Electro-Organic Synthesis and Characterization of New Dihydroxybenzene Dinitrile Derivatives with Fluorescent Properties

Abdolmajid Bayandori MOGHADDAM,[a] Mohammad Reza GANJALI,*[,a] Rassoul DINARVAND,[b] Maryam LATIFI,[a] and Parviz NOROUZI[a]

[a] *Center of Excellence in Electrochemistry, Faculty of Chemistry, University of Tehran; and* [b] *Medical Nanotechnology Research Centre, Medical Sciences/University of Tehran; P. O. Box 14155–6451, Tehran, Iran.*

Received September 9, 2007; accepted March 8, 2008; published online March 28, 2008

Novel fluorescent molecules were synthesized by designing an environmentally friendly method involving the bulk electrolysis technique. This electrochemical treatment process helps protect the environment by minimizing the toxic waste component of effluent. The electrochemical oxidation of 3,6-dihydroxybenzene-1,2-dinitrile (DBD) in the presence of benzenesulfinic acids was studied in an aqueous solution (H_2O : AN, 90 : 10), which included an acetate buffer (pH=5.0). This research utilized a variety of experimental techniques, including cyclic voltammetry, controlled-potential electrolysis as well as spectroscopic identification of compounds produced as products. In addition, our fluorescent studies offered results in line with existing findings. At the wavelength of 205 nm, DBD and compound (6) were excited and their fluorescent emissions were monitored.

Key words fluorescence spectroscopy; electro-organic synthesis; electrochemistry

Following the pioneering research of Kasha, Vavilov, Perrin, Jablonski, Weber, Stokes and Forster in 1951,[1)] fluorescence spectroscopy became a widely used research tool in pure biochemistry, biophysics and material sciences. However, in recent years a number of new applications using fluorescence spectroscopy have been developed. These developments have promoted it from being primarily a purely scientific tool to being a more routine analytical and diagnostic tool. The phenomenon of fluorescence is now exploited for accomplishing simple analytical assays in the fields of environmental science, clinical chemistry, cell identification—where it is a dominant method in flow cytometry—as well as single cell imaging in medicine. Although there has been rapid growth in the number of routinized fluorescence applications, the principles have remained the same.[2)]

Fluorescence spectrometry is a conventional but highly sensitive analytical method. The synthesis and the biological applications of fluorescent compounds have previously been investigated.[3—8)] It has been shown that the quinone/hydroquinone redox couple can interconvert reversibly by exchanging two protons and two electrons. This characteristics enables it to serve as an antenna or the control subunit, which affects the absorption or the emission optical properties of the compound. In such quinone optical molecular switches, the interconversion of the four distinct states is caused by the multiplication of the two electrochromic redox states—quinone and hydroquinone.[9—11)] Furthermore, an important pathway in the metabolism of dihydroxybenzene estrogens and catecholamines is the oxidation of their respective semiquinones and quinones. The biological activity function of such molecules is related to their ability to act both as oxidants and electrophiles.[12)] Hydroquinone oxidation in aqueous solutions is well documented.[13—20)] It involves a transfer of two electrons and two protons to create the associated quinone. The electrochemical synthesis between catechol, 4-methylcatechol, 1,4-dihydroxybenzene and benzenesulfinic acids was reported by S. M. Golabi and co-workers.[21)]

For the current work, we utilized the electrochemical method to synthesize new dihydroxybenzene dinitrile derivative compounds with fluorescent properties using benzenesulfinic acids (as nucleophile). It is assumed that the synthesis of new dihydroxybenzene dinitrile derivatives with fluorescent properties would be useful from a pharmaceutical and biological viewpoint. However, following a literature survey, it was discovered that this is the first research paper concerning dihydroxybenzene dinitrile derivative electro-organic reactions for synthesizing fluorescent molecules from such compounds. The attractive property of these new compounds opens a new entrance in electrochemical applications.

Voltammetric Studies In Fig. 1, the cyclic voltammogram (CV) (a) was related to the electrochemical behavior of benzenesulfinic acid (**3**). The CV (b) demonstrated one anodic peak (A_1) (at 0.501 V) and one corresponding cathodic peak (C_1) (at 0.455 V). These peaks corresponded to the 3,6-dihydroxybenzene-1,2-dinitrile (DBD) electro-oxidation to 3,6-dioxocyclohexa-1,4-diene-1,2-dicarbonitrile (**2**) and *vice versa* within a two-electron and two-proton process (ΔE_p= 0.046 V). The CV (c) presented the CV obtained for a 0.1 mM solution of DBD in the presence of 0.1 mM **3**. It exhibited two anodic peaks at 0.504 V (A_1) and 0.548 V (A_2) *vs.* the reference electrode, where the cathodic counterpart of the anodic peak A_1 disappeared because of the reaction between **2** and **3**, leading to the compound formation (**5**, Chart 1, Eq. 2). The anodic peak A_1 was associated with the DBD electro-oxidation (CV (b) of Fig. 1 in comparison with that of the CV (c)). A_2 was attributed to the compound electro-oxidation (**5**, Chart 1, Eq. 3). The CVs were scanned at different rates (Fig. 1, (II)). The C_1 peak height rose with the scan rate increase. In this graph, the C_1 cathodic peak corresponded to the electro-reduction of **2**. The plots of the peak current ratio (I_{PA1}/I_{PC1}) and the peak current function ($I_{PA1}/v^{1/2}$) *vs.* the different scan rates between 20 and 2500 $mV \cdot s^{-1}$ (Fig. 1, (III)) were identified. The current function for the A_1 peak ($I_{PA1}/v^{1/2}$) reduced with the scan rate increase (with the ECE mechanism).[22)] A plot of the peak current ratio (I_{PC1}/I_{PA1}) *vs.* the scan rate confirmed the reactivity of **2** towards **3**, appearing as an increase in the height of the C_1 cathodic peak at

* To whom correspondence should be addressed. e-mail: Ganjali@khayam.ut.ac.ir

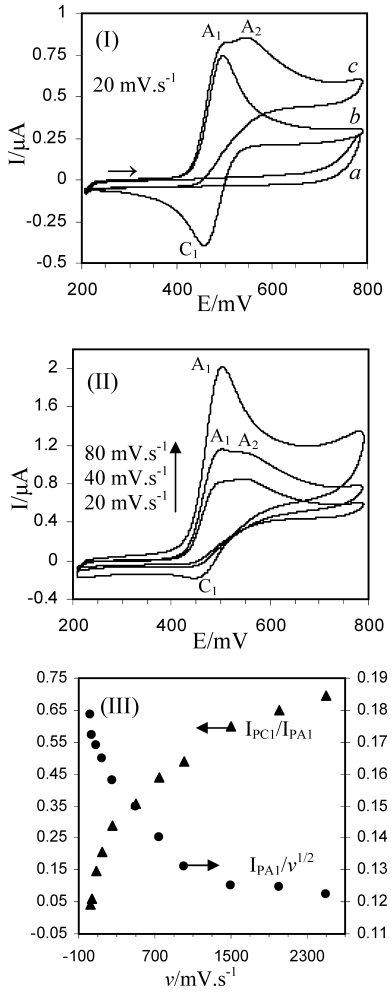

Fig. 1. (I) Comparative CVs of 0.1 mM **3** (a), DBD without (b) and with (c) **3**, at a GCE in Aqueous Solution (H_2O : AN, 90 : 10), Including Acetate Buffer (pH=5.0), (II) as (I)-(c), (III) I_{PC1}/I_{PA1} and $I_{PA1}/v^{1/2}$ *vs.* Scan Rate

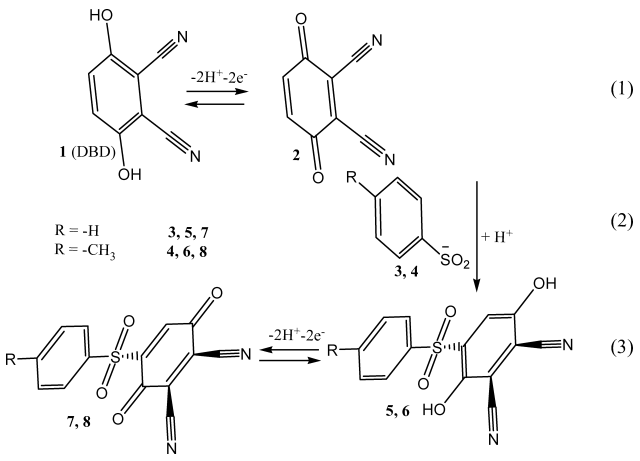

Chart 1

higher scan rates. It was evident that the A_1 and A_2 oxidation peaks converged at scan rates up to 40 mV·s^{-1} and appeared as the A_1 anodic peak (Fig. 1, (II)). This phenomenon was due to the fact that at low scan rates, the time scale for the reaction of **2** with **3** was longer and the compound formation diminished during the scan rate increase. Therefore, I_{PA1} decreased owing to the existence of smaller amounts of compound **5** for electro-oxidation in the A_1 peak potential together with DBD on the electrode surface. To determine the number of transferred electrons, controlled-potential coulometry (CPC) was performed in an aqueous solution, containing 0.1 mmol of DBD and 0.1 mmol of **3** at 480 mV *vs.* RE. Figure 2 depicts the obtained voltammograms during CPC. The volume of electricity consumed in the CPC course was 21.5 coulombs (C), which was demonstrated by the consumption of about 2e$^-$ per DBD molecule. DBD electro-organic reactions in the presence of *p*-toluenesulfinic acid (**4**) for compound **6** formation proceeded in a way similar to that of **3**. However, Fig. 1 illustrated the ECE mechanism for the DBD electro-oxidation in the presence of **3** and **4**, consisting of three consecutive steps; (i) the DBD electrochemical oxidation (E step), (ii) the chemical reaction of **3** and **4** with **2** (C step) and (iii) the electrochemical oxidation of the compounds (E step). The EC part of this mechanism was used with the application of chosen potential at 480 mV in controlled-potential electrolysis for the electro-organic synthesis of the compounds. It can be assumed that the oxidation mechanisms on the electrode and in the body share similar principles.[23] Therefore cyclic voltammograms (Fig. 3) of compound **6** were conducted. The CV of compound **6** exhibited two anodic peaks at 174 mV and 559 mV together with two corresponding cathodic peaks at 354 mV and 65 mV at 25 mV·s^{-1}. These peaks corresponded to compound **6** electro-oxidation of the related quinone within a two-electron and two-proton process, with an initial deprotonation step (Chart 2).[24] Figure 3b shows the CV of compound **6** at 100 mV·s^{-1}. It is evident from this figure that the first oxidation peak shifted to more positive potentials (appeared at 427 mV) in contrast to that of Fig. 3a. So, the oxidation peaks show a tendency to converge at higher scan rate.

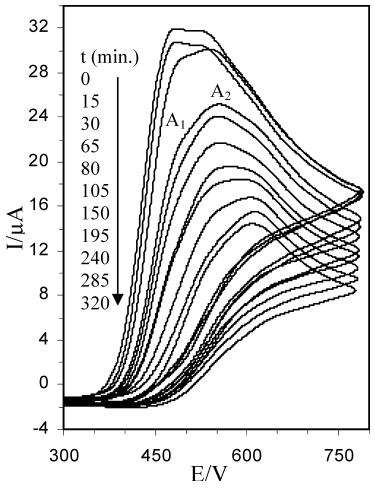

Fig. 2. CVs of 0.1 mmol **1** in the Presence of **2** (0.1 mmol) in the Course of CPC at 480 mV, Scan Rate 80 mV·s^{-1}, Other Conditions as in Fig. 1

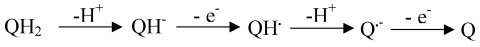

Chart 2

Fluorescent Properties of DBD and the Compounds

Figure 4 (a) shows the UV–Vis spectrum for DBD with the λ_{max} values of 205, 236 and 381 nm, while Fig. 4 (b) depicts

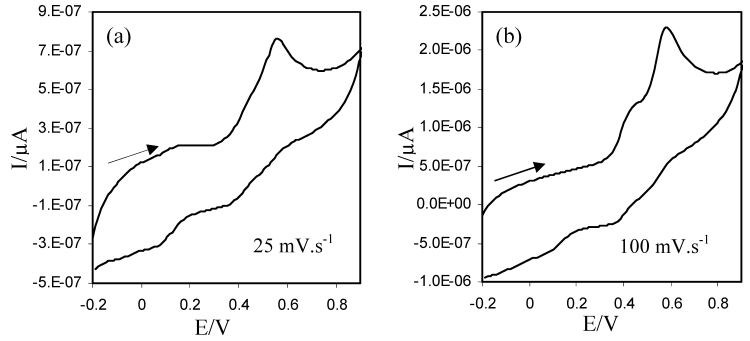

Fig. 3. CVs of 0.1 mM Product **6** at GCE in Phosphate Buffer Solution (pH=7.0)

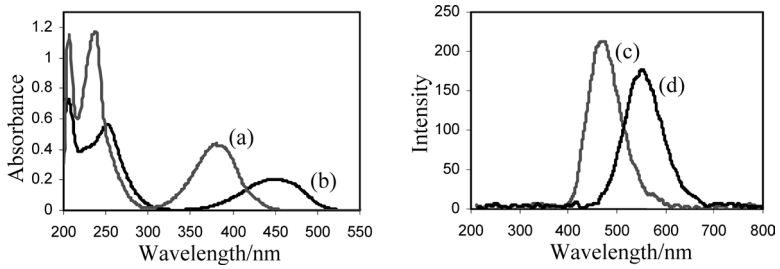

Fig. 4. UV–Vis Spectrum of 0.125 mM (a) DBD and (b) Product **6** in Phosphate Buffer Solution (pH=7.0)
Fluorescence spectrum of (c) DBD (0.125 mM, 39.25 μg/ml) and (d) product **6** (0.125 mM, 20 μg/ml). Excitation at 205 nm for the fluorescence measurements.

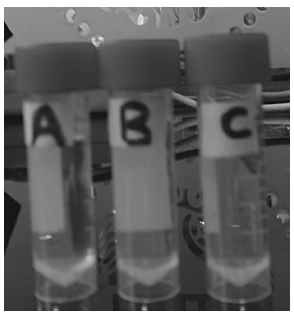

Fig. 5. (A) DBD, (B) Product **6** and (C) *p*-Toluenesulfinic Acid with the Concentration Values of 0.125 mM

the UV–Vis spectrum for compound **6** with the λ_{max} values of 205, 250 and 446 nm. In addition, to study the *in vitro* photophysical properties of the compounds, fluorescence spectral measurements were obtained in a buffer solution of pH equal to 7.0 at 25°C. Figure 4 (c) shows the typical emission spectrum of DBD, while Fig. 4 (d) displays the typical emission spectrum of compound **6**. Similar results were obtained for compound **5**. It is evident from Figs. 4 (c) and (d) that the fluorescence spectrum of compound **6** shifted to visible wavelengths in contrast to that of the starting material (DBD). This phenomenon is clear in Fig. 5, which is associated with DBD (A), compound **6** (B) and *p*-toluenesulfinic acid (C) at the concentration value of 0.125 mM.

In conclusion, the electro-organic reactions of 3,6-dihydroxybenzene-1,2-dinitrile (DBD) were studied in the presence of benzenesulfinic acids, involving the EC mechanism reaction. These reactions comprised two steps; (i) electrochemical oxidation and (ii) the chemical reaction. The reaction rate of the DBD dimerization increased with increase in pH, and the anionic formation of compounds **3** and **4** were also enhanced by the acid dissociation reaction with increased pH levels. Thus, this research studied the controlled-potential macroscale electrolysis at pH 5.0 for the synthesis of compounds **5** and **6** into new dihydroxybenzene dinitrile derivatives with fluorescent properties for the purposes of identifying new compounds with possible pharmaceutical properties. This work provides a better understanding of DBD electrochemical behavior in electro-organic reactions.

Experimental

Chemical Reagents 3,6-Dihydroxybenzene-1,2-dinitrile (Merck), benzenesulfinic acid sodium salt (Aldrich), *p*-toluenesulfinic acid sodium salt hydrate (Aldrich) were used without any further purification. For the preparation of the buffer solutions, the used salts were not purified (reagent grade materials, Merck). All solutions were prepared with acetonitrile (Merck) and deionized water.

Measurements The NMR spectra were recorded on a Bruker FT-NMR-500, while the IR spectra were recorded on a Shimadzu FT-IR-4300 Spectrophotometer. The MS spectra were obtained using an HP (Agilent Technology) GC-6890 combined with MS-5973 (EI at 20 eV and 70 eV). The fluorescence spectrophotometric studies were obtained using a Fluorescence Spectrophotometer CARY Eclipse. The elemental analyses were performed using a Heraeus CHN rapid analyzer. All electrochemical experiments were performed by the Autolab potentiostat PGSTAT 30 (Eco Chemie B.V., Netherlands), equipped with the GPES 4.9 software. A glassy-carbon disk electrode (2 mm in diameter) acted as the working electrode and a platinum wire was employed as the counter electrode (1 mm in diameter and 2 cm in length). In the controlled-potential bulk electrolysis, an assembly of three carbon rods (0.8 cm in diameter and 5 cm in length) was used as the working electrode (WE) with the total area value of 37.68 cm^2, while a platinum gauze was used as the counter electrode. Finally, the used reference electrode for the WE potential measurement was an Ag|AgCl|KCl (sat.). All electrodes were purchased from the AZAR Electrode Co. (Iran).

Electrochemical Synthesis of Compounds (5, 6) Using Bulk Electrolysis In the current trial 200 ml mixture of water–acetonitrile (90 : 10), containing acetate buffer (pH=5.0, c=0.2 M), 1.5 mmol 3,6-dihydroxybenzene-1,2-dinitrile (DBD, compound **1**) and sulfinic acids (**3**, **4**) (in equal concentration), were electrolyzed at the pre-determined potential (480 mV) in an undivided cell. For the less acetonitrile consumption as a nonaqueous solvent, the high volume cell (200 ml) was used in the controlled-potential bulk

electrolysis. The electrolysis time for the electro-organic synthesis of **5** and **6** compounds was 24 h and 16 h respectively.[19] Electrolysis stopped when thin layer chromatography (TLC with ethyl acetate) monitored very low amounts of the starting materials. Afterwards, the compounds were extracted by washing with dichloromethane. This process was repeated several times. TLC controlled both aqueous and nonaqueous media for the compound extraction. Finally the dichloromethane was air-dried at room temperature. The residual solids dissolved in the ethyl acetate following which 1 ml hexane (as anti-solvent) was added to crystallize the compounds. Then, the compounds were washed with hexane–ethyl acetate (4 : 1), air-dried and characterized by means of FT-IR, ^{1}H-, ^{13}C-NMR and MS spectrometry. The yield of compounds **5** and **6** was 48% and 63% respectively. The consumed charges were 2.23 and 2.19 F/mol for compounds **5** and **6** respectively.

Products Characteristics 3,6-Dihydroxy-4-(phenylsulfonyl)benzene-1,2-dinitrile ($C_{14}H_8O_4N_2S$, **5**): mp 168—170 °C (dec.). FT-IR (KBr): ν_{max} (cm^{-1}): 3340—3093 (OH, br), 2245 (CN), 1695, 1600, 1481, 1460, 1392, 1350, 1311, 1271, 1191, 1130, 1078, 997, 960, 840, 746, 711, 684, 601, 536, 478. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 7.57 (2H, d, J=7.56 Hz, sulfinic acid ring protons), 7.86 (2H, d, J=7.69 Hz, sulfinic acid ring protons), 7.93 (2H, m, sulfinic acid and dihydroxybenzene ring protons), 11.90 (2H, br, hydroxyl group protons). ^{13}C-NMR (DMSO-d_6, 500 MHz) δ (ppm): 106.40, 106.48, 114.30, 123.09, 129.38, 131.00, 136.70, 137.16, 140.89, 151.11, 155.04. MS (70 eV): m/z: 301 $[M+1]^+$ (10), 300 $[M]^+$ (50), 283 $[M-OH]^+$ (5), 272 (5), 253 (5), 235 (25), 208 (10), 158 (10), 141 [Phenyl-SO_2]$^+$ (50), 102 (10), 77 [Phenyl]$^+$ (100), 51 (45). *Anal.* Calcd for C, 55.99%; H, 2.69%; N, 9.33%. Found: C, 55.47%; H, 2.74%; N, 9.25%.

3,6-Dihydroxy-4-tosylbenzene-1,2-dinitrile ($C_{15}H_{10}O_4N_2S$, **6**): mp 198—200 °C (dec.). FT-IR (KBr): ν_{max} (cm^{-1}): 3224—3001 (OH, br), 2252 (CN), 1595, 1496, 1467, 1355, 1313, 1274, 1245, 1126, 1078, 1014, 966, 833, 746, 703, 682, 588, 528 and 480. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 2.41 (3H, s, Me), 7.45 (2H, d, J=8.15 Hz, sulfinic acid ring protons), 7.82 (2H, d, J=8.20 Hz, sulfinic acid ring protons), 7.93 (1H, s, dihydroxybenzene ring proton), 11.86 (2H, br, hydroxyl group protons). ^{13}C-NMR (DMSO-d_6, 500 MHz) δ (ppm): 21.99, 106.21, 106.37, 114.34, 122.94, 129.30, 130.59, 137.19, 137.48, 145.84, 151.35, 154.79. MS (70 eV): m/z: 315 $[M+1]^+$ (10), 314 $[M]^+$ (50), 297 $[M-OH]^+$ (5), 249 (25), 247 (30), 235 (15), 155 (15), 139 (20), 107 (15), 91 [Toluene]$^+$ (100), 77 [Phenyl]$^+$ (35), 65 (60), 53 (25). *Anal.* Found: C, 56.91%; H, 3.27%; N, 8.86%. Calcd C, 57.32%; H, 3.21%; N, 8.91%.

Acknowledgements The authors would like to thank Mr. G. Watson for his help and the authors wish to express their appreciation for the useful comments of the referees. Moreover, the financial support provided by the University of Tehran Research Affairs is gratefully acknowledged.

References

1) Forster T., "Fluoreszenz organischer Verbindungen. VandenHoech und Ruprecht," Gottingen, Germany, 1951.
2) Hof M., Hutterer R., Fidler V., "Fluorescence Spectroscopy in Biology," Springer, Verlag, 2005.
3) Bhown A. S., Cornelius T. W., Volanakis J. E., Bennett J. C., *Anal. Biochem.*, **131**, 337—340 (1983).
4) Suzuki Y., Yokoyama K., *J. Am. Chem. Soc.*, **127**, 17799—17802 (2005).
5) Mitsumori T., Bendikov M., Dautel O., Wudl F., Shioya T., Sato H., Sato Y., *J. Am. Chem. Soc.*, **126**, 16793—16803 (2004).
6) Grogan M. J., Kaizuka Y., Conrad R. M., Groves J. T., Bertozzi C. R., *J. Am. Chem. Soc.*, **127**, 14383—14387 (2005).
7) Bi L., Kim D. H., Ju J., *J. Am. Chem. Soc.*, **128**, 2542—2543 (2006).
8) Sun K. M., McLaughlin C. K., Lantero D. R., Manderville R. A., *J. Am. Chem. Soc.*, **129**, 1894—1895 (2007).
9) Goulle V., Harriman A., Lehn J.-M., *J. Chem. Soc., Chem. Commun.*, **1993**, 1034—1036 (1993).
10) Sutovsky Y., Likhtenshtein G. I., Bittner S., *Tetrahedron*, **59**, 2939—2945 (2003).
11) Illos R. A., Harlev E., Bittner S., *Tetrahedron Lett.*, **46**, 8427—8430 (2005).
12) Liehr F. J. G., Roy D., *Free Radic. Biol. Med.*, **8**, 415—423 (1990).
13) Golabi S. M., Pournaghi-Azar M. H., *Electrochim. Acta*, **32**, 425—431 (1987).
14) Azzem M. A., Zahran M., Haggag E., *Bull. Chem. Soc. Jpn.*, **67**, 1390—1395 (1994).
15) Bayandori Moghaddam A., Kobarfard F., Fakhari A. R., Nematollahi D., Hosseiny Davarani S. S., *Electrochim. Acta*, **51**, 739—744 (2005).
16) Bayandori Moghaddam A., Ganjali M. R., Dinarvand R., Norouzi P., Saboury A. A., Moosavi-Movahedi A. A., *Biophys. Chem.*, **128**, 30—37 (2007).
17) Ke J. N., Lu S.-S., Cheng S.-H., *Electrochem. Commun.*, **8**, 1514—1520 (2006).
18) Bayandori Moghaddam A., Ganjali M. R., Norouzi P., Latifi M., *Chem. Pharm. Bull.*, **54**, 1391—1396 (2006).
19) Bayandori Moghaddam A., Ganjali M. R., Norouzi P., Niasari M., *J. Electroanal. Chem.*, **601**, 205—210 (2007).
20) Hosseiny Davarani S. S., Najafi N. M., Ramyar S., Masoumi L., Shamsipur M., *Chem. Pharm. Bull.*, **54**, 959—962 (2006).
21) Nourmohammadi F., Golabi S. M., Saadnia A., *J. Electroanal. Chem.*, **529**, 12—19 (2002).
22) Bard A. J., Faulkner L. R., "Electrochemical Methods, Fundamentals and Applications," 2nd ed., John Wiley & Sons, New York, 2001, pp. 496—500.
23) Suzen S., Dermircigil B. T., Ozkan S. A., *New J. Chem.*, **6**, 1007—1011 (2003).
24) Cannes C., Kanoufi F., Bard A. J., *Langmuir*, **18**, 8134—8141 (2002).