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Detection of Guanine Based DNA and its Damage with Electrodes Incorporating a Polymeric film of $[Ru(v\text{-}bpy)_3]^{2+}$

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Detection of Guanine Based DNA and its Damage with Electrodes Incorporating a Polymeric film of $[Ru(v\text{-}bpy)_3]^{2+}$

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요 약. 구아닌과 ds-DNA를 검출하고 톨루엔이나 styrene oxide와 같이 DNA의 손상 유발 제로 잘 알려진 물질들 의한 화학적 손상을 검출하기 위하여 전이금속 착물로 된$[Ru(v\text{-}bpy)_3]^{2+}$ 고분자 피막으로 수식한 유리탄소전극을 제작하였다. 표면에 고정된 이 고분자 피막의 산화-환원응답을 분석신호로 사용하였다. 이 수식 전극에서 촉매 피크전류는 단순 유리탄소 전극에서 보다 6배 더 컸다. 또, 이 수식 전극의 이차 속도상수는 0.26 $M^{-1}s^{-1}$ 이였고 단순 유리탄소 전극에서 보다 4.5배 더 컸다. 검정곡선, 표면 피복 율로 규격화한 산화-환원 응답의 로그값 대 구아닌 농도의 로그값, 은 1.0×10^{-7}M에서 1.0×10^{-3}M의 구아닌농도 범위에서 탁월한 상관관계(r≥0.99)를 나타냈다. 이 수식 전극 상에 흡착한 ds-DNA의 양은 3.96×10^{-6} gcm^{-2}이였다. 흡착된 ds-DNA의 촉매 피크전류는 단순 수식전극의 것보다 6배 컸다. 이 전극 상에 회합된 ds-DNA는 styrene oxide의 경우에 2% 그리고 톨루엔의 경우 9%의 화학적 손상이 나타났다.

주제어: Guanine, ds-DNA, poly-$[Ru(v\text{-}bpy)_3]^{2+}$, DNA Damage

ABSTRACT. Glassy carbon(GC) electrodes modified by adding a poly-$[Ru(v\text{-}bpy)_3]^{2+}$ film of a transition metal complex were prepared in order to detect guanine and ds-DNA and the chemical damage caused to these molecules by the known damage inducing agents, toluene and styrene oxide. The redox response of the surface immobilized polymeric film was used as analytical signal. The catalytic peak current at the modified electrode was 6 times higher than that observed at bare GC electrode. The second-order rate constant of the modified electrode was 0.26 $M^{-1}s^{-1}$ as 4.5 times higher than that of the bare GC electrode. The calibration curve, i.e. log of the surface coverage-normalized redox response vs log[guanine], exhibited excellent correlation (r≥0.99) for guanine concentrations ranging from $1.0\times10^{-7}$to 1.0×10^{-3}M. The quantity of adsorbed ds-DNA on the modified electrode was 3.96×10^{-6} gcm^{-2}. The catalytic peak current of the adsorbed ds-DNA was 6 times larger than the modified electrode alone. The ds-DNA incorporated on the electrode surface was chemically damaged 2% in the case of styrene oxide and 9% in the case of toluene.

Keywords: Guanine, ds-DNA, poly-$[Ru(v\text{-}bpy)_3]^{2+}$, DNA Damage

INTRODUCTION

Techniques for detecting DNA and its fragments damaged by toxicities have been widely studied.[1,2] The voltammetric oxidation of DNA is a popular and inexpensive method, which can be used for the rapid detection of such damage. Rusling and coworker[3] employed alternate layer by layer adsorption of

poly (diallydimethyl ammonium chloride:PDDA) and DNA in solution containing $[Ru(bpy)_3]^{2+}$ and styrene oxide using of square wave voltammetry. They also reported detection of DNA damage in films containing DNA, ionomers and Nafion on graphite electrodes by direct oxidation using derivative square wave voltammatry[4,5]. Adsorptive voltammetry on mercury electrodes was used to detect DNA damage resulting from strong acid[6].

Catalytic electrochemical oxidation using transition metal complexes enhances voltammetric signals for DNA[7]. One of the most efficient catalyst is $[Ru(bpy)_3]^{2+}$, which specifically oxidizes guanine bases in DNA and oligonucleotides[8,9] as follows:

$$[Ru(bpy)_3]^{2+} = [Ru(bpy)_3]^{3+} + e^- \quad (1)$$

$$[Ru(bpy)_3]^{3+} + \text{DNA(guanine)} \rightarrow [Ru(bpy)_3]^{2+} + \text{DNA(guanine}^+) \quad (2)$$

The cycling of $[Ru(bpy)_3]^{3+}$ back to $[Ru(bpy)_3]^{2+}$ by the cyclic voltammetry (CV) provides catalytic current, which is greatly enhances compared to those observed with DNA alone.

In this work, employed $[Ru(v\text{-}bpy)_3]^{2+}$, which possesses a vinyl group(4-vinyl-4'methyl-2,2'-bipyridly: v-bpy), causing to give rise to polymeric films on the electrode surface[10,11]. The film of poly-$[Ru(v\text{-}bpy)_3]^{2+}$ on electrode surface exhibits a catalytic ability toward guanine bases DNA, just as the $[Ru(bpy)_3]^{2+}$ions do in solution. Also, the electrodes are covered by poly cationic films which can adsorb DNA, without the need for additional layers to be incorporated onto the electrode surface. The immobilized polycationic film cathodically polymerized could associate to with phosphate anion sites of the DNA units.

EXPERIMENTAL

Instruments and chemicals. The electrochemical experiments were performed using an EG & G 273A potentiostat/galvanostat with 270/250 software. The cyclic voltammetric data were recorded on a Philips model 8043 X-Y recorder and plotter (*hp* color pro). Potentials were measured and reported without reagents for the liquid junction sodium saturated calomel electrode(SSCE). CV was used to evaluate the redox characteristics of the reactions and for the oxidation of guanine. Quartz crystal microbalance (CHI 420) was employed for the EQCM experiments. GC electrodes, which were first polished with 1000-grid SiC paper and then with alumina (0.3 μm, Buehler), and ultrasonicated, which subsequently activated in1.0 M NaOH solution by appling a voltage of 1.20 V for 5 min, followed by potential cycling from –0.5 to 1.50 V with a sweep rate of 1 V/s in buffer solution for 5 min. The electrodes were rinsed with water and acetonitrile in order to polymerize the $[Ru(v\text{-}bpy)_3]^{3+}$. High-quality deionized water (18 MΩ) was obtained by employing a Millipore Milli-Q system. $[Ru(v\text{-}bpy)_3]^{2+}$ was synthesized by the previously reported methods[10]. 0.01 M acetate buffer solution containing 50 mM NaCl (pH=5.5) buffer was prepared from analytical grade reagents. Guanine and calf thymus (CT) ds-DNA were purchased from Sigma and used as received.

Electrode preparation. Polymeric films of $[Ru(v\text{-}bpy)_3]^{2+}$on glassy carbon electrodes were cathodically polymerized on the electrode surface, and the quantity of the film was controlled by adjusting the numbers of cycles[10]. Subsequently, guanine molecules were directly detected at various concentrations with the GC/poly-$[Ru(v\text{-}bpy)_3]^{2+}$electrodes. The GC/poly-$[Ru(v\text{-}bpy)_3]^{2+}$electrode was immersed in a solution containing 0.2 mg/mL of DNA in 0.01 M acetate buffer at pH=5.5, containing 0.05 M sodium chloride for 30 min to incorporate the DNA onto the polycationic film electrode surface, so as to give rise GC/poly-$[Ru(v\text{-}bpy)_3]^{2+}$, ds-DNA type electrodes.

DNA damages. The agents used for inducing DNA damage were styrene oxide and toluene. The GC/poly-$[Ru(v\text{-}bpy)_3]^{2+}$, ds-DNA electrode was immersed in a saturated solution of the damaging inducing agent in a temperature controlled cell at 37 °C for 30 min with constant stirring. Subsequently, a 120 mL fresh sample was added to 10 mL of the buffer solution[3]. The resultant electrode was then rinsed with water and used for the CV analysis.

RESULTS AND DISCUSSION

Catalytic ability of GC/poly-$[Ru(v\text{-}bpy)_3]^{2+}$ electrodes. *Fig.* 1A shows the CV results for the 1.06×10^{-4} M solution of guanine in 0.1 M KOH at the bare GC electrode, which produced a formal potential of 0.41 V and a peak current of 7.0 mA. The electrode was cathodically polymerized with $[Ru(v\text{-}bpy)_3]^{2+}$ to give rise to the GC/poly-$[Ru(v\text{-}bpy)_3]^{2+}$ type electrode (*Fig.* 1B), which produced the corresponding values of 0.38 V and 41 mA, respectively. From these results, it can be concluded that the modified electrode exhibited catalytic ability in the oxidation of guanine and, consequently, this polymer modified electrode could satisfy the conditions of Eqs. (1) and (2).

The kinetic parameters were measured from RDE experiments. The catalytic currents for guanine oxidation at the GC and GC/poly-$[Ru(v\text{-}bpy)_3]^{2+}$electrodes were measured in 0.1 M KOH solution containing 1.06×10^{-4} M guanine. The results obtained

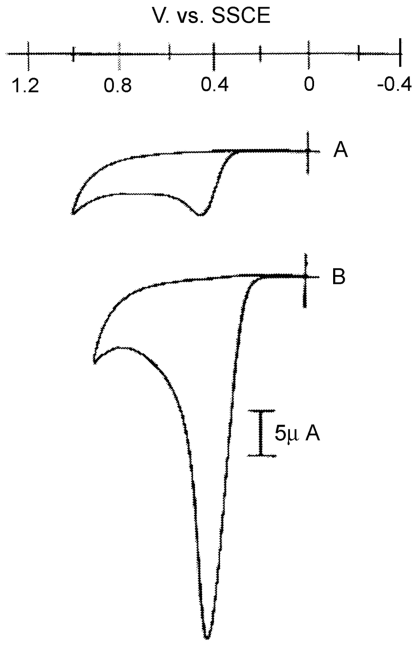

Fig. 1. The cyclic voltammetric results for the glassy carbon(A) and GC/poly-$[Ru(v\text{-}bpy)_3]^{2+}$(B) electrode, for an applied potential range of 0 to 0.9 V in 0.1 M KOH solution containing 1.06×10^{-4} M guanine, with a sweep rate of 50 mV/s, and an electrode surface area of 7.1×10^{-2} cm^2.

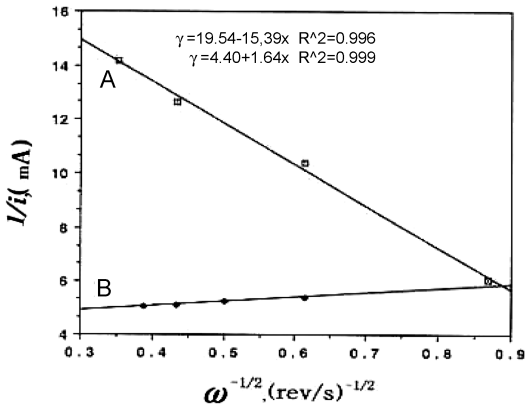

Fig. 2. The reverse Levich plots from the responses at RDE(GC): (A) and RDE(GC)/poly-$[Ru(v\text{-}bpy)_3]^{2+}$ electrodes: (B) at 50 mV/s in 0.01 M acetate buffer solution (pH=5.5) containing 0.05 M NaCl with a guanine concentration of 1.06×10^{-4} M in 0.1 M KOH solution.

using an electrode with a surface coverage of 1.67×10^{-10} $mol.cm^{-2}$. The peak current observed in increased as the revolution rate, ω, was increased up to 3,000 rpm. Also, a reverse Koutecky-Levich plot of i^{-1} vs $\omega^{-1/2}$(*Fig.* 2) was found to be linear, The corresponding equation[12] is

$$1/i_{lim}=1/(nFAk_f\Gamma C^*)+1/(0.62nFAv^{-1/6}D^{2/3}C^*\omega^{-1/2}) \quad (3)$$

where C^* is the bulk concentration of the reactant, ω is the surface coverage, n is the kinematic viscosity, ω is the rotating rate and k_f is the rate constant. From the intercepts of the plots, the values of k_f, presenting the second-order rate constants, were 5.8×10^{-2}: (A), and 0.26 $M^{-1}s^{-1}$: (B) with the rate constant of the modified electrode being 4.5 times higher than that of the bare one. In these systems, guanine is more easily oxidized by the surface confined ruthenium complex in a chemical step controlled by the rate constant, k_f. DNA (guanine$^+$) is an oxidized product of this reaction.

Determination of guanine. The CV results shown in *Fig.* 3A are the responses at a sweep rate of 50 mV/s for the GC/poly-$[Ru(v\text{-}bpy)_3]^{2+}$electrodes in 0.1 M KOH solution for guanine concentrations of 1.0×10^{-3}, 5.0×10^{-4}, 1.0×10^{-4}, 5.2×10^{-5}, 1.0×10^{-5}, 1.0×10^{-6}, and 1.0×10^{-7} M beginning from the left,

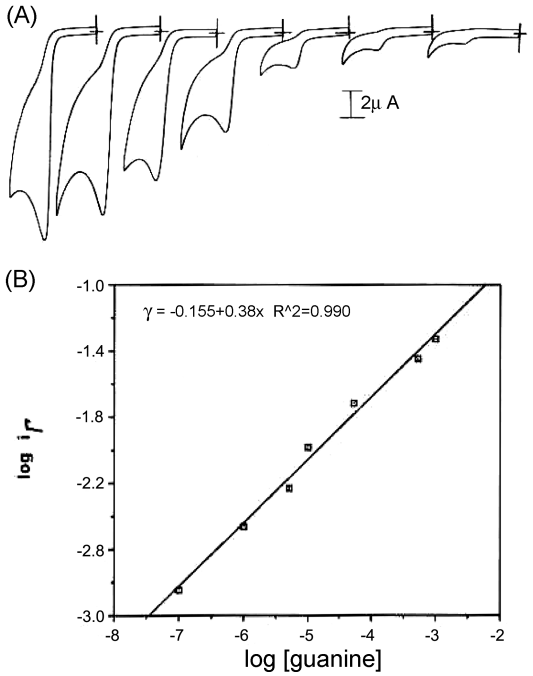

Fig. 3A, The cyclic voltammetric responses of the GC/poly-$[Ru(v\text{-}bpy)_3]^{2+}$ electrode at 50 mV/s in 0.1 M KOH solution with guanine concentration of 1.0×10^{-3}, 5.02×10^{-4}, 1.0×10^{-4}, 5.2×10^{-5}, 1.0×10^{-5}, 1.0×10^{-6}, and 1.0×10^{-7} M beginning from left, respectively, B. The calibration curve, and the plot of normalized current vs. concentration, for the data shown in *Fig.* 3A.

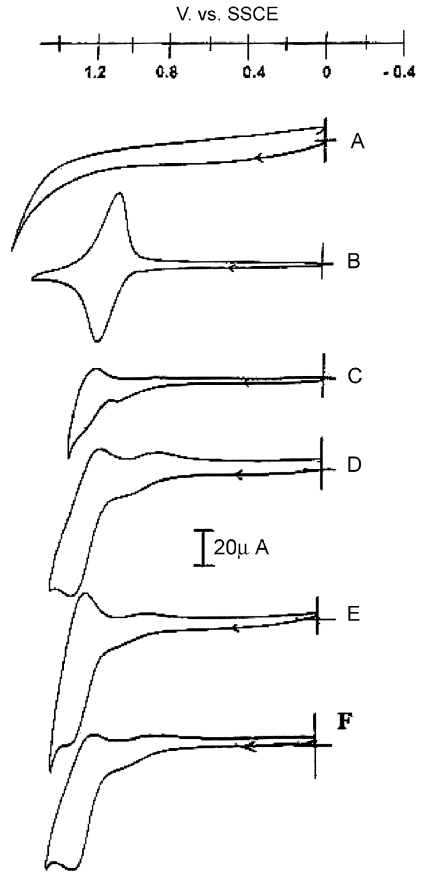

Fig. 4. The cyclic voltammograms of (A): GC, B: GC/poly-$[Ru(v\text{-}bpy)_3]^{2+}$in 0.1 M TBAP/$CH_3CN$, C: in 0.01 M acetate buffer solution containing 0.05 M NaCl(pH=5.5), D: electrode C was immobilized with ds-DNA, and E: after incubations of electrode D with toluene and F: with styrene oxide 30 min at 37 °C.

respectively. Guanine can be dissolved in strong alkaline solution. In the calibration curve, the anodic peak current is observed to increase with increasing guanine concentration, without saturation, for all of the guanine concentrations, as shown on *Fig.* 3B. Also, note that there is not significant return (cathodic) wave originating from the catalytic ability of this electrode. These types of PME electrodes are most sensitive at the concentration ranges of 1.0×10^{-5} to 1.0×10^{-4} M to adsorptive materials and, consequently, fresh electrodes are required for each measurement.

DNA detection. The surface coverage of the poly-$[Ru(v\text{-}bpy)_3]^{2+}$film on the glassy carbon electrode was differed greatly depending on the applied solution, as shown in *Fig.* 4, B and C, with the coverage being 1.68×10^{-9} mol.cm^{-2} in 0.1 M TBAT/CH_3CN and 1.67×10^{-10} mol.cm^{-2} in the buffer solution. The number of electroactive sites was reduced by a factor of 10 in aqueous acetate buffer solution, due to the incorporation of acetate ions and hydration.[13,14] The GC/poly-$[Ru(v\text{-}bpy)_3]^{2+}$electrodes containing ds-DNA, (*Fig.* 4D)[15,16], produced a wave area which was 5.7 times larger than without ds-DNA (*Fig.* 4C), but the formal potential was increased by 0.03 V. The small anodic wave of *Fig.* 4B at 1.06 V belongs to the amine groups of the v-bpy ligands of the ruthenium complex which became extended following the addition of the groups at DNA, as shown in *Fig.* 4D. From these results, showing the catalytic ability on the guanine based DNA, it can

be concluded that the surface immobilized polycationic films of $[Ru(v\text{-}bpy)_3]^{2+}$ acts in a like manner to the free ions of $[Ru(bpy)_3]^{2+}$ in solution[17,18].

DNA damages. *Figs*. 4E and 4F show the DNA damage on the GC/poly-$[Ru(v\text{-}bpy)_3]^{2+}$, ds-DNA electrodes with toluene and styrene oxide, respectively. The values of the peak potential for the oxidation process were unchanged, but their peak area was diminished by 9% at *Fig*. 4E and 2% at *Fig*. 4F compared to the values observed in *Fig*. 4D. The incorporated ds-DNA molecules were more damaged at toluene than at styrene oxide. The changes in the waveform for two damage inducing agents after incubation depended upon disturbance to the polymeric film at the incubation temperature and the damage caused to the DNA by the toxicities.[4]

EQCM results. The Pt(QCA)/ poly-$[Ru(v\text{-}bpy)_3]^{2+}$ electrodes was filled with 1.2 mg/mL ds-DNA in 0.01 M Tris/HCl buffer solution containing 0.001 M EDTA (pH=7.05)and the measurements taken for 1000 s. The value of the frequency difference at this procedure was 283 Hz (*Fig*. 5), which corresponded to the quantity of adsorbed ds-DNA on the electrode, which was calculated as being 3.96×10^{-6} g/cm^2, from the Sauerbrey equation, which be expressed as eq.(4)[19]

$$\Delta m=1.40\times10^{-8}\ gHz^{-1}\ cm^{-2}\ \Delta F \qquad (4)$$

Fig. 6 shows the changes in frequency observed in one of the CV measurements of the Pt(QCA)/ poly-$[Ru(v\text{-}bpy)_3]^{2+}$/ds-DNA electrode in 0.01 M acetate buffer solution containing 0.05 M NaCl(pH=5.5). The mass change due to hydration was 8.82×10^{-6} g/cm^2, which belonged to hydration/dehydration process involving 14 water molecules for one cycle. *Fig*. 7 showsthe frequency changes for 5 cycles of the Pt(QCA)/poly-$[Ru(v\text{-}bpy)_3]^{2+}$electrode in 0.01 M acetate buffer solution containing 0.05 M NaCl(pH=5.5). Without ds-DNA, a constant frequency change was observed after 2 cycles, which meant that the polymeric film maintained constant ion pumping during the redox process. *Fig*. 8 shows the continuous increasing in frequency observed in one CV measurements for the Pt(QCA)/poly-$[Ru(v\text{-}bpy)_3]^{2+}$/ds-DNA electrode. The terminal ds-DNA molecules, which contain the acetate ions originating from the buffer solution are electronically charged or discharged during the CV process and, consequently, the counterbalancing ions trapped on the film cannot escape. This leads to a capacitive charging or discharging of the films and the build-up of an electrostatic field. The field will eventually exceed the threshold frequency jump necessary to expel excess ions[20].

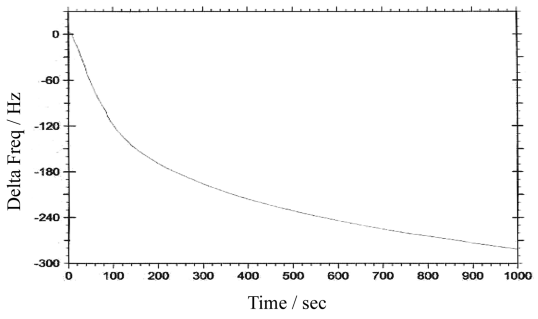

Fig. 5. Frequency-time curve for the deposition of ds-DNA on the GC/poly-$[Ru(v\text{-}bpy)_3]^{2+}$electrode.

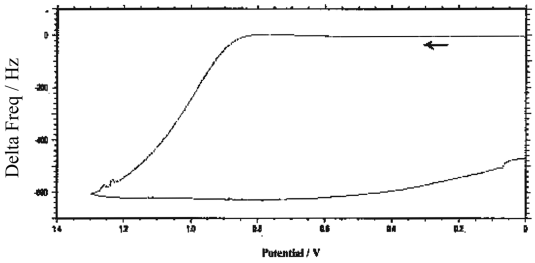

Fig. 6. EQCM of the Pt(QCA)/poly-$[Ru(v\text{-}bpy)_3]^{2+}$/ds-DNA electrode in 0.01 M acetate buffer solution (pH=5.5) containing 0.05 M NaCl corresponding to CV of *Fig*. 6D.

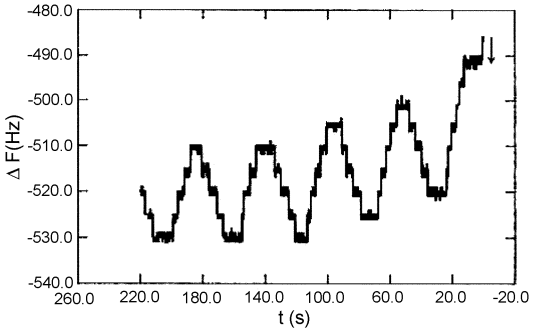

Fig. 7. Frequency-time curve for 5 cycles for the GC/poly-$[Ru(v\text{-}bpy)_3]^{2+}$electrode in 0.01 M acetate buffer solution containing 0.05 M NaCl (pH=5.5).

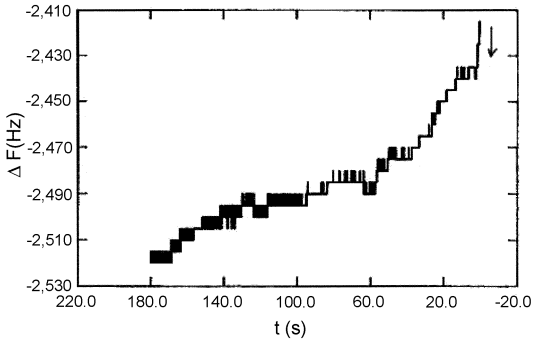

Fig. 8. Frequency-time curve for 5 cycles for the GC/poly-$[Ru(v\text{-}bpy)_3]^{2+}$/ds-DNA electrode in 0.01 M acetate buffer solution containing 0.05 M NaCl (pH=5.5).

CONCLUSIONS

The poly-$[Ru(v\text{-}bpy)_3]^{2+}$ cathodically polymerized on GC electrodes and characterized the response of the modified DNA sensitive electrodes in terms of the effects of the immobilization procedure, guanine determination, the charge transfer rate constant, the quantity of deposited ds-DNA and the amount of chemically induced DNA damage. The number of electroactive sites on the surface of the immobilized film was greatly influenced by the electrolyte solution that was employed, being 10 times higher in case of TBAP/acetonitrile than with aqueous acetate buffer solution. The modified with its integrated film of polycations and ruthenium complex is able to incorporate DNA and act as a catalyst without any additional modifications. These film covered electrodes were able to protect adsorbed ds-DNA from damage inducing agent.

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