Anal. Chem. **2005,** *77,* 3694−3699

Programmed Potential Sweep Voltammetry for Lower Detection Limits

Jung-Suk Yoo and Su-Moon Park*

Department of Chemistry and Center for Integrated Molecular Systems, Pohang University of Science and Technology, Pohang, Gyeongbuk 790-784, Korea (ROK)

We report a novel programmed potential sweep voltammetry for a much lower detection limit than those achieved by any other known electroanalyitcal techniques. In this technique, an input waveform is programmed such that the background current would become flat or any other predefined form in the potential region of interest where the peak current arising from the analyte is observed, followed by the amplification of the background subtracted peak current. The current thus obtained showed a much better signal integrity at very low analyte concentrations than those obtained by the traditional linear sweep voltammetric and other related voltammetric techniques. The technique was applied to the analysis of dopamine at a carbon ultramicroelectrode (10-μm diameter). The background-compensated currents showed excellent dynamic linearity for dopamine concentrations of more than 3 orders of magnitudes between 500 pM and 100 nM with an estimated detection limit of 127 pM. This method can provide a convenient way for determining biogenic amines in real time with a much higher sensitivity.

Linear sweep voltammetry (LSV) is a simple technique used for recording voltammograms for primarily diagnostic purposes thanks to its simplicity, although its sensitivity is rather poor as an analytical tool.[1,2] For this reason, LSV has not been considered seriously as an analytical tool. However, many investigators have been putting in efforts to improve its sensitivity. Perone et al. developed the derivative techniques to improve the resolution as well as the sensitivity, and an improvement in sensitivity by at least 1 order of magnitude has been achieved.[2−4] In efforts to improve the signal-to-noise ratio, other related voltammetric techniques such as normal and differential pulse voltammetry have been developed, in which capacitive components are minimized by either sampling the currents after the capacitive components have decayed or taking the differential currents.[6,7] Limitations of these techniques include relatively long measurement time and insufficient sensitivities for measuring small amounts of analytes such as dopamine in biological samples. Wightman et al. developed a variety of electrochemical techniques for the determination of dopamine employing a variety of methods.[8−13] The temporal resolution of cyclic voltammetric responses obtained at carbon fiber electrodes from different biogenic amines were made by deconvolution of signals based on the impulse response theory.[9,10] They also reported its selective and sensitive detection method by controlling the scan rate and signal processing, which used analog filtering and ensemble averaging, and the detection limit was 100 nM at Nafion-coated carbon fiber electrodes.[8,11−13] Nevertheless, Venton and Wightman[8] pointed out the difficulties encountered in the analysis of low-level biogenic amines in in vivo environments.

The capacitive current is a major source of noise in voltammetric measurements, particularly during the fast-scan voltammetric measurements, unless the solution contains any other impurities contributing to unwanted currents due to their redox reactions or adsorption. Elimination of the contributions from capacitive currents in LSV measurements is not trivial although background signals may be subtracted.[10,15] This is because the double layer capacitance, which is usually taken to be independent of the electrode potential, is a function of the electrode potential in an unpredictable manner, although there have been theories describing it.[14] As is well known, the capacitance of an electrode increases in different forms on both sides of the point of zero charge in a given electrolyte solution. Thus, how the double layer capacitance changes as a function of applied potential is very complex, depending on the electrode material, the type of supporting electrolyte, and its concentration. The capacitive current observed during the linear potential sweep has an expression,

$$i_c = CA(\mathrm{d}E/\mathrm{d}t) \quad (1)$$

where C is the double layer capacitance, assumed to be constant

* To whom correspondence should be addressed. E-mail: smpark@postech.edu. Phone: +82−54-279-2102. Fax: +82-54-279-3399.

(1) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods*; 2nd ed.; Wiley: New York, 2002; Chapter 6.
(2) Baoxian, Y.; Ping, X.; Lin, L. *Microchem. J.* **2000**, *64*, 125.
(3) Evins, C. V.; Perone, S. P. *Anal. Chem.* **1965**, *37*, 1061.
(4) Mueller, T. R.; Perone, S. P. *Anal. Chem.* **1965**, *37*, 1.
(5) Harrar, F. B.; Anderson, R. E.; Perone, S. P. *Anal. Chem.* **1968**, *40*, 899.
(6) Osteryoung, J. *Acc. Chem. Res.* **1993**, *26*, 77.
(7) See Chapter 7 of ref 1.
(8) Venton, B. J.; Wightman, R. M. *Anal. Chem.* **2003**, *75*, 414A.
(9) Bath, B. D.; Michael, D. J.; Trafton, B. J.; Joseph, J. D.; Runnels, P. L.; Wightman, R. M. *Anal. Chem.* **2000**, *72*, 5994.
(10) Pihel, K.; Schroeder, T. J.; Wightman, R. M. *Anal. Chem.* **1994**, *66*, 4532.
(11) Venton, B. J.; Troyer, K. P.; Wightman, R. M. *Anal. Chem.* **2002**, *74*, 539.
(12) Wiedemann, D. J.; Kawagoe, K. T.; Kennedy, R. T.; Ciolkowski, E. L.; Wightman, R. M. *Anal. Chem.* **1991**, *63*, 2965.
(13) Hochstetler, S. E.; Puopolo, M.; Gustincich, S.; Raviola, E.; Wightman, R. M. *Anal. Chem.* **2000**, *72*, 489.

10.1021/ac0481598 CCC: $30.25
Published on Web 04/21/2005

throughout the potential scanned, A the electrode area, and E the potential applied to the electrode (in V). Again, C here is a function of the potential although other parameters mentioned above are constant. The capacitive current can thus have a number of local fluctuations due to changes in double layer capacitances, depending on potentials. Finally, the capacitive current is directly proportional to the scan rate as seen from eq 1, while the faradaic current is proportional to its square root.

Nevertheless, the dopamine analysis has been carried out at high-voltage scan rates for two reasons.[9,11] First, oxidation of ascorbic acid is discriminated against by faster scan rates due to its sluggish electron-transfer rate compared to that of dopamine even though their oxidation potentials are about the same. Second, faster signal recording is required for real-time analysis in in vivo systems. In most in vivo systems, the faradaic current is small in comparison to the capacitive component due to its low concentrations and the fast-voltage scan rates used. Thus, a large amplification of the composite signal does not help much in restoring the signal integrity because the full-scale current is set to record the large total current. The situation can become even worse when this signal is presented to the input of the analog-to-digital converter (ADC); the faradaic signal may then compete for the bit resolution of the ADC. Thus, it is very important to remove a large fraction of the capacitive current before the signal is amplified. This can be attained by first making the capacitive and other background signals in a predefined form in the voltage region of interest and by removing them using a hardware method.

In this note, we propose a novel technique, programmed potential sweep voltammetry, in which the potential sweep signal is programmed such that the background currents including the capacitive component will become flat or any other predetermined shape in the potential region of interest. Then, a large fraction of the background signal is removed by hardware subtraction before the faradaic signal of interest and a small fraction of the capacitive current is subsequently amplified. This makes the faradaic current commensurate with the full scale of the A/DC input, which takes a full advantage of the high bit resolution of the A/DC. This procedure is applied to the analysis of dopamine in the presence of ascorbic acid as an interferent.[2,8,9]

EXPERIMENTAL METHOD

Dopamine (Aldrich Chemical, 98%), ascorbic acid (Samchun Pure Chemical, special grade), monobasic sodium phosphate (NaH_2PO_4, Shinyo Pure Chemical, S.P.C. grade), dibasic sodium phosphate (Na_2HPO_4, Aldrich Chemical, A.C.S. grade), hexaaminеruthenium(II) chrolide [$Ru(NH_3)_6Cl_2$, Aldrich Chemical, 98%], and potassium nitrate (KNO_3, Junsei CHemical, Extra Pure grade) were used as received. Deionized, doubly distilled water was used for the preparation of solutions. A 1.0 mM dopamine solution was prepared and used as a stock solution. An appropriate volume of the dopamine stock solution was then injected into the solution containing 10 mM ascorbic acid and 0.10 M phosphate, which served both as a buffer solution (pH 7.0) and an electrolyte for the measurements, to make up a final desired concentration. For the $Ru(NH_3)_6^{2+}$ solution, 0.10 M KNO_3 was used as an electrolyte.

For the electrochemical measurements, a glassy carbon ultramicroelectrode (UME, Bioanalytical Systems, diameter 10 μm) for dopamine and gold UME (Bioanalytical Systems, diameter 10 μm) for $Ru(NH_3)_6^{2+}$ were used at an average scan rate of ~400 V/s. A two-electrode system, in which the UME and a carbon rod of 5-mm-diameter were used as working and counter electrodes, was used because a typical three-electrode system was found to produce relatively large voltage noise signal of as much as about 100−500 μV depending on the potentiostat used due to the large input impedance of the reference electrodes. The potential drop would occur entirely at the working electrode with an insignificant contribution from the IR_u voltage drop due to relatively small uncompensated solution resistances (R_u, vide infra), because the current is very low at a nanoampere level and the solution resistance is reasonably small due to the relatively high electrolyte concentration while the counter electrode is much larger than the working electrode. The typical commercial potentiostat generated voltage noise of as high as 500 μV, and thus, we constructed a potentiostat with a low noise current-to-voltage (I/E) converter with AD549 (Analog Devices). The schematic of the potentiostat and signal processor design is shown in Figure 1.

RESULTS AND DISCUSSION

To obtain a prescribed waveform for both the capacitive and other background currents resulting from the matrix (electrolyte and ascorbic acid), we designed a feedback system as shown in Figure 2a. Here a desired form of the background current, which is flat in the potential region of interest where dopamine oxidation takes place, is first synthesized using an arbitrary waveform generator (Agilent 33120A). It is then compared with the background current output from the actual electrochemical cell, which has an electrolyte solution containing all but the analyte, by inputting a feedback-corrected sweep signal via the potentiostat. This operation is repeated automatically until the input voltage sweep signal gives the smallest possible difference between the synthesized and actual background currents. The feedback amplifier system was made of a differential amplifier constructed using a National Semiconductor LF351 operational amplifier and other passive elements including capacitors and resistors for stabilization of the feedback system. When the final voltage sweep signal that produces the desired capacitive current waveform is obtained, it is saved in a digital oscilloscope (HP 54645) and then used as a voltage sweep signal to record voltammograms of the analyte in the same electrochemical cell employing the arbitrary waveform generator via the potentiostat. It usually takes from as short as a few to as long as about 10 min to find an appropriate sweep signal that generates the desired capacitive current. In our case, the potential sweep was programmed so that a flat capacitive current was obtained in the potential region between 0.10 and 0.60 V versus Ag/AgCl (in saturated KCl) electrode in order to record voltammograms for dopamine oxidation in the presence of a large amount of ascorbic acid as an interfering agent. The dopamine oxidation has a peak current in this potential region with the current peak for ascorbic acid oxidation shifted to a much more positive potential at the average scan rate of 400 V/s due to its sluggish electron transfer in comparison to dopamine.[1]

(14) See Chapter 13 of ref 1.
(15) Howell, J. O.; Kuhr, W. G.; Ensman, R. E.; Wightman, R. M. *J. Electroanal. Chem.* **1986**, *209*, 77.

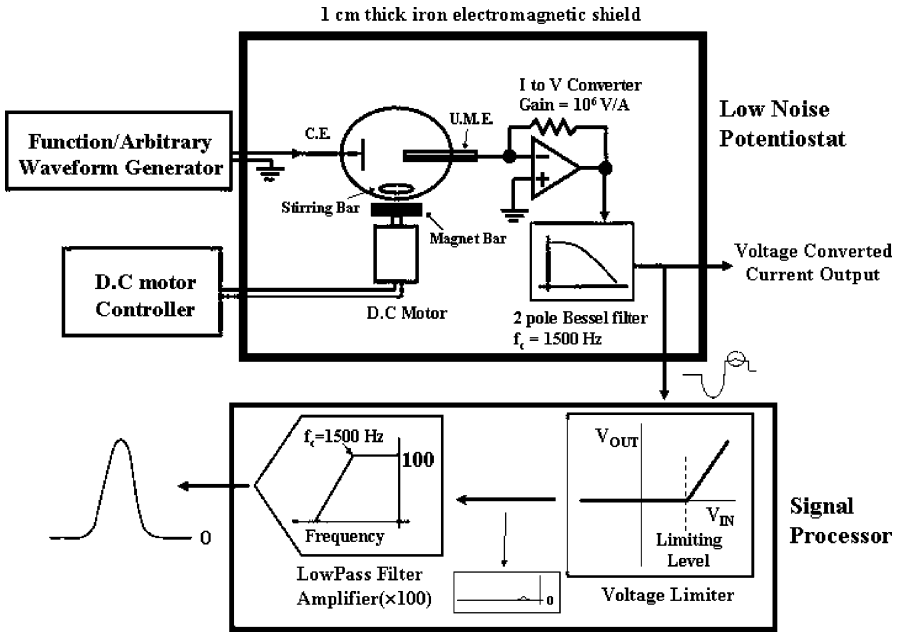

Figure 1. Schematic diagram for a low-noise potentiostat and signal processing unit.

To amplify the background-subtracted faradaic current, a large fraction of the capacitive current was removed using a voltage limiter from the composite signal obtained via the I/E converter that has an amplification factor of 10^6 V/A. The level of the voltage that the limiter used was slightly lower than the synthesized background signal. To ensure that no faradaic component would be lost, the voltage limiter signal should be set such that a small fraction of the capacitive current would be left over near the oxidation potential. Now, the faradaic signal plus a small fraction of the background current obtained from this operation was then restored using another amplifier with a gain of 100 through a low-pass filter having a cutoff frequency of 1500 Hz. This operation reduced the full-scale current significantly, which allows the faradaic component to be amplified maximally, and thus, the signal integrity as well as its sensitivity would be improved significantly.

Figure 3 shows the voltage sweep waveform (solid curve) obtained from the repeated feedback operation and the flat capacitive current (dashed curve) obtained thereof with the aforementioned setup in the actual electrochemical cell. The blank solution used for this experiment contained 0.10 M sodium hydrogen phosphate (pH 7.0), 10 mM ascorbic acid, and no analyte (dopamine) to simulate the biological fluids containing dopamine.[1,7−11] Note that the waveforms for the forward and reverse scans are quite different from each other, and the background current obtained during the forward voltage scan is flat and slightly larger than others in the potential region of interest. In other words, the microscopic changes in background currents during the potential sweep could vary wildly if the voltage sweep waveform were linear. Note also that the slopes of the sweep signal vary depending on the potential region and that the average scan rate used here is ~410 V/s; the exact scan rate varies depending on the capacitive and other responses at the working electrode in the electrolyte matrix to be used for analysis.

Figure 4 shows a series of background-subtracted voltammograms recorded for the dopamine solution with its concentrations of 0.5, 1.0, 1.5, and 2.0 nM, respectively, which were prepared by injecting an appropriate amount of the stock solution. We see clearly from these voltammograms that very low currents are recorded with the background more or less completely removed. Also, the current decay pattern shown beyond the peak potential indicates that dopamine oxidation displays the characteristics of its relatively weak adsorption,[16] not the diffusion-limited behavior, as Venton and Wightman pointed out.[8] The excellent quality of the current signals is also confirmed by the good linearity obtained in this range with a correlation coefficient of greater than 0.999. To check the dynamic linearity, solutions containing a wider range of concentrations have been examined and the results showed that a good dynamic linearity was observed (correlation coefficient of 0.996) for more than 3 orders of magnitude of the dopamine concentrations as shown in Figure 5. The detection limit estimated from the slope of the regression line and the standard deviation around the regression was ~127 pM.

We also applied the same technique to another electroactive substance, $Ru(NH_3)_6^{2+}$. The plot displayed a dynamic linearity over 2 orders of magnitude between 1 and 100 nM with a correlation coefficient of 0.992 and a detection limit of 161 pM. This is comparable to that for dopamine.

Obviously, the voltammograms shown here cannot be interpreted as the forward portion of a cyclic voltammogram or a linear sweep voltammogram is, because the voltage sweep signal comprises many segments of different scan rates depending on the potential region as shown in Figure 3 (thick line). Thus, the voltammograms observed here cannot be described by the current expression, which has been derived for a reversible electron-transfer reaction,[1]

$$i = nFAC_R^*(\pi D_R \sigma)^{1/2}\chi(\sigma t) \qquad (2)$$

where n is the number of electrons transferred, F is the Faraday

(16) Wopschall, R. H.; Shain, I. *Anal. Chem.* **1967**, *39*, 1514.

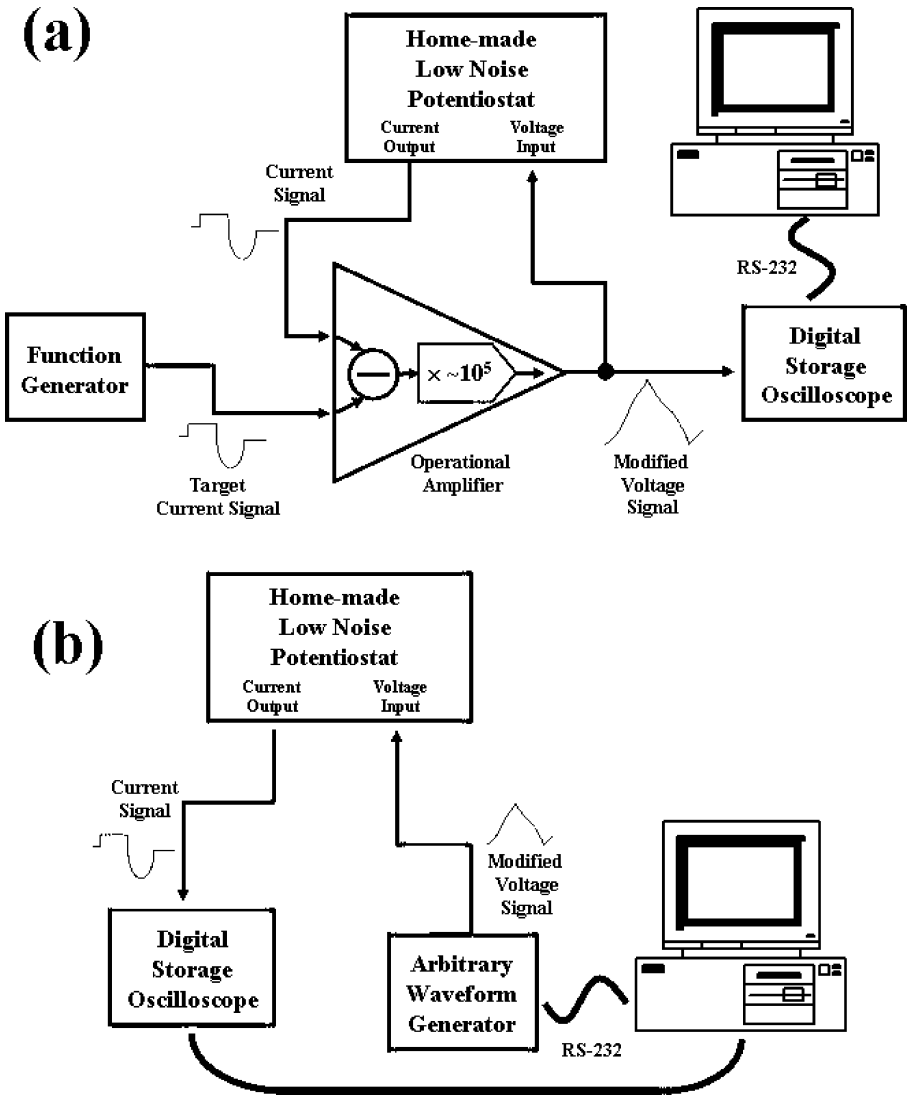

Figure 2. (a) Schematic diagram showing a feedback system to obtain a predefined background current waveform using various voltage sweep signals. (b) Operating a potentiostat employing a voltage sweep signal, which was obtained from the feedback system shown in (a), to produce a predetermined capacitive current waveform using an arbitrary waveform generator.

constant, A is the electrode area, C_R^* is the bulk concentrations of a reductant such as dopamine, D_R is the diffusion coefficient of dopamine, σ is $(nF/RT)v$, with v being the voltage scan rate, and χ (σt) is the current function. This is because σ is not constant as in LSV but varies depending on the potential region, and the contribution of the $v^{1/2}$ (or $\sigma^{1/2}$) term to the current, i, would also vary depending on the potential region. This may cause shifts in peak potentials due to the modulation of the flux of the electroactive species, e.g., dopamine, in different potential regions. However, the current will still be determined primarily by the overpotential expressed by σt, which has an expression,[1]

$$\sigma t = (nF/RT)[E(t) - E_i] \qquad (3)$$

where $E(t)$ is the potential at time t during the scan and E_i is the initial potential at $t = 0$. Thus, the concentration dependency of the current is maintained as long as the experimental conditions are not varied and the programmed potential voltammograms can be used for analytical purposes. The excellent dynamic linearity

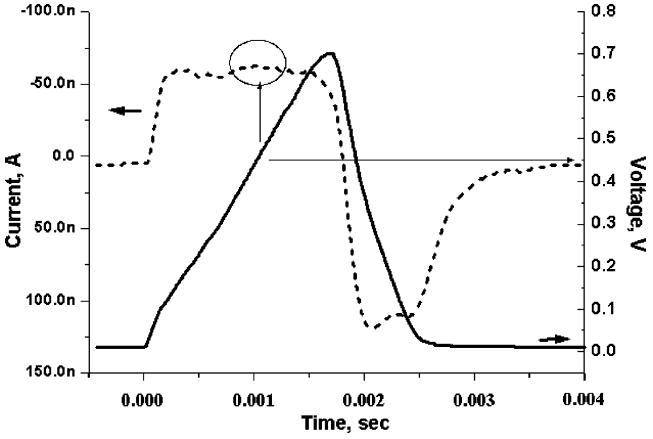

Figure 3. Typical voltage sweep waveform (solid line) such that the capacitive current would be flat by applying the voltage waveform shown (dashed line). The capacitive current was obtained at a glassy carbon UME in a cell having a solution containing 0.10 M phosphate buffer (pH 7.0) and 0.010 M ascorbic acid.

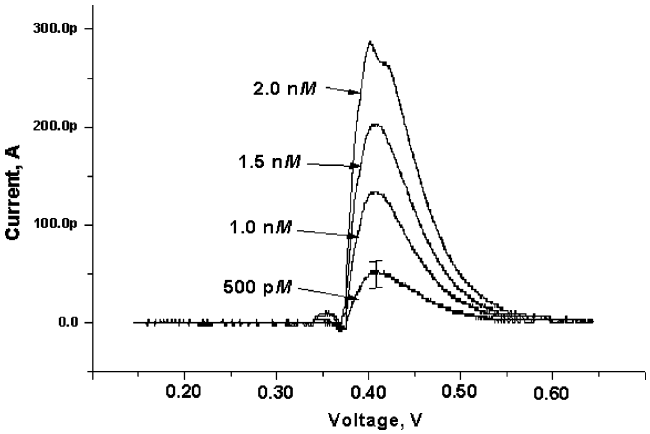

Figure 4. Oscilloscope traces of the faradaic currents from dopamine oxidation obtained after removal of capacitive currents. The currents were amplified by 100 times.

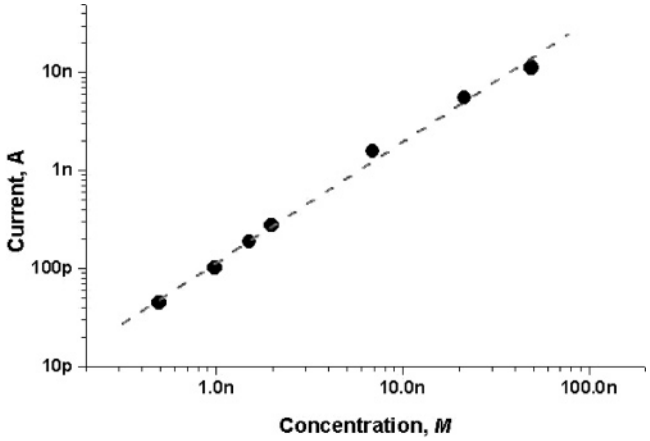

Figure 5. Dynamic linearity shown as a log−log plot for the faradaic currents obtained for different concentrations of dopamine at a 10-μm glassy carbon UME in a solution containing 0.10 M phophate buffer with its pH 7.0 and 0.010 M ascorbic acid. The scan scan rate was ~350 V/s.

shown by this system proves that the currents recorded are mainly faradaic if not all.

Another concern is the overlap of the electrical double layer with the diffusion layer due to the small dimension of the working electrode and the relatively fast scan rate. White et al. reported that a peak-shaped current would be observed due to the overlap of the double layer with the diffusion layer, when the double layer thickness is comparable with the radius of the electrode.[17] Clearly, however, this would not pose a problem in our case because the double layer thickness, estimated to be less than 1 nm from the Guoy−Chapman theory for the electrolyte concentration we used,[18] is much smaller than the radius of the electrode, 5 μm. Also, Amatore reported that a similar effect can be observed if the scan rate is very high.[19] In our case, the diffusion layer thickness, δ, estimated from the equation,[20]

$$\delta = RTD_R/Fv \qquad (4)$$

using a voltage scan rate of ~400 V/s is ~175 nm, which is still much greater than the estimated double layer thickness of ~1 nm. As was pointed out above, we thus believe that the peak shape of the voltammograms shown in Figure 4 primarily resulted from the adsorption[8] rather than from the overlap of the electrical double layer with the diffusion layer.

An uncompensated ohmic drop can also introduce a coupling between faradaic and capacitive currents. The uncompensated cell resistance, R_u, is roughly described by the equation,[21]

$$R_u = 1/4\kappa_0 r \qquad (5)$$

where κ_0 is the solution conductivity and r is the radius of the electrode. The uncompensated cell resistance is calculated to be ~10 Ω from the ionic conductivity of ~50 Ω^{-1} cm for a solution containing 0.10 M phosphate buffer at pH 7.0 and the radius of 5 μm, leading to an uncompensated ohmic drop of less than 3 nV at the most for the current we observed. Thus, the coupling between faradaic and capacitive currents would not play a role in determining the voltammetric currents under the experimental conditions described here.

CONCLUSION

We have demonstrated that the programmed potential sweep voltammetry can increase the sensitivity significantly provided the sweep signal is properly programmed. The main concept is to program its excitation waveform such that it would produce a predefined background capacitive current signal in a potential region of interest. In LSV experiments, the background current may not be flat or any other predictable form in the region where the current is to be monitored. For this reason, voltammograms can have a large contribution from the poorly defined double layer capacitance. In our work, an input waveform is programmed such that the background current would become flat or any predefined form in the potential region of interest where the peak current arising from the analyte is observed. The background-compensated peak current is then amplified by hardware. The current thus obtained pushes the detection limit down to a much lower level in comparison to any other voltammetric techniques including normal pulse and differential pulse voltammetry, not to mention the LSV method. The background-compensated currents showed an excellent dynamic linearity for dopamine concentrations between 500 pM and 100 nM. The detection limit of about 120−160 pM for electroactive species such as dopamine and $Ru(NH_3)_6^{2+}$ are unheard of thus far in electroanalytical chemistry except for stripping analysis, in which analytes are concentrated into a small volume for a lengthy period.

Although the voltammograrms reported here can hardly be interpreted in the same way as cyclic voltammograms are, the concentration dependency of the peak current is not affected by the variation of the scan rates. Thus, the concept presented here can be readily adopted for real-time analysis of various biological samples as well as for the amperometric detection of analytes in

(17) Norton, J. D.; White, H. S.; Feldberg, S. W.*J. Phys. Chem.* **1990**, *94*, 6772.
(18) See Chapter 13 of ref 1.
(19) Amatore, C.; Lefrou, C. *J. Electroanal. Chem.* **1990**, *296*, 335.
(20) Andrieux, C. P.; Savéant, J. M. In *Investigation of Rates and Mechanisms of Reactions*; Bernasconi, C. F., Ed.; Wiley: New York, 1986; Vol. 6, Part 2, Chapter 7.
(21) Wightman, R. M.; Wipf, D. O. In *Electroanalytical Chemistry*; Bard, A. J., Ed.; Marcel Dekker: New York, 1986; Vol. 15.

real time at the detection port during either chromatographic or capillary electrophoresis analyses.

ACKNOWLEDGMENT

This work was supported by a grant from the Korea Science and Engineering Foundation through the Center for Integrated Molecular Systems at POSTECH and by the BK21 program of the Korea Research Foundation.

Received for review December 14, 2004. Accepted April 4, 2005.

AC0481598