Use of disposable gold working electrodes for cation chromatography–integrated pulsed amperometric detection of sulfur-containing amino acids

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Abstract

We have prepared disposable thin-film gold working electrodes on polymeric substrates. Our microfabrication process allows for inexpensive and reproducible mass production of such electrodes. We utilize this new type of electrode in flow-through electrochemical cells to replace the conventional non-disposable gold working electrodes for integrated pulsed amperometric detection (IPAD) of compounds separated by high-performance cation-exchange chromatography. Using two S-containing amino acids (homocysteine and cysteine) as test compounds, we have modified a previously reported waveform for optimum performance with disposable gold electrodes. With the help of the same two test substances we have characterized the analytical performance of disposable gold electrodes under the new conditions. Compared to non-disposable working electrodes, the disposable working electrodes generated equal or better results in the limit of detection, linearity of calibration and reproducibility. When used with a new IPAD waveform, the disposable electrodes functioned reproducibly for 3 days. At the end of the specified usage period of 3 days, the disposable electrodes are simply replaced. Reconditioning by polishing is thus no longer required.
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1. Introduction

In 1986, Johnson and co-workers explored the possibility of pulsed amperometric detection (PAD) of S-containing compounds on a platinum electrode under alkaline conditions [1–3]. Their initial focus was on cyclic voltammetry and flow-injection analysis using thiourea as a model compound. In 1992, they also investigated cyclic voltammetry and PAD

of thiourea on a gold electrode [4]. In 1995, the same group compared the electrode reactions of cysteine on gold, both in alkaline [5] and acidic [6] media. Because of the more favorable conditions at low pH, Johnson and co-workers showed a strong preference for acidic media in all their subsequent applications of the new method for detection of sulfur-containing compounds after ion-exchange chromatography [7–9]. During the same period of time, a new type of detector became available making it possible to integrate a measured electrode current over a specified period of time within an applied cycle of potentials (integrated pulsed amperometric detection or IPAD). Owens and LaCourse demonstrated the

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utility of IPAD for the detection of thiols and disulfides in capillary electrophoresis [10]. Evrovski et al. adapted the combined use of cation-exchange separation and IPAD at gold electrodes for the analysis of homocysteine in plasma samples [11]. Existing alternative methods for the analysis of homocysteine in plasma currently include: pre-column derivatization, reversed-phase separation with fluorescence detection [12,13], reversed-phase separation with amperometric detection [14], LC–MS–MS [15] and immunoassay [16]. Recently, we described a new cation-exchange chromatography–IPAD method for a simultaneous analysis of homocysteine and seven additional S-containing compounds in plasma [17]. The new method avoids the use of column switching required by the original approach [11]. The detection waveform was optimized and a stable performance was demonstrated over a 5-day period. Following that, the detection cell has to be disassembled and the microscopic debris stemming from the use of gold working electrode under acidic conditions has to be removed by rinsing and wiping. It is interesting to note that the mechanism of corrosion of gold electrodes under acidic conditions is different from that under alkaline conditions in agreement with the reports by Johnson and co-workers [5,6] and others [18–20].

Very recently, we described the development and characterization of microfabricated gold electrodes on polymeric substrates for IPAD of amino acids in alkaline media after high-performance anion-exchange chromatography [21]. In that work, we were able to apply a slightly modified version of the waveform previously optimized for non-disposable electrodes and achieve 1 week or more of stable detection response for disposable electrodes.

Under acidic detection conditions however, the application of the optimized waveform [17] did not immediately result in an acceptably stable detection response by the disposable gold electrodes. In this report, we describe a successful optimization of the IPAD waveform for use with thin-film gold electrodes under acidic conditions of the cation-exchange chromatography–IPAD method. We also present an evaluation of the analytical parameters and demonstrate the long-term stability of detection response with optimized waveform used in conjunction with disposable gold electrodes.

2. Experimental

2.1. Disposable electrode fabrication

Polyethylene naphthalate film (0.127 mm) was purchased from DuPont Teijin Films. Before use, the polymeric films were cleaned of all particles by blowing off with filtered nitrogen gas, then thoroughly rinsed with 18 MΩ water and ethanol. After the ethanol-rinsing step, the films were left to dry in a clean area. After placing the polymeric substrate on top of a stainless steel supporting base plate, we then covered it with a stainless steel mask defining the electrode, contact lead and contact pad pattern of disposable electrodes (Fig. 1A). The assembly consisting of ground plate, polymeric film and stainless steel mask was placed into the radio frequency sputtering chamber. The chamber was then evacuated to $\sim 10^{-7}$ Torr (1 Torr=133.322 Pa). A layer of ca. 500 Å titanium was sputtered first to promote adhesion of the gold film on the polymeric film. Following that, the second layer of ca. 3000 Å gold was sputtered from a 99.99% Au target onto the titanium layer from the preceding step. The surface area of the working electrode is 0.785 mm^2.

2.2. Cation-exchange chromatography and IPAD experiments

The cation-exchange chromatography with IPAD was carried out using a Dionex BioLC system (Dionex, Sunnyvale, CA, USA). The system consisted of a GP 50 gradient pump with on-line degas, a LC 30 chromatography oven, an AS 50 autosampler (injection loop, 25 μl), a column set consisting of OmniPac PCX-500 guard (40×2 mm) and analytical OmniPac PCX-500 (250×2 mm), and an ED 50 electrochemical detector. The titanium cell body of the electrochemical detector was used as the counter electrode across the 50-μm thin-layer channel defined by a gasket cutout. A Ag/AgCl (3 *M* KCl) reference electrode was placed downstream from the thin-layer channel. The conventional ED 50 Au working electrode was removed from the detector and replaced by the microfabricated Au electrode. The disposable electrode was mounted against the cell body using a holder block and two wing nuts (Fig. 1B).
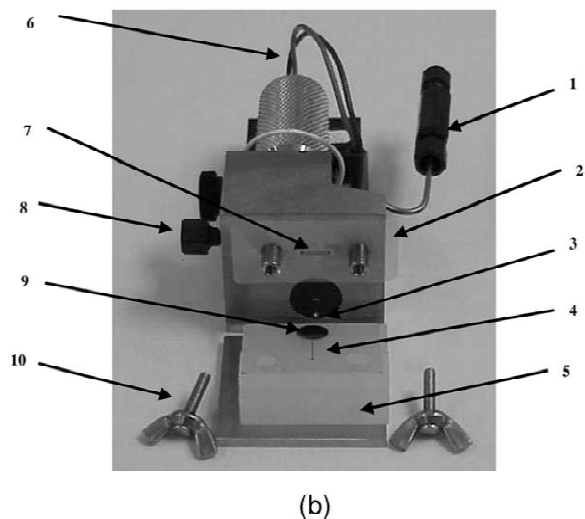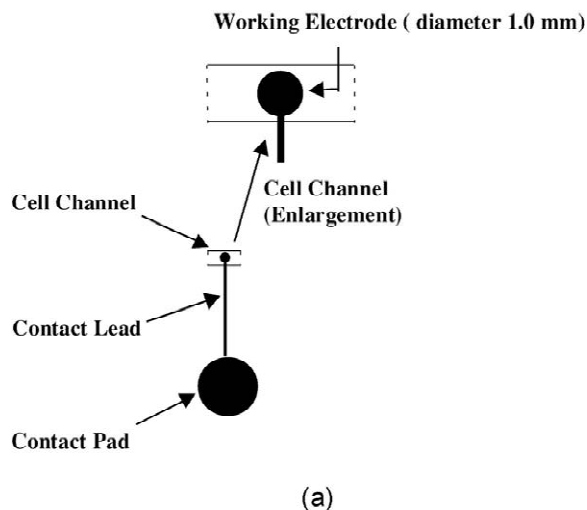
The chromatographic system control, data acquisition and analysis were carried out with the help of PeakNet 6.5 Software (Dionex). Initially, the IPAD waveform was as shown in Fig. 2 but eventually it was modified as discussed in Section 3.1. The cation-exchange chromatography of S-containing amino acids was carried out on the OmniPac PCX-500 column set at a flow-rate of 0.25 ml/min using an isocratic elution (20 mM HClO$_4$–0.15 M NaClO$_4$–5% v/v acetonitrile). Both the column set and electrochemical cell were inside the LC 30 column thermostat set at 30 °C. All standards were purchased from Sigma (St Louis, MO, USA). Anhydrous sodium perchlorate and concentrated perchloric acid for preparation of the mobile phase were obtained from Aldrich (Milwaukee, WI, USA). The acetonitrile was obtained from Allied Signal (B&J Brand, Muskegon, MI, USA). All standard solutions and mobile phases were prepared using 18 MΩ deionized water. The mobile phases were filtered through a 0.2-μm Nylon filter (VWR, West Chester, PA, USA).

3. Results and discussion

3.1. Waveform optimization

As discussed in the Introduction, the original waveform [17] with a cyclic scan (Fig. 2) makes possible 5 days of continuous use with non-disposable electrodes. After that time, the microscopic gold oxide fragments are gradually accumulating in the flow path and it is possible to observe increased levels of noise.

When we applied the same waveform [17] with disposable electrodes, the detection response and noise remained acceptable for only 1 h. With the aid of an optical microscope, we noticed that a small portion of the gold layer was etched away along the edge of the working electrode and only the titanium layer remained. Since the value and duration of the highest positive potential of a waveform were shown to cause a dissolution of gold in the past [22], we decided to optimize the size of the potentials E2, E4 of the waveform in Fig. 2. The duration of the two highest potentials (E2, E4) was already optimized (minimized) by Vandeberg and Johnson during their initial work [8]. Moreover, reducing the value of E2,

Fig. 1. (A) Pattern of a disposable electrode under evaluation. An electrode consists of the working electrode (circle, 1.0 mm diameter) located in a thin-layer flow channel (thickness×width×length, 0.050×0.9×6.0), contact lead and contact pad. The flow channel is defined by a gasket shown in (B). The exposed portion (not covered by gasket) of the contact lead contributes less than 8% of the total area. (B) The thin-layer electrochemical cell (disassembled) for use with disposable electrodes. 1: Inlet; 2: PTFE gasket with a cutout forming the thin-layer channel; 3: contact pin; 4: disposable electrode; 5: holder block for the disposable electrode; 6: reference electrode; 7: thin-layer channel; 8: outlet; 9: contact pad matching the position of contact pin; 10: wing nuts.

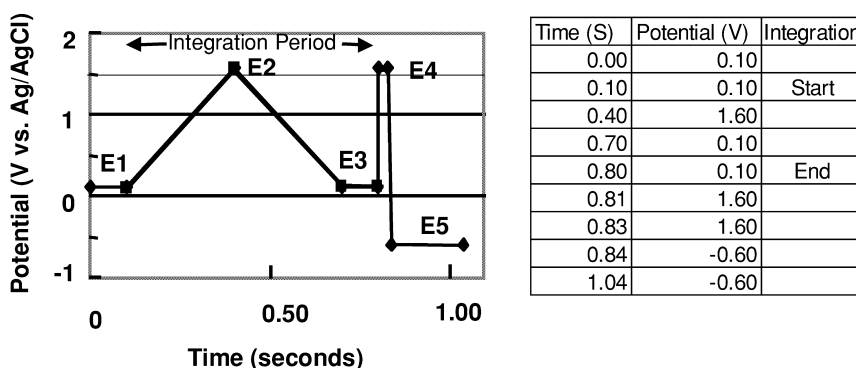| Time (S)	Potential (V)	Integration
0.00	0.10	
0.10	0.10	Start
0.40	1.60	
0.70	0.10	
0.80	0.10	End
0.81	1.60	
0.83	1.60	
0.84	-0.60	
1.04	-0.60	

Fig. 2. Integrated pulsed amperometric waveform for detecting amino acids.

E4, we were able to improve the long-term stability of performance of non-disposable electrodes in our previous work [17].

We conducted the optimization of the waveform by lowering the value of the two highest positive potentials (maximal oxidation detection potential E2 and oxidation cleaning potential E4) by 100 mV in five equal steps. The chromatograms collected during the optimization are shown in Fig. 3. During the first two steps (chromatograms B and C), we did not observe much of a change (less than 5%) of the response for homocysteine and cysteine in comparison with chromatogram A. However in the following steps (chromatograms D–F), the response of homocysteine and cysteine decreased dramatically. The corresponding values of peak heights are shown in Table 1. From the position of current

integration period within the detection waveform (Fig. 2), we can conclude that only a decrease in E2 is contributing to the response decrease. However, evaluation of peak shapes indicates that both E2 and E4 potentials are affecting the peak asymmetries of homocysteine and cysteine. In this type of effect, the oxidation cleaning potential (E4) is contributing more than the oxidation detection potential (E2). Higher peak response and better peak asymmetry are obtained for homocysteine and cysteine by waveforms with higher E2 and E4. However, in the same time, the corrosion of gold material accelerates. Clearly, a compromise solution has to be found. The values of E2 and E4 have to be as low as possible to maximize the electrode lifetime. On the other hand, these values have to be high enough to give acceptable values of response and acceptable peak shapes. Based on the results illustrated in Fig. 3 and summarized in Table 1, the best choice is that of the waveform with E2 and E4 at 1.30 V (chromatogram D).
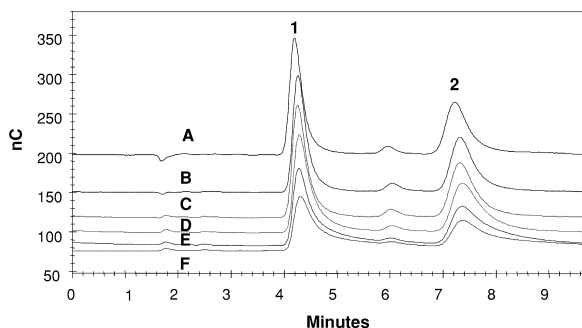

Fig. 3. Chromatograms obtained during waveform optimization. (A) E2, E4=1.60 V; (B) E2, E4=1.50 V; C: E2, E4=1.40 V; (D) E2, E4=1.30 V; (E) E2, E4=1.20 V; (F) E2, E4=1.10 V. Peak identities: 1: cysteine; 2: homocysteine; standard injections: 25 µl of 2 µM standard mixture.

Table 1
Response and peak asymmetry of cysteine and homocysteine under various maximal oxidation detection potential (E2) and oxidation cleaning (E4) potential

E2, E4 (V)	Peak height (nC)		Peak asymmetry		Life time (h)
	Cys	Hcys	Cys	Hcys	
1.60	148.06	64.64	2.18	2.00	1
1.50	147.69	67.91	2.27	2.10	2.5
1.40	141.46	67.59	2.25	2.20	16
1.30	123.13	60.64	3.17	2.55	75
1.20	96.61	46.51	4.92	4.10	160
1.10	66.38	31.72	5.97	5.05	240

3.2. Reproducibility and calibration

Reproducibility of results with the optimized waveform for disposable working electrodes was evaluated by making five consecutive injections of standard mixture over a 5-h period (25 μl of 2.0 μM of homocysteine and cysteine). The eluent was 20 mM HClO$_4$–0.15 M NaClO$_4$–5% v/v acetonitrile and the waveform of chromatogram D (Fig. 3) was used. The relative standard deviations (RSDs) of peak heights for homocysteine and cysteine in the five chromatograms are 2.57 and 1.25%, respectively (Table 2). The chromatograms from the 1st, 25th, 35th, 45th, 55th, 65th and 75th runs of the longer term evaluation are shown in Fig. 4. An evaluation of peak heights (actual values of peak heights are not shown) yields very good RSDs for homocysteine and cysteine (5.45 and 2.64%, respectively). The 75 runs used in this evaluation were generated from consecutive injections during an evaluation experiment lasting 3 days.

The detection limits calculated as three times multiple of noise are 0.40 and 0.20 pmol (or 16 and 8 nM at 25-μl injection) for homocysteine and cysteine, respectively. In our previous evaluation of non-disposable electrodes [17], we found the corresponding detection limits to be 1.01 and 0.28 pmol, respectively. This enhancement of detection sensitivity with disposable electrodes and optimized waveform is derived mainly from improvements in the baseline noise.

Because of the improved detection limits with disposable electrodes, we are able to extend the calibration range to lower concentrations while maintaining the similar upper limit of the calibration range. Consequently, the linear range of calibration is improved in going from non-disposable to dispos-
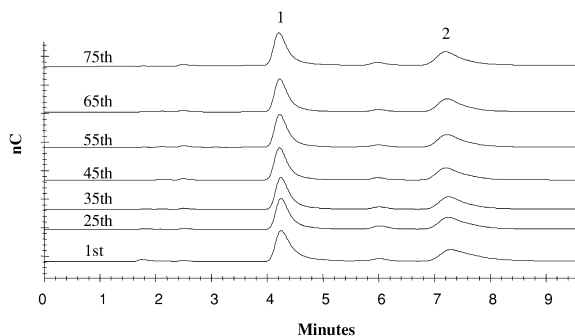

Fig. 4. Chromatograms from the long-term reproducibility test. Peak identities and chromatographic conditions were as described in Fig. 3 and Section 2. Waveform of chromatogram D in Fig. 3 was used for detection.

able electrodes. The correlation coefficients are 0.9993 and 0.9996 for homocysteine and cysteine, respectively.

4. Conclusions

The IPAD waveform, previously found suitable for detection of sulfur amino acids with non-disposable electrodes had to be modified to obtain an acceptable period of stable performance with disposable gold electrodes. In comparison with non-disposable electrodes, disposable electrodes offer improved linearity of calibration and detection limits.

Comparable or better performance of disposable electrodes makes it possible to substitute them for non-disposable electrodes in routine use. At the end of the specified period of time (3 days), the used electrode is simply replaced by another low-cost disposable gold electrode. Utilization of disposable electrodes thus eliminates the need for time consum-

Table 2
Qualitative parameters of S-containing amino acids at disposable thin-film Au electrode in 0.15 M NaClO$_4$–0.02 M HClO$_4$–5% CH$_3$CN

Amino acid	Limit of detection[a]		Linear range (μM)	R^2	RSD (%)	
	pmol	nM			Short-term[b]	Long-term[c]
Cysteine	0.20	8	0.15–20	0.9996	1.25	2.64
Homocysteine	0.40	16	0.30–20	0.9993	2.57	5.45

[a] Limit of detection was calculated as $S/N = 3$ using the lowest of injected concentrations.
[b] Short-term relative standard deviation was obtained from five injections of both cysteine and homocysteine.
[c] Long-term relative standard deviation was obtained from consecutive injections (1 h per injection) during 3 days.

ing polishing and re-equilibration that is always required with non-disposable electrodes.

References

[1] T.Z. Polta, D.C. Johnson, J. Electroanal. Chem. 209 (1986) 159.

[2] T.Z. Polta, D.C. Johnson, J. Electroanal. Chem. 209 (1986) 171.

[3] D.C. Honson, J.A. Polta, T.Z. Polta, G.G. Neuburger, J. Johnson, A.P.-C. Tang, I.-H. Yeo, J. Baur, J. Chem. Soc., Faraday Trans. 1 82 (1986) 1081.

[4] P.J. Vandeberg, J.L. Kowagoe, D.C. Johnson, Anal. Chim. Acta 260 (1992) 1.

[5] A.J. Tudos, D.C. Johnson, Anal. Chem. 67 (1995) 557.

[6] A.J. Tudos, P.J. Vandeberg, D.C. Johnson, Anal. Chem. 67 (1995) 552.

[7] P.J. Vandeberg, D.C. Johnson, Anal. Chem. 65 (1993) 2713.

[8] P.J. Vandeberg, D.C. Johnson, Anal. Chim. Acta 290 (1994) 317.

[9] M.E. Johll, D.C. Johnson, Electroanalysis 11 (1999) 534.

[10] G.S. Owens, W.R. LaCourse, J. Chromatogr. B 695 (1998) 15.

[11] J. Evrovski, M. Callaghan, D.E.C. Cole, Clin. Chem. 41 (1995) 757.

[12] A. Pastore, R. Massoud, C. Motti, A.L. Russo, G. Fucci, C. Cortese, G. Federici, Clin. Chem. 44 (1998) 825.

[13] C.M. Pfeiffer, D.L. Huff, E.W. Gunter, Clin. Chem. 45 (1999) 290.

[14] S.J. James, M. Pogribna, I.P. Pogribny, S. Melnyk, R.J. Hine, J.B. Gibson, P. Yi, D.L. Tafoya, D.H. Swenson, V.L. Wilson, D.W. Gaylor, Am. J. Clin. Nutr. 70 (1999) 495.

[15] M.J. Magera, J.M. Lacey, B. Casetta, P. Rinaldo, Clin. Chem. 45 (1999) 1517.

[16] M.T. Shipchandler, E.G. Moore, Clin. Chem. 41 (1995) 991.

[17] P. Jandik, J. Cheng, J. Evrovski, N. Avdalovic, J. Chromatogr. B 751 (2001) 145.

[18] R. Thacker, J.P. Hoave, Electrochem. Technol. 2 (1964) 61.

[19] S.H. Cadle, S.B. Bruckenstein, M. Shay, Anal. Chem. 46 (1974) 16.

[20] S.B. Bruckenstein, M. Shay, J. Electroanal. Chem. 188 (1985) 131.

[21] J. Cheng, P. Jandik, N. Avdalovic, Anal. Chem. 75 (2003) 572.

[22] R.D. Rocklin, A.P. Clarke, M. Weitzhandler, Anal. Chem. 70 (1998) 1496.