Pulsed Amperometric Detection with the ϵpsilon™ LCEC Detector

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Pulsed electrochemical detection (PED) encompasses a sophisticated set of electrochemical techniques for determining polar aliphatic compounds such as carbohydrates, polyalcohols, amines, and sulfur compounds (1). Many of these compounds lack a chromophore, so UV-Vis detection is precluded. Amperometric detection is possible, but typically leads to diminished response due to fouling of the electrode surface (2). One PED technique, pulsed amperometric detection (PAD), intersperses millisecond-long cleaning pulses with the detecting potential, thus preventing electrode fouling.

LC determination of carbohydrates and polyalcohols is of interest to both the food and medical industries. Analytes include simple sugars like glucose and galactose in plasma (3), oligosaccharides like glucuronic acid in urine (4), and complex samples such as bile acids (5) and wood pulp (6). Although PAD seems complex, the epsilon™ system makes this a relatively easy technique for the chromatographer.

T1

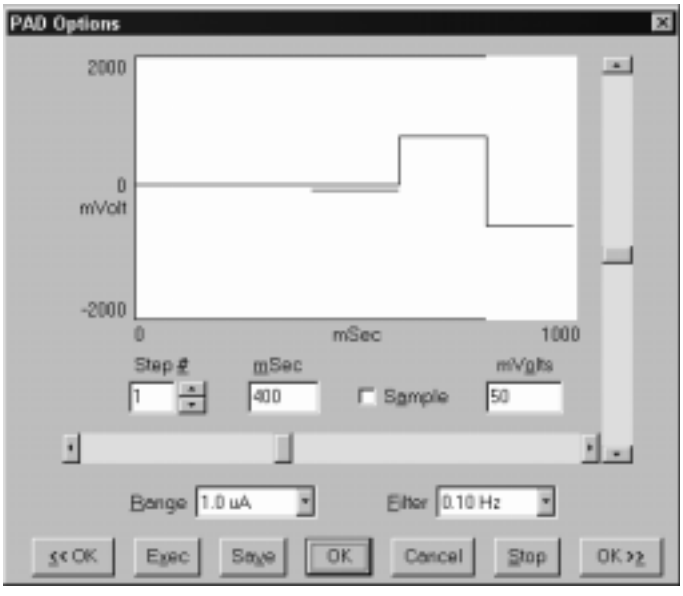
Waveform for the detection of carbohydrates and polyalcohols.

** indicates the sampling period.*

Interval (msec)	**Potential** (mV vs. Ag/AgCl)
400	50
200*	50
200	800
200	-600

F1

Control screen showing how the PAD waveform is defined. Detector output is collected only during the sampling period (underscored area).



How PAD Works

PAD is simply a series of potentials applied to the electrode. ***F1*** (the control screen from the epsilon detector) shows these potentials graphically, while ***T1*** lists them out. This is a typical waveform suitable for detection of sugars at high pH on a gold electrode. The series of potentials lasts about a second, with typically three or four discrete steps. The cycle repeats constantly.

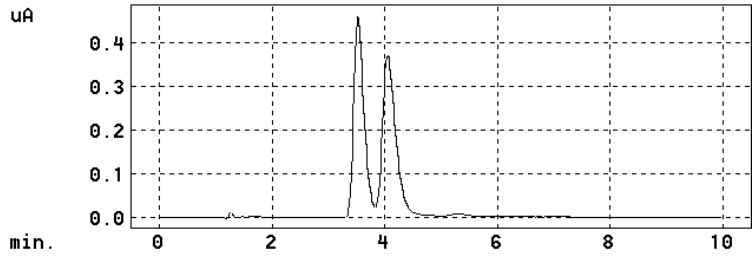
The first step of the cycle is an equilibration period, when the electrode stabilizes after the previous pulse. Step two, the detecting step, occurs next. It is the only time the output from the detector is saved as data. Step three is a cleaning step, during which any compounds adhering to the electrode surface are oxidized with a high potential. The gold surface is oxidized as well. In step four the gold oxide is reduced back to native gold, and then the cycle begins again.

Conditions

Detector: BAS epsilon system
Electrode: BAS dual 3 mm gold
Waveform: See ***T1***
Column: Hamilton RCX-10 anion exchange
Pump: BAS PM-80
Mobile Phase: 100 mM NaOH
Flow Rate: 1.5 mL/min
Injection Vol.: 10 µL

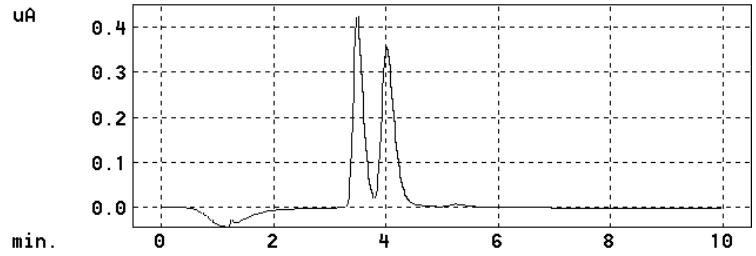
F2

Direct injection of cranberry juice diluted 1:10,000 in water. Peaks are glucose and fructose.



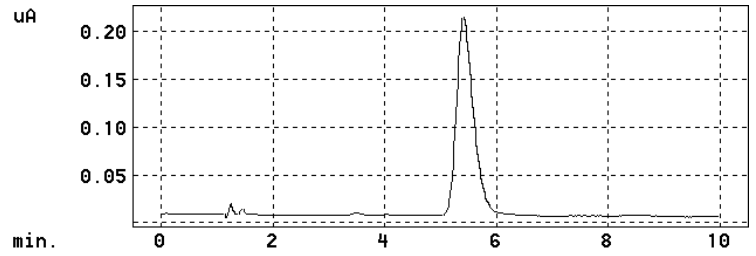
F3

Direct injection of cola soda diluted 1:10,000 in water. Peaks are glucose and fructose.



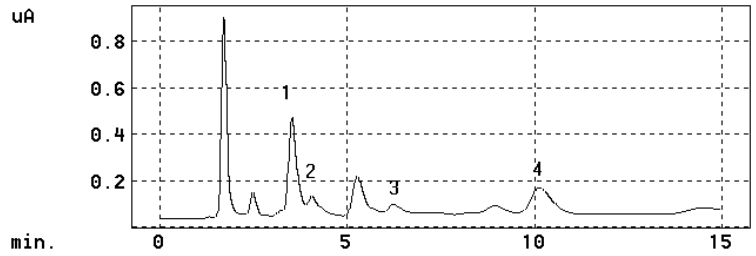
F4

Direct injection of skim milk diluted 1:10,000 in water. Peak is lactose.



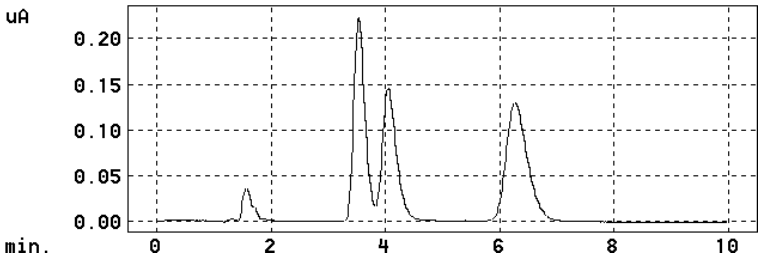
F5

Direct injection of beer diluted 1:10,000 in water. Identified peaks are glucose (1) fructose (2) sucrose (3) and maltose (4).



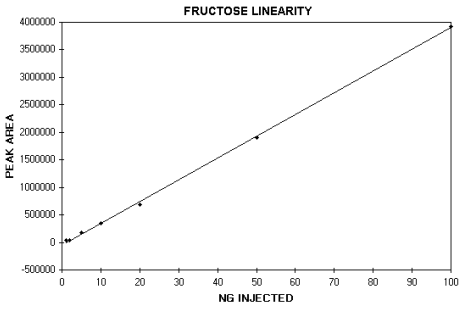
F6

Injection of orange juice diluted 1:10,000 in water and centrifuged to remove solids. Peaks are glucose, fructose, and sucrose.



F7

Linearity of detector response to injected fructose standards. $r^2 = 0.999$.



Results

Chromatograms of various samples are depicted in ***F2-F5***. Repeated injections of fructose showed a precision of 2.5%, and detector response to fructose was linear from 1 to at least 100 ng injected (***F7***).

Conclusion

The epsilon system is a flexible platform that allows a variety of electrochemical techniques to be performed. A chromatographer can switch from sensitive multichannel determinations of neurotransmitters in brain microdialysates to the determination of sweeteners in children's antibiotics, with just a few clicks of the mouse. (Well, there is the matter of changing the column and mobile phase!)

References

1. *W.R. LaCourse, "Pulsed Electrochemical Detection in High-Performance Liquid Chromatography," Wiley, New York, 1997, 324 p.*
2. *C.M. Zook, W.R. LaCourse, Current Separations 14 (1995) 48.*
3. *B.W. Griffin, G.W. Williams, Biomed. Chromatogr. 4 (1990) 87.*
4. *G.O.H. Peelen, J.G.N. de Jong, R.A. Wevers, Clin. Chem. 40 (1994) 914.*
5. *M.F. Chaplin, J. Chromatogr. B 664 (1995) 431.*
6. *J. Sullivan, M. Douek, J. Chromatogr. A 671 (1994) 339.*