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Fast Wave Forms for Pulsed Electrochemical Detection of Glucose by Incorporation of Reductive Desorption of Oxidation Products

Mark B. Jensen† and Dennis C. Johnson*

Department of Chemistry, Iowa State University, Ames, Iowa 50011

The electrochemical activity of Au electrodes held at constant potential for anodic detection of carbohydrates in alkaline media eventually decays to zero. This loss of response is a consequence of the accumulation of adsorbed oxidation products on the electrode surface. Although it is well-known that these "poisons" can be removed by oxidative desorption simultaneously with formation of surface oxide, we have discovered that electrodes fouled during the detection of glucose yield a cathodic peak at −0.77 V vs SCE resulting from reductive desorption of these species. Incorporation of the reductive desorption process into wave forms for pulsed electrochemical detection (PED) permits a significant decrease in the time periods traditionally allowed for the oxidative and reductive reactivation of the electrode with a resulting increase in wave form frequency. A 6.7-Hz wave form using $E_{red} = -1.00$ V ($t_{red} = 10$ ms), $E_{oxd} = +0.60$ V ($t_{oxd} = 10$ ms), and $E_{det} = +0.10$ V ($t_{del} = 50$ ms, $t_{int} = 50$ ms) is applied for detection of glucose in a LC-PED system and is demonstrated to yield a subpicomole detection limit with a linear dynamic range extending over three decades.

Pulsed electrochemical detection (PED) at Au electrodes in alkaline media has rapidly gained popularity for the direct and sensitive detection of carbohydrates following their separation by anion-exchange liquid chromatography (LC) and capillary electrophoresis (CE). The related literature has grown to become quite extensive, and therefore, citations are given here only for reviews of the development and applications of LC/CE-PED.[1−4]

Whereas PED is actually a generic designation covering several related techniques, the common basis of PED techniques using noble metal electrodes (Au, Pt) is the application of a repeating sequence of potential steps which systematically achieve detection with subsequent oxidative cleaning and reductive reactivation of the electrode. It is general knowledge that intermediate and/or final oxidation products from many aliphatic compounds are adsorbed at noble metal electrodes, thereby rendering the electrode surfaces inactive.[5] Therefore, the repeating pattern of surface oxidation and reduction used in PED wave forms is necessary to maintain a reproducibly high state of electrode surface activity.

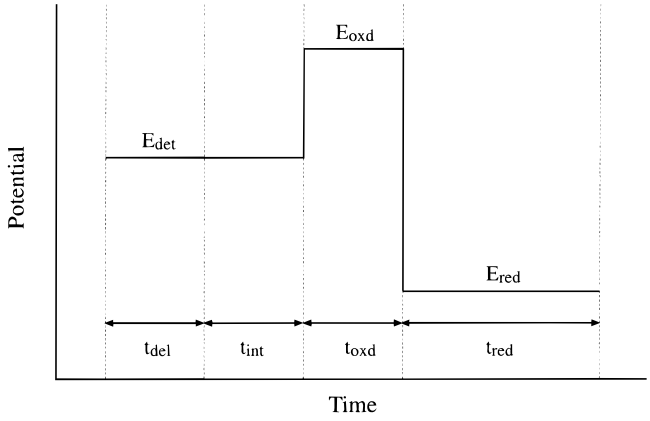

Figure 1. Three-step PED wave form. Typical values of all parameters are given in Table 1.

The most widely utilized PED wave form is the three-step wave form depicted in Figure 1. The electrode potential is first stepped to the optimum value for detection of the electroactive species (E_{det}). Following a delay time (t_{del}), during which the currents from double-layer charging and oxide formation are permitted to decay to minimal values, the analytical signal is computed by integrating the faradaic current due to analyte oxidation for a specified time period (t_{int}). The potential then is stepped to a more positive value (E_{oxd}) at which the electrode surface is oxidatively cleaned of any poisons adsorbed during the detection step. Finally, the potential is stepped to a negative value (E_{red}) to reduce the oxide layer formed at E_{oxd}, thereby regenerating the electrode activity so that the cycle may be repeated. LaCourse and Johnson described an automated procedure for optimizing the PED parameters using a large electrolysis cell and a rotating disk electrode (RDE).[6] Their optimized PED parameters for carbohydrate detection are given as wave form I in Table 1. Especially germane to this discussion is the value $E_{red} = -0.30$ V ($t_{red} = 360$ ms) and the ∼1-Hz frequency for the recommended wave form.[6]

It is generally accepted that at least 25 points are desired for proper reproduction of chromatographic peaks.[7] Whereas the 1-Hz sampling rate for wave form I (Table 1) is normally adequate for the characteristically broad peaks associated with typical separations of carbohydrate mixtures in anion-exchange columns, this frequency might not be adequate for the sharp peaks

† Present address: Department of Chemistry, St. Olaf College, Northfield, MN 55057.

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Table 1. PED Wave Form Parameters

	potentials (V vs Ag/AgCl)			time (ms)			
wave form	E_{det}	E_{oxd}	E_{red}	t_{del}	t_{int}	t_{oxd}	t_{red}
I	+0.20	+0.80	−0.30	240	200	180	360
II	+0.10	+0.60	−0.50	200	200	10	10
III	+0.10	+0.60	−1.00	200	200	10	10
IV	+0.10	+0.60	−1.00	50	50	10	10
V	+0.10	+0.60	−0.50	200	200	10	100

associated with microbore columns and CE, in which a sampling rate as high as 20 Hz has been recommended.[7]

Lu and Cassidy described application of PED for detection of carbohydrates in a mixture following their separation in a 10-μm × 60-cm capillary using a 2.6-Hz wave form applied at a 10-μm Au microdisk electrode.[8] The issue of fast PED wave forms was examined by Roberts and Johnson.[9] Seeking to determine the minimum values of t_{oxd} and t_{red} needed to effectively regenerate the electrode, they concluded that a monolayer of hydrous gold oxide (AuOH) is formed within 20 ms at $E_{oxd} = +0.50$ V in 0.1 M NaOH and that a complete monolayer of surface oxide is stripped away in 20 ms for $E_{red} < -0.50$ V. They also reported that at $t_{red} = 20$ ms the completeness of dissolution of the surface oxide is relatively insensitive to variations in E_{red} for values more negative than −0.50 V. They then went on to apply fast wave forms to PED detection of several carbohydrates separated by LC and CE with sampling frequencies ranging from 1.0 to 6.2 Hz.[9]

In the present work, we present results of an investigation into the cathodic removal of the "poisons" which result from carbohydrate oxidation at constant potential on a Au electrode. When these results are applied to the PED wave form, we find that we are able to decrease significantly the time periods for oxidative and reductive reactivation, thereby significantly increasing our sampling frequency.

EXPERIMENTAL SECTION

Reagents and Chemicals. Glucose and inositol (Fisher Scientific) were used as received. Deionized (DI) water used for preparation of all voltammetric and chromatographic solutions was purified by two D-45 demineralizing cartridges (Culligan) followed by a Milli-Q purification system (Millipore). Sodium hydroxide solutions were prepared by dilution of a 50% (w/w) stock solution (Fisher Scientific). DI water used for preparation of the 100 mM NaOH chromatographic mobile phase was prefiltered through a 0.22-μm pore size nylon membrane (MSI). The prepared eluent was deaerated with He, while NaOH solutions used in the voltammetric experiments were deaerated with N_2.

Voltammetry. Except where noted otherwise, voltammetric experiments were carried out using a AFRDE4 bipotentiostat, a AFMSRX rotator, and either a AFMD28AU rotating disk electrode (RDE) or a AFMT28AUAU rotating ring−disk electrode (RRDE, Pine Instrument). Electrodes were polished with 0.05-μm alumina (Buehler) in DI water and then rinsed with DI water. All working electrode potentials are referenced to a saturated calomel electrode (SCE). The counter electrode was a coiled Pt wire. The electrochemical cell was made of pyrex with fritted glass disks (medium porosity) separating compartments for the working, reference, and counter electrodes.

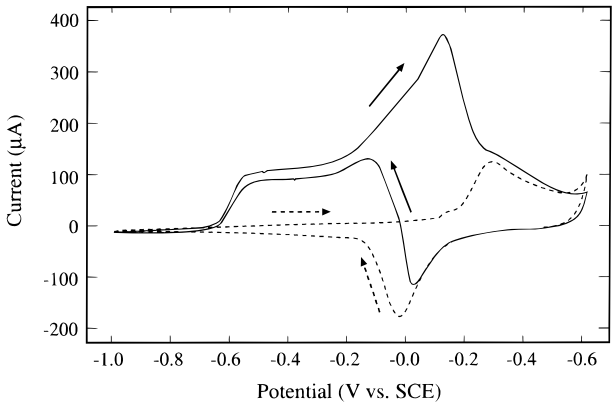

Figure 2. Voltammetric response for 1.0 mM glucose at a Au RDE in deaerated 1.0 M NaOH. The dashed line shows the residual response in the absence of glucose. Potential scan rate, 300 mV/s. Disk rotation rate, 1600 rpm.

Chromatography. All HPLC experiments were performed using chromatography equipment from Dionex Corp., except where otherwise indicated. A GPM-2 gradient pump was used with a CarboPac PA1 (4 × 250 mm) analytical column. The mobile phase was 100 mM NaOH at a flow rate of 1.0 mL/min. Sample injections were made with a pneumatically controlled injector and a 20-μL sample loop (Rheodyne). Detection was accomplished with an ED40 electrochemical detector. The thin-layer detector cell consisted of a 1.0-mm-diameter Au working electrode and a Ag/AgCl reference electrode, with the titanium cell body serving as the counter electrode.

Data Collection. Data collection for all chromatographic and voltammetric experiments was accomplished using a 486-DX personal computer (Apex) with a DT2821 data acquisition board (Data Translation). Computer programs for data collection were written using ASYST version 4.0 software (Keithley/Asyst).

RESULTS AND DISCUSSION

Reductive Desorption of Glucose Oxidation Products. Figure 2 shows the voltammetric response ($i_{disk} - E_{disk}$) for a Au RDE in deaerated 1.0 M NaOH with (−) and without (- - -) 1.0 mM glucose. The mechanism for glucose oxidation at Au electrodes in alkaline media has been examined.[10,11] As seen in Figure 2, the anodic response for glucose during the positive scan shows two distinctive steps. The first step, to generate the wave beginning at −0.65 V, is characterized by $n = 2$ equiv/mol. This step was speculated to correspond to oxidation of the aldehyde functionality of glucose to produce the gluconate anion in this alkaline medium.[10] This speculation was confirmed by analytical results obtained by Belgsir et al.[11] The current peak at +0.1 V, is characterized by $n = 8$ equiv/mol when determined for dilute solutions of glucose (<∼1 mM).[10] This was speculated to correspond to the oxidation of the aldehyde group (2 equiv/mol), followed by oxidation of the primary alcohol of the gluconate anion to produce the glucaronate dianion (4 equiv/mol), with subsequent oxidative elimination of formate anion with formation of the

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(8) Lu, W.; Cassidy, R. M. *Anal. Chem.* **1993**, *65*, 2878−2881.
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five-carbon dicarboxylate dianion (2 equiv/mol). No attempt was made by Larew and Johnson[10] to verify the identities of these oxidation products. However, it was noted that formate is not electroactive whereas the various carboxylate anions are electroactive at Au electrodes in alkaline media and can undergo further oxidation under conditions of exhaustive electrolysis. The sensitivity of the response at +0.1 V was determined to be diminished at high glucose concentrations, corresponding only to $n = \sim 2$ equiv/mol for concentrations of >10 mM.[10] This attenuation in sensitivity was speculated to be the consequence of termination of the oxidation sequence following the first step because preferential adsorption of glucose at the Au surface prevented the readsorption of gluconate and glucaronate species at catalytic surface sites as required for their subsequent oxidations. It is especially interesting in Figure 2 to note that the anodic response for glucose is sharply attenuated during the positive potential scan by the onset of oxide formation of $E > +0.1$ V. Clearly, the corresponding small amount of oxide formed during the scan to +0.3 V selectively passivates the catalytic surface sites, rendering them inactive for glucose oxidation until the oxide is cathodically reduced during the negative scan ($+0.1 > E > -0.1$ V).

Whereas formation of surface oxide quickly attenuates glucose oxidation, it is apparent in Figure 2 that a slight increase in response above the residual is observed in the presence of glucose toward the end of the positive sweep ($+0.3 < E_{disk} < +0.5$ V). This feature has been attributed to the oxidative removal of adsorbed residue produced during carbohydrate oxidation, thus forming the basis of the oxidative cleaning step (E_{oxd}, t_{oxd}) in PED wave forms (Figure 1).[12] It has become apparent recently, however, that a significant amount of the as-yet unidentified "poison(s)" also can be removed cathodically by a reductive desorption process. This process can be demonstrated clearly using a ring−disk electrode. Figure 3 shows ring−disk data obtained with the Au−Au RRDE for 1.0 mM glucose in 1.0 M NaOH with the ring potential (E_{ring}) held constant at −0.50 V. At this potential, the ring current (i_{ring}) is the result of oxidation of the aldehyde group of glucose ($n = 2$ equiv/mol) and this response exhibits virtually no attenuation as a consequence of the accumulation of oxidation products on the time scale of this experiment. Data in Figure 3A correspond to the cyclic scan of E_{disk} within the limits of −1.00 and −0.35 V; i.e., no surface oxide is generated at the disk electrode during the positive scan. The value $i_{ring} = 45\ \mu$A is observed in the absence of glucose oxidation at the disk ($E_{disk} < -0.7$ V). When aldehyde oxidation occurs at the disk, the flux of glucose at the ring electrode is shielded, thereby causing i_{ring} to decrease to 28 μA for $E_{disk} > -0.4$ V.

Data in Figure 3B were obtained after E_{disk} was held at −0.40 V for 10 min, after which the disk response had decayed to zero. It is important to note for $E_{disk} = -0.40$ V that no oxide is formed on the disk electrode; i.e., loss of disk response is due solely to accumulation of products of the glucose oxidation. The activity of the ring electrode was maintained throughout this 10-min period by continuously cycling E_{ring} between the limits −1.0 and +0.6 V. At the conclusion of the 10-min poisoning period, E_{ring} was returned to a constant value of −0.50 V. Then, E_{disk} was scanned in the negative direction and the $i_{disk} - E_{disk}$ and $i_{ring} - E_{disk}$ curves were recorded, as shown in Figure 3B by the dashed and solid lines, respectively. Initially, the i_{disk} remained at zero. However, a small cathodic peak was obtained in the region $-0.65 > E_{disk} >$

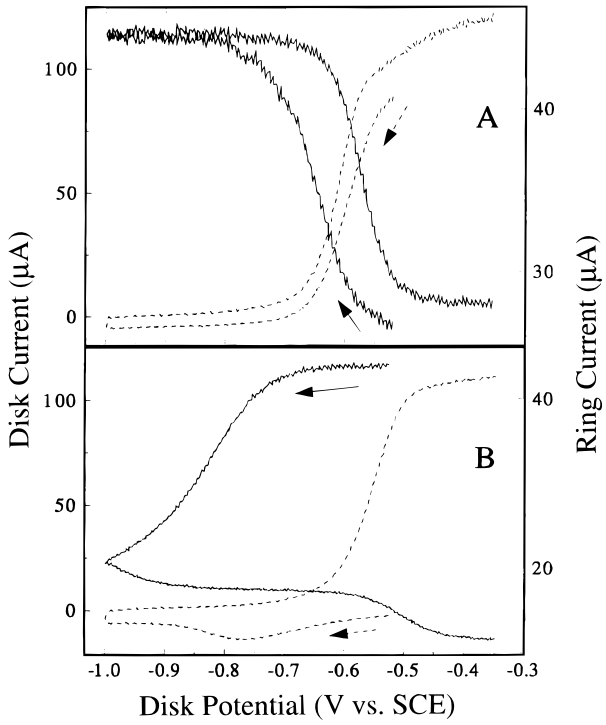

Figure 3. Ring (−) and disk (- - -) voltammetric response for 1.0 mM glucose at a Au RRDE in deaerated 1.0 M NaOH. (A) Response following normal potential cycling at the disk. (B) Response after holding disk potential at −0.40 V for 10 min. $E_{ring} = -0.50$ V. Disk scan rate, 300 mV/s. Rotation rate, 1600 revolutions/min.

−0.85 V with a peak potential (E_p) of −0.77 V. The fact that this cathodic process results in reactivation of the disk electrode is demonstrated by the appearance of the anodic wave for glucose for $E_{disk} > -0.65$ V during the subsequent positive scan from −1.0 V to give a limiting value of $i_{disk} = 110\ \mu$A, characteristic of this solution under cycling conditions (cf. Figure 2). This observation leads us to assign the cathodic process corresponding to the peak at $E_{peak} = -0.77$ V (negative scan) to the reductive desorption of oxidation products accumulated during the 10-min period at $E_{disk} = -0.40$ V.

This peak assignment is upheld by the ring data shown in Figure 3B (solid line). At the beginning of the negative scan of E_{disk} from −0.50 V, the poisoned disk allows the ring to maintain its unshielded value of $i_{ring} = 45\ \mu$A (cf. Figure 3A). However, as E_{disk} passes through the region of the cathodic peak under consideration, i_{ring} is attenuated to less than half of its original value. The only possible explanation of this decrease in i_{ring} when glucose is not being oxidized at the disk electrode is that the ring surface is poisoned by the species desorbed from the disk surface in the region $-0.65 > E_{disk} > -0.85$ V (negative scan). A further decrease in i_{ring} is seen when the disk electrode once again begins to shield glucose from the ring electrode for the oxidation reaction for $E_{disk} > -0.65$ V (positive scan). We have made no attempt to identify the fouling species. We do note, however, the phenol oxidized at a Au electrode is believed to poison the electrode surface through the accumulation of poly(phenylene oxide) films.[13] Whereas an analogous polymerization is difficult to envision for the product(s) of glucose oxidation, these adsorbed carbonaceous species are very effective in their attenuation of electrode activity.

Application of PED Wave Forms. The question arises of whether the reductive desorption process described can be

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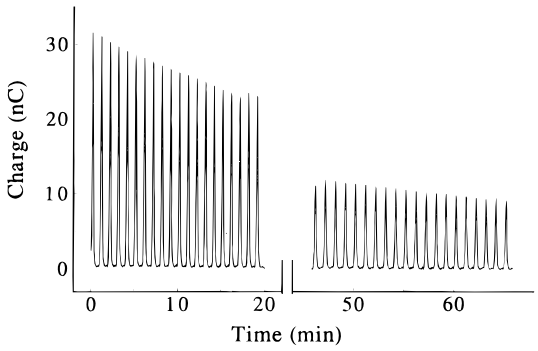

Figure 4. LC-PED results for 40 injections of 1.0 nmol of glucose, utilizing wave form II from Table I. Column, Dionex PA-1. Eluent, 0.10 M NaOH at 1.0 mL/min.

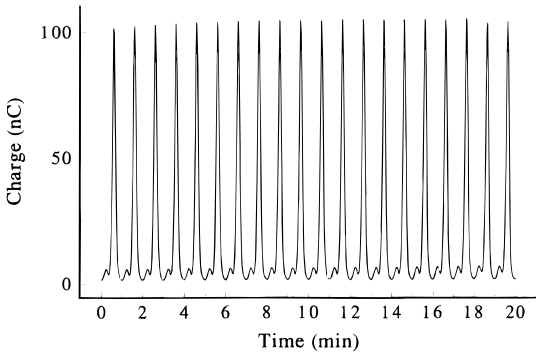

Figure 5. LC-PED results for 20 injections of 1.0 nmol of glucose, utilizing wave form III from Table I. All other conditions same as Figure 4.

incorporated into the PED wave form for carbohydrate detection. Reductive desorption cannot occur at the previously recommended value $E_{red} = -0.3$ V (wave form I, Table 1).[6] However, if E_{red} is changed to a value <-0.8 V, we do not have to rely solely on oxidative cleaning at E_{oxd} for complete removal of the adsorbed poisons. As a result, t_{oxd} and, perhaps, t_{red} can be decreased to significantly increase wave form frequencies. Figure 4 shows the PED response using wave form II (Table 1) for multiple injections of 1.0-nmol aliquots of glucose. Whereas the detection step ($t_{del} + t_{int}$) remains long (400 ms), the values of t_{oxd} and t_{red} are both 10 ms, i.e., the shortest time increment allowed by the Dionex ED40 detector. The value $E_{red} = -0.50$ V in this wave form is sufficiently positive so that reductive desorption cannot occur during application of E_{red}. It is noted that whereas 1.0 M NaOH was the medium used in Figures 2 and 3, the chromatographic mobile phase is 0.10 M NaOH. Nevertheless, glucose oxidation in either medium yields the reductive desorption peak (negative scan). Figure 4 shows the results of 40 glucose injections. Twenty consecutive injections were made at 1.0-min intervals and then, following a 25-min intermission during which application of wave form II continued, 20 more injections were made. A steady decrease in peak height is observed. Therefore, it is apparent that the extent of oxidative cleaning for $t_{oxd} = 10$ ms ($E_{oxd} = +0.60$ V) is not sufficient to maintain a reproducible and high state of electrode activity. It also is interesting that during the 25-min intermission when no injections were made, the electrode activity continued to decrease, as shown by the large discontinuity in peak decay between the 20th and 21st peaks. This observation is addressed below.

Following elution of the final peak of Figure 4, the value of E_{red} was decreased from -0.50 to -1.00 V (wave form III, Table 1); however, E_{oxd} and t_{oxd} remained the same as in Figure 4. This new reduction potential induces a contribution from the reductive desorption process at $E < -0.65$ V. Twenty minutes after the change to wave form III, 20 more injections of 1.0 nmol of glucose were made, with the results shown in Figure 5. Now we find a stable, reproducible peak signal (RSD = 0.97%).

Fast PED Wave Forms. The reproducible glucose peaks shown in Figure 5 are evidence that, for $E_{red} = -1.00$ V, the instrumental limits of 10 ms for t_{red} and t_{oxd} ($E_{oxd} = +0.60$ V) are sufficient to regenerate and maintain electrode activity. Any further modifications of the wave form parameters to increase wave form frequency must come by decreases in t_{del} and t_{int}. To discriminate against 60-Hz line noise, t_{int} is typically chosen to be an integral number of 16.7-ms increments, with larger values of t_{int} resulting in higher sensitivity.[12] We arbitrarily choose $t_{int} = 50$ ms and $t_{del} = 50$ ms (wave form IV, Table 1). The total time for one cycle of this wave form is 150 ms, not 120 ms as calculated from the parameters in Table 1 ($t_{del} + t_{int} + t_{oxd} + t_{red}$) because a 10-ms rise time is allotted in the ED40 to achieve each of the three potential steps to avoid the risk of driving the operational amplifiers to their current limits. Therefore, the resulting frequency is 6.7 Hz.

The initial peak height shown in Figure 4 and obtained, supposedly, for a fully active electrode surface (~32 nC) is substantially smaller than that shown in Figure 5 for a reproducibly clean electrode (~100 nC). This difference is explained as follows: Wave forms IV (Figure 4) and V (Figure 5) differ in the value chosen for E_{red}. At $E_{red} = -0.50$ V (Figure 4), the oxidation of glucose continues throughout the t_{red} period, and therefore, the concentration profile of glucose already has been established at approximately the steady-state value when the potential is stepped from E_{red} to E_{det}. However, for $E_{red} = -1.0$ V (Figure 4), oxidation of glucose ceases following the step to $E_{red} = -1.0$ V. Therefore, the concentration of glucose within the diffusion layer is allowed to be reestablished at the bulk value during the t_{red} period. As a result, a larger signal is registered following the potential step from $E_{red} = -1.0$ V to E_{det}.

Calibration data were obtained for injections of glucose in the range 1 pmol to 40 nmol using wave form IV. The log−log plot of peak signal (S) vs picomoles of glucose injected appeared linear in the range 5−2000 pmol (2.6 decades). Linear regression parameters a and b ($S' = a + b$(pmol)) were calculated for this region using both a traditional regression procedure, in which constant uncertainties were assumed throughout the range of picomole values and a weighted regression procedure which assumes a variance proportional to picomole.[14] Figure 6 shows log−log plots of the normalized peak signal, i.e., $|(S - a)/b(\text{pmol})|$, vs picomole. This method of graphical presentation has been recommended over customary plots of S vs picomole, or log S vs log picomole, for the benefit of placing deviations of S from S' on the same rational scale over the entire range of picomole values.[14] Accordingly, the value $\log |(S - a)/b(\text{pmol})| = 0$ is expected when $S = a + b$(pmol). Also shown in Figure 6 are intervals corresponding to relative deviations (Δ) of S and S' equal to ± 10 and ± 30%. Defining the limits of linearity (LOL) as $\Delta = 10$%, and using the regression parameters (a, b) calculated from the weighted procedure, linear response is seen to extend over slightly

(14) Johnson, D. C. *Anal. Chim. Acta* **1988**, *204*, 1−5.

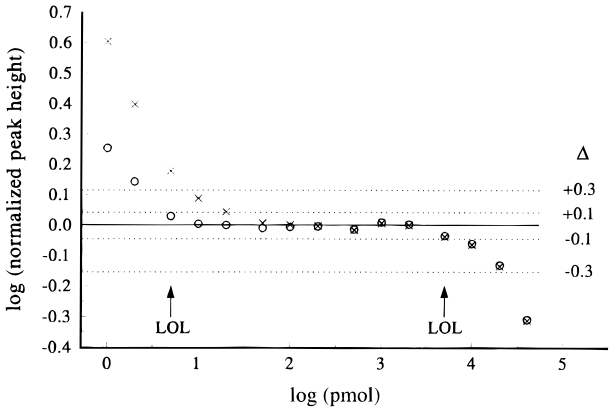

Figure 6. Calibration plot of log $|(S - a)/b(\text{pmol})|$ vs log picomole for LC-PED determination of glucose using 6.7-Hz PED sampling frequency (waveform IV, Table 1). Regression parameters a and b ($S' = a + b(\text{pmol})$) were calculated using both a traditional regression procedure (×, $a = -0.0602$, $b = +0.0255$) and a weighted regression procedure (○, $a = -0.00327$, $b = +0.0253$). Lines are included to show relative deviation (Δ) of S from S' equal to ±10 and ±30%.

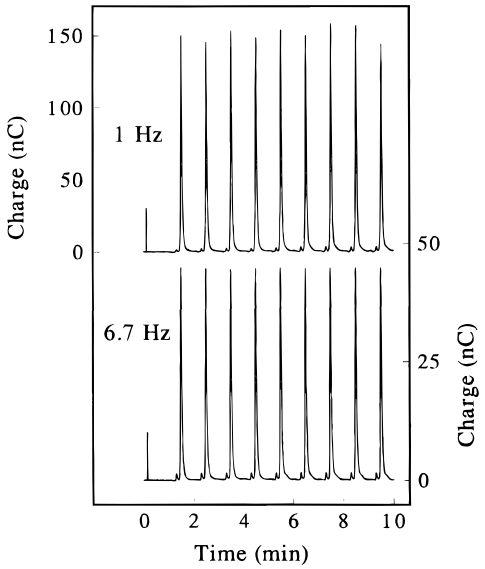

Figure 7. Effect of sampling frequency on LC-PED results for multiple injections of 0.40 nmol of inositol. 1-HZ PED wave form: $E_{det} = 0.10$ V, $t_{del} = 200$ ms, $t_{int} = 200$ ms; $E_{oxd} = 0.60$ V, $t_{oxd} = 200$ ms; $E_{red} = -1.00$ V, $t_{red} = 400$ ms. Wave form IV in Table 1 (6.7 Hz). All other conditions same as Figure 4.

more than 3 decades. A linear range of ~3.5 decades was reported previously using the same column.[12] We speculate that the slightly smaller linear range in Figure 6 results from our use of an integration period (t_{int}) of 50 ms, as compared to 200 ms in the previous study. The background signal was measured for a 3-min period and the standard deviation (σ_{bkg}) was calculated to correspond to 100 fmol. Therefore, we estimate a detection limit ($3\sigma_{bkg}$) of 300 fmol. This is surprisingly comparable to the 200-fmol detection limit reported for a 1-Hz wave form in which t_{int} = 200 ms.[15]

The LC-PED data presented in Figure 6 for a 6.7-Hz wave form show a significant improvement over previously reported results from our laboratory.[9] The fastest wave form investigated in that study, ~6.2 Hz, relied on a value of 30 ms for both t_{del} and t_{int}, yielding a detection limit of only 11 pmol. We attribute our improvement to increased values of t_{del} and t_{int}, made possible by decreased values of t_{oxd} and t_{red}, in addition to the improved noise reduction characteristics of the Dionex ED40 in comparison to its predecessor.

The obvious advantage of a fast PED wave form for sharply eluting peaks is shown in Figure 7. Peaks for multiple injections of 0.4-nmol inositol were obtained using PED wave forms with frequencies of 1 (wave form I) and 6.7 Hz (wave form IV). Under the chromatographic conditions used in Figure 7, inositol elutes with a retention time (t_r) of 1.34 min and a peak width at half-maximum ($w_{1/2}$) of 3.5 s. The 6.7-Hz sampling frequency shows a much lower degree of variability in the peak height in nine injections (RSD = 0.2%) than does the 1-Hz sampling frequency (RSD = 3.6%), but with an expected sacrifice of sensitivity resulting from the shorter integration period (t_{int}).

Contribution of Oxidative Cleaning. It is apparent in Figure 5 that cathodic desorption of carbohydrate residues at $E_{red} = -1.00$ V (t_{red} = 10 ms) makes a very effective contribution to the maintenance of electrode activity. However, there is concern that this low value of t_{red} might not be sufficient to completely reduce whatever surface oxides might be formed at $E_{oxd} = +0.60$ V (t_{oxd} = 10 ms). Therefore, a passivating oxide film might accumulate over very long operating periods. A comparison of Figures 4 and 5 shows evidence for this effect. The large difference in the heights of peaks 20 and 21 (Figure 4) shows that during the 25-min intermission period the extent of surface passivation increased even though no glucose was injected.

This observation suggests that the small amount of surface oxide formed during the 10-ms period at $E_{oxd} = +0.60$ V cannot be completely reduced within the 10-ms period at $E_{red} = -0.50$ V (wave form II). However, for $E_{red} = -1.00$ V (wave form III), the rate of oxide reduction apparently is sufficient to prevent oxide buildup (Figure 5). Further evidence for an increased rate of oxide removal at $E_{red} = -1.00$ V is apparent in Figure 8A, which contains the PED response for 0.20 mM glucose at a Au RDE in 0.10 M NaOH obtained using the ED40 potentiostat. The solution remained aerated in order to mimic the relatively high O_2 levels present in chromatographic eluates. Initially, wave form II (E_{red} = −0.50 V, t_{red} = 10 ms) was applied for 15 min. As expected, the PED signal drops continuously. A shift to wave form III (E_{red} = −1.00 V, t_{red} = 10 ms) causes the PED signal to return to its initial value and remain constant. A return to wave form II results again in a steady signal decline. At 45 min, wave form V (E_{red} = −0.50 V, t_{red} = 100 ms) is applied. The E_{red} value in this wave form is not sufficiently negative to induce reductive desorption; however, the increase in t_{red} appears to result in more complete reduction of the surface oxide. As seen from Figure 8A, wave form V causes the signal to increase and remain relatively constant over the 15 min of application. We conclude, also, that the increase in PED signal associated with the increase in t_{red} (compare V to III) is the result of a longer time period for the concentration of glucose in the diffusion layer to return to the bulk value.

The possibility that the reproducible peaks in Figure 5 are the result of faster oxide reduction at $E_{red} = -1.00$ V now requires reexamination of the role of reductive desorption in maintenance of electrode activity. Evidence that reductive desorption does contribute to surface regeneration is illustrated by data shown in Figure 8B. These PED data correspond to the detection of 0.20

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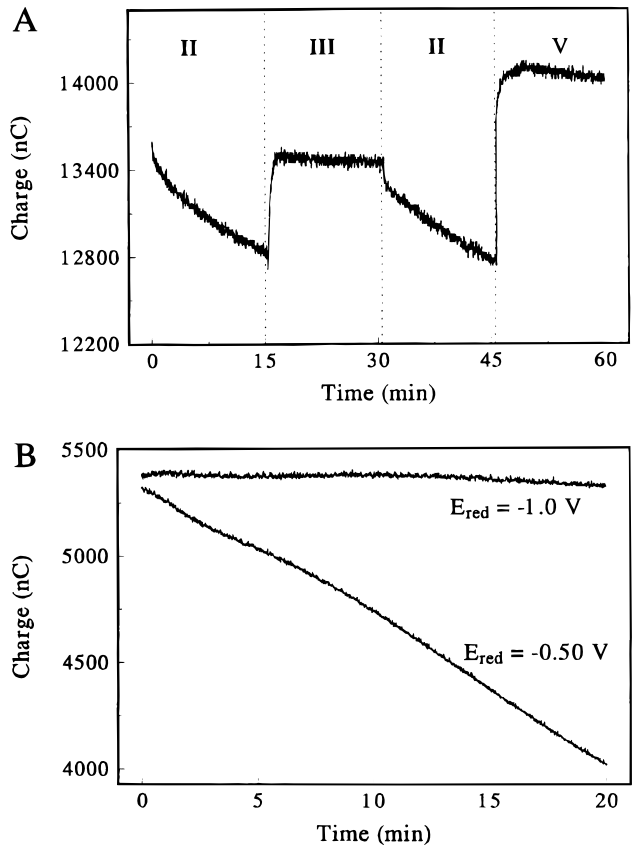

Figure 8. PED results for 0.20 mM glucose in aerated 0.10 M NaOH at a Au RDE. Rotation rate, 400 revolutions/min. (A) Response to three-step PED wave forms with Roman numerals corresponding to wave forms designated in Table 1. (B) Response to two-step PED wave forms with $E_{det} = -0.10$ V, $t_{del} = 200$ ms, $t_{int} = 200$ ms, and $t_{red} = 100$ ms.

mM glucose at the Au RDE using a two-step wave form with $E_{det} = -0.1$ V ($t_{del} = 200$ ms, $t_{int} = 200$ ms) and $E_{red} = -0.50$ and -1.0 V ($t_{red} = 100$ ms). At this value of E_{det}, glucose is detected; however, no surface oxide is formed (see Figure 2), and therefore, there is no possible contribution from oxidative cleaning of the electrode surface. For $E_{red} = -0.50$ V, as expected, the PED response shows a continuous decay over the observation period. However, for $E_{red} = -1.00$ V, the PED signal remains nearly constant at the value obtained for a fully activated electrode. These results demonstrate the significant contribution of the reductive desorption step for maintaining electrode activity during glucose detection. However, the small attenuation in signal after ~15 min indicates that reductive desorption alone is not sufficient for detection of carbohydrates in 0.10 M NaOH. Therefore, we recommend use of a three-step wave form in which brief periods of oxidative cleaning ($E_{oxd} = +0.60$ V, $t_{oxd} = 10$ ms) and reduction desorption ($E_{red} = -1.0$ V, $t_{red} = 10$ ms) are combined.

We summarize by stating that both reductive desorption and rapid oxide stripping contribute to the effectiveness of wave form IV for carbohydrate detection at 6.7 Hz, although the extent to which each of these factors contributes cannot be determined at this point.

CONCLUSIONS

The electrochemical activity of Au electrodes fouled by products of glucose oxidation in alkaline solution can be restored by reductive desorption of the accumulated poisons at $E_{red} = -1.0$ V vs Ag/AgCl. Incorporation of this reductive desorption process within PED wave forms allows t_{red} and t_{oxd} to be decreased to the instrumental limit of 10 ms, resulting in a substantial increase in wave form frequency over the previously recommended value of 1 Hz. LC-PED results for glucose using a 6.7-Hz wave form yield a linear dynamic range of 3 decades and a detection limit of ~300 fmol.

Our demonstration that PED wave forms can be successfully applied at frequencies greater than 1 Hz is significant as applications of PED move from traditional LC to microbore chromatography and capillary electrophoresis.

Results are reported here only for glucose response. Nevertheless, preliminary data support our prediction that the prescribed modification to the traditional PED wave form will be beneficial for detection of all carbohydrates. However, description of specific data must await a later communication.

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