Short Communication

Selective Detection of Uric Acid in the Presence of Ascorbic Acid and Dopamine Using Polymerized Luminol Film Modified Glassy Carbon Electrode

S. Ashok Kumar,* *Hui-Wen Cheng*, *Shen-Ming Chen**

Department of Chemical Engineering and Biotechnology, National Taipei University of Technology, No. 1, Section 3, Chung-Hsiao East Road, Taipei, 106, Taiwan (ROC)
*e-mails: smchen78@ms15.hinet.net; sakumar80@gmail.com

Received: April 9, 2009
Accepted: June 13, 2009

Abstract
Electrochemically polymerized luminol film on a glassy carbon electrode (GCE) surface has been used as a sensor for selective detection of uric acid (UA) in the presence of ascorbic acid (AA) and dopamine (DA). Cyclic voltammetry was used to evaluate the electrochemical properties of the poly(luminol) film modified electrode. Scanning electron microscopy (SEM) and atomic force microscopy (AFM) have been used for surface characterizations. The bare GCE failed to distinguish the oxidation peaks of AA, DA and UA in phosphate buffer solution (pH 7.0), while the poly(luminol) modified electrode could separate them efficiently. In differential pulse voltammetric (DPV) measurements, the modified GCE could separate AA and DA signals from UA, allowing the selective determination of UA. Using DPV, the linear range (3.0×10^{-5} to 1.0×10^{-3} M) and the detection limit (2.0×10^{-6} M) were estimated for measurement of UA in physiological condition. The applicability of the prepared electrode was demonstrated by measuring UA in human urine samples.

Keywords: Detection of uric acid, Modified electrode, Electroanalysis, Poly(luminol), Conducting polymers, Thin films, Medicinal chemistry

DOI: 10.1002/elan.200904677

Uric acid (UA) is the ultimate catabolite of purine metabolism in humans and higher primates. It is a weak organic acid that under physiologic conditions exists mainly as a monosodium salt. At a pH less than 5.75, as may occur in the urine, the predominant form is nonionized UA. The solubility of monosodium urate is about 18 times greater than UA in aqueous solutions. This solubility difference provides the therapeutic rationale for alkalinization of the urine pH to greater than 6.0 in patients forming UA stones. UA levels are influenced by age and sex. Many additional factors, including exercise, diet, drugs, and state of hydration, may result in transient fluctuations of UA levels. Extreme, abnormal levels of UA in body fluids will lead to some diseases. It is for this reason, that simple and rapid detection methods are required [1–3].

Detection of biological molecules using chemically modified electrodes is more attractive strategy since electrochemical sensors combines the specificity of biological or chemical recognition layers with the inherent advantages (sensitivity, speed, miniaturization, linearity) of electrochemical transduction [4–6]. Polymer modified electrodes (PMEs) are received considerable attention among the researchers for sensing applications. Electropolymerization is a good approach to prepare PMEs as adjusting electrochemical parameters can control film thickness, permeation and charge transport characteristics [6–8].

Dopamine (DA), UA and ascorbic acid (AA) usually coexist in physiological samples and these molecules have a same oxidation potential on the unmodified solid electrodes. Therefore it is essential to develop a simple and rapid method for their determination in routine analysis. Among many methods for determination of UA in biological samples, voltammetric method has shown to be a powerful tool [9, 10]. Recently, edge plane pyrolytic graphite electrodes [11], Ir-modified carbon working electrode with immobilizing uricase [12], hexacyanoferrate lanthanum film modified electrode [13], composite film of polyaniline nanonetworks/*p*-aminobenzene sulfonic acid modified GCE [14] and mesoporous SiO_2-modified carbon paste electrodes [15] have been applied for the determination of UA. However, a new method for selective detection of UA in the presence of DA and AA is still required. Electrochemical sensors for UA determination with the advantages of lower detection limit, wide linear range, and negligible interferences, re-useable and cheaper electrode materials are still under demand in the sensor industry for commercialization purpose [16]. Here, we attempted to develop a new electrochemical sensor for UA using polymerized luminol film as a sensing material.

Luminol (5-amino-2,3-dihydro-phthalazine-1,4-dione), is a typical strong chemiluminescence reagent, has been extensively studied in analytical chemistry and biochemistry

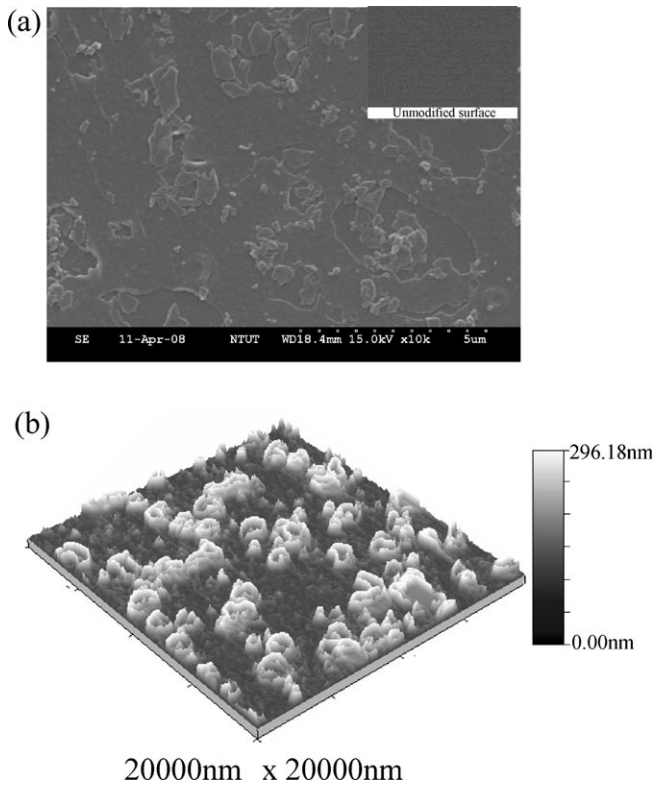

Fig. 1. SEM (a) and AFM (3-dimensional image recorded in tapping mode) (b) images of poly(luminol) film modified electrode.

[17, 18]. Electrogenerated chemiluminescences properties of luminol have been studied on various electrodes [19, 20]. Recently, polymerized luminol film modified electrodes have been used for detection of several analytes using electroanalytical methods [21–25].

The main objective of this study was to develop a rapid, selective, sensitive and convenient electrochemical method for the determination of UA utilizing the unique properties of poly(luminol) modified electrode. The electrochemical behaviors of UA at the unmodified and poly(luminol) modified GCE were investigated. It was found that the poly(luminol)-modified GCE remarkably increases the determining selectivity and sensitivity of UA due to its unique properties. To the best of our knowledge, for the first time here we report poly(luminol) modified electrode for selective and sensitive determination of UA in the presence of AA and DA. The practical application of the electrode is successfully demonstrated for the determination of UA in urine samples without any preliminary treatment.

Figure 1a shows the SEM image of poly(luminol) modified electrode as observed in this image, an adherent thin films and sheets-like polymer particles were observed in the range from 0.1 to 100 μm. Inset of Figure 1a shows SEM image of unmodified electrode which clearly revealed that polymer film has been formed on the modified electrode surface. Figure 1b depicts 3-dimensional image (20000 nm × 20000 nm) of poly(luminol) film modified electrode which was recorded in AFM tapping mode. This AFM image clearly represented the existence of poly(luminol) films on the electrode surface and the polymer particles (0.1 to 100 μm) were observed. Using AFM image analyzer, some of the important parameters of poly(luminol) modified electrode were evaluated such as film thickness (296.18 nm) and surface roughness (23.4 nm). A highly porous films were obtained for poly(luminol) grown using cyclic voltammetry (CV). The obtained roughness and the porosity of poly(luminol) films are similar to that obtained for polyaniline electropolymerized from an aqueous media [26, 27].

There was a pair of reversible redox peaks in the cyclic voltammograms (CVs) of the poly(luminol) modified GCE in phosphate buffer solution (PBS, pH 7.0), $E_{pa} = 0.2989$ V, $E_{pc} = 0.1518$ V, as shown in inset of Figure 2A. The surface coverage of poly(luminol) could be calculated from $i_p = n^2F^2\nu A\Gamma/4RT$, where i_p, ν, A, and Γ represent the peak current (in A), scan rate (in V s^{-1}), the electrode area (in cm^2), and the surface coverage of the redox species (mol cm^{-2}), respectively. Therefore, we calculated that the surface coverage for poly(luminol) film was 2.5214×10^{-10} mol cm^{-2} which indicated that polymer film attachment had occurred [28]. A GCE was cycled in 0.1 M H_2SO_4 for 10 cycles (between 0.0 and 1.0 V at 50 mVs^{-1}) and then, CVs of a treated GCE was recorded in pH 7.0 PBS and its response was featureless and the redox peak of quinine/hydroquinone groups are not generated in the potential range used for polymerization. High oxidation potentials (> 2.0 V) are required for generation of surface active quinine/hydroquinone groups on carbon electrodes [29].

The electrocatalytic behavior of the poly(luminol) modified electrode was evaluated via the oxidation of UA. Figure 2A shows the CVs of the modified electrode in 0.1 M PBS (pH 7.0) spiked with UA (curve b). It also includes a voltammogram obtained for the poly(luminol) modified electrode when placed in PBS without UA (curve a). After the addition of UA to the PBS, the anodic peak current (i_{pa}) of poly(luminol) increased, while the corresponding cathodic wave was substantially depressed on the reverse scan (Fig. 2A curve b). This behavior suggests that the electrooxidation of UA may be catalyzed by the polymer redox couple, which acts as a mediator. The CVs for UA on the poly(luminol) modified electrode and on the pretreated GCE show that UA oxidation occurs at the surface of the former electrode at a potential that is 0.140 V less positive than the potential required for oxidation on the latter electrode (curve c). At the same time, compared with the pretreated GCE, the oxidation current for UA was much higher at the modified electrode (Fig. 2A curve b). All of these factors indicate that the poly(luminol) modified electrode catalyzes the oxidation of UA. The effect of scan rate on the anodic peak current of UA was studied. As the scan rate increased, the oxidation peak current (I_{pa}) increased (Fig. 2B). The I_{pa} was directly proportional to the root of scan rate ($\nu^{1/2}$) over the range of 10–200 mVs^{-1} (Fig. 2B curves a–j), which suggested a diffusion-controlled process on the modified electrode surface [30].

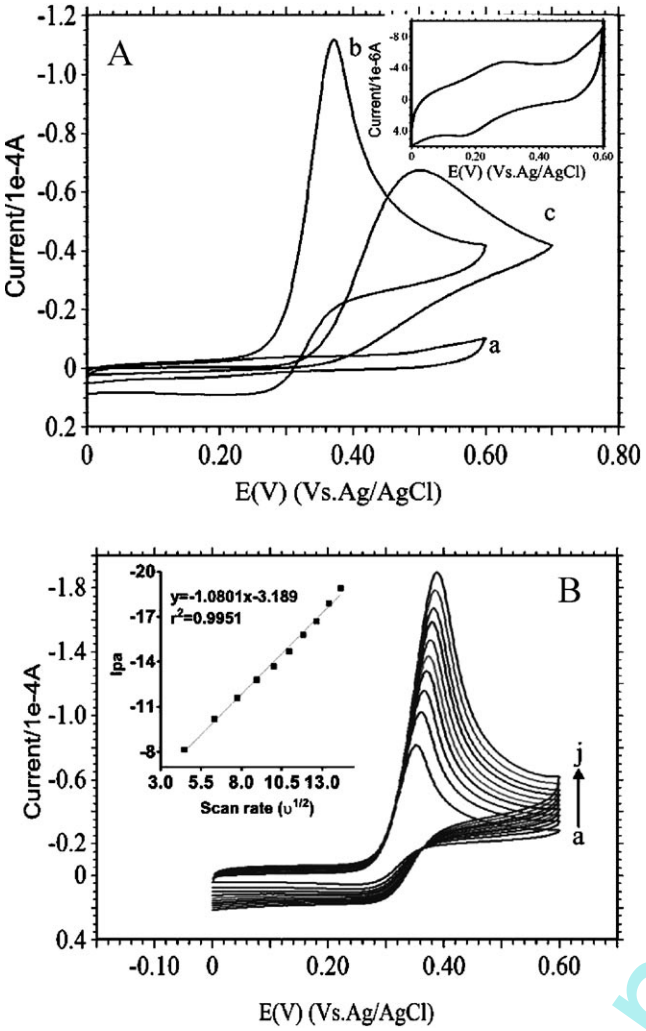

Fig. 2. (A) CVs of poly(luminol)/GCE in the absences (curve a) and presences of 5 mM UA (curve b) in pH 7.0 PBS. Unmodified GCE in pH7.0 PBS + 5 mM UA (curve c). Scan rate: 40 mV s^{-1}. Inset shows the CVs of poly(luminol)/GCE in pH 7.0 PBS. (B) CVs of poly(luminol)/GCE in PBS + 5 mM UA (pH 7.0) at different scan rates. The scan rates from inner to outer are (a to j) 0.02, 0.04, 0.06, 0.08, 0.10, 0.12, 0.14, 0.16, 0.18, and 0.20 V s^{-1}, respectively. Inset: plot of I_{pa} vs. (scan rate)$^{1/2}$.

The electrochemical polymerization mechanism of luminol was reported elsewhere and the redox reaction of poly(luminol) was involved with same number of electrons and protons as similar to polyaniline [24–26]. UA exists as anions (pKa = 5.75) in physiological pH (pH 7.0) and it is more hydrophobic than AA [31]. It was experimentally proved that conducting polymer films (e.g., polypyrrole, poly-3,4-ethylenedioxythiophene) contains a distribution of reduced and oxidized regions and reduced regions are more hydrophobic in nature [32, 33]. The same behavior is expected for poly(luminol) and accumulation of UA at the reduced region of polymer surface through hydrophobic interaction resulting in the enhancement of oxidation current. The electrochemical oxidation of UA proceeds in a $2e^-$, $2H^+$ process to lead to an unstable uric acid-4,5-diol [33, 34].

One of the main objectives of this study was to develop a modified electrode capable of selective determination of UA in the presence of AA and DA. Since differential pulse voltammetry, DPV, has a much higher current sensitivity and a better resolution than CV, it was used to estimate the lower limits of detection for UA, and to simultaneously determine AA, DA and UA. In addition, the contribution from the charging current to the background current, which was a limiting factor during the analytical determination, is negligible in DPV mode. The applicability of the poly-(luminol) modified electrode to the selective determination of UA in the presence of AA was demonstrated by simultaneously changing the concentrations of AA at fixed concentration of UA (1 mM). The DPV results showed that there were two well-distinguished anodic peaks at potentials of 29 and 295 mV, corresponding to the oxidation of AA and UA, respectively (Fig. 3A, Curves b – i). Gradual increase of anodic currents was observed at AA oxidation potential with respect to the added concentrations of AA. This made it possible to determine UA in the presence of AA at the poly(luminol) modified electrode (Fig. 3A curves a – i).

The applicability of the poly(luminol) modified electrode to the selective determination of UA in the presence of DA was also demonstrated by simultaneously changing the concentrations of DA at fixed concentration of UA (0.5 mM). The DPV results showed that there were two well-distinguished anodic peaks at potentials of 167 and 295 mV, corresponding to the oxidation of DA and UA, respectively (Fig. 3B). Gradual increase of anodic currents was observed at DA oxidation potential with respect to added concentrations of DA (Fig. 3B curves a – g). This made it's also possible to determine UA in the presence of DA at the poly(luminol) modified electrode.

As shown in Figure 3C, various concentrations of UA in the presence of 1.0 mM AA and 1 mM DA exhibit excellent DPV responses, while keeping the responses to AA and DA almost constant, indicating that the responses to AA, DA and UA at poly(luminol)/GCE are relatively concentration-independent. In the presence of AA and DA, the I_{pa} of UA was measured in pH 7.0 PBS, employing DPV as well. The dependence of the peak current on the concentration of UA is in a linear relationship in the range of 3.0×10^{-5} to 1.0×10^{-3} M (Fig. 3C, curves a – q). The linear regression equation is expressed as $I_{pa}\,(\mu A) = -0.06\,x - 20$, $r^2 = 0.9912$. The detection limit ($S/N = 3$) was 2.0×10^{-6} M. This suggests that the oxidations of UA occur independently at poly-(luminol) modified electrode. Thus, the prepared electrode allowed both selective and sensitive determinations of UA.

In order to characterize the reproducibility of this sensor, a series of repetitive measurements were carried out in 5.0×10^{-5} M UA solutions. The electrode can be renewed easily by dipping the electrode in 0.1 M PBS (pH 7.0) for potential cycling from −0.2 to 0.5 V for 10 cycles after each determination. Relative standard deviation of 2.8% was observed for 10 determinations of UA, indicating that the modified electrode had excellent reproducibility and ability to prevent the electrode from fouling by the oxidation product. The stability of the sensor was also determined. The

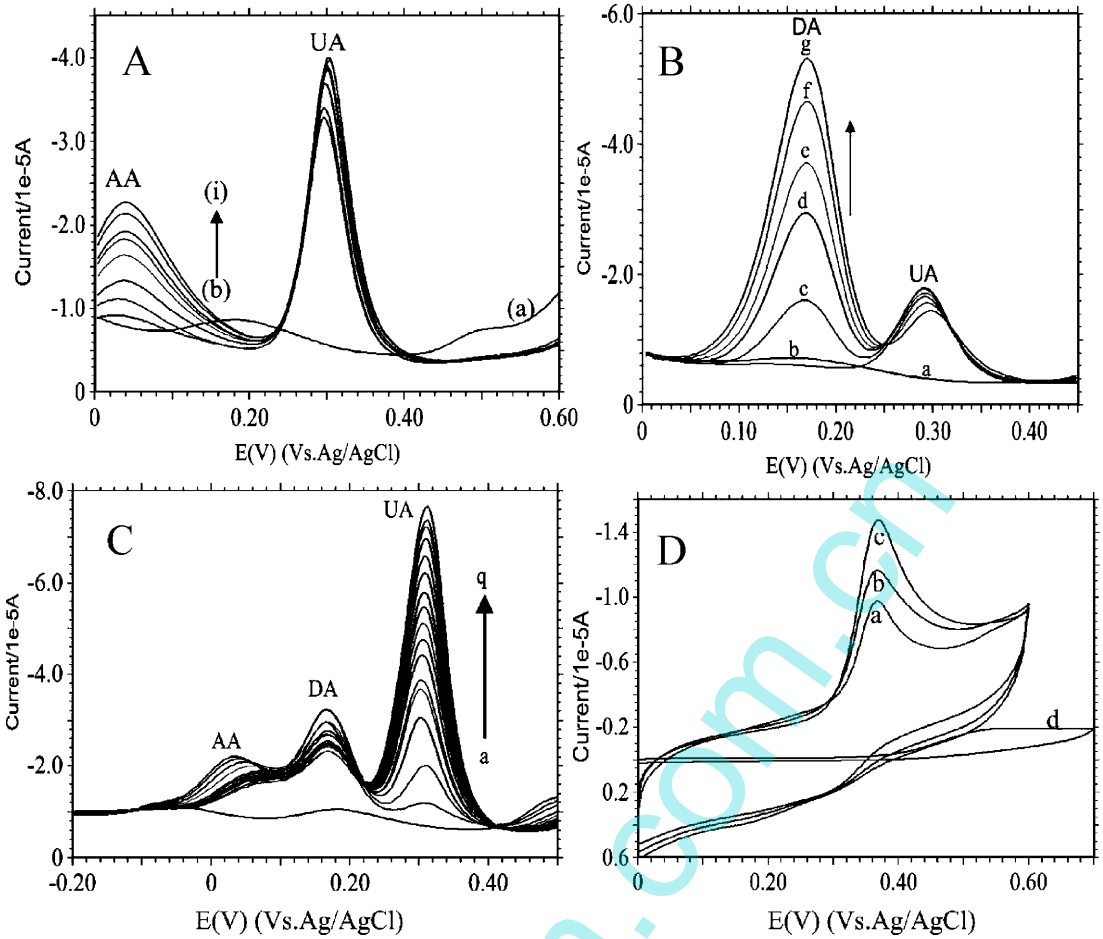

Fig. 3. (A) DPVs obtained for the determination of AA at fixed concentration of UA at poly(luminol)/GCE in 0.1 M PBS (pH 7.0). Concentration of AA: (a) 0.0, (b–i) 2.0×10^{-4}, 4.0×10^{-4}, 6.0×10^{-4}, 8.0×10^{-4}, 1.0×10^{-3}, 1.2×10^{-3}, 1.4×10^{-3}, and 1.6×10^{-3} M. Concentration of UA: (a) 0.0 M and (b–i) 1.0×10^{-3} M. (B) DPVs obtained for the determination of DA at fixed concentration of UA at poly(luminol)/GCE in 0.1 M PBS (pH 7.0). Concentration of DA: (a and b) 0.0, (c) 1.0×10^{-4}, (d) 2.0×10^{-4}, (e) 3.0×10^{-4}, (f) 4.0×10^{-4}, and (g) 1.0×10^{-3} M. Concentration of UA: (a) 0.0 M and (b) 0.5×10^{-3} M. (C) DPVs obtained for the determination of UA at fixed concentration of 1×10^{-3} M AA and 1×10^{-3} M DA using a poly(luminol)/GCE in 0.1 M PBS (pH 7.0). Concentration of UA: (a–q) 0.0, 3.0×10^{-5}, 1.3×10^{-4}, 2.0×10^{-4}, 2.7×10^{-4}, 3.3×10^{-4}, 4.0×10^{-4}, 4.7×10^{-4}, 5.3×10^{-3}, 6.0×10^{-3}, 6.7×10^{-3}, 7.30×10^{-3}, 8.0×10^{-3}, 8.7×10^{-3}, 9.0×10^{-3}, 9.3×10^{-3}, and 1.0×10^{-2} M. Amplitude: 0.05 V; pulse width: 0.05 s; pulse period: 0.2 s. (D) CVs recorded using a poly(luminol)/GCE in PBS (pH 7.0) with (a) urine sample, (b) urine sample +100 μM UA, and (c) urine sample +300 μM UA. CVs of a pretreated GCE in urine sample +300 μM UA (curve d). Scan rate: 40 mV s^{-1}.

current response to UA was no apparent decrease in the first continuous 3 weeks by every day use and only about 10% decrease in response current occurred after 6 weeks. This level of stability is acceptable for most practical applications.

The real applicability of the poly(luminol) modified electrode was tested by measuring the concentrations of UA in human urine samples collected from a normal man in our laboratory and were diluted by a factor of 100 with PBS (pH 7.0) without any further treatment. The CVs obtained for the urine sample in PBS are shown in Figure 3D, curves a–c. This shows an oxidation peak at 0.366 V, which was assigned to the oxidation of UA. To check out the observed oxidation peak at 0.366 V was UA, the sample was spiked with a specific amounts of standard UA solution and the corresponding CVs are shown in Figure 3D, curves b and c. The obvious increase in the peak current of UA after the addition of UA to the urine sample clearly shows that the observed peak in the human urine sample corresponded to the oxidation of UA. We also obtained CVs for urine sample using unmodified electrode, (see Fig. 3D, curve d), the oxidation peak observed at 0.564 and very low current was observed compared to the modified electrode. This depicts the real advantage of modified electrode for detection of UA in real samples. In addition, we determined UA in human urine samples after known amount of UA spiked in it (Table 1). The recoveries from spiked samples were determined and these ranged between 102.44% and 97.44% for UA.

This study has shown that the poly(luminol) modified GCE exhibits strong electrocatalytic activity towards the oxidation of UA, and that it can separate out the oxidation peaks from AA and DA sufficiently to be able to determine

Table 1. Determination of UA in urine samples.

Samples	UA found (mM)	Spiked (mM)	Found (mM)	Recovery [a] (%)
Urine 1	0.31	0.1	0.42	102.44
Urine 2	0.29	0.1	0.38	97.44

[a] average of three measurements

them selectively. The modified electrode was sensitive and stable, quick to respond, and good at resisting interferences from potentially interfering compounds in the simultaneous determination of DA, AA and UA. Simple fabrication procedure and low cost are the advantages of the proposed electrochemical sensor and these properties make the poly(luminol) modified electrode as an excellent option for determination of UA in real samples.

Experimental

All chemicals were of analytical reagent grade unless otherwise specified. Luminol was purchased from Aldrich (Milwaukee, WI, USA) and used as received. Dopamine hydrochloride and UA were purchased from Sigma – Aldrich (St. Louis, MO, USA). AA was received from E-Merck (Darmstadt, Germany) and used without further purification. Water was obtained from a Millipore Alpha-Q Lotun ultrapure water system. Solutions and buffers were prepared employing standard laboratory procedures. Before each experiment the solutions were deoxygenated by purging with pre-purified nitrogen gas.

Electrochemical measurements were performed with CH Instruments (TX, USA) Model-400 potentiostat with conventional three-electrode cell. GCE (Purchased from Bioanalytical systems) or GCE coated with poly(luminol) and platinum wire are used as the working electrode and auxiliary electrode, respectively. All the cell potentials were measured with respect to an Ag/AgCl [KCl (sat)] reference electrode. Hitachi scientific instruments (London, UK) Model S-3000H SEM was used for surface image measurements. The AFM images were recorded with a multimode scanning probe microscope system (CSPM4000 Instruments, Ben Yuan Ltd, China).

Prior to use, the working electrode was mechanically polished with alumina powder (Al_2O_3, 0.05 micron) up to a mirror finish. Then the electrode was cycled in 0.1 M sulfuric acid in a potential range from – 0.1 to 1.0 V at a sweep rate of 100 mVs^{-1} until a stable voltammogram obtained (Electrode pretreatment). The electrochemical deposition of poly(luminol) film was carried out by CV (between 0.0 and 1.0 V at 50 mVs^{-1}) for 10 cycles. The electrolyte consisted of 5×10^{-4} M luminol monomer in aqueous solution of 0.1 M H_2SO_4. The resulting polymer film was washed with doubly distilled deionized water before proceed electrochemical measurements. A pretreated GCE was used for comparative studies.

Acknowledgements

The authors wish to express their gratitude and appreciation for the financial support from The Ministry of Education and the National Science Council of Taiwan, Republic of China.

References

[1] S.-H. Huang, Y.-C. Shih, C.-Y. Wu, C.-J. Yuan, Y.-S. Yang, Y.-K. Li, T.-K. Wu, *Biosens. Bioelectron.* **2004**, *19*, 1627.
[2] Y.-Q. Zhang, W.-D. Shen, R.-A. Gu, J. Zhu, R.-Y. Xue, *Anal. Chim. Acta* **1998**, *369*, 123.
[3] C. R. Raj, T. Ohsaka, *J. Electroanal. Chem.* **2003**, *540*, 69.
[4] R. G. Compton, M. E. Laing, A. Ledwith, I. I. Abu-Abdoun, *J. Appl. Electrochem.* **1988**, *18*, 431.
[5] B. Šljukić, C. E. Banks, C. Salter, A. Crossley, R. G. Compton, *Analyst* **2006**, *131*, 670.
[6] A. Malinauskas, R. Garjonyte, R. Mažeikiene, I. Jurevičiute, Talanta **2004**, *64*, 121.
[7] S. A. Kumar, S.-M. Chen, *Sensors* **2008**, *8*, 739.
[8] Md. A. Rahman, P. Kumar, D.-S. Park, Y.-B. Shim, Sensors **2008**, *8*, 118.
[9] P. Ramesh, S. Sampath, *Electroanalysis* **2004**, *16*, 866.
[10] Y. Zhang, Y. Pan, S. Su, L. Zhang, S. Li, M. Shao, *Electroanalysis* **2007**, *19*, 1695.
[11] R. T. Kachoosangi, C. E. Banks, R. G. Compton, *Electroanalysis* **2006**, *18*, 741.
[12] Y.-C. Luo, J.-S. Do, C.-C. Liu, *Biosens. Bioelectron.* **2006**, *22*, 482.
[13] G. Wang, J. Meng, H. Liu, S. Jiao, W. Zhang, D. Chen, B. Fang, *Electrochim. Acta* **2008**, *53*, 2837.
[14] L. Zhang, C. Zhang, J. Lian, *Biosens. Bioelectron.* **2008**, *24*, 690.
[15] Y. Zeng, J. Xu, K. Wu, *Microchim Acta* **2008**, *161*, 249.
[16] S. Hasoň, V. Vetterl, F. Jelen, M. Fojta, *Electrochim. Acta* **2009**, *54*, 1864.
[17] A. W. Knight, TrAC – Trends Anal. Chem. **1999**, 18, 47.
[18] M. M. Richter, *Chem. Rev.* **2004**, *104*, 3003.
[19] Y.-P. Dong, H. Cui, C.-M. Wang, *J. Phys. Chem. B* **2006**, *110*, 18408.
[20] C.-M. Wang, H. Cui, *Luminescence* **2007**, *22,* 35.
[21] G.-F. Zhang, H.-Y. Chen, *Anal. Chim. Acta* **2000**, *419*, 25.
[22] K.-C. Lin, S.-M. Chen, *J. Electroanal. Chem.* **2006**, *589*, 52.
[23] A. Sassolas, L. J. Blum, B. D. Leca-Bouvier, *Anal. Bioanal. Chem.* **2009**, *394*, 971.
[24] S. A. Kumar, H.-W. Cheng, S.-M. Chen, *React. Funct. Polym.* **2009**, *69*, 364.
[25] S.-M. Chen, K.-C. Lin, *J. Electroanal. Chem.* **2002**, *523*, 93.
[26] V. Ferreira, A. C. Cascalheira, L. M. Abrantes, *Electrochim. Acta* **2008**, *53*, 3803.
[27] F. Fusalba, P. Goúerec, D. Villers, D. Bélanger, *J. Electrochem. Soc.* **2001**, *148*, A1.
[28] R. W. Murray, *Electroanalytical Chemistry* (Ed: A. J. Bard) Marcel Dekker, New York **1984**.

[29] N. de-los-Santos-Alvarez, P. de-los-Santos-Alvarez, M. J. Lobo-Castañòn, A. J. Miranda-Ordieres, P. Tuñòn-Blanco, *Anal. Chem.* **2005**, *77*, 4286.
[30] A. P. Brown, F. C. Anson, *Anal. Chem.* **1977**, *49*, 1589.
[31] P. R. Roy, T. Okajima, T. Ohsaka, *J. Electroanal. Chem.* **2004**, *561*, 75.
[32] C. R. Martin, L. S. Van Dyke, *Mass and Charge Transport in Electronically Conductive Polymers, in Molecular Design of Electrode Surfaces* (Ed: R. W. Murray), Wiley, New York **1992**, pp. 403 – 424.
[33] S. S. Kumar, J. Mathiyarasu, K. L. Phani, Y. K. Jain, V. Yegnaraman, *Electroanalysis* **2005**, *17*, 2281.
[34] C.-F. Tang, S. A. Kumar, S.-M. Chen, *Anal. Biochem.* **2008**, *380*, 174.

