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Bioelectrochemistry 70 (2007) 452–461
Bioelectrochemistry
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Electrocatalytic properties of guanine, adenine, guanosine-5′-monophosphate, and ssDNA by Fe(II) bis(2,2′:6′,2″-terpyridine), Fe(II) tris(1,10-phenanthroline), and poly-Fe(II) tris(5-amino-1,10-phenanthroline)

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Received 12 January 2006; received in revised form 27 May 2006; accepted 7 July 2006
Available online 12 July 2006

Abstract

The electrocatalytic oxidations of guanine, adenine, guanosine-5′-monophosphate(GMP) and ssDNA were performed in the presence of Fe(II) bis (2,2′:6′,2″-terpyridine) and Fe(II) tris(1,10-phenanthroline) complexes as homogeneous catalysts by cyclic voltammetric methods. The Fe(II/III) redox couple of these compounds is responsible for their catalytic properties. The electrocatalytic oxidation current of above substrates were developed from the anodic peak currents of Fe(II) bis(2,2′:6′,2″-terpyridine) and Fe(II) tris(1,10-phenanthroline) complexes at about +0.93 V and 0.97 V, respectively. The electrocatalytic oxidative properties of guanine by Fe(II) bis(2,2′:6′,2″-terpyridine) complex was measured by amperometry method using the rotating disk electrodes. Electropolymerization of Fe(II) tris(5-amino-1,10-phenanthroline) complex produced thin polymer films on gold and glassy carbon electrodes. The electrochemical quartz crystal microbalance (EQCM) and cyclic voltammetry were used to study the *in situ* growth of the polymer. The poly(FeII(5-NH_2-1,10-phen)$_3$) exhibited a good electrocatalytic oxidation towards guanine and also for the mixture of guanine and adenine too.

Keywords: DNA; Guanine; Adenine; Film-modified electrodes; Electrocatalysis

1. Introduction

Guanine and adenine are important components found in deoxyribonucleic acid (DNA). The electrochemical oxidation mechanisms of guanine and adenine were investigated along with their relevance to oxidative degradation of nucleic acids in mutagenesis, carcinogenesis and on aging. Guanine (G) can be easily oxidized into nucleic acid bases, as indicated by the lowest values of one-electron redox potentials. Determination of individual concentrations of guanine and adenine or their ratio in DNA is important for the measurement of the nucleic acid concentration itself. Measurement of the electron-transfer of guanine in solution and the oxidation of the substitution guanosine (Gs) are important and helpful in understanding the oxidation processes of DNA [1–5]. Generally, carbon paste and glassy carbon electrodes are used in the determination of the guanine and adenine by electrochemical methods [6–9].

However, these methods suffered from serious problems like irreversible adsorption of purine bases on the electrode surface and led to surface fouling [10]. To overcome these problems, the DNA-modified electrodes have been widely used to determine guanine and adenine. Since the electrochemical signals for guanine and adenine on these modified electrodes were found poor, they have been used little for direct determination [11,12].

In our work, we have developed new homogenous and heterogeneous catalytic systems for guanine, adenine, guanosine-5′-monophosphate, and ssDNA. Metal 1,10-phenanthrolines show only one redox couple, for example, Fe(II) bis(2,2′:6′,2″-terpyridine) and Fe(II) tris(1,10-phenanthroline) are with the Fe (II/III) redox couple [13–18]. Chemically modified electrodes are useful in electroanalysis and electrocatalysis too [19–28]. They find uses in bioinorganic chemistry as chemicals and biosensors [29,30]. The electrochemical and electrocatalytic properties of metal 1,10-phenanthroline or their polymer-modified electrodes are of great interest. This catalyst system can be used to develop a system that performs the electrocatalytic processes and applied to the detection of substrates in a solution by amperometric method through the redox couples.

This paper reports about the electrocatalytic oxidation of guanine, adenine, guanosine, and ssDNA by the catalysts of Fe (II) bis (2,2′:6′,2″-terpyridine), Fe(II) tris(5-amino-1,10-phenanthroline)

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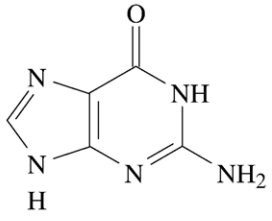
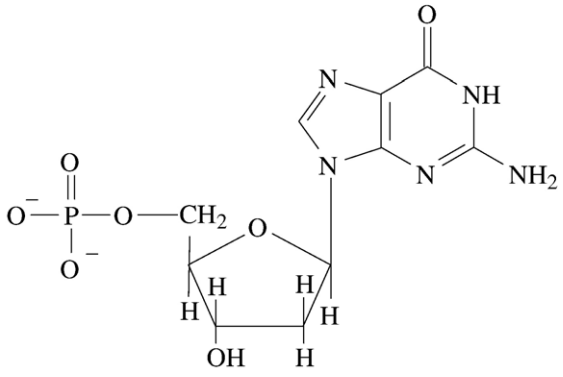
doi:10.1016/j.bioelechem.2006.07.004

complexes in solution and poly-Fe(II) tris(5-amino-1,10-phenanthroline) film-modified electrode. This paper also investigates the electrocatalytic system of Fe(II) complexes with various ligands 2,2′:6′,2″-terpyridine, and 1,10-phenanthroline in aqueous solution. The electrocatalytic oxidation of guanine was studied through $[Fe^{II}(terpy)_2]^{2+}$ by amperometry method. The electrocatalytic processes of Fe(II) complexes are discussed. The electropolymerization from the complex Fe(II) tris(5-amino-1,10-phenanthroline) was performed on various electrodes. The electrochemical quartz crystal microbalance (EQCM) and consecutive cyclic voltammetry were used to study the polymer film formation. There are a number of research programs that are going on to solve the key problems in DNA detection such as (1) covalent attachment of fluorescent, radioactive or redox-active labels to the target DNA, (2) multiplexing the detection system for the analysis of DNA and (3) achieving sufficient sensitivity to detect low concentration of DNA. The above mentioned key problems can be avoided through electrochemical detection method. Because of the recent success of electrochemical blood-glucose monitoring, there is a great enthusiasm for electrochemical detection and monitoring throughout the biotechnology industries [31]. The importance of our work is the electrocatalytic oxidation of the guanine, adenine, guanosine-5′-monophosphate and ssDNA were performed in acidic aqueous and acetonitrile solutions as well using iron complexes at low positive potential than that of earlier report. The in situ method of preparation of FeTerpy, Fe(1,10-phen), and Terpy complexes (Fe or Ru) have been reported by us first. Similarly, Fe^{2+} TerPy or 1,10-phen complexes were also prepared by in situ method. To the best our knowledge, there is no report dealing with the electrocatalytic properties of FeTerpy, Fe(1,10-phen) complex and its film towards guanine, guanosine, and ssDNA.



guanine



guanosine-5í-monophosphate2.

2. Experimental

All Fe(II) complex solutions were prepared by the reaction of ferrous chloride and ligand in a mole ratio of 1:3 (terpyridine 1:2) in aqueous solution. Fe(II) tris(5-amino-1,10-phenanthroline) was prepared by the reaction of ferrous chloride and ligand in a mole ratio of 1:3 in acetonitrile solution, and the solution was heated (at about 80 °C) for 15 min. The solution was, and then cooled to room temperature. Acetonitrile was removed *in vacuo* and the solid was then dissolved in 10 ml of water. Perchloric acid was added drop-by-drop to this solution and the resulting precipitate was filtered.

Guanine, adenine, and guanosine-5′-monophosphate(GMP) (Sigma, >99% purity) and calf thymus DNA were used as received without any further purification. Aqueous solutions were prepared with doubly distilled deionized water. Solutions were deoxygenated by purging with pre-purified nitrogen gas. Buffer solutions were prepared from H_2SO_4, potassium hydrogenphthalate (KHP), acetate, phosphate, borate, carbonate, and KOH in the pH range of 0–14.

The electrochemistry was performed using Bioanalytical Systems Model CV-50W and CH Instruments CHI-400 potentiostat. Cyclic voltammetry was conducted using a three-electrode cell in which a BAS glassy carbon electrode, a platinum electrode, and a tin dioxide electrode were used as working electrodes. The glassy carbon electrode was polished with 50-nm alumina on Buehler felt pads, and then ultrasonically cleaned for 1 min. The auxiliary compartment contained a platinum wire, which was separated by a medium-sized glass frit. All the cell potentials were taken using an Ag/AgCl/KCl (saturated solution) reference electrode.

The working electrode for the EQCM measurements was an 8-MHz AT-cut quartz crystal with gold electrodes. The diameter of the quartz crystal was 13.7 mm whereas for the gold electrode it was 5 mm. The UV–visible spectra were measured using a HITACHI Model U-3300 spectrophotometer.

The chronoamperometric and rotating ring-disk electrochemical (RRDE) experiments were performed with a Pine Instrument Co. electrode in conjunction with a CH Instruments CHI-750 potentiostat connected to a model AFMSRX analytical rotator. The RDE electrode purchased from Pine Instrument Co., consisted of a glassy carbon disk electrode.

3. Results and discussion

3.1. Electrocatalytic reaction involving guanine, guanosine, and ssDNA by Fe(II) bis(2,2′:6′,2″-terpyridine)

The electrochemical properties of Fe(II) bis(2,2′:6′,2″-terpyridine) complexes were investigated. Fig. 1 shows the reaction of Fe(II) ions with different concentrations of bis (2,2′:6′,2″-terpyridine) in a pH 4 buffer solution.

In the absence of above complexes, the cyclic voltammograms of Fe^{3+}/Fe^{2+} ions exhibited a cathodic and anodic peak at about +0.25 V and +0.80 V respectively (shown in Fig. 1A(a)). When bis(2,2′:6′,2″-terpyridine) was added to the above solution, the redox couple corresponded to Fe^{3+}/Fe^{2+} (Fig. 1A

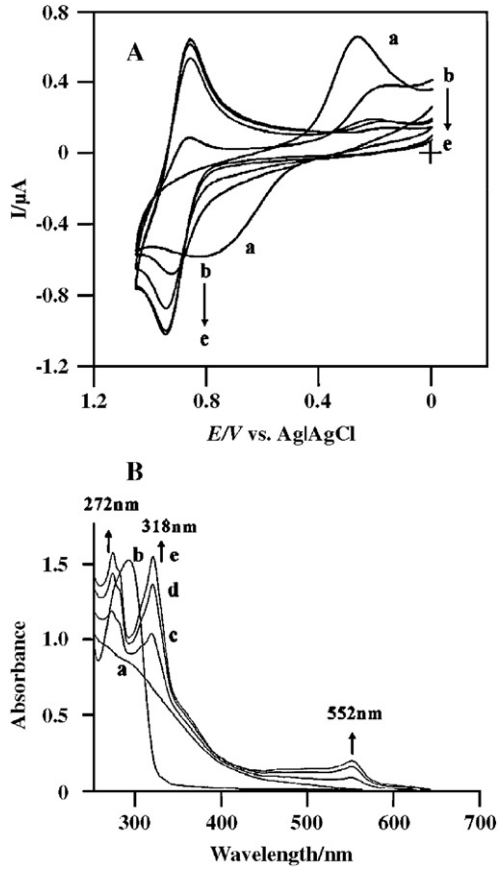


Fig. 1. (A) Cyclic voltammograms of: (a) 3.0 mM Fe^{2+} in a pH 4 buffer solution, with addition of 2,2′:6′,2″-terpyridine solution, (b) 4 mM, (c) 8 mM, (d) 12 mM and (e) 16 mM. (B) UV–visible absorption spectra of: (a) 0.5 mM Fe^{2+} and (b) 2.4 mM 2,2′:6′,2″-terpyridine. From (c–d) UV–vis spectra of 0.5 mM Fe^{2+} with addition of 2,2′:6′,2″-terpyridine in a pH 4 buffer solution: (c) 0.8 mM, (d) 1.6 mM and (e) 2.4 mM.

(a)–(e)),was shifted to a more positive side and also became highly reversible. This result indicated the formation of a new complex between these compounds with the formal potential of +0.92 V (*vs*. Ag|AgCl).

Fig. 1B shows the UV–visible absorption spectra of 2,2′:6′,2″-terpyridine in the absence and presence absence of Fe^{2+} ions at pH=4.0 aqueous solution. In the absence of Fe^{2+} ions, the 2,2′:6′,2″-terpyridine exhibited only one absorption band at about 300 nm. However, on mixing of Fe^{2+} with 2,2′:6′,2″-terpyridine formed showed absorption peaks at λ=552 nm, 318 nm and 272 nm whereas the absorption band corresponded to 2,2′:6′,2″-terpyridine at about 300 nm disappeared which may be due to formation of a new complex formation between Fe(II) and bis(2,2′:6′,2″-terpyridine). UV/Vis spectroscopy showed the ligand-centered band at 318 nm and 272 nm. At around 552 nm a weak metal-to-ligand charge transfer band (MLCT) was observed [31].

Similarly, the reaction between tris(1,10-phenanthroline) and Fe^{2+} ions was also performed through cyclic voltammetric and UV–vis spectroscopic techniques to confirm the complex formation. We obtained similar results as the one that was obtained for bis(2,2′:6′,2″-terpyridine) and Fe^{2+} system (the figures are not shown). Similarly, UV/VIS spectroscopy showed of the $[Fe^{II}(phen)_3]^{2+}$ exhibited a ligand-centered band at 266 nm. At around 508 nm a weak metal-to-ligand charge transfer band (MLCT) was observed. From the UV–vis results, the formation of $[FeII(phen)_3]^{2+}$ was confirmed.

Fig. 2A and B show the cyclic voltammograms of Fe(II) bis (2,2′:6′,2″-terpyridine) in aqueous solution with various concentrations of guanine at pH 3.0 and 5.0, respectively. In the absence of guanine, Fe(II) bis(2,2′:6′,2″-terpyridine) exhibited a redox couple with formal potential at +0.92 V. On addition of guanine to the solution the oxidation peak current of Fe(II) bis (2,2′:6′,2″-terpyridine) complex was increased. In the reverse

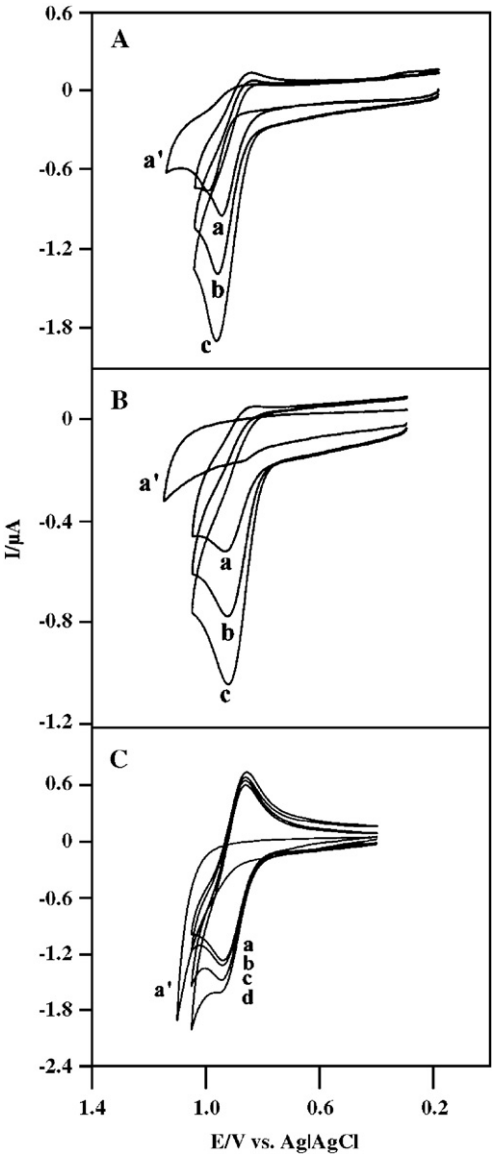
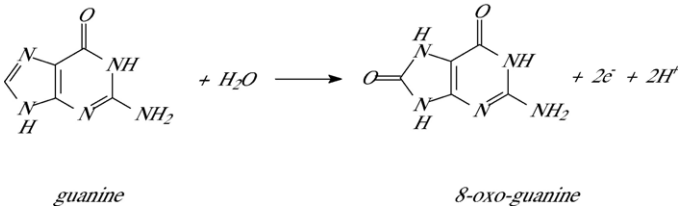


Fig. 2. (A) Cyclic voltammograms of $Fe(terpy)_2^{2+}$ in the presence of [guanine]: (a) 0, (b) 1.0 mM and (c) 2.0 mM in a pH 3 buffer solution on GC electrode and (a′) [guanine]=2.0 mM in the absence of $Fe(terpy)_2^{2+}$. (B) Cyclic voltammograms of $Fe(terpy)_2^{2+}$ in the presence of [guanine]: (a) 0, (b) 1.0 mM and (c) 2.0 mM in a pH 5 buffer solution with on GC electrode and (a′)=2.0 mM of guanine in the absence of $Fe(terpy)_2^{2+}$. (C) Cyclic voltammograms of $Fe(terpy)_2^{2+}$ in the presence of guanosine-5′-monophosphate] : (a) 0 mM, (b) 2.0 mM, (c) 4.0 mM and (d) 6.0 mM in a pH 5 buffer solution with on GC electrode and (a′) [guanosine-5′-monophosphate]=6.0 mM in the absence of $Fe(terpy)_2^{2+}$.



Scheme 1. The electrochemical oxidation of guanine to 8-oxo-guanine.

scan, the reduction peak current was decreased with the increase of concentration of guanine. This phenomenon attributed to typical homogenous mediated oxidation reaction of Fe(II) bis (2,2′:6′,2″-terpyridine) towards guanine.

The electrocatalytic oxidation process of guanine through the $Fe^{III}(terpy)_2^{2+}$ species is shown as under:

$$\mathrm{Fe(II)bis(2,2':6',2''-terpyridine)} \rightarrow \mathrm{Fe(III)bis(2,2':6',2''-terpyridine)} + e^- \quad (1)$$

$$\mathrm{Fe(III)bis(2,2':6',2''-terpyridine)} + \mathrm{guanine} \rightarrow \mathrm{Fe(II)bis(2,2':6',2''-terpyridine)} + 8-\mathrm{oxo}-\mathrm{guanine} \quad (2)$$

Hence, the oxidation of guanine may be mediated by oxidized form of (Fe(III) bis(2,2′:6′,2″-terpyridine) complex molecules present in the solution.

The electrocatalytic oxidation equation of guanine is represented below

$$\mathrm{Guanine} + H_2O \rightarrow 8-\mathrm{oxo}-\mathrm{guanine} + 2e^- + 2H^+ \quad (3)$$

and the form of structural formula [32] is represented in Scheme 1.

Fig. 2C depicts the voltammograms of $[Fe^{II}(terpy)_2]^{2+}$ in the absence and presence of guanosine-5′-monophosphate at pH 5 aqueous solution. When we used various concentrations of guanosine-5′-monophosphate, the anodic peak current responsible for $[Fe^{II}(terpy)_2]^{2+}$ complex at about +0.95 V increased proportionally to the guanosine-5′-monophosphate concentrations. The results showed that the electrocatalytic mediated oxidation of guanosine-5′-monophosphate by $FeII(terpy)_2$ present in the solution. The electrocatalytic oxidation of guanine and guanosine-5′-monophosphate by Fe^{2+}/Fe^{3+} ions (2,2′:6′,2″-terpyridine was absent) were also investigated in an acidic aqueous solution. The results demonstrated that the electrocatalytic properties of guanine and guanosine-5′-monophosphate by Fe^{2+} (or Fe^{3+}) obviously remained inactive in the above condition (the figures are not shown in the text).

The electrocatalytic activities of $[Fe^{II}(terpy)_2]^{2+}$ towards ssDNA were also investigated. Fig. 3A illustrates the cyclic voltammograms of $[Fe^{II}(terpy)_2]^{2+}$ in pH=4.0 aqueous buffered solution in the absence and presence of ssDNA. The anodic peak current of the $[Fe^{II}(terpy)_2]^{2+}$ redox couple at a potential of +0.95 V (*vs.* Ag|AgCl) was found to be increased noticeably, meanwhile, the cathodic peak current became decreased, as the concentration of ssDNA increased. This phenomenon also attributed to typical homogenous mediated oxidation reaction of $[Fe^{II}(terpy)_2]^{2+}/[Fe^{II}(terpy)_2]^{3+}$ redox couple towards ssDNA.

The interaction between $[Fe^{II}(terpy)_2]^{2+}$ and ssDNA was also characterized by a change in the UV–visible spectra (Fig. 3B). The absorption spectra of $[Fe^{II}(terpy)_2]^{2+}$ in the presence of ssDNA has been reported in the pH=4.0 buffer solution (see Fig. 3B). From the results it was understood that the absorption bands at 272 nm, 318 nm, and 552 nm of $[Fe^{II}(terpy)_2]^{2+}$ became stronger with addition of different concentration of ssDNA. This results showed that the interaction between $[Fe^{II}(terpy)_2]^{2+}$ and ssDNA might have been electrocatalytically active.

3.2. Electrocatalytic oxidation involving guanine, guanosine-5′-monophosphate, and ssDNA by Fe(II) tris(1,10-phenanthroline)

Fig. 4A shows the cyclic voltammograms of Fe (II) tris(1,10-phenanthroline) in the presence of different various concentrations of guanine at pH 3 aqueous solution. The catalytic current increased as the concentration of guanine increased noticeably. Meanwhile, the cathodic peak current became decreased, as the concentration of guanine increased. This behavior was typical of that expected for a mediated oxidation of guanine in the presence of Fe(II) tris(1,10-phenanthroline).

Similarly, the same experiment was carried out the at pH 4 of $[Fe^{II}(phen)_3]^{2+}$ in the absence and presence of [guanosine-5′-monophosphate] in aqueous solution. In these experiments also, the same type of catalytic behavior was observed that was

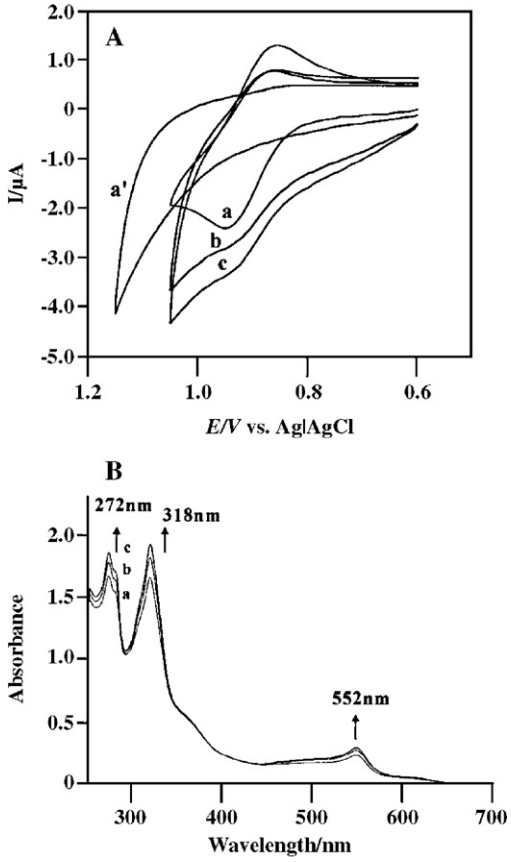


Fig. 3. (A) Cyclic voltammograms of $Fe(terpy)_2^{2+}$ in a pH 4 buffer solution with addition of [ssDNA]: (a) 0, (b) 0.001, (c) 0.002 g in 1 ml aqueous solution and (a′) [ssDNA]=0.002 g in 1 ml in the absences of $Fe(terpy)_2^{2+}$ (B) UV–visible absorption spectra of $Fe(terpy)_2^{2+}$(a) in a pH 4 buffer solution with [ssDNA]: (b) 0.001, (c) 0.002 g in 1 ml aqueous solution.

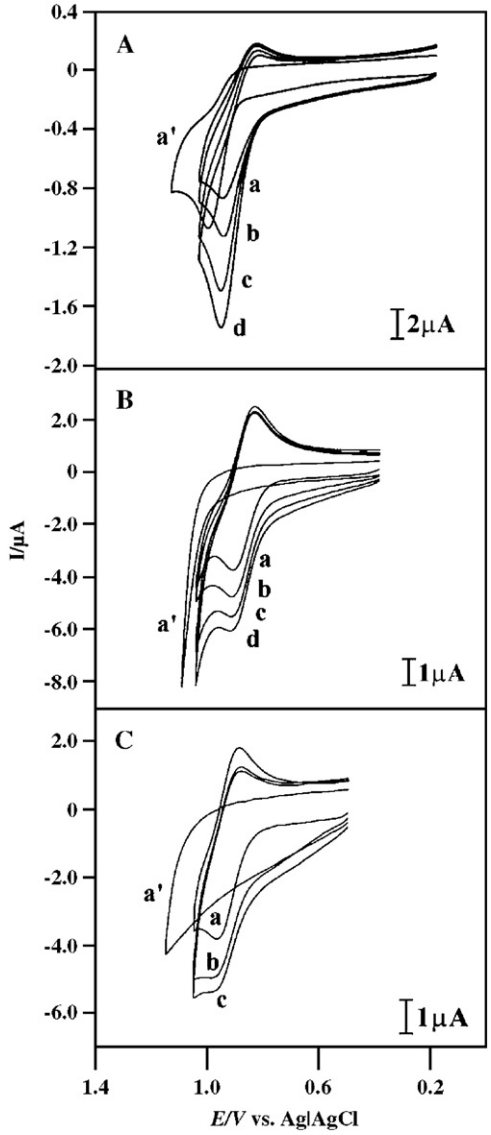


Fig. 4. (A) Cyclic voltammograms of [guanine]=3.0 mM (a′) and $Fe(phen)_3^{2+}$ in pH buffer solution with addition of [guanine]: (a) 0 mM, (b) 1.0 mM, (c) 2.0 mM and (d) 3.0 mM on glassy electrode. (B) Cyclic voltammograms of $Fe(phen)_3^{2+}$ in a pH 4 buffer solution with [guanosine-5′-monophosphate] (a) 0, (b) 1.5 mM, (c) 3.0 mM and (d) 4.5 mM and (a′) [guanosine-5′-monophosphate]=4.5 mM in the absence of $Fe(phen)_3^{2+}$ on GC surface. (C) Cyclic voltammograms of $Fe(phen)_3^{2+}$ in a pH 4 buffer solution with [ssDNA]: (a) 0, (b) 0.001 and (c) 0.002 g/1 ml aqueous solution (a′) bare glass electrode and [ssDNA]=0.002 g/1ml aqueous solution.

anodic peak current at about +0.93 V increased as the concentration of guanosine-5′-monophosphate increased (Fig. 4B). The electrocatalytic activities of $[Fe^{II}(phen)_3]^{2+}$ towards ssDNA were also investigated at different concentrations of ssDNA (Fig. 4C). The results showed that the electrocatalytic properties of ssDNA by $[Fe^{II}(phen)_3]^{2+}$ was similar to the electrocatalytic oxidation of guanosine as well as guanosine-5′-monophosphate at pH 4 buffer solution. H. H. Thorp obtained similar results for the electrochemical oxidation of DNA by using $Ru(bpy)_3^{2+}$ complex [33]. The oxidation of DNA occurred at +1.3 V in the presence of $Ru(bpy)_3^{2+}$ complex. In our system, the oxidation of DNA occurred at a less positive potential nearly at +0.95 V. Hence, the oxidation of DNA occurred at about 250 mV less positive potential using $[Fe^{II}(phen)_3]^{2+}$ complex rather than using $Ru(bpy)_3^{2+}$ complex.

The electrocatalytic oxidation process of guanosine-5′-monophosphate through $Fe^{III}(phen)_3^{2+}$ species is shown as:

$$\text{Fe(II)tris(1,10-phenanthroline)} \rightarrow \text{Fe(III)tris(1,10-phenanthroline)} + e^- \quad (4)$$

$$\text{Fe(III)tris(1,10-phenanthroline)} + \text{guanosine-5′-monophosphate} \rightarrow \text{Fe(II)tris(1,10-phenanthroline)} + \text{8-oxo-guanosine-5′-monophosphate} \quad (5)$$

The electrocatalytic oxidation of guanosine-5′-monophosphate is represented as follows

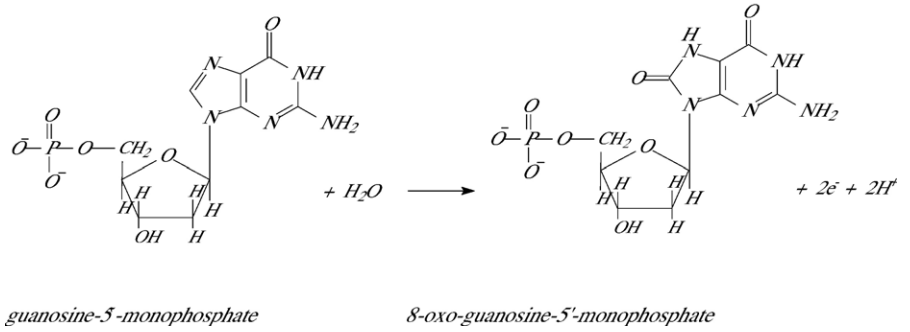
$$\text{Guanosine-5′-monophosphate} + H_2O \rightarrow \text{8-oxo-guanosine-5′-monophosphate} + 2e^- + 2H^+ \quad (6)$$

and is structurally formulated in Scheme 2.

When we compared the catalytic oxidation of guanine using Fe(II) tris(1,10-phenanthroline) and $[Fe^{II}(terpy)_2]^{2+}$ complexes, the oxidation of guanine occurred at 40 mV at a less positive side in the presence of $[Fe^{II}(terpy)_2]^{2+}$ complexes than that of Fe (II) tris(1,10-phenanthroline) complex.

3.3. Amperometric application in catalytic oxidation of guanine

The catalytic oxidation of guanine was studied through $[Fe^{II}(terpy)_2]^{2+}$ by chronoamperometry method. The obtained chronoamperometric measurements are depicted in Fig. 5A.



Scheme 2. The electrochemical oxidation of guanosine to 8-oxo-guanosine.

A typical amperometric, i–t, experiment of guanine was performed in a well-stirred solution (rotation speed = 1500 rpm) by keeping the electrode potential at 1.1 V in phosphate buffer solution (Fig. 5B). The chronoamperograms revealed that an increase in the guanine concentration was accompanied by an increase in the anodic peak current obtained for a potential step of +1.1 V (see the inset). In the amperometric experiment, a good response was obtained for 5 sequential additions of 3.0×10^{-6} M guanine by $[Fe^{II}(terpy)_2]^{2+}$ using the GC disk electrode. It was observed that the oxidation current of guanine was increased with the addition of guanine and reached the steady state within 5 s. The current responses showed a linear relationship with the concentration of guanine over the range of 0–25 μM for 1×10^{-3} M $Fe(terpy)_2^{2+}$. The electrocatalysis of guanine was carried out in the absence of $Fe(terpy)_2^{2+}$ and compared with the above results. The oxidation current of guanine in the absence of $Fe(terpy)_2^{2+}$ on the GC had started to increase very slowly and after 5 additions of guanine, the oxidation current became very weak. These results indicate that $[Fe^{II}(terpy)_2]^{2+}$ as a catalyst could be used for good and accurate measurement of guanine.

3.4. Electropolymerization from Fe(II) tris(5-amino-1,10-phenanthroline) on a gold disc electrode by consecutive cyclic voltammetry and EQCM measurements

Fig. 6(A) shows the consecutive cyclic voltammograms of Fe(II)tris(5-amino-1,10-phenanthroline) on a gold electrode. The cyclic voltammograms of the polymeric film were obtained in acetonitrile solution with 0.1 M tetrabutylammonium perchlorate as supporting electrolyte (TBAP) [34–38].

The consecutive cyclic voltammograms of Fe(II) tris(5-amino-1,10-phenanthroline) resulted in the deposition of poly (Fe(II) tris(5-amino-1,10-phenanthroline)) in 0.1 M TBAP acetonitrile solution on gold electrode. The results demonstrated that the deposition of poly-Fe(II) tris(5-amino-1,10-phenanthroline) was successful after the oxidation of monomer at potential range between +1.15 V and +1.4 V (*vs*. Ag, 0.1 M TBAP). The EQCM measurements are good for monitoring the *in situ* growth of poly(Fe(II)tris(5-amino-1,10-phenanthroline) on gold electrodes (see Fig. 6(B)). The results illustrated that the poly-Fe(II) tris(5-amino-1,10-phenanthroline) film grew steadily *vs*. time on the gold electrode. The EQCM measurements showed

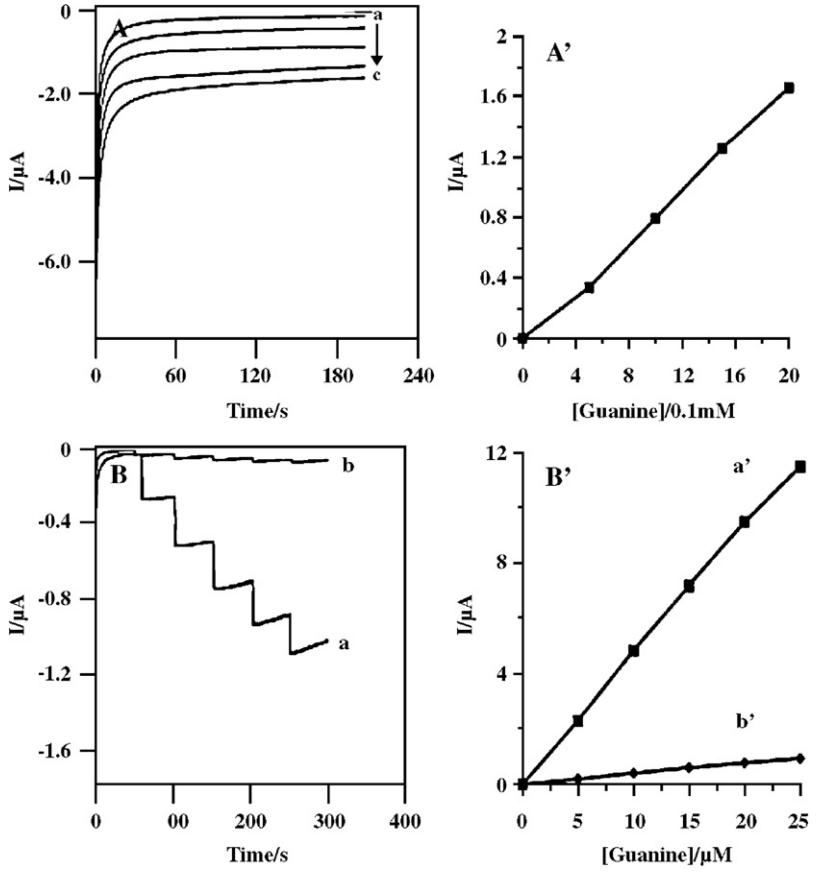


Fig. 5. (A) Chronoamperometric response of the 1×10^{-3} M $Fe(terpy)_2^{2+}$ in a pH 4.0 buffer solution containing different concentrations of guanine for a potential step of +1.1 V. The a to e corresponding to addition of guanine (each 5.0×10^{-4} M). Inset: Variation of chronoamperometric currents vs. guanine concentrations from 0 to 2 mM at t = 150 s (A′). (B) Amperometric responses of 5 sequential additions of guanine (each 3.0 μM) at (a) at glassy carbon electrode in a 1 mM $Fe(terpy)_2^{2+}$ pH 4.0 buffer aqueous solution, (b) at glassy carbon electrode in absence of $Fe(terpy)_2^{2+}$ pH 4.0 buffer aqueous solution. Inset: variation of catalytic current vs. [guanine] at (a′) in presence of 1 mM $Fe(terpy)_2^{2+}$ and (b′) absence of $Fe(terpy)_2^{2+}$ at bare disk glassy carbon in a pH 4 buffer solution (B′).Rotating speed:1500 rpm, diameter of GC disk electrode = 6.02 mM.

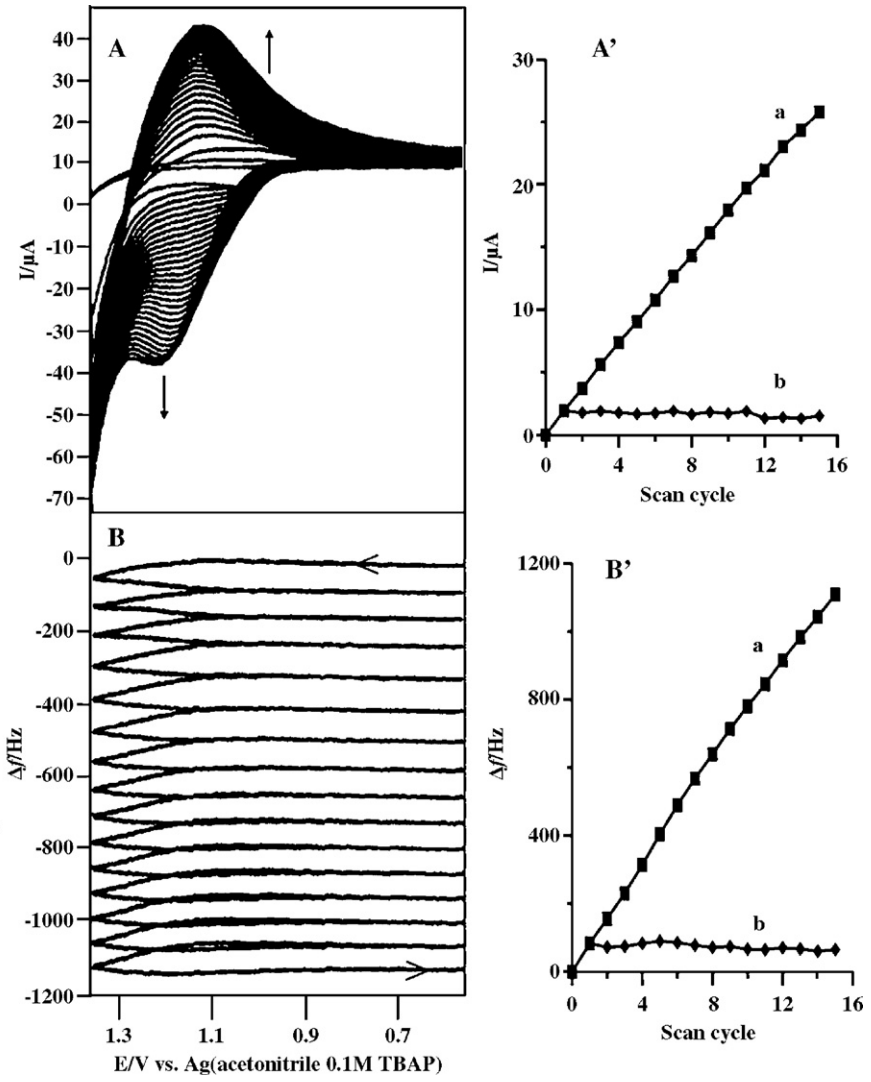


Fig. 6. (A) Consecutive cyclic voltammograms of poly-Fe(II) tris(5-amino-1,10-phenanthroline) from 0.5 mM Fe(II) tris(5-amino-1,10-phenanthroline) in 0.1 M TBAP acetonitrile solution. Electrode: gold electrode. (B) EQCM frequency change responses recorded together with the consecutive cyclic voltammograms. Scan rate: 0.02 V/s. Inset: anodic peak current (A′) and frequency change (B′) value *vs*. scan cycle.

that the deposition of the film occurred at a starting potential of about +0.8 V and then the main deposition process occurred between the potentials of +1.15 V and +1.40 V (*vs*. Ag, 0.1 M TBAP). The results, again demonstrated that before the film formation, no obvious precipitation process and adsorption for deposition occurred before the oxidation of 5-amino-1,10-phenanthroline of Fe(II) tris(5-amino-1,10-phenanthroline) as there was no obvious frequency change (mass increase). However, the EQCM results showed an obvious frequency change when the obtained cyclic voltammetry was more positive than +0.8 V (*vs*. Ag|AgCl) because of the oxidation of the monomer (Fe(II)tris(5-amino-1,10-phenanthroline)) and thereafter, polymerization began.

The EQCM experiment was performed and the mass change at the quartz crystal determined using the measurement of frequency change (Sauerbrey equation) [39,40].

$$(\Delta m) = (-1/2)(f_o^{-2})(\Delta f)A(k\rho)^{1/2} \quad (7)$$

where, Δf is the measured frequency change, A is the area of the gold disk coated onto the quartz crystal, ρ is the density of the crystal, k the shear modulus of the crystal, and f_o is the oscillation frequency of the crystal. A 1-Hz frequency change corresponded to 1.4 ng of mass change.

The inset of Fig. 6A(b) also demonstrates that the frequency change (decrease in frequency) of Fig. 6(B) was also proportional to the scan cycle. The results illustrated that the initial 15 scan cycles (per potential scan cycle of 80 s) of the film growth had stable growth rate and the efficiency was obvious by the change of film growth. Both the peak current and frequency change were almost proportional to the scan cycle and again, the peak current was also almost proportional to the mass deposition of poly-Fe(II) tris(5-amino-1,10-phenanthroline).

The insets of Fig. 6A(a) demonstrated that the anodic peak current vs. different scan of the poly-Fe(II) tris(5-amino-1,10-phenanthroline) film on a glassy carbon electrode was obtained from 0.1 M acetonitrile solution. Poly-Fe(II) tris(5-amino-1,10-

phenanthroline) TBAP film produced a reversible redox couple in 0.1 M TBAP acetonitrile buffer solution (Fe(II) tris(5-amino-1,10-phenanthroline)-free) when the cyclic voltammetry of poly-Fe(II) tris(5-amino-1,10-phenanthroline) film was performed at a lower scan rate (0.01−0.2 V/s). The anodic peak current vs. different scan rate for the film increased almost proportionally.

The ratio of I_{pa}/I_{pc} remained almost in unity as expected for a surface type behavior. These results demonstrated that the redox process was not controlled by diffusion. The peak-to-peak separation ΔE_p was only about 15 mV at a scan rate of 10 mV/s. The peak current and scan rate are related as follow [40,41].

$$Ip = n^2F^2\upsilon A\Gamma_o/4RT \quad (8)$$

where, Γ_o, υ, A, and I_p represent the surface coverage concentration, scan rate, electrode area, and peak current, respectively. This result indicated that the redox process was confined to the surface (poly-Fe(II) tris(5-amino-1,10-phenanthroline)/GC(glassy carbon electrode) and the redox process was confined to the surface, confirming the immobilized state of Fe(II) tris(5-amino-1,10-phenanthroline).

The kinetics of the potential switching responses of poly-Fe(II) tris(5-amino-1,10-phenanthroline)/GC) film used as display device in 0.1 M TBAP acetonitrile solution (Fe(II) tris(5-amino-1,10-phenanthroline)-free) were investigated (the figure is not shown). In these experiments, a square wave potential was applied over different time periods in the film oxidation state change. The experiment was performed using a square wave potential period of 0.5, 1, and 4 s, respectively. The reversibility properties of the poly-Fe(II) tris(5-amino-1,10-phenanthroline)/GC) film during the cycling of the square wave potential (+0.4 V and +1.7 V) and the charge change (chronocoulometry) were found good. This result too indicated that the redox process was confined to the surface and confirmed the stable immobilized state of poly-Fe(II) tris(5-amino-1,10-phenanthroline)/GC) during the poly-Fe(II/III) tris(5-amino-1,10-phenanthroline) oxidation state change.

The spectro-electrochemical properties of polymeric film obtained from 0.1 M TBAP acetonitrile solution was carried out in the aqueous solution without Fe(II) tris(5-amino-1,10-phenanthroline) at pH 1.0 (the figure is not shown).The spectroelectrometry of poly-Fe(II) tris(5-amino-1,10-phenanthroline) film was performed on an ITO electrode. The absorption spectra of poly-Fe (II) tris(5-amino-1,10-phenanthroline) in sulfuric acid aqueous solution at pH 1.0 were obtained when the applied working potentials were +0.5 V, +1.00 V, and +1.25 V, respectively. The results showed that the spectrum change occurred between 650 nm and 400 nm. It was similar to the spectrum change of Fe(II) tris (1,10-phenanthroline).

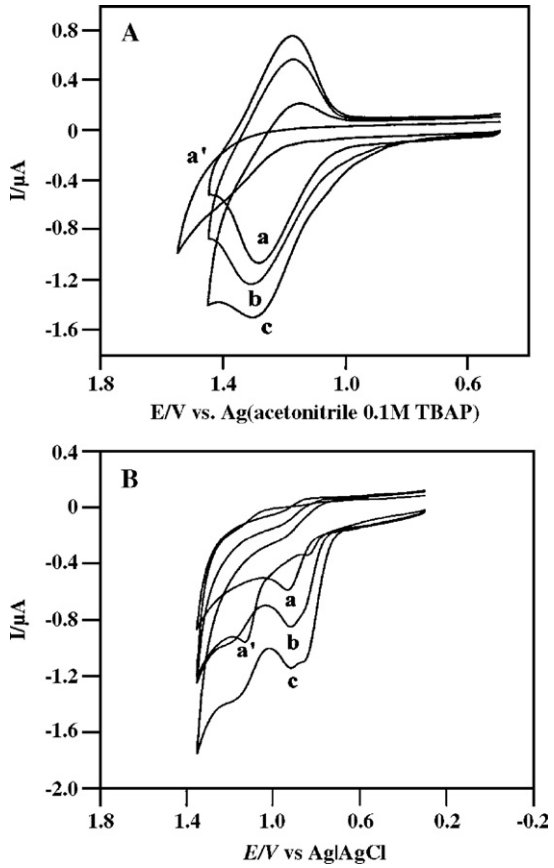


Fig. 7. (A) Cyclic voltammograms of poly-Fe(II) tris(5-amino-1,10-phenanthroline) fill in 0.1 M TBAP acetonitrile solution with: [adenine]: (a) 0 mM, (b) 1.0 mM and (c) 2.0 mM. (B). Cyclic voltammograms of 5.0×10^{-4} M $[Fe(terpy)_2]^{2+}$ in a pH 5 buffer solution with [guanine] and [adenine]: (a) 0 mM, (b) 1.0 mM and (c) 2.0 mM. (a′) [guanine] and [adenine]=2.0 mM on bare glassy electrode.

3.5. Electrocatalytic oxidation of adenine in acetonitrile solution and electrocatalytic-oxidation of guanine in acidic aqueous solution by poly-Fe(II) tris(5-amino-1,10-phenanthroline) film

Fig. 7A shows the cyclic voltammograms of poly-Fe(II) tris(5-amino-1,10-phenanthroline) in 0.1 M TBAP solution with various concentrations of adenine. Upon the addition of adenine, the anodic peak current of poly-Fe(II) tris(5-amino-1,10-phenanthroline) was found to be increased meanwhile in the reverse scan, the cathodic peak current decreased due to mediated catalytic reaction.

$$\text{poly}-\text{Fe}^{\text{II}}(5-\text{NH}_2-1,10-\text{phen}) \rightarrow \text{poly}-\text{Fe}^{\text{III}}(5-\text{NH}_2-1,10-\text{phen})+\text{ne}^- \quad (9)$$

$$\text{poly}-\text{Fe}^{\text{III}}(5-\text{NH}_2-1,10-\text{phen})+\text{guanine}\rightarrow\text{poly}-\text{Fe}^{\text{II}}(5-\text{NH}_2-1,10-\text{phen})+8-\text{oxo}-\text{guanine}. \quad (10)$$

3.6. Simultaneous determination of adenine and guanine

Simultaneous determination of adenine and guanine in the solution containing $[Fe^{II}(terpy)_2]^{2+}$ was carried out. Fig. 7B shows the cyclic voltammograms of $[Fe^{II}(terpy)_2]^{2+}$ in pH 5 aqueous solution with various concentrations of adenine and guanine. The redox couple of the film was at $E^{o\prime}=+0.92$ V. The anodic peak current of the redox couple increased noticeably, while its cathodic peak current decreased as adenine and guanine increased. From this results it is believed that the increase in the anodic peak current of $[Fe^{II}(terpy)_2]^{2+}$ corresponds to the oxidation of guanine. In the case of adenine, a small oxidation peak current was observed at 1.13 V at a glassy carbon electrode. Hence, the peak separation between adenine and guanine was

obtained approximately 200 mV. However, in the absence of $[Fe^{II}(terpy)_2]^{2+}$ at bare glassy carbon electrode a small hump corresponded to guanine oxidation was observed whereas on oxidation peak was observed for adenine in this potential range.

4. Conclusion

The electrocatalytic oxidation of guanine, adenine, guanosine, and ssDNA by the catalyst of Fe(II) bis(2,2′:6′,2″-terpyridine) complexes took place through the Fe(II/III) redox couples. Guanine was oxidized to 8-oxo-guanine with the development of E_{Pcat} from the Fe(II/III) redox couple. The electrocatalytic oxidation of guanine, guanosine-5′-monophosphate and ssDNA by Fe(II) tris (1,10-phenanthroline) was also performed. The results showed an increase in the anodic current due to the electrocatalytic oxidation of reactants (guanine, guanosine-5′-monophosphate, and ssDNA) by Fe(II) bis(2,2′:6′,2″-terpyridine) through the catalytic activity of Fe (II) tris(1,10-phenanthroline) complex. The electrocatalytic oxidation of guanine was studied through $[FeII(terpy)_2]^{2+}$ by amperometry method. The oxidation of guanine occurred at 40 mV less positive side in the presence of $[FeII(terpy)_2]^{2+}$ complexes than that of Fe(II) tris(1,10-phenanthroline) complex. The electropolymerization of the complex Fe(II) tris(5-amino-phenanthroline) was performed on various electrodes. The EQCM and consecutive cyclic voltammetry techniques were used to study the *in situ* growth of the polymer film formation. The simultaneous electrocatalytic oxidations of guanine and adenine were too performed. The electrocatalytic oxidation of guanine by the poly-Fe(II) tris(5-amino-1,10-phenanthroline) film-modified electrode was found active in strong acidic aqueous solution whereas the electrocatalytic oxidation of adenine by poly-Fe(II) tris(5-amino-1,10-phenanthroline) film-modified electrode was found active in acetonitrile TBAP solution.

Acknowledgement

This work was supported by the National Science Council of Taiwan (Republic of China).

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