Feature Article

Adsorptive Stripping Voltammetric Detection of Single-Stranded DNA at Electrochemically Modified Glassy Carbon Electrode

Huai-Sheng Wang, *Huang-Xian Ju*, *Hong-Yuan Chen**

Institute of Analytical Science, State Key Laboratory of Coordination Chemistry, Department of Chemistry, Nanjing University, Nanjing 210093, P. R. China; e-mail: hychen@netra.nju.edu.cn

Received: January 22, 2002
Final version: April 29, 2002

Abstract
Electrochemically modified glassy carbon electrode (GCE) was used to study the electrochemical oxidation and detection of denatured single-stranded (ss) DNA by means of adsorptive stripping voltammetry. The modification of GCE, by electrochemical oxidation at +1.75 V (vs.SCE) for 10 min and cyclic sweep between +0.3 V and −1.3 V for 20 cycles in pH 5.0 phosphate buffer, results in 100-fold improvement in sensitivity for ssDNA detection. We speculated that the modified GCE has a high affinity to single-stranded DNA through hydrogen bond (specific static adsorption). Single-stranded DNA can accumulate at the GCE surface at open circuit and produce a well-defined oxidation peak corresponding to the guanine residues at about +0.80 V in pH 5.0 phosphate buffer, while the native DNA gives no signal under the same condition. The peak currents are proportional to the ssDNA concentration in the range of 0–18.0 μg mL^{-1}. The detection limit of denatured ssDNA is ca. 0.2 μg mL^{-1} when the accumulation time is 8 min at open circuit. The accumulation mechanism of ssDNA on the modified GCE was discussed.

Keywords: DNA, Adsorptive-stripping voltammetry, Electrochemically modified electrode, Glassy carbon electrode, Adsorption

1. Introduction

Nucleic acid analysis requires reliable and sensitive methods for the quantitation and structural detection of DNA. Modern electroanalytical techniques have been proven to be very useful to this area [1]. The electroreduction of nucleic acid bases that occurs at rather negative potentials is attained only at mercury electrodes. Palecek and co-workers reported a series of works on detection of trace DNA or RNA and DNA damage using pulse-polarographic, adsorptive-stripping and adsorptive transfer stripping voltammetric techniques at mercury electrodes [2–9]. Their results showed that mercury electrode was an effective tool in analysis of nucleic acid with high sensitivity to small changes in the double helix. In contrast, voltammetric oxidation signals of DNA have not been so well developed at carbon electrodes, which showed only relatively small differences between single-stranded and double-stranded DNA molecules [9, 10]. Thus carbon and other solid electrodes have been seldom used in the nucleic acid analysis [11–14]. A good sensitivity of oxidation signals for DNA structure would make carbon electrodes extensively useful in contemporary DNA research. Accordingly, it is significant to exploit the potential application of carbon electrodes in this area. Recently, Wang et al. acquired remarkable success in nucleic acid analysis by using adsorptive stripping potentiometry at carbon paste electrodes [15–22]. They studied the stripping analysis of nucleic acids at electrodes of various carbon materials. Their results demonstrated that the carbon paste, pyrolytic graphite, highly oriented pyrolytic graphite and carbon strip are suitable for detecting trace levels of nucleic acids in connection with adsorptive stripping potentiometry, while carbon fiber or glassy carbon are less suitable for this task [20]. Their study opens the door to modern solid-state nucleic acid probes and motivates researchers to seek the possible use of different carbon electrodes in nucleic acid study.

The electrochemical pretreatment of glassy carbon electrodes has been widely used to improve the electrochemical response [23–30]. In our previous work [30], a pretreatment was achieved by electrochemically oxidizing the GCE at +1.75 V (vs. SCE) for 5 min under stirring in pH 5.0 phosphate buffer. Denatured DNA could be accumulated on the pretreated GCE at +0.3 V or at open circuit and gave two well-defined oxidative peaks corresponding to oxidation of guanine and adenine residues, respectively.

This work further studied the improvement of ssDNA response at the electrochemical modified GCE. The modification of GCE included the electrochemical oxidization at +1.75 V (vs. SCE) for 10 min in pH 5.0 PBS under stirring and then electrochemical reduction by cyclic sweep between +0.3 V and −1.3 V for 20 cycles. At this modified electrode, denatured single-stranded DNA gives a well-defined anodic peak at about +0.8 V in 0.1 M pH 5.0PBS corresponding to the oxidation of guanine residues, while no peak is observed for native DNA. Furthermore, the signal is about 10-fold higher than that obtained at the oxidized GCE [30], providing a sensitive method for the determination of ssDNA at low level.

  1040-0397/02/2312-1615 $ 17.50+.50/0

2. Experimental

2.1. Chemicals and Apparatus

Native calf thymus DNA (dsDNA) was obtained from Sino-American Biology Company and was used without further purification. DNA stock solutions (1 mg mL^{-1}) were prepared by dissolving appropriate amounts of dsDNA in 0.1 M pH 5.0 phosphate buffer and stored at 4 °C. All other chemicals were of analytical reagent grade. Double distilled water was used for all experiments.

Cyclic and linear sweep voltammetric measurements were carried out with 270 Electrochemistry System (EG&G, USA). The three-electrode system used in this study contained a glassy carbon working electrode (4 mm diameter), a platinum wire counter electrode and a SCE reference electrode.

2.2. Preparation of Single-Stranded DNA

Denatured single-stranded DNA (or denatured ssDNA) was produced by thermal denaturation of dsDNA. Native dsDNA solution was heated in a boiling water bath (100 °C) for about 10 min followed by rapid cooling in an ice bath. Concentrations of DNA (per nucleotide phosphate) were determined spectrophotometrically with $\varepsilon_{260} = 6600\ M^{-1}\ cm^{-1}$ [31].

2.3. Voltammetric Procedure

The electrochemical experiments were performed in 0.1 M pH 5.0 PBS with different concentrations of DNA. The accumulation of ssDNA at the working electrode was done in a stirred solution at open circuit for 5 min. After a 5 s quiet period, the anodic stripping voltammograms were recorded from +0.3 V to +1.0 V at 100 mV s^{-1}. The electrode can be used for next measurement after continuous sweep for three cycles at the same potential range.

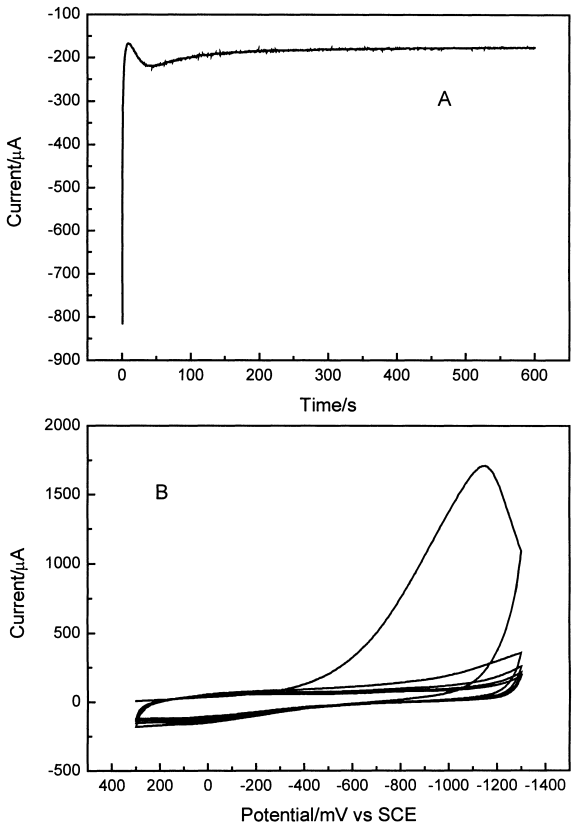

Fig. 1. Current during preanodization of a freshly polished GCE (A) and voltammogram of an oxidized GCE cycled in the potential range from 0.3 V to −1.3 V in 0.1 M pH 5.0 PBS (B).

3. Results and Discussion

3.1. Modification of the Glassy Carbon Electrode

The most common pretreatment of GCE is preanodization and precathodization. The effect of treatment procedure on electrochemical response depends on the applied voltage, pH value of the electrolyte solution and the duration. The treated GCE usually behaves as a film-modified electrode (MGC) [32]. The modification of GCE here consisted of an oxidation step at +1.75 V under stirring in 0.1 M PBS (pH 5.0) for 10 min and a reduction procedure by cyclic sweep between +0.3 V and −1.30 V for 20 cycles and then between +0.3 and +1.0 V until a steady-state curve was obtained in the same electrolyte solution. The currents through a freshly polished electrode during modification in 0.1 M pH 5.0 PBS are shown in Figure 1. The results are similar to those obtained by Engstrom [33] and Beily et al. [27]. The cathodic peak of the cyclic voltammogram (Fig. 1B) between +0.3 and −1.3 V only appears in the first cycle, suggesting that the reduction of the graphite oxide formed in anodic process was nearly finished after one cycle. The amount of charge in the cathodic peak indicates a multilayer structure [33, 34].

3.2. Cyclic Voltammograms of DNA at Glassy Carbon Electrodes

After the modified electrodes were keep in stirring solution of pH 5.0 PB containing single-stranded DNA or native DNA for 5 min at open circuit, the cyclic voltammograms of denatured single-stranded DNA (solid line) and native DNA at the modified GCE (dot line) show different electrochemical behaviors (Fig. 2). As comparison, a curve of denatured ssDNA at only oxidized GCE (dash-dot-dot line) [30] was given. It can be seen that the denatured ssDNA shows a well-defined oxidation peak at about +0.80 V in the first anodic sweep, while the native DNA hardly gives any response. Although the background current is high, the response of ssDNA at present modified electrode is about 10 times higher than that at the only

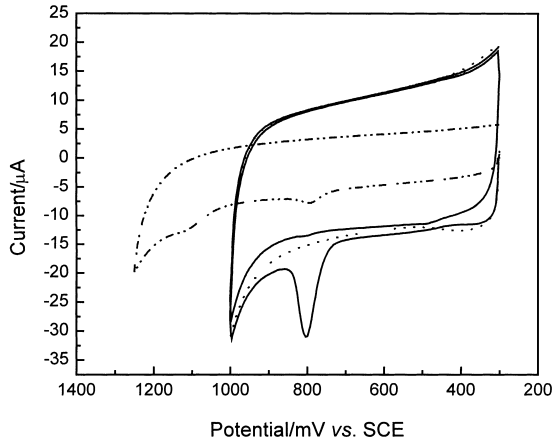

Fig. 2. Cyclic voltammograms of 18.0 μg mL^{-1} denatured ssDNA (—), 60.0 μg mL^{-1} native DNA (· · · ·) on the modified GCE and 18.0 μg mL^{-1} denatured ssDNA (–· ·– · ·) on an oxidized GCE in 0.1 M pH 5.0 PB at 100 mV s^{-1}. Preconcentration was performed at open circuit for 5 min with stirring.

oxidized GCE. The acid-denatured DNA gives a larger response than thermally denatured DNA with the same concentration (the data were not shown). It seems that the modification could not improve the adsorption of native DNA. Therefore, the modified GCE possesses high sensitivity to small changes of DNA structure in double helix and showed a large difference in response to native and denatured ssDNA. This property is very important in electrochemical analysis of nucleic acid with GCE, which may be used to detect the DNA damage at a low level. No reduction peak is observed in cathodic sweep; thus, the electrochemical oxidation of denatured ssDNA at modified GCE is also an entirely irreversible process.

When the modified electrode was immersed in a static solution of pH 5.0 PB containing the same concentration of denatured ssDNA. The voltammetric response was much smaller than that obtained above. No obvious increase of the response was observed after keeping the electrode in the static solution for 5 min. Thus stirring the solution was a necessary condition for preconcentration of ssDNA. The ssDNA-adsorbed electrode also showed an anodic peak at +0.8 V in a blank supporting electrolyte. The peak current was about 90–95% of those obtained in ssDNA solution. These results suggest that the diffusion rate of ssDNA to the electrode surface is very low and the ssDNA can adsorb at the modified GCE more firmly than at that only oxidized GCE [30]. The electrochemical activation of GCE surface could greatly enhance the signal of denatured ssDNA, thus increase the difference between the responses to native and denatured ssDNA. It can also be seen from Figure 2 that the peak of ssDNA almost disappears at the second cycle. These phenomena result from the consumption of guanine resides in ssDNA and the oxidation products of guanine residues are not reduced.

When the potential was scanned up to +1.25 V, the oxidation peak of adenine could also be observed, but the ratio of signal-to-noise would decrease gradually along with the increase of scan times. Thus in this work we scanned the potential up to +1.0 V.

3.3. Speculation About the Mechanism of ssDNA Accumulation

After reviewed the different behaviors of ds and ssDNA at mercury and carbon electrodes, Palecek et al. suggested that the large difference in the reduction signals of the ds and ssDNA at mercury electrodes was due to the inaccessibility of the primary reduction sites of adenine and cytosine. These sites were hidden in the interior of the double-helical molecule forming a part of the Watson-Crick hydrogen bonding system. In ssDNA, these reduction sites are freely accessible for interaction with the environmental molecules. Primary oxidation sites of adenine and guanine do not form the Watson-Crick hydrogen bonding system and locate close to the surface of DNA molecule. A small difference in the intensity of oxidation signals of the ds and ssDNA was attributed to their different flexibilities [9, 35]; it assumed that the more rigid dsDNA could follow the rough surface of graphite electrodes less efficiently than the ssDNA.

In our present investigation, the modification of GCE greatly increases the signal of ssDNA but does not change the response of native DNA resulting in a large difference in oxidation peak currents.

It is well known that a dielectric layer is formed on GCE during anodic oxidation. Such a layer possesses insulating properties, is electrochemically inactive and does not contribute to the double-layer capacitance. After the electrode is reduced, the whole layer becomes electrochemically active again, resulting in a significant increase in double-layer capacitance [36]. It is generally believed that the oxidation followed by reduction of GCE for a very short time produces >C=O functional group on the carbon electrode surface [33, 37], which can mediated the electron transfer reaction at the interface [38]. The experiment results indicated that the adsorptive capacity was related to the amount of surface functional groups and double-layer capacity [23, 25]. In our present study, the background and the adsorptive affinity are greatly enhanced after the reduction of the oxidized GCE. Several authors reported that the electrode area was not changed with the pretreatment in the case of the ferrocyanide oxidation [23, 33]. No increase in surface roughness was observed with AFM after oxidized and reduced [36]. Hence, the increase current is not due to an increase surface area with porous structure but to some chemical interaction between the >C=O groups and ssDNA. Therefore, the availability of ssDNA near or on the electrode surface has been increased by the presence of >C=O group on the modified GCE surface.

Paleck et al. assumed that DNA could be attached to the positively charged carbon paste electrode (CPE) surface via the negatively charged hydrophilic sugar-phosphate backbone with bases oriented toward the solution and showed only small differences between ds and ssDNA [39]. Obviously, the results obtained at the modified GCE are not

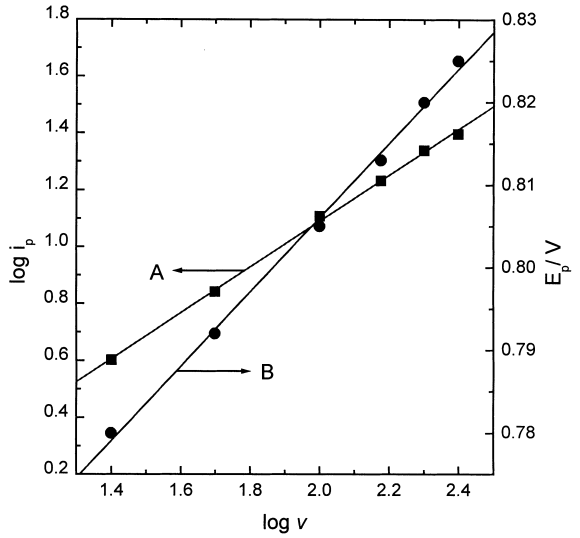

Fig. 3. Plots of log i_p – log v (A) and E_p – log v (B) for the oxidation of guanine residues in 18.0 μg mL^{-1} ss DNA. Other conditions were the same as in Figure 2.

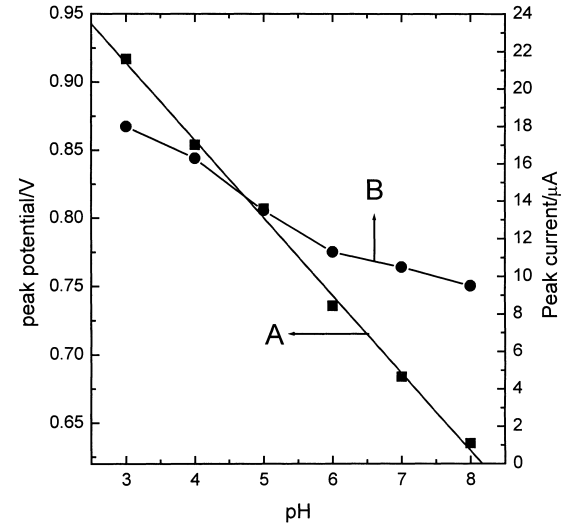

Fig. 4. Effects of pH on peak potential (A) and peak current (B) in blank supporting electrolyte after the modified electrode was immersed in a stirring solution of 18.0 μg mL^{-1} denatured ssDNA solution with stirring. Other conditions were as in Figure 2.

suitable for this assumption. Zen et al. thought that the preanodized Nafion-coated glassy carbon electrode surface has a high affinity towards uric acid through hydrogen bonding [26]. Accordingly, one possible reason for the preferential adsorption of ssDNA on the modified GCE could be the positive chemical interaction between the ssDNA and the surface produced > C=O groups. In ssDNA, all bases can be freely accessible to the electrode surface. The hydrogen bond can be formed between the more acidic −H of nucleic bases in ssDNA and the > C=O groups present on the electrode surface. As for dsDNA, the sites that can form hydrogen bond in all bases, have already formed a part of the Watson-Crick hydrogen bonding system, can not form hydrogen bond with the > C=O groups on the electrode surface. Therefore, dsDNA cannot accumulate on the modified electrodes.

3.4. Effects of Scan Rate (v)

At the modified electrode, there is no linear relation between the peak current of ssDNA in pH 5.0 PB and the scan rate, ν, or $\nu^{1/2}$. In the scan rate range of 25 – 250 mV/s, the plot of log i_p vs. log ν (Fig. 3A) follows the equation of log $i_p = -0.526 + 0.809$ log ν ($r = 0.9991$) (ν in mV/s and i_p in μA). The slop of 0.809 indicates that the electrode process is simultaneously controlled by surface adsorption and heterogeneous electron transfer rate. With increasing scan rate, the anodic peak potential shifted to more positive value. The equation of peak potential E_P vs. log ν follows (ν in mV/s) $E_p = 0.716 + 0.045$ log ν ($r = 0.9985$) (Fig. 3B). These results suggest that the oxidation of guanine residues in ssDNA is irreversible.

3.5. Effect of pH on E_p and i_p

Both the peak potential and peak current are influenced greatly by the pH of supporting electrolyte, as shown in Figure 4. The experimental results show that the pH dependence of oxidation peak potential of ssDNA obeys the equation of E_p (V) = 1.085 (0.057 pH in pH 3.0 – 8.0 (Fig. 4A). The oxidation of guanine has been shown to follow a two-step mechanism involving the total loss of four electrons and the first 2e$^-$ oxidation is the rate determining step [35]. The slope of 57 mV pH^{-1} indicates that two protons take part in the rate determining step.

The peak currents of ssDNA decreased with the increase of medium pH (Fig. 4B) due to the proton participated in the oxidative process of guanine residues. In order to avoid the depurination of DNA at the extreme pH value, we selected the pH 5.0 PBS as the supporting electrolyte.

3.6. Influence of Accumulation Time

With increasing accumulation time, the peak current initially increases and then trends to saturation value after 300 s, as illustrated in Figure 5. So 300 s was chosen as the accumulation time for all experiments.

3.7. Adsorption Feature and Detection of DNA

The effect of ssDNA concentration on peak currents is shown in Figure 6. Figure 6A presents the linear sweep adsorptive stripping voltammograms of denatured ssDNA solutions with increasing concentration (0 – 18.0 μg mL^{-1}). The peak current increases linearly with the ssDNA concentration up to 18.0 μg mL^{-1} with a slope of 0.76 μA/μg (Fig. 6B). A detection limit of 0.2 μg mL^{-1} denatured

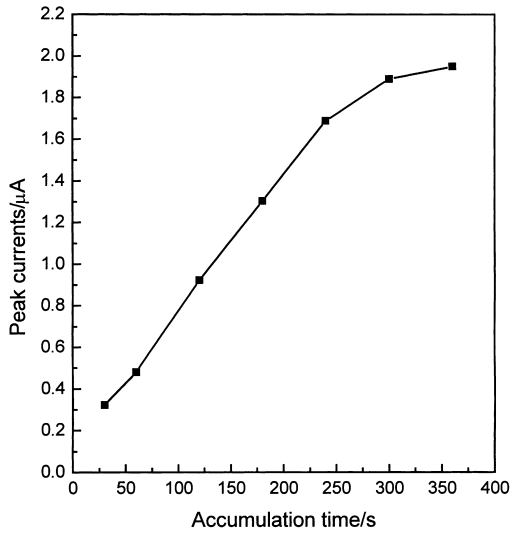

Fig. 5. Effect of accumulation time on peak current in 3.0 μg mL^{-1} ssDNA solution. Other conditions were the same as in Figure 2.

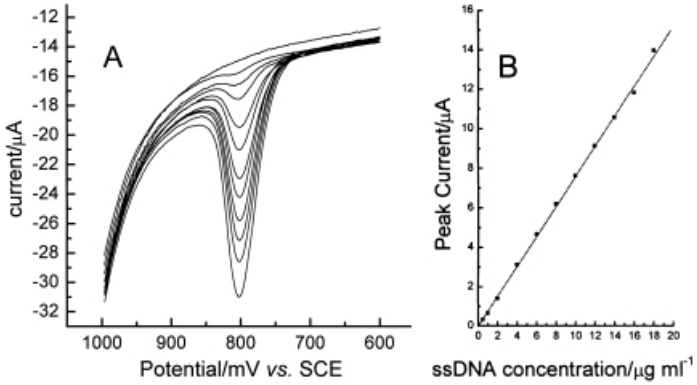

Fig. 6. Linear sweep voltammograms of ssDNA solution with increasing concentration from 0 – 18.0 μg mL^{-1} (A), along with the resulting calibration plots (B). Conditions were as in Figure 2.

ssDNA could be estimated with a 3 σ when the accumulation time is 8 min. The reproducibility of the CV response is good. A 4.5% relative standard deviation of peak currents was obtained under 6 measurements of 10.0 μg mL^{-1} ssDNA solution. Therefore, the adsorptive stripping voltammetry can be used to study the electrochemical behavior of DNA, detect the DNA structure and quantitate the denatured ssDNA.

The peak area for ssDNA oxidation can be used to estimate the adsorbed amount of ssDNA at the modified GCE. From the equation: $\Gamma = Q/nFA$, the surface coverage Γ of the adsorbed ssDNA can be calculated from the value of the charge Q (the area of the peak of ssDNA) and electron transfer number n (the value of 2 is taken according to the results of Palecek [2]). The voltammogram of 20.0 μg mL^{-1} (4.78×10^{-5} mol L^{-1} per nucleotide phosphate) ssDNA adsorbed at modified GCE gives a peak area of 7.30 μC and the surface coverage Γ of 3.01×10^{-10} mol cm^{-2} can be obtained. Obviously, the surface coverage Γ of adsorbed ssDNA is close to the theoretical value of a monolayer and is about 4 times higher than the maximum surface concentration, Γ_m, obtained at the only oxidized GCE [30]. This result indicated that the ssDNA adsorbed at the modified GCE surface more effectively than at the only oxidized GCE. Thus, the ssDNA accumulated on modified GCE surface as a monolayer maybe through the hydrogen bonding system between the $>$C=O groups and the bases in ssDNA. This result agrees with the saturation property of hydrogen bond.

4. Conclusions

The electrochemical modification of GCE resulted in an effective adsorption of ssDNA. The modified GCE shows more than 100-fold current response of guanine residues compared with that at an unmodified GCE. Furthermore, the modified GCE has a higher sensitivity to the change of DNA double helix. An accumulation mechanism of ssDNA on electrochemically modified GCE was proposed. This study provides a sensitive method for DNA immobilization, probing DNA damage and structure of nucleic acids, especially; this study could offer a basis for the extensive using of GCE in nucleic acid research.

5. Acknowledgements

The financial supports from the National Natural Science Foundation of China (No. 29835110 and 29975013), the Science Foundation of Jiangsu (No. BK99030), the foundation of the Education Ministry of China (No. 200028403, 2000143) and State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry are greatly acknowledged.

6. References

[1] E. Palecek, *Electroanalysis* **1996**, *8*, 7.
[2] E. Palecek, I. Postbieglová, *J. Electroanal. Chem.* **1986**, *214*, 359.
[3] E. Palecek, P. Boublikova. E. Jelen, *Anal. Chim. Acta.* **1986**, *187*, 99.
[4] E. Palecek, *Anal. Biochem.* **1988**, *170*, 421.
[5] E. Palecek, *Bioelectrochem. Bioenerg.* **1988**, *20*, 171.
[6] E. Palecek, F. Jelen, C. Teijeiro, V. Fucik, T. M. Jovin, *Anal. Chim. Acta* **1993**, *273*, 175.
[7] F. Jelen, M. Tomschik, E. Palecek, *J. Electroanal. Chem.* **1997**, *423*, 141.
[8] M. Fojta, E. Palecek, *Anal. Chim. Acta* **1997**, *342*, 1.
[9] F. Jelen, M. Fojta, E. Palecek, *J. Electroanal. Chem.* **1997**, *427*, 49.
[10] E. Palecek, M. Fojta, *Anal. Chem.* **2001**, *73*, 75 A.
[11] T. Yao, T. Wasa, S. Musha, *Bull. Chem. Soc. Jpn.* **1978**, *51*, 1235.
[12] C. M. A. Brett, A. O. Brett, S. H. P. Serrano, *J. Electroanal. Chem.* **1994**, *336*, 225.
[13] V. Brabec, J. Koudelka, *Bioelectrochem. Bioenerg.* **1980**, *7*, 793.

[14] V. Brabec, G. Dryhurst, *J. Electroanal. Chem.* **1978**, *89*, 161.
[15] X. H. Cai, G. Rivas, P. A. M. Farias, H. Shiraishi, J. Wang, M. Fojta, E. Palecek, *Bioelectrochem. Bioenerg.* **1996**, *40*, 41.
[16] J. Wang, X. H. Cai, C. Jonsson, E. Palecek, *Anal. Chem.* **1995**, *67*, 4065.
[17] J. Wang, E. Palecek, P. E. Nielson, G. Rivas, X. H. Cai, H. Shriraishi, N. Dontha, D. B. Luo, P. A. M. Farias, *J. Am. Chem. Soc.* **1996**, *118*, 7667.
[18] J. Wang, X. H. Cai, G. Rivas, H. Shiraishi, P. A. M. Farias, *Anal. Chem.* **1996**, *68*, 2629.
[19] J. Wang, X. H. Cai, C. Jonsson, M. Balakrishnan, *Electroanalysis* **1996**, *8*, 20.
[20] X. H. Cai, G. Rivas, P. A. M. Farias, H. Shiraishi, J. Wang, E. Palecek, *Electroanalysis* **1996**, *8*, 753.
[21] X. H. Cai, G. Rivas, H. Shiraishi, P. A. M. Farias, J. Wang, M. Tomschik, F. Jelen, E. Palecek, *Anal. Chim. Acta* **1997**, *344*, 65.
[22] J. Wang, E. Sahlin, J. L. L. Paz, *Electroanalysis* **1999**, *11*, 380.
[23] T. Nagaoka, T. Yoshino, *Anal. Chem.* **1986**, *58*, 1037.
[24] L. J. Kepley, A. J. Bard, *Anal. Chem.* **1988**, *60*, 1459.
[25] D. M. Anjo, M. Kahr, M. M. Khodabakhsh, S. Nowinski, M. Wanger, *Anal. Chem.* **1989**, *61*, 2603.
[26] J. M. Zen, J. J. Jou, G. Ilangovan, *Analyst*, **1998**, *123*, 1345.
[27] A. L. Beilby, T. A. Sasaki, H. M. Stem, *Anal. Chem.* **1995**, *67*, 976.
[28] F. Y. Song, F. S. C. Lee, K. K. Shiu, *Electroanalysis*, **2000**, *12*, 128.
[29] K. K. Shiu, K. Shi, *Electroanalysis*, **2000**, *12*, 134.
[30] H. S. Wang, H. X. Ju, H. Y. Chen, *Electroanalysis*, **2001**, *13*, 1105.
[31] D. H. Johnston, K. C. Glasgow, H. H. Thorp, *J. Am. Chem. Soc.* **1995**, *117*, 8933.
[32] C. Barbero, J. J. Silber, L. Sereno, *J. Electroanal. Chem.*, **1988**, *248*, 321.
[33] R. C. Engstrom, V. A. Strasser, *Anal. Chem.* **1984**, *56*, 136.
[34] G. E. Cabaniss, A. A. Diamantis, Jr. W. R. Murphy, R. W. Linton, T. J. Meyer, *J Am. Chem. Soc.* **1985**, *107*, 1845.
[35] E. Palecek, in *Topics in Bioelectrochemistry and Bioenergetics*, Vol. 5 (Ed: G. Milazzo), Wiley, London **1983**, p. 65.
[36] M. G. Sullivan, B. Schnyder, M. Bartsch, D. Alliata, C. Barbero, R. Imhof, R. Kotz, *J. Electrochem. Soc.* **2000**, *147*, 2636.
[37] G. N. Kamau, W. S. Willis, J. F. Rusling, *Anal. Chem.* **1985**, *57*, 545.
[38] J. P. Randlin, in *Encyclopedia of Electrochemistryof Elements*, Vol. 17 (Ed: A. J. Bard), Marcel Dekker, New York **1976**, ch. 1.
[39] E. Palecek, M. Fojta, M. Tomschik, J. Wang, *Biosens. Bioelectron.* **1998**, *13*, 621.