Journal of Electroanalytical Chemistry 703 (2013) 153–157

Contents lists available at SciVerse ScienceDirect

Journal of Electroanalytical Chemistry

journal homepage: www.elsevier.com/locate/jelechem

# Electrochemical determination of nonylphenol using differential pulse voltammetry based on a graphene–DNA-modified glassy carbon electrode

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ARTICLE INFO

*Article history:*
Received 19 December 2012
Received in revised form 26 April 2013
Accepted 29 May 2013
Available online 14 June 2013

*Keywords:*
Nonylphenol
Graphene
Modified electrode
Determination
Differential pulse voltammetry

ABSTRACT

The electrochemical determination of nonylphenol (NP) using differential pulse voltammetry (DPV) is developed based on a reduced graphene–DNA hybrid-modified glassy carbon electrode (GR-DNA/GCE). The electrochemical oxidation of NP at the GR-DNA/GCE is shown to be an one-electron and one-proton process, and due to the synergic effect of GR and DNA, the GR-DNA/GCE has 4.87, 2.76 and 2.09 times higher current response than bare GCE, DNA/GCE, and GR/GCE, respectively. Using DPV in the 0.1 mol $L^{-1}$ acetate buffer solution at 0.1 V and with a 300 s accumulation time (pH = 4.6), the GR-DNA/GCE exhibits a linear current response towards the electrochemical oxidation of NP in the concentration range of $5.0 \times 10^{-8}$ to $4.0 \times 10^{-6}$ mol $L^{-1}$ and a detection limit of $1.0 \times 10^{-8}$ mol $L^{-1}$ ($S/N = 3$). In addition, this GR-DNA/GCE also indicates high selectivity and reproducibility, and can be successfully used to determine the presence of NP in Asian Clams and natural water samples with comparative sensitivity to high-performance liquid chromatography, and with recoveries ranging from 95% to 103%, showing its practical prospects in the determination of NP in real samples.



## 1. Introduction

Nonylphenol ethoxylates, a widely used alkylphenol non-ionic surfactant, can be readily degraded in an aquatic environment to nonylphenol (NP). Recently, NP has been recognized as an important pollutant [1,2], whose bioaccumulation in the internal organs of water-dwelling organisms can reach 10–1000 times greater than in the surrounding environment [3]. In particular, due to its potential to disrupt the endocrine system, NP exhibits extreme toxicity to organisms even at moderate bioaccumulation [4]. Thus, in addition to the restriction of the use of nonylphenol ethoxylates in some materials, it is also necessary to develop rapid, simple, and efficient NP monitoring methods. Up to now, several methods, including gas chromatography (GC) [5], high-performance liquid chromatography (HPLC) [6–8], molecularly imprinted technique [9], and immunosensors [10], are used to detect NP. These techniques have high sensitivity and low detection limits, but they usually are time consuming, involve complicated instrumentation, and require a skilled operator. Electrochemical techniques exhibit predominance for the analysis of molecules with electrochemical activity due to the advantages of rapid response, low cost, high sensitivity, and real-time detection [11,12]. In addition, electrochemical techniques are also helpful for identifying the redox properties of active molecules [13]. However, the poor sensitivity and high overpotential of the electrochemical oxidation of NP molecules on bare electrodes limit the use of electrochemical techniques. Therefore, it is essential to prepare a functional film-modified electrode to extend the dynamic range of analytical determination if electrochemical techniques are to be used [14].

Deoxyribonucleic acid (DNA), a well-known natural biological macromolecule, has gained increasing attention in the field of electrochemistry [15]. The use of DNA-modified electrodes as sensitive sensors for small molecules has been demonstrated [16,17]. A DNA molecule is considered to be an array of stacked π electrons, which can provide a rapid pathway for electronic charge transport to target molecules due to intercalative and electrostatic binding. Therefore, DNA is an attractive choice as a surface modification element for sensing bioactive species [18,19]. On the other hand, as a novel modification material, graphene can greatly enhance the voltammetric response of a modified film electrode due to its unique nanostructure, excellent mechanical properties, and unusual electronic transport properties [20,21]. These tendencies are well supported by several recently published excellent reviews [22,23]. Moreover, the electrochemical properties of graphene can be effectively improved by integration with other functional materials to produce versatile electrochemical sensing properties. Considering the respective properties of DNA and graphene, their composite

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http://dx.doi.org/10.1016/j.jelechem.2013.05.029

film may exhibit unique electrochemical traits for the redox reaction of NP. In this paper, a graphene-DNA hybrid modified glassy carbon electrode (GR-DNA/GCE) is prepared, and the electrochemical behavior and the voltammetric determination of NP in the modified electrode are presented.

## 2. Experimental

### 2.1. Chemicals and reagents

Graphite powders (99%, 40 nm), NP (98.0%) and calf thymus DNA were purchased from Aladdin Chemical Reagent Co. Ltd. Other chemicals used in this study were of analytical grade. All solutions were prepared with double distilled water. The acetate buffer solution (0.1 mol $L^{-1}$) was made from HAc + NaAc, and its pH value was adjusted with 0.1 mol $L^{-1}$ HAc and 1.0 mol $L^{-1}$ NaOH.

The contents of NP in the real samples, including Asian Clams and three water samples, were detected to evaluate the performance of the modified electrode. The Asian Clams were obtained from a river known to be contaminated. They were first cut into small pieces with scissors and freeze dried, and then triturated and weighed in a small beaker to 10.0 g. The mixture was extracted in reflux distillation with dichloromethane and filtered through a glass funnel. The filter was evaporated under reduced pressure at 60 °C, and the residue was obtained. The residue was then re-dissolved in n-hexane. After passing through a reverse phase $C_{18}$ solid phase extraction (SPE) cartridge at a flow-rate of 10 ml $min^{-1}$ in a SPE workstation, the eluent was evaporated to dryness and then re-dissolved with 1 mL of ethanol. The tap water sample was collected after discharging tap water for 20 min and boiling for 5 min to remove chlorine. The river water sample was obtained from the Pearl River (Guangzhou), and the lake water sample was obtained from Yan Lake on our campus. A 500 mL aliquot of each water sample was poured into a brown bottle. Afterwards, the water sample was filtered through a cellulose acetate filter (0.45 μm). Then, 1.44 mL of HAc (36 wt.%) and 2.05 g of NaAc were added. Afterwards, the water was spiked with NP. The obtained sample solutions were used for analysis.

### 2.2. Preparation of the modified electrode

The graphene oxide (GO) was prepared using graphite powders as precursors in the modified Hummers' method [24,25], and then the reduced graphene (GR) modifier was prepared by using ascorbic acid as the reduction agent. GO and 0.1 mol $L^{-1}$ acetic acid were mixed according to a volume ratio of 1:1 and sonicated for 30 min at 60 °C. The reduction products were centrifuged at $10^4$ rpm to remove the supernatant. Then, excess 30% $H_2O_2$ was added to the black suspension to oxidize the remaining ascorbic acid under sonication for 30 min at 60 °C. After sonication, the resultant mixture was collected by centrifugation at $10^4$ rpm, washed with ethanol and water three times and dried at 60 °C. The single stranded DNA (ss-DNA) was prepared as follows: the calf thymus DNA was dissolved in twice distilled water and heated in a boiling water bath (100 °C) for 30 min, and then the solution was cooled rapidly in an ice-water bath (0 °C).

The GR and ss-DNA (GR-DNA) hybrid was prepared as previously reported [26]. Briefly, the GR (approximately 1 mg) was combined with a certain sequence of ss-DNA (5 mg $mL^{-1}$) in aqueous solution and sonicated in an ice-water bath (KQ-100E ultrasonic cleaner, Kunshan, China) for 3 h at an output power of 100 W. Immediately after the sonication, the samples were centrifuged at 5000 rpm for 90 min, and the GR-DNA hybrid was decanted and collected to obtain water-dispersed GR-DNA. Before the modification, the bare glassy carbon electrode (GCE) with a diameter of 3 mm was polished with 0.05 μm alumina slurry, followed by ultrasonication in absolute ethanol and water, respectively. After cleaning, 5 μL of GR-DNA was dropped onto the electrode and dried at room temperature.

### 2.3. Apparatus and procedures

UV–vis experiments were carried out with a UV-1800 ultraviolet–visible (UV–vis) spectrophotometer (Shimazu, Japan). Transmission electron microscopy (TEM) images were obtained with a 135 JEOL-1230 high-resolution TEM system (JEOL, Japan). Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were conducted with a CHI660B electrochemical workstation (Chenhua, China). The GCE or the modified electrode was used as the working electrode. An Ag/AgCl electrode (saturated KCl) and Pt wire were used as the reference and counter electrodes, respectively. Electrochemical impedance spectroscopy (EIS) was performed on an Autolab electrochemical workstation (Eco Chemie, Netherlands). High-performance liquid chromatography (HPLC, LC-10AT, Shimadzu, Kyoto, Japan) with a $C_{18}$ reverse phase column (5 μm, 4.6 × 250 mm) and an injection volume of 25 μL was employed as a comparative technique to test the reliability of the DPV. The mobile-phase composition was acetonitrile/water (90/10, v/v) at a flow rate of 0.2 mL $min^{-1}$. All experiments were carried out at room temperature (25 ± 2 °C).

## 3. Results and discussion

### 3.1. Characterization of the hybridization of GR and DNA

The hybridization of GR and DNA was first characterized by UV–vis spectroscopy. Fig. 1 shows the UV–vis spectra of a GR-DNA aqueous dispersion, together with a DNA solution and GR dispersion for comparison. The DNA solution exhibits an absorption peak at 260 nm, while the GR dispersion shows an absorption peak at 266 nm, which is generally regarded as the excitation of the π-plasmon of the graphitic structure [27]. The peak of the GR-DNA hybrid is observed at 268 nm, suggesting that the electronic conjugation within the graphene sheets is restored due to the coupling effect between GR and DNA, although only a little red shift (2 nm) of the absorption peak for GR-DNA hybrid is obtained compared to the GR dispersion [28]. In addition, as shown in the inset of Fig. 1, the images of GR without and with DNA clearly indicate that DNA has a high dispersion efficiency, which matches the UV–vis results well.

The combining of flake-like GR nanosheets (the insets of Fig. 2) with DNA is important to form a stable modified film with high

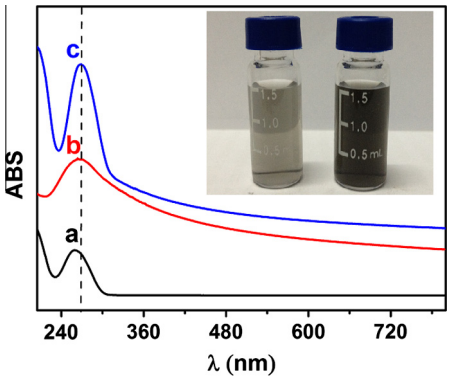


**Fig. 1.** UV–vis spectra of the DNA (a), GR (b), and GR-DNA (c) aqueous dispersion. The inset shows the digital images of the GR dispersion (1 mg $mL^{-1}$) without DNA (left) and with DNA (right).