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Electrochemical determination of bisphenol A using polyacrylamide-multiwalled carbon nanotubes modified glassy carbon electrode

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Abstract

A novel electrochemical sensor was fabricated for determination of bisphenol A (BPA) basing on a signal amplification strategy with polyacrylamide and multi-walled carbon nanotubes (PAM-MWCNTs). The modified electrode significantly enhanced the oxidation peak current and lowered the oxidation overpotential to improve the sensitivity. Several important parameters which can influence the performance of the sensor were studied and optimized. The sensor showed fast and sensitive response for the quantitative determination of BPA under the optimum condition. It exhibited a good linear relationship range from 5.0 nmol·L\(^{-1}\) to 20 µmol·L\(^{-1}\) and the detection limit was 1.7 nmol·L\(^{-1}\) (S/N = 3). The sensor exhibited remarkable sensitivity (3.830 µA (µmol·L\(^{-1}\))\(^{-1}\)). Furthermore, the fabricated sensor showed an excellent result for the detection of BPA in the milk examples.

Keywords: Bisphenol A; Polyacrylamide; Multi-walled carbon nanotubes; Electrochemical sensor; Linear Sweep Voltammetry.
1. Introduction

Bisphenol A (BPA) is a key monomer in production of polycarbonate plastic, phenol resins, epoxy resins, polyacrylates and lacquer coatings on drink and food cans\textsuperscript{1-3}. Thus, it as industrial chemical is used widely in many industries. However, BPA, belonging to endocrine-disrupting chemicals (EDCs), is one kind of endocrine disrupting chemicals. Many reports found that BPA exposed in the environment would be a threat to human health. BPA is postulated that it can cause reproductive disorders including the decrease of sperm quality in humans, birth defects due to its fetal exposure and various kinds of cancers, such as prostate, testicular, and breast cancer\textsuperscript{4}. At the same time several reports figured that BPA could migrate into environment via the manufacturing process of plastics and the degradation process of waste plastics\textsuperscript{5, 6}. BPA also can pass to the baby through the mother\textsuperscript{7}. Therefore, it is critical to establish a set of fast, simple and effective analytical methods to monitor the trace amounts of BPA.

At present, many analytical technologies have been established for BPA detection, such as high-performance liquid chromatography (HPLC)\textsuperscript{8}, gas chromatography-mass spectrometry (GC-MS)\textsuperscript{9}, liquid chromatography (LC)\textsuperscript{10}, and enzyme linked immunosorbent assays (ELISA)\textsuperscript{11}. Among these analytical technologies, chromatographic
technique exhibits good precision and high sensitivity, however, it requires quite expensive and complex equipment, skilled operators and time-consuming pretreatment steps. Thus, it is not the most ideal detection method for BPA. On this occasion, electrochemical sensor has great potential for environmental monitoring due to its advantages of cheap instruments, simple operation, low cost, short analysis time, fast response, reliability, good selectivity, and high sensitivity\textsuperscript{12}. The electrochemical sensor can be used in the work own to the good electrochemical activity of BPA, it can be detected triumphantly by electrochemical oxidation\textsuperscript{13-17}. However, the electrochemical oxidation of BPA is irreversible and requires overpotentials, it is not feasible to test the BPA directly using a bare electrode\textsuperscript{18}. Therefore, the modified electrode is a better candidate in the BPA detection.

Various modified materials have been employed to develop electrochemical sensor for determination of BPA. Among these materials, multiwalled carbon nanotubes (MWCNTs) have attracted much attention to the researchers due to their unique structures, good biocompatibility, excellent electrical conductivity, high surface area, and chemical stability\textsuperscript{19, 20}, more than that their large length-to-diameter ratio offers a large surface-to-volume ratio, leading to a good catalytic property to BPA\textsuperscript{21}. This ability is expressed as an improvement in the reversibility of electrochemical reactions and an increase in the rate of electron transfer,
leading to a reduction of working potential and an increase of current signal\textsuperscript{22}. Thus MWCNTs are chosen as the electrode material in the research.

However, it is a key point to solve the inherent hydrophobic. Polyacrylamide (PAM) is efficient and flocculant and an important water-soluble polymer. And that PAM has many excellent characteristics, such as flocculation, adsorption, bridging, surface adsorption and potentiation. It also can increase the amount of amino groups on the surface of the electrode when PAM was fixed on the electrode\textsuperscript{23, 24}. These properties can make materials dispersed more evenly, and can be a better fixed on the electrode surface. Thus, it is chosen as the electrode-modified material to disperse MWCNTs in water.

The aim of the study is to design a kind of sensor with the advantage of high sensitivity and ease to fabricate to realize the quantitative detection of BPA in milk. In the work, the addition of BPA can improve the dispersibility of MWCNTs in water. Thereby, the electrochemistry signal increased because more superficial area can contact with the solution when PAM-MWCNTs solution was drop onto the surface of GCE. Based on the above advantages, the sensor has better sensitivity and lower detection limit.

2. Experimental

2.1 Reagents and materials
BPA was purchased from Tianjin Guangfu Fine Chemical Research Institute; MWCNTs were purchased from Sigma Aldrich; PAM was purchased from Shanghai Green Chemical Technology Co.; Phosphate buffer solution (PBS) was compound by mixing the stock solution of 1/15 mol·L⁻¹ Na₂HPO₄ and 1/15 mol·L⁻¹ KH₂PO₄ to appropriate pH value. Ultrapure water was used throughout the experiment. All the chemicals were analytical reagent grade and were used directly without further purification.

2.2. Apparatus

All electrochemical measurements were performed on a CHI 760D electrochemical workstation (Shanghai CH Instruments Co., China) with a conventional three-electrode system: a modified glassy carbon electrode (GCE, 4 mm in diameter) as the working electrode, a platinum wire electrode as the counter electrode, and a saturated calomel electrode (SCE) as the reference electrode. Electrochemical impedance spectroscopy (EIS) was performed in [Fe(CN)₆]³⁻/⁴⁻ working solution in the frequency range of 0.1~10⁵ Hz. pH measurements were performed with a pH metre (pH-3c Model, Shanghai Leichi Instrument Factory, Shanghai, China). The morphology of MWCNTs and PAM-WMCNTs were obtained by a scanning electron microscope (SEM, JSM-6700F microscope, JEOL, Japan).

2.3 Fabrication of electrochemical sensor
PAM was dispersed in ultrapure water as the solvent to disperse the MWCNTs (PAM-MWCNTs). PAM has strong flocculation effect, and the surface contains a lot of amino group, can well combine with MWCNTs. A series of concentrations of PAM-MWCNTs were prepared before the experiment.

Prior to modification, a bare GCE was polished to mirror-like with Al₂O₃ powder of 1.0, 0.3 and 0.05 µm in diameter respectively, and then washed thoroughly with ultrapure water. 6.0 µL of PAM-MWCNTs was dropped onto the GCE surface and dried at room temperature. The obtained electrode was noted as PAM-MWCNTs/GCE.

2.4 Milk samples preparation

Fresh bags of liquid milk samples were purchased from a local supermarket. The milk samples were prepared as the Previous literature⁹: 10.0 mL milk was mixed 20.0 mL anhydrous alcohol. After 15 min sonication and 10 min shaking, the mixture was centrifuged for 10 min, and then the supernatant was filtrated. The filtrate was collected and added into 50 mL volumetric flask, diluted with redistilled deionised water to the mark line. There is free of BPA for the existing milk in the market, thus we can spiked certain amounts of BPA standard solution to the sample directly.

3. Results and discussion

The morphologies of MWCNTs and PAM-MWCNTs were
characterized SEM. Unadorned MWCNTs were shown in Fig 1A. As shown in Fig 1B, separated nanotubes were observed for PAM-MWCNTs in contrast to the unadorned MWCNTs. The diameter of PAM-MWCNTs was larger than the MWCNTs, demonstrating that the PAM has already attached to the surface of MWCNTs. Fig 1C showed the FTIR spectra, In the spectra of MWCNTs (curve a), there are two peaks at 3500 cm\(^{-1}\) and 1500-1700 cm\(^{-1}\), indicating that the raw MWCNTs possess a small amount of -OH and C=O functional groups\(^{25}\). A peak was found at 1650 cm\(^{-1}\) of PAM-MWCNTs (curve b), which because of the stretching vibration of the –CONH\(_2\) group. 2930, 1680, 1450 and 1398 cm\(^{-1}\) were discovered peaks in the spectra of PAM, indicating that amount –CH, -C=O, -CH\(_2\) and –CN consist in PAM\(^{26}\).

Fig 1 SEM images of MWCNTs (A), PAM-MWCNTs (B) and FTIR (C): (a)
MWCNTs, (b) PAM-MWCNTs, (c) PAM.

3.1. Electrochemical characterizations

CV and EIS were used to record each immobilization step to verify the success of fabrication of the immunosensor. Cyclic voltammetry (CV) is a kind of convenient and effect method to monitor electrochemical behaviors. In this research, the scan rate was chosen as 100 mV·s⁻¹. Fig 2A shows the CV responses of bare GCE, PAM/GCE, MWCNTs/GCE and PAM-MWCNTs/GCE respectively in the solution of [Fe(CN)₆]₃⁻/⁴⁻ (1:1, 5.0 mmol·L⁻¹) with KCl (0.1 mol·L⁻¹). Obviously, the process is a quasi-reversible process at bare GCE and modified electrodes (Fig. 2A). The peak-to-peak separation increased and the current decreased after the PAM (Fig. 2A, curve a) incubating on the electrode than bare GCE (Fig. 2A, curve b). However, the redox peak current increased after the MWCNTs (Fig. 2A, curve c) and PAM-MWCNTs (Fig. 2A, curve d) dropping on the electrode surface. EIS was also used to characterize the interfacial properties of the modified electrode.

EIS, one of the most powerful tools to measure the characteristic of the surface-modified electrodes, was also used to monitor the fabrication of the electrode. The impedance spectra were made of two parts: a semicircle at high frequencies and a linear at lower frequencies. The semicircle part at high frequencies corresponds to the electron-transfer limited process and the linear portion at lower frequencies corresponds to
the diffusion. The semicircle diameter demonstrated the electron transfer resistance \( (R_{et}) \)\textsuperscript{27, 28}. Fig. 2B states Nyquist diagrams of GCE and modified electrode in \([\text{Fe(CN)}_6]^{3-}/^{4-}\) (1:1, 5.0 mmol·L\(^{-1}\)) solution containing KCl (0.1 mol·L\(^{-1}\)). A well-defined semicircle was observed at higher frequencies. It can found that PAM-MWCNTs had a minimum resistance (Fig. 2B, curve a) and the resistance of MWCNTs increased (Fig. 2B, curve b). Their resistances were lower than the bare GCE (Fig. 2B, curve c). However, the electrode modified with PAM had the maximum resistance (Fig. 2B, curve d). The phenomenon showed the same conclusion with the CV. The PAM hindered the electronic transmission and the PAM solution has better viscosity than water, thus it can disperse MWCNTs better. It can be verified from the Fig. 2C. Thus, the resistance further reduces of the PAM-MWCNTs/GCE. It indicated that the MWCNTs have good electrical conductivity and large surface area.
Fig. 2 (A) Cyclic voltammograms of (a) PAM, (b) bare GCE (c) MWCNTs and (d) PAM-MWCNTs/GCE in 5.0 mmol·L⁻¹ Fe(CN)₆³⁻/⁴⁻ (1:1) with scan rate 100 mV·s⁻¹. (B) Nyquist plots of different modifications in 5.0 mmol·L⁻¹ Fe(CN)₆³⁻/⁴⁻ (1:1) solution containing 0.1 mol·L⁻¹ KCl: (a) PAM-MWCNTs/GCE, (b) MWCNTs/GCE, (c) GCE, (d) PAM/GCE. (C) The contrast figure of MWCNT suspension. The MWCNTs suspension was dispersed with water (a, c) and the MWCNTs suspension was dispersed with PAM solution (b, d).

3.2. Electrochemical behaviors of BPA

The structure of BPA is presented in Fig. 3A. The electrooxidation of BPA at PAM-MWCNTs/GCE is a two-electron and two-proton process²⁹. Electrochemical behaviors in different electrodes without and in the presence of 10 µM BPA were investigated by Linear Sweep Voltammetry (LSV). As shown in the Fig 3B, the electrochemical response was not
found in the environment without BPA when the electrode was modified by PAM-MWCNTs (curve a). The electrochemical response was lower than the bare GCE (curve c) when only the PAM (curve b) was dropped into the surface of the electrode, indicating that the PAM hindered the electron transfer rate. When the MWCNTs were dropped into the surface of the electrode, a larger electrochemical response was detected (curve c). The electrochemical response at PAM-MWCNTs for BPA was bigger than all other materials above under the same experimental conditions (curve d). The results revealed that PAM-MWCNTs played an important role in electrocatalytic oxidation of BPA through the addition of PAM to improve the dispersibility of MWCNTs in water.

The influence of the scan rate on the electrochemical behaviors of BPA was investigated by LSV. It was found that the current response increase linearly with the square root of scan rate from 50 to 400 mV·s⁻¹ (Fig 3C). The linear regression equation is $I_{pa} (\mu A) = 169.06 \nu^{1/2} (V·s^{-1}) - 16.793$ ($R = 0.9918$). It shows that the oxidation of BPA on PAM-MWCNTs/GCE is a representative diffusion-controlled process. For the further study, we found a liner relationship between $E_{pa}$ and $\ln \nu$ (Fig 3D). The equation is shown as followed: $E_{pa} (V) =0.029 \ln \nu + 0.553$ ($R = 0.9896$). As for a totally irreversible electrode process, $E_{pa}$ is defined by a equation$^{6,10,30}$:

$$E_{pa} = E^{\circ} + \frac{RT}{anF} \ln \frac{RTk^{\circ}/anF}{RTk^{\circ}/anF} + \frac{RT}{anF} \ln \nu$$
Where $\alpha$ is transfer coefficient, $R$, $T$ and $F$ have their usual meanings, $E^0$ is formal redox potential. $n$ is the number of transfer electron. $\alpha$ is assumed to be 0.5 in a totally irreversible electrode process. $k^0$ is standard rate constant in the reaction. Thus, the number of transfer electron ($n$) for oxidation of BPA is about 2. The pH effect on $E_{pa}$ demonstrated that the number of electrons and protons involved in the BPA oxidation process is equal. Thus, the electrochemical oxidation of BPA at PAM-MWCNTs/GCE is a two-electron and two-proton process\(^6,10,31\).

Fig 3 (A) The structure of BPA, (B) LSV of PAM-MWCNTs/GCE without BPA and LSVs of PAM/GCE (b), bare GCE (c), MWCNTs/GCE (d), PAM-MWCNTs/GCE (e) in the presence of 10 µM BPA. (C) The oxidation peak current on the square root of the scan rate. (D) The relationship between $E_{pa}$ and $\ln v$ in PBS (pH 7.0) containing 


10 µmol L\(^{-1}\) BPA (v=100 mV·s\(^{-1}\)). Other conditions are the same.

3.3. Optimization of experimental conditions

For the sensor, the concentration of PAM and MWCNTs are the critical conditions on its sensitivity. Different concentrations of PAM and MWCNTs were prepared to fabricate the sensor in pH = 7.0 PBS. As shown in Fig 4, the current changes reached to maximum at 2.5 mg·mL\(^{-1}\) of MWCNTs and 1.0 mg·mL\(^{-1}\) of PAM. This combination was chosen as the optimal condition to carry out the experiment.

Fig 4 the effect of concentration of MWCNTs (A) and PAM (B), other conditions are the same.

The pH effect of the PBS on the electrochemical response of PAM-MWCNTs/GCE to BPA was investigated over the pH ranging from 4.5 to 9.0 (Fig 5A). The oxidation peak current gradually increased with increase of pH value from 4.5 to 7.0. While the pH value exceeded 7.0, the oxidation current decreased (Fig. 5B, curve b). The maximum response of pH is lower than the p\(_{Ka}\) of BPA (p\(_{Ka}\) = 9.73), which suggests that in this way the non-dissociated BPA can be adsorbed better than the
dissociated BPA on the surface of modified electrode\textsuperscript{32}. Therefore, in consideration of the sensitivity of BPA determination, the pH value of 7.0 in PBS was chosen at all voltammetric detection.

The relationship between pH and the oxidation peak potential ($E_{pa}$) was also presented in Fig. 5B, curve a. The peak potential shifted negatively with the increase of pH value. The linear relationship was calculated between the $E_{pa}$ and pH, the equation as follows:

$$E_{pa} (V) = -0.0620 \text{pH} + 0.957 \ (R=0.9949)$$

The slope of the equation is close to the theoretical value of -0.0576 $(V \cdot \text{pH}^{-1})$\textsuperscript{6,10,33}. It indicates that the electron transfer was accompanied by an equal number of electrons and proton.

Fig 5 (A) LSV of BPA (10 µmol·L\textsuperscript{-1}) at PAM-MWCNTs/GCE under different pH. a-i: 4.5-9; (B) the potential and current response of BPA (10 µmol·L\textsuperscript{-1}) at different pH in PBS. Other conditions are the same.

3.5. Effect of accumulation time and potential

The response current of BPA linearly increases with the increase of accumulation time from 0 to 300 s. Accumulation could increase the
content of BPA on the modified electrode and then enhance the oxidation peak current of BPA. However, the oxidation signal of BPA increased slightly, along with the further increasing of accumulation time. The phenomenon shows that the amount of BPA has reached a limit value on the surface of the modified electrode. Thus 180 s was chosen as the optimum accumulation time (Fig 6A)\textsuperscript{34, 35}. Potential was another important factor for the accumulation of BPA on the electrode surface. The current response increased with the increasing of potential from -1.0 V to -0.1 V and decreased dramatically when the applied potential further increase from -0.1 V to 0.8 V (Fig 6B). Therefore, the optimal accumulation potential was chosen as -0.1 V\textsuperscript{36}.

![Fig 6 Dependence of current response for BPA (10 µmol·L\textsuperscript{-1}) oxidation on (A) accumulation time and (B) accumulation potential, other conditions are the same.](image)

3.6. Detection of BPA

In the system, LSV was chosen as the main tool to study the relationship between current response and the concentration of BPA under the optimum conditions. Fig. 7A showed the LSV curves of at the
PAM-MWCNTs/GCE at various concentrations. Fig. 7B showed the linear relation between current response and the concentrations of BPA. The oxidation peak current linearly increased linearly with the increase of concentrations of BPA (0.005-20 µmol·L⁻¹). The equation of the calibration curve is \( I (\mu A) = 3.830c (\mu mol \cdot L^{-1}) + 1.273 \) (\( R = 0.9978 \)) with a low limit of 1.7 nmol·L⁻¹. The excellent result was attributed to the factor: PAM solution was used to disperse the MWCNTs to improve the dispersibility. Thus, the sensor can take full advantage of the electroconductibility and catalytic performance of MWCNTs. The remarkable sensitivity (3.830 µA (\( \mu mol \cdot L^{-1} \))⁻¹) was more beneficial to detect the BPA at low concentrations.

![Graph](image)

Fig. 7 (A) LSV curves of PAM-MWCNTs in BPA solution at different concentrations (a-k: 5 nmol·L⁻¹ - 20 µmol·L⁻¹). (B) is the relationship between the oxidation peak current at different concentrations of BPA.

3.7. Reusability, reproducibility, selectivity, and stability

In order to verify the reusability, a electrode was used repeatedly five times to test the same concentration of BPA. The fifth
electrochemical signals drop less than 10 %, indicating a good reusability for the sensor. The reproducibility was investigated by detecting 10 μM BPA for use five PAM-MWCNTs modified electrodes. The relative standard deviation (RSD) for the oxidation peak currents is 3.68 %, revealing an acceptable reproducibility. In order to verify the selectivity of the electrochemical sensor, BPA was measured at the present of some possible interfering substances in pH 7.0 PBS buffer. As shown in table 1, it can be found that 50-fold concentrations of hydroquinone, phenol, p-nitrophenol, 1-Naphthol, pyrocatechol, 2,5-dinitrophenol, Cl$^-$, Na$^+$, Cu$^{2+}$, Mg$^{2+}$, Zn$^{2+}$, Ca$^{2+}$, Al$^{3+}$, Fe$^{3+}$, NO$_3^-$, SO$_4^{2-}$, PO$_4^{3-}$ than BPA and have no obvious influence on the signals of BPA with deviations below 10%.

The stability of PAM-MWCNTs/GCE was also checked. After two weeks, the peak current of 10 nmol·L$^{-1}$ BPA retained 97.2 % of the initial response, indicating the stability was satisfactory.

<table>
<thead>
<tr>
<th>Interferents</th>
<th>Concentration (μmol·L$^{-1}$)</th>
<th>$I_{pa}$ change (%)</th>
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<tr>
<td>Cl$^-$</td>
<td>500</td>
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<tr>
<td>Na$^+$</td>
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<td>Zn$^{2+}$</td>
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</table>
3.8. Practical application

To evaluate the sensitivity and applicability of the novel method, the fabricated PAM-MWCNTs/GCE was used to determine BPA in the milk samples. Under the optimized conditions, a certain-amount of sample solution was added into PBS, and then analyzed according to the above-described procedures. The results were represented in Table 2 in different samples. BPA was not detected in milk samples. We achieved recoveries were 95.7% - 105.0%, indicating that the fabricated sensor had excellent selective, accurate and sensitive for practical application.

Table 2 Determination of BPA in the milk samples

<table>
<thead>
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<th>Ion</th>
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<tr>
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<td>Found (10⁶mol·L⁻¹)</td>
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<td>---------------------</td>
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a Mean of seven parallel measurements.

**Conclusion**

In the study, MWCNTs and PAM were introduced to fabricate the sensor for the detection of BPA as the electrode material. PAM solution was used to disperse the MWCNTs to improve the dispersibility. Based on the strategy, the sensor can take full advantage of the electroconductibility and catalytic performance of MWCNTs. Thus, the fabricated sensor showed remarkable sensitivity (3.830 µA (µmol·L⁻¹)⁻¹), high selectivity, good reusability, excellent accuracy, wide linear range and low limit of detection. The method was also applied to detect the content of BPA in practical samples successfully. The sensor was easy to fabricate that used the raw MWCNTs directly without further purification. Thereby, the proposed method offers a kind of potential application method for monitoring the BPA in the food and environment.

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