ELSEVIER

Journal of Chromatography A, 671 (1994) 109–114

JOURNAL OF CHROMATOGRAPHY A

Maximizing signal-to-noise ratio in direct current and pulsed amperometric detection

Roy D. Rocklin *, Tom R. Tullsen, Mark G. Marucco

Dionex Corporation, 1228 Titan Way, Sunnyvale, CA 94088-3603, USA

Abstract

The magnitude of signal obtained during d.c. and pulsed amperometric detection using a thin-layer type cell is dependent on several factors, two of which are controlled by the cell design. These two factors are the surface area of the working electrode and the mobile phase velocity over the surface of the working electrode. Mobile phase velocity is controlled by the thickness and width of the thin-layer channel gasket. In this report, the effect of varying working electrode size and gasket dimensions are studied. Using 1 mm diameter working electrodes and a 25 μm $\times$ 1.3 mm gasket, the minimum detection limit for dihydroxybenzylamine is about 6 femtomoles and for glucose, about 200 femtomoles.

1. Introduction

The most commonly used amperometric detector cell design is the thin-layer cell. In a typical construction, a thin gasket with a slot cut in the middle is sandwiched between two blocks: one a metal which forms the counterelectrode and the other a flat disk working electrode surrounded by insulating plastic. The block containing the working electrode is made by force-fitting a rod of working electrode material into the plastic block and polishing the surface to a flat finish. The slot cut in the gasket forms the thin-layer channel. The column effluent enters the thin-layer channel, rapidly develops laminar flow, and flows across the surface of the working electrode where the analytes are detected. The reference electrode is placed downstream from the working electrode.

Other amperometric detector designs include the use of tubular working electrodes and the "wall-jet" design, in which the column effluent impinges directly on (perpendicular to) the working electrode. Compared to these and other designs, the thin-layer cell has several advantages: (1) Construction is simple, requiring only a means of clamping the two blocks together without the gasket leaking. (2) The thin channel produces high mobile-phase linear velocity over the surface of the working electrode, which produces high signal magnitude. (3) The working electrode is easily cleaned by polishing. (4) The internal volume of the thin-layer channel can be made very low, thus minimizing peak dispersion contributed by the detector and efficiently sweeping out of the cell high-concentration components. (5) Cell resistance is very low due to the short distance between the working and counterelectrodes. This prevents voltage drop from lowering the actual potential on the working electrode and increases the linear calibration

* Corresponding author.

0021-9673/94/$07.00
SSDI 0021-9673(93)E1234-Q

range. (6) Any conductor available in rod form can be used as the working electrode.

Several studies have been published on the signal magnitude from thin-layer type amperometric detector cells using d.c. amperometry at a glassy carbon working electrode [1–9]. Equations are derived from fundamental principles of hydrodynamics and then compared to actual data. Eq. 1 has been derived and tested [6] and appears to be an accurate predictor of cell current (i). It is applicable to a thin-layer cell with a circular working electrode whose diameter is the same as the channel width, in which flow is laminar, and for analytes where the current is diffusion controlled; *i.e.* the reaction rate is limited by the rate of diffusion of analyte molecules to the surface of the working electrode and not by the rate of the electron transfer reaction.

$$i = 1.47nFU^{1/3}(DA/h)^{2/3}C \quad (1)$$

The number of electrons transferred in the redox reaction is n, F is the Faraday, U is the volume flow-rate, D is the analyte diffusion coefficient, A is the working electrode surface area, h is the height of the thin-layer channel; *i.e.* the thickness of the gasket, and C is analyte concentration. The equation parameters can be divided into two categories. Parameters n, U, D and C are determined by the analyte and mobile phase characteristics. Working electrode surface area A and thin-layer channel thickness h are the only cell parameters which control detector response. That signal is dependent on electrode surface area is obvious; the greater the surface area, the more analyte molecules can be detected. The dependence on channel height is actually a result of the effect of height on the linear velocity of flow over the surface of the working electrode. With a constant volume flow-rate, linear velocity is inversely proportional to h, so cell current is dependent on linear velocity to the 2/3 power. The effect of increasing linear velocity is to decrease the Nernst diffusion layer thickness. This increases the analyte concentration gradient at the working electrode surface, increasing cell current.

The true goal is maximizing signal-to-noise ratio and not just signal, so determining the effect of cell parameters on noise is just as important as determining their effect on signal. Several reports have dealt with the effect of cell parameters on both signal and noise [1,3,5,7,8]. In general, noise was found to be directly proportional to working electrode surface area. Signal-to-noise ratio should actually improve slightly as the working electrode surface area is decreased. However, for very small working electrodes, electronics noise is the limiting factor [5].

In this report, the effects of thin-layer channel thickness and working electrode size on signal-to-noise ratio are studied. In addition to theoretical considerations, practical limitations on these parameters are discussed. Two amperometric detection methods are used: d.c. amperometric detection of catecholamines using a glassy carbon working electrode and reversed-phase chromatography; and pulsed amperometric detection of carbohydrates using a gold working electrode and high-pH anion-exchange chromatography.

These two methods were selected for study because they are two of the most common applications of amperometric detection. In addition, the dependence of signal-to-noise ratio on cell design parameters for pulsed amperometry had not yet been addressed. Publications discussing the determination of catecholamines in urine and plasma appear frequently. Two recent articles deal with mobile phase selection [10] and sample preparation [11]. The principles of pulsed amperometric detection and how it is used to determine carbohydrates, alcohols, amines and sulfur species are discussed in two recent reviews [12,13].

2. Experimental section

Glassy carbon working electrodes with diameters of 1 and 3 mm were tested. Smaller or intermediate sizes were not available. Three gold working electrodes were tested; their diameters were 0.5, 1 and 3 mm. Thin-layer channel gaskets ranged from 25 to 125 μm thickness.

All chromatography was performed using Dionex liquid chromatography equipment. Both

Table 1
Potential *vs.* time waveform used to detect carbohydrates

Time (s)	Potential (V)	Integrate
0.00	0.10	
0.20	0.10	Begin
0.40	0.10	End
0.41	0.70	
0.60	0.70	
0.61	−0.10	
1.00	−0.10	

an Advanced Gradient Pump and a DX-500 GP40 gradient pump were used. Flow-rate was 1.0 ml/min and injection volume was 20 μl. The detector was a prototype version of the DX-500 ED40 electrochemical detector. Catecholamines were detected at 0.65 V *vs.* Ag/AgCl reference electrode. The mobile phase for catecholamines consisted of 57 m*M* citric acid, 43 m*M* sodium acetate, 0.1 m*M* disodium EDTA, 1 m*M* octanesulfonic acid (Dionex MPIC-CR2), and 20% methanol. The column was a Zorbax C-18 5 μm, 250 × 4.6 mm. Carbohydrates were detected using the potential *vs.* time waveform listed in Table 1. A CarboPac PA1 250 × 4 mm column was used with 100 m*M* sodium hydroxide mobile phase. All chemicals were reagent grade. Catecholamines were obtained from Aldrich (Milwaukee, WI, USA). Citric acid, sodium acetate, and disodium EDTA were obtained from Fluka (Buchs, Switzerland). Sodium hydroxide was made from 50% concentrate from Fisher (Pittsburgh, PA, USA).

Noise was measured peak-to-peak over a 2-min period of flat baseline. Each point for signal and noise in the tables is the average of six measurements.

3. Results and discussion

D.c. amperometry is routinely used to detect catecholamines [10,11]. A typical example is the chromatography of urinary catecholamines as shown in Fig. 1. Dihydroxybenzylamine (DHBA) is commonly used as an internal standard, and was chosen to be used as the standard

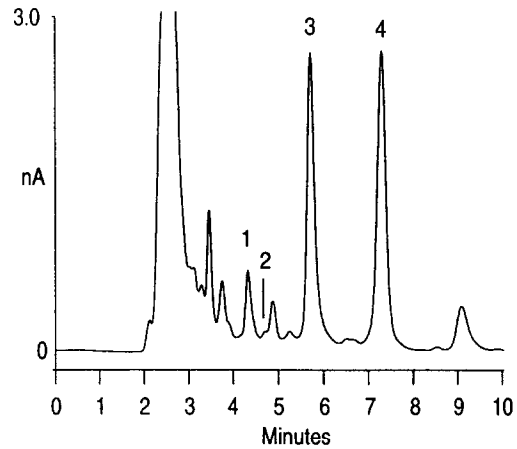

Fig. 1. Separation of catecholamines in alumina extract of urine. Catecholamines from 0.5 ml of urine diluted to 5 ml with 0.6 *M* Tris buffer at pH 8.5 plus 6.3 ng DHBA internal standard were adsorbed on 0.1 g activated alumina. Following a 10-min mixing period in an ice bath, the alumina was washed three times with 5 m*M* acetate buffer at pH 7.0. Catecholamines were released by rinsing the alumina with 0.2 ml of 0.3 *M* acetic acid. This solution was centrifuged and diluted to 1 ml for analysis. A 1 mm diameter glassy-carbon working electrode was used with a 25 μm thick by 1.3 mm wide gasket. Peaks: 1 = norepinephrine, 2 = epinephrine, 3 = DHBA, 4 = dopamine.

compound for measuring signal-to-noise ratio in this study. Glucose was used as the standard compound for pulsed amperometric detection at a gold working electrode.

3.1. Channel thickness

Signal (peak height), baseline noise, and the resulting signal-to-noise ratios for DHBA as a function of thin-layer channel thickness are listed in Table 2. Detection is by d.c. amperometry at a glassy carbon working electrode. The same measurements using glucose with pulsed amperometric detection at a gold working electrode are also listed in Table 2. For both d.c. and pulsed amperometry, noise is independent of channel thickness while signal increases as the channel is made thinner. The obvious conclusion is that the thin-layer channel should be made as thin as possible. The limit on decreasing channel thickness is a result of practical and not theoretical considerations. First, the gasket must be

Table 2
Signal and noise for d.c. amperometric detection of 2 picomoles DHBA at a 1 mm diameter glassy carbon (G.C.) working electrode and pulsed amperometric detection of 2 picomoles glucose at a 1 mm diameter gold working electrode

Channel thickness (μm)	DHBA signal (pA)	G.C. noise (pA)	DHBA S/N	Glucose signal (pC)	Gold noise (pC)	Glucose S/N
25	376	0.38	1000	264	8.4	31
50	273	0.36	760	195	7.0	28
75	209	0.38	550	153	7.5	21
100	188	0.31	610	137	8.5	16
125	161	0.39	410	124	7.8	16

Thin-layer channel width and length were 1.6 mm × 12.7 mm.

strong enough to resist damage during normal use. Gaskets which are too thin stretch and tear too easily to be practical to use. Second, the two halves of the cell tend to deform slightly when squeezed together, and if the gasket is too thin, the working electrode may short against the counterelectrode. These two factors place a practical limit of 25 μm (0.001″) on thin-layer channel thickness with this type of cell design.

Using both d.c. and pulsed amperometry, cell current as a function of thin-layer channel thickness is proportional to $h^{-2/3}$, as predicted by eq. 1. A linear fit to a plot of peak height *vs.* $h^{-2/3}$ using the data in Table 2 produces a standard error of 9 pA for DHBA and 5 pC for glucose. Inspection of the plots shows similarity in residuals between the two plots, suggesting that part of the error is caused by small deviations in gasket thicknesses from nominal values.

3.2. Electrode diameter

Signal and noise measured using glassy carbon working electrodes with two diameters are listed in Table 3. The second and third entries list data obtained using the same thin-layer channel dimensions of 125 μm thickness by 3.5 mm width, referred to as the "large" gasket in the table. Both signal and noise decrease as the working electrode diameter is decreased. Signal-to-noise ratio is similar for the two working electrodes tested. The large gasket dimensions are appropriate for the 3 mm diameter working electrode but unnecessarily large for the 1 mm diameter electrode. Using a thinner and narrower thin-layer channel gasket (25 μm × 1.6 mm) with the 1 mm diameter working electrode increases the signal with no effect on noise, as shown in the first entry. In fact, the major advantage of the smaller diameter working electrode is that the cell gasket can be made narrower, thus increasing the linear velocity of mobile phase over the surface of the working electrode, which increases signal without affecting noise. Also, thinner gaskets can be used more easily with a smaller diameter working electrode without introducing mechanical difficulties such as deformation in the channel causing the working electrode to short against the counterelectrode.

Table 4 lists signal, noise, and signal-to-noise ratio for glucose using three gold working electrodes of 0.5, 1, and 3 mm diameter. Both peak height and baseline noise are proportional to working electrode area. Data in the last three rows in Table 4 were obtained using the "large" gasket. Using this common gasket, the 1 mm

Table 3
Signal and noise for d.c. amperometric detection of 2 picomoles DHBA at glassy carbon working electrodes

Electrode diameter (mm)	Gasket	Signal (pA)	Noise (pA)	S/N
1	Small	376	0.38	1000
1	Large	124	0.38	330
3	Large	674	2.02	334

The dimensions of the small and large gaskets were 25 μm × 1.6 mm × 12.7 mm and 125 μm × 3.5 mm × 12.7 mm.

Table 4
Signal, noise, and signal-to-noise ratio for 10 picomoles glucose at a gold working electrode

Electrode diameter (mm)	Gasket	Signal (pC)	Noise (pC)	S/N
1	Small	1742	11.7	149
0.5	Large	128	4.7	27
1	Large	459	9.2	50
3	Large	3079	70.8	43

The dimensions of the small and large gaskets were 25 μm × 1.3 mm × 6.35 mm and 125 μm × 3.5 mm × 12.7 mm.

diameter electrode produces the best signal-to-noise ratio, probably because a component of electronics noise is present in the 0.5-mm working electrode baseline. It is interesting that even though there is more than one order of magnitude difference in signal between the smallest and largest electrodes, the signal-to-noise ratios are less than a factor of two apart. The large gasket dimensions of 125 μm × 3.5 mm are appropriate for the 3 mm diameter working electrode but are unnecessarily large for the smaller electrodes. Using a 25 μm × 1.3 mm gasket with the 1 mm diameter working electrode, the signal-to-noise ratio is tripled. One might expect that a further increase in signal-to-noise ratio could be achieved using the 0.5-mm working electrode and an even narrower gasket. The difficulty with this approach is machining cell parts such that the working electrode is always placed in the center of the thin-layer channel. The cell must be designed to accommodate some tolerance in the placement of the gasket relative to the working electrode, and this tolerance places a lower limit on gasket width. As a result, it is difficult to gain the advantage of a narrower gasket from the use of a 0.5 mm diameter working electrode.

There are factors to consider other than signal-to-noise ratio when selecting cell design parameters. An example is the comparison of the 3 mm and 1 mm diameter gold working electrodes, as shown in Figs. 2 and 3. A large dip appears in the chromatogram from the 3-mm electrode at approximately 13 min and is barely noticeable using the 1-mm electrode. This dip is caused by

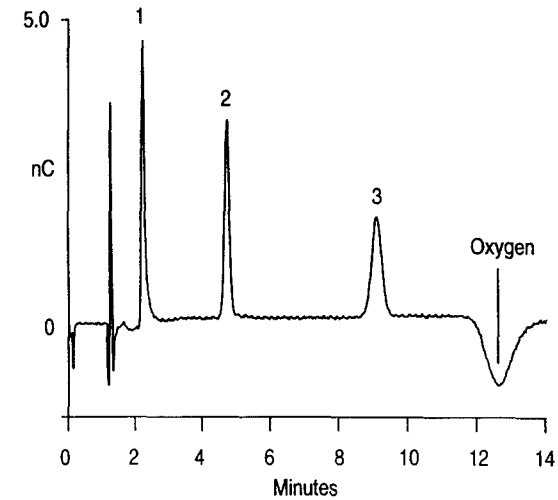

Fig. 2. Pulsed amperometric detection at a 3 mm diameter gold working electrode of 10 picomoles each of (1) sorbitol, (2) glucose and (3) sucrose. Gasket dimensions: 125 μm × 3.5 mm × 12.7 mm.

reduction of oxygen in the sample. Oxygen is reduced at negative potentials in base to hydrogen peroxide, which probably undergoes further reduction to water at the detection potential of 0.1 V. Detection occurs after a delay period of 0.2 s, which allows charging current to decay (see Table 1). It is possible that the 1 mm diameter

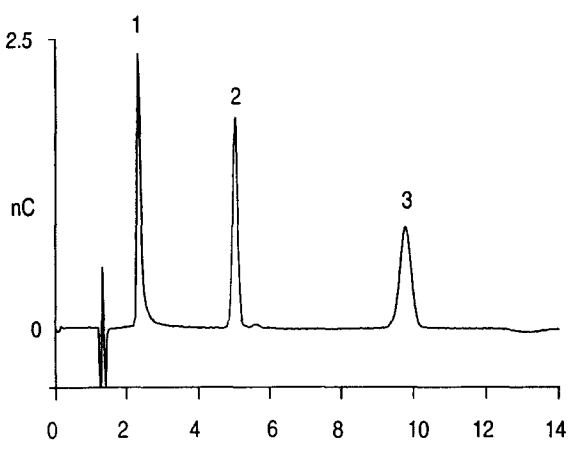

Fig. 3. Pulsed amperometric detection at a 1 mm diameter gold working electrode of 10 picomoles each of (1) sorbitol, (2) glucose and (3) sucrose. Gasket dimensions: 25 μm × 1.3 mm × 6.35 mm.

electrode is much less sensitive to oxygen because peroxide has been swept past the electrode by the time current is measured, while there is still detectable peroxide near the electrode surface with the larger 3-mm electrode. Reduced sensitivity to oxygen is further reason to select the 1 mm diameter gold electrode over the 3-mm electrode. In addition to the minimized oxygen dip, the 1 mm diameter gold electrode is much less sensitive to varying levels of oxygen in the mobile phase caused by incomplete degassing. Varying oxygen levels cause baseline wander if the electrode is sensitive to oxygen.

4. Conclusion

This study supports the conclusion that the cell should be designed with the narrowest and thinnest thin-layer channel gasket that is practical to use and with a working electrode slightly smaller than the gasket width. We have found the practical lower limit for these parameters to be a 25 μm × 1.3 mm thin-layer channel and a 1 mm diameter working electrode. Using a cell with these dimensions, extremely high sensitivities can be achieved, as demonstrated by the excellent signal-to-noise ratio for 10 picomoles each of carbohydrates shown in Fig. 3 and for 100 femtomoles each of four catecholamines shown in Fig. 4. Minimum detection limits can be calculated from the magnitudes of signal and noise in the chromatograms. Based on three times the noise levels of 12 pC using pulsed amperometry and 0.4 pA using d.c. amperometry, the minimum detection limit for glucose is about 200 femtomoles and for dihydroxybenzylamine, about 6 femtomoles.

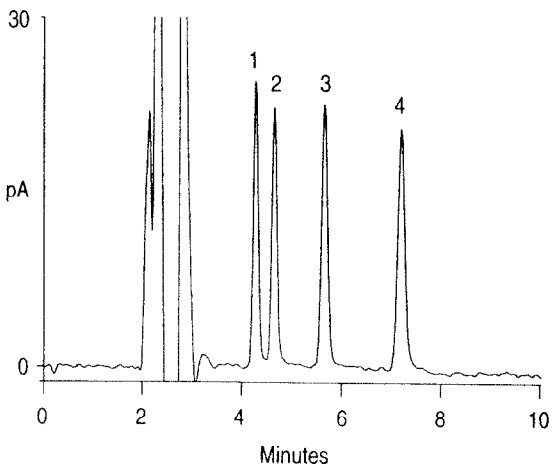

Fig. 4. Separation of 100 femtomoles each of (1) norepinephrine, (2) epinephrine, (3) DHBA and (4) dopamine. Detected using d.c. amperometry at a 1 mm diameter glassy carbon working electrode. Gasket dimensions: 25 μm × 1.3 mm × 6.35 mm.

5. References

[1] M. Righezza and A.M. Siouffi, *Analusis*, 20 (1992) 333.
[2] T.Y. Ou and J.L. Anderson, *J. Electroanal. Chem.*, 302 (1991) 1.
[3] S.G. Weber and J.T. Long, *Anal. Chem.*, 60 (1988) 903A.
[4] S. Prabhu and J. Anderson, *Anal. Chem.*, 59 (1987) 157.
[5] D.M. Morgan and S.G. Weber, *Anal. Chem.*, 56 (1984) 2560.
[6] J.M. Elbicki, D.M. Morgan and S.G. Weber, *Anal. Chem.*, 56 (1984) 978.
[7] H.W. van Rooijen and H. Poppe, *J. Liq. Chromatogr.*, 6 (1983) 2231.
[8] H.B. Hanekamp and H.J. van Nieuwkerk, *Anal. Chim. Acta*, 121 (1980) 13.
[9] S.G. Weber and W.C. Purdy, *Anal. Chim. Acta*, 100 (1978) 531.
[10] P.Y. Leung and C.S. Tsao, *J. Chromatogr.*, 576 (1992) 245.
[11] I.N. Papadoyannis, *HPLC in Clinical Chemistry (Chromatographic Science Series*, No. 54), Marcel Dekker, New York and Basel, 1990, Ch. 15.
[12] D.C. Johnson, D. Dobberpuhl, R. Roberts and P. Vandeberg, *J. Chromatogr.*, 640 (1993) 79.
[13] D.C. Johnson and W.R. LaCourse, *Anal. Chem.*, 62 (1990) 589A.