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Development and Characterization of Microfabricated Disposable Gold Working Electrodes for High-Performance Ion Chromatography and Integrated Pulsed Amperometric Detection

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We have developed a new type of microfabricated thin-film electrode on polymeric substrates. The microfabrication process allows for inexpensive and reproducible mass production of disposable working electrodes for high-performance ion chromatography and integrated pulsed amperometric detection (IPAD). These microfabricated electrodes are disposable and have been optimized for use in flow-through low-dead-volume electrochemical cells. The analytical performance of microfabricated gold electrodes was characterized with the help of the IPAD method for amino acid detection under alkaline conditions required for anion-exchange separations. When used with a new optimized six-potential IPAD waveform, the electrodes functioned properly for weeks. Compared to nondisposable working electrodes, the disposable working electrodes generated equal or better results in the limit of detection, linearity of calibration, and reproducibility. Disposable electrodes make it possible to avoid polishing and reconditioning, which are required with nondisposable electrodes.

In 1983, Polta and Johnson introduced a triple-potential pulsed amperometric detection in combination with anion-exchange chromatography to provide direct and sensitive detection of amino acids.[1] Later, Johnson and coauthors presented an optimized version of the technique utilizing integrated pulsed amperometric detection (IPAD). They described a new five-potential waveform enhancing the detection sensitivity of amino group oxidation and minimizing the baseline drift due to gold oxidation.[2] To improve long-term reproducibility of anion exchange/IPAD for carbohydrate analysis, Rocklin et al. introduced a new "quadruple-potential" waveform.[3] In the new waveform, the negative cleaning and positive activation potentials maintain a clean and active gold electrode surface without causing electrode corrosion or decreasing detection response. More recently, the cleaning and activation steps from the quadruple-potential waveform were included in a new six-potential IPAD waveform for direct detection of amino acids and amino sugars on gold electrodes after an anion-exchange separation.[4] This waveform minimizes baseline shift during the gradient and improves linearity and signal-to-noise ratio. Electrode fouling is relatively rare, and the detection response remains stable for many weeks of continuous use. When a decrease of detection response does occur, the electrode surface can be reconditioned by polishing as in the carbohydrate analysis. However, in comparison with carbohydrate analysis, reconditioning of gold electrodes for amino acid analysis usually requires more time.

This article describes the development and characterization of microfabricated gold electrodes on polymeric substrates for IPAD detection of amino acids after high-performance anion-exchange chromatography. The low cost of thin-layer gold electrodes deposited on polymeric substrates makes disposable electrodes commercially feasible. Using disposable electrodes, it is possible to avoid the time-consuming reconditioning that is required with nondisposable electrodes.

In comparison with thick-film technology, thin-film technology offers several advantages for electrode fabrication.[5] Among the advantages of thin-film technology are the following: superior dimensional characteristics of electrodes in terms of feature resolution control and minimal size, improved porosity, roughness, and purity of deposited metallic films, and absence of temperature-curing steps (125 °C and higher) that enable wider choice of substrate materials.

Consequentially, thin-film technology has been widely used to fabricate working electrodes for electrochemical detection cells. Metal electrodes made, for example, from gold and fabricated with thin-film technology have been reported on a variety of substrates ranging from silicon wafers[6,7] and glass microscope slides[8,9] to

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mica.[10,11] The gold surface was utilized as a carrier of self-assembled monolayers for electron transfer and monolayer characterization. It was demonstrated that all selected substrates[6−11] were suitable for thin-film electrodes of a high quality of surface topography (porosity, roughness, and purity) essential for assembling densely packed monolayers. More recently, several reports described metallic thin-film electrodes on polymeric substrates for use as biosensors and microdisk electrodes in a variety of applications.[12−15] To the best of our knowledge, such metallic thin-film electrodes were deposited on polyimide (Kapton) substrates in all published work.

There are relatively few reports in existence that describe the use of thin-film electrodes for electrochemical detection in liquid chromatography. Moreover, such work appears to be limited to carbon electrodes deposited on silicon wafers and to the evaluation of novel working electrode geometries suitable for dc amperometry.[16,17] We were unable to find any reports describing an optimization of performance of low-cost metal electrodes for use in liquid chromatographic IPAD detection.

The quality of IPAD detection with disposable electrodes not only depends on the proper application of thin-film technology and selection of most suitable substrate material; it also requires an adjustment of detection waveform. Thus, we also describe the necessary optimization of the six-potential waveform for use with thin-film gold electrodes in electrochemical detection in conjunction with anion-exchange chromatography.

EXPERIMENTAL SECTION

Disposable Electrode Fabrication. The evaluated polymeric substrates included polycarbonate (Lexan, GE Co., Pittsfield, MA), polyetherimide (Ultem, GE), poly(ethylene naphthalate) (Kaladex, Du Pont, Wilmington, DE), polyimide (Kapton, Du Pont), polyamide (Dartek, Du Pont), poly(ethylene terephathalate) (Merlinex, Du Pont), and poly(chlorotrifluoroethylene) (Kel-F, 3 M Co., St. Paul, MN). The thickness of the polymeric films was either 0.005 or 0.007 in. depending on availability. Before use, the polymeric films were cleaned of all particles by blowing off with filtered nitrogen gas and then rinsed with 18 MΩ water and ethanol thoroughly. After the ethanol-rinsing step, the films were left to dry in a clean space. We then placed the polymeric substrate on a stainless steel supporting base plate and covered it with a stainless steel mask that defined the electrode, contact lead, and contact pad pattern of disposable electrodes (Figure 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 ∼10^{-7} Torr. A layer of ∼500-Å titanium was sputtered first to promote adhesion of the gold film on the polymeric film. Following that, the second layer of ∼3000-Å gold was sputtered from a 99.99% Au target onto the titanium layer from the preceding step. In all our experiments, the shapes of the contact pad and contact lead (Figure 1A)

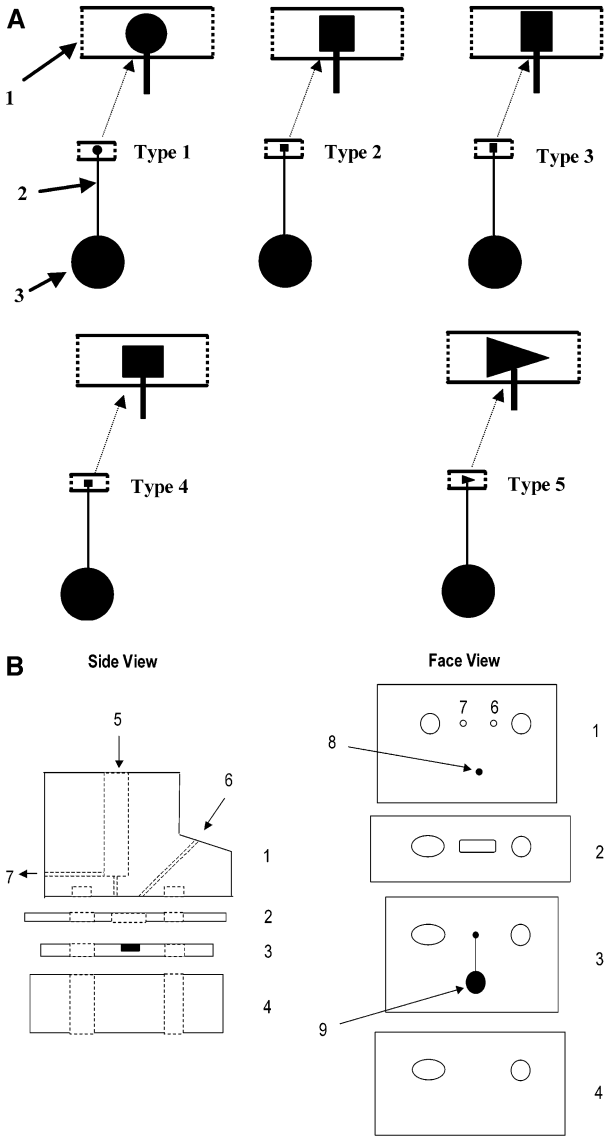

Figure 1. (A) Four shapes and five types of working electrodes under evaluation. The rectangular shape is used in two different orientations. All electrodes have identical areas. Type 1 (circle, 1.000-mm diameter); type 2 (square, 0.886 × 0.886 mm); type 3 (rectangle I, length 0.785 × width 1.000 mm); type 4 (rectangle II, length 1.000 × width 0.785 mm); type 5 (isosceles triangle, base 1.000 × height 1.570 mm). (1) Portion of the thin-layer channel near the working electrode. The flow direction is from left to right; (2) contact lead (exposed portion of the lead contributes less than 8% (except for type 5, 12,8%) to the total area); (3) contact pad. (B) Schematic drawing (not to scale) of the thin-layer electrochemical cell for use with disposable electrodes. (1) Titanium cell body (counter electrode) with inlet, outlet, contact pin to working electrode, and reference electrode chamber; (2) Teflon gasket with a cutout forming the thin-layer channel; (3) disposable electrode; (4) holder block for the disposable electrode; (5) reference electrode; (6) inlet; (7) outlet; (8) contact pin to working electrode (insulated from the cell body); (9) contact pad matching the position of contact pin.

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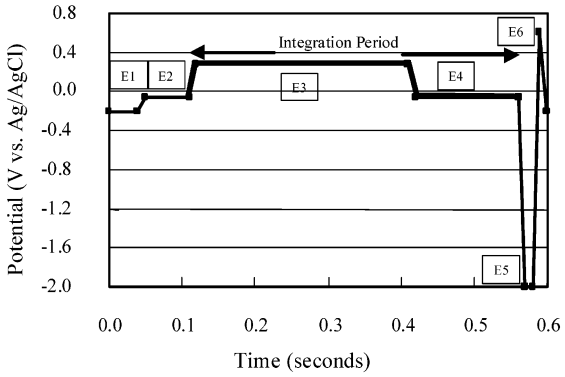
Table 1. Evaluation Summary: Suitability of Polymeric Materials as Substrates for Disposable Gold Electrodes[a]

polymer	gold bonding[b] strength[2]	deposited gold film color[c]	seal[d]	chemical compatibility[e]	limit of detection[f]	stability[g] short-term (1 day)	stability[g] long-term (3 days)
Lexan	poor	excellent	excellent	excellent	excellent	poor[h]	poor[h]
Ultem	fair	excellent	excellent	excellent	good	excellent	fair
Kaladex	excellent	good	excellent	excellent	excellent	excellent	excellent
Kapton	excellent	excellent	excellent	excellent	good	poor	poor
Dartek	excellent	poor	fair	excellent	poor	poor	poor
Merlinex	fair	excellent	excellent	excellent	good	good	poor
Kel-F	excellent	poor	poor	excellent	poor	poor	poor

[a] A 2-μm Teflon gasket (Figure 1B) and a circular gold electrode (Figure 1A) were used for all of the measurements. The waveform and gradient conditions were from Figure 2 and Table 1, gradient A of ref 18, respectively. [b] Under continuous use, the gold electrodes remained functional: not less than 3 days (excellent), not less than 2 days (good), not less than 1 day (fair), or less than 1 day (poor). [c] The color of gold electrodes on polymeric substrates was: unchanged (excellent), almost unchanged (good), somewhat changed (fair), or significantly changed (poor). [d] Absence of liquid leaks under continuous use for: not less than one week (excellent), not less than 3 days (good), not less than 1 day (fair), or less than 1 day (poor). [e] After a continuous use for 5 days, the polymeric film surface appeared: unchanged (excellent), almost unchanged (good), somewhat changed (fair), or significantly changed (poor). [f] Limit of detection (3 times multiple of noise, lysine). The LOD of the disposable gold electrode was: better or equal to that of nondisposable gold electrode (excellent), not more than 2 times that of nondisposable gold electrode (good), not more than 3 times that of nondisposable gold electrode (fair), or more than 3 times that of nondisposable gold electrode (poor). [g] During the test period, the peak height of lysine decreased: not more than 5% (excellent), not more than 10% (good), not more than 20% (fair), or more than 20% (poor). [h] Gold Film was peeling off of Lexan substrate during the stability test.



Time (S)	Potential (V)	Integration
0.00	-0.20	
0.04	-0.20	
0.05	-0.05	
0.11	-0.05	Begin
0.12	0.28	
0.41	0.28	
0.42	-0.05	
0.56	-0.05	End
0.57	-2.00	
0.58	-2.00	
0.59	0.60	
0.60	-0.20	

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

remained unchanged. Conversely, the working electrode part of the pattern was produced in a variety of different shapes (e.g., circle, square, rectangle, and triangle; see Figure 1A), each having an identical area of 0.785 mm^2.

Anion-Exchange Chromatography and IPAD Measurement. The anion-exchange chromatography with IPAD was performed using a Dionex BioLC (Dionex, Sunnyvale, CA). The system consisted of a GP 50 gradient pump with on-line degas, an AS 50 autosampler (injection loop, 25 μL), a column set consisting of an AminoPac PA 10 guard (2 × 50 mm) and analytical AminoPac PA 10 (2 × 250 mm), LC 30 column thermostat, and 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 (flow channel length and width, 8.91 × 1.27 mm). A combination reference electrode of pH and Ag/AgCl (3 M KCl) 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 (see the schematic drawing in Figure 1B).

The chromatographic system control, data acquisition, and analysis were accomplished using PeakNet 6.5 software (Dionex). Initially, the IPAD waveform was as shown in Figure 2, but eventually it was modified as discussed under Waveform Optimization. The anion-exchange chromatography of amino acids was performed on the AminoPac PA 10 column set at a flow rate of 0.25 mL/min using an elution gradient reported in Table 1, gradient A of ref 18 (ternary gradient: (A) water, (B) 0.25 M NaOH, (C) 1.0 M sodium acetate). Both the column set and electrochemical cell were inside the LC 30 column thermostat set at 30 °C. The sodium hydroxide mobile phase was prepared by dilution of 50% sodium hydroxide (Fisher Scientific, Pittsburgh, PA) with 18 MΩ water. The sodium acetate mobile phase was prepared by dissolving an aliquot of sodium acetate (Dionex) in water and by filtering through a 0.2-μm Nylon filter (VWR, West Chester, PA). All eluents were maintained under helium or nitrogen to prevent entry of atmospheric carbon dioxide. The standard mixtures of amino acids were prepared by diluting an aliquot of Standard Reference Material 2389 (NIST, Gaithersburg, MD) using a diluent containing 20 mg/L sodium azide.

RESULTS AND DISCUSSION

The main subject of this report is the design and optimization of disposable gold electrodes for replacement of nondisposable gold electrodes in an existing design of a liquid chromatographic thin-layer amperometric cell. The performance of the disposable electrodes is evaluated in the detection of amino acids following their gradient separation by anion exchange in an aqueous mobile phase containing varying proportions of sodium hydroxide and sodium acetate. The analytical performance of nondisposable gold electrodes for that application is documented in the literature.[4]

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The objective of this work is to match or exceed that performance for a defined period of time with disposable electrodes.

Initial Design Considerations. The intended use of disposable electrodes in an existing cell and for a selected application is the main determinant in the design of their geometry (Figure 1A). In the first approach, we chose a circular working electrode (1-mm diameter) with a shape that is identical to that of the nondisposable electrodes. Experiments with other shapes are discussed in one of the following paragraphs. The contacting method for the nondisposable working electrode used in the cell (directional orientation of the contact pad is the same as that of the working electrode) dictates the shape of the contact lead and contact pad of the disposable electrode (Figure 1B).

Thin-film deposits of gold on polymeric surfaces require an intermediary layer of an adhesion-promoting element. So far, a chromium layer has been used exclusively for making disposable electrodes on polymeric materials.[12−15] Portions of the adhesion-promoting element are exposed to an alkaline eluent in the thin-layer electrochemical cell during our application. We thus ruled out chromium and selected titanium because our application requires a more inert adhesive layer for prolonged use in the highly alkaline eluent.

In a series of preliminary experiments, we optimized the thickness of the titanium layer for a sufficient mechanical adhesion of a ∼3000-Å -thick layer of gold on a polyimide (Kapton) substrate. Applying the Scotch Tape test,[19] we found that the optimum adhesion of gold on polyimide was achieved with a 500-Å intermediary layer of titanium.

Selecting a Polymeric Substrate for Disposable Gold Working Electrodes. Initially, we planned to use only polyimide for our electrodes since it was the most widely reported polymeric substrate[12−15] for thin-film technology. After achieving sufficient adhesion (see the preceding paragraph), we started an evaluation of polyimide-based disposable electrodes inside the detection cell for IPAD detection of amino acids. With the polyimide-based electrodes, we found the detection response for all amino acids to be unstable and to decrease to 50% or less within hours.

That result prompted us to conduct an evaluation of the stability of detection response (both short term and long term) of gold electrodes using the largest possible choice of polymeric films as substrates. In the course of that evaluation, we added additional performance criteria such as discoloration of the gold surface, quality of seal between the cell gasket and the polymeric substrate material, chemical compatibility, and limit of detection. The evaluation results of polymeric substrates are summarized in Table 1. Results of the evaluation are expressed using the criteria defined in the footnotes to Table 1.

Polycarbonate (Lexan), polyetherimide (Ultem), and poly(ethylene naphthalate) (Merlinex) did not provide sufficient adhesion for deposited gold films, and consequently, we could not achieve the required detection performance for continuous long-term use. We subjected all evaluated polymeric materials to the same type of pretreatment (see Experimental Section). The varying quality of bonding was thus most likely attributable to surface conditions (roughness, surface tension, etc.) and chemical properties (additives, functional groups, etc.) of evaluated materials. However, we did not try to identify a single property or group of properties responsible for poor bonding on some of the substrates.

With two of the polymeric materials, we observed a strong discoloration on the surface of the gold electrode (column 3, Table 1). That change in color was matched in both cases by poor results in sensitivity and stability (long term and short term) of detection response (columns 6−8, Table 1). This test outcome supports the conclusion of a possible interaction of the polymeric material, or of a substance contained in the polymeric material, with the metallic layer.

Only the chemical compatibility (column 5, Table 1) in alkaline eluents could be described as excellent for all seven materials. To function properly in an assembled detection cell, the polymeric substrate materials must possess a suitable combination of dimensional stability (stiffness) and elasticity. Insufficient stiffness can lead to deformations of the thin-layer channel (Figure 1B). Lack of elasticity leads to a poor seal between the gasket and substrate materials, as was observed for two of the materials (column 4, Table 1).

Polymeric materials can influence both the noise and signal contributions to limit of detection results. We speculated that surface roughness under the electrode layer and elsewhere in the thin-layer channel may contribute to noise levels, but we did not observe significant differences in noise levels among the seven materials being evaluated. On the other hand, chemical impurities from a substrate can affect the limit of detection, either instantaneously (Dartek, Kel-F) or gradually, due to their slow rate of extraction from the polymer (Merlinex). Chemical contamination of the electrode can either be accompanied by discoloration (Dartek, Kel-F) or occur without any visually detectable change (Kapton).

Of all evaluated materials, only Kaladex rated good to excellent in all of the tests and was therefore selected as the most suitable substrate material.

Different Geometric Shapes of Working Electrodes. In addition to discussions of relative merits of different amperometric cell designs,[20−22] there are several reports in the literature that discuss theoretical and practical aspects of working electrode shapes in thin-layer amperometric cells.[23−25] Experimental data were obtained for various sizes of rectangular[23−25] and circular[23] electrodes. The rectangular electrodes were also studied in various orientations versus the direction of flow.[21,22] At least one published account discusses theoretical aspects of triangular electrodes.[23] Although most reports agree in their predictions of signal decrease in going from rectangular shapes to circular and triangular ones, they differ in the predicted size of signal differences among the investigated working electrode geometry. While some authors emphasize the influence of working electrode areas,[24] others consider the electrode length (electrode dimension parallel with the direction of flow) to be more important.[25]

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Table 2. Detection Response[a] (Peak Height, nC) and Peak Asymmetry[b] for Electrodes of Different Geometric Shapes

	circle	square	rectangle		triangle
	type 1	type 2	type 3	type 4	type 5
Val (height)	28 ± 2	32 ± 1	30 ± 1	31 ± 1	28 ± 1
His (height)	386 ± 19	420 ± 16	407 ± 3	423 ± 3	376 ± 35
Phe (height)	147 ± 10	156 ± 10	156 ± 5	155 ± 1	145 ± 7
Asp (height)	57 ± 4	67 ± 5	59 ± 5	68 ± 4	60 ± 4
Cys (height)	144 ± 8	157 ± 7	150 ± 7	152 ± 2	144 ± 9
Val (asym)	1.38 ± 0.04	1.41 ± 0.02	1.41 ± 0.01	1.41 ± 0.01	1.40 ± 0.05
His (asym)	3.24 ± 0.24	3.33 ± 0.48	2.64 ± 0.26	3.29 ± 0.14	3.60 ± 0.25
Phe (asym)	1.39 ± 0.07	1.32 ± 0.04	1.32 ± 0.06	1.31 ± 0.04	1.36 ± 0.10
Asp (asym)	1.21 ± 0.05	1.18 ± 0.04	1.18 ± 0.02	1.15 ± 0.04	1.17 ± 0.04
Cys (asym)	1.28 ± 0.03	1.28 ± 0.01	1.31 ± 0.01	1.32 ± 0.01	1.31 ± 0.03

[a] Each data point was collected from three independent measurements and reported as the response at the surface area after correction for the contribution from the electrode connection bars, which is from 4.6 to 6.5% over that of electrodes (See Figure 1A.). The peak height of each amino acid was taken from the chromatograms obtained with the injection of 25 μL of 8 μM amino acid standards (See text.). [b] Peak asymmetry (asym) was calculated from (RW5% + LW5%)/(2LW5%). RW5% and LW5% denote the right width and left width at 5% of peak height, respectively. Each data point was collected from three independent measurements.

Unable to obtain clear guidance from the literature, we decided to conduct our own evaluation of electrode shapes under our specific instrumental conditions and for the selected application.

The nondisposable gold electrode discussed in our preceding work[3−4,18] was prepared using a gold rod of 1-mm diameter embedded in a block of Kel-F material. The resulting circular electrode had a surface area of 0.785 mm^2. Utilizing the benefits of thin-film technology, we were able to prepare working electrodes of four different shapes (circle, square, rectangle, and triangle; Figure 1A), each with an identical surface area of 0.785 mm^2. To evaluate the influence of different lengths and widths, rectangular electrodes were prepared in two different orientations to the direction of flow in the thin-layer channel (types 3 and 4, Figure 1A).

To begin with, we evaluated the effects of geometric shapes on chromatographic detection performance by means of peak heights of five representative amino acids. We chose peak heights rather than detection limits because we found the baseline noise values to be identical for all five electrode types (~40 pC). The selected amino acids were Val, His, Phe, Asp, and Cys, representing aliphatic, basic, aromatic, acidic, and sulfur containing amino acids, respectively.

As shown in Table 2 (first five rows), the peak height values of all five amino acids are in rough agreement with the predictions published in the literature. All of the analytes show the highest values of peak heights with rectangular or square electrodes.[23,24] Also, the longer of the two rectangular electrodes (type 4) appears to produce slightly higher peaks for most of the five amino acids than the shorter one (type 3). However, the differences among all electrode types can be considered negligible from a practical point of view, as most of the differences are less than the measurement error.

Weitzhandler et al.[26] compared UV and IPAD recordings of amino acid separations in an alkaline medium and presented clear evidence of peak shape distortion of some amino acids at gold electrodes. Based on that observation, we decided to conduct an evaluation of peak asymmetry with all five types of microfabricated disposable electrodes. With one exception, all peak asymmetry values in Table 2 (last five rows) are comparable with those observed on circular nondisposable electrodes[4] and independent of electrode geometry. The single exception is that of histidine. The peak asymmetry for that compound appears to be significantly improved on the type 3 (rectangular, short, Figure 1A) disposable electrode. While the observed improvement of peak asymmetry is important for our future work, we continued our evaluation focusing on disposable electrodes of circular shape.

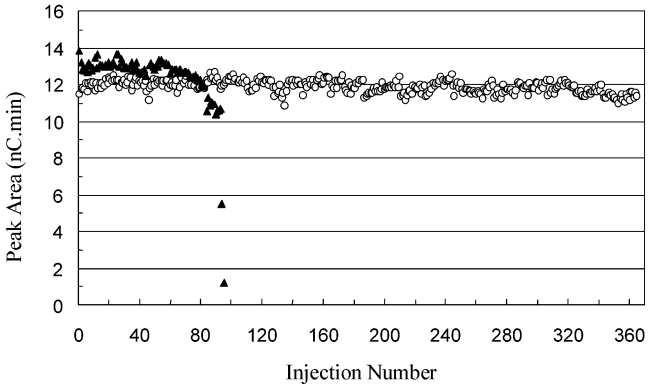

Figure 3. Peak areas for lysine versus injection number. Triangles, original waveform; circles, new optimized waveform (see Waveform Optimization). The data were generated by injecting 25 μL of 8 μM lysine in the dilution of NIST SRM 2389 (amino acid standards) 12−14 times a day. Additional gradient and detection conditions are described in the Experimental Section.

Waveform Optimization. In the initial approach of the evaluation of different polymeric substrate materials, we used the waveform originally developed for circular, nondisposable electrodes. As discussed in the introduction, some IPAD waveforms have been found to gradually dissolve the gold working electrodes. The original six-potential waveform (Figure 2) makes possible weeks of continuous usage with nondisposable electrodes. However, when we used the same waveform with disposable electrodes (type 1, Kaladex), we observed a sudden decrease of response for all amino acids after only 3−4 days (Figure 3). Inspecting the failed electrodes under a microscope, we noticed that the gold layer was etched away at the edge of the working electrode and only the titanium layer remained. Since the highest

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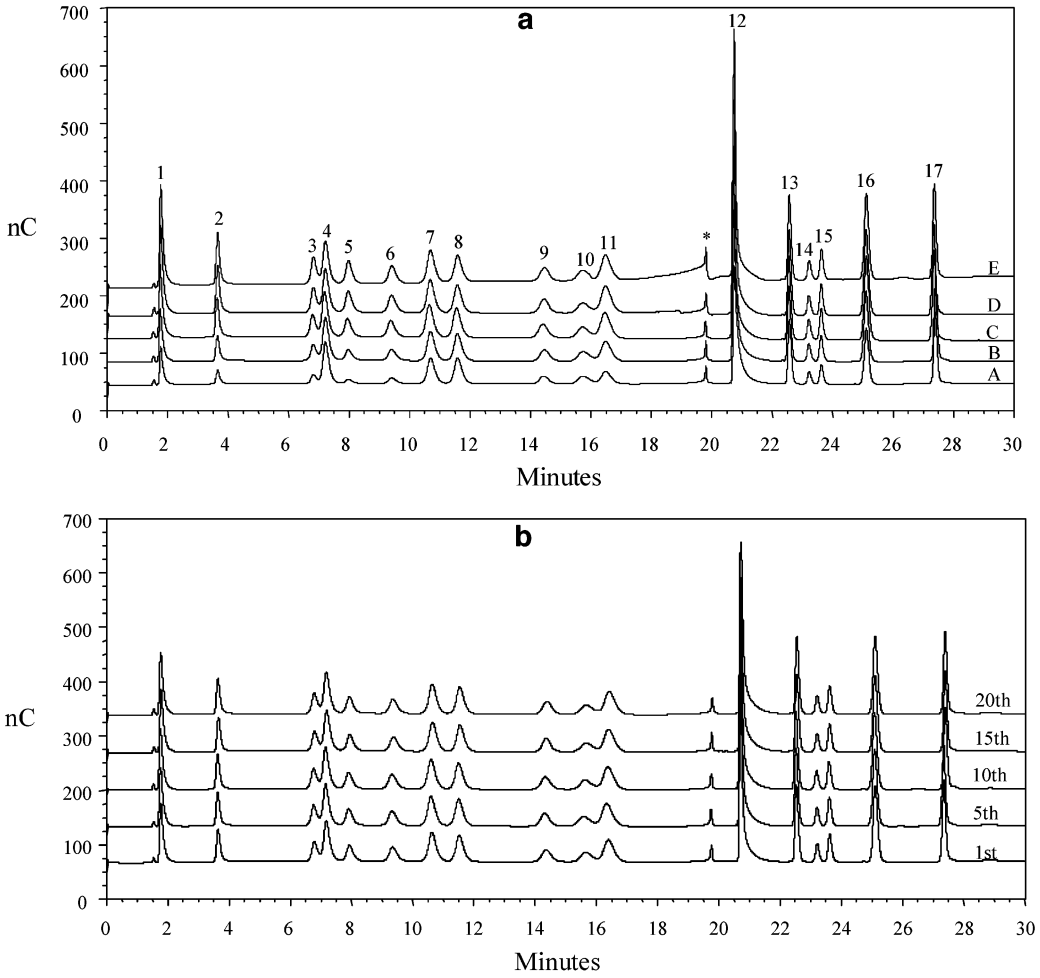

Figure 4. (a) Chromatograms obtained during waveform optimization. (A) E3, 180 (−100) mV; (B) E3, 205 (−75) mV; (C) E3, 230 (−50) mV; (D) E3, 255 (−25) mV; (E) E3, 280 (original value) mV. Peak identities: 1, arginine; 2, lysine; 3, alanine; 4, threonine; 5, glycine; 6, valine; 7, serine; 8, proline; 9, isoleucine; 10, leucine; 11, methionine; 12, histidine; 13, phenylalanine; 14, glutamate; 15, aspartate; 16, cystine; 17, tyrosine; *, system peak. Standard injections: 25 μL of 312.5-fold dilution of NIST SRM 2389 (See text for additional conditions). (b) Chromatograms from the short-term reproducibility test. Peak identities and chromatographic conditions are as described in (a). Waveform of chromatogram C in (a) was used for detection.

positive potential of a waveform (potential E2, Figure 1A, ref 3) has been known to cause a dissolution of gold, and a modification of the value and duration of that potential minimized that problem in the past,[3,4] we concluded at this time that an additional optimization of the E6 potential in the waveform of Figure 2 could improve the long-term performance of disposable electrodes. We conducted two experiments lowering the value of E6 by 50 and 100 mV; no improvement of long-term reproducibility was observed using the two lower values of the E6 potential. Since we were unable to further reduce the duration of E6 potential and further decreases of that potential would interfere with signal stability,[3,4] we turned our attention to the second highest potential of significant length, the potential E3. In the next series of experiments, we used four otherwise identical waveforms differing only in a decreased value of E3 potential (by 25, 50, 75, and 100 mV, E3 duration unchanged and as shown in Figure 2). The resulting gradient chromatograms of a protein hydrolysate standard are shown in Figure 4a. A careful inspection reveals not only an overall gradual change in response but also changing ratios of size for some of the peaks. In our judgment, two of the waveforms (E3 potential reduced by 75 and 100 mV) caused an excessive decrease of sensitivity for some of the amino acids (i.e., Lys, Ala, Gly, and Val). Because of that, we decided not to include those waveforms in the long-term experiments. The two other waveforms (E3 lower by 25 and 50 mV, Figure 4a chromatograms D and C, respectively) did not cause any significant loss of sensitivity and were included in the subsequent long-term evaluation.

From the two remaining modified waveforms, only the one with the E3 potential lowered by 50 mV delivered an improved long-term performance with circular disposable electrodes on Kaladex substrate. The detection response for all 17 amino acids remained stable over the entire test period of four weeks. Figure 3 includes the peak area values of lysine from that long-term evaluation overlaid with the lysine peak area data obtained with the original waveform. We concluded that the decreased E3 potential (by 50 mV) improves the practical usefulness of microfabricated electrodes and proceeded using that modified waveform in the evaluation of analytical performance discussed in the following paragraph.

Reproducibility and Calibration. We evaluated the short-term reproducibility of circular disposable electrodes prepared on Kaladex substrate (type 1, Figure 1A) by making 20 consecutive

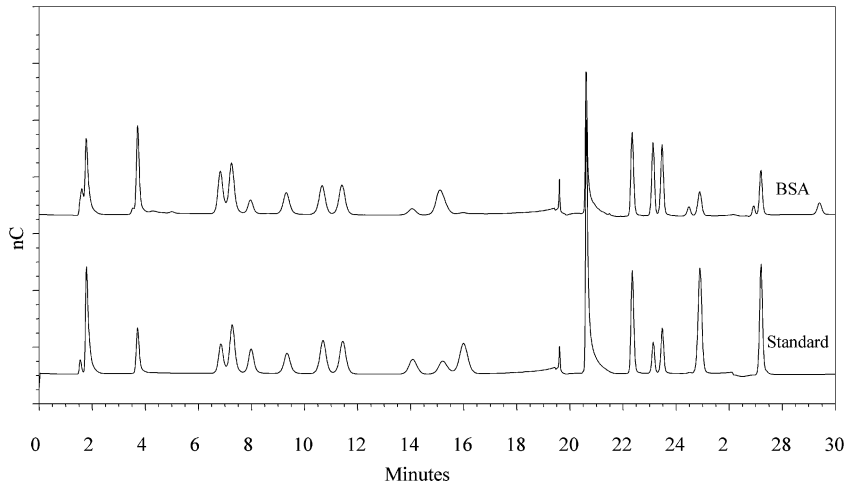

Figure 5. Comparison of chromatograms of hydrolyzed bovine serum albumin (BSA) and 312.5-fold diluted NIST SRM 2389 (Standard). Conditions of hydrolysis: 16 h, 110 °C, 6 N HCl. Protein hydrolysis was carried out with 4.80 μg of BSA. Following the hydrolysis, HCl was removed by evaporative centrifugation, the hydrolysate was redissolved in 200 μL of water, and 25 μL of reconstituted sample was injected. Chromatographic conditions and peak identities are same as in Figure 4a. Waveform of chromatogram C in Figure 4a was used for detection.

injections of hydrolysate standard over a 24-h period. The gradient conditions were as reported previously (gradient A, Table 1, ref 18), and the optimized waveform from the preceding section was used as part of the detection method. The relative standard deviations of peak areas for all amino acids in the 20 chromatograms are in the range of 0.6−2.0%. The chromatograms from the 1st, 5th, 10th, 15th, and 20th run shown in Figure 4b compare favorably (minimal baseline fluctuations, absence of artifacts and peak distortion) with those obtained with nondisposable electrodes.[3,4]

The calibration experiments with the Kaladex-based, type 1 disposable electrode produced excellent linearity results for the range of amounts injected from 1 pmol to 1 nmol. The correlation coefficients were equal to or greater than 0.998. The only exception was the basic amino acid histidine for which we observed a lowered upper limit of calibration range of 250 pmol.

It should be noted that a lowered upper limit of calibration range for histidine and other basic amino acids (arginine, lysine) is also observed with nondisposable gold electrodes.[4]

Because of the improved detection limits with disposable electrodes (see below) and good reproducibility of peak areas for injection amounts of 0.25 pmol of histidine, the lowered upper limit of calibration does not automatically result in a narrower calibration range. Even with the lowered upper limit, the linearity range of histidine still extends over 3 orders of magnitude (0.25−250 pmol, $R^2 \geq 0.991$). Overall, the linearity of calibration is thus improved in going from nondisposable to disposable electrodes.[27] Arginine and lysine also exhibit linear ranges similar to those of the majority of amino acids. The calibration range for histidine is also similar and merely transposed to lower values.

The detection limits (3 times noise level) with type 1 electrodes on Kaladex substrates ranged from the tens of femtomoles for peaks exhibiting high chromatographic efficiency (His, Phe, Cys, Tyr) to hundreds of femtomoles for slightly broader peaks in the middle of the chromatogram. In our previous evaluation of nondisposable electrodes,[4] we found the detection limits to be between the low hundreds of femtomoles to ~4 pmol. This enhancement of detection sensitivity for disposable electrodes is derived mainly from improvements in the baseline noise.[28] The lower levels of noise observed with disposable electrodes are most likely due to a greater smoothness of sputtered metal layers. Due to the use of polishing compounds, the surface of nondisposable electrodes may be scratched as deep as 0.5−1 μm. (The particle size of final polishing compound recommended for our electrodes is 1 μm.) In contrast, the total thickness of the sputtered gold layer is only ~0.3 μm on our disposable electrodes. Good smoothness and uniformity of sputtered gold layers are documented in the literature.[5]

Disposable gold electrodes have already been used to detect separated amino acids from many types of samples. One example of a sample analysis is included in Figure 5. It is a separation of a hydrolyzed sample of bovine serum albumin.

CONCLUSIONS

Polyester (Kaladex) was found to be the best substrate material for the new thin-film gold working electrodes evaluated for use as disposable electrodes in thin-layer amperometric detection cells. Evaluation of different working electrode shapes revealed similar detection sensitivity for all electrodes. With the exception of

(27) The calibration study of this report was carried out under conditions that were not identical with those of ref 4. To optimize the liquid seal, the disposable electrodes described here were used exclusively with 50-μm Teflon gaskets. On the other hand, all calibration data for the nondisposable electrode were generated using a 25-μm Ultem gasket. Also, the calibration with disposable electrodes was carried out using the optimized value (see Waveform Optimization) of potential E3 of the original waveform, whereas an unmodified version of the waveform was applied with nondisposable electrodes. All other experimental condition were the same (i.e., eluent composition, flow rate, temperature, length, and width of the thin-layer channel). When trying out the optimized version of the waveform and 50-μm Teflon gaskets with nondisposable electrodes, we found calibration parameters that were similar to those reported in ref 4.

(28) The detection limit study of this report was also done using conditions that were different from those in ref 4 (50-μm Teflon gasket, optimized waveform vs 25-μm Ultem gasket, and original waveform). The noise measured in this report with disposable electrodes was 40 ± 6 pC. Applying the same conditions (50-μm Teflon gasket, optimized waveform) to nondisposable electrodes, we found the noise to be 128 ± 34 pC. This value is comparable to that measured with 25-μm Ultem gasket and original waveform for nondisposable electrodes.

histidine, observed peak asymmetry values were also independent of electrode geometry. The IPAD waveform, previously found suitable for nondisposable electrodes, had to be modified to expand the period of stable performance with disposable gold electrodes. In comparison with nondisposable 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 nondisposable electrodes. In addition, utilization of disposable electrodes eliminates the need for time-consuming polishing and reequilibrating of working electrodes.

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