Anal. Bioanal. Electrochem., Vol. 8, No. 5, 2016, 566-577

Analytical & Bioanalytical Electrochemistry

2016 by CEE
www.abechem.com

Full Paper

Electroanalytical Determination of Atorvastatin in Pharmaceutical Formulations based on Edge-Plane Pyrolytic Graphite Electrode

Omid Fazlolahzadeh,[1] Ahmad Rouhollahi,[1,*], Mojtaba Hadi[2]

[1]*Department of Chemistry, Faculty of Sciences, K.N. Toosi University of Technology, Tehran, Iran*

[2]*Department of Chemistry, Faculty of Sciences, University of Qom, Qom, Iran*

*Corresponding Author, Tel.: +98 21 2285-3308; Fax: +98 21 2285-3650

E-Mail: rouhollahi@kntu.ac.ir

Received: 8 May 2016 / Accepted: 31 July 2016 / Published online: 15 August 2016

Abstract- An electrochemical sensor employing edge-plane pyrolytic graphite electrode (EPPGE), without any need for surface pretreatment or surface chemical modification, for the sensitive detection of atorvastatin (AT) are reported using voltammetric techniques. The results indicated that EPPGE remarkably enhanced the electro-oxidation of AT, which led to enhanced peak currents and decrease in peak potentials to less positive values. Compared with basal-plane pyrolytic graphite electrode (BPPGE) and glassy carbon electrode (GCE), EPPGE showed better electrochemical responses both in terms of sensitivity and detection limit. The repeatability of the responses was found good and the method was applied successfully for sensing of AT in pharmaceutical formulations with good recoveries.

Keywords- Atorvastatin electro-oxidation, Basal-plane pyrolytic graphite, Edge-plane pyrolytic graphite

1. INTRODUCTION

Atorvastatin (AT) is used as a cholesterol lowering agent [1]. Several analytical techniques such as spectrophotometry, high performance liquid chromatography with ultra-violet detection and liquid chromatography-tandem mass spectroscopy have been devised for its determination [2-14], however, complicated time-consuming procedures, need for

derivatization or extraction, and expensive instrumentation are the main problems with such methods. The electrochemical techniques may offer an attractive alternative due to their inherent advantages such as low cost, simplicity, high sensitivity, and speed. These methods have been used successfully for sensing and detection of several drug compounds with the advantages of no need for derivatization and less sensitivity to the complex matrix effects [15-19]. It is well-known that the selection of working electrode material, in electroanalytical techniques, is a key factor that determines the success of analysis [15-17]. Among different electrode materials that can be used, carbon-based materials are widely utilized as working electrode due to their low cost, wide potential window and a high electron-transfer activity [16, 20-22]. The electrochemistry of traditional graphitic carbon-based electrode materials such as glassy carbon electrode (GCE) and pyrolytic graphite electrode (PGE) have been thoroughly investigated and it has been well-known that their electrochemical activities are significantly influenced by the surface microstructures so that, the surface density of electrochemically active edge sites may be a prominent factor influencing the electrode kinetics [20, 21, 23]. For many redox systems, the electron-transfer rate constants observed at the edge-plane sites have been found to be about 10^3 times more than that observed at the basal-plane graphitic sites [24]. Recently, the application of carbon nanostructured materials such as carbon nanotubes (CNTs) and nano-graphenes has been the subject of wide interest and detailed research [25-29]. The enhanced electron-transfer kinetics observed with these materials have been attributed to their outstanding microstructures; a high edge to basal plane ratio due to a high density of electrochemically active edge sites at the open end of nanotubes (as opposed to the nanotubes' side walls) or at the exposed edges of nano-graphene layers provide a high electron-transfer rate; However, high background currents and time consuming electrode preparation procedures are the major drawback with these materials. Lately, Compton and coworkers have reported EPPG electrode as an alternative electrode material and shown this electrode can be used successfully for detection of some important compounds [23, 30-34]. The superior electroanalytical performances of EPPG should be attributed to its surface microstructure that is wholly composed of electrochemically active edge-plane sites compared with the GCE and BPPGE with the surface composed of both electrochemically inert basal-plane and edge-plane sites. This provides such a high electrode activity that EPPG has been applied for electroanalytical determination of many important compounds based on direct electro-oxidation or electro-reduction without needing to apply any pretreatment methods or immobilize electron-transfer mediators on the surface [30, 34-38]. This electrode has been also used successfully for the direct electroanalysis of some important drug compounds such as diclofenac [39], hydrocortisone [40], epinephrine, and norepinephrine [41]. So far, the determination of AT has been reported at the GCE by stripping techniques [42] and at the boron-doped diamond electrode (BDDE) by DPV and SWV techniques [43]. In this paper, a simple procedure to quantify AT is presented using

EPPGE to acquire an enhanced sensitivity, repeatability, and low detection limits by using DPV and SWV techniques. The proposed method has been applied successfully to the electroanalytical determination of AT in bulk form, pharmaceutical formulations.

2. EXPERIMENTAL SECTION

2.1. Reagent and Solutions

Pure atorvastatin calcium (AT) was from Aldrich. All other reagents were purchased from Merck. All solutions were prepared using double-distilled water. 0.1 M phosphate buffer solutions (PBSs) were used as supporting electrolyte solutions; whenever necessary, the pH was adjusted either with concentrated HCl or NaOH solutions to change the pH in the area from 1 to 9. Stock solutions of AT (1×10^{-4} M) were prepared in methanol and to prepare the desired concentration, the stock solutions were diluted with the supporting electrolyte.

2.2. Apparatus and electrodes

The electrochemical techniques were applied in an electrochemical cell having a three-electrode set-up using an AUTOLAB (EcoChemie) model PGSTAT 30 potentiostat. EPPGE, BPPGE and GCE (surface area of 0.062 cm^2) were used as working electrode and a 2-mm diameter platinum rod was used as auxiliary electrode. The potentials are referred to an Ag/AgCl (3 M KCl) reference electrode. The GC electrodes were polished manually with aqueous slurry of alumina powder (0.05 μm) on a polishing pad before each measurement and then cleaned in ethanol using ultrasound. Pyrolytic graphite pieces were machined to prepare the EPPG and BPPG electrodes with the faces parallel with the edge or basal directions [23]. The EPPG electrode was first polished on fine size polishing papers to obtain a smooth surface and then treated in the same manner as described for the GC electrode. The surface of BPPG was renewed by applying a cellotape strip onto the electrode surface and peeling away some surface few layers of graphite [23].

2.3. Procedure

Supporting electrolyte (25 mL) was placed in the electrochemical cell and the required amount of standard AT solution was added by a micropipette. Before analysis the solution was deoxygenated by nitrogen gas purging for 8 min.

2.4. Analysis of Tablets

5 AT tablets (amount declared of AT per tablet was 10.0 mg) were grounded in an agate mortar. An appropriate amount of powder was dissolved in methanol by sonication for 1 min and the excipient was separated by filtration. The solution was transferred into a calibrated

flask and diluted with methanol to a final volume of 100 mL and the test solutions were prepared by diluting this solution with the PBS (0.1 M).

3. RESULTS AND DISCUSSION

3.1. Voltammetric behavior of Atorvastatin

The cyclic voltammograms recorded for 2 μM AT at the EPPG and BPPG electrodes in 0.1 M PBS (pH 3) are shown in Fig. 1. A well-defined oxidation peak at 1.026 V was obtained at the EPPG electrode, which shifted to a more positive value (1.092 V) at the BPPG electrode with a significant decrease in peak current (Fig. 1), which clearly disclose that edge-plane sites act as a better substrate and an efficient promoter for increasing the electron transfer rate of AT electro-oxidation. At the GC electrode, however, a defined oxidation peak was obtained at least at a concentration of 80 μM (inset A of Fig. 1). The increasing of current response and the decrease in peak potential are apparent proofs of the high electro-activity of edge-plane sites towards the oxidation of AT. The repetitive CVs for AT which are shown in the inset B of Fig. 1indicate high response repeatability at the EPPG electrode.

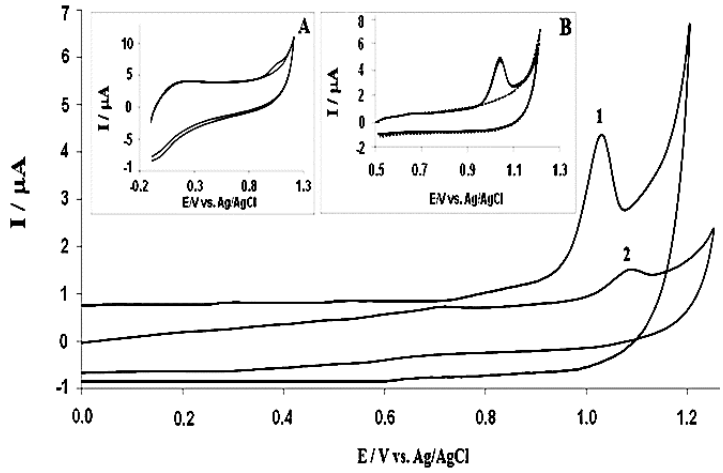


Fig. 1. Cyclic voltammograms for 2 μM AT in PBS (pH 3, 0.1 M) at (1) EPPG, (2) BPPG, and (inset A) GC electrode for 80 μM AT at the scan rate of 100 mV s^{-1}. Inset B shows the repetitive CVs for 2 μM AT at the EPPG electrode

The dependence of peak current (I_p) on the scan rate (υ) was also studied in the υ range from 10 to 140 mV s^{-1} and it was found that the I_p is proportional to the $\upsilon^{1/2}$. The linear relationship between I_p and $\upsilon^{1/2}$, which can be seen in Fig. 2, suggests that the electro-oxidation of AT at the EPPG electrode is a diffusion-controlled process. Furthermore, a slope of 0.45 for the straight line of the logarithm of I_p versus logarithm of υ plot, which is very

close to the expected value for diffusion-controlled current (0.5), indicates that adsorption doesn't contribute to the electrode reaction. The influence of equilibration time on I_p was also studied. As indicated in the inset of Fig. 2, equilibration time has little influence on the electrode response, which may be expectable as the results of scan rate study indicate no any adsorption behavior for AT electro-oxidation.

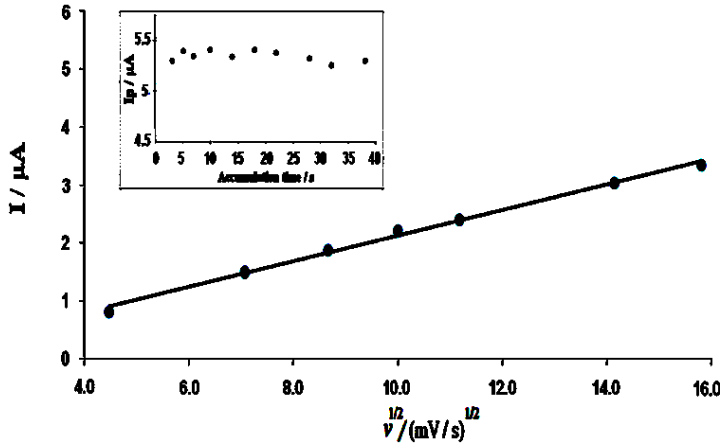


Fig. 2. Oxidation peak current versus scan rate and (inset plot) oxidation peak current versus accumulation time for 2 µM AT at the EPPG electrode in PBS (pH 3, 0.1 M)

3.2. Influence of pH

The effect of pH on the peak potential (E_p) and I_p was studied by recording CV in a solutions of AT at the EPPG electrode at different pH in the range from 1 to 9.

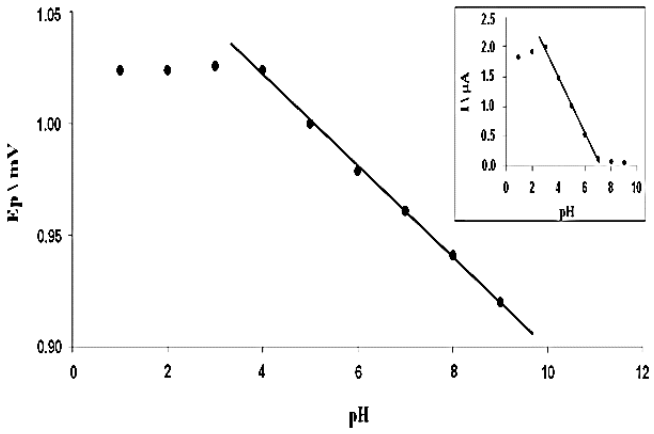


Fig. 3. E_p and (inset plot) I_p versus pH for 2 µMAT at EPPGE and at the scan rate of 100 mV s^{-1}

As can be seen from Fig. 3, though at the pH values lower than about 4.0, the peak potential shows pH-independent behavior, at the pH range from 4.0 to 9.0, it is strongly

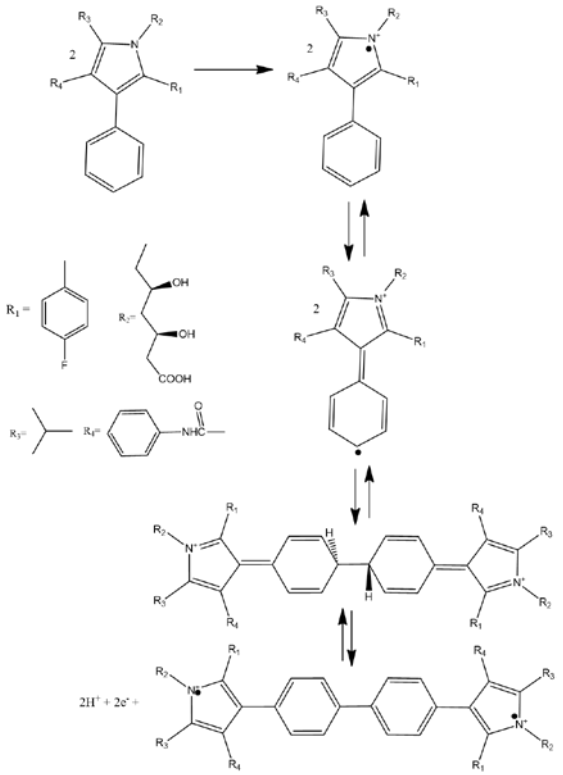
influenced by the pH and shifts linearly towards less positive values with pH increasing, so that the relationship between $-E_p$ and pH may be represented by the following equations:

$$E_p = 1106 - 27.2\ \text{pH}$$

The slope value of −27.2 mV/pH are close to the theoretical value of −30 mV/pH that indicates the number of protons released in oxidation process should be half the number of transferred electrons to the electrode [15,44].

A possible reaction mechanism is shown in Scheme 1 that supposes the oxidation occurs on the nitrogen atom of the indole ring, similar to those supposed for the first step of electro-oxidation mechanism for some drugs with indole ring in the central part of the molecule [45-48]. As shown in Scheme 1 dimerization of the radical-cation intermediate product may happen at the end step, which to some extent, is similar to that proposed by Veiga et. al [49] for the electro-oxidation of carbamazepine. In the supposed mechanism, in each dicationic dimer formation, four electrons and two protons are involved. The two steps in Scheme 1 may happen simultaneously and cannot be distinguished in the voltammograms [50].

The plot of I_p versus the pH values is shown in the inset of Fig. 3. The maximum I_p is observed at the pH range of about 1–3 and then at the higher pH values, there is a decrease in I_p. To obtain a higher sensitivity, further experiments were conducted at pH 3.0.



Scheme 1. Probable reaction mechanism for electro-oxidation of AT at the EPPG electrode

3.3. Concentration study

On the basis of the electrochemical oxidation of AT, EPPG electrode was applied for the successive determination of AT. To develop a sensitive voltammetric method, DPV and SWV techniques were selected since, as can be seen in Fig. 4, the peaks were sharper and better defined than that obtained by CV, resulting in improved resolution and sensitivity.

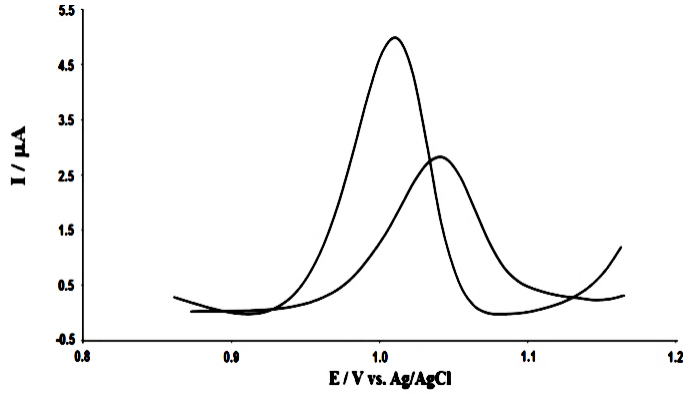


Fig. 4. DPV and linear sweep voltammetry (LSV) voltammograms for 2 µM AT in PBS (pH 3, 0.1 M) at EPPGE

To further improve the sensitivity of determination, the optimum conditions were obtained from the studies of the variation of the I_p values on pulse amplitude, pulse width, and potential step. For DPV method, it was found that the I_p value increased with pulse amplitude increasing from 10 to 85 mV/s and then decreased disorderly with further increasing of pulse amplitude (Fig. 5). Therefore, pulse amplitude of 85 mV was chosen for DPV experiments.

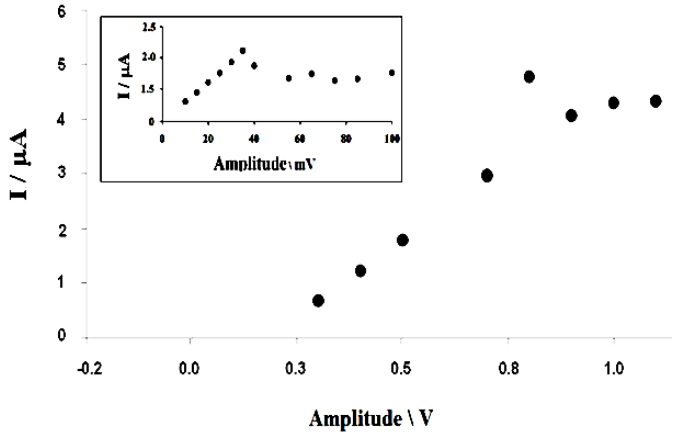


Fig. 5. Peak current versus pulse amplitude for 2 µM AT in PBS (pH 3, 0.1 M) at EPPGE for DPV and (inset plot) SWV

Others optimum operating conditions were: pulse width 50 ms; potential step 10 mV; scan rate 33 mV. For SWV technique, the peak current shows increase with increasing pulse amplitude from 10 to 35 mV/s, but then decreased and changed disorderly (inset of Fig. 5). Optimum operating conditions found for SWV experiments were as follows: potential step 4 mV; pulse amplitude 35 mV/s; frequency 10 Hz.

Using the optimum conditions, the voltammograms for various concentrations of AT were recorded by DPV and SWV techniques. Typical square wave voltammograms that show increase in peak current with AT concentration increasing in the range from 96 to 7400 nM and the corresponding calibration curve are shown in Fig. 6, with a correlation coefficient of 0.999.

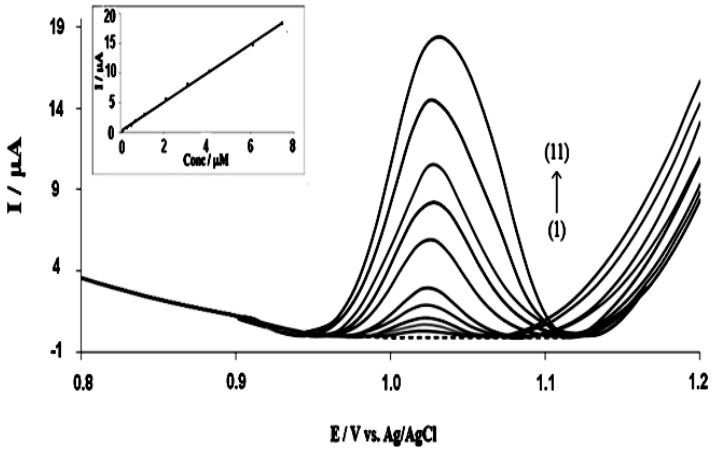


Fig. 6. DPV recorded in PBS (pH 3, 0.1 M) (1) at EPPGE (dashed line) and increasing concentration of AT ((2) 96 nM, (3) 320 nM, (4) 720 nM, (5) 1120 nM, (6) 2120 nM, (7) 3120 nM, (8) 4120 nM, (9) 4960 nM, and (10) 5600 nM) and (inset plot)the corresponding calibration graph

Validation of the procedure for AT determination was also examined by evaluation of the limit of detection (LOD), limit of quantification (LOQ), and precision for the applied methods (Table 1). Both the methods show figures at the same range except sensitivity which is significantly higher for DPV. The relative standard deviation (RSD) values in Table 1 were calculated by repeating seven independent determinations of 2.0 μM AT that indicate adequate precision and good reproducibility for the developed assay.

Table 1. Calibration characteristics at EPPGE

Parameter	SWV	DPV
Linear range (µM)	0.096-7.74	0.096-5.60
Limit of Detection (nM)	23.3	20.5
Limit of quantitation (nM)	77.6	68.8
Sensitivity (µA/µM)	2.51	8.20
Correlation coefficient (r)	0.999	0.998
Precision (RSD%)	1.35	1.56

3.4. Analysis of commercial tablet

To evaluate the applicability of the method in the real sample analysis for pharmaceutical industry, commercial AT tablets were analyzed and the validity was examined by applying both the calibration curve and the standard addition methods with SWV and DPV techniques. Solution obtained by dissolving AT tablets (see the experimental section) was diluted so that the concentration falls in the linear range of the calibration plot. A typical standard addition calibration graphs for DPV methods have been also shown in Fig. 7. The values of experimentally determined AT and the reported AT amounts in tablet and the corresponding recovery and bias values for both DPV and SWV methods are shown in Table 2. It was found that the concentration of AT which is determined for the tablet by using this method are in good agreement with the content marked in the label, with 98-100% recovery.

Table 2. Results Obtained by DPV and SWV techniques and recovery experiments for AT tablets in PBS (pH 3, 0.1 M) at EPPGE

Parameter	**SWV**	**DPV**
Labeled claim (mg)	10.00	10.00
Amount found (mg) by calibration curve method	9.84	9.72
Recovery%	98.4	97.2
Bias%	1.6	2.8
Amount found (mg) by standard addition method	10.07	9.82
Recovery%	100.7	98.2
Bias%	0.7	1.8

4. CONCLUSIONS

The aim of the present study was to develop a robust method for AT determination in pharmaceutical formulations by using EPPGE. An enhanced peak current, less positive peak potential, and higher response stability of AT electro-oxidation at EPPGE indicate that it is a better substrate for electroanalysis of AT compared to GC and BPPGE, which was explained based on the edge-plane sites that constitute most of the surface area of this electrode. A suitable mechanism for AT electro-oxidation was proposed. SWV and DPV methods were optimized and successfully applied for quantification of AT and the peak current was linear to AT concentration over a certain range. The EPPGE can be used successfully for determination of AT in pharmaceutical samples with good recovery and precision.

REFERENCES

[1] R. G. Bakker-Arkema, J. Best, R. Fayyad, T. M. Heinonen, A. D. Marais, J. M. Nawrocki, and D. M. Black, Atherosclerosis 131 (1997) 17.
[2] P. O. Edlund, P. Bowers, J. Henion, and J. R. Covey, J. Chromatogr. 497 (1989) 49.
[3] J. J. Avery, D. Y. Mitchell, F. C. Falkner, and H. G. Fouda, Biol. Mass Spectrom. 21 (1992) 353.
[4] D. Wang-Iverson, M. E. Arnold, M. Jemal, and A. I. Cohen, Biol. Mass Spectrom. 21 (1992) 189.
[5] T. G. Altuntas, and N. Erk, J. Liq. Chromatogr. Rel. Tech. 27 (2004) 83.
[6] N. Erk, Anal. Lett. 36 (2003) 2699.
[7] G. Bahrami, B. Mohammadi, S. Mirzaeei, and A. Kiani, J. Chromatogr. B 826 (2005) 41.
[8] H. Freddy, and V. Chaudhari, Asian J. Chem. 17 (2003) 2502.
[9] A. Zarghi, A. Shafaati, S. M. Foroutan, and A. Khoddam, Arzn. Forsch. Drug Res. 55 (2005) 451.
[10] T. G. Altuntas, and N. Erk, J. Liq. Chromatogr. Rel. Tech. 27 (2004) 83.
[11] S. Erturk, E. S. Aktas, L. Ersoy, and S. Fıcıcıoglu, J. Pharm. Biomed. Anal. 33 (2003) 1017.
[12] V. Borek-Dohalsky, J. Huclova, B. Barrett, B. Nemec, I. Ulc, and I. Jelinek, Anal. Bioanal. Chem. 386 (2006) 275.
[13] W. W. Bullen, R. A. Miller, and R. N. Hayes, J. Am. Soc. Mass Spectrom. 10 (1999) 55.
[14] X. S. Miao, and C. D. Metcalfe, J. Mass Spectrom. 38 (2003) 27.
[15] P. T. Kissenger, and W. R. Heineman, Laboratory Techniques in Electroanalytical Chemistry, Marcel Dekker, New York (1996).
[16] B. Uslu, and S. A. Ozkan, Anal. Lett. 40 (2007) 817.

[17] B. Uslu, and S. A. Ozkan, Comb. Chem. High T. Scr. 10 (2007) 495.
[18] S. A. Ozkan, B. Uslu, and H. Y. Aboul-Enein, Crit. Rev. Anal. Chem. 33 (2003) 155.
[19] B. D. Topal, S. A. Ozkan, and B. Uslu, Open Chem. Biomed. Methods J. 3 (2010) 56.
[20] R. L. McCreery, Chem. Rev. 108 (2008) 2646.
[21] C. E. Banks, and R. G. Compton, Analyst 131 (2006) 15.
[22] A. Qureshi, W. P. Kang, J. L. Davidson, and Y. Gurbuz, Diam. Relat. Mater. 18 (2009) 1401.
[23] C. E. Banks, and R. G. Compton, Anal. Sci. 21 (2005) 1263.
[24] R. L. McCreery, in Electroanalytical Chemistry, (A. J. Bard, ed.), Marcel Dekker, New York (1991).
[25] J. Wang, Electroanalysis 17 (2005) 7.
[26] G. G. Wallace, J. Chen, D. Li, S. E. Moulton, and J. M. Razal, J. Mater. Chem. 20 (2010) 3553.
[27] M. Pumera, A. Ambrosi, A. Bonanni, E. L. K. Cheng, and H. L. Poh, TrAC, Trends Anal. Chem. 29 (2010) 954.
[28] Y. Shao, J. Wang, H. Wu, J. Liu, I. A. Aksay, and Y. Lin, Electroanalysis 22 (2010) 1027.
[29] M. Pumera, Mater. Today 14 (2011) 308.
[30] R. R. Moore, C. E. Banks, and R. G. Compton, Analyst 129 (2004) 755.
[31] D. S. Shishmarev, N. V. Rees, and R. G. Compton, Electroanalysis 22 (2010) 31.
[32] R. T. Kachoosangi, C. E. Banks, and R. G. Compton, Electroanalysis, 18 (2006) 741.
[33] F. Wantz, C. E. Banks, and R. G. Compton, Electroanalysis 17 (2005) 1529.
[34] F. Wantz, C. E. Banks, and R. G. Compton, Electroanalysis 17 (2005) 655.
[35] C. E. Banks, and R. G. Compton, Analyst 130 (2005) 1232.
[36] C. E. Banks, A. Goodwin, C. G. R. Heald, and R. G. Compton, Analyst 130 (2005) 280.
[37] E. R. Lowe, C. E. Banks, and R. G. Compton, Electroanalysis 17 (2005) 1627.
[38] F. Wantz, C. E. Banks, and R. G. Compton, Electroanalysis 17 (2005) 1524.
[39] R. N. Goyal, S. Chatterjee, and B. Agrawal, Sens. Actuat. B 145 (2010) 743.
[40] R. N. Goyal, S. Chatterjee, and A. R. S. Rana, Talanta 83 (2010) 149.
[41] R. N. Goyal, and S. Bishnoi, Talanta 84 (2011) 78.
[42] N. Erk, Crit. Rev. Anal. Chem. 34 (2004) 1.
[43] B. D. Topal, B.Uslu, and S. A. Ozkan, Comb. Chem. High T. Scr. 10 (2007) 1514.
[44] M. R. Smyth, and J. G. Vos, Analytical Voltammetry, Elsevier Science Publication, Amsterdam (1992).
[45] S. A. Ozkan, and B. Uslu, Anal. Bioanal. Chem. 372 (2002) 582.
[46] S. Yilmaz, B. Uslu, and S. A. Ozkan, Talanta 54 (2001) 351.
[47] K. Sagar, J. M. Fernandez-Alvarez, C. Hua, M. R. Smyth, and R. Munden, J. Pharm. Biomed. Anal. 10 (1992) 17.

[48] K. Humphries, and G. Dryhurst, J. Pharm. Sci. 76 (1987) 839.
[49] A. Veiga, A. Dordio, A. J. P. Carvalhoa, D. M. Teixeira, and J. G. Teixeira, Anal. Chim. Acta 674 (2010) 182.
[50] H. R. Thirsk, and O. R. Brown, Electrochemistry 5 (1975) 220.