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A new voltammetric sensor for sensitive and selective determination of xanthine based on DNA and polyaniline composite Langmuir–Blodgett film

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ABSTRACT

DNA–polyaniline (PAn) complex Langmuir–Blodgett film modified glassy carbon electrode (GCE) was used as a new voltammetric sensor (DNA/PAn-LB/GCE) for xanthine (XA) detection. The characteristic of DNA/PAn-LB film was studied by electrochemical impedance spectroscopy and scanning electron microscope. Electrochemical behaviors of XA at the sensor were studied in pH 7.0 phosphate buffer solutions by cyclic voltammetry and differential pulse anodic voltammetry. The results showed that this new modified electrode exhibited an excellent immunity from uric acid and hypoxanthine interference and a new sensitive and selective electroanalytical method for XA was proposed with wider linear range. Under the optimum conditions, the calibration curve for XA was obtained over the range of 7.0×10^{-8}–1.0×10^{-5} mol L^{-1}, with the detection limit of 3.0×10^{-8} mol L^{-1}. The practicability of this method was demonstrated by determining the concentration of XA in human serum samples.

1. Introduction

In recent years, much attention has been focused on developing electrochemical DNA sensors. It can be used for molecular recognition not only for DNA hybridization but also for sensing of bioactive species [1–4]. Meanwhile, interaction of DNA with some kinds of heavy metal ions has been reported [5,6]. Therefore, DNA sensors have a wide range of applications, including disease diagnosis, environmental monitoring and pharmaceutical research [7–9]. In the research field of DNA sensors, immobilization and assembly of DNA on a solid supporter are very important, which directly affects the biosensor performance, such as natural life, sensitivity and stability. Typical methods for immobilization of DNA are self-assembly technology [10], covalent bonding [11] and adsorption [12]. Another effective approach is Langmuir–Blodgett (LB) technique, by which functional and organized ultrathin multilayer of biomolecule-containing films can be constructed in predefined ways [13,14].

Up to now, immobilizing DNA on Langmuir monolayer is well known. A variety of materials, such as cationic surfactant [15], polycation [16], ionic discogen [17] and lipid [18] have been reported to be capable of complexing with DNA. However, there have been no reports on the interaction of DNA with polyaniline at the air–water interface. Moreover, to the best of our knowledge, successful modification of this DNA-containing LB films onto a solid substrate as voltammetric sensor is rarely reported.

In previous reports, we have demonstrated DNA–octadecylamine LB film modified electrode as voltammetric sensors for the recognition of small molecule [19–21]. We extend our studies in the direction where the DNA can be immobilized at the air–water interface with a polyaniline monolayer through the electrostatic interaction between the cationic polyaniline and anionic DNA [22]. In this approach, the DNA–polyaniline complex LB film modified electrode (DNA/PAn-LB/GCE) was used to determine xanthine. Xanthine (XA) is a metabolite of adenine nucleotides in human beings. Its content level in body fluid can reflect the state of body's immune, metabolism, and endocrine function [23]. So, the determination of XA level in blood and tissue is essential for diagnosis and medicine management of various diseases like hyperuricemia, gout, xanthinuria and renal failure [24]. At present, the XA analysis technique involving chemical modified electrodes has attracted much attention. Various modifying materials have been used, such as enzymes, carbon nanotube, graphene and nano-particles

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[23–27]. However, there is no report about the determination of XA on DNA modified electrode. Therefore, we investigated the electrochemical behavior of XA on DNA/PAn-LB/GCE in detail and a new voltammetric method for determination of XA was proposed. The results show that this electrode has the comparable sensitivity and detection limit for the determination of XA.

2. Experimental

2.1. Equipments and reagents

A JML-04 LB trough with an area of 357 cm^2 (Shanghai Zhongchen Company, China) was used. Electrochemical measurements (CV) were performed with a computer-controlled RST5000 electrochemical system (Zhengzhou Shiruisi Instrument Technology Co., Ltd, China) in a conventional three-electrode electrochemical cell using a bare GCE ($d=3$ mm) or modified electrode as the working electrode, platinum wire as the auxiliary electrode, and Ag/AgCl (saturated KCl) as the reference electrode. The morphology analysis of the layers was obtained by scanning electron microscope (SEM, Nanoscope IIIa, USA). SEM images used silicon wafer as substrate which were prepared as the previous report [28].

Polyaniline (PAn) (Sigma Aldrich; Mw ca. 10,000) was doped with p-toluene sulfonic acid (PTSA) using methanol as solvent [29,30]. Fish sperm DNA was purchased from Shanghai Sangon Company (Shanghai, China). Xanthine (XA) was received from Sigma. Uric acid (UA) was purchased from Shanghai Chemical Reagents Company and Hypoxanthine (HXA) was obtained from Aladdin Chemistry Company. Human blood serum was supplied by The First Affiliated Hospital of Zhengzhou University which was pretreated.

2.2. Procedure of DNA/PAn-LB film modified electrode

DNA is soluble in water. In order to avoid the DNA adsorbed on the electrode surface before modified, for preparation of DNA/PAn-LB films, 1500 μL doped PAn was firstly spread over the subphase in the Langmuir trough. After 30 min time for evaporating of solvent and diffusing of PAn, 500–1500 μL DNA (1 mg mL^{-1}) was injected into PAn coated subphase. The monolayer formed at the interface was left for at least 1 h before compression. The monolayer was compressed (10 mm min^{-1}) until a steady surface pressure of 30 mN m^{-1} was reached. With the horizontal attachment method, films were transferred onto pretreated GCE or Silicon wafer which were immersed in the subphase previously. Scheme 1 show the process of electrode modified with DNA/PAn-LB film. This fabricated electrode was named DNA/PAn-LB/GCE. For comparison, a PAn modified GCE (denoted as PAn-LB/GCE) was prepared using the same method without spreading DNA over the subphase surface. Prior to use, the modified electrode was thoroughly rinsed with pure water, and stored in 0.01 mol L^{-1} phosphate buffer (pH=7.0) at 4 °C when not in use.

2.3. Analytical procedure

For the analysis of XA, phosphate buffer solution (PBS) was selected as supporting electrolytes. Cyclic voltammetry (CV) or differential pulse voltammetry (DPV) were used as qualitative or quantitative electrochemical tools for the detection of XA. The renewal of the modified electrode was achieved in blank PBS (pH 7.0) solution with stirring for 60 s to eliminate memory effect. For EIS experimental, the EIS was performed in 0.2 mol L^{-1} KCl containing 5×10^{-3} mol L^{-1} $Fe(CN)_6^{3-/4-}$ with an equilibrium potential of 0.279 V (vs. Ag/AgCl), a perturbation amplitude of 5 mV and a frequency range from 100 kHz to 0.01 Hz.

3. Results and discussions

3.1. π–A isotherms of DNA/PAn-LB films

The surface pressure–mean molecular area (π–A) isotherm of PAn monolayer and DNA/PAn film are shown in Fig. 1. As the shown in curve a, the limiting mean molecular area (A_{lim}) of PAn observed in this investigation was calculated to be 0.48 nm^2, and the collapse pressure was nearly 45 mN m^{-1}. When 1000 μL DNA was added on the subphase (curve b), the isotherm shifted to larger areas per molecule. A_{lim} was calculated to be 0.80 nm^2. Such a dramatic change suggested that the DNA penetrated into the PAn Langmuir film. In order to achieve compact and obtain a more stable DNA/PAn-LB film, transfer pressure was chosen as 30 mN m^{-1}.

3.2. Electrochemical characterization of DNA/PAn-LB/GCE

Electrochemical impedance spectroscopy (EIS) is also a powerful technique to investigate the interface properties of modified electrodes. The semicircle diameter of Nyquist equals the R_{et}, called reaction resistance, which can be used to describe electron transfer resistance at the electrode surface. Fig. 2 shows the Nyquist plots of EIS using different electrodes in 5×10^{-3} mol L^{-1} $Fe(CN)_6^{3-/4-}$ (containing 0.2 mol L^{-1} KCl). It was clearly seen that the R_{ct} of these electrodes was in a sequence of: bare GCE < PAn-LB/GCE < DNA/PAn-LB/GCE (Fig. 2 curves a–c). This is because the DNA acted as a blocking layer for electron and mass transfer,

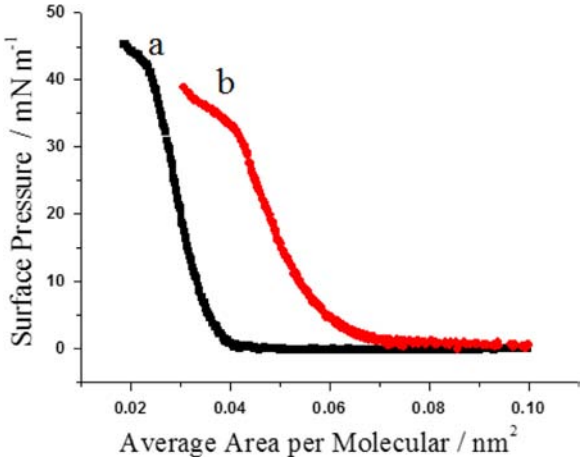
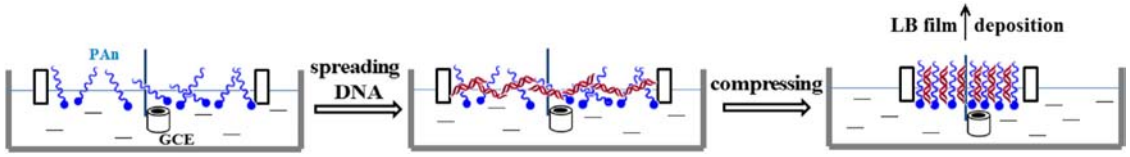


Fig. 1. π–A isotherms of PAn monolayer on subphase (pH=5) with absence (a) and presence of 1000 μL (b) of DNA.



Scheme 1. Schematic illumination of the DNA/PAn-LB/GCE fabrication process.

which hinders the diffusion of ferricyanide toward the electrode surface. These data demonstrated that the electrode was successfully modified as intended.

3.3. The morphology of DNA/PAn-LB

Fig. 3 shows SEM images of one layer of PAn-LB and DNA/PAn-LB film on Si surface. Under the selected transfer pressure, the PAn-LB film (Fig. 3a) shows well-spread particle-like surface morphology. At the DNA/PAn-LB film coated Si wafer (Fig. 3b), the density of the particles become lower. This is because the A_{lim} of PAn becomes larger after interacting with DNA, as mentioned in Section 3.1. Meanwhile, as in the compression process, DNA and PAn twined each other, particles in Fig. 3b appear to be of flocculent structure.

3.4. Electrochemical behavior of XA

3.4.1. Interaction between XA and DNA in solution

Differential pulse voltammetry was employed to investigate the interaction between XA and DNA. Fig. 4 shows the DPV curves in the presence of 2.0×10^{-5} mol L^{-1} XA (curve a) at different concentrations of DNA (curves b and d). With the addition of DNA, the peak of XA shifted to higher potential from 688 to 732 mV, which suggested that the interactive mode was intercalation, if a peak shift to lower potential was to be observed, then electrostatic binding could be inferred. As the XA can be bonded to DNA, we speculated that XA could be more effectively enriched on the surface of DNA modified electrode to improve detection sensitivity.

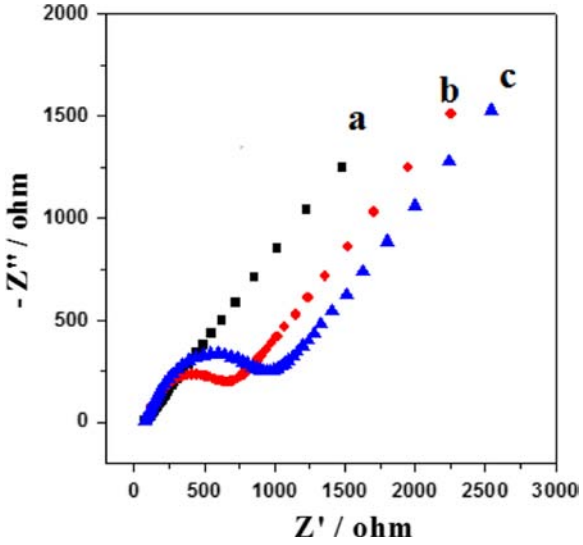


Fig. 2. Nyquist plots of EIS with different electrodes: (a) bare GCE; (b) one layer of PAn-LB/GCE;and (c) one layer of DNA/PAn-LB/GCE.

3.4.2. Cyclic voltammetric behavior of XA on DNA/PAn-LB/GCE

The CV curves of XA on different electrodes were presented in Fig. 5. At bare GCE, XA exhibited poor electrochemical response (curve b), whereas at the DNA/PAn-LB/GCE (curve d), a well-defined oxidation peak of XA was obtained and the peak current was about 3-fold and 25-fold large as that at PAn-LB/GCE (curve c) and bare GCE (curve b). These results indicated that DNA/PAn-LB/GCE possessed a more excellent electrochemical response to XA. This phenomenon could be explained by the interaction between XA and the surface-confined DNA, by which XA could be accumulated on the electrode surface effectively. Moreover, as Barton's researches pointed, the DNA molecule is considered as an array of stacked π electrons, which could provide a rapid pathway for electronic charge transport [31–33]. The oxidation process is not accompanied by a reduction wave, which indicates that the electrode reaction of XA is totally irreversible.

For all we know, the determination of XA usually encounters interference from UA and HXA. The response of XA with coexistence of UA and HXA was also investigated using the DNA/PAn-LB/GCE. As shown in Fig. 6, the anodic peak potentials of UA (0.37 V) and HXA (1.12 V) are far away from that of XA (0.75 V) at the proposed sensor, and the presence of UA and HXA does not modify the signal for XA significantly. So, we can conclude that the DNA/PAn-LB/GCE can be used for sensing XA selectively and eliminating the interference of UA and HXA effectively.

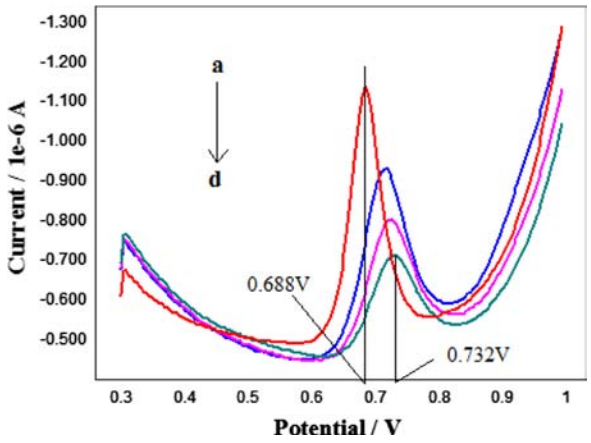


Fig. 4. DPV voltammograms of XA in the presence of DNA. C_{DNA}=0, 10, 20, 30 μg mL^{-1} for curves a–d; $C_{XA}=2.0\times10^{-5}$ mol L^{-1}.

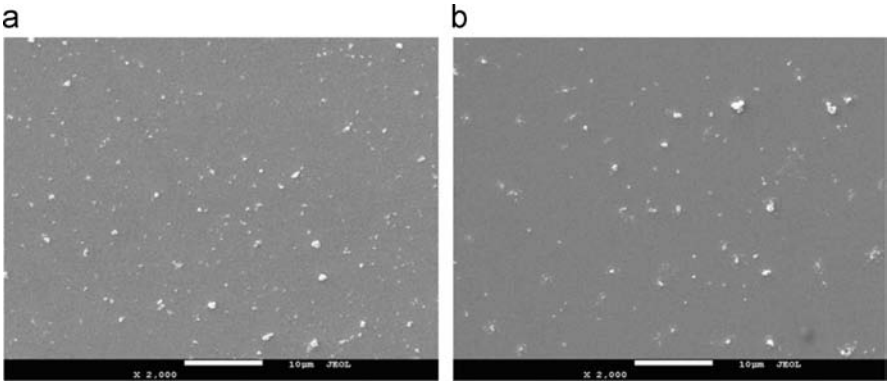


Fig. 3. SEM images of one layer of PAn-LB (a) and DNA/PAn-LB film (b) deposited on silicon wafer.

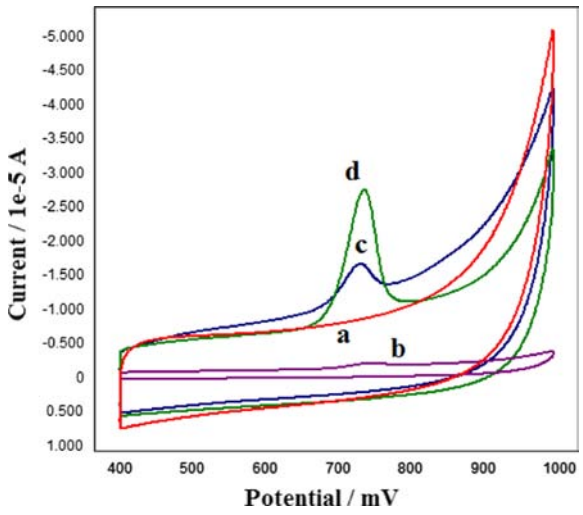


Fig. 5. Cyclic voltammograms of XA (5.0×10^{-5} mol L^{-1}) at different electrodes. (a) DNA/PAn-LB/GCE in blank PBS (pH 7.0); (b) bare GCE; (c) PAn-LB/GCE; and (d) DNA/PAn-LB/GCE. Scan rate: 100 mV s^{-1}.

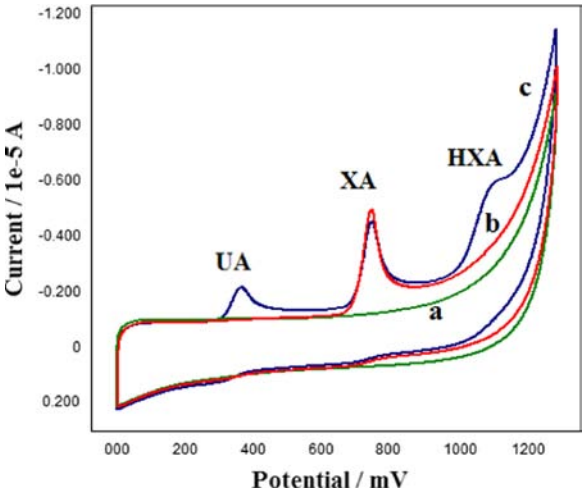


Fig. 6. Cyclic voltammograms of blank solution (curve a), 1.0×10^{-5} mol L^{-1} XA (curve b) and 3.0×10^{-5} mol L^{-1} UA+1.0×10^{-5} mol L^{-1} XA+4.0×10^{-5} mol L^{-1} HXA at DNA/PAn-LB/GCE in PBS solution (pH 7.0).

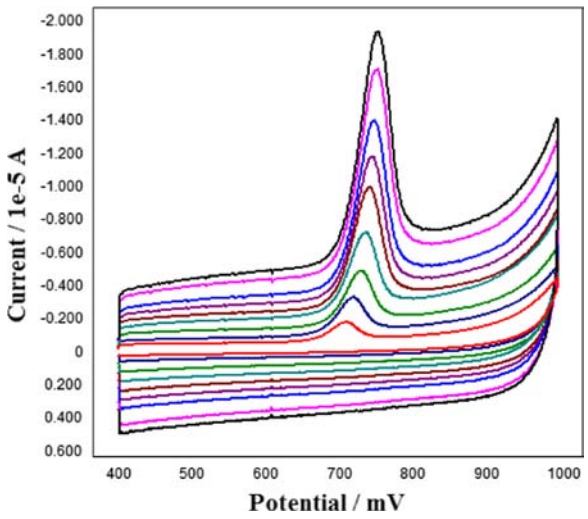


Fig. 7. Cyclic voltammograms of 2.0×10^{-5} mol L^{-1} XA in pH 7.0 PBS solution at DNA/PAn-LB/GCE with different scan rates from 10 to 250 mV s^{-1} (from inner to outer).

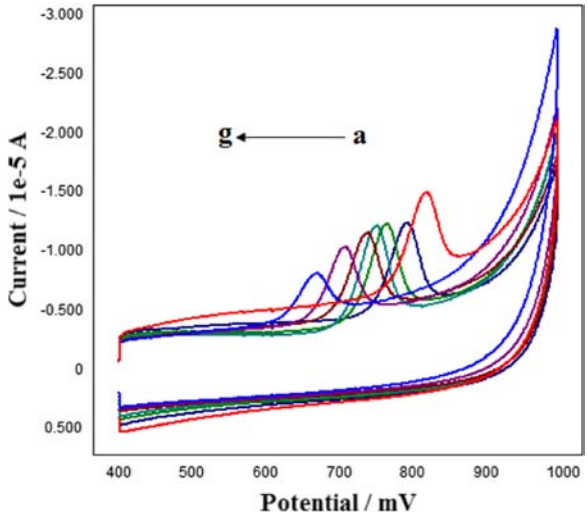


Fig. 8. Cyclic voltammograms of 2.0×10^{-5} mol L^{-1} XA at different solution pH of PBS. pH: (curve a to g) 5.8, 6.2, 6.6, 7.0, 7.4, 7.8, 8.0.

Next, reaction characters of XA on the DNA/PAn-LB/GCE were investigated in detail. CV was performed and scan rate (ν) was changed in a stationary concentration of XA solution (2.0×10^{-5} mol L^{-1}). From Fig. 7 we can see that, the anodic peak potentials were positive shifted slightly with increasing ν, and the anodic current was linear with ν in the range of 10–250 mV/s with linear equation of ipa=1.011+0.0708 ν (R=0.995). These data demonstrate that the redox of XA at DNA/PAn-LB/GCE is an adsorption-controlled irreversible process. Based on Laviron's theory for an adsorption-controlled irreversible electrode process, the E_p–ν relation can be described using the following equation: $E_p = E^0 - (RT/(1-\alpha)nF)\ln\nu$. Here, the E_p–ln ν relation of XA was described as E_p=0.781+0.015 ln ν (r=0.999). From this relation, a value of 4 was determined as the electron transfer number of XA at DNA/PAn-LB/GCE.

The pH value of the solution affects the sensitivity of determination and changing the solution pH can be used to detect the H^+ number taking part in the electrode reaction. In this system, investigations were performed in the pH range of 5.8–8.0 PBS solutions and the effect on the CV voltammograms is shown in Fig. 8. The anodic potential of XA was shifted negatively with increasing solution pH, indicating that the H^+ taken part in the electrode reaction of XA at the DNA/PAn-LB/GCE and the anodic process of XA was a losing of H^+ reaction. The relationship between the anodic peak potential and pH could be fitted into a regression equation of E_p (V)=1.213−0.065pH, with a correlation coefficient of 0.998. A value of the slope is −65 mV per pH unit (theoretical value of −59 mV), indicating equal number of electrons and protons involved in the electrode process of XA. That is, XA lose 4 electrons and 4 protons during its oxidation process at the sensor surface. It was also found that the current of XA was stable in the pH range of 6.2–7.4, considering that healthy human blood is weakly alkaline, pH 7.0 PBS solution was chosen for the following study.

3.4.3. Chronocoulometry studies

For an adsorptive controlled electrode process, it is necessary to calculate the saturated absorption capacity of analyte on the electrode surface. The chronocoulometry is the best technique to do this work. In this system, the DNA/PAn-LB/GCE was immersed in XA solution (5.0×10^{-6} mol L^{-1}) for several minutes to achieve saturated absorption. And then, a step potential from 0.4 V to 0.96 V was applied and the Q–t relation was recorded

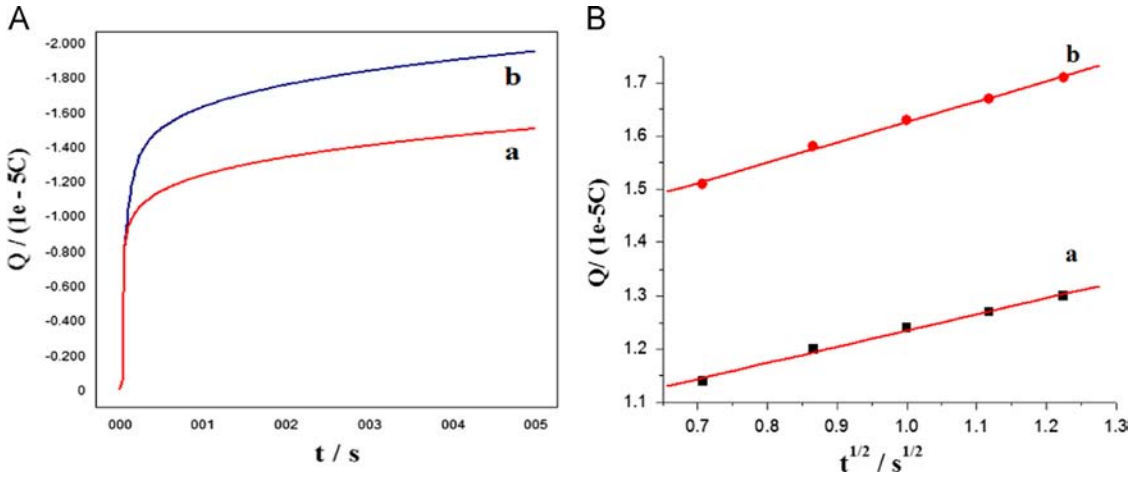


Fig. 9. (A) Chronocoulogram of XA at DNA/PAn-LB/GCE and (B) corresponding Q–$t^{1/2}$ plots. Concentration of XA: (a) 0 and (b) 5.0×10^{-6} mol L^{-1}.

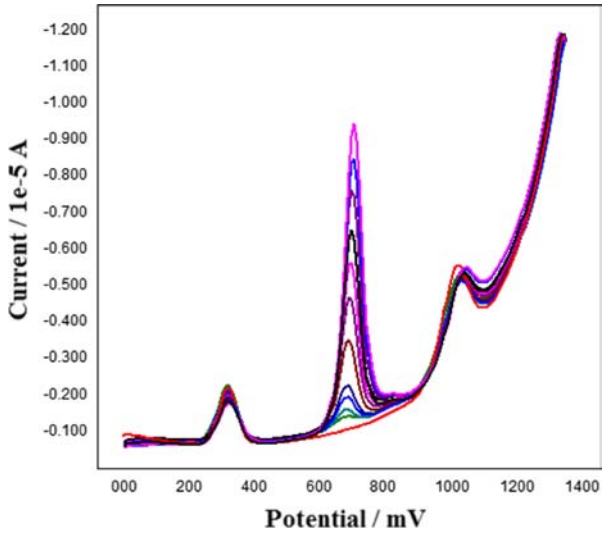


Fig. 10. Differential pulse anodic voltammograms recorded at DNA/PAn-LB/GCE in the XA concentrations of 7.0×10^{-8}–1.0×10^{-5} mol L^{-1}, containing 7.0×10^{-6} mol L^{-1} UA and 1.0×10^{-5} mol L^{-1} HXA in pH 7.0 PBS.

(Fig. 9A, curve b). For control, Q–t curve was recorded in blank PBS solution, too (Fig. 9A, curve a). The corresponding Q–$t^{1/2}$ plots were also performed and are shown in Fig. 9B. As shown in Fig. 10B, the control and XA plots have the same slope values, meaning no XA diffusion occurred on the electrode surface. The intercept difference between curve a and b in Fig. 9B was the electric quantity (Q) from the oxidation of adsorbed XA at the sensor surface. According to Laviron's theory, $Q=nFA\Gamma^*$, here, n is the electron transfer number and A is the electrode area, so the Γ^* value of XA on the DNA/PAn-LB/GCE was 1.15×10^{-10} mol cm^{-2}.

3.5. Analytical applications

3.5.1. Accumulation conditions

In consideration of detection sensitivity and the fact of XA adsorbed on the DNA/PAn-LB/GCE surface, differential pulse voltammetry (DPV) coupled with accumulation procedure was employed in researching the analytical method. For the system containing 1.0×10^{-6} mol L^{-1} XA, the accumulation potential (E_{acc}) was no more effective on the response of peak currents, so the accumulation of XA was carried out under open circuit. And the DNA/PAn-LB/GCE surface reached adsorption saturation under 60 s of accumulation time (t_{acc}). For consideration to the detection limit and linear range, we chose 30 s as the t_{acc} in the following study.

Table 1
Comparison of voltammetric response of different modified electrodes for XA determination.

Electrode	Linear range (mol L^{-1})	Detection limit (mol L^{-1})	Refs.
ERGO/GCE	5.0×10^{-6}–3.0×10^{-4}	1.1×10^{-7}	[23]
Poly-BCP/GCE	1.0×10^{-7}–1.0×10^{-4}	6.0×10^{-8}	[24]
Co_3O_4/MWCNTs/CS/GCE	2.0×10^{-7}–1.6×10^{-5}	2.0×10^{-7}	[25]
Lt/fMWCNT/MGCE	1.0×10^{-6}–7.0×10^{-5}	6.5×10^{-7}	[26]
XOD/l-Cys/AgNPs/Au	2.0×10^{-6}–1.6×10^{-5}	1.5×10^{-7}	[27]
DNA/PAn-LB/GCE	7.0×10^{-8}–1.0×10^{-5}	3.0×10^{-8}	This work

3.5.2. Determination of XA in the presence of UA and HXA

In the presence of large amount of UA (7.0×10^{-6} mol L^{-1}) and HXA (1.0×10^{-5} mol L^{-1}), DPV i–E curves exhibited a linear relationship between the anodic peak current and XA concentration within the range of 7.0×10^{-8}–1.0×10^{-5} mol L^{-1} (Fig. 10). The linear regression equation and correlation coefficient are:

$$i_p(\mu A) = 0.790 + 0.064\times10^6 C \quad (r = 0.998)$$

The lowest detectable concentration of XA was estimated to be 3.0×10^{-8} mol L^{-1}. For testing the electrode reproducibility, we renew the modified electrode surface in blank PBS (pH 7.0) solution for stirring 60 s to eliminate memory effect and then test the response again in XA solution (1.0×10^{-6} mol L^{-1}). The comparison of this method with other electrochemical methods for the determination of XA was listed in Table 1. From Table 1, it can be seen that this method has the comparable sensitivity and detection limit. The R.S.D. of five successive measurements was calculated to be 2.51%, which demonstrated the good reproducibility of the proposed sensor. To assess the consistency of DNA/PAn-LB/GCE, cyclic voltammetric scanning was recorded on day one and a week later respectively in a same XA solution. Peak currents decreased by 5.8%, which indicated that DNA/PAn-LB/GCE has good stability.

3.5.3. Interference studies

The main interference in real sample, UA and HXA, were discussed above. Other various possible interfering species were evaluated also, with a fixed XA concentration. The experiment results showed that no interference could be observed for the

Table 2
Determination results of XA in human blood serum.

Sample	Original found (10^{-6} mol L^{-1})	Added (10^{-6} mol L^{-1})	Total found (10^{-6} mol L^{-1})	Recovery (%)	R.S.D. (%)
1	1.51	1.00	2.53	101.74	1.92
2	1.38	1.50	2.90	101.16	1.29
3	1.66	2.50	4.21	101.99	2.23

following organic compounds: ascorbic acid (up to 100-fold), glucose (up to 100-fold), dopamine (up to 10-fold), epinephrine (up to 5-fold), theophylline (up to 5-fold), caffeine (up to 5-fold) and adenine (up to 5-fold). At the same time, some inorganic species, such as 50-fold excess of Fe (III), Pb (II) and 100-fold excess of Ca (II), Mg (II) and Zn (II) had no interference. The results indicate the present method was adequate for the determination of XA in real samples.

3.5.4. *Determination of XA in samples*

For the possible use of the current method in the physiological sample and evaluation of its background interference, human blood serum was selected as a sample for testing XA. The standard addition technique was used for the determination of XA in serum samples. The human blood serum samples were diluted to 50 times using 0.2 mol L^{-1} PBS solution (pH 7.0). Some XA standard solution was added in the blood serum before analysis. And the recovery was determined by the standard addition method. The results of determination are listed in Table 2. The mean recovery of XA was 101.63% for three independent determinations.

4. Conclusion

In conclusion, a new DNA voltammetric sensor, based on DNA–polyaniline complex film modified glassy carbon electrode, was fabricated by Langmuir–Blodgett technique. The sensor has a sensitive electrochemical response to xanthine with excellent stability and a wide linear range. At the same time, the modified electrode exhibits excellent selectivity for XA detection with good repeatability, being free of interference from excess UA and HXA. The method was further utilized for the detection of XA in human blood serum samples with satisfactory results. This research suggested that DNA modified electrode with LB techniques might be a promising platform for determination of purine derivatives in serum samples.

Acknowledgments

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