Technical Note 21

Optimal Settings for Pulsed Amperometric Detection of Carbohydrates Using the Dionex ED40 Electrochemical Detector

INTRODUCTION

Pulsed amperometry is a powerful detection method for carbohydrates that requires no sample derivatization. Pulsed amperometric detection (PAD) is particularly useful in combination with high-performance anion-exchange chromatography (HPAE). For a full description of HPAE-PAD, refer to Dionex Technical Note 20. For an introduction to amperometry refer to *Conductivity and Amperometry*, by Roy D. Rocklin, Ph. D. (P/N 34358), or Chapter 6 of the *ED40 Manual.* All are available from your Dionex representative.

At high pH, carbohydrates are electrocatalytically oxidized at the surface of the gold electrode by application of a positive potential. The current generated is proportional to the carbohydrate concentration, and therefore carbohydrates can be detected and quantified. If only a single potential is applied to the electrode, oxidation products gradually poison the electrode surface. This electrode surface poisoning causes a loss of analyte signal. To prevent signal loss, the electrode surface is cleaned by a series of potentials that are applied for fixed time periods after the detection potential. A series of potentials applied for defined time periods is referred to as a waveform. Repeated application of a waveform is the basis of pulsed amperometry. The potentials of a waveform are designated E_1, E_2, E_3, etc., where E_1 is the detection potential. The remaining potentials clean and restore the electrode for subsequent detection. Potentials are maintained for time periods t_1, t_2, t_3, etc. The first time period (t_1) is subdivided into t_{del} and t_{det}. The delay period, t_{del}, is the time that is allowed for the charging current (produced when changing potentials) to decay so that only current from analyte oxidation is measured during the detection period, t_{det} (see Figure 1).

Optimal values for all waveform parameters are determined by systematic variation of one parameter while holding the other parameters constant. An excellent discussion of the optimization of pulsed amperometry waveforms was published by LaCourse and Johnson .[1]

Dionex supports three waveforms for carbohydrate analysis. This Technical Note discusses each waveform with special emphasis on their benefits and liabilities. Figure 1 shows a schematic representation of each waveform. The ED40 electrochemical cell is equipped with a combination pH-Ag/AgCl reference electrode. The potentials presented in this Technical Note require use of the Ag/AgCl half of the reference electrode.

WAVEFORM A

This Technical Note introduces Waveform A (Table 1 and Figure 1, Panel A), a new waveform for carbohydrate analysis that dramatically improves long term reproducibility. This waveform differs from Waveforms B and C in that it uses negative rather than positive potentials for electrode cleaning. When positive cleaning potentials are used there is a gradual decrease in carbohydrate peak areas over time due to working electrode wear (recession below the plastic housing). Waveform A minimizes electrode wear. This is demonstrated by comparing Figures 2 and 3. Figure 2 shows a two week repetitive analysis of monosaccharide standards (100 pmol each) using a new working electrode and Waveform B which uses a positive potential for electrode cleaning. Figure 3 shows the same analysis using Waveform A. While there is a gradual decrease in peak area response using Waveform B, response is constant using Waveform A. Waveform A provides the best reproducibility of absolute electrochemical response.

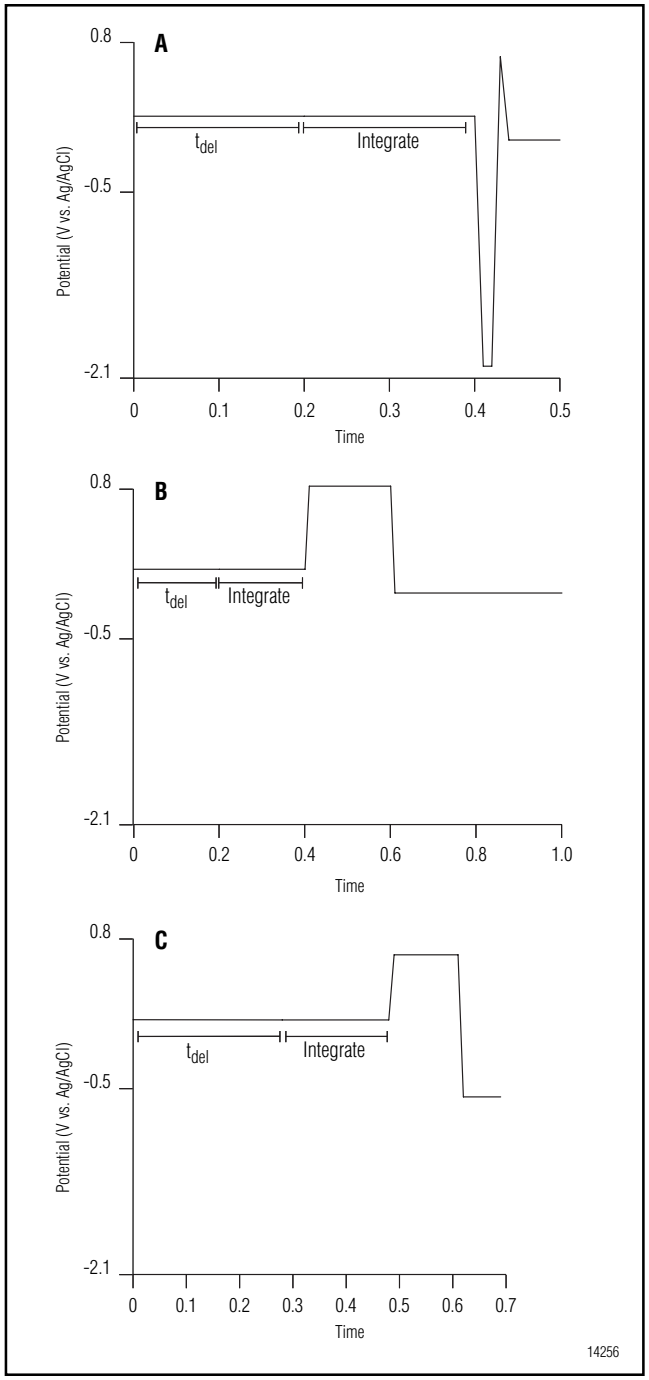

Figure 1 *Schematic representation of Waveforms A, B, and C.*

The detection potential, E_1, of Waveform A is 0.1 V. This potential is maintained for 400 ms. The first 200 ms is t_{del} and the second 200 ms is t_{det}. The working electrode is cleaned for 10 ms at -2.0 V (E_2). This is followed by a quick excursion to 0.6 V (E_3). The latter potential forms a small amount of gold oxide that was found to be necessary to maintain an active working electrode surface. These two steps, reductive cleaning and gold oxide formation, require only 40 ms. The final potential of this waveform, E_4, is -0.1V, and it is required to reduce the small amount of gold oxide formed in E_3. This potential is maintained for 60 ms and therefore this waveform requires a total of 500 ms. The development of Waveform A is described in a publication by Rocklin et al.[2] For more details on the theory of cleaning at negative potentials see the publication by Jensen and Johnson.[3]

The greatest benefit of using Waveform A is consistent long term peak area response. This is beneficial when comparing two or more systems that are analyzing monosaccharides. Because Waveform A requires only 500 ms, data can be collected at 2 Hz. This is twice the data collection rate of Waveforms B and C. Because Waveform A allows twice as many data points per peak, sharp, early eluting peaks (e.g., fucose) are detected with greater reproducibility. A further advantage of Waveform A is that it is less subject to interference from electroactive amino acids than Waveforms B and C.

There are some small disadvantages associated with Waveform A. Though the response is, in some cases, higher with Waveform A compared to Waveform B, the noise is also higher. Taken together, the minimum detection limits using Waveform A are usually not as low as those found with Waveform B. Waveform A has a greater sensitivity to dissolved oxygen and therefore higher backgrounds (16–22 nC) and higher noise. This may be apparent when using the CarboPac™ PA1, where the baseline dip, due to reduction of dissolved oxygen, is between glucosamine and mannose. The CarboPac PA10 places the baseline dip, due to dissolved oxygen, after the carbohydrate elution window.

When using Waveform A, there is occasionally a small dip observed after amino sugars (see Figure 4). This dip is largest after glucosamine, but is also observed after

Table 1 Waveform A

Time	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	

galactosamine and mannosamine. Dips are not observed after the acetylated amino sugars *N*-acetylglucosamine and *N*-acetylgalactosamine. Increasing E_1 to 0.15 V minimizes this dip, though the long term (> 1 month) effect of this change on peak area reproducibility has not been measured.

Waveform A requires a GP40 or GP50 pump with the vacuum degas option installed and a new working electrode. Polish the working electrode before installing it in the ED40 cell. The procedure for polishing the working electrode can be found in the ED40 manual. Waveform A can be programmed with PeakNet software (release 5.0 or higher) or through the detector's front panel. Set the data collection rate to 2 Hz. Set the pump to degas for 30 s every 4 min. During the first day peak areas may increase as the working electrode surface is activated. This increase may be observed anytime the electrode is polished. Only polish new working electrodes and those believed to be fouled. Evidence of fouling is visible electrode discoloration or a decrease in peak area response that occurs without working electrode wear.

WAVEFORM B

Waveform B (Table 2 and Figure 1, Panel B) has been a recommended waveform since the introduction of the ED40 in 1993. This waveform was developed to increase sensitivity, minimize the sensitivity to dissolved oxygen, and minimize baseline drift when separating oligosaccharides with sodium acetate gradients. Waveform B provides the greatest carbohydrate sensitivity, the least sensitivity to dissolved oxygen, and is equivalent to Waveform A in baseline drift using sodium acetate gradients. Because Waveform B uses oxidative cleaning (E_2 = 0.75V), there is working electrode wear and a gradual decrease in carbohydrate peak area over time (Figure 2). Even with this peak area decrease, quantitative carbohydrate analyses are possible by using internal standards and regularly spaced external standards.[4, 5]

Table 2 Waveform B

Time	Potential (V)	Integration
0.00	+0.05	
0.20	+0.05	Begin
0.40	+0.05	End
0.41	+0.75	
0.60	+0.75	
0.61	-0.15	
1.00	-0.15	

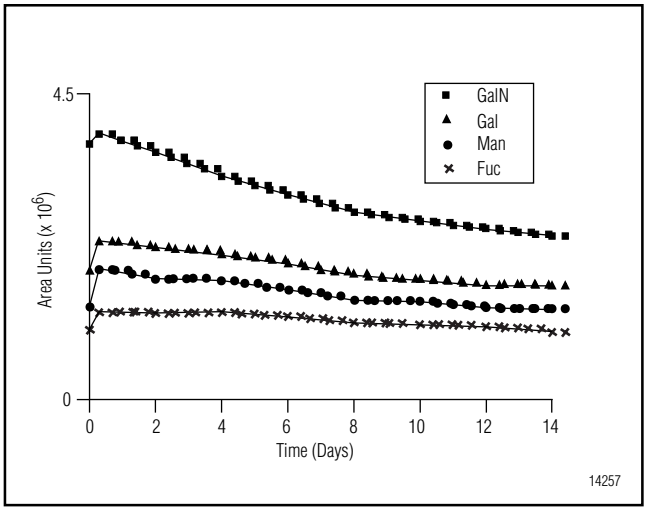

Figure 2 *Carbohydrate response as a function of time for a freshly sanded and polished electrode, using Waveform B.*

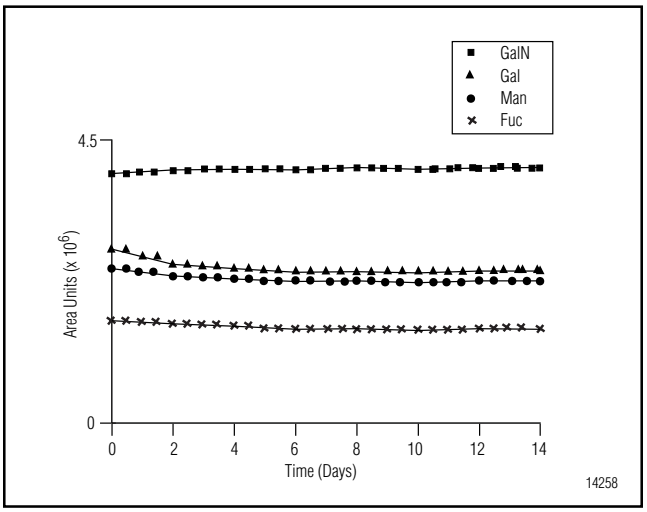

Figure 3 *Carbohydrate response as a function of time for a freshly sanded and polished electrode, using Waveform A.*

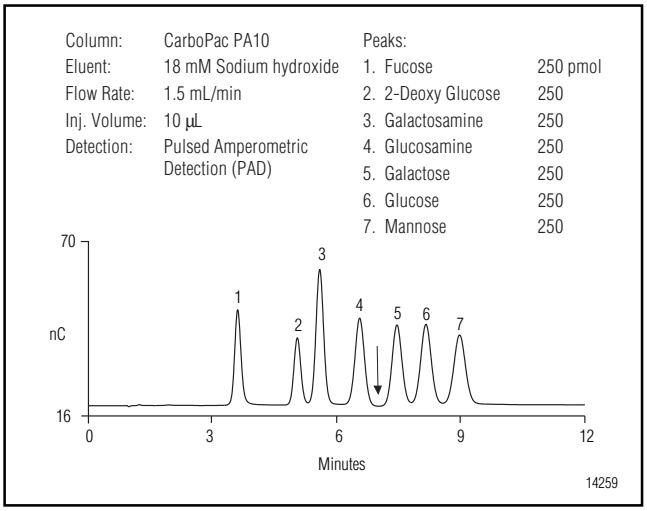

Figure 4 *A small dip is occasionally observed after amino sugars when using Waveform A.*

WAVEFORM C

Waveform C (Table 3 and Figure 1, Panel C) has been a recommended waveform since 1990. This waveform is in widespread use and gives good results when used with standard isocratic HPAE conditions and where maximum sensitivity is not needed. Because Waveform C uses oxidative cleaning (E_2 = 0.60 V), there is working electrode wear and a gradual decrease in carbohydrate peak area over time, but this does not preclude quantitative carbohydrate analyses.[4,5] Using this waveform, the baseline dip due to dissolved oxygen is similar to that found with Waveform A.

Table 3 Waveform C

Time	Potential (V)	Integration
0.00	+0.05	
0.28	+0.05	Begin
0.48	+0.05	End
0.49	+0.60	
0.61	+0.60	
0.62	-0.60	
0.69	-0.60	

SUMMARY OF WAVEFORM CHARACTERISTICS

Long Term Peak Area Reproducibility	A >> C > B
Carbohydrate Detection Limits	B > A > C
Lack of Sensitivity to Dissolved Oxygen	B > C = A
Reproducibility for Early Eluting Peaks	A > B = C
Baseline Drift During Sodium Acetate Gradient	B ≥ A > C

OTHER CONSIDERATIONS

For best results the reference electrode should be changed every six months. When the reference electrode will not be used for a few weeks, it should be stored in its container in a saturated KCl solution.

REFERENCES

1. LaCourse, W.R. and Johnson, D. C. *Anal. Chem.* **1993**, *65*, 50–55.
2. Rocklin, R.D., Clarke, A.P., and Weitzhandler, M. *Anal. Chem.* **1998**, *70*, 1496–1501.
3. Jensen, M.B. and Johnson, D. C. *Anal. Chem.* **1997**, *69*, 1766–1781.
4. Rohrer, J., Thayer, J., Avdalovic, N., and Weitzhandler, M. *Techniques in Protein Chemistry VI.* **1995**, 65–73.
5. Rohrer, J. S., Thayer, J., Weitzhandler, M., and Avdalovic, N. *Glycobiology*. **1998**, *8*, 35–43.

Dionex Corporation
1228 Titan Way
P.O. Box 3603
Sunnyvale, CA
94088-3603
(408) 737-0700

Dionex Corporation
Salt Lake City Technical Center
1515 West 2200 South, Suite A
Salt Lake City, UT
84119-1484
(801) 972-9292

Dionex U.S. Regional Offices
Sunnyvale, CA (408) 737-8522
Westmont, IL (630) 789-3660
Houston, TX (281) 847-5652
Atlanta, GA (770) 432-8100
Marlton, NJ (609) 596-0600

Dionex International Subsidiaries
Austria (01) 616 51 25 *Belgium* (015) 203800 *Canada* (905) 844-9650 *France* 01 39 46 08 40 *Germany* 06126-991-0
Italy (06) 66030052 *Japan* (06) 6885-1213 *The Netherlands* (0161) 434303 *Switzerland* (062) 205 99 66 *United Kingdom* (01276) 691722
* Designed, developed, and manufactured under an NSAI registered ISO 9001 Quality System.

http://www.dionex.com

LPN 034889-03 3.5M 10/98