

## Electrochemical Determination of Bisphenol A at Carbon Nanotube-Doped Titania-Nafion Composite Modified Electrode

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A highly sensitive electrochemical detection method for bisphenol A (BPA) has been developed by using multi-walled carbon nanotube (CNT)-doped titania-Nafion composite modified glassy carbon (GC) electrode. The CNT-titania-Nafion/GC electrode exhibited excellent electrocatalytic activity towards BPA. Therefore, the CNT-titania-Nafion/GC electrode showed improved voltammetric responses for BPA compared to that obtained with bare GC electrode. In addition, cetyltrimethylammonium bromide (CTAB), a cationic surfactant, was added into the BPA sample solution in order to accumulate BPA through hydrophobic interaction between CTAB and BPA. The CNT-titania-Nafion/GC electrode gave a linear response ( $r^2 = 0.999$ ) for BPA from  $1.0 \times 10^{-8}$  M to  $5.0 \times 10^{-6}$  M with a detection limit of  $9.0 \times 10^{-10}$  M ( $S/N = 3$ ). The modified electrode showed good selectivity against interfering species and also exhibited good reproducibility. The present electrochemical sensor based on the CNT-titania-Nafion/GC electrode was applied to the determination of BPA in food package samples.

**Key Words :** Bisphenol A, Titania, Carbon nanotube, CTAB, Linear sweep voltammetry

### Introduction

Bisphenol A (2,2-bis(4-hydroxyphenyl) propane, BPA) is a typical endocrine disrupting compound causing abnormal hormone activity,<sup>1</sup> and widely used as a major component for the industrial production of polycarbonate and epoxy resins. Polycarbonate and epoxy resins are commonly used to produce plastic food containers, inner surface coating of food and beverage cans, from which BPA can leach into food and environment.<sup>2</sup> Therefore, it is very important to develop highly sensitive and simple detection method for the determination of BPA in food and environment. A number of analytical methods such as high-performance liquid chromatography (HPLC),<sup>3</sup> liquid chromatography coupled with mass spectrometry (LC-MS),<sup>4</sup> gas chromatography-mass spectrometry (GC-MS),<sup>5</sup> fluorescence detection method,<sup>6</sup> flow injection inhibitory chemiluminescence method,<sup>7</sup> and enzyme-linked immunosorbent assay<sup>8</sup> have been reported for the detection of BPA. These methods are quite sensitive, but they are very complicated and require sophisticated instruments. So, many researchers have tried to develop simple and sensitive analytical methods for the detection of BPA. In particular, electrochemical detection methods are quite promising since they are simple, fast, and cost-effective. Up to date, electrochemical sensors based on modified electrodes with surfactant,<sup>9</sup> phthalocyanine,<sup>10,11</sup> PAMAM dendrimer,<sup>12,13</sup> layered double hydroxide,<sup>14</sup> carbon nanotube (CNT),<sup>15</sup> and graphene<sup>16</sup> have been reported and they provided good sensitivity for BPA. However, the development of electrochemical sensors for BPA with good sensitivity is still needed in order to expand the applicability of the electrochemical methods in real analytical problems.

In this paper, we describe the characteristics of the CNT-

doped titania-Nafion composite-modified glassy carbon (GC) electrode for the electrochemical detection of BPA with cyclic voltammetry (CV) and linear sweep voltammetry (LSV). Due to the excellent electrocatalytic activity, CNT has been widely used in the fabrication of electrochemical biosensors.<sup>17</sup> Previously, we reported that the composite film of the sol-gel derived titania and Nafion has greater pore size (3.14 nm) than that of the pure Nafion film (2.82 nm), thus leading to faster diffusion of analyte into the composite film.<sup>18</sup> In addition, the titania-Nafion composite can disperse the CNT.<sup>19</sup> Therefore, the CNT-doped titania-Nafion mesoporous composite has been advantageously used for the fabrication of dopamine sensors<sup>20</sup> and also biosensors of phenol,<sup>21</sup> alcohol<sup>22</sup> and glucose.<sup>23</sup> They exhibited good sensitivity and improved long-term stability. The present CNT-doped titania-Nafion mesoporous composite has an ion exchange interaction with cetyltrimethylammonium bromide (CTAB), a cationic surfactant, which was used for the pre-concentration of BPA in the present study as reported for the determination of estrogens at Nafion-modified GC electrode.<sup>24</sup> The attractive performance of the CNT-based BPA sensor was evaluated over other reported BPA sensors. The CNT-titania-Nafion/GC electrode was applied to the determination of BPA in food samples.

### Experimental

**Reagents.** Titanium(IV) isopropoxide ( $\text{Ti}(\text{OR})_4$  ( $\text{R} = \text{CH}(\text{CH}_3)_2$ , 99.999%)), Nafion<sup>®</sup> (perfluonated ion-exchange resin solution, 5 wt % in mixture of lower aliphatic alcohols and water), Cetyltrimethylammonium bromide ( $((\text{C}_{16}\text{H}_{33})\text{-N}(\text{CH}_3)_3\text{Br})$ ), Bisphenol A ( $\text{C}_{15}\text{H}_{16}\text{O}_2$ ), Ethanol ( $\text{C}_2\text{H}_6\text{O}$ ,  $\geq 99.8\%$ ), Multiwalled CNT (95% purity, diameter:  $30 \pm 15$

nm, and length: 5–20  $\mu\text{m}$ ) were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). All working solutions were prepared with the water purified through a Milli-Q system (Millipore, Bedford, MA).

**Instrumentation.** Electrochemical experiments were performed with EG&G 263A potentiostat (Princeton, NJ, USA) and frequency response detector (model 1025, Oak Ridge, TN, USA) with a conventional three-electrode system in a 15 mL electrochemical cell. A conventional three-electrode system was employed with a platinum-wire counter electrode, glassy carbon working electrode ( $A = 0.07 \text{ cm}^2$ ) and Ag/AgCl (3 M NaCl) reference electrode. Cyclic voltammograms and linear sweep voltammograms were recorded in BPA solution prepared in 0.05 M phosphate buffer (pH 7.0) and CTAB.

**Preparation of CNT-Titania-Nafion/GC Electrode.** The titania ( $\text{TiO}_2$ ) sol was prepared by the hydrolysis and condensation by mixing 44.3  $\mu\text{L}$  of 0.15 M  $\text{Ti}(\text{OC}_4\text{H}_9)_4$  dissolved in 955.7  $\mu\text{L}$  of 2-propanol and 5 mL of deionized water and followed by 7.531  $\mu\text{L}$  of 0.1 M HCl solution under vigorous stirring for at least 1 h similar to the previous report.<sup>18</sup> The titania sol was mixed with diluted Nafion (EtOH:Nafion = 1:1, v/v) to form the titania–Nafion composite solution (1:1, v/v). A 1.2 mg CNT was dispersed in 1.0 mL titania–Nafion composite solution. Then, the mixture was agitated in an ultrasonic bath for 90 minutes, giving a black dispersion. GC electrode was polished before each experiment with 0.05  $\mu\text{m}$   $\gamma$ -alumina powder, rinsed thoroughly with distilled water. An aliquot of 3  $\mu\text{L}$  CNT-doped composite solution was handcast on the surface of a pre-cleaned GC electrode. The thin composite films were dried for 10 minutes at room temperature and the modified electrode was stored in 0.05 M phosphate buffer at pH 7.0 for 15 minutes in order to swell the composite modified GC electrode.

**Electrochemical Detection of BPA.** Prior to the electrochemical experiments, the CNT–titania–Nafion modified GC electrode was soaked in 8 mL of 0.05 M phosphate buffer solution (pH 7.0), and then 1 mL of BPA standard solution and 1 mL of  $1.0 \times 10^{-5}$  M CTAB were added in an electrochemical cell. The electrode was kept in the solution for 4 minutes in order to accumulate the BPA into the modified electrode. Cyclic voltammetric experiments were carried out at a scan rate of 100 mV/s in the potential range of +0.3 ~ +0.8 V vs. Ag/AgCl (3 M NaCl). Linear sweep voltammetry was used for the determination of BPA at the same condition with cyclic voltammetry experiments. In the LSV experiment, peak current was recorded at 0.55 V. All experiments were performed at room temperature.

**Real Sample Test.** Food packaging container has been used for real sample test. Distilled water (20 mL) was put into the food packaging container and it was placed in the microwave oven for 5 minutes at 1100W. After microwave operation, 1 mL solution sampled from the food package container was spiked into an electrochemical cell containing 1 mL of  $1.0 \times 10^{-5}$  M CTAB and 8 mL of phosphate buffer solution at pH 7. Linear sweep voltammetry was carried out with the CNT–titania–Nafion modified GC electrode at a

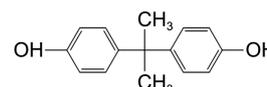
scan rate of 100 mV/s in the scan range of +0.3 ~ +0.8 V. For recovery test, 1 mL of  $5.0 \times 10^{-7}$  M BPA standard solution was added into the above real sample solution in the electrochemical cell.

## Results and Discussion

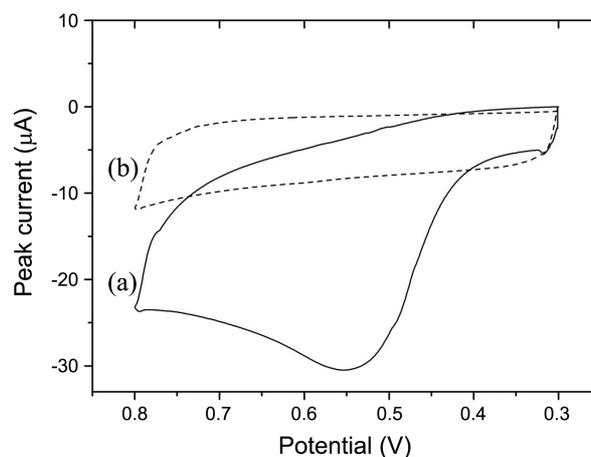
**Electrochemical Behavior of BPA at the Modified Electrode.** As shown in Figure 1, BPA has two phenolic hydroxyl groups that can be easily oxidized at the modified electrode. Therefore, cyclic voltammetry and linear sweep voltammetry were employed to characterize the electrochemical behavior of BPA.

Cyclic voltammogram of  $1.0 \times 10^{-4}$  M BPA in 0.05 M phosphate buffer (pH 7.0) was obtained at the CNT–titania–Nafion modified GC electrodes in the presence of  $1.0 \times 10^{-6}$  M CTAB. As shown in Figure 2(a), oxidation peak was obtained at +0.55 V with the peak current of  $-20.4 \mu\text{A}$ , and the reduction peak was not observed in the reverse potential sweep, indicating the electrode reaction of BPA is an irreversible process. Meanwhile, the cyclic voltammogram of CNT–titania–Nafion/GC electrode was obtained in 0.05 M phosphate buffer (pH 7.0) in the absence of  $1.0 \times 10^{-4}$  M BPA (Figure 2(b)). Obviously, no redox peak was observed, indicating that the oxidation peak at +0.55 V is related to the oxidation of BPA.

Linear sweep voltammetry has also been employed in the potential range from 0.3 V to 0.8 V in order to quantitate BPA in a simple and convenient manner. As shown in Figure 3, the linear sweep voltammogram of  $1.0 \times 10^{-4}$  M BPA at a bare GC electrode in the presence of  $1.0 \times 10^{-6}$  M CTAB (a) shows a small oxidation peak of  $-5.6 \mu\text{A}$  at 0.566 V vs. Ag/AgCl (3 M NaCl). While, the linear sweep voltammogram



**Figure 1.** Chemical structure of BPA.



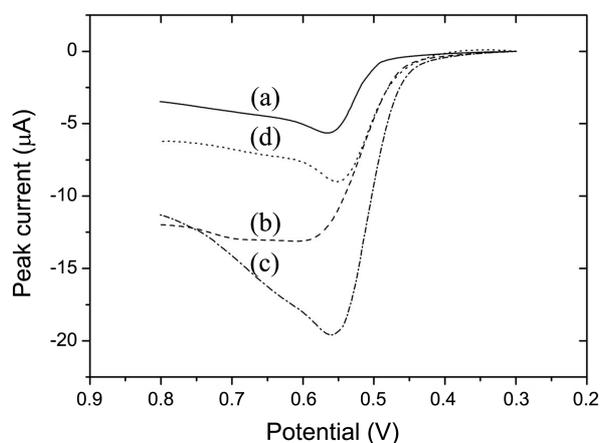
**Figure 2.** Cyclic voltammograms obtained at the CNT–titania–Nafion modified GC electrode in  $1.0 \times 10^{-6}$  M CTAB and 0.05 M phosphate buffer (pH 7.0) in the presence (a) and in the absence (b) of  $1.0 \times 10^{-4}$  M BPA at a scan rate of 100 mV/s.

of  $1.0 \times 10^{-4}$  M BPA at the CNT–Nafion/GC electrode in the presence of  $1.0 \times 10^{-6}$  M CTAB (b) shows 2-fold increased oxidation currents than that at bare GC electrode, and the oxidation potential is positively shifted to 0.600 V vs. Ag/AgCl (3 M NaCl). On the other hand, the linear sweep voltammogram of  $1.0 \times 10^{-4}$  M BPA at the CNT–titania–Nafion/GC electrode in the presence of  $1.0 \times 10^{-6}$  M CTAB (c) shows more than 3-fold increased oxidation currents than that at bare GC electrode, and the oxidation potential is negatively shifted to 0.560 V vs. Ag/AgCl (3 M NaCl). These results indicate that CNT-modified electrode has obviously enhanced the oxidation of BPA as reported previously.<sup>15</sup> The enhanced electrochemical signal at the CNT-modified electrode could be due to the enhanced conductivity and increased surface area for electron transfer at the CNT-modified electrode. From the electrochemical impedance spectroscopic experiment at the modified electrode interface in the presence of redox probe ions,  $[\text{Fe}(\text{CN})_6]^{3-/4-}$ , the electron transfer resistances at the CNT–titania–Nafion/GC as well as the CNT–Nafion/GC electrodes were found to be 151 k $\Omega$  and 1302 k $\Omega$ , respectively, which were smaller than at a Nafion-modified GC electrode.<sup>20</sup> The experimental results at Figure 3 also suggest that the titania–Nafion composite is a good immobilization medium for the construction of the CNT-based electrochemical and electrochemiluminescent sensors.<sup>19</sup> When CNT is doped in titania–Nafion composite, the CNT–titania–Nafion/GC electrode (c) exhibited 57% higher oxidation current for BPA than that at the CNT–Nafion/GC electrode (b). This result appears to indicate that the titania–Nafion composite is much more effective medium than the pure Nafion for the fabrication of CNT-based electrochemical sensor for BPA. The larger oxidation current for BPA at the CNT–titania–Nafion/GC electrode than at the CNT–Nafion/GC electrode in the present study could be due to the greater mass transport rate in the CNT–titania–Nafion composite film than in the CNT–Nafion composite film as observed for the electrochemical dopamine sensor.<sup>20</sup> In our

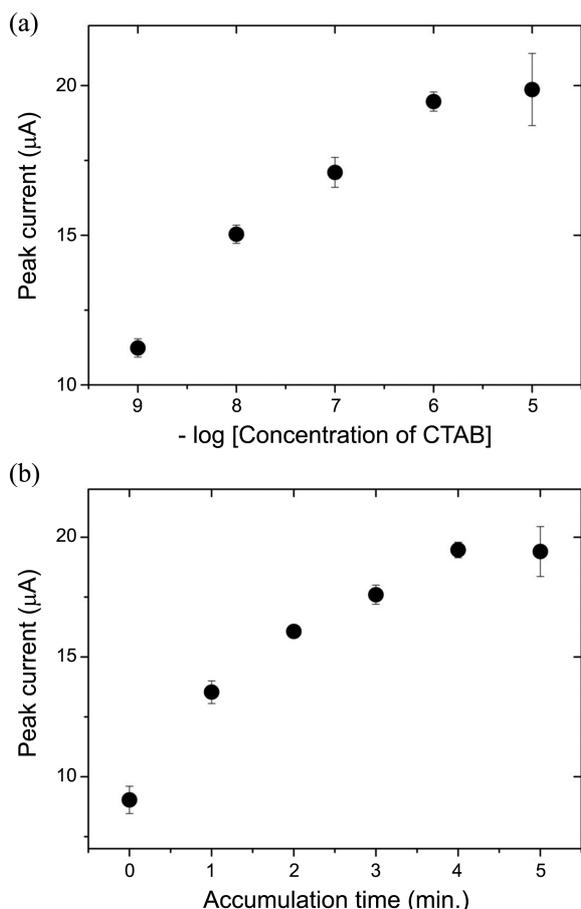
previous work, the pore size (3.14 nm) of the titania–Nafion composite film determined from BET test was larger than that (2.82 nm) of pure Nafion film.<sup>18</sup> In addition, the titania–Nafion composite film (pore volume = 0.28 cm<sup>3</sup>g<sup>-1</sup>) was more porous than the pure Nafion film (pore volume = 0.020 cm<sup>3</sup>g<sup>-1</sup>). Therefore, the BPA can easily diffuse into the CNT–titania–Nafion/GC electrode surface through the porous channels in the composite film.

CTAB was used for the preconcentration of BPA in the present study as reported for the determination of BPA at GC electrode<sup>9</sup> and estrogens at the Nafion-modified GC electrode.<sup>24</sup> CTAB is a hydrophobic and cationic surfactant so that it can have ion-exchange interaction with Nafion, a cation exchanger polymer. Therefore, CTAB can be immobilized on to the present CNT–titania–Nafion/GC electrode and the immobilized CTAB can interact with hydrophobic BPA by hydrophobic interaction, thus inducing BPA to be accumulated on the electrode surface as observed for the determination of estrogens at the Nafion-modified GC electrode.<sup>24</sup> As shown in Figure 3, the linear sweep voltammograms of  $1.0 \times 10^{-4}$  M BPA at the CNT–titania–Nafion/GC electrode in the presence of  $1.0 \times 10^{-6}$  M CTAB (c) showed 2.2-fold larger oxidation currents compared to that in the absence of  $1.0 \times 10^{-6}$  M CTAB (d). This result indicates that CTAB successfully works as a preconcentration medium for BPA on the CNT–titania–Nafion/GC electrode. In the present experiments, the CNT–titania–Nafion modified GC electrode was soaked in the solution mixture containing equal volume (1.0 mL) of a certain concentration of BPA standard as well as  $1.0 \times 10^{-5}$  M CTAB for 4 minutes prior to the electrochemical measurement. However, the CNT–titania–Nafion modified GC electrode can be first soaked in  $1.0 \times 10^{-5}$  M CTAB solution for 4 minutes and then be placed again in a certain concentration of BPA standard solution for the electrochemical measurement. The two methods produced almost similar electrochemical signals for BPA.

**Optimization of Experimental Conditions.** The effect of CTAB concentration on the oxidation current of BPA was studied at a concentration range lower than the CMC ( $9.0 \times 10^{-9}$  M) of CTAB. As shown in Figure 4(a), the oxidation peak current of  $1.0 \times 10^{-4}$  M BPA was increased gradually in the CTAB concentration range from  $1.0 \times 10^{-9}$  M to  $1.0 \times 10^{-5}$  M. However, the peak current was not reproducible at the CTAB concentration of  $1.0 \times 10^{-5}$  M. Therefore, the concentration of CTAB at  $1.0 \times 10^{-6}$  M was used in all subsequent experiments. Since the CTAB works as a preconcentration medium for BPA, the accumulation time of CTAB onto the CNT–titania–Nafion/GC electrode strongly affects the oxidation current of BPA. As shown in Figure 4(b), the oxidation current sharply increased as a function of accumulation time up to 4 minutes and then no longer increased with the accumulation time. The curvature presumably indicates that a limiting amount of BPA is accumulated on the modified surface owing to surface saturation. This kind of saturation behavior was also observed for the determination of estrogens at Nafion-modified GC electrode



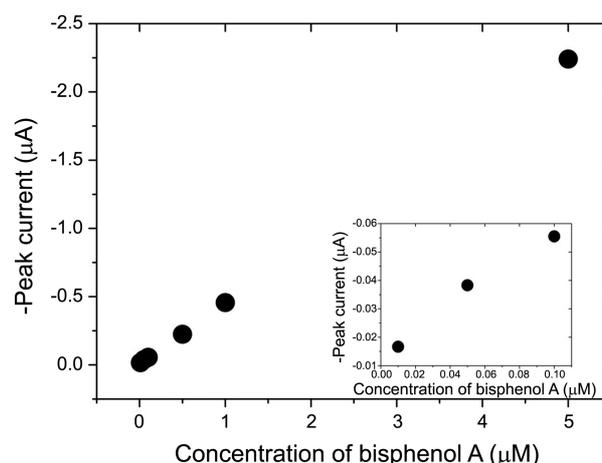
**Figure 3.** Linear sweep voltammograms of  $1.0 \times 10^{-4}$  M BPA at a bare GC electrode in the presence of  $1.0 \times 10^{-6}$  M CTAB (a), at the CNT–Nafion/GC electrode in the presence of  $1.0 \times 10^{-6}$  M CTAB (b), at the CNT–titania–Nafion/GC electrode in the presence (c), and the absence of  $1.0 \times 10^{-6}$  M CTAB (d), Scan range = 0.3–0.8 V, Scan rate = 100 mV/sec, CTAB accumulation time = 4 minutes.



**Figure 4.** (a) Effect of CTAB concentration on the oxidation peak current of  $1.0 \times 10^{-4}$  M BPA. Scan range: 0.3–0.8 V, Scan rate = 100 mV/sec, CTAB accumulation time = 4 minutes. (b) Effects of accumulation time on the oxidation peak current of  $1.0 \times 10^{-4}$  M BPA in the presence of  $1.0 \times 10^{-6}$  M CTAB.

in the presence of CTAB.<sup>24</sup> Therefore, the CNT–titania–Nafion/GC electrode was soaked in BPA solutions with  $1.0 \times 10^{-6}$  M CTAB for 4 minutes prior to the LSV experiments.

**Detection of BPA.** Under the optimal conditions studied previously, a calibration curve for BPA has been constructed by plotting the anodic peak current as a function of BPA concentration. As shown in Figure 5, the CNT–titania–Nafion/GC electrode gave a linear response ( $r^2 = 0.999$ ) to BPA from  $1.0 \times 10^{-8}$  M to  $5.0 \times 10^{-6}$  M with a detection limit of  $9.0 \times 10^{-10}$  M.



**Figure 5.** Calibration curves of BPA at the CNT–titania–Nafion/GC electrode from  $1.0 \times 10^{-8}$  M to  $5.0 \times 10^{-6}$  M in the presence of  $1.0 \times 10^{-6}$  M CTAB, Scan range: 0.3–0.8 V, Scan rate: 100 mV/sec. Inset: Calibration curves of BPA at the CNT–titania–Nafion/GC electrode from  $1.0 \times 10^{-9}$  M to  $1.0 \times 10^{-8}$  M.

The detection limit obtained with the present method is lower than those reported values in the literature as summarized in Table 1. For example, the detection limits obtained with cobalt phthalocyanine–CPE/DPV<sup>10</sup> and PAMAM–Fe<sub>3</sub>O<sub>4</sub>–GCE/amperometry method<sup>13</sup> were  $1.0 \times 10^{-8}$  M and  $5.0 \times 10^{-9}$  M, respectively. While, the detection limits obtained with CTAB–CPE/DPV<sup>9</sup> and CNT–GCE/LSV<sup>15</sup> were  $7.5 \times 10^{-9}$  M and  $5.0 \times 10^{-9}$  M, respectively.

The reproducibility of this method was relatively good with a relative standard deviation (RSD) of 3.2% for the repeated measurement of  $1 \times 10^7$  M BPA ( $n = 7$ ).

**Interference and Real Sample Test.** In order to verify the selectivity of the present electrochemical BPA sensors, the effect of various possible interferents on the electrochemical response of BPA has been examined. As shown Table 2, some ions such as Na<sup>+</sup>, Fe<sup>2+</sup>, Ca<sup>2+</sup>, K<sup>+</sup>, NO<sub>3</sub><sup>-</sup>, Cl<sup>-</sup> with concentrations 20 times higher than that of BPA ( $1.0 \times 10^{-5}$  M) exhibited negligible effect on the detection of BPA (signal change < 2%). When the concentrations of interfering ions were 200 times higher than that of BPA, the oxidation current of BPA decreased to 78% of the initial current. This result could be attributed to that the ionic exchange sites in Nafion might be occupied by interfering cations, which prevent CTAB, preconcentration medium for BPA, from being

**Table 1.** Performance comparison of bisphenol A detection at different detection methods

Electrode material / Method	Linear range (M)	Detection limit (M)	Reference
PAMAM–Fe <sub>3</sub> O <sub>4</sub> / GCE* / Amperometry	$1.0 \times 10^{-8}$ – $3.1 \times 10^{-6}$	$5.0 \times 10^{-9}$	13
Cobalt phthalocyanine / CPE* / DPV*	$8.8 \times 10^{-8}$ – $1.3 \times 10^{-5}$	$1.0 \times 10^{-8}$	10
MWCN* / GCE / LSV*	$1.0 \times 10^{-9}$ – $1.0 \times 10^{-8}$	$5.0 \times 10^{-9}$	15
CTAB / CPE / DPV*	$2.5 \times 10^{-8}$ – $1.0 \times 10^{-6}$	$7.5 \times 10^{-9}$	9
Nickel tetraamino phthalocyanine / GE* / CV*	$7.0 \times 10^{-4}$ – $3.0 \times 10^{-2}$	$3.7 \times 10^{-9}$	11
Titania–Nafion–MWCN / LSV	$1.0 \times 10^{-8}$ – $5.0 \times 10^{-6}$	$9.0 \times 10^{-10}$	This work

GCE = Glassy carbon electrode, CPE = Carbon paste electrode, MWCN = Multi wall carbon nanotube, LSV = Linear sweep voltammetry, DPV = Differential pulse voltammetry, GE = Gold electrode, CV = Cyclic voltammetry.

**Table 2.** Interference effect of other species on the detection of  $1.0 \times 10^{-5}$  M BPA

Interference	Concentration (M)	Current change (%)
Na <sup>+</sup>	$2.0 \times 10^{-4}$	-0.60
Fe <sup>3+</sup>	$2.0 \times 10^{-4}$	-1.79
Ca <sup>2+</sup>	$2.0 \times 10^{-4}$	-1.80
K <sup>+</sup>	$2.0 \times 10^{-4}$	1.16
NO <sub>3</sub> <sup>-</sup>	$2.0 \times 10^{-4}$	-1.78
Cl <sup>-</sup>	$2.0 \times 10^{-4}$	-1.80
Phenolphthalein	$2.0 \times 10^{-4}$	-1.23
β-phenylethylamine	$2.0 \times 10^{-4}$	1.01
Ascorbic acid	$2.0 \times 10^{-4}$	1.81

immobilized in Nafion. It was also found that 20-fold concentrated phenolphthalein and β-phenylethylamine having similar structure to BPA had no influence on the BPA detection. In addition, ascorbic acid also did not affect the BPA detection, thus could be used for the determination of BPA in biological fluids. To demonstrate the practical usage of the present electrochemical BPA sensor, the recovery test of BPA in plastic food packaging samples has been performed using standard addition method. The test for the spiked BPA of  $5.0 \times 10^{-7}$  M was satisfactory with the recovery of  $99.57 \pm 2.01\%$ . From these results, it is expected that the present electrochemical sensor can be employed to the determination of BPA in real samples.

### Conclusions

A sensitive and simple electrochemical detection method has been proposed for the determination of BPA. Due to the large pore size of the composite film of sol-gel-derived titania–Nafion film as well as the excellent electrocatalytic activity of CNT towards BPA, the present BPA sensor based on the CNT–titania–Nafion/GC electrode displayed a reasonably good sensitivity along with a good detection limit of  $9.0 \times 10^{-10}$  M (S/N = 3), which is lower compared to those obtained at the other detection methods. The present BPA sensor shows good selectivity and thus the practical application of BPA in food package samples was satisfactory with good recovery. The good sensitivity of the present BPA sensor along with its good selectivity and reproducibility might allow us for its potential use in the determination of BPA as well as estrogen hormones having similar chemical structure in real biological and environmental samples.

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