Full Paper

Zinc Oxide/Zinc Hexacyanoferrate Hybrid Film-Modified Electrodes for Guanine Detection

Hung-Wei Chu, *R. Thangamuthu*, *Shen-Ming Chen**

Department of Chemical Engineering and Biotechnology, National Taipei University of Technology, No. 1, Section 3, Chung-Hsiao East Road, Taipei, Taiwan 106
*e-mail: smchen78@ms15.hinet.net

Abstract
An electroactive polynuclear hybrid films of zinc oxide and zinc hexacyanoferrate (ZnO/ZnHCF) have been deposited on electrode surfaces from H_2SO_4 solution containing $Zn(NO_3)_2$ and $K_3[Fe(CN)_6]$ by repetitive potential cycling method. Simultaneous cyclic voltammetry and electrochemical quartz crystal microbalance (EQCM) measurements demonstrate the steady growth of hybrid film. There are two redox couples present in the voltammograms of hybrid film and it is obvious in the case of pH 2. Surface morphology of hybrid film was investigated using scanning electron microscopy (SEM) and atomic force microscopy (AFM). Energy dispersive spectrometer (EDS) data confirm existence of zinc oxide in the hybrid film. The effect of type of monovalent cations on the redox behavior of resulting film was investigated. In pure supporting electrolyte, electrochemical responses of modified electrode resemble with that of a surface immobilized redox couple. The electrocatalytic activity of ZnO/ZnHCF hybrid film was investigated towards guanine using cyclic voltammetry and rotating disc electrode (RDE) techniques. Finally, feasibility of using ZnO/ZnHCF hybrid film-coated electrodes for guanine estimation in flow injection analysis (FIA) was also investigated.

Keywords: Modified electrodes, Hybrid film, Electrocatalysis, Guanine, RDE, FIA

DOI: 10.1002/elan.200703966

1. Introduction

Guanine (2-amino-6-oxypurine) is one of the two principal purines found in nucleic acids, and as such is involved in many biological processes [1, 2]. For example, guanine is believed to play a key role in the oxidation of DNA by various types of oxidants and free radical. Thus, oxidative electrochemistry of guanine appears to be of particular interest to compare with biological pathways. In addition, determining individual concentrations of guanine in DNA is important for the measurement of nucleic acid concentration itself. Therefore, considerable efforts have been focused recently on the development of electrochemical methods for the detection of nucleic acid in biological samples based on quinine oxidation [3 – 5].

It is generally believed that direct oxidation of guanine at bare electrodes is totally irreversible and therefore require a high overpotential [4, 5]. Also, direct oxidation of guanine often suffers from pronounced surface fouling, which results in poor reproducibility. The high overpotential and poor reproducibility usually limit the use of the bare electrodes for direct detection of guanine in biological matrices [6, 7]. Different approaches have been proposed to overcome these problems, which include electrochemical pretreatment to glassy carbon electrode [8] and use of sonovoltammetry [9]. As another strategy, various electrocatalytic approaches were adopted to enhance the electrode kinetics

of guanine oxidation. For example, Thorp proposed a few homogeneous electrocatalytic schemes for the detection of nucleic acids using the catalytic nature of $Ru(bpy)_3Cl_2$ toward oxidation of guanine in nucleic acids [10]. Similarly, Rusling and co-workers demonstrated that there is a direct correlation between the catalytic current of guanine oxidation by ruthenium-bipyridine complexes and the degree of DNA damage [11 – 14]. Zen et al. [15] have applied Nafion-ruthenium oxide pyrochlor-modified electrode for the simultaneous determination of guanine, adenine contents in DNA and RNA and synthetic oligonucleotides. Recently, carbon paste electrodes modified with cobalt phthalocyanine [16], cobalt hexacyanoferrate [17] and sodium montmorillonite clay [18] were used for the electrocatalytic detection of guanine. Another example is the modification of carbon electrodes with carbon nanotubes [19, 20]. The resulted electrodes lower the overpotential and enhance the sensitivity as well as reproducibility. However, one serious problem associated with the above-mentioned modified electrodes is that the operating potential is relatively high. In a recent investigation, redox polymer-modified indium tin oxide has been demonstrated for the detection of guanine at lowest oxidation potential ever reported [21].

Metal hexacyanoferrates have attracted considerable attention as excellent surface modifier ever since the first report by Neff [22] on deposition of a thin film of Prussian blue (PB) on an electrode surface. The electrode surfaces

can be modified with metal hexacyanoferrate by different ways such as electrodeposition, adsorption, entrapping into a polymer matrix, electroless deposition, mechanically attaching the insoluble metal-hexacyanoferrate or by the reaction between metal hexacyanoferrate with self-assembled monolayer of an organosulfur compound on a gold electrode [23–28]. The metal hexacyanoferrate-modified electrodes have been widely used in electrochemistry to electrocatalyze the oxidation of dopamine [29], ascorbic acid [30], NADH [31], glutathione [32, 33], cysteine [32], guanine and single-strand DNA (ss-DNA) [17] and H_2O_2 [34] and the reduction of Fe^{3+} [35], and hydrazine [36]. When these inorganic complexes combine with glucose oxidase, metal hexacyanoferrate-modified electrodes can also be applied as a biosensor for glucose [37].

Among the transition metal hexacyanoferrates, zinc hexacyanoferrate (ZnHCF) has not received much attention. For the first time, Joseph et al. [38] have reported the formation of ZnHCF film on a carbon electrode. They have found that modification of ZnHCF is different from other PB analogues, as it can be formed only in a certain ratio of modification species. Out of this ratio, electrocrystallization phenomena are observed instead of stable surface modification. Eftekhari [39] has chemically deposited a thin solid film of ZnHCF onto zinc electrode and the electrocatalytic properties of the modified electrode for oxidation of thiosulfate were also investigated. Recently, ZnHCF film has been used as an effective protecting layer to prevent the anodic dissolution of zinc substrate [40].

In the present paper, we are reporting the modification of GC electrode with a thin film of ZnO/ZnHCF. The modified electrode was characterized using different techniques. Finally, the electrocatalytic behavior of ZnHCF-modified electrode was investigated towards oxidation of guanine. To evaluate the utility of present modified electrodes for analytical application, they have been used for guanine determination in flow injection analysis (FIA).

2. Experimental

All the chemicals used were of analytical grade and used without further purification. Aqueous solutions of pH 1 to 3 were prepared from H_2SO_4. The aqueous solutions were prepared using doubly distilled deionized water and then deaerated by purging with high purity nitrogen gas for about 20 min before performing electrochemical experiments. Also, a continuous flow of nitrogen over the aqueous solution was maintained during measurements.

The electrochemical experiments were carried out with a CH Instruments (Model CHI-400) using CHI-750 potentiostat. Cyclic voltammograms were recorded in a three-electrode cell configuration, in which a BAS glassy carbon electrode (area = 0.07 cm^2) was used as working electrode. The auxiliary compartment contained a platinum wire that was separated by a medium-sized glass frit. Cell potentials were recorded using an Ag | AgCl (sat KCl) electrode. The working electrode for electrochemical quartz crystal micro-

balance (EQCM) measurements was an 8 MHz AT-cut quartz crystal coated with gold. RDE experiments were performed using PINE Instrument (USA) in conjunction with CHI-750 potentiostat. HITACHI Model S-3000H scanning electron microscope was used for surface analysis of the modified electrodes. CSPM 4000 (Ben Yuan Ltd, China) scanning Probe Microscope was used to record AFM images in tapping mode. Flow injection analysis system consisted of a carrier reservoir, a Cole Parmer Masterflex microprocessor pump drive, a Rehodyne 7125 sample injection valve (20-μL loop), interconnecting Teflon tubing, and a BAS CC-5 type electrochemical injector (West Lafayette, IN). The flow injection analysis using H_2SO_4 (pH 2) as a mobile phase was carried out at an applied potential of $+1.1$ V (vs. Ag/AgCl). The flow rate was 1.2 mL min^{-1}. The solutions of increasing concentration of guanine, prepared in the same medium (H_2SO_4), were injected successively. A series of measurements were performed after allowing the transient current to decay to steady state before analysis. Note that screen-printed electrode (SPE) with a working area of 0.196 cm^{-2} and a conductive track radius of 2.5 mm, purchased from Zensor R&D (Taichung, Taiwan), was used as working electrode in FIA. The SPE was modified with ZnO/ZnHCF hybrid film by adopting similar procedure used to modify glassy carbon electrode (GCE). For comparison, FIA measurements were also carried out with unmodified SPE.

Prior to film deposition, the GCE was polished with 0.05 μm alumina on Buehler felt pads and then ultrasonically cleaned for about a minute in water. Finally, the electrode was washed thoroughly with double distilled water and used. The electrochemical deposition of zinc oxide/zinc hexacyanoferrate films was accomplished by potentiodynamic cycling of the working electrode between preset potential range in a suitable aqueous solution containing Zn^{2+} and $Fe(CN)_6^{3-}$ ions. After film formation, the electrode was rinsed with distilled water and used for further characterization.

3. Results and Discussion

3.1. Preparation of Zinc Oxide/Zinc Hexacyanoferrate Hybrid Film-Modified Electrodes

Figure 1A – C illustrates repetitive cyclic voltammograms recorded during electrodeposition of zinc oxide/zinc hexacyanoferrate (ZnO/ZnHCF) hybrid film at pH 1, 2 and 3, respectively. The film was deposited on GC electrode from H_2SO_4 solution containing $Zn(NO_3)_2$ and $K_3[Fe(CN)_6]$. The progressive increase of both anodic and cathodic peak currents demonstrates continuous deposition of hybrid film. Two redox couples representing ZnHCF and ZnO could be noticed, however, the second redox couple could be seen clearly in pH 2 compared to pH 1 and 3. Among these two, the redox transition occurring at more positive potential represents the ZnHCF while the redox peaks of ZnO appeared at less positive region. ZnO is deposited during the
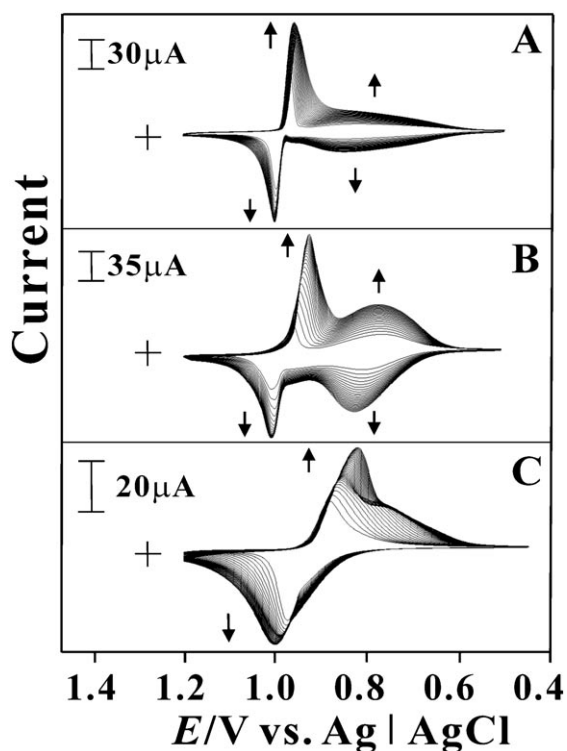
Fig. 1. Consecutive cyclic voltammograms of ZnO/ZnHCF hybrid film growth on GCE from aqueous solution of different pH containing 1.0×10^{-4} M $Zn(NO_3)_2$ and 1.0×10^{-3} M $Fe(CN)_6^{3-}$. A) pH 1, B) pH 2, and C) pH 3. Scan rate $= 0.1$ V s^{-1}.

potentiodynamic cycling due to base generation by the reduction of nitrate [41] (i.e. the reduction reaction causes an increase in the pH of the electrolyte close to the electrode [42]). The electrochemical reaction relevant to growth of ZnO film can be written as

$$Zn^{2+} + NO_3^- + 2e^- \rightarrow ZnO + NO_2^- \qquad (1)$$

In order to evaluate the composition of the coating, EDS measurement was carried out at a number of locations throughout the coatings, and the representative EDS pattern data were shown in Figure 2. The EDS results demonstrate the existence of ZnO and ZnHCF in the hybrid coating.

3.2. In Situ EQCM Study of ZnO/ZnHCF Hybrid Film Growth

In order to study the growth of ZnO/ZnHCF hybrid film, we have performed simultaneous voltammetric and microgravimetric experiments. Simultaneous cyclic voltammetry and electrochemical quartz crystal microbalance (EQCM) measurements permit continuous monitoring of surface mass changes without disturbing experimental conditions. Figure 3A and B show cyclic voltammograms and simultaneously recorded microgravimetric spectrograms of ZnO/ZnHCF hybrid film on gold electrode prepared from H_2SO_4
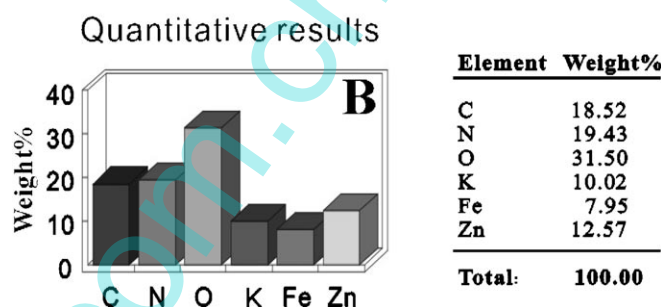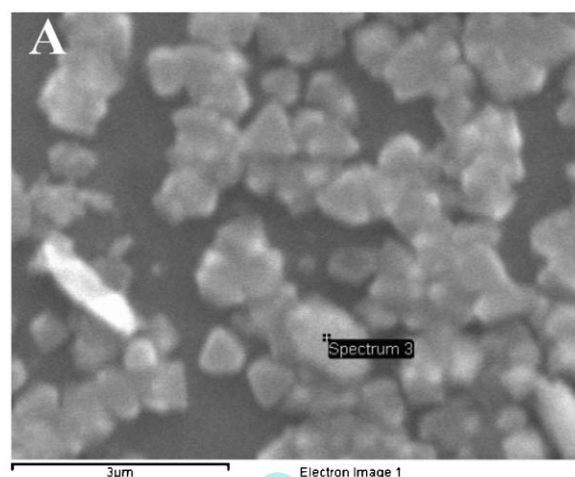

Fig. 2. SEM photograph and EDS data of electrodeposited thin films of ZnO/ZnHCF hybrid film on ITO electrode. The film was deposited from the aqueous solution containing 1.0×10^{-4} M $Zn(NO_3)_2$ and 1.0×10^{-3} M $Fe(CN)_6^{3-}$ (pH 2).
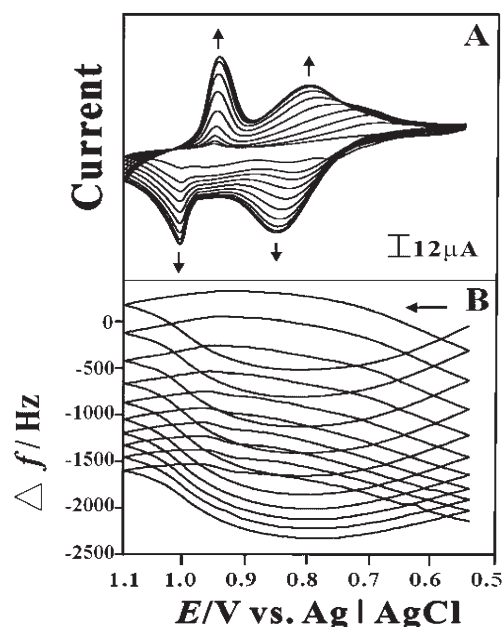

Fig. 3. A) Repeated cyclic voltammograms of ZnO/ZnHCF hybrid film growth on a gold electrode from the aqueous solution containing 1.0×10^{-4} M $Zn(NO_3)_2$ and 1.0×10^{-3} M $Fe(CN)_6^{3-}$ (pH 2). Scan rate $= 0.02$ V s^{-1}. B) Change in the EQCM frequency recorded concurrently with the first ten repeated CVs between the potentials of 0.5 and 1.2 V.

solution (pH 2) containing 1×10^{-4} M $Zn(NO_3)_2$ and 1×10^{-4} M $Fe(CN)_6^{3-}$. It is obvious that peak current in cyclic voltammograms increases with scanning number, while frequency decreases during microgravimetric measurement. According to Sauerbrey equation [43], frequency drop corresponds to mass gain. The results demonstrate that ZnO/ZnHCF hybrid film grows steadily with time.

In the EQCM experiments, mass change (Δm) at quartz crystal was calculated from the change in frequency using Sauerbrey equation [43]

$$\Delta m = (-1/2)(f_o^{-2})(\Delta f)\ A\ (k\varrho)^{1/2} \qquad (2)$$

Where, A is the area of the gold disk coated onto the quartz crystal, ϱ is the density of the crystal, k is the shear modulus of the crystal, Δf is the measured frequency change, and f_o is the oscillation frequency of the crystal. A frequency change of 1 Hz is equivalent to 1.4 ng change in mass. In the present study, about 17857 ng cm^{-2} of ZnO/ZnHCF film was deposited on gold electrode after ten cyclic voltammetric scans. Similarly, the observed mass change during ZnO/ZnHCF film deposition under pH 1 and 3 (not shown) were 19764 and 18571 ng cm^{-2}, respectively.

3.3. Surface Characterization of ZnO/ZnHCF Hybrid Films

The morphology and structure of ZnO/ZnHCF hybrid films were examined with atomic force microscopy (AFM) and scanning electron microscopy (SEM). Figure 4 displays the typical AFM image of ZnO/ZnHCF hybrid films prepared on ITO electrode from pH 2. It can be seen that ZnO/ZnHCF hybrid coating was uniform. SEM image presented in Figure 2 also confirm this conclusion.

3.4. The Electrochemical Characteristics of ZnO/ZnHCF Hybrid Film-Modified Electrodes

The formation of ZnO/ZnHCF hybrid film on the glassy carbon electrode surface was also verified by immersing the modified electrode in H_2SO_4 supporting electrolyte of pH 1, 2 and 3 and performing repetitive potentiodynamic cycling between the pre-set potential limits. Figure 5A–C show the cyclic voltammetric responses of ZnO/ZnHCF hybrid film-modified electrodes at different scan rates in H_2SO_4 solution of pH 1, 2 and 3, respectively. The voltammetric traces confirm the formation of ZnO/ZnHCF hybrid film. Both anodic and cathodic currents of the first redox couple increase linearly with scan rates up to 200 mV s^{-1} as shown in the insets of Figure 5. This is an indication that the electron transfer process involves a surface confined species. However, the ratio of anodic to cathodic peak currents is not exactly unity and the peak-to-peak separation (ΔE_P) is not zero. For an ideal surface confined redox species, the peak separation should be zero and current ratio is expected to be unity [44].
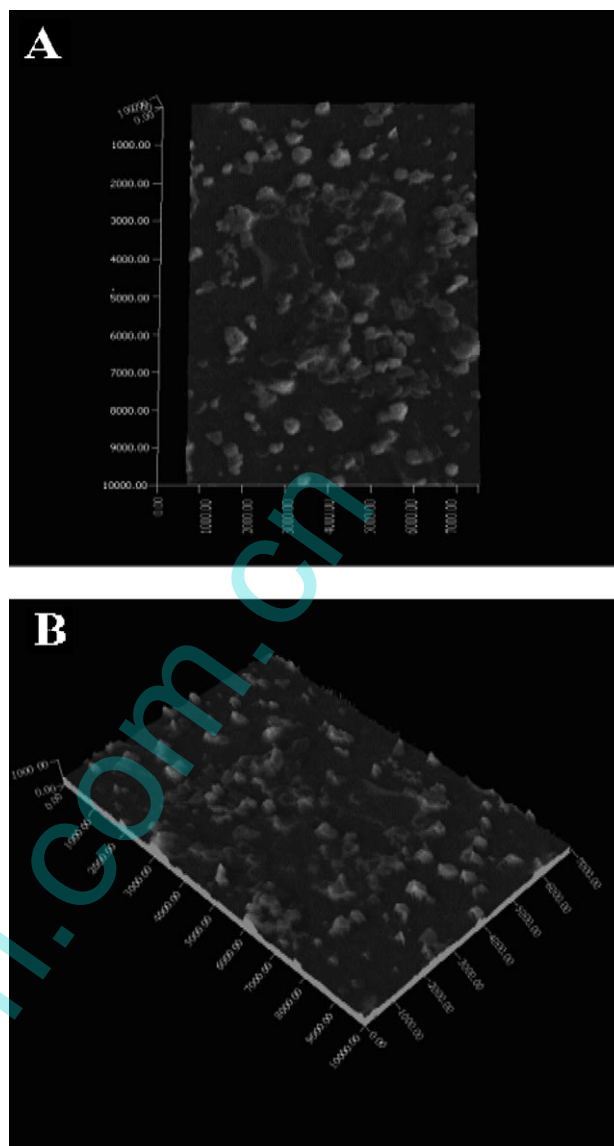
Fig. 4. AFM image of electrodeposited thin films of ZnO/ZnHCF hybrid film on ITO electrodes. The film was deposited from the aqueous solution containing 1.0×10^{-4} M $Zn(NO_3)_2$ and 1.0×10^{-3} M $Fe(CN)_6^{3-}$ (pH 2).

The electrochemical response of ZnO/ZnHCF hybrid film-coated electrodes in supporting electrolyte (H_2SO_4) of different pH values was investigated using cyclic voltammetric technique. As shown in Figure 6A, our results exhibited pH dependent voltammetric peak potentials, i.e. the anodic and cathodic peak potentials of the modified electrode were shifted to a less positive region with increasing pH of supporting electrolyte. In addition, the peak currents also decreased. The formal potential ($E^{\circ\prime}$) of the ZnO/ZnHCF hybrid film was evaluated as the mean of the anodic and cathodic peak potentials [45] of the cyclic voltammograms recorded at various pH values. The $E^{\circ\prime}$ vs. pH plot yields straight line with a slope of 33 mV per unit change in pH of supporting electrolyte as shown in the inset
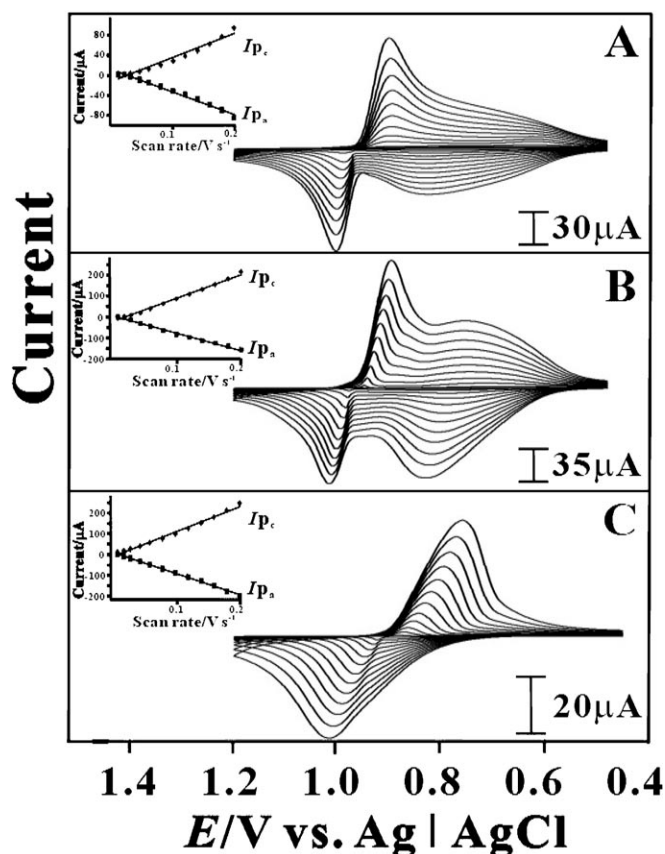
Fig. 5. Cyclic voltammetric responses of ZnO/ZnHCF hybrid film-modified GCE recorded at different scan rates (inner to outer voltammograms correspond to scan rates of 10, 20, 30, 45, 60, 80, 100, 120, 140, 160, 180 and 200 mV s^{-1}, respectively) in aqueous solution of different pH: A) pH 1, B) pH 2, and C) pH 3. The modified electrodes were prepared as in Figure 1. Insets show variation of anodic and cathodic peak currents with scan rate.

of Figure 6A. It suggests the involvement of proton in the overall redox reaction of the film.

In order to assess cation effect on the cyclic voltammetric response of ZnO/ZnHCF hybrid film-coated electrode, voltammograms were recorded in 0.1M solutions of Li$_2$SO$_4$, Na$_2$SO$_4$, and K$_2$SO$_4$ and the results are shown in Figure 6B. Note that pH of all the solutions are maintained at 2 with sulfuric acid. It is obvious that the features of the voltammograms are strongly affected by the nature of cations and follows the order K$^+$ > Na$^+$ > Li$^+$ as reported in the literature for ZnHCF film directly formed on Zn electrode [39]. The observed changes are attributed to the differences in the magnitude of the association constants of ion-pairs containing a cation and oxidized or reduced hexacyanometalate [46].

3.5. Electrocatalytic Behavior of ZnO/ZnHCF Hybrid Film towards Oxidation of Guanine

The electrocatalytic behavior of ZnO/ZnHCF hybrid film-modified electrode towards oxidation of guanine was
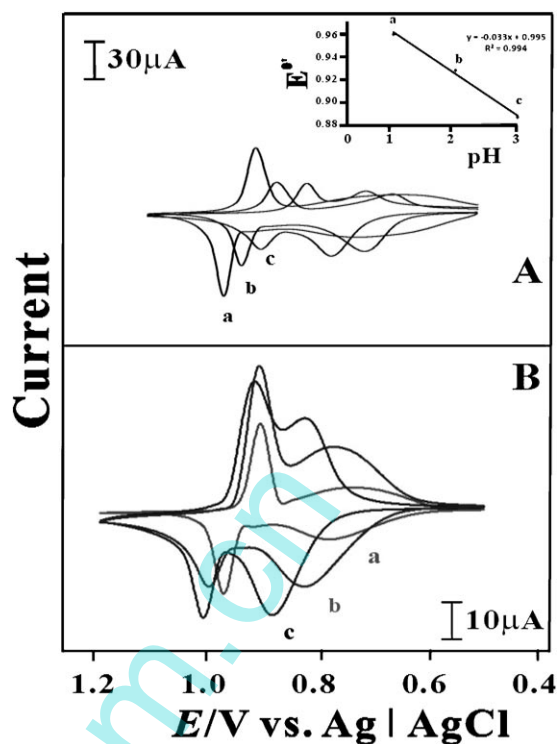

Fig. 6. A) Cyclic voltammetric responses of ZnO/ZnHCF hybrid film-modified electrode in aqueous solutions of different pH: a) pH 1, b) pH 2, c) pH 3. Inset shows variation of formal potential with solution pH. B) Cyclic voltammetric responses of ZnO/ZnHCF hybrid film-modified electrode in 0.1M solution of different cations (pH 2): a) Li$_2$SO$_4$, b) Na$_2$SO$_4$, c) K$_2$SO$_4$.

investigated using cyclic voltammetry and rotating disc electrode (RDE) techniques.

Figure 7 shows cyclic voltammetric responses of ZnO/ZnHCF hybrid film-modified electrode, in H$_2$SO$_4$ solution
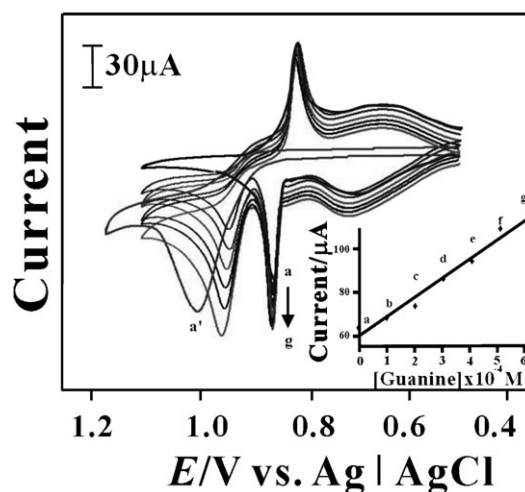

Fig. 7. Cyclic voltammograms of ZnO/ZnHCF hybrid film-modified electrode in H$_2$SO$_4$ (pH 2) aqueous solution containing different concentration of guanine. a) 0, b) 1 × 10^{-4}, c) 2 × 10^{-4}, d) 3 × 10^{-4}, e) 4 × 10^{-4}, f) 5 × 10^{-4}, and g) 6 × 10^{-4} M. a′ corresponds to the response of 6 × 10^{-4} M guanine on bare GCE. The inset shows a plot of oxidation current of guanine vs. its concentration.

(pH 2), in the absence (curve a) and presence of different concentration of guanine in the range of 1 to 6×10^{-4} M (curve b–g). Curve a' represents oxidation of 6×10^{-4} M guanine on bare GC electrode. It can be seen that guanine oxidized on unmodified GC electrode around 1.0 V. On the other hand, the anodic peak current of ZnO/ZnHCF hybrid film-modified electrode was found to increase in the presence of guanine, i.e. on ZnO/ZnHCF hybrid film-modified electrode guanine oxidizes at a less positive potential compared to that of bare GCE. When guanine was added successively, the anodic current increased while that of cathodic current decreased. Both, the decrease of oxidation potential and increase of oxidation current clearly indicate that ZnO/ZnHCF hybrid film can catalyze the oxidation of guanine. Note that apart from catalytic oxidation peak, second peak that was very close to the peak observed for the oxidation of guanine at unmodified electrode, and in a potential region where the modified electrode shows no electrochemical activity. These observations suggest that the first electrochemical process observed for the oxidation of guanine at ZnO/ZnHCF hybrid film-modified electrode corresponds to the mediated reaction. The second peak can be attributed to the direct oxidation of guanine at the underlying GC electrode surface. It is a clear indication that guanine has facile penetration through the ZnO/ZnHCF hybrid film as in the case of deoxyguanosine molecule through ruthenium oxide hexacyanoferrate (RuOHCF) film [47].

In order to investigate the electrocatalytic process in detail, the modified electrode was used in RDE voltammetric experiments. Figure 8A shows a set of current-potential curves of ZnO/ZnHCF hybrid film-coated electrode in H_2SO_4 solution (pH 2) containing different concentration of guanine. As shown in the inset, limiting current of guanine oxidation was increased linearly with guanine concentration. Figure 8B shows RDE voltammograms of ZnO/ZnHCF hybrid film-coated electrode in 5×10^{-4} M guanine at different rotation rate. The limiting current of guanine oxidation increases with increasing electrode rotation rate. As shown in the inset of Figure 8B, the Koutecky–Levich is linear with net intercept, which indicates kinetic limitation. The rate of catalytic reaction between oxidized form of catalyst and guanine can be estimated from the Koutecky–Levich plot using the following equation:

$$1/i_l = 1/I_{Lev} + 1/I_k \tag{3}$$

$$I_{Lev} = 0.62 \, n \, FA \, D^{2/3} \, \omega^{-1/2} \, v^{-1/6} \, c_0 \tag{4}$$

$$I_k = n \, F \, A \, k \, \Gamma \, c_0 \tag{5}$$

where c_0 is the bulk concentration of guanine, ω is the angular velocity of electrode, v is the kinematic viscosity, Γ surface coverage of the catalyst (6.5×10^{-10} mol cm^{-2}), k is the rate constant, and all other parameters have their usual meanings. The rate constant evaluated from the intercept of
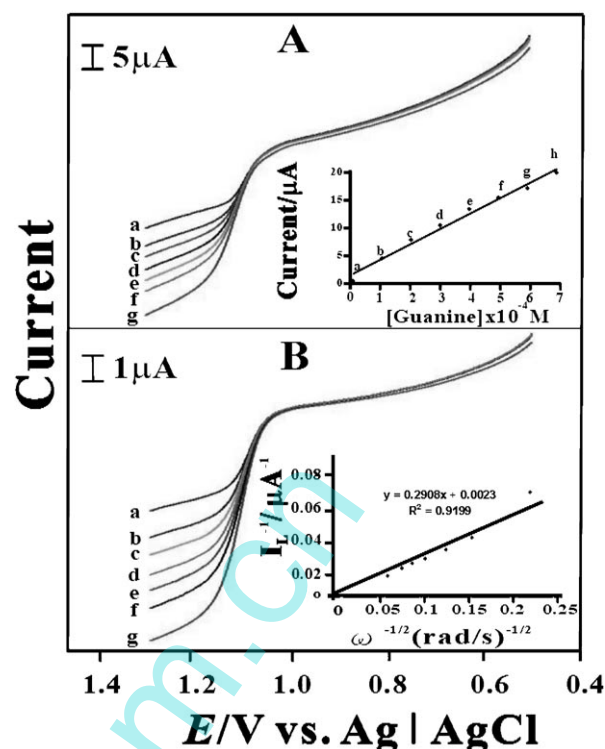

Fig. 8. A) RDE responses of ZnO/ZnHCF hybrid film-modified electrode in H_2SO_4 (pH 2) solution containing different concentration of guanine: a) 1×10^{-4}, b) 2×10^{-4}, c) 3×10^{-4}, d) 4×10^{-4}, e) 5×10^{-4}, f) 6×10^{-4}, and g) 7×10^{-4} M. Electrode was rotated at a speed of 200 rpm. Scan rate = 0.02 V s^{-1}. B) RDE responses of 5×10^{-4} M guanine in H_2SO_4 (pH 2) solution at ZnO/ZnHCF hybrid film-modified electrode. Electrode rotation speeds were a) 200; b) 400; c) 600; d) 900; e) 1200; f) 1600; and g) 2500 rpm. Inset shows Koutecky–Levich plot.

Koutecky–Levich plot was found to be 2.86×10^4 M^{-1} s^{-1} (by considering two-electron mechanism).

3.6. Analytical Application of ZnO/ZnHCF Hybrid Film-Modified Electrodes – Flow Injection Analysis

The ZnO/ZnHCF hybrid film-modified electrode was used for the determination of guanine by flow injection analysis. Figure 9A displays the FIA signals of guanine at an applied potential of 1.1 V by the injection of various concentration of guanine from 1 to 5×10^{-4} M into aqueous H_2SO_4 (pH 2) solutions. FIA responses increased rapidly just after injection of the sample and returned to baseline within about 5 s. Similar experiment was carried out with unmodified electrode, which resulted in poor response as shown in Figure 9B. The resulting calibration plots for the modified electrode (curve A') as well as that of unmodified electrode (curve B') were shown in the inset. The modified electrode displays a linear relationship between guanine oxidation current and concentration in the entire concentration range studied (0.1 – 0.5 mM), which is relative higher compared to the linear range obtained with {MeReO(edt)}$_2$-modified sol-gel screen-printed carbon electrode by differential pulse
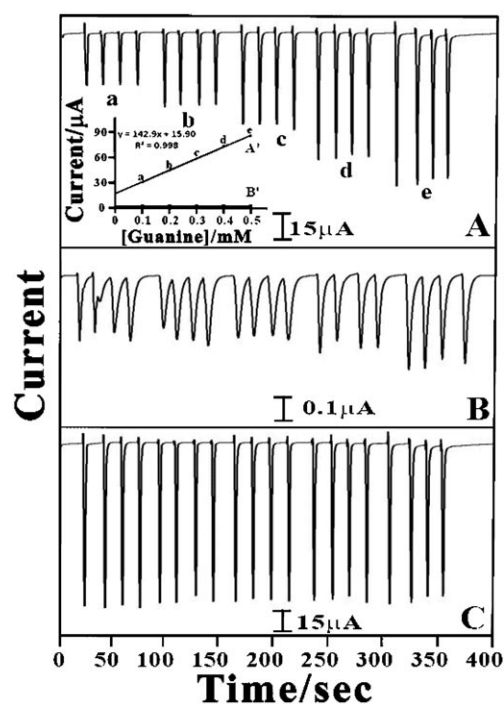
Fig. 9. FIA chronoamperogram for guanine oxidation at A) ZnO/ZnHCF hybrid film-modified SPE. B) Unmodified SPE as a function of guanine concentration: a) 1×10^{-4}, b) 2×10^{-4}, c) 3×10^{-4}, d) 4×10^{-4}, and e) 5×10^{-4} M. C) FIA chronoamperogram of ZnO/ZnHCF hybrid film-modified electrode for 17 successive additions. Each step represents 6×10^{-4} M guanine. Applied potential = 1.1 V (Ag/AgCl).

voltammetry [48]. The calibration plot yielded a sensitivity of 143 µA mM^{-1} with correlation coefficient of 0.998 ($n = 5$).

In order to evaluate the influence of foreign species on determination of guanine using ZnO/ZnHCF hybrid film-modified electrode, an indirect study was carried out. In which, electrocatalytic activity of present modified electrode was tested toward some biologically important compounds such as adenine, uric acid, dopamine, ascorbic acid, L-cystenine, and NADH. It was found that none of them oxidized at ZnO/ZnHCF hybrid film-modified electrode. Hence, present modified electrode can be used to estimate guanine in the presence of other compounds.

3.7. Stability, Reproducibility of ZnO/ZnHCF Hybrid Film-Modified Electrodes

An important requirement of modified electrode surfaces for analytical applications is its stability when in contact with the supporting electrolyte or sample. The stability of the ZnO/ZnHCF hybrid film-modified electrode was evaluated in pure supporting electrolyte by scanning the electrode potentiodynamically. The results indicated that ZnO/ZnHCF hybrid film-modified electrode was relatively stable as the peak current of the modified electrode remains nearly unchanged (not shown) for reasonable period of time.

The reproducibility of the ZnO/ZnHCF film-modified electrode response was investigated by measuring the current signal for repetitive injection of standard solution. As shown in Figure 9C, the reproducibility of the system was satisfactory for 17 continuous injections of 6×10^{-4} M guanine. The stability and the reproducibility of ZnO/ZnHCF film-modified electrode are as good as that of some of the modified electrodes reported in the literature for guanine determination [21, 48]. Thus, the proposed modified electrode promises for the development of an automated detection device for guanine.

4. Conclusions

A stable and electroactive hybrid film of zinc oxide and zinc hexacyanoferrate was successfully prepared on electrode surface by cycling repetitively in H$_2$SO$_4$ solution containing Zn(NO$_3$)$_2$ and K$_3$[Fe(CN)$_6$]. EQCM reveals steady growth of hybrid film with increasing scan number. EDS results on the composition of hybrid coating confirm the existence ZnO/ZnHCF. The surface analysis by SEM and AFM shows that the hybrid film was uniform. The electrochemical characterization of modified electrode in pure supporting electrolyte indicated that the redox process was confined to the surface, confirming the immobilized state of the redox species. The pH dependence redox response of modified electrode yields a slope of 33 mV per unit change in pH in the plot of $E^{\circ\prime}$ vs. pH. The electrocatalytic oxidation of guanine on ZnOZnHCF film-modified GC electrode was investigated using CV and RDE techniques. A rate constant of 2.86×10^4 M^{-1} s^{-1} was obtained from RDE analysis by considering two-electron mechanism. Finally, use of ZnOZnHCF film-modified electrodes was demonstrated by utilizing them in flow injection analysis.

5. Acknowledgement

6. References

[1] W. H. Brown, *Introduction to Organic and Biochemistry*, 4th ed., Brooks Cole, Monterey **1987**, p. 418.
[2] E. Palecek, *Top. Bioelectrochem. Bioenerg.* **1983**, *5*, 65.
[3] J. Wang, *Anal. Chim. Acta* **2002**, *469*, 63.
[4] H. H. Thorp, *Top. Curr. Chem.* **2004**, *237*, 159.
[5] E. Palecek, F. Jelen, *Crit. Rev. Anal. Chem.* **2002**, *3*, 261.
[6] J. Langmaier, Z. Samec, E. Samcova, P. Hobza, D. Reha, *J. Phys. Chem. B* **2004**, *108*, 15896.
[7] V. A. Szalai, H. H. Thorp, *J. Phys. Chem.* **2000**, *104*, 6851.
[8] H.-S. Wang, H.-X. Ju, H.-Y. Chen, *Anal. Chim. Acta* **2002**, *461*, 243.
[9] A. M. Oliveira Brett, F. M. Matysik, *Bioelectrochem. Bioenerg.* **1997**, *42*, 111.
[10] H. H. Thorp, *Top. Curr. Chem.* **2004**, *237*, 159.
[11] L. Zhou, J. F. Rusling, *Anal. Chem.* **2001**, *73*, 4780.

[12] A. Mugweru, J. F. Rusling, *Anal. Chem.* **2002**, *74*, 4044.

[13] B. Wang, J. F. Rusling, *Anal. Chem.* **2003**, *75*, 4229.

[14] L. Zhou, J. Yang, C. Estavillo, J. D. Stuart, J. B. Schenkman, J. F. Rusling, *J. Am. Chem. Soc.* **2003**, *125*, 1431.

[15] J.-M. Zen, M.-R. Chang, G. Ilangovan, *Analyst* **1999**, *124*, 679.

[16] A. Abbaspour, M. A. Mehrgardi, R. Kia, *J. Electroanal. Chem.* **2004**, *568*, 261.

[17] A. Abbaspour, M. A. Mehrgardi, *Anal. Chem.* **2004**, *76*, 5690.

[18] W. Huang, S. Zhang, Y. Wu, *Russ. J. Electrochem.* **2006**, *42*, 178.

[19] K. Wu, J. Fei, W. Bai, S. Hu, *Anal. Bioanal. Chem.* **2003**, *376*, 205.

[20] Z. Wang, S. Xiao, Y. Chen, *Electroanalysis* **2005**, *17*, 2057.

[21] Z. Gao, *Sens. Actuators B* **2007**, *123*, 293.

[22] V. D. Neff, *J. Electrochem. Soc.* **1978**, *125*, 886.

[23] Z. Gao, E. Wang, P. Li, Z. Zhao, *Electrochim. Acta* **1991**, *36,* 147.

[24] C. X. Cai, H. Y. Ju, H. Y. Chen, *J. Electroanal. Chem.* **1995**, *397,* 185.

[25] O. Ikeda, H. Yoneyama, *J. Electroanal. Chem.* **1989**, *265,* 323.

[26] M. H. Pournaghi-Azar, H. Razmi-Nerbin, *J. Electroanal. Chem.* **1998**, *456,* 83.

[27] S. Jayarama Reddy, A. Dostal, F. Scholz, *J. Electroanal. Chem.* **1996**, *403,* 209.

[28] J. J. Xu, C. Wang, H. Y. Chen, *Anal. Sci.* **2000**, *16,* 231.

[29] M. Jiang, X. Y. Zhou, Z. F. Zhao, *Ber. Bunsenges. Phys. Chem.* **1991**, *95*, 720.

[30] C. X. Cai, H. X. Ju, H. Y. Chen, *Anal. Chim. Acta* **1995**, *310*, 145.

[31] G. Y. Shi, J. X. Lu, F. Xu, W. L. Sun, L. T. Jin, K. Yamamoto, S. G.Tao, J. Y. Jin, *Anal. Chim. Acta* **1999**, *391*, 307.

[32] S. Zhang, W. L. Sun, W. Zhang, W. Y. Qi, L. T. Jin, K. Yamamoto, S. G. Tao, J. Y. Jin, *Anal. Chim. Acta* **1999**, *386*, 21.

[33] A. Eftekhari, *Microchim. Acta* **2003**, *141*, 15.

[34] B. D. Humphrey, S. J. Sinha, A. B. Bocarsly, *J. Phys. Chem.* **1987**, *91*, 586.

[35] K. Ogura, M. Nakayama, C. Kusumoto, *J. Electrochem. Soc.* **1996**, *143*, 3606.

[36] S. M. Golabi, F. Noor-Mohammadi, *J. Solid State Electrochem.* **1998**, *2*, 30.

[37] S. Milardovic, I. Kruhak, D. Ivekovic, V. Rumenjak, M. Tkalcec, B. S. Grabaric, *Anal. Chim. Acta* **1997**, *350*, 91.

[38] J. Joseph, H. Gomathi, G. P. Rao, *J. Electroanal. Chem.* **1997**, *431*, 231.

[39] A. Eftekhari, *J. Electroanal. Chem.* **2002**, *537*, 59.

[40] M. H. Pournaghi-Azar, H. Nahalparvari, *Electrochim. Acta* **2005**, *50*, 2107.

[41] T.Yoshida, K. Terada, D. Schlettwein, T. Oekermann, T. Sugiura, H. Minoura, *Adv. Mater.* **2000**, *12*, 1214.

[42] G. Helen Annal Therese, P. Vishnu Kamath, *Chem. Mater.* **2000**, *12*, 1195.

[43] G. Sauerbrey, *Z. Phys.* **1959**, *155*, 206.

[44] H. Jaegfeldt, A. B. C. Torstensson, L. G. O. Gorton, G. Johansson, *Anal. Chem.* **1981**, *53*, 1979.

[45] A. J. Bard, L. R. Faulkner, *Electrochemical Methods*, Wiley, New York **1980**, p 521.

[46] I. Markovich, D. Mandler, *J. Electroanal. Chem.* **2000**, *484*, 194.

[47] Thiago R. L. C. Paixao, M. Bertotti, *Electrochim Acta* **2007**, *52*, 2181.

[48] A. Abbaspour, L. Baramakeh, S. Masoud Nabavizadeh, *Electrochim. Acta* **2007**, *52*, 4798.