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Detection of hydrazine, methylhydrazine and isoniazid by capillary electrophoresis with a 4-pyridyl hydroquinone self-assembled microdisk platinum electrode

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Abstract

Capillary electrophoresis (CE)/electrochemical detection (EC) for the simultaneous detection of hydrazine, methylhydrazine, and isoniazid has been developed with a 4-pyridyl hydroquinone self-assembled microdisk platinum electrode. Such an electrode has very high catalytic ability for hydrazines and they could be detected even at 0.0 V. The responses for hydrazine, methylhydrazine, and isoniazid are linear over 3 orders of detected concentration and of magnitude of 0.2–400 μM, 0.2–400 μM, 0.5 μM–2 mM, with correlation coefficients of 0.9998, 0.9991, and 0.9982, respectively. And they could be detected to levels of 0.1, 0.1 and 0.2 μM, respectively. This modified electrode was found to be very stable and reproducible when continuously used as detector for capillary electrophoresis for period of at least 4 weeks with no apparent loss of response.

Keywords: Capillary electrophoresis; Microdisk platinum electrode; Hydrazine; Methylhydrazine; Isonazid

1. Introduction

Since capillary electrophoresis (CE) was introduced decades ago by Mikkers et al. [1] Jorgenson and Lukacs [2,3] it has become an active research and development area of considerable interest in analytical chemistry due to its small sample volume requirement, short analysis time and high separation efficiencies [4] as compared to high performance liquid chromatography. Many detection techniques can be used with CE, such as UV-vis absorbance spectroscopy, fluorescence, indirect techniques, refractive index detectors, radioisotope, mass spectrometry, electrochemical detection (EC), and so on. Moreover, originally introduced in 1987 by Wallingford and Ewing [5], high sensitivity combined with high efficiency and an ability to handle ultrasmall volume samples has made this technique an attractive method for many separations while offering a high degree of selectivity toward electroactive species with wide linear range, and low cost [6]. But it is very difficult to detect those analytes which exhibit

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high overpotentials. Recently, lots of chemically modified electrodes (CMEs) have been exploited in CEEC for the determination of biological compounds. Zare et al. have tried two types of copper modified carbon fiber electrodes [7]. O'Shea and his coworkers have detected free thiols with cobalt phthalocyanide carbon paste microelectrode, glucose with a glucose oxidase/1,1′-dimethylferrocene-modified carbon paste microelectrode [6]. Lunte et al. have used a mixed-valent ruthenium cyanide-modified microelectrode to detect disulfide, and a D-amino acid oxidase modified electrode to detect amino acids [8,10]. Lu has detected some metal ions with a Hg/Au electrode [9].

Hydrazines are important compounds of interests in chemical industry and pharmaceutical processes. They are applied in many areas such as fuel cells, herbicides, catalysts, rocket propellants, and so on. When they are oxidized, they produce nitrogen and water which do not cause environmental pollution. Thus, numerous approaches have been taken to develop effective detection methods for this family. Amperometric detection is a powerful technique for monitoring easily oxidized species. Unfortunately, it is difficult to detect hydrazines because of their high overpotentials toward electrooxidation at ordinary solid electrodes. In order to enhance the amperometric detection for hydrazines, a lot of effort has been made, including preanodized glassy carbon electrode [11] and various chemically modified electrodes, such as CoPc-modified carbon paste electrode [12], oxymanganese film-modified electrode [13], inorganic mixed-valent Prussian Blue (PB) and its analogous ruthenium cyanide film-coated electrode [14,15], and Nafion/ruthenium oxide pyrochlore-modified electrode [16], polymeric porphyrin film electrode [17], single crystal metal electrode [18], metallophthalocyanines electrode [19], and so on.

In previous work, we have reported on a Pt-modified carbon fiber electrode [20] and a Pd-modified carbon fiber microdisk array electrode [21] for the determination of hydrazines by CEEC. Here, the design and application of 4-pyridyl hydroquinone on platinum electrode by self-assembling technique for CEEC are reported. The modified electrode exhibited excellent stability and highly catalytic activity toward hydrazines. Utilizing these merits, hydrazine, methylhydrazine, and isoniazid were successfully separated and detected by capillary zone electrophoresis (CZE) even at zero potential.

2. Experimental section

2.1. Reagents

Hydrazine sulfate (HZ) was obtained from Beijing Institute of Chemicals, methylhydrazine (MHZ) was purchased from Fluka (Buchs, Switzerland), and isoniazid (ISO) was obtained from the Shanghai Reagent Factory. All chemicals were analytical reagent grade and used as received. All solutions were freshly prepared with doubly distilled water (passed through a 0.45 μm membrane filter).

2.2. Apparatus

Electrophoresis in the capillary was driven by a high-voltage power supply (Spellman CZE 1000R, Plainview, NY). A 37 cm length of 25 μm i.d. 320 μm o.d. uncoated fused-silica capillary was used (Yongnian Optical Fiber Factory, Hebei, China). New separation capillary was flushed with 0.1 mol l^{-1} sodium hydroxide solution overnight before use. Before each run, the capillary was rinsed with 0.1 M sodium hydroxide solution, doubly distilled water, and running buffer for 2, 2, and 5 min, respectively.

The construction of the complete CE system with electrochemical detection was built in the laboratory. End-column detection was employed by using a wall-jet configuration. Detection was operated in the amperometric mode using a three-electrode, with a microdisk (25 μm diameter) working electrode, an Ag/AgCl (saturated KCl) reference electrode and a platinum auxiliary electrode. Potential control and current output were provided by a PAR Model 400

amperometric detector (USA). The data collection of electropherogram was provided by a Philip's computer configured as a Gilson 715 HPLC system controller software.

Cyclic voltammetry was carried out with a Model 660 computerized voltammetric analyzer (CH Instruments, TN) in a three-electrode system cell with a 1 mm microdisk Pt working electrode, an Ag/AgCl reference electrode (saturated KCl aq.) and a platinum wire auxiliary electrode.

Sample introduction was accomplished by an electromigration system and the volume injected was calculated in the continuously filling mode by recording the time required for the sample to reach the detector.

2.3. Formation of 4-pyridyl hydroquinone self-assembled monolayer (SAM) Pt microdisk electrode

The platinum microdisk electrode was electrochemically cleaned before each modification [22,23] and immersed into a 10 mM KF/HF (pH = 3) buffer solution containing 5 mM 4-pyridyl hydroquinone (4-PHQ) while the potential was kept at −0.1 V for 5 min. Then the electrode was completely washed with 10 mM KF/HF buffer and distilled water. Thus 4-pyridyl hydroquinone can be easily assembled on platinum surface based upon strong covalent binding of Pt and N interatoms.

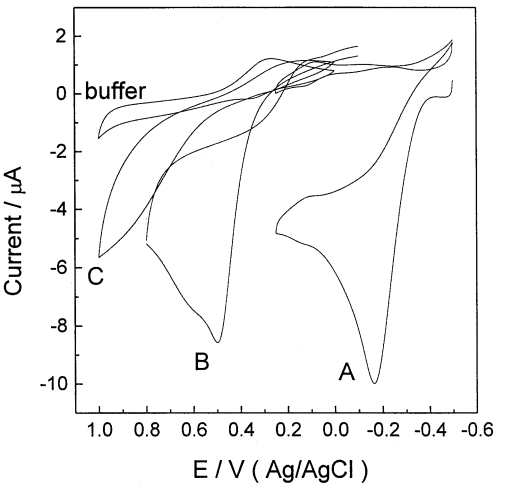
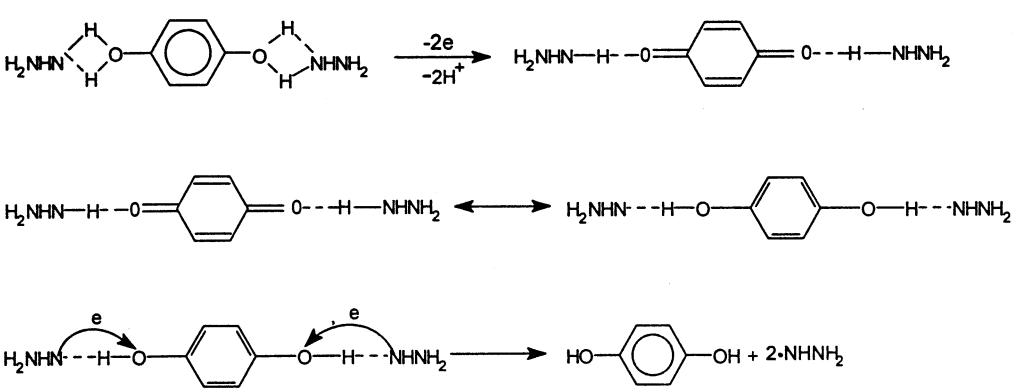


Fig. 1. Cyclic voltammograms of 1 mM HZ (A), MHZ (B) and ISO (C) at the 4-pyridyl hydroquinone SAM platinum electrode in 0.1 M phosphate buffer (pH, 7.2). Scan rate: 50 mV s^{-1}.

3. Results and discussion

3.1. Voltammetric behaviors of hydrazines

Fig. 1A shows the cyclic voltammograms of hydrazine obtained at 4-PHQ SAM modified Pt electrode. It can be seen that a defined oxidation peak occurred at −0.16 V (1 mM) versus Ag/AgCl while it often occurred above 1.0 V at ordinary electrode. The lower potential of electrooxidation and higher electrocatalytic current indicated the dramatically electrocatalytic activity of SAM.



Scheme 1. Schematic illustration of the possible reaction steps of hydrazin occurred at 4-PHQ modified platinum electrode.

It is supposed that there is a two-step reaction of the oxidation of hydrazine at the 4-pyridyl hydroquinone SAM electrode [24]. As shown in Scheme 1, the first step is that 4-PHQ loses its electrons, and the second step is that the product of the first step oxidizes hydrazine.

Firstly, the hydrazine was adsorbed on the modified electrode surface. The SAM electrode modified by incorporating quinone-containing materials was greatly effective to promote proton transfer of the adsorbed species. So the possible overall reaction of hydrazine oxidation at the 4-pyridyl hydroquinone SAM electrode involves the following steps [24].

According to the above steps, firstly, 4-PHQ loses its electrons and becomes quinone. The hydrazine molecule adsorbed on the monolayer surface formed surface complexes by hydrobinding. The SAM electrode modified by incorporating quinone-containing materials was greatly effective in promoting proton transfer of the adsorbed species. Then the hydrazine molecule is successively dehydrogenated through proton transfer from hydrazine to quinone, so quinone is reduced to hydroquinone. After that, another cyclic reaction starts again. Such transfer through adsorption especially decreases the free energy of oxidation reaction and easily makes very fast oxidation. It can be seen in Fig. 1B,C that the SAM modified electrode can also produce electrocatalytic response for MHZ and ISO, decreasing the overpotentials of MHZ and ISO. However, the catalytic ability of SAM-modified electrode for HZ is much higher than that of MHZ or ISO, due to the presence of the readily electrooxidized amine group with excellently coordinated configuration. The varying of the currents of hydrazines with the concentration variation was also investigated (data not shown). When the concentration of hydrazine becomes higher, the maximum peak potential shifts more positive because it takes a longer time to approach their limited currents.

3.2. CEEC using the 4-PHQ SAM modified microdisk Pt electrode

Compared to the ordinary solid electrode, the microelectrode has many advantages including rapid mass transport towards the electrode surface, very rapid relaxation, very small ohmic drop values, and in flow measurements the signal-to-noise ratio is further enhanced by the effect of lateral diffusion (edge effect). Such, a 4-PHQ SAM modified Pt electrode exhibits high catalytic ability towards hydrazines. Therefore, a 4-PHQ SAM modified 25 μm Pt microdisk electrode was used in our work.

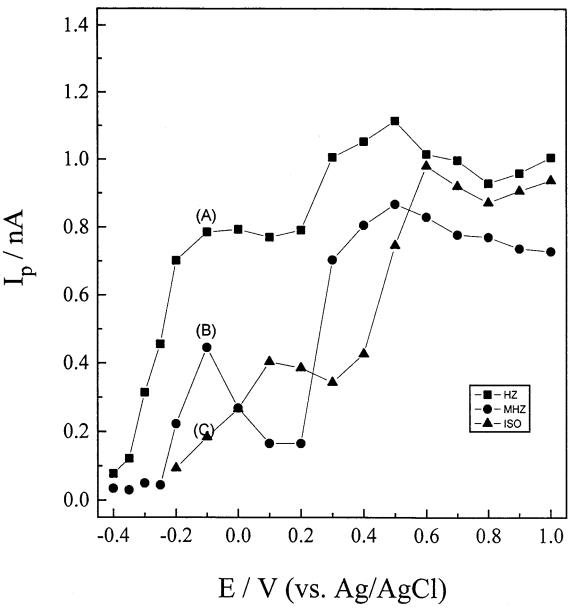


Fig. 2. Hydrodynamic voltammograms of 50 μM HZ (A), MHZ (B) and 1 mM ISO (C) at the 4-pyridyl hydroquinone SAM 25 μm platinum electrode. Buffer, 10 mM sodium phosphate, pH, 7.02. Separation capillary, 25 μm i.d. 37 cm long. Operating voltage, 15 KV. Sample injection, 2 s at 15 KV.

To determine the optimum potential for electrochemical detection, hydrodynamic voltammograms (HDVs) of hydrazines on the SAM modified electrode were shown in Fig. 2. In the potential range investigated from -0.4 to 1.0 V versus Ag/AgCl, the hydrazines exhibit similar HDV behavior. In the interval from -0.4 to 0.2 V, peak-shaped curves are observed with a peak potential of 0, -0.1, and 0.1 V for hydrazine, methylhydrazine, and isoniazid, respectively. The response of hydrazine and isoniazid reaches a plateau, which represents the second reaction equation in Scheme 1. After that, the response of hydrazine increases slowly while that of methylhy-

drazine and isoniazid increases sharply with more positive potential. Isoniazid exhibits another identical peak potential at + 0.6 V, whereas hydrazine and methylhydrazine have a peak potential of 0.5 V, and increase again at higher potentials. As a compromise of high sensitivity and low background current, a 0.5 V value was selected for simultaneous detection of hydrazine, methylhydrazine, and isoniazid. From HDVs, it can also be obtained that the response of hydrazine is higher than that of methylhydrazine and isoniazid. Because the naked hydrogen atom linked N atom of hydrazine can be excellently adsorbed with the hydroxyl group of 4-PHQ on the platinum electrode surface. They can form a good coordination with each other in space position while methylhydrazine and isoniazid have a big spacial hindrance and resulted in a bad coordination.

As illustrated in Fig. 3, the effect of pH of phosphate running buffer on the separation efficiency, peak current, and retention time with the CEEC of hydrazines at 4-PHQ modified electrode was investigated over a range of pH 3.06 ~ 8.92. The separation efficiency was satisfied and had no apparent changes during the pH values. The electroosmotic flow and the mass-charge ratio of analytes change according to the pH values. Because of the interactions of these two factors, the retention time becomes shorter and then becomes longer again after pH > 6.03 as pH increases from 3.06 to 8.92. Thus a pH 7.02 value was selected for CEEC.

Electropherograms of hydrazine, methylhydrazine, and isoniazid in phosphate pH 7.02 at the 4-PHQ SAM modified 25 μM Pt microdisk electrode under 0.5 V are shown in Fig. 4(A). The hydrazines can be separated and easily detected in 3 min. Under optimum conditions, the detection limits for hydrazine, methylhydrazine and isoniazid are 0.1, 0.1 and 0.2 μM with efficiencies of 282600, 202400 and 191900, respectively. The linear ranges are over 3 orders and of magnitude of 0.2–400 μM, 0.2–400 μM and 0.5–2 mM, with correlation coefficient of 0.9998, 0.9991, and 0.9982, respectively. Compared with the results we have reported before [20,21], the potential applied for detection at the 4-PHQ SAM modified electrode was lower, and the detection limit obtained

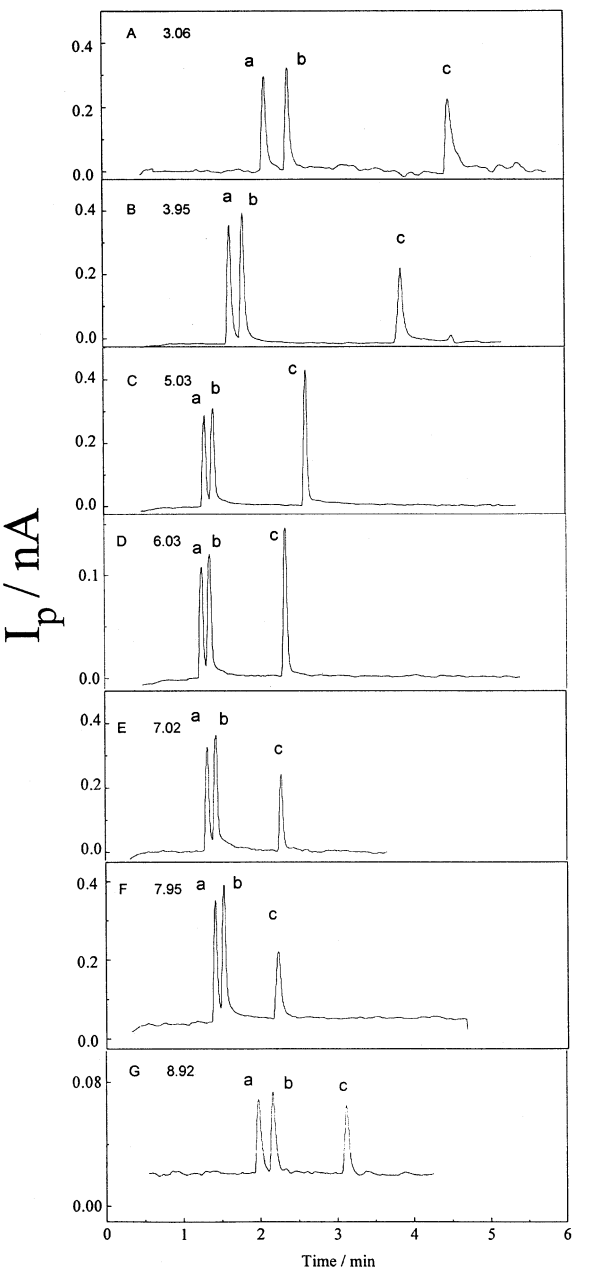


Fig. 3. Electropherograms of (a) 25 μm HZ, (b) 50 μm MHZ, and (c) 0.4 mM ISO at different pH values of 10 mM sodium phosphate at the 4-pyridyl hydroquinone SAM 25 μm platinum electrode. (A) 3.06, (B) 3.95, (C) 5.03, (D) 6.03, (E) 7.02, (F) 7.95 and (G) 8.92. Others conditions are same as Fig. 2.

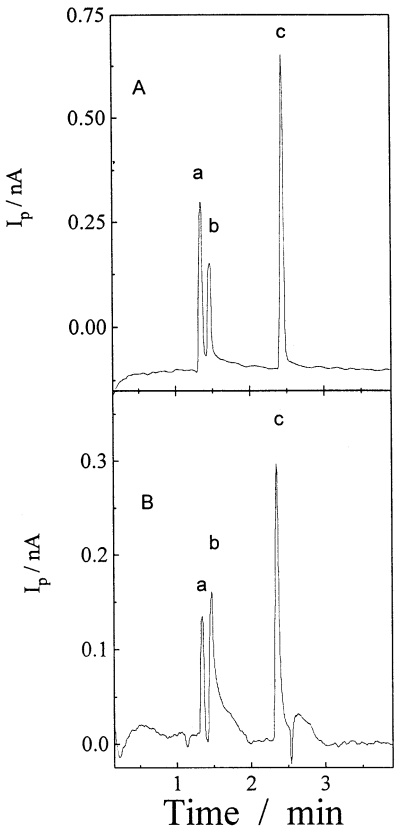


Fig. 4. Electropherograms of (a) 20 μm HZ, (b) 20 μm MHZ and (c) 0.8 mM ISO at the 4-pyridyl hydroquinone SAM 25 μm platinum electrode (A) at 0.5 V (B) 0.0 V versus Ag/AgCl. Other conditions are same as Fig. 2.

was 1 order lower. Moreover, the 4-PHQ SAM modified electrode has dramatic catalytic ability such that the three species can be easily detected even at the potential of 0.0 V. Fig. 4(B) illustrates electropherograms of three species detected at 0.0 V. It shows that the modified electrode has high a catalytic ability towards hydrazines. Because lots of species are not easily oxidized at such a low potential, this modified electrode used for detector can avoid interference of some other species and improve selectivity towards hydrazines for potential practical application.

Finally, the response reproducibility of the modified electrode in the CEEC flow system was investigated. The reproducibility of the method was calculated by making 12 replicate injections of 50 μM hydrazine, 100 μM methylhydrazine, and 200 μM isoniazid. The relative standard deviations of the current response were 5.00, 5.24, and 2.67%, respectively. And the relative standard deviation of retention time were 0.926, 0.355, and 0.524%, respectively. The day-to-day variability experiments were also done as shown in Fig. 5. From Fig. 5, it can be seen that the 4-PHQ SAM modified electrode was very stable toward oxidation of hydrazines during 4 weeks in CEEC.

It is well known that property of specific adsorption is steady and the sensitivity in SAM is high. Throughout the experiments, the 4-PHQ SAM modified electrode maintained a very high and stable catalytic activity. The electrode can be continuously used in flow system for about 4 weeks and the responses of hydrazines are stable. When the responses decrease apparently, the electrode should be remodified again. To improve reproducibility, between each run, the modified electrode was cycled between $-0.2 \sim 1.0$ V for 2 min to refresh the surface of the electrode and the capillary was rinsed with 0.1 M sodium hydroxide solution, doubly distilled water, and running buffer for 2, 2, and 5 min, respectively.

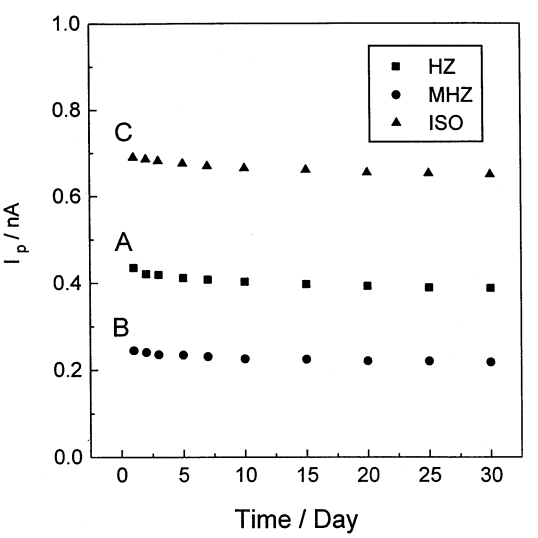


Fig. 5. Day-to-day variability of currents of 20 μm HZ (A), 20 μm MHZ (B) and 0.8 mM ISO (C) at the 4-pyridyl hydroquinone SAM 25 μm platinum electrode. Other conditions are same as Fig. 2.

4. Conclusion

In this work, the preparation and use of the 4-pyridyl hydroquinone self-assembled monolayer modified Pt electrode for simultaneous detection of hydrazine, methylhydrazine, isoniazid by CEEC have been described. The modified electrode exhibits so highly stable catalytic capability toward hydrazines which can be detected even at 0.0 V in CEEC.

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References

[1] F.E.P. Mikkers, F.M. Everaerts, Th.P.E.M. Verheggen, J. Chromatogr. 169 (1979) 11–20.
[2] J.W. Jorgenson, K.D. Lukacs, J. Chromatogr. 218 (1981) 209–216.
[3] J.W. Jorgenson, K.D. Lukacs, Anal. Chem. 53 (1981) 1298–1302.
[4] C.A. Monning, R.T. Kenendy, Anal. Chem. 66 (1994) 280R–314.
[5] R.A. Wallingford, A.G. Ewing, Anal. Chem. 59 (1987) 1762–1786.
[6] T.J. O'Shea, S.M. Lunte, Anal. Chem. 66 (1994) 307–311.
[7] L.A. Colon, R. Dadov, R.N. Zare, Anal. Chem. 65 (1993) 476–481.
[8] J. Zhou, T.J. O'Shea, S.M. Lunte, J. Chromatogr. A 680 (1994) 271–277.
[9] W. Lu, R.M. Cassidy, A.S. Baranshi, J. Chromatogr. 640 (1993) 433.
[10] J. Zhou, S.M. Lunte, Anal. Chem. 67 (1995) 13–18.
[11] K. Pavichandran, R.P Baldwin, Anal. Chem. 55 (1983) 1782–1786.
[12] K.M. Korfhage, K. Pavichandran, R.P Baldwin, Anal. Chem. 56 (1984) 1514.
[13] Z. Taha, J. Wang, Electroanalysis 3 (1991) 215.
[14] W. Hou, E. Wang, Anal. Chim. Acta 257 (1992) 275–280.
[15] J. Wang, Z. Lu, Electroanalysis 1 (1989) 517.
[16] J. Zen, J. Tang, Anal. Chem. 67 (1995) 208–211.
[17] J.E. Bennett, T. Mallinski, Chem. Mater. 3 (1991) 490.
[18] R.G. Mez, J.M. Orts, J.M. Feliu, A. Aldaz, J. Electroanal. Chem. 358 (1993) 287.
[19] J.H. Zagal, Coord. Chem. Rev. 119 (1992) 89.
[20] W. Zhou, L. Xu, M. Wu, Li. Xu, E. Wang, Anal. Chim. Acta 299 (1994) 189–194.
[21] J. Liu, W. Zhou, T. You, F. Li, E. Wang, S. Dong, Anal. Chem. 68 (1996) 3350–3353.
[22] D.C. Zapien, J.Y. Gui, D.A. Stem, A.T. Hubbard, J. Electroanal. Chem. 330 (1992) 469–487.
[23] M.P. Soriaga, A.T. Hubbard, J. Am. Chem. Soc. 104 (1982) 2735.
[24] L. Niu, T. You, J.Y. Gui, E. Wang, S. Dong, Electrocatalytic oxidation of hydrazines at 4-pyridyl hydroquinone self-assembled platinum electrode and its application to amperometric detection in capillary electrophoresis, J. Electroanal. Chem. (in press).