Analyst

RSC Publishing

PAPER

View Article Online
View Journal | View Issue



Cite this: *Analyst*, 2013, **138**, 1141

# A novel dsDNA/polydiphenylamine-4-sulfonic acid electrochemical biosensor for selective detection of the toxic catechol and related DNA damage

Pingping Wang,[ab] Yongnian Ni*[ab] and Serge Kokot*[c]

A novel biosensor consisting of a glassy carbon electrode (GCE) modified by a polydiphenylamine-4-sulfonic acid (PDPASA, conjugated polymer) film and double-stranded DNA (dsDNA), *i.e.* dsDNA/PDPASA/GCE, was researched and developed for the analysis of catechol – a potentially toxic substance for humans and the environment. The surface properties of the PDPASA film, particularly after dsDNA was immobilized on it, were characterized with the use of atomic force microscopy (AFM), electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). The surfaces of the novel DNA/PDPASA/GCE biosensor changed during the fabrication process and displayed high sensitivity for catechol. The oxidation potential of catechol decreased significantly and the corresponding current increased substantially as compared with the values obtained at the GCE alone and at the dsDNA/GCE. Also, with the addition of hydroquinone, two well discriminated CV peaks were obtained, and it was demonstrated that hydroquinone did not interfere with catechol. DPV analysis produced a linear catechol calibration (range: 0.750 to 8.25 $\times$ $10^{-6}$ mol $L^{-1}$; detection limit: 6.48 $\times$ $10^{-7}$ mol $L^{-1}$), and thus, various water samples were analysed successfully by this novel method. In addition, the DNA/PDPASA/GCE was used to study DNA damage in the presence of catechol with the use of the $Co(phen)_3^{3+}$ electroactive probe. Results indicated that the potentially toxic catechol and its metabolites were all responsible for DNA damage.

Received 26th September 2012
Accepted 3rd December 2012

DOI: 10.1039/c2an36389e

**www.rsc.org/analyst**

## 1 Introduction

In general, many chemical, biochemical and physical properties of deoxyribonucleic acid (DNA) are well known and documented,[1] and as a result, DNA-based diagnostics have been widely investigated in fields such as genetics, toxicology, pharmacology, food safety and pathology.[2–5] Consequently, a variety of DNA biosensors based on different techniques have been researched and developed, *e.g.* surface plasmon resonance spectroscopy,[6,7] fluorescence,[8] electrochemiluminescence,[9] and conventional aerosol technology.[10] Other important analytical applications involve electrochemical DNA biosensors; these have received particular attention because they can be adapted for many different analytes, and also because they can be miniaturized. The associated analytical methods are usually quite fast and comparatively cheap. Since DNA is electroactive,[11] some electrochemical methods, especially those involving electrochemical DNA biosensors, have been reported and applied.[12–15]

In recent years, conducting polymers have been used in conjunction with electrochemical DNA biosensors in polymer-based sensing devices.[16–18] The semiconducting polymer, polyaniline (PANI) has been particularly noted because it is highly electro-active, biocompatible, relatively stable in the environment, and readily available.[19,20] It was reported that the positively charged amino group of PANI provided the ability to form polyelectrolyte complexes with DNA by electrostatic interactions,[21] and the PANI polymer can be covalently linked to the DNA probes by forming phosphoramidate bonds between the amino group of the PANI and the phosphate group of the DNA.[22] Hence, DNA/PANI biosensors were investigated and used for further experiments.[23–25] However, it was also discovered that the electropolymerization of PANI only occurs in acidic, aqueous or non-aqueous media,[26] and this restriction limits its application as a DNA biosensor. Furthermore, other potential applications of PANI have been restricted by its insolubility, infusibility as well as its hygroscopic nature.[27] Introduction of substituents into the benzene ring or at the nitrogen atom of aniline were shown to improve the polymeric properties of PANI,[28–30] *e.g.* (1) the introduction of sulfonic acid groups into the benzene ring of the aniline improved the plasticity and

[a]*State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, China*

[b]*Department of Chemistry, Nanchang University, Nanchang 330031, China. E-mail: ynni@ncu.edu.cn; Fax: +86 791 83969500; Tel: +86 791 83969500*

[c]*School of Chemistry, Physics and Mechanical Engineering, Science and Engineering Faculty, Queensland University of Technology, Brisbane 4001, Australia. E-mail: s.kokot@qut.edu.au*

protonation so as to facilitate the electropolymerization of PANI in weakly acidic or neutral solutions;[31] (2) poly(diphenylamine), which is formed by the addition of an aromatic substituent on the nitrogen atom of aniline,[32] has a better solubility and processability, and was used to fabricate a new glucose biosensor.

In this study, the aniline derivative diphenylamine-4-sulfonic acid (DPASA, Fig. 1)[33] was investigated as a possible alternative coating to PANI to fabricate a novel DNA biosensor. This polymer is positively charged in acid solution. Hence, as a water-soluble cationic conjugated polymer, it could be readily grafted onto DNA by an electrostatic interaction.[34]

1,2-Dihydroxybenzene or catechol is a phenolic compound, which exists naturally in fruit, vegetables, and in some traditional Chinese medicines (TCM).[35] Its applications include photography, cosmetics, antiseptics and pharmaceuticals.[36] Nevertheless, catechol is regarded as an organic pollutant with toxic implications for human health and the environment.[37] Hence, simple, sensitive and inexpensive analytical methods would be useful. Electrochemical methods of analysis are well known for their rapid and sensitive detection procedures as well as for their low operational costs. Consequently, analytical methods involving various types of electrode have been developed for the determination of substances such as catechol. Wang *et al.*[38] developed a poly(diallyldimethylammonium chloride)-functionalized graphene-modified electrode for the simultaneous determination of catechol and hydroquinone (linear concentration range and limit of detection (LOD) of catechol were 1 to 500 $\times$ $10^{-6}$ mol $L^{-1}$ and 0.2 $\times$ $10^{-6}$ mol $L^{-1}$, respectively). Hu *et al.*[37] applied a reduced graphene and multiwall carbon electrode for the detection of catechol, hydroquinone, *p*-cresol and nitrite (the LOD of catechol was 1.8 $\times$ $10^{-6}$ mol $L^{-1}$). Kong *et al.*[39] described an expanded graphite electrode modified with intercalated montmorillonite for the determination of catechol (the LOD was 1.13 $\times$ $10^{-6}$ mol $L^{-1}$). From these studies it was found that catechol is often together with hydroquinone in the environment, and since the latter molecule is structurally similar to catechol, it often interferes in its analysis.[40] This problem has been recently addressed with the use of electrochemical sensors, which were able to analyse simultaneously the catechol and hydroquinone analytes.

In addition, catechol has been classified as a possible human carcinogen (group 2B) by the International Agency for Research on Cancer (IARC).[41] Although many studies have investigated its genotoxicity, the mechanism of DNA damage induced by catechol is still unclear although it has been suggested that the oxidation products of catechol, semiquinone and quinone, were more damaging than their precursor and, also, could damage DNA.[42,43]

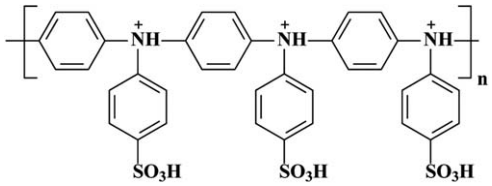


**Fig. 1** Structure of PDPASA.

The aims of this study were:

(1) to construct and characterize a novel dsDNA/polyDPASA electrochemical biosensor;

(2) to apply this modified electrode for the analysis of catechol in the presence of hydroquinone, and evaluate the performance of the method;

(3) to use this biosensor for the evaluation of DNA damage caused by both catechol and its redox products with the use of $Co(phen)_3^{3+}$ as an electrochemical probe.

## 2 Experimental

### 2.1 Reagents and chemicals

Stock solutions of catechol and hydroquinone (5.0 $\times$ $10^{-3}$ mol $L^{-1}$) were prepared by dissolving each crystalline compound (Analytical grade reagent, 99%, Aladdin Co., Shanghai, China) in 100.0 mL distilled water, and mixed thoroughly. Phosphate buffer solution (PBS, pH = 6.80) was prepared by mixing suitable amounts of 0.1 mol $L^{-1}$ $KH_2PO_4$ with $Na_2HPO_4$. Double-stranded calf thymus DNA (dsDNA) solution (0.5 mg $mL^{-1}$) was obtained by dissolving solid DNA (50.0 mg; Type 1, D1501, Sigma Chemical Co., St. Louis, MO, USA) in 100 mL PBS containing 25.0 mmol $L^{-1}$ sodium chloride. Sodium diphenylamine-4-sulfonate stock solution (2.0 $\times$ $10^{-3}$ mol $L^{-1}$) was prepared by dissolving its crystals (Sigma Chemical Co., St. Louis, MO, USA) in 100 mL 0.1 mol $L^{-1}$ PBS. $Co(phen)_3^{3+}$ solution (1 $\times$ $10^{-4}$ mol $L^{-1}$) was prepared by dissolving a suitable amount of $[Co(phen)_3](ClO_4)_3 \cdot 2H_2O$ (yellow crystals, synthesized according to ref. 44) in 100 mL PBS solution. All other chemicals were Analytical grade reagents and doubly distilled water was used throughout. Indium tin oxide (ITO) was purchased from Zhuhai Kaivo Electronic Components Co., Zhuhai, China.

### 2.2 Instrumentation

Cyclic voltammetry (CV), differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) experiments were performed using a CHI 660A electrochemical workstation (CH instruments, Shanghai, China). A three-electrode system was used, in which a glassy carbon electrode (GCE; 3 mm diameter) or a modified GCE served as the working electrode, a saturated calomel electrode as the reference electrode and a platinum wire as the auxiliary electrode. Atomic force microscopy (AFM) imaging was carried out with the use of an AJ-V model in the tapping mode (Aijian Nanotechnology Co., Shanghai, China). Pyramidal cantilevers with probes were used at a scanning frequency of 1 Hz. All experiments were carried out at 25 $\pm$ 0.5 °C.

### 2.3 Immobilization of dsDNA/PDPASA on the GCE or indium tin oxide

Prior to the modification treatment, the GCE was polished sequentially to a mirror-like shine with 1.0, 0.3 and 0.05 μm alumina slurry on polishing pads; then, the electrode was sonicated, in turn, in 0.5 mol $L^{-1}$ dilute nitric acid, ethanol and deionized water, for 15 min in each medium. Such a polished

 

GCE was subjected to potential cycling (−1.0 to 1.0 V) with a scan rate of 100 mV $s^{-1}$ in 0.25 mol $L^{-1}$ sulfuric acid until reproducible CV profiles were obtained. This electrode was then immersed into the PBS containing $2 \times 10^{-3}$ mol $L^{-1}$ sodium diphenylamine-4-sulfonate. The PDPASA film was then electrochemically deposited by the CV method between −0.5 and 2.2 V with a scan rate of 100 mV $s^{-1}$ for 16 cycles (these conditions were investigated and optimized beforehand). During the electropolymerization process, diphenylamine-4-sulfonate is known to produce the amine radical cation, and this species can form a carbon–nitrogen bond on the carbon electrode. Consequently, PDPASA could be stabilized on the GCE by forming a C–N covalent bond.[45] After electropolymerization, the modified electrode was rinsed thoroughly with distilled water in readiness for further application. Subsequently, a 20.0 μL aliquot of 0.5 mg $mL^{-1}$ dsDNA solution was deposited on the modified film and dried for 8 h in air at 25 ± 0.5 °C. Finally, this new fabricated biosensor was washed with deionized water and immersed in PBS (pH = 6.80) for 2 min to remove any loosely adsorbed DNA before further experiments. Then, CV and EIS analyses were performed to characterize the fabrication process of the electrode in the $[Fe(CN)_6]^{3-/4-}$ solution ($5.0 \times 10^{-3}$ mol $L^{-1}$, containing 0.1 mol $L^{-1}$ KCl).

The ITO substrate also required cleaning prior to deposition, and generally it was submitted to ultrasonication in ethanol (10 min), acetone (10 min) and finally deionized water (15 min). After drying in air, the immobilization of PDPASA and dsDNA on ITO was performed similarly to the above process with the GCE.

### 2.4 Quantitative analysis of catechol

**Different electrodes – sensitivity check.** 100 μL of $5.0 \times 10^{-3}$ mol $L^{-1}$ catechol was transferred into a 10.0 mL electrolysis cell containing 7.0 mL PBS (pH = 6.80) and 3.0 mL deionized water to obtain a $5 \times 10^{-5}$ mol $L^{-1}$ solution. CV was performed from −0.2 to 0.8 V with a scanning rate of 100 mV $s^{-1}$ to detect the catechol, with the use of the GCE, dsDNA/GCE and dsDNA/PDPASA/GCE, in turn.

**Determination of catechol in the presence of hydroquinone.** The dsDNA/PDPASA/GCE was immersed in 10.0 mL solution (7.0 mL PBS at pH = 6.80 and 3.0 mL deionized water) containing suitable amounts of catechol, hydroquinone or a mixture of components, and then CV analysis was performed over the potential range of −0.2 to 0.6 V with a scanning rate of 100 mV $s^{-1}$.

**Calibration and analysis of catechol in water samples.** A series of catechol solutions with different concentrations were prepared by mixing the suitable $5 \times 10^{-4}$ mol $L^{-1}$ catechol solution with 10 mL electrolyte (7.0 mL PBS at pH = 6.80 and with 3.0 mL deionized water added); then, a calibration for the analysis of catechol was developed with the use of the dsDNA/PDPASA biosensor and DPV (from 0 to 0.3 V, with a pulse width of 60 ms and a 50 mV pulse amplitude).

Three kinds of water sample (tap water, Ganjiang river water, and Aixi lake water) collected from Nanchang city sources, were boiled for 5 min to remove any dissolved carbon dioxide. After cooling, these samples were filtered (filter paper: quantitative, medium speed #202, Wohua Co., Hangzhou, China) and stored in a refrigerator (4 °C) for further analysis. Each treated water sample (3.0 mL) was added to the cell containing 7.0 mL PBS, and then each sample was spiked with a series of catechol standard solutions (for the added aliquots see Table 1, columns two and three). These samples were then analysed for catechol with the use of the DPV method (from −0.4 to 0.4 V, with a pulse width of 60 ms and a 50 mV pulse amplitude).

### 2.5 Indirect investigation of DNA damage induced by catechol and its products

The dsDNA/PDPASA/GCE was immersed in 10 mL of $1.0 \times 10^{-4}$ mol $L^{-1}$ $Co(phen)_3^{3+}$ solution (pH = 6.80) for 10 min, and then DPV analysis was carried out in the 0.4 to −0.1 V range with a pulse width of 60 ms and a 50 mV pulse amplitude.

This electrode was rinsed by deionized water three times and then either immersed for 10 min or cycled 40 times from −0.2 to 0.6 V in a catechol solution ($5.0 \times 10^{-3}$ mol $L^{-1}$). Finally, the electrode was subjected to DPV analysis as described above.

## 3 Results and discussion

### 3.1 Morphology of modified electrodes: AFM studies

The dsDNA/PDPASA/ITO was prepared as previously described (Section 2.3) and AFM images of the morphology of this modified electrode were obtained with the use of the ITO substrate. Two- and three-dimensional AFM images of the ITO alone, PDPASA/ITO and DNA/PDPASA/ITO films were recorded (Fig. 2). The surface of the ITO was quite rough, consisting of many irregular grains with diameters in the range of about 40–70 nm (Fig. 2A-1 and B-1); similar grain sizes on ITO samples have been reported elsewhere.[20] After electropolymerization of PDPASA on the ITO for 16 cycled, potential scans, the surface morphology (Fig. 2A-2 and B-2) changed from an irregular, spiky surface to a flattened one. This observation was consistent with a film, in this case PDPASA, being deposited on the ITO because of the electrostatic effect,[34] and DNA could be successfully immobilized on the PDPASA film. The smoother but apparently thicker, more layered morphology (Fig. 2A-3 and B-3) was observed after the reaction with DNA, and this image was consistent with the immobilization of this biopolymer.

### 3.2 Electrochemical characterization of the modified electrode: CV and EIS

Electrochemical stepwise modification of the GCE electrode was characterized with the use of CV; the electrode was immersed in

**Table 1** Recoveries (%) of the added catechol in water samples at the DNA/PDPASA/GCE

| Samples | Added/ μmol $L^{-1}$ | | Found/ μmol $L^{-1}$ | | Recovery (%) | |
|---|---|---|---|---|---|---|
| Tap water | 3.00 | 7.00 | 2.80 | 6.90 | 93.3 | 98.6 |
| Ganjiang river water | 4.00 | 7.00 | 3.90 | 7.20 | 97.5 | 103 |
| Aixi lake water | 4.00 | 8.00 | 3.80 | 8.10 | 95.0 | 101 |

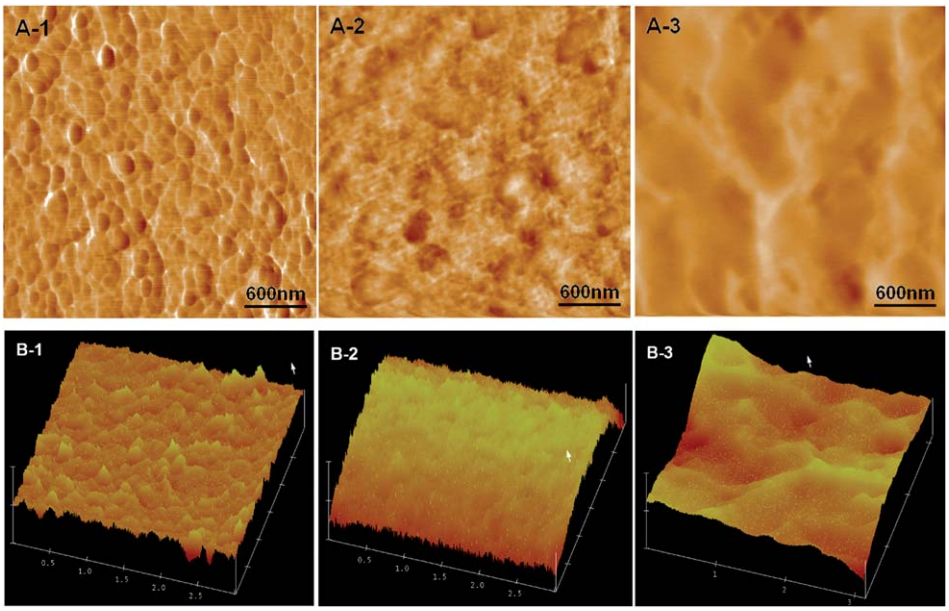


**Fig. 2** Two-dimensional (A) and three-dimensional (B) AFM images for surfaces of (1) untreated ITO, (2) PDPASA/ITO, and (3) dsDNA/PDPASA/ITO.

0.1 mol $L^{-1}$ KCl solution containing $5.0 \times 10^{-3}$ mol $L^{-1}$ $[Fe(CN)_6]^{3-/4-}$, and the CV profile (−0.1 to 0.5 V) displayed two well formed redox peaks reflecting oxidation (0.24 V) and reduction (0.16 V) reactions (Fig. 3A, curve a), *i.e.* the peaks were about 80 mV apart. However, after the electropolymerization of PDPASA on the GCE, the redox peak currents of $[Fe(CN)_6]^{3-/4-}$ clearly decreased (Fig. 3A, curve b); this probably can be attributed to the repulsion between the sulfonic groups of PDPASA.[46] Also, another rather unexpected profile was observed (Fig. 3A, curve c), and was attributed to the redox responses of a DNA-modified electrode interacting with $[Fe(CN)_6]^{3-/4-}$ ions in solution; the small but definite signal was most likely due to the negatively charged DNA biosensor. The charge distribution on the PDPASA film (Fig. 1), suggested that the whole DNA/PDPASA/GCE interaction may be understood if it is considered that the positive charges on the amino groups are embedded in the polymer structure, and the existence of the hydrogen bond between PANI and DNA ensures the immobilization of the DNA on the PDPASA film. The peak current profiles of $[Fe(CN)_6]^{3-/4-}$ at the DNA/PDPASA/GCE (Fig. 3A, curve d), which were different from the peaks at the DNA/GCE and PDPASA/GCE, demonstrated that DNA was attached to the PDPASA film.

Further support for the above argument was provided by an EIS investigation of the electron transfer properties of the differently modified electrodes. EIS Nyquist plots (Fig. 3B) of the GCE (curve a), PDPASA/GCE (curve b), dsDNA/GCE (curve c) and dsDNA/PDPASA/GCE (curve d) in 0.1 mol $L^{-1}$ KCl solution containing $5.0 \times 10^{-3}$ mol $L^{-1}$ $[Fe(CN)_6]^{3-/4-}$ in the frequency range of 0.1 to 100 000 Hz reflected the different behavior of the electrodes. In such diagrams, the curvature of the EIS profiles at higher frequencies corresponds to an electron transfer limited process and the linear behavior at lower frequencies is indicative of a diffusion process. Also, the larger the curvature is, the slower is the electron transfer.[47] For the GCE alone (Fig. 3B, curve a), almost a straight line was observed. For the PDPASA/GCE (Fig. 3B, curve b), the plot is a curve and the measured $Z$ (ohm) values are relatively quite high ($R_{ct} \geq 8000\ \Omega$); presumably, these observations are the result of the repulsion between the sulfonic groups of PDPASA and the redox probe. The $R_{ct}$ of

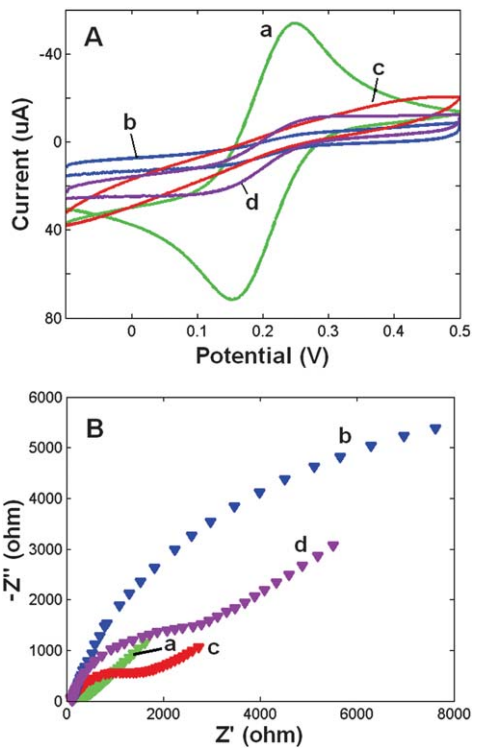


**Fig. 3** Cyclic voltammograms (A) and EIS (B) of (a) untreated GCE, (b) PDPASA/GCE, (c) dsDNS/GCE and (d) dsDNA/PDPASA modified GC electrodes recorded in 0.1 mol $L^{-1}$ KCl solution containing $5.0 \times 10^{-3}$ mol $L^{-1}$ $[Fe(CN)_6]^{3-/4-}$. Scan rate: 100 mV $s^{-1}$.

the DNA/GCE was estimated to be 800 Ω higher than that of the GCE, which indicated that the negatively charged phosphate backbone of the DNA could repel $[Fe(CN)_6]^{3-/4-}$ at the surface of the electrode. When the DNA interacted with the PDPASA film, the $R_{ct}$ value reached 1300 Ω, and this supported the view that the DNA was immobilized on the PDPASA film.

### 3.3 Electrochemical behavior of catechol and hydroquinone

The CV profiles of the $5 \times 10^{-5}$ mol $L^{-1}$ catechol at the GCE, DNA/GCE and DNA/PDPASA/GCE in 0.1 mol $L^{-1}$ PBS (pH = 6.80; Fig. 4A) indicated that catechol had sharp reversible peaks (0.2 V) on the profile obtained from the DNA/PDPASA/GCE (curve c). In comparison, the CV profiles from the GCE alone also had two reversible peaks, but in this case, the electrochemical response of catechol was very weak (curve a). The CV profile from the DNA/GCE indicated two weak, hitherto undetected peaks of catechol (curve b); all of these observations demonstrated that the analysis of catechol at the DNA/PDPASA/GCE produced significantly more sensitive results as indicated by the relatively high peak intensities of the CV responses. This could have been facilitated by the comparatively high conductivity of PDPASA.

Cyclic voltammograms of the mixture of $5 \times 10^{-5}$ mol $L^{-1}$ catechol and $1.1 \times 10^{-4}$ mol $L^{-1}$ hydroquinone in PBS at the GCE, DNA/GCE and DNA/PDPASA/GCE (Fig. 4B) indicated that at the GCE and DNA/GCE (Fig. 4B, curve a and curve b), two broad oxidation and reduction peaks were present at 0.35 and −0.12 V, respectively. However, two reversible peaks were observed on the CV profiles when the DNA/PDPASA/GCE (Fig. 4B, curve c) was used. It has been demonstrated (Fig. 4A) that peak 2 at 0.2 V represents the oxidation of catechol, and, therefore, the reversible peak 1 at 0.1 V can be attributed to the oxidation of hydroquinone. These observations indicated that the DNA/PDPASA/GCE has a significant electrocatalytic effect on catechol; this effect may be facilitated by the hydrogen bond between the PDPASA film and catechol. The existence of the hydrogen bond (N···H–O) between PDPASA and catechol played an important role. It weakened the effect of the hydroxyl groups, and because there are two adjacent hydroxyl groups on a catechol molecule, this substance is more easily oxidized than hydroquinone. Thus, the novel biosensor, DNA/PDPASA/GCE has a significant selectivity for catechol, and this can be useful for the analysis of this substance.

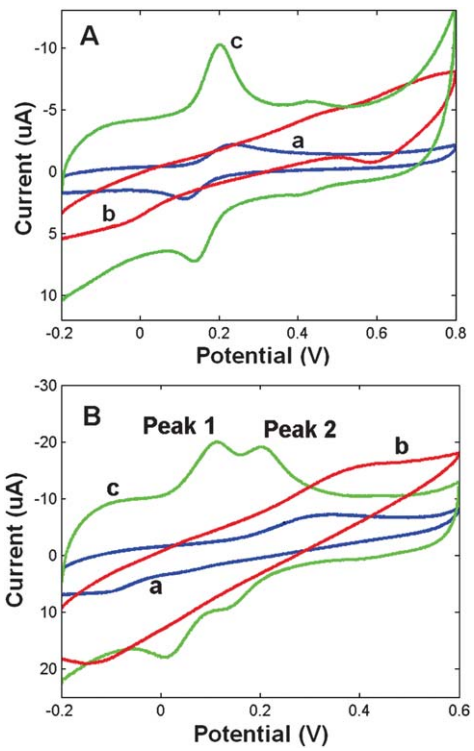


**Fig. 4** Cyclic voltammograms of $5 \times 10^{-5}$ mol $L^{-1}$ catechol (A) and $1.1 \times 10^{-4}$ mol $L^{-1}$ hydroquinone (B) in pH = 6.80 PBS at different electrodes: (a) untreated GCE, (b) DNA/GCE and (c) DNA/PDPASA/GCE; scan rate: 100 mV $s^{-1}$.

### 3.4 Interference study, quantitative analysis, and analytical application of catechol

Although the DNA/PDPASA/GCE demonstrated electrocatalytic activity, which discriminated between the catechol and hydroquinone peaks, it was still unclear whether or not hydroquinone was an interferent in the analysis of catechol. Therefore, the CV behavior of the $5 \times 10^{-5}$ mol $L^{-1}$ solutions of catechol, hydroquinone and their mixture was investigated at the DNA/PDPASA/GCE in the PBS (pH = 6.80). The overlay of the three types of sample profile, respectively demonstrated that the catechol profile (Fig. 5A, peak 2, green) was discriminated from the hydroquinone profile (Fig. 5A, peak 1, blue) and both of these profiles corresponded well with the two peaks evident in the mixture profile (red), *i.e.* the mixture profile was the sum of the two single component profiles (Fig. 5A). In addition, when a series of hydroquinone samples of different concentrations was added to a solution of a given concentration of catechol, the peak current profile remained effectively constant (Fig. 5B). It has been reported that the voltammogram of another isomer of benzenediol, resorcinol, could be completely separated from the voltammograms of catechol and hydroquinone at pH = 7.0 (PBS) on an untreated glassy carbon electrode.[48] For other related phenolic compounds such as phenol, *p*-cresol, *p*-nitrophenol, and aminophenol, their peak potential was reported at 0.65, 0.62, 0.06, 0.58–0.907 V in pH 7.0, 7.0, 6.0, 6.0 buffer solution, respectively on the GCE.[37,49–52] Thus, most phenolic compounds should not interfere in the determination of catechol (pH 6.8, 0.2 V, in this work, on the GCE). This indicated that DPV calibrations could be constructed for the determination of catechol in water samples irrespective of the presence of hydroquinone in the samples.

The DPV calibration set from 12 catechol samples was collected at the DNA/PDPASA/GCE (Fig. 6), and it was demonstrated that the peak current increased linearly with the catechol concentration in the range of 0.750 to $8.25 \times 10^{-6}$ mol $L^{-1}$. A linear calibration equation ($I_p$ (μA) = 2.17 + 0.224*c* (μmol $L^{-1}$); $R = 0.9971$) was obtained, and the detection limit for catechol was $6.48 \times 10^{-7}$ mol $L^{-1}$. These results compared well with those previously recorded in the voltammetric literature, *e.g.* (1) reduced

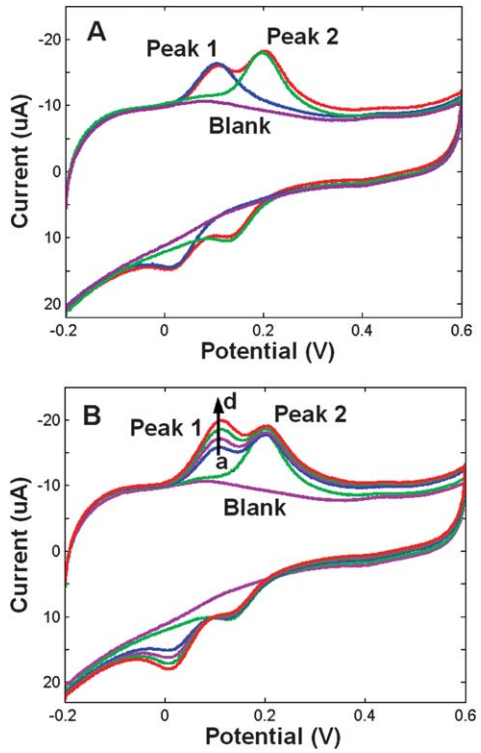


**Fig. 5** (A) Cyclic voltammograms of $5 \times 10^{-5}$ mol $L^{-1}$ catechol, $5 \times 10^{-5}$ mol $L^{-1}$ hydroquinone and their mixtures at the DNA/PDPASA/GCE in pH = 6.80 PBS. (B) Cyclic voltammograms of $5 \times 10^{-5}$ mol $L^{-1}$ catechol at the DNA/PDPASA/GCE in the presence of (from a to d) $5.0 \times 10^{-5}$, $7.0 \times 10^{-5}$, $9.0 \times 10^{-5}$, $1.1 \times 10^{-4}$ mol $L^{-1}$ hydroquinone concentrations.

graphene and multi-wall carbon electrode (LOD of catechol: $1.8 \times 10^{-6}$ mol $L^{-1}$),[37] (2) expanded graphite electrode modified with intercalated montmorillonite (LOD: $1.13 \times 10^{-6}$ mol $L^{-1}$).[39]

The above method and the novel biosensor, DNA/PDPASA/GCE, were applied for the analysis of catechol in water samples (tap water, Ganjiang river water and Aixi lake water; pretreatment – Section 2.4). However, the standard addition method (see Table 1) was adopted for this analysis. The concentrations of added catechol can be estimated by the above calibration.

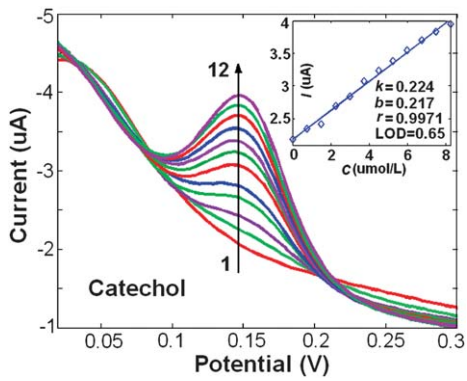


**Fig. 6** DPV voltammograms of catechol at different concentrations (1–12): 0, $7.5 \times 10^{-7}$, $1.50 \times 10^{-6}$,... in steps of $0.75 \times 10^{-6}$..., $8.25 \times 10^{-6}$ mol $L^{-1}$ obtained at the novel DNA/PDPASA/GCE.

Estimates of the catechol in various river waters (Table 1) suggested that the method was satisfactory with the recoveries in the range of 93.3–103%.

### 3.5 DNA damage induced by catechol and its redox products

The DNA/PDPASA/GCE biosensor was also tested for dsDNA damage in the presence of catechol, and this effect was monitored with the use of the $Co(phen)_3^{3+}$ complex as the electrochemical probe. Previous work has suggested that much more $Co(phen)_3^{3+}$ bonded to undamaged rather than damaged DNA because of the intercalative binding of $Co(phen)_3^{3+}$ to the double helix of the DNA.[53] Thus, the strong DPV peak (Fig. 7, curve a) can be attributed to the reduction of $Co(phen)_3^{3+}$ at the DNA/PDPASA/GCE biosensor immersed in pH = 6.80 PBS. After this biosensor was treated in a catechol solution for 10 min, the peak current decreased somewhat (Fig. 7A, curve b), *i.e.* catechol must have damaged the DNA. Since, in general, a different or newly prepared DNA/PDPASA/GCE will be used in repetitive experiments, it is useful to monitor electrode-to-electrode variation, and thus, the reduction peak current ratio ($I_{pi}/I_{pa}$; where the subscript 'i' refers to another peak current profile such as 'b' or 'c', Fig. 7) would be more appropriate to reflect the degree of DNA damage. Through repeated experiments, the value of $I_{pb}/I_{pa}$ was estimated to be 94.0 ± 0.3%. If catechol in contact with the DNA is indeed responsible for the damage to the biomolecule, then greater exposure of the DNA/PDPASA/GCE to catechol

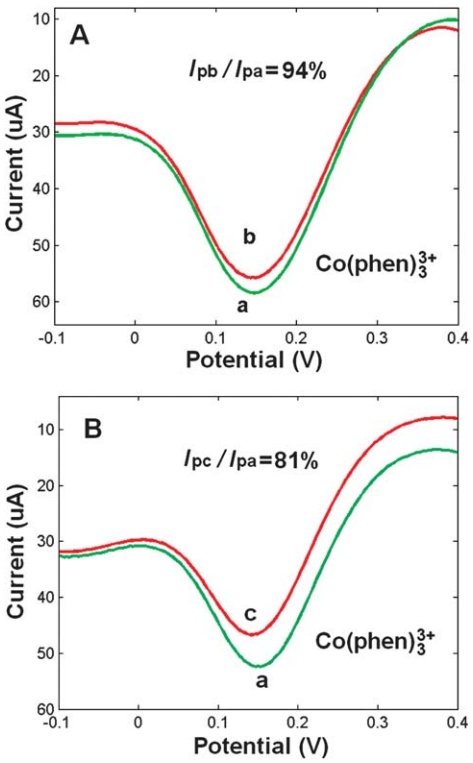


**Fig. 7** DPV voltammograms of $1 \times 10^{-4}$ mol $L^{-1}$ $Co(phen)_3^{3+}$ in 0.1 mol $L^{-1}$ pH = 6.80 PBS obtained at the DNA/PDPASA/GCE: (a) before and (b) after immersion in a $5 \times 10^{-3}$ mol $L^{-1}$ catechol solution for 10 min, and (c) after 40 scans from −0.2 to 0.6 V in a $5 \times 10^{-3}$ mol $L^{-1}$ catechol solution.

should result in a lower $Co(phen)_3^{3+}$ peak current than that observed in Fig. 7A, b, *i.e.* a smaller $I_{pc}/I_{pa}$ value is expected. Thus, the working electrode was cycled 40 times in the potential range of −0.2 to 0.6 V while immersed in a $5 \times 10^{-3}$ mol $L^{-1}$ catechol solution, and the $I_{pc}/I_{pa}$ value dropped to 81.0 ± 0.3%, which is significantly lower than the 94.0% result noted above, *i.e.* the DNA on the biosensor was damaged considerably more than in the previous experiment. This observation suggested that the metabolism of catechol *in vivo* may produce intermediates, which are more damaging to DNA than catechol itself. This view was supported by a study of a metabolic pathway of catechol, which involved its oxidation to semiquinones and quinines, respectively; importantly, it was found that all of these oxidative products could damage DNA.[42,43]

### 3.6 Reproducibility and stability of the DNA/PDPASA electrode

In order to investigate the reproducibility of the DNA/PDPASA electrode, five different GC electrodes were modified with DNA and PDPASA and their performance was compared with the peak current of $5 \times 10^{-5}$ mol $L^{-1}$ catechol solution. The RSD was 8.1% for catechol, indicating that the reproducibility of this modified electrode was satisfactory. To assess the stability of the modified electrode, the CV peak was recorded every 5 min in $5 \times 10^{-5}$ mol $L^{-1}$ catechol solution; only a small decrease of the catechol peak current was observed with a satisfactory RSD value of 2.3%; this indicated that the electrode was quite stable.

## 4 Conclusion

A GCE was covered with a film of PDPASA and subsequently reacted with DNA to realize a novel DNA/PDPASA/GCE biosensor for the analysis of potentially toxic catechol, particularly in different types of water. Importantly, it was demonstrated with the use of CV that the presence of hydroquinone, which could also be detected with this biosensor at similar concentrations, did not interfere with the analysis of catechol. Consequently, with the use of the standard addition method (catechol concentration range: 0.750 to $8.25 \times 10^{-6}$ mol $L^{-1}$) and the DPV technique, several different waters were analysed for catechol. The results were satisfactory within the % recovery range of 93–103 and an LOD value of $6.48 \times 10^{-7}$ mol $L^{-1}$. The PDPASA, as the conjugated polymer, was biocompatible with the DNA, and this polymer may be particularly useful for future biological and medicinal analyses. The application of the electroactive catalyst $Co(phen)_3^{3+}$ provided an *in vitro*, indirect method to mimic a pathway to DNA damage, and effectively demonstrated that products of the catechol metabolism may be responsible for the detected changes attributed to damaged DNA.

## Acknowledgements

The authors gratefully acknowledge the financial support of this study by the National Natural Science Foundation of China (NSFC-21065007), and the State Key Laboratory of Food Science and Technology of Nanchang University (SKLF-MB-201002 and SKLF-TS-200919).

## References

1 D. Borchiellini, M. C. E. Grimaldi, J. Thariat and G. Milano, *Cancer Treat. Rev.*, 2012, **38**, 737–759.
2 A. L. McCarthy, Y. C. O'Callaghan, A. Connolly, C. O. Piggott, R. J. FitzGerald and N. M. O'Brien, *Food Chem.*, 2012, **134**, 641–646.
3 M. Kikuyama, H. Takeshima, T. Kinoshita, E. O. Takada, M. Wakabayashi, S. A. Tanaka, T. Ogawa, Y. Seto and T. Ushijima, *Cancer Lett.*, 2012, **322**, 204–212.
4 O. D. AlAmri, A. B. Cundy, Y. Di, A. N. Jha and J. M. Rotchell, *Environ. Pollut.*, 2012, **168**, 107–112.
5 R. Levicky and A. Horgan, *Trends Biotechnol.*, 2005, **23**, 143–149.
6 W. J. Zhou, A. R. Halpern, T. H. Seefeld and R. M. Corn, *Anal. Chem.*, 2012, **84**, 440–445.
7 S. Miura, S. Nishizawa, A. Suzuki, Y. Fujimoto, K. Ono, Q. Gao and N. Teramae, *Chem.–Eur. J.*, 2011, **17**, 14104–14110.
8 X. H. Zhao, Q. J. Ma, X. X. Wu and X. Zhu, *Anal. Chim. Acta*, 2012, **727**, 67–70.
9 J. Zhang, H. L. Qi, Y. Li, J. Yang, Q. Gao and C. X. Zahng, *Anal. Chem.*, 2008, **80**, 2888–2894.
10 R. Amin, A. Kulkarni, T. Kim and S. H. Park, *Curr. Appl. Phys.*, 2012, **12**, 841–845.
11 E. Palecek, *Talanta*, 2002, **56**, 809–819.
12 H. W. Wu, S. C. Liu, J. H. Jiang, G. L. Shen and R. Q. Yu, *Chem. Commun.*, 2012, **48**, 6280–6282.
13 L. Hlavata, K. Benikova, V. Vyskocil and J. Labuda, *Electrochim. Acta*, 2012, **71**, 134–139.
14 C. L. Bian, Q. X. Zeng, L. J. Yang, H. Y. Xiong, X. H. Zhang and S. F. Wang, *Sens. Actuators, B*, 2011, **156**, 615–620.
15 L. Wang, X. H. Chen, X. L. Wang, X. P. Han, S. F. Liu and C. Z. Zhao, *Biosens. Bioelectron.*, 2011, **30**, 151–157.
16 Y. X. Fang, D. Zhang, X. Qin, Z. Y. Miao, S. Takahashi, J. Anzai and Q. Chen, *Electrochim. Acta*, 2012, **70**, 266–271.
17 Y. B. Zeng, D. J. Yu, Y. Y. Yu, T. S. Zhou and G. Y. Shi, *J. Hazard. Mater.*, 2012, **217–218**, 315–322.
18 C. Qin, C. Chen, Q. J. Xie, L. H. Wang, X. H. He, Y. Huang, Y. P. Zhou, F. Y. Xie, D. W. Yang and S. Z. Yao, *Anal. Chim. Acta*, 2012, **720**, 49–56.
19 P. Mukherjee and A. K. Nandi, *J. Colloid Interface Sci.*, 2011, **356**, 145–150.
20 E. C. Venancio, C. A. R. Costa, S. A. S. Machado and A. J. Motheo, *Electrochem. Commun.*, 2001, **3**, 229–233.
21 M. Yousel Elahi, S. Z. Bathaie, S. H. Kazemi and M. F. Mousavi, *Anal. Biochem.*, 2011, **411**, 176–184.
22 Y. Bo, H. Y. Yang, Y. Hu, T. M. Yao and S. S. Huang, *Electrochim. Acta*, 2011, **56**, 2676–2681.
23 J. Yang, X. L. Wang and H. Q. Shi, *Sens. Actuators, B*, 2012, **162**, 178–183.
24 X. Li, M. X. Wan, X. N. Li and G. L. Zhao, *Polymer*, 2009, **50**, 4529–4534.
25 J. Yano, T. Kohno and A. Kitani, *J. Solid State Electrochem.*, 2009, **13**, 1441–1447.
26 A. A. Karyakin, I. A. Maltesev and L. V. Lukachova, *J. Electroanal. Chem.*, 1996, **402**, 217–219.

27 T. Nakajima and T. Kawagoe, *Synth. Met.*, 1989, **28**, 629–638.
28 A. Benyoucef, S. Boussalem, M. I. Ferrahi and M. Belbachir, *Synth. Met.*, 2010, **160**, 1591–1597.
29 S. Bhadra, D. Khastgir, N. K. Singha and J. H. Lee, *Prog. Polym. Sci.*, 2009, **34**, 783–810.
30 S. Bhadra, N. K. Singha and D. Khastgri, *Eur. Polym. J.*, 2008, **44**, 1763–1770.
31 R. K. Paul and C. K. S. Pillai, *Synth. Met.*, 2000, **114**, 27–35.
32 P. Santhosh, K. M. Manesh, S. Uthayakumar, A. I. Gopalan and K. P. Lee, *Biosens. Bioelectron.*, 2009, **24**, 2008–2014.
33 T. C. Wen, C. Sivakumar and A. Gopalan, *Electrochim. Acta*, 2001, **46**, 1071–1085.
34 X. R. Duan, L. B. Liu, F. D. Feng and S. Wang, *Acc. Chem. Res.*, 2010, **43**, 260–270.
35 H. G. Lin, T. Gan and K. B. Wu, *Food Chem.*, 2009, **113**, 701–704.
36 M. F. Chen, X. Li and X. Y. Ma, *Int. J. Electrochem. Sci.*, 2012, **7**, 2616–2622.
37 F. X. Hu, S. H. Chen, C. Y. Wang, R. Yuan, D. H. Yuan and C. Wang, *Anal. Chim. Acta*, 2012, **724**, 40–46.
38 L. T. Wang, Y. Zhang, Y. L. Du, D. B. Lu, Y. Z. Zhang and C. M. Wang, *J. Solid State Electrochem.*, 2012, **16**, 1323–1331.
39 Y. Kong, Y. Y. Xu, H. H. Mao, C. Yao and X. F. Ding, *J. Electroanal. Chem.*, 2012, **669**, 1–5.
40 Q. H. Guo, J. S. Huang, P. Q. Chen, Y. Liu, H. Q. Hou and T. Y. You, *Sens. Actuators, B*, 2012, **163**, 179–185.
41 Internation Agents for Research on Cancer (IARC), *Agents Classified by the IARC Monographs, Volumes 1–102*, World Health Organization, Lyon, 2011.
42 E. L. Cavalieri, K. M. Li, N. Balu, M. Saeed, P. Devanesan, S. Higginbotham, J. Zhao, M. L. Gross and E. G. Rogan, *Carcinogenesis*, 2002, **23**, 1071–1077.
43 J. L. Bolton, *Toxicology*, 2002, **177**, 55–65.
44 L. S. Dollimore and R. D. Gillard, *J. Chem. Soc., Dalton Trans.*, 1973, 933–940.
45 R. S. Deinhammer, M. Ho, J. W. Anderegg and M. D. Porter, *Langmuir*, 1994, **10**, 1306–1313.
46 Y. Gu and M. T. Lai, *Biosens. Bioelectron.*, 2012, **31**, 124–129.
47 X. Liu, L. Q. Luo, Y. P. Ding, Z. P. Kang and D. X. Ye, *Bioelectrochemistry*, 2012, **86**, 38–45.
48 H. S. Yin, Q. M. Zhang, Y. L. Zhou, Q. Ma, T. Liu, L. S. Zhu and S. Y. Ai, *Electrochim. Acta*, 2011, **56**, 2748–2753.
49 I. E. Mulazimoglu, A. D. Mulazimoglu and E. Yilmaz, *Desalination*, 2011, **268**, 227–232.
50 L. Chu, L. Han and X. L. Zhang, *J. Appl. Electrochem.*, 2011, **41**, 687–694.
51 Z. L. Wang, Y. Kong, C. Yao, Y. Wang, X. F. Ding and Y. S. Zhou, *Appl. Clay Sci.*, 2012, **55**, 173–176.
52 H. S. Yin, X. M. Meng, Z. N. Xu, L. J. Chen and S. Y. Ai, *Anal. Methods*, 2012, **4**, 1445–1451.
53 W. Zhang, T. Yang, W. J. Li, G. C. Li and K. Jiao, *Biosens. Bioelectron.*, 2010, **25**, 2370–2374.

 