with or without the complete system cleanup), confirm the normal detection sensitivity. Should the response be still too low, immediately remove the new working electrode from the system.

5.3. Column Problems

The guard column protects the main column not only from contamination but also from excessive pressure fluctuations caused by the instrument or by operator errors. Have the guard column installed at all times, disconnect it only during some of the testing described in this section, or when priming the pump to prevent accidental over pressure.

The column set is causing the high background if the background reading decreases after the column is replaced by a section of PEEK tubing, as described in Section 5.1 item 3, "Column Contamination."

5.3.1. Column Set Causing High Background

If the column has been determined to be the cause of the high background, as described above, replace the column.

5.3.2. Peak Efficiency and Resolution are Decreasing

Always have a spare guard available. Sometimes samples can foul the column. In this case a guard column may be used.
Peak deformations may sometimes be caused by sample matrix.

NOTE	***For the purpose of Beta testing a guard has not been supplied. Please contact the Product Manager if you feel you are experiencing column fouling issues and suspect a guard column may help.***

Run a standard separation with the Guard column removed from the system. Install a new Guard column should the separation improve with the old Guard removed. It is quite common to replace the Guard column several times during the lifetime of the analytical column.

Verify that only the 0.005" I.D. tubing is installed for all connections between injector and detector.

Check for proper installation of ferrules on all PEEK tubing starting with the injector outlet and all other connectors to the detection cell inlet. Check the quality of cuts at the end of each tubing.

Check temperature settings in your method and/or actual temperature in your column oven.

The column may be overloaded. Try to inject a smaller amount of your sample or dilute the sample more.

If all of the above does not lead to an improved separation, the resin bed of the main column has been damaged and the main column must be replaced.

5.4. System Problems

5.4.1. High Detection Background Caused by the System

Verify the problem is neither the detector (see Section 5.1 item 1, "Incorrect detection parameter" and item 2, "Compromised working electrode surface") nor the column (see Section 5.1 item 3, "Column Contamination") related.
With injector, column and detector cell installed (cell voltage off) carry out the 2M NaOH wash as described in Section 5.5, "Sodium Hydroxide Cleanup."
Prepare a new aliquot of filtered water and transfer it into a rinsed out, inert gas blanketed eluent bottle.
Rinse eluent lines with the freshly filtered water (at least 40 mL by priming speed).

5.4.2. No Peaks, Poor Peak Area Reproducibility or Too Small Peak Areas

1. Check the position and filling levels of sample vials in the autosampler.
2. Check injector needle-height setting.
3. Check each line of the schedule for proper injector parameters.
4. Service the injection valve (check for leaks, Tefzel fragments, or sediments inside the valve)

5.4.3. Large Baseline Dip in the Chromatogram

A large baseline dip appearing between 7 and 8minutes at 1.5ml/min, 45˚C, without the guard column, is usually caused by oxygen in the sample injected. The 'oxygen dip' is normal and usually the magnitude is 1-2nC.

5.4.4. Incorrect or Variable Retention Times

1. Check the operational parameters of your CR-ATC.
2. Prime the pump if necessary.
3. Measure the flow rate by weighing out the eluent collected during exactly sixty minutes of flow. Recalibrate the pump if necessary.
4. The re-equilibration period at the end of the program with a 100-200 mM KOH rinse is too short.
5. Samples containing high salt content (>50 mM) will decrease the retention times.

5.4.5. Unidentified Peaks Appear Alongside the Expected Analyte Peaks

During the 100-200 mM KOH rinse, a number of small peaks may appear. These peaks are usually due to trace contaminants in the water supply. The contaminants accumulate on the column during the isocratic section of the chromatogram and are released, frequently as irregular baseline deformations or sharp spikes, with the increasing eluent strength. Some trace contaminants can co-elute with monosaccharides, compromising accuracy of quantitation at lower concentrations.

If extraneous peaks are observed even after the water supply is excluded as a possible cause, clean the autosampler lines and sample loop. The autosampler should be cleaned using the following protocol:

Disconnect the column and detector cell from the autosampler.

Set the pump to 100% deionized water.

Place the following solutions in the autosampler and inject in sequence:

1. 1 M NaOH
2. Deionized water
3. IPA
4. Deionized water
5. 1 M HCl
6. Deionized water

5.5. Sodium Hydroxide Cleanup

The sodium hydroxide (2 M) rinse used to decrease column or system-related elevated background is essentially identical to the rinse performed during an installation of a new system, Section 2.5.1, "System Rinse." Following the rinse, check the background again while pumping the 10 mM potassium hydroxide and repeat the rinse at least once if necessary. Leave the old gold working electrode in place during the first and second checking of the detection background. Use a new or reconditioned electrode only if the background remains high even after the second rinse. Should the new electrode also produce a reading of > 30 nC, remove it from the system within 30 minutes, rinse it with water and reinstall the old electrode. In case the repeated rinse does not lower the background, perform the EDTA cleanup described in Section 5.6, "EDTA Cleanup." Then try the background with the old electrode first and if necessary only briefly with the new electrode again. In case the new electrode delivers < 30 nC, leave it in the system, and if non-disposable electrodes are used, recondition the old electrode using the chemical cleanup described in Section 5.7.1, "Mechanical Polishing."

5.6. EDTA Cleanup

1. Install new Pump Seals (P/N 064946)
2. Wash System with 6.5 mM (2.4 g/L) Na2EDTA (must be disodium EDTA) MW 372 g/mol
3. Remove the column.
4. Remove gold electrode from the cell, close the cell again using an empty holder block over a gasket.
5. Restore liquid connection between injector valve and detection cell (column has been removed)
6. Empty the contents of eluent container, rinse it with 1 L of 18megohm water and discard the water.
7. Filter 1 L of 18megohm water through 0.2 um Nylon filter.

NOTE	***Do not use any other material than Nylon for eluent filtration.***

8. Transfer filtered water into eluent container. Pump at least 30 mL of water into pump waste at priming flow rate.
9. Stop the pump. Close the priming valve. Pump at least 50 mL of water from eluent container through the system into detector waste at 1 mL/min.
10. Toward the end of the water rinse, turn the injection valve at least 3 times.
11. Prepare 1L of 6.5 mM Na2EDTA (must be the disodium form) and filter it through a 0.2 um Nylon filter. Discard water from eluent container and replace it by the filtered aliquot of 6.5 mM EDTA.
12. Pump at least 30 mL of EDTA into pump waste at priming flow rate.
13. Stop the pump. Close the priming valve. Pump (1 mL/min) at least 100 mL of EDTA from eluent container through the system into detector waste.
14. Toward the end of the EDTA rinse, turn the injection valve at least 3 times.
15. Carry out steps d to h again. Rinse the system (column remains out of the system) with the initial eluent composition (10 mM KOH).
16. Re-Install the column set and using the rate of 0.25 mL pump for at least 10 minutes from the column to waste.
17. Open the working electrode side of the cell, remove the gasket and rinse the sealing surface with 18 megohm water.
18. Reassemble cell with a new disposable electrode and a new gasket.
19. Reconnect the detection cell to the column and perform separation. Repeat cleanup and install a new column if the peak area is still lower than normal.

5.7. Reconditioning of Gold Electrodes

NOTE	***The following procedures apply only to non-disposable gold working electrodes.*** ***Do not recondition disposable electrodes.***

5.7.1. Mechanical Polishing

1. Polish with coarse polishing compound (P/N 36319) for 10 minutes with as much strength as you can sustain.
2. Apply several mL of water to a fresh polishing pad (P/N 36121) and 'polish' for one minute. This step removes the coarse polishing powder particles imbedded in the gold material.
3. Polish with fine polishing compound (P/N 36318) for at least 10 minutes.
4. Apply several mL of water to a fresh polishing pad and 'polish' for 1 minute. This step removes the fine polishing powder particles imbedded in the gold material.
5. Reassemble the ED cell and apply an appropriate Table 1 waveform under initial conditions. If necessary, wait for at least 24 hours for the response to stabilize. In many cases, it is useful to wait overnight. Repeat the entire polishing procedure until the background drops below 30 nC, or glucose response increases above 2 nC/min

5.7.2. Sanding of Receded Gold Working Electrodes

NOTE	***This entire procedure should be used only for seriously damaged or receded non-disposable gold working electrodes. Do not sand disposable gold electrodes.***

Sanding off of the gold electrodes is always done with a subsequent coarse and fine polishing as described above.

The only reason to sand off an electrode is to make the gold electrode flush with the KEL-F surface.

Use a fresh 600-grit sand paper. Make sure that the KEL-F surface remains planar. If the surface is not planar, the ED cell will leak. The cell gasket will not have the required uniform seal around the entire flow path inside the assembled cell.

Sand for less than 1 minute (continuous sanding only to bring the KEL-F to the same level as gold), rinse off the powder residue with deionized water. Polish the rinsed electrode on a clean polishing pad (P/N 36121) with deionized water to remove last traces of the powder residue. Rinse the water again.

5.7.3. Chemical Reconditioning of Gold Working Electrodes

The chemical method of reconditioning removes chemical contamination from the non-disposable working electrode surface and restores the electrode performance. Disposable electrodes should simply be replaced. If the electrode has been passivated by excessive gold oxide formation (see, for example, section 5.2), the chemical cleaning will not restore the electrode performance.

5.7.3.1. Preparation of Chromic Acid

SAFETY	***Chromic acid is corrosive and carcinogenic.*** ***Follow all usual precautions and proper disposal procedures.*** ***Wear gloves and safety glasses whenever handling chromic acid solutions.***

Dissolve/suspend 1 gram of sodium chromate in 1 mL water in a 100 mL glass beaker, slowly add 10 mL of concentrated sulfuric acid with constant stirring. Store the solution in a suitable glass vessel. When used for the first time, transfer approximately 10 mL of chromic acid from the glass vessel into a 20 mL glass scintillation vial, then screw the cap on. After that, the chromic acid solution can be returned to the closed glass vessel and stored for future use.

5.7.3.2. Reconditioning of Electrodes

WARNING	***Before, during and after the reconditioning, avoid any skin contact with the gold electrodes.***

Put the working electrode on a clean filter resting on a horizontal surface. Using a fresh glass transfer pipette, apply one or two droplets of chromic acid to the electrode surface. The chromic acid should form a hemisphere (approximately 2-3 mm in diameter) covering the entire gold surface and surrounding polymeric material. Leave the reagent in place for 10 minutes. Rinse the chromic acid off with DI water, then rinse the entire electrode with water again and dry it with a clean airflow.

5.7.4. Failed pH/Ag/AgCl Reference Electrode

The first indication that a pH/Ag/AgCl reference electrode has failed is a pH readout outside of the expected range of pH 12-13. A reference electrode can be irreversibly damaged by drying out. This happens most frequently by leaving the reference electrode inside a disconnected ED. Always remove the reference electrode when the system is not in proper use (i.e. cell inlet and out let are not plugged or connected to a flowing eluent). After removal from the ED cell, keep the pH/Ag/AgCl reference electrode immersed in 3 M KCl solution (224 g KCl/L) at all times. The PdH electrode is stored dry.

With a "known good" reference electrode it is possible to carry out one of the following checks of the reference electrode being used in the ED cell:

Immerse the "known good" reference electrode and the tested electrode into the same 0.1 M KCl solution. Using a voltmeter, measure the potential between the two electrodes. Discard and replace any tested electrode that differs by more than 30 mV from the "known good" Ag/AgCl reference.

Simply replace the electrode you wish to check with a "known good" reference electrode inside the electrochemical detector. Apply the voltage to the cell. Discontinue using the checked electrode if insertion of the "known good" electrode decreased the background from > 20 nC to <20 nC.

NOTE	***Improper storage of electrodes will cause damage to the electrode***

Immediately remove the "known good" electrode and store it properly. This referencing procedure will work as long as you do not leave your "known good" electrode inside the ED cell for more than a few minutes at a time and store it properly (immersed in 3 M KCl) in the intervening periods of time.

SECTION 6 - GOOD PRACTICES FOR SUCCESSFUL HPAE-PAD

6.1. Good Practices

1. Always use a guard column
2. Keep tubing lengths to a minimum and change tubing from the injector to the column, between columns, and to the detector at least once a year.
3. Change the autosampler needle every 6 months to a year.
4. Regularly test the autosampler to ensure that it is functioning properly (see TN40, "Glycoprotein Monosaccharide Analysis Using High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD)," for an example)
5. Develop all new methods with waveform A, the quadruple waveform (see TN21, "Optimal Settings for Pulsed Electrochemical Detection of Carbohydrates Using the Dionex ED40 Electrochemical Detector.").
6. Establish a system suitability standard, such as the mix of 6 monosaccharide's used by Dionex for the test chromatogram.
7. Change the reference electrode every 3 months and be sure to calibrate it.
8. Disconnect the column from the cell when cleaning the column
9. Control the column temperature to 45 °C and the cell temperature to 25 °C.
10. Calibrate the pump flow rate every three months and repair (pump seals, check valves) as necessary.

6.2. System Parameters to Monitor

When your system has been installed and optimized, note the values of the following parameters. These values can be checked periodically to ensure that the system is still functioning optimally.

6.2.1. System backpressure

1. System background under your running conditions. This value is typically 20 –35 nC using the quadruple waveform.
2. Baseline noise for 1 minute intervals, should range from 30–100 pC peak-to-peak.
3. The pH reading of the pH/Ag/AgCl reference electrode should be between 11 and 12.5 for 10 mM KOH. If there is no reading, the reference electrode has failed.
4. Mannose asymmetry should be less than 1.2, typically. If it is greater than 1.2, then the probable source of the problem is the water source. This can be confirmed by installing a BorateTrap to see if the asymmetry improves

6.3. Shutting Down an HPAE-PAD System

1. For short-term (a few days) turn off the cell, stop the flow or flow a strong eluent (100 mM KOH or stronger) at a low flow rate.
2. For long-term, fill the column with strong eluent, remove from the system and plug the ends.
3. Remove the reference electrode and put it in its container with a saturated KCl solution (see the detector manual for more information). PdH reference electrodes are stored dry.
4. Pump water through the system (each channel used). This can be done at 2 mL/min when no column is in-line. Flush the autosampler.

6.4. Restarting an HPAE-PAD System

1. Pump water at 2.0 mL/min (no column in-line) through each eluent channel, or withdraw at least 20 mL from each line using a priming flow rate.
2. Replace the water with the appropriate eluent and pump the eluent through each line at 2 mL/min to replace the water in the lines, or withdraw at least 20 mL from each line using a priming flow rate.
3. Install the column, wash with strong eluent at 1 mL/min for 30 minutes into waste
4. Reinstall the reference electrode.
5. Flush the autosampler, make sure the flush line is free of air bubbles.
6. Run the system with strong eluent and evaluate the background.

SECTION 7 - INSTRUMENT CONFIGURATION FOR HIGH-CONCENTRATION SAMPLES

It is highly recommended that the 400nL injection valve (P/N 072050) with a 15 mil gasket be used for high concentration samples (>80g/L).

A 2µL sample loop and 15mil ED gasket can be used on the system (instead of the 400nL low volume injection valve),for lower concentration samples. High-concentration samples can still be analyzed on the column, however, greater dilutions will be needed to stay in the linear region.

Dilution accuracy can be improved by diluting by weight rather than volume.

7.1. How to change the sample loop

1. Stop the pump flow.
2. Loosen the sample loop end-fittings. Take out the original sample loop.
3. Install the new sample loop with provided fittings and ferrules. Tighten the fittings.
4. Turn on the pump. Gradually increase the flow rate to check whether there is a liquid leakage. If leakage is found, loosen the fittings, take out the sample loop and install it again.
5. Tighten the fittings till no leakage is observed.
6. Change the sample loop size in the system.
   For Dionex AS:
   1) Disconnect the AS from Chromeleon.
   2) Press Menu.
   3) Press 5 Module Set-up menu.
   4) Press 3 Plumbing Configuration.
   5) Change the loop size to 2µL, press Enter.
   6) Press Menu to go back to the main screen.

7.2. How to change the ED gasket

1. Turn off the ED cell voltage if it is on. Stop the pump flow.
2. Uninstall the ED cell assembly. Loosen the knob (for ED), or the wing nuts (ED40 and ED50) to disassemble the cell. Carefully remove the gold electrode and take out the regular gasket.
3. Carefully attach the 15mil gasket onto the cell body in the same way as for the regular gasket. Both sides of the 15mil gasket are the same so there is no orientation. Install working gold electrode, spacer block (for disposable electrode) and then tighten the knob or wing nuts.
4. Install the ED cell. Turn on ED cell voltage and pump flow to start analysis.
5. The regular gasket and the 15mil gasket can be saved and reused.

SECTION 8 - HPAE-PAD CARBOHYDRATE RESOURCES

None of these resources specifically use the CarboPac SA10; however, the following Technical Notes, Application Notes and articles can be used to determine starting conditions for separations on the CarboPac SA10. The CarboPac SA10 is designed to have shorter run times and higher efficiencies.

8.1. Basic HPAE-PAD Resources

1. Dionex Technical Note 20, "Analysis of Carbohydrates by High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD)"
2. Dionex Technical Note 21, "Optimal Settings for Pulsed Electrochemical Detection of Carbohydrates Using the Dionex ED40 Electrochemical Detector"
3. Rocklin R.D., et al. (1998), Anal. Chem., 70, 1498-1505 (Quadruple waveform)

8.2. HPAE-PAD Monosaccharide Resources

1. Dionex Technical Notes 30, 40, 53
2. Dionex Application Notes 117, 122
3. Application Update 125
4. Weitzhandler, M., et al. (1996) Anal. Biochem., 241, 128-134 (AminoTrap Paper)
5. Weitzhandler M., et al (1996) Anal. Biochem., 241, 135-136 (BorateTrap Paper)
6. Hanko V. and Rohrer J., (2000), Anal. Biochem., 283, 192-199 (Fermentation Broths)
7. Rohrer J., (2000), Anal. Biochem., 283, 3-9 (sialic acids)
8. Rohrer J., et al. (1998), Glycobiology, 8, 35-43 (sialic acids)

